

European Society of Veterinary Pathology

26TH ANNUAL MEETING

PROGRAMME AND BOOK OF ABSTRACTS

Veterinary Faculty, University of Zagreb



Hotel Palace, Dubrovnik, Croatia, 17 - 21 September 2008

European Society of Veterinary Pathology

26th Meeting

17 -21 September, 2007

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Organizers:

European Society of Veterinary Pathology;
Veterinary Faculty, University of Zagreb

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Welcome from the Chair of the Local Organizing Committee

On behalf of the Organizing and Scientific Committees, Veterinary Faculty of the Zagreb University, European Society of Veterinary Pathology, Ministry of Science, Education and Sports and Ministry of Agriculture, Fisheries and Rural Development of the Republic of Croatia it is my honor to welcome you to Dubrovnik for 26th Meeting of the European Society of Veterinary Pathology.

Our Meeting was and it still is the most important scientific event in the field of Veterinary Pathology which is recognized every year by couple of hundred veterinary pathologists not only from Europe but from all over the world. This tendency is especially present this year, and all of us are very pleased because of that. All organizers made an effort to review the scientific contributions of the submitted papers and to organize it in the adequate sections. The best and/or most interesting works will be orally presented, and the Scientific Committee decides to give more time for the oral lectures. We also hope that you will find that invited key lectures reflect our goal considering most challenging current topics in the veterinary pathology.

City of Dubrovnik almost needs no presentation. Conference venue is close to the old city and I hope that every participant will find some extra time for him- or herself to visit and see what Dubrovnik offers to those who decide to give little bit more attention to this mystic, ancient place.

Local Organizing Committee did everything in its power to make your stay here pleasant, scientifically stimulating and socially important because every one and each of us is looking forward to meet old friends and to get new ones.

Chair of the Local Organizing Committee

Željko Grabarević

**Programme of the 26th Annual Meeting of the European Society
of Veterinary Pathology
Hotel Palace, 17-21 September 2008, Dubrovnik, Croatia**

Wednesday, 17.09.2008.	
16:00	Registration (Floor T)
18:30	Opening ceremony at the Conference venue, main meeting room, welcome speech. (Floor TT, Hotel Dubrovnik Palace)
19:00	Welcome party (Sunset lounge bar, Hotel reception floor)
21:00	End of the party

Thursday, 18.09.2008.	
Hall A	Hall B
8:30-9:30	Keynote lecture - Ted van den Ingh: Morphological Classification of Parenchymal Disorders of the Liver / Hall A

9:30-10:30	<p>Poster Tour A - <u>Haemopathology, Cardiovascular System, Modern Methods</u></p> <p>1. The role of <i>Bartonella henselae</i> in cats with immunocompromising conditions <u>Olivia Kershaw</u>, Alexandra U. Buchmann, Achim D. Gruber P1</p> <p>2. A case of "extranodal NK/T-cell lymphoma, nasal type" in a Rottweiler <u>Chiara Brachelente</u>, Elvio Lepri, Monica Sforna, Giovanni Ricci, Alfredo Dentini, Daniela Mignacca, Luca Mechelli P2</p> <p>3. Differentiation of canine lymphatic and blood vascular endothelial tumours by co-expression of LYVE-1 and CD31 <u>Christiane Krudewig</u>, Sheena Warman, Jan Rybnicek and Michael J. Day P3</p> <p>4. Expression of eosinophil chemotactic factors eotaxin and IL-5 in mast cell tumours: relation with stromal eosinophilia and prognosis Justina P. Oliveira, Anabela Alves, <u>Laura Peña</u> P4</p> <p>5. Prognostic significance of matrix metalloproteinases MMP-2 and MMP-9 immunoexpression in canine mast cell tumors Justina P. Oliveira, Anabela Alves, <u>Laura Peña</u> P5</p> <p>6. Malignant lymphoma with intrahepatocellular invasion of neoplastic cells in the cats <u>Isao Narana</u>, Naoko Ano, Kohji Nomura and Kiyokazu Ozaki P6</p> <p>7. Pathological findings in 11 dogs with naturally occurring fatal heatstroke <u>Emmanuel Loeb</u>, Itamar Aroch, Joseph Saragusty and Yaron Bruchim P7</p> <p>8. Immunohistochemical demonstration of homeobox proteins in canine vascular tumours <u>Atsushi Kodama</u>, Hiroki Sakai, Mami Murakami, Takashi Mori, Kohji Maruo, Tokuma Yanai and Toshiaki Masegi P8</p> <p>9. Myocardial lesions and objective evaluation of arteriolar hypertrophy in Wistar rats with streptozotocin-induced diabetes mellitus type 1 and adriamycin-induced congestive cardiac failure <u>Emilia Ciobotaru</u>, Manuella Militaru, Teodoru Soare, Georgeta Dinescu, Daniela Elena Braslasu P9</p>	<p>Poster Tour B - <u>Gastrointestinal Tract, Infectious Diseases</u></p> <p>1. Blackhead disease-like typhlohepatitis in two Red-breasted Mergansers (<i>Mergus serrator</i>) caused by tetratrichomonads <u>Barbara Richter</u>, Christoph Schulze, Jens Kämmerling, Herbert Weissenböck P22</p> <p>2. Colorectal hamartomatous polyp associated with diffuse ganglioneuromatosis in a puppy: a canine model of Cowden's syndrome in humans? Ingrid Bemelmans, Sébastien Küry, Olivier Albaric, Frédérique Nguyen, Catherine André, <u>Jérôme Abadie</u> P23</p> <p>3. A case of gastric carcinoma with osseous metaplasia in a dog <u>Irina Amorim</u>, Célia Lopes, Augusto Rui M. Gil da Costa, Fátima Gärtner, M. R. Faustino, Patrícia Dias Pereira P24</p> <p>4. E-cadherin-β-catenin expression in canine colorectal adenocarcinoma: a suggested role upon local invasion <u>Luca Aresu</u>, Bartolomeo Biolatti, Paola Pregel, Laura Cavicchioli, Rossella Zanetti, Massimo Castagnaro P25</p> <p>5. Feline oral squamous cell carcinoma: an 'osteogenic' type? <u>Elvio Lepri</u>, Chiara Brachelente, Leonardo Leonardi, Monica Sforna, Giovanni Vitellozzi P26</p> <p>6. Metastatic salivary gland adenocarcinoma in a dog <u>Tolga Guvenc</u>, Ahmet Özak, Mustafa Yavuz Gulbahar, Murat Yarim, Yonca Betil Kabak, Onder Karayigit P27</p> <p>7. Histology of inflammatory bowel diseases (IBD) in Interleukin-10 deficient and TNFαARE mutant mice <u>Gabriele Hölzlwimmer</u>, Irene Esposito, Dirk Haller, Leticia Quintanilla-Martinez P28</p> <p>8. Gastrointestinal stromal tumour in a guinea pig <u>Frantisek Jelinek</u>, Pavel Hron, Frantiska Hozmanova P29</p> <p>9. Pathological findings associated with physalopterid larvae in <i>Cordylus tropidosternum</i> (Tropical Girdled Lizard) MJ Ruiz, CR Jiménez, R Zafra, <u>E Mozos</u> P30</p> <p>10. An unusual squamous cell carcinoma in a sheep <u>Namjo, A.</u>, Nourani, H. and Farid, M. P31</p> <p>11. Distribution of AA amyloid in naturally affected sheep and goats: The gut as a target organ <u>Neila Alvarez</u>, Tatiana Crespo, Eider Salazar, Marta Pérez, Esther Biescas, Lluís Luján P32</p>
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<p>10:30-10:45</p>	<p>10. Objective evaluation of the intrinsic cardiac response in Wistar rats with streptozotocin-induced diabetes mellitus type 1 and adriamycin-induced congestive cardiac failure Georgeta Dinescu, Emilia Ciobotaru, Manuella Militaru, Teodoru Soare, Cornel Mihai Brăslășu P10</p> <p>11. Septic pericarditis and cardiac tamponade associated with pulmonary botryomycosis in a dog Domingo Casamian, Jon Shippam, Becky Woodward, Dominique Fournier P11</p> <p>12. Myointimal cell formation in canine pulmonary arteriosclerosis Udo Hetzel, Simon Swift, Sonja Fonfara, Julie Wayne P12</p> <p>13. NMDA inhibits kainate-induced arteritis in hearts of mice through activation of NMDA receptor Kiyokazu Ozaki, Tomoya Sano, Tetsuro Matuura, Isao Narama P13</p> <p>14. Accumulation of advanced glycation end products in canine atherosclerosis Valerie Vandenberghe, Sara Van der Heyden, Koen Chiers, Richard Ducatelle P14</p> <p>15. Detection and characterization of chondroid metaplasia in canine atrioventricular valves Heike Aupperle, Imke März, Heinz-Adolf Schoon P15</p> <p>16. The expression of transforming growth factor β1, β2, and β3 in normal canine mitral valves and their role in chronic valve disease Heike Aupperle, Imke März, Jens Thielebein, Heinz-Adolf Schoon P16</p> <p>17. Role of CLCA proteins in mucus homeostasis in diseases with secretory dysfunctions. Melanie K. Bothe, Josephine Braun, Friederike Range, Lars Mundhenk, Achim D. Gruber P17</p> <p>18. The immunohistological detection of feline CD8⁺ T Cells in paraffin-embedded lymphoid tissues using the HOPE fixation technique Gisa Schmiedeknecht, Manfred Reinacher P18</p> <p>19. Zebrafish as animal model for human mismatch repair deficiency Harma Feitsma, Raoul Kuiper, Jeroen Korving, Isaac Nijman, Edwin Cuppen P19</p> <p>20. Immunocytochemical evaluation of the cell-cycle regulatory proteins (p53, p21, p16) in a case of multiple primary tumors in a Boxer dog Carlos M. Martínez, Alberto Benito, Juan Manuel Corpa P20</p> <p>21. Flow cytometric analysis of dendritic cells in peripheral blood of healthy and BLV infected cattle. Maria Szczołka, Jacek Kuźmak P20</p>	<p>12. Acanthocephalans in southern california pinnipeds Suzana Tkalcic, Vanessa Fravel, Jennifer Boardman, Lauren Palmer P33</p> <p>13. First asian report of spontaneous chytridiomycosis in captive frogs Yumi Une, Syou Kadekaru, Kenichi Tamukai, Kouichi Goka, and Toshiro Kuroki P34</p> <p>14. Investigation on histopathological findings and serological methods for evidence of leptospiral abortion in Tehran dairy herds Moayer Fariborz, Ourang Ataei, Farhad Moosakhani P35</p> <p>15. Evaluation of a three-dimensional culture of equine guttural pouches to study interactions between <i>Streptococcus equi</i> and mucopolysaccharides: preliminary results Maria Arias, Gian Enrico Magi, Francesco Parillo, Vincenzo Cuteri, Giacomo Renzoni P36</p> <p>16. Ectopic paragonimiasis in a dog Hiroo Madarame, Haruhiko Suzuki, Masahiro Tachibana, Yasuhide Saitoh, Shigehisa Habe, Akihiko Uchida, Hiromu Sugiyama P37</p> <p>17. Preliminary findings in a case of Onchocercosis in Italian wild red deer (<i>Cervus elaphus</i>). Federico Morandi, Roberta Galuppi, Sandro Nicoloso, Barbara Brunetti, Barbara Bacci, Giuseppe Sarli, Renato Giulio Zanoni, Maria Paola Tampieri, Cinzia Benazzi P38</p> <p>18. A rare case of septicemia due to <i>Bacteroides melaninogenicus</i> in a Holstein cow Ahmad Reza Movasseghi, Mehrnaz Rad P39</p> <p>19. Quality and quantity assessment of potentially pathogenic bacteria in aquaculture Agnieszka Skowrońska, Karol Korzekwa, Ewelina Sosnowska and Izabella Zmysłowska P40</p> <p>20. <i>In vivo</i> and <i>in vitro</i> study of phagocytic function during ovine paratuberculosis Giacomo Rossi, Laura Bonfili, Mauro Angeletti, Gian Enrico Magi, Ennio Taccini, Giacomo Renzoni, and Anna Maria Eleuteri P41</p>
	Coffe break	

10:45-12:15	Oral presentations/parallel sessions - Liver pathology	Oral presentations/parallel sessions - Infectious diseases-general
10:45	1. Pathological and morphometric study of the liver of goats immunized with recombinant thioredoxin peroxidase and challenged with <i>Fasciola hepatica</i> . <u>Alejandro Pérez-Écija</u> , Rafael Zafra, Leandro Buffoni, Ricardo Mendes, Francisco-Javier Martínez-Moreno, Alvaro Martínez-Moreno, José Pérez	1. Studies on the coinfection with scrapie and visna-maedi virus in ovine natural cases. <u>Eider Salazar</u> , Pérez Marta, Marta Monzón, Crsitina Acín, Rosa Bolea, Neila Álvarez, Eva Monleón, Beatriz Amorena, Juan José Badiola, Lluís Luján
11:00	2. Advanced glycation end products and their involvement in Turquoise Killifish (<i>Nothobranchius furzeri</i> , Jubb 1971) liver pathologies. <u>Emiliano Di Cicco</u> , Alessandro Cellerino, Eva Terzibasi, Giacomo Renzoni and Giacomo Rossi	2. Pathology of influenza virus H5N1 infection in resistant and susceptible laboratory mice. <u>Mutien-Marie Garigliany</u> , Karine Cloquette, Mickael Leroy, Mélanie Palm, Etienne Baise, Daniel Desmecht
11:15	3. Evaluation of the local immune response in goats immunized with a synthetic peptide of the Sm14 antigen and challenged with <i>Fasciola hepatica</i> . <u>José Pérez</u> , Alejandro Pérez-Écija, Leandro Buffoni, Alvaro Martínez-Moreno, Rafael Zafra	3. Immunodepression does not affect innate resistance of SJL mice to respiratory syncytial virus. <u>Glineur Stephanie</u> , Dermine Martin and Desmecht Daniel
11:30	4. Response of pet iguanas liver to injury. <u>Roman Halouzka</u> , Vladimír Jekl, Zdeněk Knotek	4. Systemic coronavirus-associated disease resembling feline infectious peritonitis in the domestic ferret (<i>Mustela putorius</i>). <u>Matti Kiupel</u> , Michael M. Garner, Annabel Wise, Katrina D. Ramsell, Roger Maes
11:45	5. Effect of fish technology production on the pathomorphological pattern of the liver in carp (<i>Cyprinus carpio</i> L.). Szarek Jozef, Skibniewska Krystyna, Guzior Janusz, <u>Mieszczynski Tomasz</u> , Babinska Izabella, Gesek Michal	5. Pathogenesis of a highly pathogenic avian influenza virus (H7N1) in chickens. <u>A. Ramis</u> , N. Busquets, F.X. Abad, R. Dolz, R. Valle, A. Chaves, A. Darji and N. Majó
12:00	6. The pathomorphological pattern of the liver in black-striped field mice living near the pesticide tomb, in Ilawskie Lake District, during its existence and three years after its liquidation. <u>Szarek Jozef</u> , Andrzej ewska Anna, Skibniewska Krystyna, Grzybowicz Mirosław, Guzior Janusz, Sawicka Kapusta Katarzyna, Babinska Izabella, Zakrzewska Marta, Zmyslowska Izabela	6. Early events of jaagsiekte sheep retrovirus infection in the ovine lung. <u>Henny Martineau</u> , Chris Cousens, Stuart Imlach, Massimo Palmarini, David J.Griffiths
12:15-13:45	Buffet light lunch	

13:45-14:45	<p style="text-align: center;">Poster Tour C - <u>Genital Tract</u></p> <ol style="list-style-type: none"> 1. The effect of astaxanthin in chemically induced mammary carcinogenesis in immature Wistar female rats <u>Gal Adrian Florin</u>, Sanda Andrei, Alecsandru Ioan Baba, Catoi Cornel, Rus Ioan, Taulescu Marian P42 2. Histological examination of the rat mammary gland: impact of tissue sampling and estrous cycle <u>Henning Hvid</u>, Inger Thorup, Martin Oleksiewicz, Henrik Jensen P43 3. Effect of growth hormone on recovery from testicular damage induced by methotrexate in rats. <u>Arash Khaki</u>, Marefat Ghaffari Novin, Amir Afshin Khaki, Mohamad Nouri, Chelar Cozanci P44 4. Immunohistochemical profile of one case of canine endometrial carcinoma Rita Payan-Carreira, Fernanda Seixas, <u>Maria dos Anjos Pires</u> P45 5. Feline endometrial carcinoma: evaluation of cytokeratins, vimentin and Ki 67 expression <u>Maria dos Anjos Pires</u>, João Ribas, Carlos Augusto, Rita Payan-Carreira P46 6. Identification of molecular phenotypes in canine mammary carcinomas with clinical implications: application of the human classification <u>Adelina Gama</u>, Anabela Alves, Fernando Schmitt P47 7. Cav-1 immunoexpression in canine mammary tissues <u>Irina Amorim</u>, Célia Lopes, Rui M. Gil da Costa, Augusto M. R. Faustino, Patrícia Dias Pereira P48 8. Survivin expression in canine mammary tumours <u>Laura Bongiovanni</u>, Daniela Malatesta, Alessandra D'Andrea, Leonardo Della Salda P49 9. Immunophenotypical changes associated with feline endometrial neoplastic transformation <u>Rui M. Gil da Costa</u>, Marta Santos, Irina Amorim, Célia Lopes, Patrícia Dias Pereira, Augusto Faustino P50 10. Immunodetection of CK5 and CK19 in normal and neoplastic canine mammary tissue <u>Cristina Cartuccia</u>, Giacomo Renzoni, Francesca Mariotti P51 11. A case of a complex mammary carcinoma in a male cat <u>Célia Lopes</u>, Irina Amorim, Patrícia Dias Pereira, Augusto Faustino P52 12. MMP-9 and TIMP-1 expression in feline endometrial carcinoma <u>Maria dos Anjos Pires</u>, Carlos Augusto, João Ribas, Rita Payan-Carreira P53 	<p style="text-align: center;">Poster Tour D - <u>Urinary Tract, Genital Tract, Retrospective Studies and Current Trends</u></p> <ol style="list-style-type: none"> 1. Immunohistochemical characterization of canine renal cell carcinomas <u>Rui M. Gil da Costa</u>, Justina P. Oliveira, Ana L. Saraiva, Fernanda Seixas, Fátima Faria, Fátima Gärtner, Maria A. Pires, Carlos Lopes P62 2. Acquired polycystic kidney disease in 4 African black rhinoceroses (<i>Diceros bicornis michaeli</i>) with renal haemochromatosis <u>Ruetten Maja</u>, Steinmetz Hanspeter, Claus Markus, Hatt Jean-Michel, Ossent Pete, Pospischil Andreas P63 3. Histopathological effects of morphine on kidney in rat embryo. <u>Afshin Zahedi</u>, Arash Khaki, Tahmineh Payrovi P64 4. Blockade of E- and P- selectin reduces neutrophil infiltration into the ischemia-reperfusion induced murine testis <u>Muzaffer Celebi</u>, Alberta G.A.Paul P65 5. Rhabdomyosarcoma in the urinary bladder of a dog <u>Milijan Jovanovic</u>, Zivka Eri, Aleksandar Spasojevic, Vladimir Kukolj, Milijana Knezevic P66 6. Changes in systemic and placental cytokine expression during infection with <i>Chlamydophila abortus</i> in two murine strains <u>Jose A Navarro</u>, Antonio J Buendía, Carlos M Martínez, Joaquin Sánchez, Maria R Caro, Nieves Ortega, Jesus Salinas P67 7. Histological and immunohistochemical development of methods to evaluate endometrial biopsies in mares with infertility <u>Nilsson, Ebba</u>, Westergren Ewa, Gavier-Widen Dolores P68 8. Pathological findings of primary xanthinuria in a family of wire-haired Dachshunds <u>Paola Roccabianca</u>, Paola Scarpa, Stefano Faverzani, Carolina Ramirez-Sanchez, Pietro Riccaboni, Giancarlo Avallone P69 9. Effects of unilateral nephrectomy and high-fat diet on diabetic nephropathy in alloxan-induced diabetic WBN/Kob rats <u>Mai Sato</u>, Kiyokazu Ozaki, Tomoya Sano, Tetsuro Matsuura, Isao Narama P70 10. Intersexuality in the dog – pathomorphological findings in true hermaphrodites and literature survey <u>Tanja Stassen</u>, Wolfram Breuer, Beate Kienzle, Werner Hecht, Walter Hermanns P71 11. Histopathological survey of cervicovaginal samples from Holstein dairy cows with or without reproductive tract disorders <u>Massoud Talebkhan Garoussi</u>, Farhang Sassani, Parviz Hovareshti P72
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	<p>13. Magnal adenocarcinoma in a budgerigar (<i>Melopsittacus undulatus</i>) <u>Murat Yarim</u>, M.Yavuz Gulbahar, Ahmet Ozak, Tolga Guvenc, Ozlem Nisbet, Yonca B. Kabak, Onder Karayigit P54</p> <p>14. Ovarian neoplasia in European bison (<i>Bison bonasus</i>). Case reports <u>Barbara Osirńska</u>, Maria Katkiewicz P55</p> <p>15. Immunohistochemical findings of uterine tumours in the rabbit <u>Annachiara Vinci</u>, Barbara Bacci, Cinzia Benazzi, Giuseppe Sarli P56</p> <p>16. Histopathological classification and immunohistochemical features of canine mammary tumors <u>Gye-Hyeong Woo</u>, Ha-Young Kim, Jung-Won Park, You-Chan Bae, Yi-Seok Joo, Cheong-Up Choi P57</p> <p>7. Estrogen receptors (ER) dependent breast cancer in mice and cellular model <u>Kishor Kumar S</u> and Mahesh Kumar M.J. P58</p> <p>18. Expression of leptin and leptin receptor (OB-R) in normal, hyperplastic, and neoplastic canine mammary tissues Ressel Lorenzo, Finotello Riccardo, Vannozi Iacopo, Innocenti Maria Viola, and <u>Poli Alessandro</u> P59</p> <p>19. Expression of PTEN in mammary tumours of dog and cat <u>Ressel Lorenzo</u>, Millanta Francesca., Caleri Elvanessa., Poli Alessandro P60</p> <p>20. Cyclooxygenase-2, EP2 receptor and microsomal prostaglandin E₂ synthase-1 expression in canine healthy, hyperplastic, and neoplastic mammary tissues <u>Millanta Francesca</u>, Canale Alberto, Ressel Lorenzo, Lorenzi Davide, Citi Simona, Poli Alessandro P61</p>	<p>12. First report of acute toxic nephrosis by leaves of Boxelder or Manitoba Maple (<i>Acer negundo</i>) in Greek sheep and goats <u>Dimitrios Tontis</u>, Dimitrios Doukas, John Edwards, Fotios Lykotrafitis, Maria Kritsepi-Konstantino P73</p> <p>13. Retrospective assessment of veterinary cytopathology and histopathology correlation studies of various organs <u>George Reppas</u>, Paul Canfield P74</p> <p>14. Spontaneous tumors in domestic hamsters <u>Hirota Kondo</u>, Mamoru Onuma, Hisashi Shibuya, and Tsuneo Sato P75</p> <p>15. Descriptive epidemiology of canine neoplasia: a retrospective study of a histopathology laboratory database (2000-2005, France, 67087 dogs). <u>Nguyen Frédérique</u>, Dessimoulie Anne-Sophie, Lagadic Marie, Albaric Olivier, Poujade Agnès, Abadie Jérôme P76</p> <p>16. Cancer registry of dogs and cats living in Venice and Vicenza provinces (Veneto region, north-eastern Italy) <u>Marta Vascellari</u>, Elisa Baioni, Antonio Carminato, Giuseppe Ru, Franco Mutinelli P77</p> <p>17. General overview of ocular lesions in dogs <u>Luliana Ionascu</u>, Manuella Militaru, Emilia Ciobotaru, Georgeta Dinescu, Teodoru Soare P78</p> <p>18. Current trends in veterinary pathology education: evidence based medicine and problem based learning <u>Suzana Tkalcic</u> P79</p> <p>19. Aspects of conflicts in poultry breeding in veterinary expert opinions in Poland <u>Gesek Michal</u>, Szarek Jozef, Babinska Izabella, Wojtacka Joanna, Mieszczynski Tomasz P80</p> <p>20. External quality assurance [EQA] in veterinary histopathology technique <u>Brian Kelly</u> P81</p>
14:45-15:45	Keynote lecture - Massimo Castagnaro: Feline mammary tumors in comparative oncology / Hall A	
15:45-16:00	Coffe break	
16:00-17:30	<p>Oral presentations/parallel sessions - Mammary tumors</p> <p>16:00</p> <p>1. Quantitative expression analyses of key molecular targets in microdissected canine mammary tumors and their metastases. <u>Robert Klopffleisch</u>, Achim D. Gruber</p> <p>2. Angiogenesis in canine inflammatory mammary cancer: increased lymphangiogenesis and overexpression of vascular endothelial growth factors A and D and COX-2. Mónica Clemente, Ana Rodríguez Sánchez-Archidona, Francesco Sassi, Paloma Jimena de Andrés, M^a Dolores Pérez-Alenza, Gema Silván, Juan Carlos Illera, Susana Dunner, <u>Laura Peña</u>.</p> <p>16:15</p>	<p>Oral presentations/parallel sessions - Infectious diseases-porcine</p> <p>1. Occurrence of porcine circovirus type 2 (PCV2) in cases of antibiotic non-responsive diarrhoea in pigs. <u>Anna Szczotka</u>, Katarzyna Podgórska, Jacek Żmudzki, Wojciech Kozaczyński, Zygmunt Pejsak, Tomasz Stadejek</p> <p>2. Ultrastructural findings of lymphoid tissues from postweaning multisystemic wasting syndrome affected pigs.t <u>Carolina Rodriguez-Cariño</u>, Joaquim Segales</p>

16:30	3. Galectin-3 expression in canine malignant mammary tumours. <u>Joana de Oliveira</u> , Joana Gomes, Augusto de Matos, Eva Héllmen, Emerson Bernardes, Fátima Gartner	3. Porcine necrotizing enteritis revisited: cellular targets for <i>Clostridium perfringens</i> beta-toxin. <u>Esther Sutter</u> , Marianne Wyder, Julien Miclard, Horst Posthaus
16:45	4. Dynamic relationship between Sialyl-Lewis x and E-cadherin expression in canine malignant/invasive mammary tumour phenotype. <u>Salomé S Pinho</u> , Augusto J F Matos, Célia Lopes, Nuno T. Marcos, Júlio Carvalheira, Mary L Alpaugh, Celso A Reis, Fátima Gärtner	4. Comparison of tissue and serum expression of IFN α and IFN γ during acute EU PRRSV infection. <u>Jaime Gómez-Laguna</u> , Francisco Javier Salguero, Inmaculada Barranco, María del Mar Fernández de Marco, Filip Barbé, Francisco José Pallarés, Librado Carrasco
17:00	5. Immunophenotypes of canine mammary carcinomas. Sassi F., Benazzi C., <u>Sarli G.</u>	5. Detection of porcine circovirus type 2 and viral replication in primary lymphoid organs from postweaning multisystemic wasting syndrome affected pigs.
17:15	6. Immunomarkers expression in feline mammary tumours. <u>Valentina Zappulli</u> , Diego Caliari, Roberta Rasotto, Massimo Castagnaro	<u>Mette S. Hansen</u> , Ole L. Nielsen, Llorenç G. Roma, Joaquim Segalés 6. Inflammatory reactions in lung tissue of pigs infected with <i>Pasteurella multocida</i> : An immunohistochemical study <u>Susanne E. Pors</u> , Tine Iburg, Mette S. Hansen, Ole L. Nielsen, Magne Bisgaard and Henrik E. Jensen
17:30-18:30	ECVP Annual General Assembly	
19:00	Meeting of the ECVP	

Friday, 19.09.2008.

	Hall A	Hall B
8:30-9:30	Keynote lecture - Robin Franklin: Regeneration in the Nervous System/ Hall A	
9:30-10:30	<p>Poster Tour E - <u>Neuropathology, Endocrine System</u></p> <p>1. Pathological study of experimental lead poisoning in sheep <u>Ahmad Reza Movassehgi</u>, Mohammad Reza Aslani, Hadi Mohebalian P82</p> <p>2. An immunopathological study of naturally occurring ovine louping-ill encephalitis <u>Julio Benavides</u>, Mark Dagleish, Clare Underwood, David buxton, Francesca Chianini P83</p> <p>3. Intracerebral immune response in naturally occurring listeric encephalitis of small ruminants <u>Stefano Di Palma</u>, Barbara Brunetti, Ursula Forster, Monika Hilbe, Andreas Zurbriggen, Marc Vandevelde, Anna Oevermann P84</p> <p>4. Glia cytoarchitecture in the brain and spinal cord of bearded dragon (<i>Pogona vitticeps</i>) CR Jiménez, R Zafra, MJ Ruiz, E Mozos P85</p> <p>5. Late onset cerebellar cortical abiotrophy in a koala <u>Mitsuru Kuwamura</u>, Fumi Murai, Shin Nishioka, Mica Aoki, Fumihito Ohashi, Jyoji Yamate, Takao Kotani, Brian A. Summers P86</p> <p>6. Ultrastructural study of cerebellum with methylmercury chloride poisoning in newborn rats <u>Pejman Mortazavi</u>, Taki-Altirahi P87</p> <p>7. Gene expression profiling of spleens of SJL/J mice experimentally infected with Theiler's murine encephalomyelitis virus María José Navarrete-Talloni, Arno Kalkuhl, Reiner Ulrich, Ulrich Deschl, Wolfgang Baumgärtner, <u>Andreas Beineke</u> P88</p> <p>8. Phenotypic spectrum of listeric encephalitis in ruminants: a comprehensive study <u>Anna Oevermann</u>, Carlos Abril, Andreas Zurbriggen, Marc Vandevelde P89</p> <p>9. Isolation of <i>Streptococcus suis</i> from a cat with meningo-encephalitis <u>Stefan Roels</u>, Olivier Devroye, Herma Buys, Hilde Smith, Patrick Butaye P90</p> <p>10. Ovine neosporosis and toxoplasmosis <u>Farhang Sasani</u>, Parvaneh Seifori, Gholamreza Habibi P91</p> <p>11. An unusual encephalomyelopathy in a litter of Middle White pigs <u>Alexandra Schock</u>, J. Paul Hutchinson P92</p>	<p>Poster Tour F - <u>Respiratory Tract, Musculoskeletal System, Sensory Organs</u></p> <p>1. Study on ovine and caprine pneumonia : ovine pulmonary adenomatosis <u>Abbas Ezzi</u> P103</p> <p>2. The bovine brachyspina syndrome: a congenital malformation in Holstein calves <u>Klaas Peperkamp</u>, Stefania Testoni, Jørgen S. Agerholm P104</p> <p>3. Expression of inducible nitric oxide synthase (iNOS), nitrotyrosine (NT) and manganese superoxide dismutase (Mn-SOD) in lungs of calves experimentally infected with <i>Mycoplasma bovis</i> <u>Kathrin Hermeyer</u>, Björn Jacobsen, Joachim Spergser, Renate Rosengarten, Marion Hewicker-Trautwein P105</p> <p>4. Ultrastructural study of naturally occurring ovine pulmonary adenocarcinoma in sheep <u>Azizollah Khodakaram Tafti</u>, Alireza Hematian P106</p> <p>5. Eosinophils, macrophages and respiratory epithelial cells and the prepatent phase of <i>Dictyocaulus viviparus</i> infection of cattle John Graham-Brown, Andrew Tomlinson, Vad Lazari, Diana Williams, Jacqui Matthews, <u>Anja Kipar</u> P107</p> <p>6. Microscopic screening pulmonary lesions in European bison from Białowieża Primeval Forest. <u>Barbara Osirńska</u>, Magdalena Matuszewska, Wojciech Bielecki, Magdalena Skrzypczak, Krzysztof Anusz P108</p> <p>7. Localisation of <i>Pasteurella multocida</i> in experimentally infected immunosuppressed pigs <u>Susanne E. Pors</u>, Jens Nielsen, Magne Bisgaard and Henrik E. Jensen P109</p> <p>8. Histopathological changes in fish gills – preliminary observations Jerzy Antychowicz, Marek Matras, <u>Michał Reichert</u>. P110</p> <p>9. Immune response to cavitary pulmonary tuberculosis in the goat <u>Joquin Sánchez</u>, Antonio J Buendía, Laura Tomás, José A. Navarro P111</p> <p>10. Tracheal rupture with subcutaneous emphysema and unilateral pneumothorax in an adult sheep <u>Alexandra Schock</u>, Jackie Willmington P112</p> <p>11. Metastasis of primary lung carcinoma in skeletal muscle of a cat <u>Antonio Carminato</u>, Marta Vascellari, Marcella Paoli, Claudio Pasolli, Wendy Marchioro, Franco Mutinelli P113</p>

	<p>12. Investigation upon the role of apoptosis in Theiler's murine encephalitis virus-infected SJL/J and C57BL/6 mice <u>Stephanie Kristin Klein</u>, Ingo Gerhauser, Reiner Ulrich, Suliman Elmarabet, Wolfgang Baumgärtner, Andreas Beineke P93</p> <p>13. Altered myelinogenesis and glial responses in the attractin-deficient <i>mv</i> rat. <u>Takeshi Izawa</u>, Shigeo Takenaka, Takao Kotani, Jyoji Yamate, Mitsuru Kuwamura P94</p> <p>14. A canine spinal cord meningioma with two unusual features: amianthoid collagen fibres and secretory pattern <u>Lucy Woolford</u>, John J. Kepes, F. Clarke Berryman, Alexander de Lahunta, Sandra Schoeniger, Brian A. Summers P95</p> <p>15. Head injury in New Zealand sea lion neonates: is there a shaken pup syndrome? <u>Wendi Roe</u>, Joe Mayhew, Christine Thomson, Robert Jolly P96</p> <p>16. Malignant peripheral nerve sheath tumor of mediastinum in horse <u>Petr Fictum</u>, Miša Škorič, Roman Halouzka, Barbora Bezděková, Petr Jahn P97</p> <p>17. Malignant peripheral nerve sheath tumor in the spleen of a dog <u>Wilhelmina Bergmann</u>, Iwan Burgener, Paola Roccabianca, Monika Welle P98</p> <p>18. Malignant neuroendocrine tumour in a horse <u>Barbara Bacci</u>, Mario Pischedda, Barbara Brunetti, Giuseppe Sarli, Federico Morandi, Cinzia Benazzi P99</p> <p>19. C-cell thyroid adenoma in a horse: clinical, histological and immunohistochemical characterisation <u>Jaime Gómez-Laguna</u>, Elisa Díez, Alejandro Suárez-Bonnet, Jose María Santiesteban, Yolanda Millán, José Carlos Estepa, Ana Isabel Raya, Escolástico Aguilera, Antonio Espinosa de los Monteros, Juana Martín de las Mulas P100</p> <p>20. Histological findings in the adrenal glands of slaughtered cattle <u>Frantisek Jelinek</u>, Roman Konecny P101</p> <p>21. The localization of histochemical activity of 3α, 3β and 17α-HSD in the adrenal cortex of cows (<i>Bos taurus L.</i>) <u>Daut Rexhepaj</u> P102</p>	<p>12. Morphological alterations in horse skeletal muscles associated with <i>Purpura Hemorrhagica</i> <u>Dominique Cassart</u>, Cédric Thomas, Sandra Jolly, Sandra Berthemim, Freddy Coignoul, Daniel Desmecht P114</p> <p>13. Retroperitoneal epithelioid leiomyosarcoma with disseminated metastasis in a donkey. <u>Daniela Malatesta</u>, Mariarita Romanucci, Chiara Palmieri, Augusto Carluccio, Laura Bongiovanni, Leonardo Della Salda P115</p> <p>14. Myostatin is overexpressed and binds decorin in canine masticatory muscle myositis <u>Orlando Paciello</u>, Francesca Trapani, Serenella Papparella P116</p> <p>15. Local muscular tolerance of titanium nickel alloy implants coated with oxides and polymers: acute experiment in the rabbit <u>Teodoru Soare</u>, Emilia Ciobotaru, Iuliana Ionascu, Manuella Militaru, Dan Batalu, Georgeta Dinescu, Georgeta Cosmeleata P117</p> <p>16. Histopathological evaluation of a dose-responsive reaction associated with wov-formulated vaccine in chickens <u>Vlasta Herak-Perković</u>, Marina Tišljar, Suzana Tkalčić, Neda Ergotić P118</p> <p>17. Discospondylitis in mink (<i>Mustela vison</i>): a preliminary study in Spanish mink farms <u>Nieto JM</u>, López-Peña M, Failde LD(1), Astorga J(2), Barreiro A, Espino L, Fernández-Antonio R P119</p> <p>18. Morphogenesis of ocular changes and microphthalmia in Royal College of Surgeons (RCS) rats <u>Naho Tsuji</u>, Tetsuro Matsuura, Kiyokazu Ozaki, Isao Narama P120</p> <p>19. Poorly differentiated carcinoma of the external ear canal with chondroid metaplasia at metastatic site in a dog <u>Mariarita Romanucci</u>, Daniela Malatesta, Alessia Marinelli, Pierluigi Di Lorenzo, Leonardo Della Salda P121</p> <p>20. Primitive neuroectodermal tumor (PNET) in the left eye of a bulldog <u>Eva Ludwig</u>, Ekkehard Schaeffer, Dirk Emmelmann, Albrecht Fenger, Walter Hermanns P122</p>
10:30-10:45	Coffe break	

<p>10:45-12:15</p> <p>10:45</p> <p>11:00</p> <p>11:15</p> <p>11:30</p> <p>11:45</p> <p>12:00</p>	<p>Oral presentations/parallel sessions - CNS pathology</p> <p>1. Pathological effects of quinazolinones on the brain of newborn Balb/C mice. <u>Moayer Fariborz</u>, Maryam Shams Lahijani, Elham S. Hossein Pour</p> <p>2. Distribution of Theiler's murine encephalomyelitis virus in acute and chronic brain lesions of SJL/J and C57BL/6 mice. <u>Maren Kummerfeld</u>, Reiner Ulrich, Ingo Gerhauser, Stephanie Klein, Wolfgang Baumgärtner, Andreas Beineke</p> <p>3. Cholesterol, <i>Chlamydomytila pneumoniae</i>, Alzheimer's disease: expanding the horizons of pathogenesis. <u>Nikolaos G. Papaioannou</u>, Dimitra Psalla, Victoria I. Siarkou, Paschalis Fortomaris, Michael Patsikas and Ioannis Vlemmas</p> <p>4. Primary and metastatic spinal cord tumours in dogs – a study of 26 cases. <u>Simon Priestnall</u>, Emma Rayner, Nanny Wenzlow, Brian Smyth, Brian Summers and Sandra Schöniger</p> <p>5. Plexiform neurofibroma and diffuse neurofibroma as subtypes of canine peripheral nerve sheath tumours. <u>Sandra Schöniger</u>, Brian A. Summers</p> <p>6. Bluetongue virus serotype 8 (BTV-8) associated hydranencephaly in calves. Griet Vercauteren, Cora Miry, Frank Vandenbussche, Richard Ducatelle, Sara Van der Heyden, Elise Vandemeulebroucke, Ilse De Leeuw, Piet Deprez, Koen Chiers, Kris De Clercq</p>	<p>Oral presentations/parallel sessions-Non-infectious conditions</p> <p>1. The expression of matrix metalloproteinases (MMPs) and their tissue specific inhibitors (TIMPs) in idiopathic canine dilative cardiomyopathy – immunohistochemistry and mRNA analyses. <u>Heike Aupperle</u>, Imke März, Andreas Schubert, Gregor Dinges, Jens Thielebein, Heinz-Adolf Schoon</p> <p>2. A gross and histopathological assessment of feline appendicular osteoarthritis. <u>Pamela Johnston</u>, Matthew Keifer, David Bennett</p> <p>3. New acquisitions about the diagnosis of proventricular dilatation disease (PDD) in parrots. <u>Stefano Pesaro</u>, Renato Ceccherelli, Raffaello Gambi, Giacomo Rossi</p> <p>4. Possible role of oxidative stress in the pathogenesis of canine zinc-responsive dermatosis. <u>Mariarita Romanucci</u>, Laura Bongiovanni, Anita Russo, Silvia Capuccini, Luca Mechelli, Laura Ordeix, Leonardo Della Salda</p> <p>5. New acquisitions about canine lymphangiectasia during IBD. <u>Giacomo Rossi</u>, Gian Enrico Magi, Simona Cortese, Chiara Tarantino, Graziano Pengo, Giacomo Renzoni</p> <p>6. Induced hyperuricaemia results in severe nephropathy in Green Iguanas (<i>Iguana iguana</i>). <u>E.J.B.Veldhuis Kroeze</u>, P.Zwart, S.J.Hernandez-Divers, D.Martinez-Jiminez, S.Bush, K.S.Latimer</p>
<p>12:15-13:45</p>	<p>Buffet light lunch</p>	
	<p>Poster Tour G - Skin, Liver</p> <p>1. Immunohistochemical examination of MHC class II expression in canine skin samples with particular consideration of keratinocytes Maïke Huisinga, Manfred Reinacher P123</p> <p>2. Skin tumours of dogs in Grenada Alfred Chikweto, M. Iqbal Bhaiyat, Ravindra Sharma, Claude de Allie, Pauline E. McNeil P124</p> <p>3. Specific mutations of the c-kit proto-oncogene observed in feline cutaneous mast cell tumors Jérôme Abadie, Vincent Fort, Sonia Becavin, Lionel Martignat P125</p> <p>4. Lymphangiogenesis does not influence progression and outcome of canine mast cell tumours Barbara Brunetti, Rosario Preziosi, Federico Morandi, Valentina Zagonara, Paolo Paolo Degliesposti, Cinzia Benazzi, Giuseppe Sarli P126</p>	<p>Poster Tour H - Infectious Diseases</p> <p>1. Inflammatory response to bovine and avian tuberculin and to phytohaemagglutinin skin testing in wild Iberian red deer M^a Paz Martín-Hernando, M^a Isabel G. Fernández de Mera, Joaquín Vicente, Raquel Jaroso, Christian Gortazar P144</p> <p>2. Endemic bovine tuberculosis by Mycobacterium caprae in a Tyrolian valley Joachim Weikel, Walter Glawischnig P145</p> <p>3. Detection of frequency rate of reactive amyloidosis in affected cows with tuberculosis Abbas Tavasoly, Piman Mohammadi P146</p> <p>4. Black wild pigs of Nebrodi Park in Sicily: evidence of their possible role as reservoir of Mycobacterium tuberculosis complex infection Di Marco Vincenzo, Marianelli Cinzia, Russo Miriam, Aronica Vincenzo, Bollo Enrico, Amedeo Stefano, Valenza Federico, Capucchio Maria Teresa P147</p>

<p>5. Skin mast cells and endogenous fatty acid amides in canine atopic dermatitis: a pilot study <u>Francesca Abramo</u>, Francesco Albanese, Vincenzo Miragliotta, Stefania Petrosino, Vincenzo Di Marzo P127</p> <p>6. Beta-catenin expression in canine normal skin and epithelial skin tumours Vincenzo Miragliotta, Michela Gregori, Saverio Meini, Fabrizio Fabbri, Maria Paola Ricciardi, <u>Francesca Abramo</u> P128</p> <p>7. <i>In vitro</i> anti-tumour activity of two <i>Pteridium aquilinum</i> extracts <u>Rui M. Gil da Costa</u>, Joana Pereira, Júlia Bessa, Maria São José Nascimento, Fátima Gärtner, Anake Kijjoo, Carlos Lopes P129</p> <p>8. Atrophic dermatosis in a coatimundi (<i>Nasua nasua</i>) <u>Maike Huisinga</u>, Christiane Herden, Manfred Reinacher P130</p> <p>9. Panniculitis in dogs and cats; a retrospective study of 115 cases <u>Monir Majzoub</u>, Andriy Synilo, Daniela Denk, Walter Hermanns P131</p> <p>10. p63 expression in canine primary cutaneous adenocarcinomas <u>Ana Laura Saraiva</u>, Fátima Gärtner, Maria dos Anjos Pires P132</p> <p>11. Pododermatitis in farmed mink (<i>Mustela vison</i>) in Spain <u>JM Nieto</u>; L D Fraílde; AP Losada; M López-Peña; S Vázquez; R Bermúdez; MI Quiroga; R Fernández-Antonio P133</p> <p>12. Feline post-vaccinal sarcoma: an immunohistochemical study <u>Tatiane Watanabe</u>, Rui M. Gil da Costa, Irina Amorim, Alexandra Rema, Célia Lopes, Eduarda Matos, Manuela Duarte, Fátima Gärtner P134</p> <p>13. Effect of feeding with conventional or organic wheat on morphology of the liver and muscles in pigs <u>Babinska Izabella</u>, Szarek Jozef, Tyburski Jozef, Skibniewska Krystyna P135</p> <p>14. Molecular pathological analysis of the late stage of tumor promotion by oxfendazole in a rat two-stage hepatocarcinogenesis model <u>Yasuaki Dewa</u>, Jihei Nishimura, Meilan Jin, Masaomi Kawai, Yukie Saegusa, Makoto Shibutani, Kunitoshi Mitsumori P136</p> <p>15. Cholangiocarcinoma in cockatiel (<i>Nymphicus hollandicus</i>): case report <u>Gesek Michal</u>, Stenzel Tomasz, Szarek Jozef, Babinska Izabella, Mieszczynski Tomasz P137</p>	<p>5. Occurrence of bacterial pathogens and porcine circovirus type II in colitis of post-weaning pigs in Switzerland <u>Miclard Julien</u>, Mamani Javier, Titus Sydler, Goy Naomi, Speiser Stephanie, Zeeh Friederike, Albini Sarah, Posthaus Horst P148</p> <p>6. Immunohistochemical diagnosis of porcine circovirus from pigs with postweaning multisystemic wasting syndrome <u>Trpe Ristoski</u>, Slavco Mrenoski, Iskra Cvetkovic P149</p> <p>7. Histopathological and immunohistochemical findings in lungs and lymph nodes of pigs naturally affected with post-weaning multisystemic wasting syndrome and porcine reproductive and respiratory syndrome <u>Novosel Dinko</u>, Lipej Zoran, Mihaljević Željko, Deždek Danko P150</p> <p>8. Histological, ultrastructural and molecular pathological investigations on the kidney of a pig displaying porcine dermatitis and nephropathy syndrome (PDNS) <u>Zoltán Bago</u>, Zsuzsanna Bago-Horvath, Susanne Richter, Sandra Revilla-Fernandez, Karin Priessner, Herbert Weissenbock P151</p> <p>9. Haptoglobin expression in blood and liver of pigs infected with a european PRRSV field isolate <u>Inmaculada Barranco</u>, Jaime Gómez-Laguna, Francisco José Pallarés, Francisco Javier Salguero, Silvia Martínez-Subiela, Ana Gutierrez, Librado Carrasco P152</p> <p>10. Ultrastructural findings of PK-15 cells infected with porcine circovirus type 2 <u>Carolina Rodriguez-Cariño</u>, Maria Fort, Joaquim Segales P153</p> <p>11. Comparative pathology and viral distribution of experimental infection with highly pathogenic avian influenza H5N1 A/turkey/turkey/05 (Asian lineage) in chicken, turkey and Pekin duck <u>Alejandro Núñez</u>, Angela Richard, Brandon Londt, Yvonne I. Spencer, Ian H. Brown and Jill Banks P154</p> <p>12. Effects of natural transplacental infection of bluetongue virus serotype 8 in cattle: abortion and hydranencephaly Willem Wouda, <u>Klaas Peperkamp</u>, Thijs Roumen, Jan Vos, Evert van Garderen, Jan Muskens P155</p> <p>13. Immunohistochemical detection of E2 protein (gp55) of classical swine fever virus in brain and ileoocaecal valve of experimentally infected pigs <u>Vladimir Polaček</u>, Nikola Vasković, Jasna Prodanov, Milijana Knežević, Sanja Aleksić Kovačević P156</p> <p>14. An experimental study on early pathogenesis of a very virulent isolate of Infectious Bursal Disease virus, using histopathology <u>Minoosh Siavosh</u>, Abbas Tavassoly, A. Shoshtry, M.A. Bahmani nejad, S.H. Marjanmehr P157</p>
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14:45-15:45	Keynote lecture - Antonio Fernandez: Whale strandings linked to sonar / Hall A	
15:45-16:00	Coffe break	
<p>16:00-17:30</p> <p>16:00</p> <p>16:15</p> <p>16:30</p>	<p>Oral presentations/parallel sessions-Wildlife pathology</p> <p>1. Differentiation among egg stages of <i>Angiostrongylus vasorum</i> in the lungs of experimentally infected foxes. Pia Webster, Sigridur O. Magnusdottir, Pia Petersen, Jesper Monrad, <u>Henrik E. Jensen</u></p> <p>2. Pathological studies of host-parasite interactions: morpho-pathological and biochemical aspects (field cases). <u>Laura Urdes</u>, Cristiana Diaconescu, Hangan Marius, Cornila Nicolae, Vasile Petrica</p> <p>3. Diagnosis of bovine tuberculosis in wild ungulates from Spain: culture and pathology. <u>M^a Paz Martín-Hernando</u>, M^a José Torres, Joaquín Vicente, Manuel Reglero, Jose de la Fuente, Javier Aznar-Martín, Juan José Negro, Christian Gortazar</p>	<p>Oral presentations/parallel sessions - Tumor pathology</p> <p>1. PTEN, PI3K and NFκ-B expression in feline post-vaccinal fibrosarcoma. <u>Gian Enrico Magi</u>, Giacomo Rossi, Chiara Bertani, Giacomo Renzoni</p> <p>2. Clinical, histological and immunohistochemical analysis of feline squamous cell carcinoma in situ. <u>Franco Guscelli</u>, Marianne Heimann, Monika Welle, Dale L. Godson, Claude Favrot</p> <p>3. A PCR-based assay for the assessment of clonality in feline B-cell lymphomas with characterization of a new family of heavy chain variable region genes. <u>Manfred Henrich</u>, Werner Hecht, Alexander Weiss, Manfred Reinacher</p>

16:45	4. Re-emergence of Morbillivirus infection in the Mediterranean sea. <u>Sara Soto</u> , Jorge Martínez, Iván José Galindo, Rocío González, Beatriz González, Toni Raga, Mariano Domingo	4. Pathology of alimentary B-cell lymphoma in juvenile Miniature Dachshunds. <u>Hiroki Sakai</u> , Atsushi Kodama, Keiya Kobayashi, Tokuma Yanai, Toshiaki Masegi
17:00	5. Epidemiological investigation and characterization of <i>Chlamydia</i> -like organisms in brown trout (<i>Salmo trutta</i>) over a two year period. Adam Polkinghorne, Heike Schmidt-Posthaus, Angelika Lehner, Helmut Segner, <u>Lloyd Vaughan</u>	5. Expression of BAG3 protein in the urothelial tumour in cattle. <u>Valeria Russo</u> , Roberto Brun, Sante Roperto, Chiara Urraro, Alessandra Rosati, Maria Caterina Turco, Franco Roperto
17:15	6. Pathological findings and viral protein expression in the central nervous system of harbour seals (<i>Phoca vitulina</i>) infected with Phocine Distemper virus. <u>Lev Stimmer</u> , Jean-Jaques Fontaine, Wolfgang Baumgärtner, Andreas Beineke	6. Immunohistochemical analysis of survivin and X-linked inhibitor of apoptosis protein (XIAP) expression in canine lymphoma. Julia Wimmershoff, Paula Grest, Adam Polkinhorne, <u>Franco Guscetti</u>
17:30-18:30	ESVP Annual General Assembly	
19:45	Departure by buses from the hotels to BANJE Beach, Beach party/dinner	
23:30	Shuttle buses from Pile gate to the hotels	
24:30	Last shuttle bus	

	Saturday, 20.09.2008.
	Hall A
9:00-10:30	Workshop - Sven Seiwerth: Telepathology – possible use in veterinary pathology
10:30-11:00	Coffe break
11:00-12:30	Workshop - Fabio del Piero: Indirect immunohistochemistry for the diagnosis and pathogenical study of viral diseases
12:30	Closing ceremony

**Abstracts of
the Keynote Lectures**

Morphologic Classification of Parenchymal Disorders of the Liver

Ted S.G.A.M. van den Ingh, DVM, PhD, Diplomate ECVP
TCCI Consultancy BV, Utrecht, The Netherlands; (t.s.g.a.m.vandeningh@wanadoo.nl)

Parenchymal disorders can be classified as 1) hepatocellular degeneration and lipidosiis, 2) hepatic amyloidosis, 3) hepatocellular death: apoptosis and necrosis, 4) acute and chronic hepatitis and cirrhosis, 5) granulomatous hepatitis, hepatic abscesses and non-specific reactive hepatitis, and 6) hepatic metabolic storage diseases.

HEPATOCELLULAR DEGENERATION AND LIPIDOSIS are two patterns of reversible cell injury of the liver. Grossly they are characterized by focal or diffuse swelling, focal or zonal or diffuse pallor, and a decreased consistency with increased fragility of the liver.

Hepatocellular swelling and hydropic change. Hepatocellular swelling (synonym: cloudy swelling) is the first manifestation of almost all forms of injury to cells and appears whenever cells are incapable of maintaining ionic and fluid homeostasis and accumulate water. In more advanced cases the hepatocytes will show a more severe hydropic change with marked swelling and very pale staining cytoplasm arranged in thin strands or vacuolar change of the cytoplasm

Feathery degeneration is a form of hydropic degeneration of hepatocytes associated with cholestasis and likely caused by the intracellular accumulation of bilirubin and bile acids. The lesion is characterized by enlarged hepatocytes with pale staining cytoplasm arranged in thin strands in combination with the intracytoplasmic presence of bilirubin.

Steroid induced hepatopathy is a specific disorder only recognized in dogs and characterized by excessive hepatic accumulation of glycogen. Typical features are swollen hepatocytes with cleared cytoplasm due to glycogen accumulation, fine strands of eosinophilic cytoplasm, and usually without displacement of the nucleus. Fragmentation of needle biopsies is common in livers with marked steroid induced hepatopathy.

Hepatocellular lipidosiis / steatosis/ fatty change. These terms (lipidosiis, steatosis, fatty change or fatty liver) are used interchangeably by some, but others have strong opinions about the use of these terms. Hepatic steatosis is the abnormal accumulation of lipids as clear vacuoles in the cytoplasm of hepatocytes. Hepatic steatosis can be differentiated according to the size of the cytoplasmic vacuoles in microvesicular and macrovesicular steatosis, or mixed types. As various causes may have a different type and distribution of the lesion and severe steatosis may affect the function of the liver, it is necessary to communicate to the clinician not only the type of vacuolation but also the severity and the distribution of the steatosis.

HEPATIC AMYLOIDOSIS

Amyloidosis is usually secondary or reactive (AA) amyloidosis with a systemic distribution. The hepatic lesion is characterized by the deposition of hyalin eosinophilic material in the space of Disse and is frequently associated with atrophy of the adjacent hepatocytes.

HEPATOCELLULAR DEATH: APOPTOSIS AND NECROSIS

Knowledge about the various forms of hepatocellular death i.e. apoptosis and necrosis, their morphological substrate and the subsequent response of the liver are essential to understand the development and various morphological aspects of acute and chronic hepatitis and cirrhosis.

Hepatocytes may be killed by various insults including hypoxia, toxins, microorganisms, immunological events and severe metabolic disturbances. Classically cell death has been considered

to occur through apoptosis or necrosis; however, recent evidence suggests overlap between both processes as moderate exposure to some toxins causes apoptosis whereas greater exposure may result in necrosis and that necrosis and apoptosis are morphologic expressions of a shared biochemical network of both caspase dependent mechanisms as well as non-caspase dependent effectors.

Apoptosis is a caspase-dependent active process of programmed cell death which results in shrinkage of the cell without loss of integrity of the cell and nuclear membrane, and subsequent fragmentation.

Necrosis involves cytoplasmic swelling and loss of integrity of the cell membrane and may result in coagulative necrosis or liquefactive (lytic) necrosis. **Coagulative necrosis** is the result of sudden and catastrophic denaturation of the cytosolic protein and appears as swollen hepatocytes with acidophilic cytoplasm, preservation of the basic outline of the coagulated cell, and karyopyknosis, karyorrhexis or karyolysis. The acute phase of coagulative necrosis is followed by proliferation of Kupffer cells and infiltration of mononuclear and polymorphonuclear phagocytes and subsequent resorption and lysis of the necrotic cells. **Liquefactive or lytic necrosis** is the result of osmotic swelling and disintegration of hepatocytes and appears as loss of hepatocytes with subsequent collapse of the residual reticulin network and/or replacement by erythrocytes and eventually the presence of ceroid-laden macrophages. The outcome of a given hepatic insult depends on the nature, extent and duration of the insult, and of course survival of the host.

MORPHOLOGICAL PATTERNS OF APOPTOSIS AND NECROSIS.

Apoptotic bodies (acidophil bodies) are shrunken, intensely eosinophilic hepatocytes with condensed nuclei and surrounded by an empty halo. After subsequent fragmentation, the remnants are phagocytosed by adjacent Kupffer cells and hepatocytes, and visible as small cytoplasmic eosinophilic inclusions that are rapidly degraded.

Focal and multifocal necrosis refer to coagulative or liquefactive necrosis of small aggregates of hepatocytes, mostly attended and recognized by the secondary inflammatory reaction of Kupffer cell proliferation and infiltration of mononuclear and, or polymorphonuclear phagocytes.

Confluent and bridging necrosis may be coagulative or liquefactive necrosis and comprises larger areas of hepatocytes, in a random or zonal distribution. Confluent necrosis linking vascular structures is called bridging necrosis. Bridging at the periphery of acini links terminal hepatic venules to each other and is called central-central bridging. Bridging linking terminal hepatic venules and portal tracts is called central-portal bridging, whereas bridging necrosis with a periportal distribution is called portal-portal bridging. When confluent necrosis is more extensive and involves complete acini or lobules, the process is described as panacinar or panlobular necrosis.

Massive necrosis represents the most severe form of necrosis and generally is used when the liver shows extensive diffuse panlobular and multilobular coagulative and, or liqueactive necrosis. The sequel of massive necrosis often is collapse of the reticulin and fibrous network so that portal areas and hepatic venules are approximated and the connective tissues subsequently condenses (post-necrotic scarring).

Piecemeal necrosis, recently called **interface hepatitis** can be defined as death of hepatocytes at the interface of parenchyma and (newly formed) connective tissue and most likely the pathogenetic process involved is apoptosis.

Response of the liver to hepatocellular apoptosis and necrosis

Following destruction of hepatic parenchyma, regeneration of parenchyma, fibrosis, and ductular proliferation may occur. When hepatocytic destruction is limited and the reticulin network remains intact, regeneration with almost complete restitution of the liver structure can occur.

Severe parenchymal destruction with extensive loss of hepatocytes often is followed by ductular proliferation. Many of these structures contain both liver-cell and bile-duct elements and may reflect regenerative proliferation of an hepatic stem cell population analogous to oval cells in the rat, or transformation of regenerating hepatocytes into ductular structures. These structures generally are most prominent in the periportal areas, and their location would correspond to the former canals of Hering now transformed into complex arborizing networks of proliferating cells. With persistent parenchymal damage or extensive loss of hepatocytes fibrosis and postnecrotic scarring may occur with the formation of intrahepatic portovenous shunts; in these cases prolonged regenerative effort will result in regenerative parenchymal nodules.

HEPATITIS AND CIRRHOSIS

There is an intricate relationship between inflammation in the liver parenchyma and hepatocellular apoptosis and necrosis, the latter often being the initiating event of the inflammation. Despite, considerable discussion exists in the literature about the preferred nomenclature in cases of acute hepatic necrosis without or with minimal inflammation in non-infectious, particularly toxic or ischaemic insults, i.e. acute hepatic necrosis versus acute hepatitis. However, also infectious causes of hepatocellular necrosis, traditionally referred to as hepatitis, may show extensive hepatic necrosis with minimal or even without inflammation in the acute stage. No disagreement exists about the term chronic hepatitis, which is used irrespective of the cause of the lesion and which is characterized by the presence of fibrosis, inflammation and hepatocellular apoptosis and necrosis. Although some still will hesitate or will decline to consider hepatocytic apoptosis and necrosis about to be included under the heading of hepatitis, it is the only way in which phenomena common in acute and chronic conditions can be fitted together, irrespective of the cause, into an understandable and coordinated pattern.

Most important however, irrespective of the nomenclature used, is the necessity to include data about the type, pattern and extent of the necrosis and inflammation, and the possible cause, as well as in more prolonged disease about the presence, pattern and extent of fibrosis and regeneration.

Acute hepatitis is characterized morphologically by a combination of inflammation, hepatocellular apoptosis and necrosis, and possibly regeneration. The proportion and detailed nature of these components vary widely according to the cause, the host response and the passage of time and it is necessary to include in the diagnosis the type, pattern and extent of the necrosis and inflammation as well as the possible etiology. The lesions are usually sufficiently diffuse within the liver to be diagnosed with confidence on small biopsy samples. However, it might often be difficult to distinguish a cause for hepatitis by morphological means alone although there may be histological clues for a specific etiology.

Chronic hepatitis is characterized by hepatocellular apoptosis or necrosis, a variable mononuclear or mixed inflammatory infiltrate, regeneration and fibrosis. The proportion and distribution of these components vary widely and it is necessary to include in the diagnosis the activity and stage of the disease as well as the possible etiology. The activity of the disease is determined by the quantity of inflammation and extent of hepatocellular death which may be present as interface hepatitis and in a randomly focal and, or confluent pattern within the lobule. The stage of the disease is determined by the extent and pattern of fibrosis and possible presence of architectural distortion and development of cirrhosis. Fibrosis may be present in and extending from the portal areas usually in association with interface hepatitis and may result in portal-portal bridging fibrous septa. Fibrous septa may also develop following collaps and condensation of the reticulin network and the resulting scars are usually hypocellular. In addition, regeneration of hepatic parenchyma may be seen as well as proliferation of ductular-like structures at the periphery of the parenchyma and within fibrous septa.

Copper-Associated Chronic Hepatitis. Progressive chronic hepatitis associated with accumulation of copper has been described in Bedlington terriers, West Highland White terriers and Dalmatians, all of which have an inherited metabolic disorder of copper metabolism. In these animals copper continuously accumulates in hepatocytes, starting in the centrolobular regions, and with progressive accumulation results in hepatocellular necrosis, inflammation with copper-laden macrophages and finally chronic hepatitis and cirrhosis. Chronic hepatitis with excessive copper accumulation has also been described in other breeds, including the Skye terrier, Doberman pinscher (females are predisposed), American and English cocker spaniel, and Labrador retriever.

Cirrhosis is the end-stage of chronic hepatitis and is defined as a diffuse process characterized by fibrosis of the liver and the conversion of normal liver architecture into structurally abnormal nodules, and the presence of portal-central vascular anastomosis. Like in chronic hepatitis it is essential to include in the diagnosis the extent of the fibrosis, the activity of the disease and the possible etiology. Portal-portal fibrosis without other architectural changes does not constitute cirrhosis, but instead represents biliary-type fibrosis.

In cirrhosis two morphological categories can be distinguished i.e. micronodular cirrhosis with nodules less than 3 mm (the size of a normal lobule) and regular in size and macronodular cirrhosis with nodules greater than 3 mm (up to several centimeters) and irregular in size. Whereas micronodular cirrhosis develops from regular and diffuse alteration and fibrosis of the acini, macronodular cirrhosis develops from irregularly distributed larger areas of necrosis with secondary collaps and scarring and the development of portal-central vascular connections.

Lobular Dissecting Hepatitis is a form of cirrhosis seen in young or young adult dogs as isolated cases or in groups of dogs from the same litter or kennel with a rapid clinical course. The liver usually has a normal size with a smooth capsular surface or some small nodules of regeneration. Microscopically bands of fibroblasts and thin strands of extracellular matrix are seen between individual and small groups of hepatocytes which cause dissection of the original lobular architecture. Connective tissue stains (especially for reticulin) are helpful in demonstrating the pattern of connective tissue alterations. Inflammation and hepatocellular apoptosis / necrosis are usually slight to moderate. A similar morphologic pattern is sometimes seen in newborn or aborted calves and foals.

GRANULOMATOUS HEPATITIS, HEPATIC ABSCESES AND NON-SPECIFIC REACTIVE HEPATITIS

Granulomatous hepatitis is a distinctive pattern of inflammation in the liver characterized by accumulations of epithelioid macrophages and/or multinucleated giant cells and can be initiated by a variety of infectious and non-infectious agents. The presence of poorly digestible material,

T – cell mediated immunity to the material or both appears to be necessary for granuloma formation. Granulomatous hepatitis is infrequently seen and may be caused by mycobacterial infections and fungal infections or associated with degradation of indigestible material for instance parasitic cuticula and eggs.

Hepatic abscesses usually are the result of bacterial infections evoking intense accumulation and subsequent lysis of neutrophilic granulocytes at the infection site. They can reach the liver via different routes including the portal vein or umbilical vein, as an ascending infection of the biliary system, and by direct contact and penetration of the liver capsule.

Non-specific reactive hepatitis is a morphological entity widespread within the liver, representing a non-specific response to a variety of extrahepatic disease processes, especially febrile illnesses and inflammation somewhere in the splanchnic bed, or representing the residual lesion of previous inflammatory intrahepatic disease. The lesion is characterized by an inflammatory infiltrate in portal areas and in the parenchyma without evident hepatocellular necrosis.

HEPATIC METABOLIC STORAGE DISORDERS

Hepatic metabolic storage disorders, usually associated with inherited, sometimes acquired, metabolic enzyme deficiencies, can have a variety of morphologic appearances. The most common finding is the presence of clear vacuoles, vacuoles with granular or hyaline material, or pigmented granules in hepatocytes and, or Kupffer cells and macrophages.

WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases. WSAVA Liver Standardization Group. Elsevier, Edinburgh, 2006

Feline mammary tumors in comparative oncology

Massimo Castagnaro and Valentina Zappulli

Department of Public Health, Comparative Pathology and Veterinary Hygiene, Faculty of Veterinary Medicine, University of Padua, Italy

Spontaneous and induced animal models have been extensively used to improved our knowledge of human normal biology and etiology, pathophysiology and therapy of human diseases (1,2). Among spontaneous models, naturally occurring tumors in domestic animals have been recognized as an interesting opportunity for comparative oncology studies (3,4). In USA and Europe, cancer is the second most frequent cause of death in humans, after cardiovascular disease, and the first one in dogs and cats (4,5,6). However, cancer still accounts for more deaths than heart disease in persons under age 85 years (5). Age-adjusted overall cancer incidence per 100,000 individuals is 485 in humans, 381 in dogs and 264 in cats (5,7).

Although cancer incidence rates for dogs, cats and people based on site show several differences (4,5,7), mammary cancer is the most frequent in women and dogs, and the third in queens.

Based on age incidence, risk factors, histopathology, prognostic aspects, metastatic pattern and response to therapy feline mammary carcinomas has been proposed by many authors as a good model for breast cancer (8,9,10). In this presentation we summarized the common features shared by feline and human mammary carcinomas.

Secretory lobules of the feline mammary gland are situated in the subcutis and are formed by tubuloacinar glands and intralobular ducts, both lined by a luminal cuboidal epithelial layer resting on a basal layer of actin-positive myoepithelial cells. Secretory lobules are drained by arborized interlobular ducts, characterized by a double-stratified epithelium of cuboidal to tall columnar cells and scattered peripheral myoepithelial cells, which in turn lead to sinuses and the teat canal (11). Similar features are observed in the human breast (12). Significant differences in the blood venous drainage and lymphatic communications are present between human and feline mammary glands (MGs). A unique feature of feline MGs is the presence of veins which cross the midline eventually allowing metastatic dissemination between paired glands (13). Recent studies on the lymphatic drainage of the mammary glands in female cats (14,15) revealed a more complex situation than what was reported previously (16). In human MG part of the lymph drainage from a gland may go to the other breast where a controlateral metastatic tumor may develop (17).

The influence of steroid hormones on the onset of mammary tumors is well established in both human and feline mammary tumors. In cats, intact females have a significantly higher risk of developing mammary cancer than early ovariectomized subjects and the regular and prolonged use of progestagens has been associated with increased risk of mammary tumor development (8, 18, 19, 20). In humans, young age at menarche increases the risk by up to 20%, as does late menopause, and postmenopausal hormone therapy may also slightly increase the risk in women (21, 22, 23). A marked decreased risk of breast tumors is associated with oophorectomy (24).

Although the specific cell type of origin in human and feline mammary tumors is still uncertain, human (HMCs) and feline carcinomas (FMCs) are generally classified into in situ and infiltrative carcinomas (25,26). Most of FMCs, which shows at the time of diagnosis extensive infiltration of adjacent tissues, are of the papillary, tubular/cribriform or solid type (11). Special types such as mucinous, squamous cell and invasive micropapillary have been described both in FMC and HMC (27). In cats, lobular and medullary invasive

carcinomas are unreported. The grading system for HMCs and FMCs is based on identical criteria and it evaluates the degree of tubules formation, nuclear and cellular pleomorphisms and mitotic count (28,29). When these parameters are scored and the values used to allocate each tumors into well differentiated (grade I), moderately differentiated (grade II) and poorly differentiated (grade III), a similar pattern of distribution is obtained for HMC (grade I, 20%; grade II, 42%; grade III, 32%) and FMC (grade I, 16%; grade II, 50%; grade III, 27%).

Prognosis in FMCs is generally assessed as the 1-year post-surgical rate of survival/remission, which is comparable to the 10-year post-surgical survival/remission rate generally used in HMCs (30). Among prognostic factors, tumor size and node metastases are the most important in both HMCs and FMCs (31-32). Histologic subtype (in situ versus invasive carcinomas), grading system, extent of surgery and chemotherapy, proliferative rate (AgNOR, Ki-67 and PCNA indexes) have been also used as prognostic factors (28,-30, 33-36).

Changes in the expression of many genes at the mRNA and/or protein level have been reported in mammary carcinomas (32). Important genes commonly mutated in breast cancer important and recently studied also in FMCs are the human epidermal growth factor receptor-2 (HER2 or c-erb-2 or neu) and RON gene (tyrosine kinase MET receptor gene) (37-39). Both genes have been shown to be overexpressed in FMCs. Other molecules such as p53, cyclin A, metallothioneins, VEGF, chemokine receptor CXCR4, E-cadherin and BCAR1/p130C, confirmed that FMCs are to many extent similar to HMCs (40-48).

Female steroid hormones are associated with mammary tumour development both in domestic animals and in humans. In mammary gland, both normal and neoplastic tissues show concomitant expression of different hormone receptors (49). Oestrogens can directly stimulate growth mainly of both interlobular and intralobular ducts and induce progesterone receptors (PR) expression (50). Progesterone stimulates development of the tubular-alveolar units and regulates growth hormone expression (51). In HMCs, ER+/PR+ tumors (70-80%) respond to hormonal treatments, they are usually well differentiated do not express proliferation markers (52, 53) whereas ER- carcinomas are poorly differentiated, more aggressive and generally do not respond to tamoxifen therapy (54). An interesting situation is presented in cats that tend to have ER- highly aggressive mammary tumours (80%) and therefore might represent a good model for late-stage HBC (55).

A specific feature of the feline species similar to what is seen in human but unreported in any other experimental or spontaneous model, is the presence of splicing isoforms of ERalpha (56,57). Progesterone receptor expression analysis in FMC led to controversial results.

In this presentation we summarize some of the features of feline mammary tumors suggesting that mammary carcinogenesis in cats may be a good model to study from different point of view human breast cancer.

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Regeneration in the CNS

Professor Robin Franklin

Department of Veterinary Medicine and Cambridge Centre for Brain Repair, University of Cambridge, Cambridge CB3 0ES, UK

In this talk I will focus on remyelination, the process by which new myelin sheaths are restored to demyelinated axons. Remyelination represents one of the most compelling examples of adult multipotent stem/precursor cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in both clinical disease and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. Identifying potential targets will depend on a detailed understanding of the cellular and molecular mechanisms of remyelination. This talk will review 1) the nature of the cell or cells that respond to demyelination and generate new oligodendrocytes, identifying current areas of uncertainty and addressing the role of adult CNS stem and progenitor cells, 2) intrinsic factors regulating precursor differentiation and 3) how an environment favourable to remyelination is generated, and will introduce the concept of a matrix of signalling events critical for the successful completion of remyelination.

Whale strandings linked to sonar

Prof. Antonio Fernández

Research University Institute of Animal Health (IUSA), Division of Histology and Pathology, Department of Morphology - Veterinary School, University of Las Palmas de Gran Canaria Canary Islands, Spain, (afernandez@dmor.ulpgc.es)

The Veterinary School and the Research University Institute of Animal Health and Food Safety (IUSA) are located in Gran Canaria, one of the seven Canary Islands and both belong to University of Las Palmas de Gran Canaria. This young University (40 years old) has been putting much attention in developing education and research on marine issues, and therefore, the Veterinary School (20 years old) and the research institute (5 years old) have implemented resources on basic and applied marine animal sciences in order to approach that aim at a competitive level. In this regard, our unit of Veterinary Histology and Pathology has set up the grounds for covering the morphology and pathology of marine animals (especially, fishes, cetaceans, turtles and avian species).

In the Canary Islands waters, 28 cetacean species have been identified of which 24 species have been found stranded. A regional stranding network has established 10 years ago, involving biologists and veterinarians who attend stranded cetaceans beached in the seven islands. A rehabilitation small center and the Veterinary School work together when a cetacean strands, being our unit responsible for the corresponding pathological studies. Around 40 to 50 necropsies of different cetacean species have been carried out every year, and from 1999 an specific stranded cetacean health project was launched.

For a better information, we include here a short summary of this project. During a 6 year-period (1999-2005), 233 stranded whales and dolphins of 19 different species were recovered for scientific studies. Using a systematic standardized necropsy protocol, 138/233 stranded cetaceans were subjected to a complete or partial necropsy. Of these, 71/138 (51.45%) carcasses were in a very fresh or fresh status, 28/138 (20.29%) in moderate autolysis and 39/138 (28.22%) in advanced autolysis.

From a total of 233 cetaceans stranded in the Canary Islands (1999-2005), in 59,23% of the cases a morphological diagnosis was done, in 56,22% an etiological diagnosis could be established and, finally, we were able to classify 51,07% within natural or anthropogenic pathological entities.

Etio-pathologically, 62,32% of the 138 studied cetaceans were diagnosed as natural (i.e. non-anthropogenic) pathological entities that included infectious diseases, neonatal pathology, intra- and interspecific interactions and typical mass strandings. Another 33,33% of cases were diagnosed as anthropogenic entities including fishing interaction (by-catch), atypical mass-stranding linked to naval exercises, ship collisions, and other anthropogenic-related pathology. A cause of death could not be ascribed in only 4,35% of the 138 animals examined.

Within the group including cetacean mortalities linked to human activities it has been taking a considerable importance those which has been linked to military naval manouevres in which high intensity- mid-frequency sonar was used. In the present lecture we will deal this complex issue following the historical sequence of our involvement in this kind of "Atypical beaked whale (BWVs) mass stranding", considering mainly the pathological studies in addition to other biological, epidemiological, oceanographic, technological etc aspects which are also relevant for a better understanding of these events.

As veterinary pathologists we have really had to learn much from many different scientific fields for a better understanding and a wider view in order to try to answer the main questions about "what, how and why?"....healthy beaked whales (1000 to 2000 kg weight) die and / or strand within few hours after the beginning of military naval excersises in which "high intensity mid-frequency antisubmarine sonar" is being used. Why do mainly members of the Family *Ziphiidae* (rarely detected at sea and

scarcely known until those mortality events) massively strand and no other cetacean species which were also present in the area where naval exercises took place.

Typically, strandings of BWs prior to 1963, when certain types of midfrequency sonar equipment began to be employed, involved single individuals. Subsequently, mass strandings of BWs linked with naval exercises have been described in several locations: Bonaire in 1974, the Canary Islands in 1985, 1988, and 1989, Greece in 1996, and the Bahamas in 2000.

A mass stranding of BWs in the northern Bahamas Islands causally incriminated the use of tactical, midfrequency sonar because of its close temporal association with such naval maneuvers. Hemorrhage in the brain, ears, and acoustic fat was reported as the main lesion found in some BWs in the Bahamas stranding. A number of acoustically mediated behavioral modifications and pathophysiologic pathways were proposed to have caused these lesions; however, conclusive evidence linking a mechanism to the lesions was lacking. Past research efforts on the potential for anthropogenic sound to affect marine mammals have focused on auditory effects and behavior modifications following sound exposure. Nonauditory consequences of exposure to sound have received less attention.

A hypothesized, nonauditory link between strandings and sonar exposure is proposed to occur when tissues are supersaturated with dissolved nitrogen gas, and bubble growth–facilitated diffusion is stimulated within tissues. Bubble growth could result in emboli induced tissue separation and increased localized pressure in tissues, the presumed cause of decompression sickness (DCS) in human divers.

Following the initial stimulation of bubble growth in tissue that has been highly supersaturated with gas, growth could continue in the absence of a persistent acoustic stimulus.

It is believed that marine mammals have evolved adaptations to prevent deleterious, nitrogen bubble formation. However, to our knowledge, no studies have specifically addressed whether nitrogen bubble formation in tissue occurs in diving marine mammals. Recently, researchers have presented evidence of chronic, gas bubble lesions in the liver and kidney of different stranded cetacean species. These lesions suggest that gas bubbles formed *in vivo* can persist and generate fibrosis in diving cetaceans. Such emboli presumably cause ischemia and would explain the formation of chronic, bony lesions, consistent with those of dysbaric osteonecrosis (DON), described in sperm whales (*Physeter macrocephalus*).

DCS is the result of the supersaturation of body tissues with nitrogen gas and the subsequent release of nitrogen gas bubbles. In human divers, DCS is typically caused by rapid decompression following dives using compressed air and also has been reported during repetitive, breath-hold dives. Until the lungs collapse during a dive, alveolar gases are absorbed into the blood proportional to hydrostatic pressure. The amount of gas dissolved in specific tissues depends on dive depth and duration, descent and ascent rates, lipid content of the tissue, and surface time between successive dives. A number of anatomic, physiologic, and behavioral adaptations of marine mammals have been proposed to guard against bubble formation.

Due to alveolar collapse, gas exchange does not occur at depths greater than 70 m in dolphins or at depths greater than 30–50 m in seals. BWs are theoretically at a greater risk of developing deleterious tissue–nitrogen levels because their deep prolonged dives increase during the period in which nitrogen uptake can occur prior to lung collapse. Thus, deep-diving cetaceans may be more susceptible to the action of high-intensity acoustic energy on preexisting gas nuclei. The presence of gas emboli is an important finding in human DCS, but pulmonary fat emboli have also been reported with DCS-related, severe cardiorespiratory disturbances. Systemic fat embolism is a secondary effect of the abrupt pressure changes observed with DON, a condition initiated by the evolution of gas bubbles in nitrogen-supersaturated fatty marrow after inadequate decompression.

Forensic pathologists associate fat emboli with bone fractures, diabetes mellitus, burns, acute pancreatitis, fat and soft tissue injury, and DCS with acute DON. These entities are underdiagnosed clinically and at postmortem examination. The pathogenesis of fat embolism is not fully understood, and it is likely multifactorial. Two mechanisms have been proposed for the development of fat emboli. First, direct entry of fat emboli into the bloodstream after trauma may cause direct, toxic injury in the lung and produce respiratory insufficiency when free fatty acids are released from fat tissues. A second mechanism involves the generation of fat emboli from plasma lipoprotein disruption and coalescence of lipid at the intravascular gas bubble interface.

Fat emboli have not been reported in stranded cetaceans, and reports of gas bubble lesions are uncommon. Here we will describe the pathologic findings in BWs involved in a mass stranding that occurred coincidentally with naval exercises in the Canary Islands as in other places where an “atypical beaked whales” has taken place. Both gas bubble lesions and fat emboli are documented in these whales, and a hypothesis is presented to explain the association of mass stranding of BWs and sonar exposure.

Among potential mechanisms proposed for these stranding events, theoretical mechanisms for in vivo bubble formation in marine mammals mediated by exposure to loud anthropogenic sound sources (e.g. naval sonar) have been proposed. Linked pathological findings demonstrate that cetaceans can suffer tissue injury associated with gas bubble development, most probably through a mechanism similar to DCS. Emerging data from beaked whale dive profiles suggest that these species may be adapted to deep-diving through a combination of slow ascent rates and short surface intervals. As a consequence, there is a growing scientific consensus that an initial behavioural disruption to normal beaked whale dive profiles (e.g. accelerated ascent or extended surface interval) induced by loud acoustic exposure such as naval sonar may precipitate a potentially fatal physiological response resulting in bubble formation in tissues and mass stranding events. The confirmation of in vivo bubble formation in cetaceans as a mechanism in sonar-induced beaked whale mass strandings, including the quantification of received levels of acoustic sonar activity necessary to trigger a specific and adverse behavioural response, undoubtedly necessitates an experimental approach.

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Introduction to Telepathology

Sven Seiwerth MD, PhD

Institute of Pathology Faculty of Medicine University of Zagreb

INTRODUCTION

Pathology, as a morphologic discipline offers large application possibilities for computerized tools, both in science and routine. In the past decades great progress has been made in the field of patient data archiving and retrieval, but the most intriguing advances have been made in image storage, manipulation and communication.

Telepathology, as a branch of telemedicine transfers patient data and images to remote sites for diagnostic, teaching or research purposes. It depends on many technical and human resources and is one of the most demanding telemedical fields

GENERAL REMARKS

The diagnostic application of telepathology goes from intraoperative frozen sections, over second opinion of fellow pathologist to consultations with an expert. It is mostly used in everyday routine work in areas with few pathologists covering several hospitals, in diagnostic work with remote hospitals without pathologist or in expert consultation in difficult diagnostic cases.

As telepathology operates with images (mostly full color) it generates lots of data, transfer of which represents one of the major technical problems.

Communication channels currently used worldwide for telepathology range from simple telephone lines to sophisticated satellite-based connections. Although communication possibilities generally improve, most of developing countries will have to rely on ordinary telephone lines for years to come.

STILL VERSUS LIVE IMAGES

One of the major controversies in telepathology is whether still or live image transfer and analysis gives better results. Live images communication gives to the receiving pathologist an opportunity of on-line scanning the material in a way similar to that on the microscope. This is especially true if the system includes a remote robotic microscope. By this way a high level of user comfort is achieved, combined with high diagnostic quality. To operate such a system, triple ISDN or broad band communication channels are needed. If we transmit high resolution, high quality images, only broad band channels can provide ample capacity for approximately live transmission.

Still image systems which can be operated also on ordinary telephone lines or using Internet seem to be economically feasible in many instances. One of the major drawbacks of this approach is that it can be used for transmission and analysis of limited number of high quality images coupled with a limited, time consuming, capability of scanning the specimen, actually not used in practice. This can result in serious diagnostic difficulties or even errors due to not representative sampling (*keyhole phenomenon*). So one of the major challenges in still image telepathology is to ensure for the receiving pathologist the possibility of reviewing the whole specimen before rendering his diagnosis.

INTERACTIVE VERSUS STORE AND FORWARD TELEPATHOLOGY

Still image telepathology can be performed in two ways – on-line (interactive) or by store and forward concept. Interactive communication means that the referring pathologist and the consultant are both simultaneously present an exchange notes (via separate telephone line or on a “chat” bar). Store and forward concept understands that they send/analyze the material

upon convenience (certainly within reasonable limits). As most experts are professionals with tight schedules this concept seems to be more appropriate in many instances.

TOOLS FOR SPECIMEN SCANNING

One of the most widely used concepts is to scan the specimen in low resolution and then to focus, with high resolution, on specific areas. This approach is time consuming and to make it halfway working demands interactive communication.

Ten years ago we developed and tested a tool called "patchwork" - enabling automatic arrangement of collected images. The patchwork tool arranges automatically an unlimited number of collected images in a 3x2, 4x3, etc. manner. When the patchwork is assembled the visible size of the individual images is in reverse to their number but each image can be expanded to its full size.

Introduction of virtual slides rapidly changes the field of telepathology. Although still their routine application in diagnostic work is seriously limited through enormous data volume (hundreds of MB per slide) they are introduced primarily in teaching institutions. For the moment they represent the ultimate technology for digitalization of histological slides. Their feasibility in diagnostic pathology will primarily depend on development of transmission and storage technology as well as possible shortage of pathologists.

IMAGE DATABANK AS AN IMPORTANT PART OF TELEPATHOLOGY SYSTEM

Most of the studies and programs dealing with telepathology are so far concentrated on "how to transmit as many as possible representative images in the shortest time possible?" Although this question is the basis for all telepathological endeavors it is by far not the only one. Issues such as communication protocol (chart), safety of patient data and documenting the final diagnosis are of great practical (legal) importance and each system having aspiration on routine use must comply with it.

Teaching is one of the major goals of today's communication. The described system has also been successfully applied in the field of under and postgraduate teaching. The program we use is the ISSA/Pharos (VAMSTECH, Zagreb, Croatia). This is a PACS (picture archiving and communication system) coupled with patient database and telepathology/telemedicine program working under Windows. It is designed for simultaneous transmission and storage of text and images and can be used on standard telephone lines, via Internet or using broadband communication channels.

CONCLUSION

Telepathology represents in many technical aspects the high end of telemedicine. In our work so far we have tried to solve some of the most crucial problems in telepathology in order to render this discipline applicable as possible. As acquisition and transmission of images is the most time consuming part of telepathology, the store and forward concept gives both the sending and receiving side a better possibility of organizing their work so that telepathology activity does not collide with other engagements. This request is, from our point of view, crucial in introducing telepathology as a routine tool. In addition, the patchwork tool or virtual slides give the possibility of much better insight in the architecture of the specimen reducing possible misdiagnosis and fostering positive attitude toward telepathology. Linking patient/image database to telepathology system gives additional data safety and comfort in managing simultaneously patient data and images. As far as I know, the use of this tool in the field of veterinary pathology could significantly improve and fasten the process of standardization and classification of the histopathological lesions and be of great help for veterinary pathology students.

Indirect Immunohistochemistry for the study of the pathogenesis and the diagnosis of viral diseases

Fabio Del Piero, DVM, PhD, Dipl. ACVP

University of Pennsylvania, School of Veterinary Medicine, Department of Pathobiology and Clinical studies New Bolton Center, United States of America

Indirect immunohistochemistry (IHC) is widely used for the detection of viruses in fixed tissue for the study of the pathogenesis and the diagnosis of viral diseases. In addition, IHC is used for the detection of other agents such as bacteria, protozoa and fungi and for the detection of cell proteins. The technique is rapid, inexpensive, sensitive, specific, safe, and characterized by permanent staining because infectious agents, with the exception of prions, are inactivated by common fixatives. In addition, IHC allows the simultaneous visualization of histologic and histopathologic features and their correlation to intracellular viral localization. Monoclonal and polyclonal antibodies can be produced against any infectious agent and used for their detection. Dextran polymers and enzyme polymerization are used to increase sensitivity and increase the quality of the staining. The selective or combined use of IHC, PCR, in situ hybridization and conventional virus isolation will give the best results, since the techniques are often complementary. Here we briefly describe tissue localization and lesions during natural infection of several viruses which are diagnosed and studied by veterinary pathologist. Viruses can be classified in polyspecific (tropism for multiple species), species specific with similar lesions and distribution and species specific with variation of lesions and distribution. The following viruses are polyspecific. *Rabies rhabdovirus* causes polioencephalomyelitis with Negri bodies and ganglionitis and abundant intracytoplasmic virus can be detected within neurons, fibers, glial cells, particularly within the CNS gray matter, but also within retinal cells and less frequently within the corneal epithelium and in some carnivores within the skin adnexa. Intensity of inflammation does not correlate with viral distribution. *West Nile flavivirus* (WNV) is a mosquito transmitted arbovirus which causes polioencephalomyelitis in mammals with rhombencephalic tropism, sparing the neocortex. Intracytoplasmic WNV can be detected within neurons, fibers and glial cells, particularly in glial nodules. It rarely causes a productive infection in dogs, where it has been also identified within the cytoplasm of renal tubular epithelial cells. Birds are a natural host of the virus and any cell type of receptive avian species can be colonized. Lesions may be mild to severe and may include acute hemorrhage, encephalitis, and enteritis with gland crypt necrosis. Virus distribution does not correlate with lesions.

Eastern equine encephalitis alphavirus (EEV) geographic distribution follows the presence of the carrier *Culiseta melanura*. EEV and the related North American viruses (WEE, VEE) cause severe polioencephalomyelitis with neuronal necrosis and neutrophils in mammals. In horses and humans, lesions are severe and correlate with abundant virus distribution, whereas in camelids, lesions are less intense and do not necessarily correlate. Viral targets are neurons, fibers, glial cells, but also cardiomyocytes, smooth muscle cells and renal dendritic interstitium. The extraneural locations contain small viral quantities. Horses and pheasants present very similar viral localization but lesions are more severe in horses, which also present smooth muscle and myocardium necrosis. *Borna disease virus* causes polioencephalitis and retinochorioidopathy in mammals and localizes within the nucleus and cytoplasm of neurons and fibers. *Canine distemper morbillivirus* is able to infect canids, felids, procionids, mustelids, viverrids and marine mammals. The virus is pantropic being detectable within the cytoplasm and nucleus of almost any cell type. It causes

multisystemic lesions which may include enteritis, pneumonia, dermatitis, encephalitis and ophthalmitis and dysplasias of enamel and retina. *Pseudorabies suid herpesvirus 1* causes abortion, pneumonia and encephalitis in pigs but is able to cause encephalitis in other mammals, particularly in cats and other carnivores. As many other herpesvirus the targets are epithelia, endothelia and white cells, and in addition this virus is characterized by a very strong neuronotropism. For this reason it has been extensively used to study neural pathways. *Influenza orthomyxoviruses* are able to colonize nucleus and cytoplasm of numerous cell types, in particular epithelia, endothelia, white cells and neurons. Depending on their pathogenicity, they can produce mild to very severe lesions in vertebrates, which include multiple organ necrosis, pneumonia, enteritis and encephalitis. *Foot and mouth disease aphthovirus* infects numerous cloven foot species and others and can be localized within the cytoplasm of rapidly replicating squamous epithelia where it is associated with the formation of vesicles. In cases of myocarditis the virus is present in cardiomyocytes and interstitial cells. *Vesicular stomatitis rhabdovirus* is very similar. The followings are species specific viruses with similar pathogenesis and distribution. *Papillomaviruses* are common epithelial pathogens and often associated with normal and neoplastic squamous epithelia. They can be detected within the nucleus of squamous cells in a process of apical progressive maturation and condensation. *Parvoviruses* cause enteric glandular crypt necrosis, lymphoid tissue necrosis, conceptus loss and malformations. They can be detected within the nucleus and cytoplasm of enterocytes and other epithelia, white cells and in young animals in several cell types including cardiomyocytes. Canine parvovirus 1 is a sporadic pathogen of puppies able to induce pneumonia with pneumocyte and bronchiolar epithelium colonization. *Rotaviruses* colonize the proximal part of the small intestinal villi epithelium in young animals producing enteric disease. *Coronaviruses* are able to colonize the large intestine as well, and may be detected within the cytoplasm of epithelium and in a few mucosal macrophages. They have been associated with interstitial pneumonia, but reports generally lack convincing morphologic evidence of lung localization. *Adenoviruses* are able to cause enteritis, bronchiolitis, rhinitis, laryngopharyngitis, multiple organ necrosis and frequently necrotizing hepatitis. They colonize the cell nucleus only. *Lentiviruses* responsible of pneumonia, mastitis, polyarthritis and myeloencephalitis can be found in the cytoplasm of macrophages of lung, bone marrow, mammary gland, lymph node, spleen, synovium, brain, and spinal cord, frequently in association with lymphocyte infiltrates. The followings are species specific viruses with some variations in viral distribution and induced lesions. *Equine herpesvirus 1* (EHV-1) is able to cause abortion associated with fetal necrotizing bronchiolitis and multifocal necrosis of liver, adrenal gland and thymus, mild respiratory disease, pulmonary vasculotropic infection with acute fatal lung edema and hemorrhage, and myeloencephalopathy. In the abortigenic form, EHV-1 colonizes nucleus and cytoplasm of epithelia, endothelia and white cells. In the fatal pulmonary vasculotropic form it infects the vascular pulmonary endothelia. CNS endothelial colonization produces the thrombosis, necrosis and hemorrhage which characterize the EHV-1 myeloencephalopathy. EHV-1 is not a neuronotropic herpesvirus like the pseudorabies virus. The vascular system is the principal but not unique target of *equine arteritis arterivirus* (EAV). Equine viral arteritis has variable presentations including interstitial pneumonia, panvasculitis with edema, thrombosis and hemorrhage, lymphoid necrosis, renal tubular necrosis, abortion and inflammation of male accessory genital glands. EAV can be demonstrated within the cytoplasm of epithelial cells such as alveolar pneumocytes, enterocytes, adrenal cortical cells, trophoblast, thymus stroma, renal tubular cells and male accessory genital glands. It can also be demonstrated within endothelia, in vascular, myometrial and cardiac myocytes, macrophages, dendritic-like cells of lymphoid organs and chorionic mesenchymal stromal

cells. The aborted fetus rarely contains diagnostic levels of EAV and the diagnostic tissues are chorion and maternal endometrium. *African horse sickness orbivirus* causes fatal pulmonary edema and localizes in a few sparse endothelial cells, but also monocytes, macrophages and dendritic cells. *Bluetongue orbiviruses* target the cytoplasm of the same cells, but are characterized by additional lesions such as epithelial erosions, ulcers and necrosis of cardiac papillary muscles. *Bovine diarrhea virus*, *border disease virus* of small ruminants and *classical swine fever virus* are *pestiviruses* able to cause conceptus loss, malformations, natimortality, enterotyphlocolitis, pneumonia, lymphoid tissue necrosis and persistent infection. They are pantropic viruses and in persistently infected animals they are able to colonize any cell type, perhaps with the exception of skeletal muscle. In no persistently infected animals, cell targets are epithelia, endothelia and white cells. IHC on skin biopsy is a very good diagnostic technique to detect persistently infected cattle. *Bovine herpesvirus 1* causes abortion, laryngotracheitis and bronchopneumonia, lymphoid tissue necrosis and vulvovaginitis. Like in other herpesviral infections, intranuclear and intracytoplasmic virus can be detected within epithelia, endothelia and white cells. The fetal lesions and viral distribution are very similar to EHV-1, with the difference that BHV-1 heavily infects the chorionic endothelia. For this reason, the placental chorion is an excellent diagnostic tissue. *Bovine respiratory syncytial pneumovirus* (BRSV) cause syncytial bronchointerstitial pneumonia with inclusion bodies. BRSV can be detected within pneumocytes and macrophages. *Parainfluenza 3 virus* has a similar distribution, but lesions are less severe. *Rinderpest morbillivirus* causes enterotyphlocolitis with lymphoid tissue necrosis and syncytia with intranuclear and intracytoplasmic epithelio- and lympho-tropism. *Pest des petite ruminants morbillivirus* causes similar lesions and virus distribution in small ruminants, in addition bronchointerstitial pneumonia with syncytia can be observed. *Rift Valley fever bunyavirus phlebovirus* and *Wesselbron's disease flavivirus* are African zoonotic arboviruses able to cause abortion, natimortality, malformations, hepatic necrosis with non specific inclusion bodies and encephalitis. Viruses can be detected within the cytoplasm of hepatocytes and neurons. *Porcine reproductive and respiratory syndrome arterivirus* causes conceptus loss, sometimes with funisitis, lymphoid tissue necrosis, vasculitis, pneumonia, and rarely encephalitis. The virus can be detected within the cytoplasm of macrophages, but also endothelial cells and vascular myocytes. At least four samples of affected lung should be examined to significantly increase the possibility to obtain a diagnosis. *Porcine circovirus 2* causes postweaning wasting syndrome (PMWS), pneumonia, dermatitis and nephritis, granulomatous enteritis, rarely conceptus loss. Histologically syncytial cells and inclusion bodies can be identified. In PMWS, the virus colonizes nucleus and cytoplasm of macrophages, histiocytes, multinucleated giant cells and dendritic cells of tonsil, lymph nodes, spleen and other lymphoid areas *African swine fever virus* causes systemic and lymphoid tissue hemorrhages and abortion. The virus can be detected in intracytoplasmic viral factories within macrophages and sometimes, later in the infection, within endothelial cells. *Canine herpesvirus 1* causes natimortality in puppies. As other herpesviruses, it induces multifocal necrosis of organs, such as liver, lung and lymphoid tissue, but also a very characteristic multifocal to coalescing renal necrosis with hemorrhages and inclusion bodies. The virus colonizes nucleus and cytoplasm of epithelia, endothelia and white cells. *Feline herpesvirus 1* sporadically produces similar lesions with the addition of keratoconjunctivitis and dermatitis and with a similar viral cell colonization pattern. *Feline infectious peritonitis coronavirus* causes pyogranulomatous systemic disease with vasculitis in felids. The virus localized within the cytoplasm of macrophages forming granulomas. IHC examination of multiple affected organs is often necessary to detect the agent. *Feline calicivirus* causes ulcerative stomatitis and virulent isolates are able to spread systemically inducing

vasculitis, ulcerative stomatitis and multiple organ necrosis. The virus is present within the cytoplasm of epithelia and endothelia of the affected tissues. *Rabbit hemorrhagic disease calicivirus* causes systemic hemorrhages thrombosis and necrosis. The virus can be detected within liver, lung, spleen and lymph nodes cells and intravascularly in cells of macrophage lineage. The followings are viruses infecting poultry. *Avian infectious bronchitis coronavirus* causes sinusitis, laryngotracheitis, pneumonia and nephritis. The virus is intracytoplasmic and epitheliotropic and it colonizes the respiratory tract and the renal tubular epithelium. *Newcastle disease avian paramyxovirus 1* colonizes epithelia, lymphoid tissue, neurons and is able to cause lymphoid tissue necrosis and necrotizing proventriculitis and gastroenterotyphlocolitis. *Infectious bursitis (Gumboro) byrnavirus* causes lymphoid tissue necrosis and the virus targets the cytoplasm of macrophages dendritic cells and lymphocytes. *Avian pneumovirus* colonizes the cilia and the apical cytoplasm of the upper respiratory epithelium. It has been associated with sinusitis and rhinolaryngotracheitis. There is a progressive expansion of IHC test for the detection of infectious agents in pet birds and reptiles. *Ophidian paramyxovirus* causes pneumonia with occasional epithelial syncytial cell formation and intraepithelial eosinophilic intracytoplasmic inclusions and the virus localizes within the nucleus and cytoplasm of epithelial cells and phagocytes. There is also a progressive expansion in the IHC for the detection of piscine viral diseases such as *nodavirus retinopathy and encephalopathy, channel catfish herpesvirus, herpesvirus disease of salmonids, turbot herpesvirus, koi herpesvirus, carp pox cyprinid herpesvirus, viral hemorrhagic septicemia of rainbow trout novirhabdovirus, salmonid infectious hematopoietic necrosis rhabdovirus, spring viremia of carp rhabdovirus carpio, pike fry rhabdovirus disease, infectious pancreatic necrosis of salmonid fry and fingerlings birnavirus, lymphocystis disease iridovirus, epizootic erythropoietic necrosis iridovirus ranavirus, erythrocytic necrosis iridovirus ranavirus, largemouth bass iridovirus ranavirus, Atlantic salmon infectious anemia orthomyxovirus.*

References available upon request – Fabio Del Piero – UPenn New Bolton Center – 382, W. Street Road – Kennett Square – PA – 19348-1692 – USA – fdp@vet.upenn.edu.

**Abstracts of
the Oral and Poster Presentations**

Specific mutations of the C-KIT protooncogene observed in feline cutaneous mast cell tumors

Abadie, Jérôme, Vincent Fort, Sonia Becavin, Lionel Martignat

Unité d'Anatomie Pathologique – Ecole Nationale Vétérinaire de Nantes, Nantes cedex 03
- FRANCE

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Introduction: Feline cutaneous mast cell tumors (MCTs) are frequent and challenging tumors because of often unpredictable biologic behavior. Regarding mast cell neoplasia, great interest has been focussed on the proto-oncogene *c-kit* and its product the KIT protein, a transmembrane tyrosine-kinase receptor which is known to play a critical role in normal mast cell growth and differentiation. In recent years, mutations of the *c-kit* gene, resulting in constitutive activation of KIT have been reported in human and canine mast cell malignancies. Our aim was to look for occurrence and type of *c-kit* mutations in feline cutaneous MCT

Material and methods: A total of 35 formalin-fixed paraffin embedded samples of feline cutaneous MCT were analysed. Specific PCR primers were designed to amplify the exon 8, the exon 11 and intron 11 and the exon 17 of the feline *c-kit* gene. Amplification products were visualised with agarose or polyacrylamide gels. Analyses of dissociation curves followed by sequencing were used to detect short mutations (exon 11).

Results: In 6/27 samples, 2 different amplified sequences were unambiguously detected for exon 11 due to a 6 pb deletion that has been confirmed by sequencing. Furthermore, 1/5 feline cutaneous MCT presented a deletion of intron 11, similar to what has been described in canine MCT and 5/35 samples, presented an 15 pb insertion in exon 8, which has never been reported before in the canine or feline *ckit* gene. None of our 35 samples revealed any internal tandem duplication in exon 11, or any mutation in exon 17.

Discussion: In a comparative perspective, *c-kit* is frequently mutated in either the juxtamembrane domain (exon 11/intron 11) or in the tyrosine kinase domain (exon 17) in human mastocytosis. Furthermore, internal tandem duplication in the juxtamembrane domain (exon 11) is the most common mutation observed in canine MCT. A single study reported the occurrence of duplication in the exon 8 in a feline case of systemic mastocytosis.

In our study, we described specific mutations in exon and intron 11 and in exon 8 in 12/35 cases of feline MCT. Further studies are in process to correlate the above mutations of the *ckit* gene and the prognosis in feline cutaneous MCT.

Skin mast cells and endogenous fatty acid amides in canine atopic dermatitis: a pilot study

Abramo, Francesca, Francesco Albanese, Vincenzo Miragliotta, Stefania Petrosino, Vincenzo Di Marzo

Department of Animal Pathology – University of Pisa, Pisa, Italy

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Introduction: Atopic dermatitis (AD) is an itchy chronic skin disease. Langerhans cells, lymphocytes and mast cells (MCs) have been shown to play a pivotal pathogenetic role, together with a defective skin lipid barrier. On the other hand, the increase in tissue concentrations of endogenous fatty acid amides (FAAs) has been demonstrated to play an “autoprotective” role in some inflammatory and degenerative disorders of the skin. The purpose of this study was i) to investigate the number of MCs (morphometry) and their granule content (densitometry) in dogs affected by AD and ii) to analyse skin levels of the aliamide palmitoylethanolamide (PEA) and other FAAs in atopic skin.

Material and methods: Five healthy dogs and 5 dogs affected by AD were included in the study. Clinical diagnosis of AD was based on Willemse’s criteria. Six mm biopsy punches were collected in duplicate from adjacent sites (lesional areas in AD subjects), one was stained with toluidine blue (TB), the other was snap frozen in liquid nitrogen for lipid extraction. MC morphometry and densitometry were evaluated by using the Lucia (Nikon, Japan) analyser system. The distribution of MCs and their granule content were assessed at high power field (magnification x400) from the superficial (subepidermal) and perifollicular dermis. Tissues were homogenized and lipid-containing organic phase was dried down, weighed and pre-purified by open-bed chromatography on silica gel. The amounts of PEA and other FAAs were determined by isotope dilution liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry (LC-APCI-MS). The amounts of lipid extracts and FAAs were compared by the Wilcoxon signed-rank test. MCs counts and densitometry values were compared by the unequal variance t-test.

Results: MCs in normal skin were 53.90 ± 16.64 cells/mm² and 34.34 ± 5.06 cells/mm² in the subepidermal dermis and in the perifollicular areas, respectively. In the same selected areas dogs with AD showed higher values (131.75 ± 45.69 cells/mm² and 101.58 ± 21.84 cells/mm²). The difference was statistically significant ($p < 0.01$). In the perifollicular areas, a statistically significant decrease in MC granule content was observed in atopic compared with normal canine skin ($p < 0.0001$). The amount of lipid extract was significantly reduced in the lesional skin of AD dogs, as compared to control dogs (5.79% vs 35.63%, $p < 0.05$). Conversely, the levels of all analysed FAAs were significantly elevated ($p < 0.05$). In particular PEA levels showed the highest increase, being more than 30-fold higher in AD lesional skin than in normal non-atopic skin.

Discussion: The present data represent the first demonstration of a significant difference in the skin lipid content and skin levels of the aliamide PEA and other FAAs between normal and atopic dogs. Although there is still some disagreement over the increase of MC count in lesional skin from atopic dogs, our data clearly show a significant higher number of MCs both in superficial and perifollicular dermis. The decrease in perifollicular MC granule content is a further indirect proof of the so-called MC hyper-releasability observed in the skin of dogs with AD.

The effect of the astaxanthin in chemically induced mammary carcinogenesis in immature Whistar female rats

Adrian Florin, Gal, Sanda Andrei, Alecsandru Ioan Baba, Catoi Cornel, Rus Ioan, Taulescu Marian

University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Romania

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Introduction: The aim of the study is to notice the effect of an antioxidative agent (astaxanthin-ASTA) in the prevention of the chemically induced mammary carcinogenesis in immature Whistar rats. The specialty literature mentions that methyl-nitrosourea is the election chemical carcinogen used in mammary tumor induction in laboratory animals. Astaxanthin (ASTA) is a red pigment that belongs to carotenoid family and could be encountered in marine algae and aquatic animals. There are several reports that indicate the involvement of the reactive oxidative species (ROS) in the induction of mutations. The studies realized in other types of tumors indicate that, a diet reached in antioxidative agents could help in the prevention of cancer, but there are no studies that use ASTA in mammary tumor prevention.

Material and methods: To induce the mammary tumors in female immature rats had been used chemically induced carcinogenesis, respectively methyl-nitrosourea (MNU). There were established 5 groups of 37 days old Whistar immature rats, such as: MNU group (7), MNU+ASTA group (8), oil group (5), ASTA group (5), and absolute free group (5). The dose of carcinogen utilized was 55 mg MNU/kg body weight, intraperitoneally inoculated. The antioxidative agent was administered orally in a dose of 50 µg astaxanthin/rat/day, during 7 months. The rats were sacrificed after 14 months by narcosis.

Results: Our results indicate a reduced incidence of mammary tumors in Whistar immature female rats, respectively of 5,26% in MNU group and 37,5% in MNU+ASTA group, but there were diagnosed a lot of other non-mammary tumors in both groups. The necropsy revealed different tumor types in MNU group (liposarcoma 6 tumors; nephroblastoma–4 tumors; hemangiosarcoma–2 tumors; bronchial gland carcinoma–2 tumors; lipoma–1 tumor; squamous carcinoma–1 tumor; sebaceous gland carcinoma–1 tumor; small cell lung carcinoma–1 tumor; simple mammary adenoma–1 tumor) and MNU+ASTA group (nephroblastoma–2 tumors; liposarcoma–1 tumor; bronchial gland carcinoma–1 tumor; colangiocarcinoma–1 tumor; simple tubulo-papillary mammary carcinoma–1 tumor; mammary fibroadenoma–1 tumor; anaplastic mammary carcinoma–1 tumor). All the female rats from the MNU group developed 1 or several tumors in different organs, the mean tumor number/rat being of 2,71. In the case of MNU+ASTA group had been encountered the following situation: 3 rats without any tumors, 2 rats with 1 tumor each one and the others 3 rats developed 3 tumors each female. The mean tumor number in MNU+ASTA group was 1 neofomation/rat. The tumor size varied from 0,1-4 cm in MNU+ASTA group and 0,3-7,5 cm in MNU group.

Discussions: Comparatively with bibliography that indicate a mammary tumor induction rate of 86-95%, in our experiment the mammary tumors were encountered in 5,26% of MNU group and 37,5% in MNU+ASTA group. Regarding the protective effect of astaxanthin in mammary tumors, the results indicate a partially protection, but not only in mammary tumors but in a lot of other tumors that were diagnosed in our experiment induced by MNU. Despite of that, the protection is not complete but reduce significantly the tumor incidence that could be noticed in our dates (mean tumor number/rat being of 2,71 in MNU+ASTA group comparative with 1 tumor/rat in MNU group). Our dates are confirmed statistically by Student T-test.

Distribution of AA amyloid in naturally affected sheep and goats: The gut as a target organ

Alvarez, Neila, Tatiana Crespo, Eider Salazar, Marta Pérez, Esther Biescas, Lluís Luján.

University of Zaragoza, Zaragoza, Spain

P32

Introduction: AA amyloidosis type AA is an important pathological process in the local ovine livestock. It is characterized by desposition of fibrillar proteins, which structure is responsible for the affinity for Congo Red staining, showing green birefringence when it is viewed in cross-polarized light. This deposits produce important disfunctions, and eventually the death of the animal. Reactive, AA amyloidosis is related to persistent infections, neoplasms and chronic inflammation. AA amyloid is derived from a protein of the acute phase of the inflammation called Serum Amyloid A (SAA), sintetized in the liver. When the concentration of this molecule is increased persistently, certain isoforms of SAA are partially cleaved into fragments that form fibrillar aggregates that are deposited in tissues.

Our group had already described the pathology of AA amyloidosis in kidney, but the complete histopathology of affeted animals is not known.

The objective of the present study is to describe the tissue distribution in AA amyloidosis naturally-affected sheep and goats.

Material and methods: Tissues from 18 naturally affected sheep and 6 goats were studied. Slides were collected from previous necropsies files, reviewed since 2002, attending to the amyloid-associated macroscopic pathology described for each reported case: gangrenous pneumonia, other chronic inflammations and the presence of tubulonephrosis.

Tissues studied were: kidney, rumen, reticulum, omasum, abomasum, duodenum, large intestine, spleen, lymphnode, liver, pancreas, heart and lung.

Slides were stained with Hematoxilin- Eosin, and also with specific Congo Red and immunohistochemistry stain. Presence of amyloid in samples either by CR staining or by IHC was classified as - (absent), w+ (weak positive), + (mild), ++ (moderate), and +++ (severe).

Results: The most important finding was the gastrointestinal tract and specially the duodenum. The gastrointestinal tract had a severe to moderate desposition of amyloid, with a massive affection of duodenum and Brunner Glands. In many cases, the affection of the digestive was more severe than kidneys. All prestomachs were moderate to severe affected, but amyloid was absent in abomasum in all cases. In spleen, amyloid deposited diffusely or in ring-forms around the follicles.

Discussion: The digestive tract is a main target for natural sheep and goats AA amyloidosis. When lesions of tubulonephrosis can be observed macroscopically, the digestive tract is already affected. Duodenum and prestomachs seem to be the primary tissue where amyloid deposits although the affection of the digestive tract is not associated to a macroscopic pathology.

Cav-1 immunoexpression in canine mammary tissues

Amorim, Irina, Célia Lopes, Rui M. Gil da Costa, Augusto M. R. Faustino, Patrícia Dias Pereira

ICBAS-UP, Oporto/Portugal

P48

Introduction: Caveolins are integral membrane proteins of caveolae which seem to be involved in several physiological processes, including regulation of signal transduction cascades. Recent investigations have implicated caveolins in a variety of human diseases, namely breast cancer. Several studies documented a down-regulation of caveolin-1 (one of the members of the caveolin family) in breast cancer, suggesting that it may play a tumour-suppressive role, while others investigators reported caveolin-1 overexpression in mammary neoplasms. Despite those contradictory results, it is consensual that caveolin mediate the tumour surveillance process, constituting therefore an important factor when considering cancer research. The role of caveolins in the development of canine mammary tumour is still unknown and, so far, there are no reports on its immunoexpression in canine normal or neoplastic mammary gland. The purpose of this study is to evaluate the immunoexpression of caveolin-1 in normal, benign and malignant neoplastic canine mammary tissue, in neoplastic emboli and metastatic lesions.

Material and methods: Samples of 5 normal canine mammary tissue, 24 benign and 49 malignant mammary neoplasms, 10 metastatic lesions and 10 cases with neoplastic emboli were collected. Consecutive sections were obtained from each case and used for haematoxylin-eosin staining and for the immunohistochemical study with polyclonal antibody Cav-1, diluted 1:400. The immunoexpression of Cav-1 was evaluated according to the grade, intensity and pattern of staining.

Results: A weak focal immunostaining was found in luminal epithelium in 40% of normal mammary samples and in 30% of benign neoplasms. In 91% of malignant tumours, 90% of metastatic lesions and 90% of neoplastic emboli, a stronger and more extensive immunostaining was observed in luminal epithelial cells. Myoepithelium exhibited moderate to strong immunoreactivity in normal as well as in benign and malignant neoplasms.

Discussion: Our results support the hypothesis that caveolin-1 may play an important role in the neoplastic transformation process, especially concerning the acquisition of a malignant and metastatic phenotype. Further research would be very helpful in order to identify signalling molecules and modifying factors that interact with caveolins facilitating tumourigenesis.

A case of gastric carcinoma with osseous metaplasia in a dog

Amorim, Irina, Célia Lopes, Augusto Rui M. Gil da Costa, Fátima Gärtner, M. R. Faustino, Patrícia Dias Pereira

ICBAS-UP, Oporto/Portugal

P24

Introduction: Gastric tumours account for less than 1% of all reported neoplasms in dogs. Carcinoma is the most frequent canine gastric neoplasm, followed by smooth muscle tumours and lymphomas. Most gastric carcinomas are located in the smaller curvature and pylorus often progressing to involve most of the stomach body. The average age of dogs with gastric carcinoma ranges from 7,5 to 10,2 years. Some authors report a higher incidence in males. This report describes an unprecedented case of a gastric carcinoma with osseous metaplasia in a 8-year-old male standard poodle.

Material and methods: An exploratory laparotomy confirmed a firm yellowish nodular mass, located in the pyloric region, with irregular borders and indistinct limits. The lesion was surgically removed and submitted for current histological examination. Immunohistochemistry was also performed employing the monoclonal antisera pan-cytokeratin, vimentin, muscular α -actin and BMPs -2 and -4.

Results: Histologically, the mass consisted of a multinodular neoplastic proliferation of well-differentiated epithelial cells, arranged in a tubular or acinar pattern and supported by scirrhous stroma. Mitotic figures were frequent (6 mitotic figures per high power field) some of which were atypical. Multiple foci of metaplastic ossification, consisting of osteoid and fully matured bone tissue, surrounded by neoplastic epithelial cells, were identified both in the mucosa and muscular layers. Neither cytological atypia nor mitotic figures were found in those metaplastic osseous foci. Multiple neoplastic epithelial emboli were also observed. Histopathological and immunohistochemical findings suggested a diagnosis of gastric carcinoma with osseous metaplasia.

Discussion: Osseous metaplasia is a rare finding in gastric epithelial neoplasms, with only very few cases reported in humans. Its pathogenesis remains unclear, however there are some recognized tumour related factors with osteogenic properties, namely BMPs that may be involved in the formation of osseous areas both within the tumour and in metastatic lesions. Histopathological and immunohistochemical features of this particular lesion suggested that the bone tissue may be primarily originated from neoplastic epithelial cells which directly circumscribe the osseous metaplasia foci.

Immunohistochemical findings of uterine tumours in the rabbit

Annachiara, Vinci, Barbara Bacci, Cinzia Benazzi, Giuseppe Sarli

Department of Veterinary Public Health and Animal Pathology, Faculty of Veterinary Medicine, Bologna, Italy

P56

Introduction: Uterine neoplasms are the most common spontaneous tumours in the rabbit and most cases are adenocarcinomas of the endometrium (Weisbroth, 1974). The incidence of this tumour increases with age and has been reported to reach 60% in animals over 4 years (Brown, 2002). The neoplastic proliferation of endometrial epithelium is influenced by estrogen and progesterone. Pseudopregnancy is commonly seen in the mature rabbit and is characterized by prolonged maintenance of the corpora lutea and secretion of progesterone in the absence of conceptus (Knobil, 1998). This suggests that non-neutered female rabbits are predisposed to the development of uterine cancer.

Material and methods: Nine uterine tumors (4 adenomas and 5 adenocarcinomas) were selected and submitted for immunohistochemistry (IHC) with antibodies to: CK19, Progesterone (PR), MIB-1 (anti Ki67) and telomerase (TEL).

Results: All four cases of adenoma were positive to PR, with moderate to strong nuclear staining; all cases were negative to CK19; staining with MIB-1 was observed in all cases with 2 showing less than 25% of positive nuclei and 2 more with 25-50% positive nuclei; 2/4 were positive to TEL. Among adenocarcinomas 4/5 cases were positive to PR; all were moderately to intensely positive to CK19; staining with MIB-1 was observed in all cases with 4/5 cases showing less than 25% of positive nuclei and 1/5 cases with 25-50% positive nuclei; all cases were negative to TEL.

Discussion: It is well-known that hormones play a major role in the development of abnormal proliferative lesions of the endometrium and in women with uterine adenocarcinomas several studies have shown that the presence and quantity of steroid receptors are correlated with histologic differentiation and survival (Silverberg et. al., 1992). In the present study the expression of progesterone was similar in both adenomas and adenocarcinomas. This suggests that progesterone promotes neoplastic transformation, but does not influence the behaviour of the tumour. Proliferative activity was evaluated by means of the expression of MIB-1. Proliferation was low in both benign and malignant tumors; in most cases (7/9) the percentage of proliferating cells was lower than 25%. This indicates that the proliferative activity is not correlated with malignancy. Telomerase expression suggests that it plays a key role in the progression of several tumours. In this study nuclear telomerase expression was detected in only two cases of adenoma. CK19 expression was investigated and resulted positive in all malignant cases, whereas it was completely absent in benign tumors. On the basis of the obtained results some tumours do not seem to arise from the uterine glands as appears from the negativity to CK19, but from the luminal epithelium. Hormonal therapy of uterine carcinomas in rabbits can be considered more appropriate than the antiproliferative therapy, considering the low proliferative activity of endometrial malignant tumors. The low expression of telomerase in adenomas and its absence in adenocarcinomas does not support the importance of telomerase dependence of malignant transformation and the employment of anti-telomerase therapeutic approaches.

Histopathological changes in fish gills – preliminary observations

Antychowicz, Jerzy, Marek Matras, Michal Reichert

National Veterinary Research Institute, Pulawy, Poland

P110

Introduction: The gills differ from all other fish organs firstly because they contact indirectly with water environment and could be damaged easily by various physical, chemical and biological factors, secondary because gill damage has immediate consequences for the rest of the body and for the fish survival. Gills delicate met-like structure provide for gaseous exchange as well as acid-base balance, osmoregulation, excretion of nitrogenous waste products (in teleost fishes in form of ammonia). Consequences of the functional gill impairment and pathological changes in morphology are quite severe for the fish though not always wholly understood.

Material and methods: The samples of the gills from carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) were taken for histological examination when clinical symptoms in these fishes were observed.

Results: Various types of pathological lesions in gills were documented and described. In some fishes the infectious and parasitic diseases were diagnosed in other stress or negative environmental factors were suspected. Stress and toxic substances induced gill damages which predispose fish to viral infections and also secondary bacteria and water moulds colonization.

Discussion: Histological examination of the gills have paramount importance in investigation the cases of fish abnormal mortality as well as pathogenesis of fish diseases.

E-cadherin- β -catenin expression in canine colorectal adenocarcinoma: a suggested role upon local invasion

Aresu, Luca, Bartolomeo Biolatti, Paola Pregel, Laura Cavicchioli, Rossella Zanetti, Massimo Castagnaro

Dipartimento di Sanità Pubblica, Patologia Comparata e Igiene Veterinaria, Facoltà di Medicina Veterinaria, Università di Padova, Legnaro (PD), ITALIA

P25

Introduction: E-cadherin and its associated cytoplasmic proteins including beta-catenin play a pivotal role in the maintenance of normal tissue architecture and as suppressor of cancer invasion. The molecular mechanisms through which the tumor acquires the invasive potential remain poorly understood. The pathogenesis of colorectal tumors in domestic animals seems to be correlated to a multistage process, beginning as a benign lesion progressing in carcinoma through invasion of basement membrane. The purpose of this study was to evaluate the expression of E-cadherin and β -catenin in colorectal cancer, and to examine their relation with various clinicopathologic variables.

Material and methods: Paraffin wax-embedded canine intestinal adenocarcinoma (n= 44) from the Veterinary Pathology Diagnostic Service of Padua and Turin University were included in this study. The tumours were classified on haematoxylin and eosin sections following the diagnostic criteria of the World Health Organization classification of tumours in domestic animals. The human grading for the extension of the tumor was applied T1 (invading submucosa), T2 (invading *muscularis propria*), T3 (extending beyond *muscularis propria*), T4 (invading free surface of adjacent organs). E-Cadherin and β -Catenin immunohistochemical analysis was performed. The percentage of tumor cells with normal membranous expression of E-cadherin and β -catenin in each specimen was graded semi-quantitatively into one of the five-ties scale scoring system: (-) no staining, (+) 1-20%, (++) 20-50%, (+++) 50-80%, (+++++) 80%. For β -catenin, tumors were additionally subdivided into either cytoplasmatic or nuclear localization.

Results: In normal intestinal mucosa, E-cadherin and β -catenin expression was located on cell membranes along intercellular interfaces. Expression of E-cadherin membrane was preserved in 11 cases (25%) and reduced in the rest of tumours examined (75%). Expression of β -catenin at cell-cell junctions was reduced in 81.8% of cases. In addition, for β -catenin, a cytoplasmic expression was observed in 6.8% of cases and also nuclear staining was detected in 20.5% of colon-rectal carcinoma. A significant correlation emerged between the reduction of E-cadherin expression and the worsening of tumour grade (depth of the tumour). Results for β -catenin could be superimposed. A marginally significant correlation was also detected between the increase of tumour size and the reduction of the β -catenin expression.

Discussion: Down-expression of membrane staining of E-cadherin and β -catenin, observed in adenocarcinomas, was correlated with different transmural invasion compared to tumors limited to submucosa and muscular layer. The dysfunction of the E-cadherin-catenin complex occurs in early stages of carcinogenesis and the disruption of tissue architecture is progressively associated with the invasion of the tumor. Variable cytoplasmic and nuclear staining for β -catenin was associated with its activity directly with the cytoplasmic domain of E-cadherin, which mediates the interaction with the actin filament network. The translocation of β -catenin from membrane to nucleus disrupts the interaction between E-cadherin and α -catenin. Blocking E-cadherin down-regulation in tumors is

one of the important features in the new gene therapy; dogs might become important animal models to approach therapies in this direction.

Evaluation of a three-dimensional culture of equine guttural pouches to study interactions between *Streptococcus equi* and mucopolysaccharides: preliminary results

Arias, Maria, Gian Enrico Magi, Francesco Parillo, Vincenzo Cuteri, Giacomo Renzoni

Department of Veterinary Science, University of Camerino, Matelica, Italy

P36

Introduction: Some bacteria usually use mucopolysaccharides as a kind of receptors for adhesion to host cells. Streptococci interact with glycosaminoglycans (GAGs) through some surface M-like proteins; *Streptococcus pyogenes* M-like proteins bound to GAGs in vitro, whereas deficient M-like proteins strains did not bound them. It was suggested that SeM protein of *Streptococcus equi* subsp. *equi* (*S. equi*), the causative agent of Strangles, may interact as adhesin with mucopolisaccharides produced by guttural pouches mucosa. Then the mucopolisaccharides secretion along 5 days of tissue culture was studied. In addition, the validity of guttural pouches biopsies as model to study interactions between mucopolisaccharides and *S. equi*. was assessed.

Material and methods: Six 1cm² biopsies were taken from guttural pouches of healthy slaughtered horse and cultivated for one to five days in normal RPMI 1640 with l-glutamine, 10% FCS and 1% penicillin-streptomycin medium. Biopsies culture were stopped daily during 5 days, fixed in Carnoy fluid, paraffin embedded and stained with haematoxylin and eosin (HE), periodic-acid Schiff (PAS) and alcian-blue (AB) at pH 1.0 and pH 2.5 to evaluate tissues viability and mucopolisaccharides secretion. For experimental infection, biopsies were washed and incubated for two hours at 37°C as follows: 2 with RPMI as controls, 2 with RPMI + *S. equi* CF32 ATCC (bacteria concentration: 10⁷) and 2 with RPMI + *S. equi* + 0,25 heparin µU. Then, biopsies were washed and divided in two aliquotes: one was fixed, paraffin embedded and stained with HE and Gram. The second one was weighted, homogenized and 100 µl of 10-fold serial dilution was spread on Columbia Blood Agar with and without Streptococcus supplement and incubated at 37°C for 48 hours.

Results: Histochemical procedure showed secretion of mucopolysaccharides by goblet cells and submucosal glands until 5 days of culture although the architecture of tissue was slightly altered after 2 days of culture, as was evidenced by PAS and AB at pH 1,0 and 2,5 positivity. The best time to infect tissues was established within 24 h of culture, when epithelium is stil well conserved. Microbiology procedure showed that all control samples were negative. biopsies infected with *S. equi* had a significant higher bacterial count (3.0 x 10⁸ CFU) than biopsies treated with heparin (4.5 x10⁻² CFU).

Discussion: In our previous work we argued that some glycosaminoglycans as heparin, heparan sulphate and chondroitinsulphate B may be involved in pathogenesis of Strangles, acting as receptors for *S. equi* surface SeM protein. In this study, the well preservation and functionality of biopsies demonstrated that these might be used as three-dimensional culture model to study interactions between GAGs and *S. equi*; This was was corroborated by the fact that tissues infected with *S. equi* showed bacterial adherence. Furthermore, the finding that exogenous heparin could inhibited *S. equi* adhesion suggest that GAGs operate as receptors for SeM protein.

Immunohistochemical demonstration of homeobox proteins in canine vascular tumours

Atsushi, Kodama, Hiroki Sakai, Mami Murakami, Takashi Mori, Kohji Maruo, Tokuma Yanai and Toshiaki Masegi

Gifu University, Gifu, Japan

P8

Introduction: Hemangiosarcoma (HSA) is one of the aggressive neoplasms found in dogs, and its prognosis is very poor. Previously, we reported that HSA produced endothelial growth factors and expressed receptors similar to those of active endothelial cells (ECs) in the angiogenic condition. During angiogenesis, proliferation, migration, tube formation and vascular maturation are controlled by many transcriptional factors such as homeobox proteins, etc. Homeobox proteins regulate the sets of genes that determine cellular fates in embryonic morphogenesis and maintain adult tissue architecture by regulating cellular motility and cell-cell interaction. Angiogenic ECs express several members of homeobox proteins, suggesting a role for these morphoregulatory mediators during migration, proliferation, tube formation and vascular maturation. Considering the similarity between angiogenesis and malignant growth with regard to the growth factors produced, we hypothesized that the expressions of homeobox genes were associated with malignant characteristics. Therefore, in this study, we examined the immunohistochemistry of homeobox proteins in canine vascular tumours.

Material and methods: We investigated 83 canine primary HSA and 30 canine primary hemangioma (HA) tumour samples collected between August 1998 and April 2007. Immunohistochemistry was performed to demonstrate the expression of homeobox proteins related to angiogenesis (proangiogenic Hox: HoxA9, HoxB3, HoxB7 and HoxD3; antiangiogenic Hox: HoxD10; non-Hox homeobox: Meox2 and Hex; and cofactors for Hox: Pbx1 and Meis1).

Results: HoxA9 was detected in 57 cases of HSA (68.7%), but it was negative in all HAs. Positive reactions for HoxA9 protein were observed in the cytoplasm and nucleus. The positive immunoreactivities of HoxB3, HoxD3, HoxB7, and HoxD10 were noted in 80 (96.4%), 80 (96.4%), 76 (91.6%) and 74 (89.2%) HSAs, and 27 (90.0%), 28 (93.3%), 29 (96.7%) and 24 (80.0%) HAs, respectively. HoxD3 produced intense immunoreactivity in the cytoplasm and nuclei of HSA and HA cells. Meis1 and Hex were detected in almost all HSA and HA samples. However, Pbx1 was only detected in 10 cases of HSA, while Meox2 was detected in 7 cases of HSA and 1 case of HA.

Discussion: In this study, HoxA9 was detected in the malignant ECs of HSA but not in those of HA. Previously, HoxA9 was reported to play a specific role in EC migration and tube formation *in vitro*. Therefore, HoxA9 may contribute to the invasive potential of malignant ECs of HSA. Moreover, HoxD3 is necessary to initiate vascular endothelial sprouting and migration in the early phase of angiogenesis. In contrast, HoxD10 expression inhibited the transcriptional function of HoxD3 protein *in vitro*. Further, although HoxD3 was detected in HSA and HA samples, the functions of HoxD3 protein might be inhibited by HoxD10 expression in canine vascular tumours. In addition, Meis1 protein, which is a co-factor of HoxA9, was detected in all HSA and HA samples, suggesting that HoxA9, which collaborated with Meis1, contributed to the malignant potency of HSA.

The expression of matrix metalloproteinases (MMPs) and their tissue specific inhibitors (TIMPs) in idiopathic canine dilative cardiomyopathy – immunohistochemistry and mRNA analyses

Aupperle, Heike, Imke März, Andreas Schubert, Gregor Dinges, Jens Thielebein, Heinz-Adolf Schoon

Institut für Veterinär-Pathologie, Leipzig, Germany

Introduction: While the pathogenesis of canine idiopathic dilative cardiomyopathy (DCM) remains unclear, alterations in the activity of specific metalloproteinase enzymes (MMPs) and their inhibitors (TIMPs) within the myocardium are suspected to be involved.

Material and methods: This study describes the clinical and pathological findings in canine DCM. Furthermore, the immunohistochemical distribution patterns of MMP-2, -9, -14 and TIMP-2, -3 in the the left ventricular wall of 28 canine hearts (healthy controls n=16; DCM n=12) were investigated. Myocard samples from 10 dogs (healthy controls n=7; DCM n=3) were available for mRNA analyses of MMP-2, MMP-9 and TIMP-2, TIMP-3 using Realtime-PCR.

Results: Clinical and pathological investigations showed a decreased fractional shortening (4.2-9.7%), increased cardiac size and relative heart weight (controls 0.67%; DCM 0.85%; P=0.006) in the DCM group compared to the controls. Histopathologically the “attenuated wavy fibre type” (wType; n=6) and the “fatty infiltration-degenerative type” (fType; n=6) of DCM were diagnosed. Surprisingly, the number of fibrocytes did not vary significantly among the controls (62.5 fibrocytes/0.1 mm²) and/or the DCM groups (“wType”: 54 fibrocytes/0.1 mm²; “fType”: 74.5 fibrocytes/0.1 mm²). Immunohistochemistry showed a significant increase of MMP-9 (controls 1.0; DCM 2.0) and a decrease of MMP-14 (controls 3.0; DCM 2.0) expression intensity in cardiomyocytes in the DCM group. In all dogs, cardiomyocytes mildly expressed MMP-2, TIMP-2, and TIMP-3. The percentage of TIMP-3 positive fibrocytes was significantly increased in the DCM groups (fType 16.4%, P=0.015; wType 15.0%; P=0.028) compared to the healthy controls (6.6%). No significant variation in the percentage (about 40%) of fibrocytes expressing TIMP-2 was measured. The immunohistochemical findings did not vary between the two histopathological types of DCM. The mRNA analyses revealed a significant increase of MMP-9 mRNA in the DCM group (48.89 MMP9/GAPDH) compared to the controls (0.09 MMP9/GAPDH; P=0.02). The mRNA level of TIMP-3 was also increased in the DCM group, but the difference was not confirmed statistically.

Discussion: In conclusion, the present study showed a selective dysregulation of MMPs and TIMPs in canine idiopathic DCM. Interestingly, the expression patterns of MMPs and TIMPs did not vary between the “wType” and “fType” of DCM. The increased MMP-9 expression may lead to a diminished consistency of the myocardium, as described in non-ischemic DCM in humans. However, the decreased expression of MMP-14 and the increase in TIMP-3 positive fibrocytes have not been described in other species before. This may indicate the presence of a profibrotic compensatory mechanism, which however seems to be insufficient, resulting in an altered composition of the extracellular matrix.

The expression of transforming growth factor $\beta 1$, $\beta 2$, and $\beta 3$ in normal canine mitral valves and their role in chronic valve disease

Aupperle, Heike, Imke März, Jens Thielebein, Heinz-Adolf Schoon

Institut für Veterinär-Pathologie, Leipzig, Germany

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Introduction: The pathogenesis of chronic valve disease (CVD) in dogs remains unclear, but activation and proliferation of valvular stromal cells (VSC) and their transdifferentiation into myofibroblast-like cells have been described. These alterations may be influenced by transforming growth factor- β (TGF- β) which is a potent cytokine in extracellular matrix (ECM) regulation and mesenchymal cell differentiation.

Material and Methods: The present study investigates the expression patterns of TGF- $\beta 1$, - $\beta 2$, - $\beta 3$ and smooth muscle alpha actin (α -SMA) in normal canine mitral valves (MVs) ($n=10$) and in dogs with mild ($n=7$), moderate ($n=14$) and severe ($n=9$) CVD by use of immunohistochemistry.

Results: In normal mitral valves TGF- $\beta 1$ was expressed in about 10% of VSC, only 1-5% labelled for TGF- $\beta 2$ and about 50% expressed TGF- $\beta 3$, α -SMA expression was not found.

In mild CVD, the affected atrialis contained activated and proliferating α -SMA positive VSC, which intensively expressed TGF- $\beta 1$ and TGF- $\beta 3$, but only 10% were mildly positive for TGF- $\beta 2$. In the remaining unaffected areas of the leaflet, the expression of TGF- $\beta 1$ and TGF- $\beta 3$ increased significantly, whereas TGF- $\beta 2$ did not change.

In advanced CVD, the activated subendothelial VSC intensively expressed α -SMA, TGF- $\beta 1$ and TGF- $\beta 3$. The inactive VSC within the centre of the nodules showed a diminished expression intensity of TGF- $\beta 1$ and TGF- $\beta 3$. TGF- $\beta 1$ was markedly present in the deposited ECM.

Discussion: It may be hypothesized that TGF- $\beta 1$ was secreted by the stromal cells and bound to fibronectin within the accumulations of extracellular matrix in CVD. It was described in earlier studies that the expression of MMP-2 decreased, and the TIMP-2 and -3 expression increased in advanced CVD. As TIMPs are potent inhibitors of TGF- $\beta 1$ activation, increased TIMP levels may lead to an accumulation of inactive TGF- $\beta 1$. The decrease of TGF- $\beta 1$ activation itself may result in a negative feed back down-regulation of its intracellular expression. Furthermore, the decrease of TGF- $\beta 1$ may be associated with the transformation of the active myofibroblasts into the inactive cell type. Thus, in advanced stages of CVD the increased expression of TIMPs is probably one key factor, which suppress ECM degradation and TGF- $\beta 1$ activation. However, it can not be concluded through this study if these mechanisms are parallel or consecutive events. In conclusion, TGF- β appears to play an important role in the pathogenesis of CVD by inducing myofibroblast-like differentiation of VSC and ECM secretion. Changed hemodynamic forces and MMP expression patterns are plausibly to be involved in the regulation of TGF- β expression.

Detection and characterization of chondroid metaplasia in canine atrioventricular valves

Aupperle, Heike, Imke März, Heinz-Adolf Schoon

Institut für Veterinär-Pathologie, Leipzig, Germany

P15

Introduction: The present study aimed to describe the morphology and the echocardiographic characteristics of cartilage foci in the canine atrioventricular valves and to investigate the correlation to chronic valve disease (CVD).

Material and methods: The hearts of 103 dogs of different breeds and age were investigated. Atrioventricular valves were examined grossly, histopathologically (HE, PAS-alcian-blue, picrosirius red) and immunohistochemically (collagen types I, III, VI, fibronectin, laminin).

Results: In 25 cases (24.3%) foci of cartilage were seen in the tricuspid septal leaflet (sTV). These foci were positioned within the fibrosa (n=21) or spongiosa (n=3). There was a significant predisposition in large breeds (P=0.011) and those older than five years of age (P=0.0045) and the foci correlated positively to CVD in the parietal tricuspid leaflets (P=0.01). In five of these dogs echocardiographic findings were available, showing a hyperechogenic structure in three cases, corresponding to the cartilage/bone foci (0.1, 1.12 and 5.63 mm² in size). The expression patterns of extracellular matrix components varied between hyaline and fibrocartilage. The fibrocartilage was mainly composed of collagen I and VI, whereas, hyaline cartilage consisted of laminin, collagen III and VI.

Discussion: The diagnostic value of the cartilaginous foci is still unclear, but for the clinician it may be of interest to know what causes such echogenic foci in the septal leaflet. The exact mechanism of valvular chondro- and osteogenesis in dogs remains uncertain. In general, depending on the forces affecting the mesenchymal cells, they changed their shape and produced different types of cartilage. In contrast with genetically programmed skeletal osteogenesis, the heterotopic valvular cartilage in adult dogs seems to be largely determined by breed, size and other mechanical and metabolic factors as CVD.

Effect of feeding with conventional or organic wheat on morphology of the liver and muscles in pigs

Babinska, Izabella, Szarek Jozef, Tyburski Jozef, Skibniewska Krystyna

University of Warmia and Mazury, Faculty of Veterinary Medicine, Department of Forensic and Administration of Veterinary Medicine, Olsztyn/Poland

P135

Introduction: It is considered that organic food is of higher nutritional and health value, than the produced conventionally one. It becomes more and more popular. Lower total protein value is a consequence of not-using of nitrogen fertilizers in organic corn. The aim of the work was to compare the influence of feeding with organic (of lower protein content) and conventional wheat on morphology of lever and skeletal muscles of swine.

Material and methods: Three groups of porkers (n = 6) were used in the experiment: a control group (K), group A fed with organic wheat and group B fed with conventional corn (25% more of total protein). The feed did not contain any additives (synthetic amino acids, probiotics, growth stimulators, antibiotics) and the wheat origin was the only difference. Lever and muscle samples was analyzed macro- and microscopically (HE, HBFPS staining).

Results: Liver: the organ of swine of all groups was congested in different extent, the most in animals from group B. Damage of blood vessel wall was found in 1 swine of group A. Parenchymatous degeneration was observed most often as a degenerative change: focal character of the lesion was noted predominantly in animals from group K and A and wide one in group B. Focal steatosis was found in 1 of A group and in 2 of group B. Necrosis of individual hepatocytes was noted in all groups. Most animals had small infiltration of lymphoid cells, that were localized also near blood vessels in group K and B swine. Hepatocytes with big cell nucleus were detected in 2 swine of group K and A and in 4 of group B. Two-nucleus cells were found sporadically in most animals, and only in 1 animal of group A and B were numerous. No clear differences in number of lymphocytes and Browicz-Kupffer cells in liver parenchyma were stated; small increase in lymphocyte number was determined only in 5 animals and it did not correlate with kind and intensification of histopathological changes. Muscles: Disturbances in blood circulation as small extravasations were observed only in 2 animals of group K. Parenchymatous degeneration of muscle fibres were found in individual animals (2 – 3) of each group, and in group A was the slightest (only in 1 animal). Degradation of fibres was observed in all animals of group K, in 3 ones of group A and in 1 of group B, and in group K the intensity of lesions was higher than in the other groups. No significant differences in number of miogenic cells and young fibres were noted. Individual and small infiltrations of lymphoid cells were observed in some swine of group K and A. Visibly less of fat tissue was found between bundle of fibres in swine of group A and B.

Discussion: It can be concluded that feeding with organic and conventionally produced wheat did not cause significant histopathological changes in liver and skeletal muscles. The observed lesions were similar in all animals and differed only slightly in intensification; the lesions observed in liver were slightly more intensive in swine of group B.

Malignant neuroendocrine tumour in a horse

Bacci, Barbara, Mario Pischedda, Barbara Brunetti, Giuseppe Sarli, Federico Morandi, Cinzia Benazzi

Department of Veterinary Public Health and Animal Pathology, Faculty of Veterinary Medicine, Bologna, Italy

P99

Introduction: A gelding argentin horse, 25 years of age, was presented to a private veterinary clinician with a history of 3 months masticatory problems. The oral cavity examination revealed a large ulcerated mass 12 x 5 x 5 cm that affected the upper 2nd and 3rd premolar teeth, and involved as well the nasal cavity where it formed a bulging mass. Multiple samples were obtained from the mass and were submitted to histopathological and immunohistochemical (IHC) examinations.

Material and methods: Serial tissue samples were processed routinely and stained with hematoxylin & eosin. IHC stainings were performed with the following antibodies: anti-pancytokeratin, anti-vimentin, anti-NSE, anti-synaptophysin, anti-chromogranin A, anti-CD3, anti-CD79, anti-S-100 and anti-GFAP.

Results: The mass was submucosal, not encapsulated, multilobular, ulcerated, with invasive growth and was composed by packed nests of polygonal to round cells embedded in a fine fibrovascular stroma. The cells showed indistinct cell borders, moderate amount of clear cytoplasm, round nucleus with coarsely stippled chromatin and two to three evident nucleoli. The mitoses were 5-6 for high power field. Anisocytosis and anisokaryosis were moderate. The neoplastic cells were strongly positive for NSE and vimentin, variably positive for chromogranin A, and negative for synaptophysin, pankeratin, CD3, CD79, S-100 and GFAP.

Discussion: Neuroendocrine tumours occur in tissues that contain cells derived from the embryonic neuronal crest, neuroectoderm and endoderm. Thus they may develop at many sites in the body. The immunohistochemical results confirm the present case as a neuroendocrine tumour. The exact primary origin of the tumour cannot be established, since both oral or nasal neuroendocrine tumour have the same features and the horse described was affected at both sites. Differential diagnosis included neuroendocrine tumours (derived from APUD cells), neuroendocrine carcinoma (from Merkel-like cells) and olfactory neuroblastoma (from olfactory membrane). According to the WHO classification of tumours in domestic animals, olfactory neuroblastomas are characterized by the presence of typical rosettes and NSE and S-100 positivity; neuroendocrine carcinomas are positive to cytokeratin and Chromogranin A. In the present case the pankeratin negativity excluded the diagnosis of neuroendocrine carcinoma, and the absence of rosettes and S-100 negativity excluded that of olfactory neuroblastoma. The morphology and the positivity of the neoplastic cells to vimentin, NSE and mildly to chromogranin A suggested the origin of this tumour from the dispersed neuroendocrine system (APUD).

Histological, ultrastructural and molecular pathological investigations on the kidney of a pig displaying porcine dermatitis and nephropathy syndrome (PDNS)

Bago, Zoltán¹, Zsuzsanna Bago-Horváth², Susanne Richter¹, Sandra Revilla-Fernández¹, Karin Priessner², Herbert Weissenböck³

¹AGES, Institute for Veterinary Disease Control Mödling, Austria

²Clinical Institute of Pathology, Medical University of Vienna, Austria

³Institute of Pathology and Forensic Veterinary Medicine, University of Veterinary Medicine Vienna, Austria

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Introduction: Porcine dermatitis and nephropathy syndrome (PDNS) is a common condition in PCV2 infected pigs. Although the etiology of the disease is not completely understood, immune complexes have been demonstrated to play an important role in the pathogenesis of the disease. PCV2 is commonly present in infected animals, but involvement of PRRSV, *Pasteurella multocida* or its dermonecrotic toxin, *Streptococcus* sp., *Actinobacillus pleuropneumoniae* or the lipopolysaccharides of gram-negative bacteria has been implicated as well. Some authors even found a significantly increased prevalence of PDNS in stocks vaccinated against PRRS. A systemic type III hypersensitivity reaction plays a crucial role in the pathogenesis of PDNS. Here we present the results of pathomorphological and ultrastructural analyses of a porcine kidney displaying PDNS-typical morphology.

Material and methods: Lymph nodes and kidneys of a fattening pig were submitted for pathological examination, in particular to exclude classical swine fever. The kidneys were investigated pathomorphologically including special stains as well as ultrastructurally. In situ hybridisation (ISH) and PCR analyses for PCV2 and immunohistochemistry (IHC), ISH and PCR for PRRSV were also performed.

Results: Histologically an exudative-necrotising glomerulonephritis and a moderate multifocal interstitial nephritis were detected. Using electron microscopy, we could diagnose an immune-complex glomerulonephritis with mesangial, subendothelial and few subepithelial deposits. In some cells circovirus-particles and PRRSV-like particles were demonstrated, respectively. Using in situ hybridisation moderate amounts of PCV2 were found in lymph node and single positive signals were present in interstitial inflammatory cells of the kidney. IHC and ISH for PRRSV of both, lymph node and kidney did not provide unequivocal results, but were most likely negative. PCV2-DNA was detected in the infected animals by PCR. CSF was excluded virologically.

Discussion: Our morphological findings were concordant with those in the cited references. Ultrastructural investigations confirmed an immuno-complex-glomerulonephritis resembling the early stages of membranoproliferative glomerulonephritis in humans. The detected virus (PCV2) and virus-like-(PRRSV)-particles raise new questions regarding the etiology of the disease and point out the necessity of new, controlled studies.

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Haptoglobin expression in blood and liver of pigs infected with a european PRRSV field isolate

Barranco, Inmaculada, Jaime Gómez-Laguna, Francisco José Pallarés, Francisco Javier Salguero, Silvia Martínez-Subiela, Ana Gutierrez, Librado Carrasco.

Cordoba University, Córdoba, Spain

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Introduction: Acute phase proteins (APPs) are liver-derived proteins whose normal concentration may change after different events just as infection, tissue injury, trauma or an imbalanced homeostasis. Although APPs levels are not suitable for establishing a specific diagnosis, the changes in their concentrations can provide objective information about the extent of ongoing lesions in individual animals, becoming in an alternative tool for monitoring animal health. The main aim of this study was to correlate the gross and histopathological lesions of the lung with the kinetics of Haptoglobin (Hp) in tissue and serum of pigs inoculated with a European PRRS field isolate.

Material and methods: Twenty eight specific pathogen free, five weeks old pigs from a PRRSV seronegative farm were randomly distributed in batches of four and inoculated by intramuscular route with PRRSV field isolate 95/05 and humanely killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). Other four pigs, were used as controls, inoculated with 1ml of sterile medium and humanely killed at the end of the study (24dpi). Porcine Serum Hp concentrations were quantified by using a spectrophotometric method with a commercial kit. Samples from the liver were fixed in 10% buffered formaldehyde and embedded in paraffin-wax for immunohistochemical study.

Results: Gross lesions displayed a significant increase from 7dpi until the end of the study, being affected almost the 50% of the pulmonary parenchyma at 7, 14 and 24 dpi. Infected animals developed very significant microscopic lesions with respect to the control group, describing a similar trend as for gross lesions. The most severe lesions were observed at the cranial and medial pulmonary lobes at 14 and 24 dpi. Inoculated animals showed higher levels than control animals for Hp concentration analysed in sera and liver throughout the study, showing an undulating expression. Serum Hp concentration was peaked at 10 dpi ($p < 0,01$). Hepatocytes showed a diffuse or vacuole-like cytoplasmic immunostaining presenting a panlobulillar pattern. Hepatic Hp concentration displayed an increase from 10 to 21 dpi, decreasing at the end of the study.

Discussion: Gross and microscopic lesions presented a higher index of lesion at 7, 14 and 24 dpi. Serum and hepatic Hp levels were enhanced at 10 dpi, just after the first increase of gross and microscopic lesions were observed (7 dpi). The Hp tissue expression was kept on the liver whereas gross and microscopic lesions (14 and 17 dpi). Therefore, Hp expression was correlated with lung lesions becoming an alternative tool for monitoring animal health.

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Colorectal hamartomatous polyp associated with diffuse ganglioneuromatosis in a puppy: A canine model of Cowden syndrome in human?

Bemelmans, Ingrid, Sébastien Küry, Olivier Albaric, Frédérique Nguyen, Catherine André, Jérôme Abadie

Unité d'Anatomie Pathologique – Ecole Nationale Vétérinaire de Nantes, Nantes cedex 03
- FRANCE

P23

Introduction: A 5-month-old female Great Dane puppy was referred for evaluation of hematochezia, tenesmus and rectal prolapse. Surgical removal and subsequent gross pathologic examination of a 10-cm colorectal segment revealed a diffuse and significant thickening of the mucosa and submucosa, with multiple exophytic nodules, the surface of which showed erosions.

Material and methods: Histopathological evaluation on Hematoxylin-Eosin-Saffron stained section and immunohistochemistry (KI67 proliferation marker and NeuN neuronal marker) were performed.

Results: On microscopic examination two different lesions were identified. First the mucosa showed a multinodular polypous epithelial proliferation with a tubular architecture and cystic glands embedded in a hyperplastic stroma and inflammatory infiltrate. Most of the proliferative tubules were well differentiated, with only few of them showing dysplasia while breaking through the muscularis mucosae. A second lesion was characterized by hypertrophy and hyperplasia of the Meissner and Auerbach plexus in the submucosa and the muscularis respectively and, more interestingly, by a diffuse invasion of the mucosa by ectopic neuronal bodies, which was confirmed by the NeuN immunohistochemical marking. To our knowledge, the association of these two distinct hamartomatous colorectal lesions, a polyposis on the one hand and a diffuse ganglioneuromatosis on the other hand, has never been described in veterinary medicine.

Discussion: Hamartomatous polyps are found in several uncommon paediatric entities like the Peutz-Yeghers syndrome and the Juvenile polyposis syndrome which are inherited as autosomal-dominant traits but can also be sporadic. The causative mutated genes are well identified and correspond to *STK11* and the *SMAD4/BMPR1A* genes respectively. Intestinal ganglioneuromatosis is a genetically heterogenous disorder found in several syndromes such as the Von Recklinghausen's disease (associated with *NF1* gene mutations), the Multiple Endocrine Neoplasia type IIB and the Familial Ganglioneuromatosis (both associated with *RET* gene mutations). In humans, the co-existence of the two lesions is an extremely rare condition described in the Cowden syndrome. It is an autosomal dominant disorder that results most commonly from a mutation in the *PTEN* (phosphatase and tensin homolog) gene. Considering the strong genetic homology between human beings and dogs, a search for a *PTEN* gene mutation in our dog is in process, as well as investigations on relatives.

An immunopathological study of naturally occurring ovine louping-ill encephalitis

Benavides, Julio, Mark Dagleish, Clare Underwood, David Buxton, Francesca Chianini

Moredun Reserach Institute, Edinburgh, Scotland (UK)

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Introduction: Louping-ill, a zoonotic flavivirus encephalitis, is widespread in large parts of Britain. While it chiefly affects sheep and grouse it can also infect other animals and humans. Initial pathological studies of natural ovine cases defined it as a non-purulent encephalitis with associated neurone necrosis in several brainstem nuclei and the Purkinje cell layer of the cerebellum. Subsequent experimental studies put more emphasis on the inflammatory component and appeared to play down the importance of neurone necrosis, especially that occurring in the cerebellum. In this study we evaluate the histopathological changes and distribution of viral antigen in the brains of naturally occurring cases of ovine louping-ill, and compare them with the previous observations in both natural and experimental infections.

Material and methods: 17 cases of natural louping-ill in lambs and adult sheep were studied. Histopathological evaluation was made of coronal sections at different levels of the brain and a sagittal cerebellar section. In order to characterize the distribution and intensity of lesions, the same criteria used in the previous experimental studies were followed. In addition the distribution of the virus was evaluated by means of immunohistochemical labelling of the viral antigen in adjacent sections.

Results: The non-purulent meningoencephalitis was characterized by mononuclear perivascular cuffs and focal microgliosis. Inflammatory lesions appeared in all sections but were more severe in the brainstem, and mainly involved nuclei such as the red, vestibular, hypoglossal and olivary. A degree of neurone necrosis was also present in these different nuclei but it was more evident in the Purkinje cell layer of the cerebellum, sometimes with a dramatic loss of these neurones, along with pyknosis of cells in the associated granular layer and white matter. Lesion distribution and intensity was similar in both young and adult animals. Positive immunolabelling of the virus was mainly observed in the cytoplasm of neurones, but also in other type of cells associated with the perivascular cuffs. Although the degree of positivity correlated with the intensity of the inflammation, it did not always appear to correlate with the number of necrotic neurons, especially in the cerebellum.

Discussion: When compared to previous descriptions of experimental infections, the inflammatory lesions in this study showed a similar character in both distribution and intensity. However, the discrepancy in the prevalence and intensity of the cerebellar lesions between this and previous studies is noteworthy as lesions in this location may be a significant feature in the pathogenesis of natural louping-ill. The relative paucity of an inflammatory reaction despite the severe damage to the Purkinje cells, along with an absence of immunolabelling for the virus in some of the affected cells permits the suggestion that a mechanism other than the direct replication of the virus may be involved in their death and therefore in the development of clinical disease

Malignant peripheral nerve sheath tumor in the spleen of a dog

Bergmann, Wilhelmina, Iwan Burgener, Paola Roccabianca, Monika Welle

Institute of animal pathology, Berne, Switzerland

P98

Introduction: Peripheral nerve sheath tumors (PNST) are classified in veterinary medicine into benign and malignant schwannoma, neurofibroma and neurofibrosarcoma. Based on morphological features it may be difficult to differentiate a PNST from other soft tissue neoplasms and therefore the true incidence of this neoplasm is unknown. Immunohistochemistry, electromicroscopy or genetical analysis are necessary for a definitive diagnosis. PNSTs are seen mostly in the skin or in the nervous system of dogs, and less frequently in cattle and cats. However, 11 cases of PNSTs in the thoracic cavity, 8 cases of PNST in the abdomen and one intraocular PNST have been described. To the best of our knowledge no PNST has been described in the spleen. Here we describe the histological and immunohistochemical findings in a dog with a PNST in the spleen.

Material and methods: The spleen of a 8 years old mixed breed dog contained a non-encapsulated multilobulated, infiltrative growing, marmorated mass of 5 x 7.5 x 11cm of soft consistency. The mass was processed according to a routine protocol and stained with hematoxylin and eosin. Immunohistochemistry for Factor VIII, S100, Desmin, α -Smooth muscle actin, Vimentin and GFAP was performed.

Results: Histologically the mass is non encapsulated, poorly demarcated and infiltrative growing into the splenic parenchyma, the surrounding adipose tissue, and the mesentery. The mass replaces the majority of the splenic tissue. The mass is biphasic and is composed of either highly cellular areas of interlacing bundles and streams of spindle shaped cells, which are growing in a storiform pattern or as whorls around blood vessels and nerves. The cells have poorly defined eosinophilic, fibrillar cytoplasm and spindle shaped, basophilic nuclei and are embedded in a collagenous stroma of variable extent (Antoni A). Other less cellular, more loosely arranged areas are composed of spindle to oval cells with inconspicuous cytoplasm and a small hyperchromatic round nucleus, surrounded by a copious myxoid, often microcystic matrix (Antoni B). There is less than one mitotic figure per high power field. In the areas where the cells are more loosely arranged numerous lymph vessels are severely dilated. Immunohistochemistry revealed a cytoplasmatic labeling for vimentin, S-100 and GFAP. No immunolabeling was found for desmin, α -smooth muscle actin and Factor VIII. Based on these findings a morphological diagnosis of a locally malignant PNST in the spleen was made.

Discussion: Unfortunately no specific marker for PNSTs is available. Therefore a definitive diagnosis can only be made by a combination of several markers. All PNST are positive for vimentin and most are S-100 positive. Variable positivity for GFAP, collagen IV, laminin, NGFR, GFAP and NSE has been documented. Tumours of vascular or myofibroblastic origin can be ruled out by factor VIII or desmin and α -smooth muscle actin, respectively.

Survivin expression in canine mammary tumours

Bongiovanni, Laura, Daniela Malatesta, Alessandra D'Andrea, Leonardo Della Salda

Department of Comparative Biomedical Science, Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy

P49

Introduction: Survivin, which belongs to the inhibitor of apoptosis protein family (IAP), is ubiquitous expressed during tissue development, but absent or expressed at low levels in most normal tissues. Survivin has been demonstrated to be re-expressed in most human cancers, as well as in about 60-70% breast cancers, and its expression has been associated with tumour aggression, poor prognosis and poor response to therapy.

Material and methods: This retrospective study included 13 canine mammary tumours (1 solid carcinoma, 5 tubulo-papillar carcinomas, 2 mixed carcinomas, 4 complex carcinomas) and 2 pre-neoplastic lesions (epithelial hyperplasia). Four samples of human colon carcinomas, a tumour that frequently expresses survivin, were used as positive controls. Immunohistochemical expression of full-length survivin was determined using commercially produced antibody (rabbit polyclonal antibody, 0,8 g/ml, NOVUS Biologicals). Mitotic index was evaluated by counting positive mitosis in 10 high power fields (HPF).

Results: Survivin expression was observed in 73% of cases, with more intense immunolabeling in malignant neoplasms than preneoplastic lesions. The staining was mainly cytoplasmic; nuclear immunolabeling and positive mitosis were also observed in 8 samples. Three cases showed intense cytoplasmic survivin staining and high mitotic index (3-4HPF). Preneoplastic lesions, normal mammary epithelium, as well as myoepithelium, were negative.

Discussion: The present results indicate for the first time that in dogs, as in human beings, both cytoplasmic and nuclear survivin is frequently expressed in malignant mammary tumours, suggesting a direct correlation with histological malignancy and biological behavior of canine breast carcinoma, consistent with anti-apoptotic and proliferative functions of the molecule.

Role of CLCA proteins in mucus homeostasis in diseases with secretory dysfunctions

Bothe, Melanie K., Josephine Braun, Friederike Range, Lars Mundhenk, Achim D. Gruber_

Department of Veterinary Pathology, Freie Universität Berlin, Germany

P17

Introduction: Mucus homeostasis is out of balance in many diseases with secretory dysfunctions like chronic obstructive bronchiolitis (COB), asthma or cystic fibrosis (CF). CLCA proteins are thought to play a role as modulators of mucus homeostasis by contributing to different aspects of processing of the mucus. Some CLCA family members are expressed in goblet cells and are thought to play a role in mucus secretion while others are expressed in non-goblet cell epithelial cells where they might affect mucus hydration. In contrast to the fully secreted CLCA proteins of goblet cells, the CLCA proteins in non-goblet cell epithelial cells possess a transmembrane domain at the carboxy-terminal cleavage product. The carboxy-terminal cleavage product might therefore be the key to distinguish between different functions of CLCA family members. Here we present immunohistochemical and biochemical characteristics of the murine mCLCA3 and mCLCA6 proteins with a focus on their membrane association and their cellular distribution patterns.

Material and methods: Computational prediction of possible transmembrane domains of mCLCA3 and mCLCA6 were performed and verified using newly generated polyclonal rabbit antibodies, including immunoblotting, deglycosylation, acid release, immunohistochemistry, immune electron microscopy and co-localization via confocal laser scanning analyses. Furthermore, the results of the immunolocalization were corroborated on the mRNA level with laser capture microdissection (LCM) followed by RT-qPCR.

Results: In contrast to the fully secreted murine mCLCA3, mCLCA6 possesses a transmembrane domain within the carboxy-terminal cleavage product. Unlike mCLCA3, which is expressed in intestinal goblet cells, mCLCA6 is exclusively located at the apical plasma membrane of non-goblet cell enterocytes in both the small and large intestine. In the large intestine, mCLCA6 co-localizes with the cystic fibrosis transmembrane conductance regulator (CFTR) channel, one of the most important chloride channels which is defective in cystic fibrosis patients. Furthermore, both mCLCA3 and mCLCA6 appear to be upregulated in the intestine of CF mouse models.

Discussion: Both mCLCA3 and mCLCA6 are expressed in different intestinal cellular microenvironments. Co-localization of mCLCA6 with the CFTR channel and differential expression in CF mouse models suggest that at least one of the murine CLCA or both might play a role in modulating the CF phenotype. The functional significance and structural details of this co-localization have to be determined in the future.

A case of «extranodal NK/T-cell lymphoma, nasal type» in a Rottweiler

Brachelente, Chiara, Elvio Lepri, Monica Sforza, Giovanni Ricci, Alfredo Dentini, Daniela Mignacca, Luca Mechelli

Dept. of Biopathological Sciences and Hygiene of Animal and Alimentary Production, Perugia, Italy

P2

Introduction: Nasal tumors in the dog have a very low incidence, ranging from 0.3 to 1% of all canine tumors. The more common diagnosed neoplasia are adenocarcinomas and transitional cell carcinomas and, amongst the mesenchymal types, chondrosarcomas, followed by fibrosarcomas, osteosarcomas and lymphomas. Therefore, different from the situation in the cat, nasal lymphomas are rare in dogs.

Case Report: A 9-year-old, male, Rottweiler dog was submitted for a clinical examination due to the appearance of a cauliflower, 1 cm in diameter, apparently well-circumscribed, greyish-white mass with smooth surface, at the right nostril. Cytological examination prompted a diagnosis of a poorly differentiated, malignant mesenchymal tumor, possibly of lymphohistiocytic origin. The histopathological examination of the mass revealed the presence of neoplastic round cells, with marked atypia and a high mitotic index. Tumor cells were variable in size and had round to indented nuclei with vesicular chromatin and evident nucleoli. Numerous multinucleated giant cells were interspersed within the neoplastic cells. At the periphery of the mass, tumor cells were arranged in perivascular cuffs. Large areas of necrosis were also observed together with nodular, eosinophil-rich infiltrates and granulomatous reactions around eosinophilic, amorphous material. Immunohistochemistry using antibodies against CD3, CD79, c-Kit, tryptase and Mac387 was negative for the histiocytic and mast cell markers; on the other side, neoplastic cells showed a multifocal and strong positive reaction to CD3 (T lymphocytes), with rare CD79+, infiltrating cells (B lymphocytes). The histopathological and immunohistochemical findings were compatible with the so called “extranodal NK/T-cell lymphoma, nasal type”.

Discussion: The extranodal, nasal NK/T-cell lymphoma, formerly known as angiocentric lymphoma, is described in human medicine as a lymphoma with a NK phenotype, rarely T, which co-expresses Epstein-Barr (EBV) virus in 80-100% of cases. The predominantly NK-phenotype has a worse prognosis than the mixed NK/T-phenotype. It often occurs in the nasal cavity and paranasal sinuses. Other locations may be skin, gastrointestinal tract, testicles, kidney, upper respiratory tract and rarely eye/orbit. The histopathological peculiar aspect of this tumor consists of a marked, although not constant, angiocentric/angiodestructive behaviour, causing extensive areas of necrosis, and the presence of many inflammatory cells such as plasma cells, histiocytes and eosinophils. Based on the clinical and histopathological features, our case is consistent with what is described in the human literature and spread the knowledge about this subtype of lymphoma with unusual prognostic and diagnostic implications.

Detection of Canine Oral Papillomavirus (COPV) in Conjunctival Plaque and Papillomas in Three Dogs

Brandes, Kristin¹, Jens Fritsche², Nina Mueller³, Birgit Koerschgen³, Barbara Dierig⁴, Guenther Strebelow⁵, and Jens-Peter Teifke⁵

¹Animal Pathology Augsburg, Germany, ²Veterinarian Practice for Ophthalmology, Munich, Germany, ³Veterinary Eye Specialists, Pohlheim, Germany, ⁴Veterinary Practice, Neusaess, Germany, ⁵Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Isle of Riems, Germany

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Introduction: Papillomavirus infections are responsible for epithelial plaques and papillomas in various locations on the skin and in mucous membranes. Viral conjunctival papilloma of the third eyelid or conjunctiva is not yet included in the proposed WHO histological classification of ocular tumors of domestic animals. The aim of this presentation is to describe distinct and different morphological features of a viral pigmented conjunctival plaque and two conjunctival squamous papillomas in three dogs, and to investigate these lesions for the presence of papillomavirus DNA by means of polymerase chain reaction (PCR), DNA sequence analysis and in situ hybridization (ISH).

Material and methods: The entire conjunctival neoplastic tissue was fixed in 4 % formaldehyde, routinely processed, embedded in paraffin wax, cut at 3 µm and stained hematoxylin-eosin (HE). To investigate the paraffin-embedded material for the presence of papillomavirus antigens, PCR, DNA sequence analysis and ISH were applied.

Results:All three conjunctival neoplasms revealed various degrees of epithelial hyperplasia, acanthosis and hyperkeratosis with hypergranulosis and koilocytosis in the upper epithelial layers. Occasionally, basophilic intranuclear inclusions were present. Based on the different morphological aspects, including the growth pattern and surface aspect conjunctival plaque and squamous papillomas were diagnosed. In all three lesions PCR for COPV DNA yielded amplification products each with the predicted size for the E6, E7 and L1 genes. Sequencing of the amplicons revealed for all three fragments 100% identity with the published DNA sequence of COPV. In all samples, ISH revealed COPV DNA in a highly specific pattern within nuclei of the hyperplastic epithelium.

Discussion: Based only on the different morphology, consideration must be given to whether the plaque is a preliminary stage of an exophytic papilloma or an entity on its own. Molecular results, however, support the assumption that the plaque is the initial lesion progressing into papilloma with time. Since COPV was isolated from squamous cell carcinomas, a progression from virus-induced papilloma or plaque to this malignant variant must be also taken into consideration. This is the first time that the oncogenic lambdapapillomavirus COPV has been detected in ocular epithelial hyperplastic lesions.

Lymphangiogenesis does not influence progression and outcome of canine mast cell tumours

Brunetti, Barbara, Rosario Preziosi, Federico Morandi, Valentina Zagonara, Paolo Paolo Degliesposti, Cinzia Benazzi, Giuseppe Sarli

Department of Veterinary Public Health and Animal Pathology, Faculty of Veterinary Medicine, Bologna, Italy

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Introduction: Mast cell tumours often metastasize to the skin, regional lymph nodes and internal organs. Prodromic for metastasis is the increase in the haematic and lymphatic network, driven by VEGF receptors and ligands, in particular VEGFR3 influencing lymphangiogenesis. In malignant mastocytoma an increase is known in the number of blood vessels (angiogenesis) that acquires prognostic value. The aim of this study was to evaluate whether tumoral lymphangiogenesis develops and if it has prognostic value.

Material and methods: Thirty-one primary cutaneous mast cell tumours, single or multiple and with one year follow-up, have been classified according to the Patnaik's grading system. An immunohistochemical (IHC) anti-laminin/anti-VEGFR3 double stain has been used to identify lymphatics when negative for laminin. IHC stained sections have been evaluated for blood vessels (showing positive laminin stain), lymphatic vessels (negative laminin stain), then further subgrouped for presence/absence of VEGFR3 positivity. The count has been carried out on 5 intratumoral (fields randomly chosen amid the tumour stroma) and extratumoral areas (between the external border of the neoplasm and surrounding derma/subcutaneous tissue). The randomly chosen areas were of 0.789 square millimeters each. The data did not have a normal distribution, therefore they were processed for statistical analysis with the Spearman rank correlation test.

Results: According to Patnaik's grading system 15 out of 31 were graded I, 11 were graded II and 5 of 31 were graded III. Four were multiple and 27 single tumours. Twelve dogs had died and 19 were still alive at one year post surgery. IHC double stain evidenced laminin in the basement membrane stained brown, and the blue positivity to VEGFR3 in the cytoplasm/cell membrane of endothelial cells. In all the cases, the quantity of micro vessels of the four typologies (haematics: laminin+/VEGFR3+ or laminin+/VEGFR3-; lymphatics: laminin-/VEGFR3+ or laminin-/VEGFR3-) have been compared. Spearman's test has pointed out that the number of lymphatics with or without VEGFR3 expression did not vary between intratumoral vs extratumoral fields, Patnaik grade progression, single vs multiple tumours, dead vs alive animals at one year follow-up. The only significant changes included an increase in the haematic vessels without VEGFR3 expression in extratumoral (median 1.6) vs intratumoral (median 0.4) stroma ($P=0.041$), in multiple (median 3.2) vs single (median 0.6) neoplasms ($P=0.013$) and in dead (median 1.7) vs alive (median 0.4) animals at one year follow-up ($P=0.005$).

Discussion: The expression of VEGFR3 indicates a response to angiogenetic factors produced during tumour growth in vessel endothelium (mainly of lymphatics). On the basis of the obtained results, no objective data support a true increase in lymphatics at the tumour borders, where the functionality of lymphatic vessels is better preserved than in intratumoral areas, nor the number of lymphatics was enhanced in multiple than single tumours or in dead compared with alive animals. It seems that lymph node involvement in mastocytomas is due to the spread through the preexisting lymphatics and true lymphangiogenesis does not happen. This study confirms the role of haemangiogenesis, but not of lymphangiogenesis, as prognostic factor in canine mast cell tumours.

Metastasis of primary lung carcinoma in skeletal muscle of a cat

Carminato, Antonio, Marta Vascellari, Marcella Paoli, Claudio Pasolli, Wendy Marchioro, Franco Mutinelli

Istituto Zooprofilattico Sperimentale delle Venezie, Italy

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Introduction: Primary lung tumors are less common than metastatic lung lesions in cats. Adenocarcinomas account for 70% to 80% of primary pulmonary neoplasia in dogs and cats. Metastasis of primary lung tumors can occur to other areas of the lungs, or to the bones, liver, spleen, pancreas, kidneys, adrenal glands, heart, brain, esophagus, abdominal and mediastinal lymphnodes, eyes or digits in cats. The present case report describes a case of pulmonary papillary adenocarcinoma with skeletal muscle metastasis.

Material and methods: A 9 year-old domestic long hair female cat was evaluated because of a history of progressive lameness by the referring veterinarian. Complete physical examination followed by a total body x-ray imaging and aspiration biopsy of subcutaneous masses were performed. After a few days supportive treatment the cat was euthanized. At necropsy tissues from lung and skeletal muscles were collected and fixed in 10% buffered formalin. Samples were routinely processed for histology and stained with haematoxylin and eosin. Histochemical Periodic acid-Schiff (PAS) staining was performed on all sections. Immunohistochemical staining for pan-cytokeratin (CKAE1- AE3, DAKO) and Factor VIII (HISTO-LINE) were performed using the EnVision™ Peroxidase - Dual Link System detection system and DAB as chromogen.

Results: Physical examination revealed the presence of multiple subcutaneous masses affecting both hind and forelimbs and the left mandible. Radiographic assays showed a moderately radiodense nodular lesion in the thoracic cavity caudally to the heart. Cytology was suggestive of a neoplastic epithelial lesion with squamous differentiation. At necropsy, multiple nodular proliferative masses, measuring 0.5 to 5 cm, invading the muscles of the limb, the back and the masseter muscle were detected. Furthermore, two proliferative masses involved the caudal lobe of the right lung. Microscopic examination highlighted multifocal infiltration of the lung by neoplastic tubulo-papillary structures composed of cuboidal/columnar epithelial cells. Similar neoplastic epithelial pattern was observed on muscular tissues. Strong immunohistochemical cytoplasmic staining for pan-cytokeratin antigens revealed by neoplastic cells confirmed their epithelial origin. Neoplastic cells did not contain PAS positive secretory material. Factor VIII demonstrated neoplastic emboli within lymphatic vessels of the lung. A diagnosis of papillary pulmonary adenocarcinoma with multiple muscular metastasis was made.

Discussion: Rare skeletal muscle metastasis of lung carcinoma are reported in veterinary medicine in opposite to the more common distal bone manifestation. Cats presenting soft tissue masses should have metastatic pulmonary neoplasia added to the list of differential diagnoses. Most cats with pulmonary neoplasia are middle age or geriatric, presenting signs of cough, dyspnoea, weight loss, anorexia, and lethargy. Diagnosis is usually established based on radiographs, cytology and histopathology.

Immunodetection of CK5 and CK19 in normal and neoplastic canine mammary tissue

Cartuccia, Cristina, Giacomo Renzoni, Francesca Mariotti

Department of Veterinary Sciences, University of Camerino, Matelica (AN), Italy

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Introduction: Mammary tumours are the most commonly occurring neoplasm in bitches. A characteristic of all cancers is a striking variability among the neoplastic cells within a single tumour. In human medicine, two current ideas attempt to describe the establishment and maintenance of tumour heterogeneity: the clonal evolution model and the cancer stem cell hypothesis. The cancer stem cell hypothesis states that a particular subset of tumour cells with stem cell-like properties, called “cancer stem cells”, drives tumour initiation, progression, and recurrence. Cancer stem cells are widely believed to arise from normal stem or progenitor cells of an organ and are thought to persist as a small fraction of the cells into the tumour. Although it is not possible to identify a unique marker for a given stem cell type, these generally express certain proteins in quantities which stand out from all other cells in the body. Several authors indicate the cytokeratin (CK) 5 like a marker of mammary stem cells during differentiation. On the other hand, the intermediate or suprabasal population suggested to be breast stem cells seems to comprise ER/PR+ cells that coexpress the CK19. An undifferentiated subpopulation of stem cells called “side population” (SP) seems to express CK19 also. In veterinary medicine, poor investigation has been performed concerning stem cell of mammary neoplasms. The aim of this work is to verify the immunohistochemical expression of CK19 and CK5 in canine mammary tumours.

Material and methods: Thirty-nine canine mammary tumours, classified according to standard diagnostic criteria by WHO (5 simple adenomas, 2 complex adenomas, 12 simple carcinomas solid-type, 9 simple carcinomas tubular-type and 11 complex carcinomas) were selected. Four normal canine mammary glands were analysed too. For immunohistochemical investigations 4µm-serial-sections were cut from archived paraffin blocks and analyzed in accordance with a standardized protocol. After microwaves antigen retrieval and inhibition of endogenous peroxidase activity, the slides were incubated overnight with mouse Mab anti-CK19 (DAKO) and Mab anti-CK5 (Novus Biologicals). The reaction was detected using an avidin-biotin peroxidase method and the DAB was used as chromogen.

Results: Specific cytoplasmic stain for CK19 was present in the myoepithelial cells in normal tissue. In all cancers analysed, a strong reaction was found in spindle-shaped cells with an oval nucleus situated in the peripheral regions of mammary acini. These cells resembled to neoplastic myoepithelial and/or basal cells. In adenomas as well as normal gland scattered epithelial cells reacted to CK19. In carcinomas, only few neoplastic epithelial cells were positive. Specific stain to CK5 was found in myoepithelial normal cells. In the cancers only little clusters of neoplastic cells with vesicular nuclei appeared positive. These cells seemed to be poorly differentiated epithelial elements and were located in the peripheral portion of lobuli especially.

Discussion: In this study the immunoreactivity to CK19 seems more evident in myoepithelial/basal cells than epithelial cells in normal as well as neoplastic tissue. Moreover, CK5 appears detected only in normal myoepithelial cells and in neoplastic poorly differentiated epithelial cells whereas it is not evident in differentiated, normal and neoplastic, epithelial cells. As it is demonstrated that human mammary stem cells, particularly SP, can expressed CK19 and CK5, in canine mammary tissue also these proteins could be markers of stem cells. Obviously other studies are needed to confirm this hypothesis.

Septic pericarditis and cardiac tamponade associated with pulmonary botryomycosis in a dog

Casamian, Domingo, Jon Shippam, Becky Woodward, Dominique Fournier

Finn pathologists, Weybread, Norfolk, UK

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Introduction: The term botryomycosis describes a chronic granulomatous disease caused by non-branching bacteria, usually *Staphylococcus sp*, characterized on histology by the presence of eosinophilic granules. Most cases of botryomycosis in dogs involve the skin or muscle.

Material and methods: A two year-old neutered male springer spaniel was presented with a history of acute collapse. Echocardiography showed severe pericardial effusion resulting in cardiac tamponade. Pericardiocentesis was performed and fluid was submitted for cytology and bacterial culture. Subtotal pericardiectomy and lobectomy of the affected pulmonary area were later performed and samples from the affected tissue were submitted for histopathology and bacterial culture.

Results: Cytology results were consistent with a septic effusion with several degenerated neutrophils showing intracytoplasmic cocci. Histopathology showed severe necrotizing and pyogranulomatous inflammation centered around amorphous eosinophilic material containing radiating clubs (Splendore-Hoeppli) with a number of intra-lesional gram positive cocci bacteria. Aerobic and anerobic bacterial cultures were inconclusive.

Discussion: Septic pericarditis is an uncommon cause of pericardial effusion in dogs. Differential diagnoses of botryomycosis include filamentous bacteria (especially *Actinomyces sp*) and fungal mycetomas. In this case the use of gram stain was especially useful to tentatively identify the bacteria as *Staphylococcus sp*, as the bacterial culture was inconclusive. To the author's knowledge, this is the first report of pulmonary botryomycosis in a dog.

Morphological alterations in horse skeletal muscles associated with *Purpura Hemorrhagica*

Cassart, Dominique, Cédric Thomas, Sandra Jolly, Sandra Berthemin, Freddy Coignoul, Daniel Desmecht

Faculty of veterinary medicine, Liège, Belgium

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Introduction: Known as being responsible for strangles, *Streptococcus equi* can also give rise to 3 immune-mediated diseases affecting horses muscles: *Purpura Hemorrhagica*, rapid muscle atrophy and acute rhabdomyolysis. *Purpura Hemorrhagica*, the most common form, is a vasculitis associated with immune complexes composed of IgA and streptococcal M protein; it is a type III hypersensitivity reaction. *Purpura Hemorrhagica* is characterized by infarcts involving mainly skeletal muscles but sometimes other organs such as skin, lung and intestine. Here, the morphopathological changes in striated muscles of 2 ponies dead after presenting signs of acute myopathy, identified as *Purpura Hemorrhagica* at necropsy, are illustrated.

Material and methods: In spring 2007, 2 young ponies from different farms (P1 : a 4 years old female saddle pony and P2 : a 5 years old neutered Shetland pony) were referred to the necropsy clinic of the Liège Faculty of Veterinary Medicine with an history of fatal acute myopathy. For P1, the anamnestic data reported an episode of strangles, with antibiotic therapy for 2 weeks, followed by clinical signs of myopathy as stiffness, muscle pain, abnormal gait, recumbency, increased CK and death 5 days after the beginning of the muscular clinical signs. P2 showed concomitantly slight clinical signs of strangles and severe clinical signs of acute myopathy and was treated with antibiotics for 5 days till death. The ponies were necropsied following a standard procedure and tissue samples were collected for histologic examination and bacteriological investigation.

Results: Both ponies showed many irregular foci of hemorrhagic necrosis suggesting infarcts from 1 to about 4 cm diameter in different skeletal muscles. In P1, the bladder contained blackish-red urine, retropharyngeal lymph nodes were enlarged with central necrosis in the right one and lesions of alveolar pneumonia were observed in apical lobes. In P2, laryngeal muscles were affected with hemorrhagic necrosis, an infarct of about 60 cm length was present in the proximal duodenum and inflammatory lesions were observed in the upper respiratory tract with an hemorrhagic, fibrinous and purulent exudate in nasal cavities, sinus and larynx. Histological examination of affected muscles confirmed hemorrhagic infarcts with vasculitis and fibrinoid necrosis of vascular walls. The histopathological muscular picture was central ischemic necrosis, with fragmentation of muscle fibers, surrounded by many erythrocytes and some inflammatory cells at the periphery of the lesion. *Streptococcus equi* was isolated from laryngeal exudate of P2 ; no conclusive result was obtained from bacteriological diagnosis on retropharyngeal lymph node of P1.

Discussion: *Purpura Hemorrhagica*, a complication of *Streptococcus equi* infection, should be included in the differential diagnosis of horses with clinical signs of acute myopathy if there is a history of clinical strangles or if horses live on farms where infection is endemic.

Blockade of E- and P- selectin reduces neutrophil infiltration into the ischemia-reperfusion induced murine testis

Celebi, Muzaffer, Alberta G.A.Paul

University of OMU, Veterinary Faculty, Samsun, Turkey

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Introduction: Ischemia-reperfusion (IR) injury of the testis results in germ cell specific apoptosis which is dependent on neutrophil recruitment to the testis. Adhesion molecules, including E- and P-selectins play a critical role in this pathology. The present study sought to characterize the inhibitory effect of function-blocking monoclonal anti-mouse E- and P- selectin antibodies on the migration of neutrophils into the IR-induced testis of the mouse.

Material and methods: Mice were subjected to a 2 hr period of testicular torsion (ischemia) followed by detorsion (reperfusion). Ten minutes after the onset of reperfusion mice received either a mixture of 200 µg function-blocking monoclonal E-selectin and P- selectin antibody (FBMAB group; 100 µg; each) intravenously or 200 µg of isotype-matched control- antibody (IMCA group). Separate groups of mice underwent sham-operation (SO group) or received 500 ng of TNF α (IF group) to induce inflammation. Mice were sacrificed 24 hr after reperfusion and testicular interstitial cells were isolated and analyzed for the presence of neutrophils by means of flow cytometry.

Results: The results of flow cytometric analysis showed a significant reduction in the percentage of neutrophils present in the testicular cells isolated from the mice in the FBMAB group as compared to the IMCA group (Figure 1; 21 ± 6 vs. 52 ± 10 % Gr-1+CD11b+ of total leucocytes; $P=0.001$). The percentage of neutrophils in the testicular cells of the IF (positive control) and SO (negative control) groups were 63 ± 12 and $8\pm3\%$ respectively.

Discussion: Germ cell-specific apoptosis occurs contemporaneous with an increase in neutrophil margination and diapedesis in the mouse or rat. Neutrophil recruitment to the affected organs is one of the hallmarks of IR injury and the precise role of each selectin (E-, P-, and L-) may vary depending on the particular inflammatory stimulus and species. In this study our aim was to reduce neutrophil infiltration into the IR-induced testis with a mixture of function-blocking monoclonal E- and P- selectin antibody. In the present study, flow cytometric results of neutrophil recruitment to the IR-induced testis in FBMAB and IMCA groups were 21 and 52 , respectively. These results demonstrate that neutrophil infiltration into the IR-induced testis was reduced significantly, by 60% with the administration of FBMab. In conclusion, these data demonstrate that blocking both E- and P- selectin even after onset of reperfusion with function blocking monoclonal anti-mouse E- and P- selectin antibodies inhibit neutrophil recruitment to the IR-induced murine testis. Combined antibody therapy inhibiting both P- and E- selectin may be a promising strategy for testis protection against ischemia-reperfusion injury.

Skin tumours of dogs in Grenada

Chikweto, Alfred, M. Iqbal Bhaiyat, Ravindra Sharma, Claude de Allie, Pauline E. McNeil

St. George's University, Dept. Paraclinical Studies, School of Veterinary Medicine St. George's, GRENADA West Indies

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Introduction: Skin tumours are common in dogs and a number of surveys have been published from various geographical locations indicating that the most common is the mast cell tumour. Evaluation of skin masses from dogs in Grenada suggested that the prevalence of several tumour types differed from that reported elsewhere.

Material and methods: Histological sections of skin masses found in biopsy and necropsy samples from dogs in Grenada were reviewed in a retrospective study that included 200 dogs over a period of six years.

Results: Proliferative and neoplastic vascular growths were relatively common whilst mast cell tumours were found less frequently. Haemangiosarcoma was the most common tumour, comprising 14% of the neoplastic masses, and various forms of haemangioma and angiomas were also seen.

Discussion: This study raises questions about the genetic and environmental factors that contribute to the development of neoplastic lesions and highlights difficulties in the diagnosis and classification of vascular growths in particular. We suggest that exposure to sunlight might be contributing to the apparent high prevalence of haemangiosarcomas in dogs in Grenada and ask if the genetic make-up and/or the presence of other skin diseases in the Grenadian pothounds might influence the prevalence of other growths in our series.

Myocardial lesions and objective evaluation of arteriolar hypertrophy in Wistar rats with Streptozotocin induced diabetes mellitus type 1 and Adriamycin induced congestive cardiac failure

Ciobotaru, Emilia, Manuella Militaru, Teodoru Soare, Georgeta Dinescu, Daniela Elena Braslasu

Faculty of Veterinary Medicine, Bucharest, Romania

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Introduction: Clinical and experimental research of the last years proved the associated *diabetes mellitus* type 1 or 2 and cardiac failure in the same patient induced by streptozotocin administration. There have been also created experimental models of congestive cardiac failure using Adriamycin as inductor agent. Morphological investigations of the heart included in previous studies revealed hypertrophy of myocardium, apoptosis and necrosis of myocardiocytes, myocardial fibrosis, important lesions of coronary arteries and their branches as acellular capillaries, microaneurisms, poor formation of collateral vessels in myocardium, hypertrophy of arteriolar media together with a reduced lumen of arterioles. Our research is based on an experimental model which induces streptozotocin *diabetes mellitus* type 1 cardiomyopathy and Adriamycin cardiac failure.

Material and methods: *Diabetes mellitus* type 1 and congestive cardiac failure were experimentally induced in 55 Wistar adult rats. The animals were divided in four unequal groups and treated as follows: group 1 (streptozotocin and Adriamycin), group 2 (streptozotocin), group 3 (Adriamycin), group 4 (healthy control rats). 70 days after administration, the rats were euthanized. Heart was entirely harvested and fixed in 10% formaldehyde solution. Paraffin embedded samples were previously sectioned and stained using Masson trichromic and Azan. Hypertrophy of arteriolar media was objectively evaluated using computerized morphometry (AxioVision programme). The values of thickness of media/arteriolar diameter ratio were statistically evaluated. All data were expressed as mean \pm standard error of mean (SEM). Significant differences between mean values of multiple groups were evaluated using one way analysis of variance ANOVA. Significant differences between mean values of two groups were evaluated by Student's t test. In both tests, values of $P < 0.05$ were considered statistically significant.

Results: Group 1 exhibited mostly focal multicentric necrosis of myocardiocytes, interstitial oedema and vacuoles in sarcoplasm. Subendothelial insudation, vacuolization and hyperplasia of leiomyocytes of media were encountered in the majority of animals. The heart of group 2 presented scattered necrotizing myocardiocytes and discrete interstitial fibrosis. Arterioles presented vacuoles in leiomyocytes of media and inconstant insudation. Focal multicentric necrosis was also recorded in heart of group 3, together with a vacuolization of sarcoplasm and obvious interstitial fibrosis. The lesions of arterioles were resumed to sclerosis of media, discrete insudation in intima and eosinophilic cytoplasm and vacuoles of leiomyocytes. The values of thickness of media/arteriolar diameter ratio were: 0.38 ± 0.16 in group 1, 0.24 ± 0.09 in group 2, 0.21 ± 0.11 in group 3 and 0.14 ± 0.03 in control. There were no significant differences between group 2 and group 3.

Discussion: Previous studies proved that increased endothelial and adventitial cell proliferations are early events in diabetes associated vascular hypertrophy. Furthermore, we consider that increased values of thickness of media/arteriolar diameter ratio is probably induced by increased quantities of extracellular matrix within the media and insudation, these features being an important lesion of diabetes associated vascular hypertrophy.

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Angiogenesis in canine inflammatory mammary cancer: increased lymphangiogenesis and overexpression of vascular endothelial growth factors A and D and COX-2

Clemente, Mónica ¹, Ana Rodríguez Sánchez-Archidona², Francesco Sassi³, Paloma Jimena de Andrés¹, M^a Dolores Pérez-Alenza¹, Gema Silván⁴, Juan Carlos Illera⁴, Susana Dunner², Laura Peña¹.

Veterinary Faculty, Complutense University of Madrid, Spain.

¹Dept. . Animal Medicine Surgery and Pathology

²Dept. Animal Physiology

³Laboratory of Animal Genetics

⁴Dept. Veterinary Public Health and Animal Pathology, Bologna, Italy. Clemente, Mónica, Ana Rodríguez Sánchez-Archidona, Francesco Sassi, Paloma Jimena de Andrés, M^a Dolores

Introduction: Canine inflammatory mammary cancer (IMC) is a distinct type of mammary cancer with particular aggressive behavior and prognosis. The pathogenesis of IMC is unknown, although distinct mechanisms related to the inflammatory phenotype have been described. It is characteristically angioinvasive. Besides the typical clinical signs, the observation of massive tumor embolization in superficial dermal lymphatic vessels, is necessary to confirm a diagnosis of IMC. Vascular endothelial growth factor- A (VEGF-A) is a potent inducer of new blood vessels (angiogenesis) and it has been indicated to participate in canine malignant mammary tumors, whereas VEGF-D specifically induces lymphangiogenesis and has not been previously studied in canine tissues. By the other hand, it is known that COX-2 (cyclooxygenase-2) plays an important role in angiogenesis and endothelial progenitor cell mobilization. In this study the expression of VEGF-A, VEGF-D, COX-2 and tumor angiogenesis in IMC versus comparable non-inflammatory malignant canine mammary tumors were evaluated.

Material and methods: In this prospective study, dogs with mammary tumors were included. Twenty-two cases with IMC (both clinical and pathological diagnosed) (group IMC) and 20 histological grade III non-inflammatory malignant mammary tumors, with tumor embolization and or distant metastases (group MMT) were selected. Adjacent tissue samples were taken and: a) frozen (VEGF-A enzymeimmunoassay-EIA), b) formalin fixed and paraffin embedded (histopathology and CD31, Lyve-1, VEGF-A, VEGF-D, COX-2 and Ki-67 immunohistochemistry-IHC) and c) immersed in RNA-later (VEGF-D and COX-2 RT-PCR). Serum content of VEGF-A was assayed by EIA. In each case, the microvascular density (MVD) (CD31 stained-capillaries) and lymphatic capillary endothelial cells proliferation Ki-67 index was obtained.

Results: VEGF-A (tumor and serum) and VEGF-D expression were higher in IMC group. COX-2 expression was significantly increased in IMC specimens compared with MMT; COX-2 immunostaining was intensely positive in highly malignant migrating individual epithelial cells and endothelial-like neoplastic cells (vasculogenic mimicry) of IMC cases. VEGF-A immunohistochemical expression was significantly associated ($p= 0.01$) with COX-2 in MMT but not in IMC. Lymphangiogenesis (Ki-67 index) was significantly increased in IMC cases, being MVD similar in both groups. Human Lyve-1 antibody showed no cross-reaction in canine endothelial lymphatic cells.

Discussion: There is a differential profile of VEGF-A, VEGF-D and COX-2 in IMC, possibly associated to the increased lymphangiogenesis and the presence of the vasculogenic mimicry phenomenon. VEGF-A could be participating in the angiogenetic process in combination with COX-2 in MMT, but

not in IMC. In IMC, VEGF-A, VEGF-D and COX-2 might play an important role in the endothelial phenotype and vasculogenic mimicry.

Lethal herpesvirosis in captive snakes - horned viper (*Vipera ammodytes ammodytes*) and water snake (*Natrix natrix*)

Cornel, Catoi, Gal Adrian, Taulescu Marian, Catoi Adriana Florinela

University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania

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Introduction: Horned viper (*Vipera ammodytes ammodytes* - VAA) is a Mediterranean snake and is the most venomous snake from Europe. In Romania is found in the western Carpathian Mountains. Common European viper (*Vipera berus* -VB) is a common and venomous viper in Europe. These species are listed as protected under the Berne Convention. Water Snake (*Natrix natrix* - NN) is no venomous snake very common in Europe.

Material and methods: In 2006 year, we found the same gross lesion at the necropsy of seventeen captive snakes. Sixteen VAA came from a flock that all died in two weeks. Interesting, in the same room there was many VB but these snakes don't show any signs of disease. One NN snake came from a didactic reptile collection. The gross examination was followed by microbiology, histology (five VAA and one NN relative fresh cadavers) and transmission electron microscopy (one really fresh cadaver of VAA).

Results: The gross examination showed hemorrhagic diathesis especially on the serous and mucous membranes. Hemorrhages were also observed in musculature, fatty body, kidney and myocardium. Serous or hemorrhagic exudates with fibrin clots were observed in the celomic and pericardial cavities. The kidneys were enlarged and discolored with precipitation of urates. Gastric wall was thick, deep red, and hemorrhagic. The gastric content was blood colored and the intestinal one was black (melena). The spleen was enlarged and firm. The liver was yellow with multiple focal hepatic necroses in almost cases. The most extensive microscopic lesions were fatty degeneration and diffuse and focal hepatic necrosis. Mononuclear cells dominated the inflammatory infiltrate. The most striking lesion was endothelial intranuclear acidophil inclusions, endothelial necrosis and thrombosis of the sinusoid capillaries. Occasionally, the same endothelial lesions were seen in other tissues - myocardium, fatty body, kidney, and spleen. Viral particles morphologically typical for herpesviruses were demonstrated in endothelial cells by electron microscopy. Intranuclear particles, capsids, have ranged from 110 to 120 nm in diameter. These particles have form large conglomerates which corresponds to inclusion body observed in optic microscopy. Outside of nucleus the enveloped capsids have 170 nm.

Discussion: According the localization and morphology of the body inclusions and the viral particles we consider that there is a herpesvirosis. Adenovirosis was a differential diagnosis based on the histopathologic results, but adenoviruses are smaller (70-80nm), unenveloped, hexagonal outlined and arranged in paracrystalline arrays. In addition, inclusion bodies induced by adenoviruses are generally basophilic. Herpesviruses have been shown in association with venom gland infection in Indian Cobra (*Naja naja*), Banded Krait (*Bungarus fasciatus*), Siamese Cobras (*N.n. kaouthia*) and Mojave Rattlesnakes (*Crotulus scutulatus*). Infection has been associated with multifocal necrosis of columnar glandular epithelium and infiltration with mixed inflammatory cells. A fatal herpesvirus infection has been reported just once in two juvenile boa constrictors. The described lesions were similar to those seen in our report. So, the present study seems to be the second report of fatal herpesvirosis in snake and first report of fatal herpesvirosis in *Vipera ammodytes ammodytes* and in *Natrix natrix*.

Galectin-3 expression in canine malignant mammary tumours

De Oliveira, Joana, Joana Gomes, Augusto de Matos, Eva Héllmen, Emerson Bernardes, Fátima Gartner

IPATIMUP, Porto, Portugal

Introduction: Canine mammary tumours affect mainly older female dogs and comprise approximately 25-50% of all their tumours, 40-50% being malignant. Galectin-3 (Gal-3), is a member of beta-galactoside-binding animal lectins, which has been implicated in a number of possible important biological functions, including the regulation of cell growth and association with tumour transformation. Gal-3 expression has been described to protect cells from apoptosis induced by loss of cell adhesion, and from oxidative stress-induced apoptosis during metastasis. The aim of this work was to assess the expression of Gal-3 in CMMT and metastasis, and in a previously established canine malignant mammary tumour (CMMT) cell line in vitro and in vivo.

Material and methods: CMTU27 derived from a ductular carcinoma was cultured in RPMI medium with 10% foetal bovine serum and gentamycin and kept at 37°C in 5% CO₂ atmosphere. Western blot. Protein extracts were analysed by standard SDS-PAGE, and blotted with anti-Gal-3 monoclonal antibody (M3/38). In vivo assay. Six weeks old female N:NIH Y(s)II-nu/nu mice were inoculated subcutaneously in the fat mammary pad with a suspension of 10⁶ cells. Tumours and metastatic target organs were collected. Immunohistochemistry. CMMT and metastasis paraffin sections from the files of the Companion Animals Department of ICBAS-UP and paraffin tumour and organ sections of the mice were examined for Gal-3 expression.

Results: Gal-3 was heterogeneously detected in all CMMT cases, mainly expressed by tumour cells in the periphery of necrotic areas. Even though CMMT solid metastasis did not express galectin-3, clusters of galectin-3-positive tumour cells were found within lymphatic vessels located at the tumour periphery. CMTU27 cell line expressed Gal-3 in vitro when analyzed by Western blot. However, in nude mice-derived tumours and metastasis Gal-3 expression was negative.

Discussion: Our data seems to indicate that galectin-3 expression in CMMT may be related to cell death protection and metastatic potential. The absence of galectin-3 in mice-derived tumours may indicate that a competent immune system may somehow affect its expression. Finally, CMTU27 cell line may constitute an excellent model to assess the role of this galectin in tumour cell adaptation to different microenvironmental stress factors.

Molecular pathological analysis at the late stage of tumor promotion by oxfendazole in a rat two-stage hepatocarcinogenesis model

Dewa, Yasuaki, Jihei Nishimura, Meilan Jin, Masaomi Kawai, Yukie Saegusa, Makoto Shibutani, Kunitoshi Mitsumori

Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo, Japan

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Introduction: Oxfendazole (OX) is a benzimidazole anthelmintic that has been widely used for the treatment and prevention of gastrointestinal parasites in livestock animals. OX is also known as a tumor promoter in rat livers and have a potential to induce cytochrome P450 1A regardless of its ligand binding to aryl hydrocarbon receptor (AhR); however the precise mechanism of its tumor promoting activity is still unclear. Previously, we reported that OX enhanced oxidative stress responses such as reactive oxygen species (ROS) production through its metabolism and preneoplastic lesions in an early stage of promotion in a rat two-stage hepatocarcinogenesis model. In this study, we performed molecular pathological analyses in preneoplastic and neoplastic lesions of the livers in rats induced by OX.

Material and methods: Male F344/N rats aged 6 weeks were treated with 0 or 0.05% of OX in the diet after DEN initiation. Two-third partial hepatectomy was applied one week after the start of OX treatment. After 28 weeks of tumor promotion, the livers were subjected to mRNA expression analyses by real-time RT-PCR and following immunohistochemical examinations for confirming the localizations of corresponding molecules.

Results: Histopathological examinations revealed that OX significantly enhanced the multiplicity of hepatocellular adenomas, the number and area of glutathione S-transferase placental form (GST-P)-positive foci, and the number of PCNA-positive hepatocytes as compared with DEN-alone animals. In mRNA expression analyses, OX significantly induced expressions of AhR-regulated xenobiotic enzymes (*Cyp1a1*, *Cyp1a2* and *Nqo1*), Nrf2-regulated anti-oxidant enzymes (*Gpx2*, *Afar*, *Yc2* and *Gstm1*), and decreased the levels of cell cycle regulators (*p21* and *p27*). Immunohistochemically, adenomas and some of the GST-P-positive foci showed decreased immunoreactivity of CYP1A1, p21 and its positive regulator C/EBP alpha, but increased that of AFAR and the number of PCNA-positive hepatocytes as compared with the surrounding tissues.

Discussion: These results suggest that OX elicits persistent oxidative responses through its own metabolism during the promotion. In addition, some altered foci and neoplastic lesions might acquire a phenotype to escape from the oxidative stress and normal cell cycle regulation.

Advanced glycation end products and their involvement in Turquoise Killifish (*Nothobranchius furzeri*, Jubb 1971) liver pathologies

Di Cicco, Emiliano, Alessandro Cellerino, Eva Terzibasi, Giacomo Renzoni and Giacomo Rossi

Department of Veterinary Science, Camerino, Italy

Introduction. Currently the Turquoise Killifish is considered the best animal model suitable for aging studies. This annual fish, from south east Africa, shows an exceptionally adaptive behaviour to dry periods: bearing in mind this feature, it's easy to understand that the average lifespan of *Nothobranchius furzeri*, just about 8-9 weeks, makes this species (more similar to highly evolved vertebrates than nematodes or fruit flies) highly practical for aging researches. Furthermore, because of its high metabolic activity, the liver represents one of the first target organs to evaluate aging damages. Taking into account the occurrence of age-related liver alterations in *N. furzeri*, the presence and the differences of AGE products expression was evaluated in comparison to the age and the strain of each group of fishes.

Materials and Methods. 34 fish were divided in 6 groups, three age classes (5, 11 and 21 weeks) and three strains (GRZ, MZM-3 and MZM8/10pl) characterized by different lifespan. A calories restriction regimen was applied to one group of MZM3 strain fishes. Animals were euthanized at different times and liver were routinely processed for morphological and immunohistochemical evaluations. AGEs were immunohistochemically evidenced by using an anti-AGEs polyclonal Ab (Biologo, GE).

Results. Morphological data evidenced a positive correlation between the presence of hepatic alterations, as fat degeneration or neoplasia, and fish age. Interestingly, the timing of lesions insurgence was also influenced by the strain. AGEs were described for the first time in fish and their accumulation in liver showed a trend very similar to other pathologies occurrence. Finally, strong AGEs accumulation was observed especially in neoplastic areas.

Discussion. Advanced glycation end products (AGEs) are a heterogeneous group of molecules, formed in vivo both by non-oxidative and oxidative reactions of sugars and their adducts to proteins and lipids. It is now well established that formation and accumulation of AGEs progress during normal aging and cancer, as well other degenerative pathologies. By the way, to date these molecules have been studied only in human or lab animals as mice, therefore this study amplify the range of sensible species. Moreover, the results obtained by immunohistochemistry confirmed the trend of accumulation of AGEs products in correlation to the age of the fish and also to the strain. In fact, the different expected lifespan between the strains influenced the timing of comparison of AGEs products, with long lifespan strains which showed later the same alterations and pattern of AGEs accumulation than short lifespan strains. In conclusion, we demonstrated AGEs may accumulate in the liver from all the vertebrates with a direct relationship with the age.

Black wild pigs of Nebrodi Park in Sicily: evidence of their possible role as reservoir of *Mycobacterium tuberculosis* complex infection

Di Marco, Vincenzo¹, Marianelli Cinzia², Russo Miriam¹, Aronica Vincenzo¹, Bollo Enrico³, Amedeo Stefano³, Valenza Federico³, Capucchio Maria Teresa³

¹. Istituto Zooprofilattico della Sicilia, Barcellona (ME), Italy; ². Istituto Superiore di Sanita', Roma, Italy; ³. Dipartimento Patologia Animale, Torino, Italy

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Introduction. Bovine Tuberculosis (TB) is caused by several mycobacteria species of the *Mycobacterium tuberculosis* complex. One of the most important obstacle to eradication of this infection in bovine livestock is the implication of other species and especially of wildlife in the cycle of the bacterium. The identification of the reservoir hosts is crucial for the effective control measures. The aim of this paper was to investigate the role of the Black wild pigs living in the Nebrodi Park of Sicily as probable TB reservoir.

Material and methods. During 2008, 149 Nebrodi Black wild pigs were examined. Animals aged between 7 and 24 months were classified as young and those more than 2 years old as adult. During necropsies, lymph nodes and organs harbouring lesions suggestive of tuberculosis were collected for histological and molecular biological investigations.

Results. Macroscopically TB compatible granulomatous lesions were found in 9.4% of animals involving head, thorax and/or abdomen. These lesions were observed especially in young pigs (76%). Respectively 76% and 24% of the lesions were classified as large lesions (more than 1 cm in size) and small lesions (less than 1 cm). Lesions affecting more than one anatomical region were the most frequent (48%), indicating a generalized TB infection. Sixteen percent of the animals showed macroscopical lesions confined in the head only (mandibular lymph nodes). Histologically the typical tuberculous granuloma with necrotic-calcified core surrounded by a mixed population of epithelioid and giant cells, macropahages and lymphocytes were detected in 88% of the collected samples. PCR investigations revealed the presence of the *Mycobacterium* genome in all samples.

Discussion. This study reports the high incidence of the generalized TB infection in the Nebrodi Black wild pigs and supports their possible role as TB reservoir in the Nebrodi Park. Further investigations aimed to verify the ability of pigs to transmit the disease to other species living in the same area are needed, together with the prevalence and distribution of tuberculosis in cattle sharing the pasture. The data suggest also the need to control this species for the eradication of tuberculosis in the Park.

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Objective evaluation of intrinsic cardiac response in heart failure of Wistar rats with streptozotocin induced diabetes mellitus type 1 and adriamycin induced congestive cardiac failure

Dinescu, Georgeta, Emilia Ciobotaru, Manuella Militaru, Teodoru Soare, Cornel Mihai Brăslășu

Faculty of Veterinary Medicine, Bucharest, Romania

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Introduction: Rats with streptozotocin induced *diabetes mellitus* represent the most used experimental model in the cardiovascular research. These models followed clinical and morphological investigations, focused on cardiac activity and morphology, integrity of peripheral microvascular structure and function and response to various protocols of therapy. Gross assessment of heart in experimental animals represents one of the important issues, lesions as cardiac hypertrophy or cardiac dilation being objectively determined. This study aims to assess and correlate some parameters for an objective characterization of diabetic and Adriamycin cardiomyopathy and its consequences.

Material and methods: Diabetes mellitus type 1 were experimentally induced in 55 Wistar adult rats. The animals were divided in four unequal groups and treated as follows: group 1 (streptozotocin and Adriamycin), group 2 (streptozotocin), group 3 (Adriamycin), group 4 (healthy control rats). 70 days after administration, the rats were euthanized. Body weight, cardiac weight and liver weight were assessed. Cardiac gravimetric values were completed with the assessment of longitudinal diameter and transversal diameter of the heart, interventricular sept and free walls of left ventricle and right ventricle. All values were used for calculation of longitudinal cardiac diameter/transversal cardiac diameter ratio, heart weight/body weight ratio, ventricular ratio, liver weight/body weight ratio.

Results: Rats from group 1 and group 2 presented the highest degree of hypertrophy of left ventricle, ventricular ratio being 8.33 ± 2.53 and 6.25 ± 1.85 respectively ($P < 0.05$). Increased values of heart weight/body weight ratio and liver weight/body weight were also recorded in group 1 and group 2, with $0.37(10^2) \pm 0.06$ and $4.19(10^2) \pm 0.79$ respectively in group 1 ($P < 0.05$), $0.40(10^2) \pm 0.04$ and $3.73(10^2) \pm 0.74$ respectively in group 2 ($P < 0.05$). Transversal diameter/longitudinal diameter ratio recorded no significant differences between all groups, but significant differences occurred between group 3 and control.

Discussion: The rats with streptozotocin induced diabetes type 1 included in groups 1 and 2 presented hypertrophy of left ventricle, revealed by increased values of ventricular ratio, comparing with control group. Same groups exhibited significant increasing of heart weight/body weight ratio and liver weight/body weight ratio, comparing with control group. These figures were smaller than mean value of Adriamycin treated rats. Longitudinal cardiac diameter/transversal cardiac diameter ratio did not recorded significant differences between all experimental groups, excepting group 3 treated with Adriamycin, comparing with control rats. This result expresses cardiac dilation in this group. Cardiac dilation is not always correlated with increasing of cardiac diameters and a significant increasing of longitudinal diameter/transversal diameter ratio implicitly. It is possible that a cardiac remodelling process occurred in group 3, the heart being spherically shaped, without significant increasing of its dimensions.

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Intracerebral immune response in naturally occurring listeric encephalitis of small ruminants

Di Palma, Stefano, Barbara Brunetti, Ursula Forster, Monika Hilbe, Andreas Zurbriggen, Marc Vandeveldel, Anna Oevermann

Institute of Animal Pathology, University of Bern, Bern, Switzerland

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Introduction: *Listeria monocytogenes* (LM) is a facultatively intracellular Gram positive bacterium causing fatal food-borne infections in humans and animals, and ruminants have been implicated as a reservoir for human infection. A recent study identified a high percentage of listeric encephalitis in the fallen stock population of small ruminants being by far the most frequent cause of CNS disease in small ruminants in Switzerland. Therefore, the disease is of high importance for veterinary public health. Within the framework of a host-pathogen interaction study, the inflammatory response in naturally occurring listeric encephalitis was characterized. This poster describes the local cerebral immune response in small ruminants.

Material and methods: Brains were collected from sheep and goats with listeric encephalitis. The etiology was confirmed either by positive bacteriological culture or by immunohistochemistry for listeriolysin O. Lesions were evaluated by light microscopy on HE-stained sections, classified into acute or subacute encephalitis, and their severity was graded. Frozen and formalin-fixed, paraffin-embedded brainstem sections were examined by immunohistochemistry with antibodies against CD3, WC1, CD79, CD20, MAC, CD68, Lysozyme, MHC I, MHC II, listeriolysin O and GFAP. The LSAB (Labelled Streptavidin Biotin)-AEC method was used. Immunoreactive cells were counted in 20 areas (0.06 mm² each) of perivascular cuffs, microabscesses and surrounding brain parenchyma.

Results: LM was mainly observed within phagocytes and extracellularly in microabscesses. Furthermore, in some animals, LM was present in neurons and axons. Preliminary results indicate that T-lymphocytes represent a significant cell population infiltrating the affected brain independent of the age of the lesion. In particular, also animals with an acute encephalitis had a high amount of perivascular T-lymphocytes. Furthermore, T-cells were observed in subacute microabscesses and diffusely migrating through the surrounding parenchyma. Only single WC1⁺ γ/δ T lymphocytes were observed in few animals. B-lymphocytes were present in much lower numbers than T-cells in perivascular cuffs, but almost absent in microabscesses and the surrounding parenchyma. Astrocytes were strongly activated at the periphery of microabscesses, but also in the surrounding parenchyma.

Discussion: Preliminary results indicate that the cell-mediated immune response plays an important role during natural listeric encephalitis of small ruminants, reflecting experimental models in laboratory animals. Macrophages, neutrophils and T-cells are the predominant cells populations involved. Albeit WC1⁺ γ/δ T lymphocytes are described to occur during CNS infections and to have a propensity to respond to bacterial antigens, we could not find indications that they are significantly involved in the immune response during listeric encephalitis.

Study on ovine and caprine pneumonia: Ovine pulmonary adenomatosis

Ezzi, Abbas

RAZI INSTITUTE, Karaj, Iran

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Introduction: Ovine Pulmonary Adenomatosis (OPA) has been named firstly as Jaagsiekte by Cowdry in 1925 Then in 1938 as Epizootic Adenomatosis (Dungal 1938). The etiology was definitely explained as retrovirus (Meuten 2002).Affected lung is consolidated with patchy nodules mostly involved at the right cranial lobes .The tumors are solid ,grey or whitish with bulging miliary foci smaller than 1 mm. (Jubb et al 1985 ,Martin & Aitken 1991,Meuten 2002)

Material and methods: Bacteriology : The lung tissue specimen were cultured and grown on blood agar incubating in 37°C and checking every 24-48 hours . The colonies were confirmed by oxidase tests .Then cultured on McCankey-s agar plates incubating in 37°C .The lung tissue specimen were cultured and grown on blood agar incubating in 37°C and checking every 24-48 hours . The colonies were confirmed by oxidase tests .Then cultured on McCankey-s agar plates incubating in 37°C .Histopathology : Lung tissue samples were fixed in 10% formalin saline and embedded in paraffin , sectioned at 5µm in thickness and stained by Haematoxylin & Eosin.

Results: 282 cases of pneumonia out of 12168 sheep and goats were inspected and collected at the Ziaran abattoir within 2005-2006 . Nine out of 282 cases were diagnosed as Ovine Pulmonary Adenomatosis . Five of them in sheep and one in goat were in winter time and the rest of them were in the other seasons individually. The predilection of lesions were mostly at the right cranial (1.77%) and raised .The epithelial cells of the lining alveolar were replaced by columnar and forming acinar or papillary architecture filled by alveolar macrophages, plasma cells and neutrophils (figure 1 & 2) .Also two cases of OPA were intermingled by Verminous Pneumonia and were associated with fibrinous bronchopneumonia . Two cases were mixed with Oat Shaped neutrophil cell infiltration .

Discussion: Our data indicated that the predilection ,place of lesions and seasons were in accordance with Meuten 2002 and Martin &Aitken (1991). The latter authors interpreted that the old condition and close contact are the major factors for transmission of the tumor (Martin & Aitken1991) .According to the our science OPA in goat is reported for the first time in Iran

Zebrafish as animal model for human mismatch repair deficiency

Feitsma, Harma, Raoul Kuiper, Jeroen Korving, Isaac Nijman, Edwin Cuppen

Utrecht University, Utrecht, The Netherlands

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Introduction: Whereas heterozygous mutations in mismatch repair (MMR) genes predispose to non polyposis colorectal cancer in humans, rare biallelic disorders result in development of brain tumors, neurofibromas, café au lait spots, and lymphoproliferative disorders. Available mouse models develop lymphomas but neurectodermal tumors are relatively infrequent. Here we show that homozygous mutations in the major MMR genes *mlh1*, *msh2*, and *msh6* in zebrafish result in development of neurofibromas/peripheral nerve sheath tumors that morphologically resemble their human counterparts.

Material and methods: Mutant zebrafish were selected by resequencing *mlh1*, *msh2*, and *msh6* genes after ENU-driven mutagenesis. Individual fish carrying tentative loss-of-function mutations in *mlh1*, *msh2*, and *msh6* were outcrossed and subsequently inbred to generate homozygotes. Lengths of selected microsatellites were analyzed in offspring from male homozygous *msh2* and *msh6* mutants to indicate genome instability (homozygous *mlh1* mutant males were sterile). Fish were euthanized when developing tumors, or at 24 months of age, and fixed in 4% paraformaldehyde for 4 days. Following decalcification in EDTA they were routinely processed and H&E stained tissue sections were prepared.

Results: Sequence analyses showed a nonsense mutation in both *mlh1* and *msh6*, resulting in loss of mlh1 protein, and a reduction to only 15% of the *msh6* transcript present in homozygous mutants. Mutated *msh2* was alternatively spliced resulting in loss of the 5th exon. Microsatellite lengths were altered in respectively 3 and 4% of offspring from male *msh2*^{-/-} and *msh6*^{-/-}, but not heterozygous or wildtype males, crossed with wildtype females. Homozygous *mlh1* (n=29), *msh2* (n=16), and *msh6* (n=31) mutants started developing tumors from 6 months of age, with most tumors occurring in the second year of life; respective tumor incidences were 45, 6, and 35%. No tumors were observed in wildtype or heterozygous fish. 76% of all tumors were composed of whorls of elongate cells and showed morphology consistent with neurofibroma; ocular or abdominal localization was most frequent. Other tumors of neurectodermal origing included 1 olfactory neuroblastoma and 1 primitive neurectodermal tumor. Remaining non-neurectodermal tumors included 1 squamous cell carcinoma, 1 tentative thyroid carcinoma and 2 hemangiosarcomas.

Discussion: Zebrafish were generated with homozygous mutations in orthologues of the human MMR genes *mlh1*, *msh2*, and *msh6*. Altered microsatellite lengths in offspring from homozygous mutant males indicate loss of MMR function in germline cells of these fish. Incidences of tumors resembling the neurofibromas observed in human MMR deficiency syndromes are increased in these MMR deficient zebrafish. As currently available mouse models characteristically develop lymphomas, MMR deficient zebrafish could provide an additional animal model for human MMR deficiency.

Pilot whales (*G. melas*) mortality due to Morbillivirus in Mediterranean Sea

Fernández, Antonio, Manuel Arbelo, Fernando Esperón, Pedro Herraéz, Antonio Espinosa de los Monteros, Cristina Clavel, Antonio Bernabé, J. Manuel Sánchez-Vizcaino, Philippe Verborgh, Renaud DeStephanis, Francisco Toledano, Alejandro Bayón

Unit of Cetacean Research. Division of Histology and Veterinary Pathology. Institute of Animal Health. University of Las Palmas de Gran Canaria, Arucas, Gran Canaria, Spain

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Introduction: Morbilliviruses have emerged as significant pathogens of cetaceans and pinnipeds worldwide. Two cetacean morbilliviruses have been identified and named porpoise morbillivirus (PMV) and dolphin morbillivirus (DMV). Although, morbillivirus outbreaks have not been previously reported in pilot whales, antibodies to morbilliviruses have been reported in 86% of two species of pilot whales (*Globicephala melas* and *macrorhynchus*) in the western Atlantic. Interestingly, molecular evidences from one stranded pilot whale suggested that the long-finned pilot whale is host of a different, novel type of cetacean morbillivirus (called pilot whale morbillivirus or PWMV), and distinct from both PMV and DMV.

Material and methods: During a period of six months (November, 2006 to April, 2007) more than 25 long finned pilot whales (*G. melas*) died along the southern Spanish Mediterranean coast and Balearic Islands. Nine pilot whales were fresh or moderate autolytic and they were completely or partially necropsied and sampled. A histological, immunohistochemical and virological study was performed on frozen and formalin fixed tissues. RT-PCR detection of cetacean morbillivirus (CetMV) was carried out on samples of brain, lung, spleen, lymph nodes, liver and kidney, from 7 pilot whales.

Results: Main macroscopical findings detected during the necropsy were related to moderate to severe cachexia. No content was found in the stomachs. Internally, a marked subcutaneous yellowish edema (icterus), enlarged edematous lymph nodes which showed parenchymal multifocal necrosis (especially, digestive tract lymph nodes), erosive stomatitis and erosive to ulcerative necrotizing esophagitis was observed. Microscopically, the main lesions were found in the lymph nodes which showed a multifocal necrotizing lymphadenitis with the presence of multinuclear syncytial cells. A non-purulent encephalitis with syncytial cells and intranuclear and/or intracytoplasmic inclusions bodies were detected in animals from which nervous samples analyzed microscopically. Mild to severe erosive to ulcerative necrotizing esophagitis was detected microscopically in most of the analyzed whales. An immunohistochemical staining, using a polyclonal antibody, demonstrated morbilliviral antigen in bronchiolar epithelium, in syncytial cells, monocytic-like cells and cell debris of affected lymph nodes and brain, often containing positive intracytoplasmic globular and/or granular positive immunoreaction. A morbillivirus was detected by RT-PCR in brain, spleen, kidney, lymph nodes, liver and lung from 7 pilot whales virologically analyzed. Sequencing revealed the same sequence in all positive samples. The novel sequence obtained was closely related to DMV and more divergent to PWM was observed.

Discussion: This represents the first morbillivirus infection inducing high mortality of long-finned pilot whales (*M. melas*). The first morbillivirus epizootic described in cetaceans involved striped dolphins in the Mediterranean Sea in the 1990s when a DMV was described by the first time. Both the pilot whale and the striped dolphin mortalities share a very closely related virus phylogenetically and the potential for interspecific transmission must be considered. Although it has been reported

that pilot whales may be enzootically infected world-wide by morbillivirus, the virus involved in the present mortality is different to the PWMV, supporting previously evidence that different strains of CetMV may be infecting dolphins and whales.

Malignant peripheral nerve sheath tumor of mediastinum in horse

Fictum, Petr, Miša Škorič, Roman Halouzka, Barbora Bezdělková, Petr Jahn

University of Veterinary and Pharmaceutical Sciences, Brno, Czech republic

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Introduction: Thirteen-year old gelding was sent to the Clinic of Equine Diseases with anamnesis of loss of weight, weakness, limb edema diffusing to the thorax and abdomen. By ultrasonograph we detected bigger amount of effusion in the thoracic cavity and presence of mass pushing away the heart in a dorsocaudal way. In terms of these finding the euthanasia was performed.

Material and methods: The necropsy was performed and tissue samples taken for the histopathological examination. The specimens were fixed in 10 % buffered formalin, processed by paraffin technique and stained by haematoxylin-eosin, by Verhoeff van Gieson, also the immunohistochemical examination was performed. For the immunohistochemical examination we used following antibodies: Vimentin (Clone Vim 3B4, dilution 1:50, DAKO), Cytokeratin (Clone AE1/AE3, dilution 1:50, DAKO), GFAP (Clone GF2, dilution 1:50, DAKO), S-100 protein (polyclonal rabbit anti S-100, dilution 1: 200, DAKO), collagen IV (Clone CIV 22, dilution 1:50, DAKO), NSE (Clone 5E2, dilution 1:50, Novocastra) and laminin (clone LAM-89, dilution 1:50, Novocastra). For the detection of antibodies binding we used detection systems EnVision™+/HRP, Mouse (DAKO) and EnVision™+/HRP, Rabbit (DAKO).

Results: During the necropsy we detected multilobular, whitish, partially heamorrhagic mass (40x25x25 cm), externally enclosing cranial lung lobes and infiltrating outer pericardial sheet. In the diaphragm lobe of the lungs we detected whitish nodule of size 5x5 cm. Histopathological examination of the mass from mediastinum and lung nodule revealed neoplastic tissue composed of spindle shaped cells showing cellular abnormalities, the cells were organized in interwoven bundles, locally there was presence of neoplastic cells forming whorles and locally indication of palisade-like setting of nuclei. Also numerous mitotic figures and angioinvasion were present. Similar neoplastic tissue was histopathologically found in the lung nodule. Staining by Verhoeff van Gieson showed only one positivity of fibrous stroma in tumor tissue. Immunohistochemical examination revealed positivity of vimentin, S-100 protein and collagen IV. In examination, where antibodies against GFAP, cytokeratins, NSE and laminin were used, were negative. Based on performed examinations the tumor was classified as malignant peripheral nerve sheath tumor.

Discussion: Malignant peripheral nerve sheath tumors belong to rarely occurring malignant neoplasms in horses. Their presence is described in skin, GIT, periocular tissues and intracranially (with extradural localization). One case of this tumor is described in horse in heart and one case of mediastinal malignant schwannoma in horse. In our case, except the presence of tumor in mediastinum, there was metastazing of tumor to the lungs.

Identification of molecular phenotypes in canine mammary carcinomas with clinical implications: application of the human classification

Gama, Adelina, Anabela Alves, Fernando Schmitt

Universidade Trás Montes e Alto Douro, Departamento Ciências Veterinárias, Vila Real, Portugal

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Introduction: Mammary gland tumours are the most commonly occurring neoplasm in the female dog and represent a remarkably heterogeneous group in terms of morphology and biological behaviour. About half of canine mammary tumours are considered malignant and the identification of reliable prognostic factors is essential in order to estimate the individual risk of unfavourable clinical outcome. Recently, gene expression profiling and immunohistochemical analyses have redefined human breast cancer taxonomy and identified molecular subtypes associated with distinct clinical outcomes. In the present study, we sought to identify similar phenotypical subtypes in canine mammary cancer with possible clinical implications.

Material and methods: In the present study we characterized a series of a hundred and two canine mammary carcinomas based on this new classification, by using an immunohistochemistry surrogate panel based on five molecular markers (estrogen receptor, HER2, cytokeratin 5, p63 and P-cadherin).

Results: Canine mammary carcinomas were classified into four different subtypes: luminal A (ER+/HER2-; 44.8%), luminal B (ER+/HER2+; 13.5%), basal (ER-/HER2- and a basal marker positive; 29.2%) and HER2 overexpressing tumours (ER-/HER2+; 8.3%). Luminal A-type tumours were characterized by lower grade and proliferation rate, whereas basal-type tumours were mostly high grade, high proliferative and positive for CK5, p63 and P-cadherin. Follow up data revealed that basal subtype was significantly associated with lower overall ($P=0.002$) and disease-free ($P=0.01$) survival rates, whereas the other groups showed higher survival rates, including the HER2-overexpressing group.

Discussion: In this study we found similar findings to the ones described in human breast cancer studies and we have also identified distinct phenotypical subtypes by using an immunohistochemical panel which included five molecular markers (ER, HER2, CK5, p63 and P-cadherin). These results are in contrast to a previous study which only identified luminal A and B subtypes in canine mammary tumours. However, the tumour series was smaller and, although a similar terminology was used, the subtype definition was not identical. Moreover, we have identified a basal-like subtype representing almost 30% of our series, which was associated with a more aggressive clinical behaviour. We believe that canine mammary carcinomas would be suitable natural models for the study of this particular subset of human breast carcinomas. However, more studies are needed regarding the prognostic value of these immunohistochemically determined subtypes in canine mammary cancer.

Pathology of influenza virus H5N1 infection in resistant and susceptible laboratory mice

Garigliany, Mutien-Marie, Karine Cloquette, Mickael Leroy, Mélanie Palm, Etienne Baise, Daniel Desmecht

Faculty of Vet Med – University of Liège, Liège, Belgium

Influenza A virus is a main human pathogen and it is estimated that approximately 10-15% of people get influenza around the world each year, during annual influenza epidemics. Besides, it was responsible for different pandemic outbreaks over the 20th century, among which the 1918 Spanish influenza pandemic was accountable for more than 50 millions deaths. The current H5N1 avian influenza virus is considered a serious candidate for the next pandemic. In 1962, the A2G inbred mouse line was shown to survive influenza A virus infections that were fatal to other inbred lines. This resistance trait was later shown to cosegregate with the type I interferon-dependent expression of a ~70 kDa protein, the so-called mouse MX1. Ongoing studies in the laboratory consist in the delineation of the antiviral function of several domestic animal homologues of the mouse MX1. Here, we compare the morphological alterations seen in the lungs of wildtype (susceptible) and in-house constructed MX-transgenic mice (anticipated to be resistant from in vitro data) infected with a murinized strain of the current H5N1 virus. Severity of histopathological lesions, virus tropism at different time points after inoculation and virus lung titres are reported and put in perspective with body weight loss and lethal-dose 50 data.

Cholangiocarcinoma in cockatiel (*Nymphicus hollandicus*): case report

Gesek, Michal, Stenzel Tomasz, Szarek Jozef, Babinska Izabella, Mieszczynski Tomasz

University of Warmia and Mazury, Faculty of Veterinary Medicine, Department of Forensic and Administration of Veterinary Medicine, Olsztyn, Poland

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Introduction: Cholangiocarcinoma (intrahepatic bile duct carcinoma) is a malignant tumour, composed of cells resembling biliary epithelium. It is diagnosed in different animal species, though rather seldom in birds. It can be found as large single mass or as multiple nodules.

Material and methods: Cockatiel (*Nymphicus hollandicus*) at the age of 9 with respiratory difficulties. Chlamydiophylosis was suspected. Medical examination, that is always a stressogenic situation for wild birds, caused death of the bird. During necropsy the samples of the liver, kidney and lungs were taken for microscopic examination. Material for histopathological evaluation was fixed in 10% neutralized formalin. Paraffin slides were stained with hematoxylin and eosine (HE).

Results: Medical examination revealed dyspnoea, leanness, abdomen enlargement, of reduction muscle strength. Post mortem examination showed in the liver presence of multiple nodules. Locally they were grown together, with dimensions of 1 to 5 mm. They were of yellow to brown colour and firm consistency. Presence of tumour cells agglomerations of cuboidal to columnar shape, organized in tubular and acinar pattern resembling biliary epithelium were observed microscopically in liver parenchyma. Nuclei of the cells were of various dimensions: some were big, round and oval-shaped, with reticular chromatin. Cytoplasm of the tumour cells was light, eosin absorbing. A small area of mucin was sporadically visible in the tumour tissue. Numerous extravasations and inflammatory cells were found inside the neoplasms. Congestion, blood stasis, oedema and hemosyderosis was stated in lung. In kidney congestion, bloody haemorrhage, parenchyma degeneration of kidney tubules epithelium and focuses of their necrosis and amyloidal degeneration were observed. Cholangiocarcinoma was diagnosed due to analysis of macro- and microscopical examinations.

Discussion: Cholangiocarcinoma was found in many animal species. Nevertheless, occurrence of the tumour was not reported yet in cockatiel (*Nymphicus hollandicus*). Moreover, descriptions of the cases usually concentrated on the tumour itself putting the concomitant lesions aside.

Aspects of conflicts in poultry breeding in veterinary expert opinions in Poland

Gesek, Michał, Szarek Jozef, Babinska Izabella, Wojtacka Joanna, Mieszczynski Tomasz

University of Warmia and Mazury, Faculty of Veterinary Medicine, Department of Forensic and Administration of Veterinary Medicine, Olsztyn, Poland

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Introduction: The aim of the study was to determine the degree of conflictogenity of poultry industry and to show the reasons of the conflictogenity that result in issuing veterinary expert opinion.

Material and methods: The research was carried out on 110 expert opinions relating poultry, collected from Poland, drew up by the authors of this publication, between 1995 and 2008.

Results: The analysis of expert opinions showed that broiler chicken were the most frequent subject of the conflicts (64 cases). Turkeys were the subjects in 20 cases, hens (18), geese (7), and ostrich in 1 case. The most serious looses in poultry were caused by bacterial infections. *E. coli* was the most common cause of the looses: 16 cases in broilers, 6 cases in turkeys and 1 case in laying hens. *Clostridium sp.* was relatively common cause of deaths in the birds and decreased gaining of body mass: 13 cases in broilers, 3 cases in laying hens and 2 cases in turkeys. 12 expert opinions showed *Salmonella sp.* as the reason of looses on the poultry farms. The hatchery was the source of infection four times. In 5 cases (2 in broilers, 1 in laying hens and 1 in turkeys and geese) *Salmonella* infection was the effect of breeder action and in 3 other cases (2 in broilers and 1 in laying hens) the source of bacteria was found in feed. Single expert opinions concern presence of *Mycoplasma* (laying hens and turkeys), *Pasteurella* (geese) and *Ornithobacterium* in turkeys.

Problem in broiler breeding resulted from coccidiosis that was showed in 3 cases.

Marek's disease was the reason of looses in breeding of laying hens in 3 expert opinions. It was also established that reoviruses were the cause of looses in broiler breeding in 6 cases, Gumboro disease in 2 cases and AE and leukemia viruses in single cases. In one case concerning laying hens it was confirmed that wrong feed was accompanied by secondary viral infection (ILT, EDS and reoviruses). Two conflicts aroused based on Derzsy's disease confirmed in geese and related significant looses.

Fungi and their toxins were relatively frequent causes of breeding failure in the poultry farms. Their negative effect on the birds was noted in 13 expert opinions concerning broiler chickens, 9 – laying hens and 3 - turkeys. In 7 cases intoxication with ochratoxin (including 3 in broilers, 4 in laying hens and 1 in turkeys) and 1 with aflatoxin (in laying hens) were stated.

The feed was the most common cause of breeding failure in poultry in the 90'. It was destined for broiler chicken – 21 cases, laying hens – 4 cases and turkeys – 3 cases. Despite microbiological pathogens (bacteria, fungi) improper content and quality of the feed were noted (increased NaCl level, rancid fat, vitamin A deficiency, protein surplus). This kind of conflicts showed decreasing tendency in the years analyzed.

The mistakes in veterinary art and in hatching and breeding were also noted. The breeders had also made false decisions and actions.

Conclusion: The analysis of the expert opinions showed that the number of conflicts concerning bacterial and viral infections in poultry in Poland has increased and conflicts regarding the feed are not as numerous as it was noted previously.

Effects of oral administration of *Silybum marianum*(L.Gaertn.) extract (silymarin) on histopathological and biochemical changes caused by Aflatoxin in broiler

Gholamhoseini, Banafshe¹, HamidReza Ahmadi-Ashtiani², Farzaneh Tajdini³, Saeed Bokaei⁴

Islamic Azad University Karaj baranch, Iran

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Introduction: The most important mycotoxin in poultry industry is Aflatoxine and the most common aflatoxin is aflatoxin B1. The effect of AflatoxinB1 on different body organs in different species of animals has been well established. Enzymatic changes of blood, increasing FCR, histopathological defects and sometimes mortality were observed in cases that their diet was contaminated by more than 10 ppm aflatoxin B1. Using effective and inexpensive substances is the target of most of the researchers. The issue of silymarin effects in preventing various liver and kidney damage has been addressed by some writers. Although little is known about how would be useful in decreasing the pathological and biochemical defects of this mycotoxin in liver, kidney and muscle in 1ppm contamination of diet in Broilers. The aim of this study was to study preventing effect of silymarin on the AflatoxinB1 defects by in 1ppm dosage in Broilers

Material and methods: An experimental study was designed in 56 ordinary Ross Broiler one-day chickens were randomly selected and assigned into 4 groups contains 14 chickens and kept for 42 days. First group were fed with normal allotment, second group with normal allotment and 1mg/Kg aflatoxin B1, third group with normal allotment and 800 mg silymarin per Kg body weight and fourth group with normal allotment, 800 mg silymarin per Kg body weight and 1mg/Kg aflatoxin B1. After 6 weeks blood samples were taken from chicks of each group, then chicks euthanized and pathological samples of liver, kidney and muscle were taken. AST, ALT, uric acid were measured and pathological slides of liver, kidney and muscle were studied by light microscope.

Result: The results of ANOVA test in the comparison between groups indicated that enzyme rates and pathological defects in silymarin treated in fourth group were significantly lower than in second group. less mitosis in nucleus of liver cell, vacoulation degeneration heterophiles in liver, glumerular cells increasing, tubular degeneration, hyperemia in kidney were observed. ALT, AST and uric acid in blood serum were also low.

Discussion: According to the reviewed data and comparing the results of studied groups, silymarin can be recommended for decreasing the aflatoxin B1 damages during the period of breeding broilers.

Immunohistochemical characterization of canine renal cell carcinomas

Gil da Costa, Rui M., Justina P. Oliveira, Ana L. Saraiva, Fernanda Seixas, Fátima Faria, Fátima Gärtner, Maria A. Pires, Carlos Lopes

ICBAS - UP, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Portugal.

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Introduction: Canine renal cell carcinomas (RCCs) are uncommon, aggressive tumours that occur mainly in middle-aged, male dogs. The histological classification of these tumours bears no relation with their prognosis and, so far, little information is available concerning their immunohistochemical properties.

Material and methods: RCCs from 13 dogs of different breeds, averaging 8 years of age, were retrieved from the archive. RCCs were classified as papillary, tubulo-papillary, papillary-cystic, solid or sarcomatoid and were analyzed immunohistochemically for immunoexpression of cytokeratins (AE1,AE3, CAM 5.2 and wide-spectrum screening (WSS) anti-cytokeratins antibodies), vimentin, c-KIT, CD10 and CEA.

Results: Out of 13 canine RCCs, 3 were classified as tubulopapillary, 4 as papillary, 2 as papillary-cystic, 1 as sarcomatoid and 3 as solid. All 3 solid RCCs showed vimentin, c-KIT and CEA immunoexpression and were negative for cytokeratins. All 7 papillary and tubulo-papillary tumours showed immunostaining for vimentin, while 6/7 were also positive for cytokeratins and c-KIT. Both papillary-cystic RCCs were negative for vimentin, positive for cytokeratins and c-KIT, and, interestingly, showed lower-than-average mitotic indices. Only 3 out of all 13 RCCs showed immunostaining for CD10, with no association with specific histologic types.

Discussion: These results show an association between certain canine RCC histological types and particular immunohistochemical profiles, but the histological and immunohistochemical phenotypes of canine and human RCCs do not seem to be super imposable. The histological pattern has been considered, so far, irrelevant for the prognosis of canine RCCs. Further studies are needed to determine whether some of these immunohistochemical markers correlate with these tumours's prognosis

***In vitro* anti-tumour activity of two *Pteridium aquilinum* extracts**

Gil da Costa, Rui M., Joana Pereira, Júlia Bessa, Maria São José Nascimento, Fátima Gärtner, Anake Kijjoo, Carlos Lopes

ICBAS - UP, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto., Porto, Portugal

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Introduction: Bracken (*Pteridium aquilinum*) is a ubiquitous fern of the *Pteridaceae* family. Bracken ingestion is associated with disease in several domestic animals, particularly in cattle where it produces urinary bladder and digestive tract tumours. Ptaquiloside, a *nor*-sesquiterpene glycoside with strong DNA alkylating properties, is considered a major bracken carcinogen. While some studies have addressed the carcinogenicity of bracken and its toxins, only one report suggested that ptaquiloside might show anti-tumour properties. The present report summarizes preliminary data on the anti-tumour properties of *P. aquilinum*, based on its capacity to inhibit *in vitro* the growth of four tumour cell lines.

Material and methods: Young *P. aquilinum* leaves and stems were collected in northern Portugal (Matosinhos council). Samples were air-dried, milled, and extracted with methanol and chloroform. Stock solutions of both methanol (MeOH) and chloroform (CHCl₃) extracts were prepared in DMSO and kept at -20°C. Appropriate dilutions were freshly prepared before each assay. Three human tumour cell lines (cutaneous melanoma A375-C5, mammary carcinoma MCF-7, lung carcinoma NCI-H460) and one mouse melanoma cell line (B16F10) were used. Monolayer cultures were maintained either in RPMI-1640 supplemented with 5% FBS (for A375-C5, MCF-7 and NCI-H460) or in MEM supplemented with 10% FBS and 1% non-essential aminoacids (for B16F10), 2mM glutamine, penicillin 100 U/ml and streptomycin 100 µg/ml, at 37°C in a moist atmosphere with 5% CO₂. The effects of both extracts on the growth of tumour cell lines were evaluated according to the procedure adopted by the National Cancer Institute (USA) in their *in vitro* anticancer drug discovery screen, that uses the protein-binding sulforhodamine B (SRB) to assess cell growth. Exponentially growing cells were exposed to 5 serial concentrations of each extract for 48 h. Adherent cells were then fixed *in situ* with 50% trichloroacetic acid, washed and stained with 0.4% SRB in 1% acetic acid. Bound stain was solubilised in 10 mM Tris and absorbance was measured at 492 nm in a microplate reader. For each cell line a dose-response curve was obtained and the growth inhibition of 50% (GI₅₀), corresponding to the concentration of each extract that inhibited 50% of the net cell growth was calculated

Results: For each cell line, the GI₅₀ (µg/ml) concentrations of each extract (CHCl₃/MeOH) ± SEM were as follows: A375-C5 63.2 ± 5.9/637.0 ± 32.0, B16-F10 50.4 ± 6.1/183.0 ± 6.0, NCI-H460 94.7 ± 2.7/693.0 ± 23.0, MCF-7 92.0 ± 4.0/907.0 ± 17.0.

Discussion: The GI₅₀ concentrations of the chloroform extract were consistently lower than those of the methanol extract for each cell line, probably reflecting higher concentrations of active compounds, obtained by chlorophyll elimination process. Further tests are warranted to determine the activity of different bracken compounds (ptaquiloside, quercetin, shikimic acid), both separately and in associations.

Immunophenotypical changes associated with feline endometrial neoplastic transformation

Gil da Costa, Rui M., Marta Santos, Irina Amorim, Célia Lopes, Patrícia Dias Pereira, Augusto Faustino

ICBAS - UP, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto., Porto, Portugal

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Introduction: Feline endometrial adenocarcinomas are infrequent, little studied tumours with an aggressive biological behaviour. Immunohistochemical studies of human endometrial carcinomas revealed markers related to increased cell proliferation, loss of cell adhesion, cytoskeleton changes and increased cell mobility. This study describes the epithelial immunophenotype of 6 feline endometrial adenocarcinomas in respect of pancytokeratins (AE1,AE3), cytokeratin 14, vimentin, -actin, cyclooxygenase-2 (COX-2), E-cadherin, β -catenin, progesterone receptor (PgR), oestrogen receptor (OR) and caveolin-1, compared with 6 normal feline uteri.

Material and methods: 2 μ m-thick serial sections from each case were used for haematoxylin-eosin (H&E) staining and for immunohistochemistry following an avidin-biotin peroxidase complex method. Each case was ascribed to one of 5 classes (negative, 0-25%, 26-50%, 51-75%, 76-100% of stained cells) for each marker, considering the number of immunostained epithelial cells.

Results: All normal uteri and 4/6 adenocarcinomas showed moderate to strong pancytokeratins immunostaining in 75-100% of cells. In 2/6 tumours only 0-25% of cells were immunostained. Moderate to strong vimentin immunostaining in normal uteri was detected in 0-25% (2/6 cases) or in 75-100% cells (1/6 cases). Moderate cytoplasmic vimentin immunostaining was also present in 1/6 adenocarcinomas in 25-50% cells. All normal uteri presented membrane-associated, apical COX-2 immunostaining in 75-100% cells. All tumours presented membrane-associated COX-2 immunostaining without defined polarity, and cytoplasmic staining in 0-25% (5 cases) or 25-50% cells (1 case). All normal uteri and all tumours presented diffuse, membrane-associated E-cadherin and β -catenin immunostaining in 75-100% of cells. Moderate to strong PgR immunostaining was present in 75-100% of cells in 5/6 normal uteri and also in 3/6 (0-25% cells), 2/6 (75-100% cells) or 1/6 (25-50% cells) tumours. OR immunostaining was detected in all normal uteri (50-75% cells in 3 samples, 75-100% cells in another 3) and in 1/5 tumours (0-25% cells). Anti-CK14 antibodies did not label any tumoral or normal structures. Normal and neoplastic epithelium was negative for caveolin-1, but endothelial cells and smooth muscle cells around blood vessels and in the myometrium were consistently immunostained. Immunostaining for -actin was detected in the myometrium and the muscular layer of vessels but not in tumour cells.

Discussion: Results suggest that COX-2 and PgR may be involved in feline endometrial neoplastic transformation. Loss of cell adhesion does not seem to require E-cadherin down-regulation, and β -catenin nuclear translocation does not seem to occur in these tumours. Despite some cytoskeleton changes, no clear shift towards a basal epithelial or a mesenchymal phenotype was observed.

Immunodepression does not affect innate resistance of SJL mice to respiratory syncytial virus

Glineur, Stephanie, Dermine Martin and Desmecht Daniel

University of Liege, Faculty of Veterinary Medicine, Liege, Belgium

Introduction: Respiratory syncytial viruses (RSV) are a main cause of respiratory illness among mammals. Whereas human RSV is known to infect ~ 85% of children under 18 months, bovine RSV is causing enormous economical costs in bovine production. Recently, mouse RSV (PVM, for *Pneumonia Virus of Mice*) proved to be a reliable model for studying human as well as bovine RSV-associated disease. Innate susceptibility/resistance patterns to PVM infection were compared among six inbred mouse strains from different lineages: BALB/c, DBA/2, 129/Sv, SJL/J, C3H/HeN and C57BL/6 (Bui Tran Anh et al., 2006). Based on exhaustive functional, histological and virological examinations, SJL/J and 129/Sv were identified as being the most resistant and susceptible strains respectively. As a first step toward the understanding of the cause underlying this difference in susceptibility, we examined the effect of acute immunosuppression on the development of PVM-associated disease in SJL/J mice. According to preliminary studies, the most dramatic immunodepression after gamma total-body irradiation (γ -TBI) of SJL mice (9.02 Gy) is typically observed on day 4 after exposure. Therefore, the study consisted in the follow-up of PVM infection in a cohort of 129/Sv (immunocompetent/susceptible) and two cohorts of SJL/J, either without pretreatment (immunocompetent/resistant) or starting 4 days after irradiation (immunodepressed/unknown status).

Material and methods: The experience was conducted with specific pathogen-free 12 to 14 wk-old female mice of the SJL/J (n=30) and 129/Sv (n=10) inbred strains. The mice were distributed as follows: group No I = 1 x 10 129Sv and groups No II, III & IV = 3 x 10 SJL/J. γ -TBI was performed on SJL from Groups No II and III. Four days after the exposure, mice from groups I, II and IV were inoculated with 50 μ l of the PVM suspension (10^3 PFU) and mice from group III with 50 μ l of a placebo solution. The animals were studied for seven days after inoculation during which body weight data were daily collected. Double-chamber plethysmography was also achieved from day 5 to day 7. Finally, mice were sacrificed at day 6 and 7 post inoculation (p.i.) for the lung titration and histological examinations.

Results: The body weight follow-up yielded a statistically significant difference between positive (group No I) and negative controls (group No. IV) on days 6 and 7 p.i., whereas the body weight course was similar among groups II, III and IV (SJL/J). Results from immunohistochemical examination of the viral spread throughout the lungs paralleled body weight loss data: extensive in group I and restricted to a few foci in other groups. Respiratory pattern/function values and lungs titrations are in progress.

Discussion: The study reported here was designed to bring a first insight over the origin of the genetic resistance of the SJL/J strain to the PVM. TBI did not result in any change in the course of the disease associated to PVM infection, despite a dramatic immunosuppression. We therefore suggest that the innate resistance opposed by the SJL strain is probably not driven by a better/faster development of acquired immunity.

C-cell thyroid adenoma in a horse: clinical, histological and immunohistochemical characterisation

Gómez-Laguna, Jaime, Elisa Díez, Alejandro Suárez-Bonnet, Jose María Santiesteban, Yolanda Millán, José Carlos Estepa, Ana Isabel Raya, Escolástico Aguilera, Antonio Espinosa de los Monteros, Juana Martín de las Mulas

Cordoba University, Cordoba, Spain

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Introduction: Nodular foci in the thyroid gland of horses are considered to be a common finding in older horses. Most of these foci have been classified as nodular hyperplasia and thyroid follicular adenomas. However, thyroid tumours may present parafollicular (C-cell) differentiation either singly or in combination with follicular differentiation more frequently than formerly suspected. An immunohistochemical profile is needed to be able to identify the specific type of thyroid tumour to determine clinical significance and prognosis.

Material and methods: A 17-year-old Andalusian gelding was presented to the Internal Medicine Service of the Veterinary Teaching Hospital of the University of Córdoba for the investigation of a mass located at the cervical region, which had been present for several months. Clinical examination, blood work, and ultrasonography and excisional biopsy of the mass were performed. On clinical examination a nodular movable lesion was identified at the retropharyngeal level. Clinical pathology findings included only mild anaemia. Ecographic examination of the left lobe of the thyroid gland revealed the existence of a mass (2.0 x 4.0 cm) of heterogeneous appearance and less echogenic than the adjacent enlarged thyroid gland (6.6 x 2.7 cm). The excisional biopsy was fixed in 10% buffered formalin and several tissue samples were embedded in paraffin wax, sectioned and stained with haematoxylin and eosin (HE). In addition, the immunophenotype of the tumour cells was analysed using a panel of selected antibodies.

Results: On gross examination, the retropharyngeal mass showed a multinodular or smooth aspect, white to grey in colour, homogeneous and white surface of section and mild consistency. Microscopically, a highly cellular neoplasia composed of clusters and cords of neoplastic cells was observed. Most of the neoplastic cells were fusiform in shape although there were some cuboidal cells forming medium to small, colloid-containing acini or follicles. The cellular density was homogenous and mitosis were scarce. The majority of tumour cells expressed calcitonin while the immunohistochemical expression of neuron specific enolase, chromogranin A and synaptophysin and intermediate filament proteins was more heterogeneous.

Discussion: The diagnosis of parafollicular, C-cell thyroid adenoma suggested by the histologic picture was confirmed by the immunohistochemical characterization of the neoplasm. Our findings show the possibility of use of commercially available antibodies in the characterization of proliferative lesions of the thyroid of the horse. In addition, they add a new case to the growing list of specifically identified equine parafollicular adenomas, which may have been under diagnosed because of the lack of overt clinical functionality.

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Comparison of tissue and serum expression of IFN α and IFN γ during acute EU PRRSV infection

Gómez-Laguna, Jaime, Francisco Javier Salguero, Inmaculada Barranco, María del Mar Fernández de Marco, Filip Barbé, Francisco José Pallarés, Librado Carrasco

Introduction: Porcine Reproductive and Respiratory Syndrome (PRRS) is clinically characterized by reproductive failure in gilts and interstitial pneumonia in growing pigs. PRRS virus (PRRSV) is known to replicate mainly in macrophages and to persist in lungs and lymphoid organs. Viral infections are regulated by interferons (IFNs), which constitute a family of antiviral cytokines considered to play a significant role in the innate and adaptive immune responses. In this work the serum and tissue expression of IFN- γ and IFN- α during a European PRRSV infection is described.

Material and methods: Twenty eight specific pathogen free, male, five weeks old pigs from a high-healthy farm seronegative to PRRSV were randomly distributed in batches of four, inoculated by intramuscular route with PRRSV field isolate 95/05 and humanely killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi). Other four pigs, used as controls, were inoculated with sterile medium and humanely killed at the end of the study (24 dpi). Blood samples were taken at 0, 3, 7, 10, 14, 17, 21 and 24 dpi. Sera samples were analysed for viraemia and IFN- γ and IFN- α expression. Samples from the lung and the mediastinal lymph node were fixed in 10 % buffered formalin and in Bouin solution and embedded in paraffin-wax for immunohistochemical study.

Results: Viraemia showed a progressive increase from 3 to 10 dpi, decreasing by the end of the study. IFN- α enhanced progressively in sera displaying a peak at 14 dpi, and decreased at the end of the study. Serum IFN- γ expression was undulating and increased progressively towards the end of the study. PRRSV antigen was detected in macrophages from the lung and lymph node parenchyma from 3 dpi, reaching a maximum expression at 7 dpi. IFN- α antigen was expressed in macrophages and lymphocytes, showing a higher number of positive cells at 7 and 10 dpi and decreasing at 17 dpi. IFN- γ antigen was lightly expressed in both macrophages and lymphocytes, presenting an erratic and undulating expression through the study.

Discussion: Both serum and tissue IFN- α expression were increased coinciding with the drop of the viraemia or PRRSV-antigen positive cells, respectively. However, the delayed and low levels of IFN- α expression compared to other porcine respiratory viral diseases, reflects that IFN- α expression is not an efficient tool in PRRSV clearance. IFN- γ protects macrophages *in vitro* against PRRSV replication, however in our study it was poorly expressed, not developing an effective antiviral immune response. Alveolar and interstitial macrophages and macrophages from the lymph node were the main cells expressing both IFN- γ and IFN- α , pointing to a modulation and activation of the macrophages by PRRSV.

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Eosinophils, macrophages and respiratory epithelial cells and the prepatent phase of *Dictyocaulus viviparus* infection of cattle

Graham-Brown, John, Andrew Tomlinson, Vad Lazari, Diana Williams, Jacqui Matthews, [Anja Kipar](#)

University of Liverpool, Faculty of Veterinary Science, Department of Veterinary Pathology, Liverpool, UK

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Introduction: Parasitic bronchitis (“husk”) caused by the nematode *Dictyocaulus viviparus* in cattle, has a worldwide distribution and economic importance. It is most important and often fatal in naïve calves in their first grazing season. Pulmonary disease is caused initially by migrating L₄ larvae (early prepatent phase; day 7-14 post infection), then by maturing larvae (late prepatent phase; days 14-25 p.inf.) and latterly through the aspiration of eggs and L₁ larvae into the alveoli (patent phase; day 25-55 p.inf.). The main pathological finding in the prepatent phase is a multifocal lobular eosinophil-dominated bronchiolitis and alveolitis in response to L₄ and L₅ larvae, with a substantial number of macrophages and multinucleated giant cells (MGC). This is associated with a rise in the transcription of cytokines, such as IL-4, IL-5, IL-13, IL-10 and IFN- γ . The aim of this study was to further characterise the pulmonary immune response to *D. viviparus* in the prepatent phase with particular emphasis on the interplay between eosinophils, macrophages and epithelial cells.

Materials and methods: Naïve calves were experimentally infected with *D. viviparus* and euthanased at days 14 and 21 p.inf. Formalin-fixed and paraffin-embedded lung tissue was examined by light microscopy, using H&E and Lendrum’s stains and immunohistology for specific cell markers and cytokines IFN- γ , TNF- α and IL-13.

Results: In general, the inflammatory reaction was dominated by eosinophils and macrophages, with evidence of a shift from eosinophils to macrophages on day 21 p.inf. Alveolar infiltrates contained a significant proportion of T cells and generally exhibited MGC in variable numbers. In addition, progressive activation of the lymphoid tissue (BALT) was observed. In areas of inflammation, the respiratory epithelium showed MHC-II, myeloid/histiocyte antigen (m/h Ag) and IL-13 expression. All inflammatory cells were MHC-II positive. Macrophages and MGC also stained for both lysozyme and m/h Ag. They expressed IL-13 and TNF- α , whilst eosinophils expressed IL-13 only. In addition, strong extra-cellular staining for IL-13 and IFN- γ was observed in the oedema fluid of alveoli within the inflammatory foci.

Discussion: Results suggest direct recruitment of eosinophils by the parasites and their role in killing L₄ larvae, thus explaining the lower number of eosinophils on day 21 p.inf., when the majority of parasites are maturing adults (L₅). Eosinophils, due to the release of e.g. IL-13, may contribute to both macrophage recruitment and MGC formation, which may at a later stage be promoted by macrophages in an autocrine manner, via IL-13 and TNF- α production. The latter may also promote further eosinophil recruitment. The respiratory epithelium becomes activated during the inflammatory processes and, via IL-13 production, may stimulate mucus secretion in an autocrine manner, whilst its expression of MHC-II and m/h Ag, which might be a consequence of IFN- release by inflammatory cells, suggests its further immunological and anti-microbial functions.

The role of heat shock proteins in the pathogenesis of myocarditis associated with foot-and-mouth disease virus type O in lambs

Gulbahar, M.Yavuz, Tolga Guvenc, Murat Yarim, Unal Parlak, Yonca B. Kabak, Onder

Karayiğit University of Ondokuz Mayıs, Faculty of Veterinary Medicine, Samsun/Turkey

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Introduction: Foot-and-mouth disease (FMD) is characterized by the mortality in young animals, in particular lambs and piglets, may be due to acute myocarditis. Heat shock proteins (HSPs) are a family of highly conserved, protective proteins expressed in all cells and their well-established roles in cell survival (necrosis-apoptosis) as well as chaperone functions. Moreover, immunoregulatory functions of certain in activation of innate immune system of HSP have been described. There were limited studies on the pathogenetic mechanisms of myocarditis associated with foot-and-mouth disease virus type O (FMDV) in lambs. The role of HSPs in pathogenesis of myocarditis in FMD has not been studied. The aim of the present study was to determine expressions of some HSPs in myocardium of FMDV-infected lambs.

Material and methods: Myocardial tissues were obtained at necropsy from 9 native Karayaka lambs died during an outbreak of FMD in Samsun Province in northern Turkey. The studies associated with virus isolation and identification with partial 1D gene sequence analysis and in situ reverse transcription (RT) for FMDV type O mRNA on heart sections were made. For immunohistochemistry, the sections were reacted with HSP90, HSP70, HSP60, alpha basic-crystallin (alpha-BC) and desmin antibodies.

Results: FMDV type O was detected in all heart samples tested, as determined by ELISA, sequencing and in situ RT. HSP90 immunostaining was detected in the cytoplasm of some affected cardiomyocytes in coarse granular pattern, with localization to sub-endo/epicardial areas in FMDV-infected lambs. Control groups revealed weak and diffuse labeling for HSP90 in cardiomyocytes and other cellular components. HSP70 immunostaining were seen especially in subendocardial cardiomyocytes damaged in hearts. Immunolabeling was diffuse and intracellular in myocardial components along the plasma membrane and perinuclear in the cardiomyocytes in coarse granular pattern. Many interstitial spindle cells had dense immunolabeling for HSP70 whereas immunolabeling was weak in the areas of inflammatory cells. HSP60 expression in infected hearts was abundant than control lambs and they had an appearance as granules or particles dispersed in cytoplasm of the cells along diffuse staining. The interstitial spindle cells and inflammatory cells had nuclear and cytoplasmic immunostaining for HSP60. In severe cases, the presence of extracellular and coarse granular accumulations among/on cardiomyocytes and other cellular components was determined. Alpha-BC was present in cardiomyocytes with diffusely and/or linearly in desmin co-localized Z band area. The strongest immunostaining for alpha-BC among the myocardial tissues was encountered in purkinje fibers in both infected and control hearts compared with other HSPs. Alpha-BC had nuclear immunostaining in some sections.

Discussion: The present study showed that HSPs may have a role in pathogenesis of myocarditis associated with FMDV in lambs. The mechanism by which HSPs protect cardiomyocytes against apoptosis and role by which HSPs have in immune response to FMDV remains to be fully elucidated. Further studies could be included expressions of other adaptor proteins involved in apoptotic pathway, and interactions of apoptosis adaptor proteins and HSPs with cytoskeleton molecules of cardiomyocytes in pathogenesis of FMDV-related myocarditis in young animals as well as epithelial damage in older animals.

Clinical, histological and immunohistochemical analysis of feline squamous cell carcinoma in situ

Guscetti, Franco, Marianne Heimann, Monika Welle, Dale L. Godson, Claude Favrot

Institute of Veterinary Pathology, Zürich, Switzerland

Introduction: Actinic keratosis (AK) and Bowenoid in situ carcinoma (BISC) are two distinct forms of in situ squamous cell carcinoma in felines. They usually occur on different locations and present with specific clinical and histological features. However, in some cases, these diseases cannot be distinguished either clinically or histopathologically. The aim of the present study was to determine the accuracy of diagnosis based on clinical and / or histological criteria alone, and whether immunohistochemistry for papillomavirus or p53 can improve the accuracy of diagnosis.

Material and methods: A series of in situ squamous cell carcinoma cases (n=45) were selected according to their location, classified as AK (n=22) or BISC (n=23) according to clinical criteria and histological sections were reevaluated independently by 2 dermatopathologists. The clinical and histopathological diagnoses were compared in each case. In addition, immunohistochemistry for p53 and papillomavirus antigen was performed.

Results: All BISC cases and most of the AK cases (n=15) were confirmed histologically. In 7 cases clinically classified as AK this diagnosis was not unanimously confirmed histologically due to the presence of overlapping features. P53 immunoreactivity was observed in 11/14 (79%) confirmed AK cases and in 4/22 (18%) BISC cases, while papillomavirus antigen was not detected in any confirmed AK case but was detected in 11/23 (48%) BISC cases.

Discussion: It was concluded that BISC can usually be reliably diagnosed histologically. The histological diagnosis of lesions clinically suggestive of AK might sometimes be difficult. Results of immunohistochemistry for p53 and papillomavirus antigen were supportive for a role of sun exposure and papillomavirus in the pathogenesis of AK and BISC, respectively. In addition, immunohistochemistry for these antigens may be helpful in unclear cases.

Metastatic salivary gland adenocarcinoma in a dog

Guvenc, Tolga, Ahmet Özak, Mustafa Yavuz Gulbahar, Murat Yarim, Yonca Betil Kabak, Onder Karayigit

Ondokuz Mayıs University, Faculty of Veterinary Medicine Department of Pathology, Samsun, Turkey

P27

Introduction: Salivary gland adenocarcinoma is uncommon in dogs. Tumors may be arised both major and minor salivary glands. Although, several types of salivary gland tumors have been reported, adenocarcinoma is the most common malignant tumors in dogs. There is no breed or sex predilection has been reported.

Material and methods: A 12-year-old female terrier dog was presented to the University of Ondokuz Mayıs, Faculty of Veterinary Medicine, Department of Surgery because of dyspnoea. On physical examination, cyanosis of mucosa and nonpainful mass at the cervical region was observed. Radiographic examination revealed a lot of radiodense area at the lungs. Six month later, because of the poor prognosis the dog was euthanized upon the owners' request and necropsied. Samples of tissues were collected, fixed in 10% buffered formalin, and embedded in paraffin wax. The sections were stained with H&E, periodic acid-Schiff (PAS), Phosphotungstic Acid Hematoxylin (PTAH). In addition, immunohistochemical staining for desmin, S-100, cytokeratin, vimentin, alpha-smooth-actin was performed using avidin-biotin-peroxidase complex procedure.

Results: At necropsy, on the radix linguade a whitish gray mass (5x2.5x2.5) was observed. A whitish-gray 1 cm in diameter nodule on the spleen and several nodules 0.5-1 cm in diameter on the lung parenchyma and surface were seen. Left ventricule of the heart was hypertrophic. Microscopically, tumor tissue was surrounded with thinly connective tissue and lobulated with fibrous septa. Tumor cells were round in shape with marked pleomorphism. The cytoplasm was large, lightly eosinophilic and granulated. Necrosis or marked mitotic figures were not observed. Tumor cells were not stained with PAS and PTAH. Immunohistochemically tumor cells were not labeled with S-100, desmin, alpha-smooth-actin and vimentin, but positive with cytokeratin. Metastatic tumor cells at the lung and spleen were similar to the cells of main mass. The gross, histopathologic and immunohistochemical findings were consistent with diagnosis of sublingual salivary gland adenocarcinoma with multi organ metastases.

Discussion: Primary tumors of major or minor salivary glands are infrequent in animals and most of the malign neoplasms are adenocarcinoma. In our case, tumor cells histochemically were not stained with PTAH. This finding was thought to tumor was not an oncocytoma, but further electronmicroscopic investigation should be needed. On the other hand S-100, desmin, vimentin and alpha-smooth-actin was negative, but cytokeratin was found positive. This immonohistochemical characterization was strongly supported that this case was an adenocarcinoma. It has been reported that mean survival time of dogs affected by salivary gland tumors is about 550 days. However, our case was euthanized six month later because of poor prognosis and radiographically widespread metastasis to many organs.

Response of pet iguanas liver to injury

Halouzka, Roman, Vladimír Jekl, Zdeněk Knotek

Veterinary and Pharmaceutical University, Brno, Czech Republic

Introduction: Response of the mammalian liver to injury depends on the nature of the causal factor and its duration. The outcome hepatic lesions are characterized by the different ratio of the regeneration capacity, fibrosis and biliary hyperplasia. The aim of the work is to present the interesting uniformity of the pet iguana liver as the general response to injury.

Material and methods: Dead or euthanized pet iguanas with the average age of 5,5 years (1,5 – 9 years) with unspecific clinical signs, depression, loss of appetite, some of them with icterus were necropsied and sampled for histopathology. The tissue blocks were routinely processed by parafin method, stained by HE, VG, Gömori, PAS and alcian blue.

Results: All animals included in our set appeared with hepatomegaly, fine granular surface of the liver with grey-pink or grey-green discoloration, evident pigmentation and hard consistency. Histopathological finding corresponds to the tubular gland of unregular structures. Some glands were of cystadenomatous character. Hepatocytes were vacuolized, necrotic and atrophic. Some residual tubular glands were formed only by basement membrane. Diffuse intertubular fibrosis and black pigmentation were conspicuous. Small nests of hepatocytes were only sporadically found.

Discussion: Presented uniform hepatic lesions of pet iguanas are the most frequent lesions (approx. 40 %) observed in the livers of necropsied iguanas, followed by diffuse hepatic lipidosis (approx. 25 %), bacterial and mycotic granulomas (approx. 15 %) and others (gout, neoplasms). The observed lesions seemingly reminded the biliary hyperplasia of mammalian liver. Recently is known that the biliary hyperplasia in mammals is due to pluripotent (oval) cells proliferation in biliary capillaries. Considering pathogenesis of bile duct hyperplasia as the component of hepatic response to injury in mammals and the uniformity of the observed lesions in iguana livers, we regard it as the general response of the liver to injury not dissimilar to abortive liver regeneration in mammals. Pet iguanas are very sensible to the diet disbalance and the metabolic disorders, that might be a cause of the observed lesions. It is interesting that the lesions of iguana liver are identical with the analogous lesions observed in parrots.

A PCR-based assay for the assessment of clonality in feline B-cell lymphomas with characterization of a new family of heavy chain variable region genes

Henrich, Manfred, Werner Hecht, Alexander Weiss, Manfred Reinacher

Institut fuer Veterinaer-Pathologie, Giessen, Germany

Introduction: The differentiation of neoplastic lymphatic proliferation from benign hyperplasia can be challenging in some cases. This problem, which is also well known in human medicine is especially important in species with high incidence of lymphatic tumors like the cat. Clonality is a key feature of malignant tumors and can be a useful tool for the diagnosis of these. Analysis of clonality in cases of lymphatic neoplasia can be achieved by demonstrating identical rearrangement of the antigen receptor genes of the neoplastic lymphocytes by use of the polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE).

Material and methods: To develop a PCR-based assay for the assessment of clonality in feline B-cell lymphomas, we analyzed cDNA transcripts of B-cell mRNA as well as the NCBI Trace Archive of the cat. Ten cases of B-cell lymphomas and ten cases of feline hyperplastic lymphatic tissue were examined.

Results: Analysis of cDNA transcripts created by use of the SMART™ RACE technique preferentially revealed the 3' parts of the heavy chain variable region genes. Analysis of the NCBI Trace archive by comparing the sequences to human sequences revealed 11 traces significantly similar to the human heavy chain variable region gene family 1 (*IGHV-1*) as well as 137 traces significantly similar to the human heavy chain variable region gene family 3 (*IGHV-3*). By determining the family-specific leader region of the immunoglobulin within these sequences two family-specific forward primers were designed based on these sequences. These primers in combination with a reverse primer against the constant region were able to amplify the genes of the heavy chain variable part of both families from cDNA transcripts of B-cell mRNA by use of reverse transcriptase (RT)-PCR. According to the human counterpart, we denominate these feline families *IGHV-1* and *IGHV-3*. Within the amplicons we identified the conserved parts (framework regions) of the heavy chain variable genes. A diagnostic assay with family-specific primers designed to bind these parts was able to detect monoclonality in seven of ten cases of B-cell lymphomas. In two of the seven samples the exclusive rearrangement of the *IGHV-1* genes was demonstrable. Another sample showed rearrangement of both alleles, with *IGHV-1* rearrangement on one allele and *IGHV-3* on the other. The remaining three samples showed either mono- or biallelic rearrangement of *IGHV-3*. In ten samples of hyperplastic feline lymphatic tissue only polyclonal populations could be observed.

Discussion: The demonstration of clonality in feline B-cell lymphomas by use of PCR has been performed before; however, these authors described only cDNA transcripts which were homologous to the *IGHV-3* transcripts found in our study. To our knowledge the feline *IGHV-1* family of genes has not been described before. By use of primers directed against this family (*IGHV-1*) we were able to detect monoclonality in three of ten cases examined, demonstrating that rearrangement of these genes in lymphomas occurs as well. Therefore, the detection of this new family of heavy chain variable genes is an important finding, which extends a very helpful diagnostic tool for the support of a diagnosis of feline lymphomas.

Detection of porcine circovirus type 2 and viral replication in primary lymphoid organs from postweaning multisystemic wasting syndrome affected pigs

Hansen, Mette S., Ole L. Nielsen, *Llorenç G. Roma, *Joaquim Segalés

Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

*Centre de Recerca en Sanitat Animal, Universitat Autònoma de Barcelona, Bellaterra, Spain

Introduction: Porcine circovirus type 2 (PCV2), a circular single-stranded (ss) DNA virus, is recognized as the causative agent of postweaning multisystemic wasting syndrome (PMWS). In the last decades several circular ss DNA viruses have been identified in birds and mammals, including man. The viruses belong to the Circoviridae or Anellovirus and share various molecular biological features; e.g. an intermediate, circular double-stranded DNA form (RF) is generated during the replication process. Furthermore these viral infections have many epidemiological, clinical and histopathological parallels, and most of these viruses infect and/or replicate specifically in primary lymphoid organs. The present study was conducted to assess the role of the primary lymphoid organs in PMWS affected pigs. Thymus and bone marrow samples were examined by histopathology and *in situ* hybridization (ISH) for the presence of PCV2 nucleic acids and the specific presence of PCV2 RF.

Material and methods: Formalin fixed samples of thymus and bone marrow from 33 pigs with PMWS and 29 clinically healthy age-matched control animals were included. Thymus was not available from 13 animals with PMWS and in one control animal. Different histochemical stainings were carried out for histopathological characterisation. For ISH two complementary digoxigenin labelled oligonucleotide probes (CP and RFP) were used. The CP probe was complementary to ORF1 (encoding for the replicase proteins) of the viral genome and hybridized with viral ss DNA, the RF form and mRNA, and thus detected PCV2 irrespective of replicative status. The RFP probe was identical to ORF1 of the viral genome and therefore solely hybridized with the RF form, which only is present during viral replication.

Results: Increased amounts of mucopolysaccharides were identified by histochemistry in 26/33 (79%) of the bone marrows from cases and in 7/29 (24%) of the controls. No PCV2 was detected by CP ISH in thymus or bone marrow of the control animals. The CP ISH was positive in 26/33 (79%) of the PMWS affected pigs: 19/20 (95%) thymuses and 16/33 (48%) bone marrows were positive (9/20 (45%) pigs had positive results in both thymus and bone marrow). Of the PCV2 positive animals 8/26 (31%) also showed low grade of replication (4 were positive only in thymus, one only positive in bone marrow (animal with non-available thymus) and 3 were positive in both thymus and bone marrow). The primary cell types positive for PCV2 (CP and RFP probes) were identified as macrophages by morphological criteria.

Discussion: The finding of mucopolysaccharides in the bone marrow of the PMWS affected pigs was regarded as low grade of serous fat atrophy. The inability to find thymus in 39% of the PMWS affected animals was due to the pronounced thymus atrophy. In general the finding of PCV2 in the primary lymphoid tissues was correlated with high amounts of PCV2 in other lymphoid tissues (data not shown). Replication was only detected when the general virus load was high, which corresponds with previous studies. In conclusion the present study did not find evidence for the primary lymphoid organs being apparently important in regard to PCV2 replication in advanced cases of PMWS.

Histopathological evaluation of a dose-responsive tissue reaction associated with wow-formulated vaccine in chicken

Herak-Perković, Vlasta¹, Marina Tišljarić², Suzana Tkalčić³, Neda Ergotić¹

¹VETERINA Inc. Product Development, 10436 Rakov Potok, Croatia

²Poultry Center, Croatian Veterinary Institute, 10000 Zagreb, Croatia

³College of Veterinary Medicine, Clinical Pathology, Pomona, CA, USA

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Introduction: To date, worldwide, most killed vaccines used in poultry are formulated as water-in-oil emulsions and contain oil-based adjuvants. This study investigates the tissue responses of a novel double-phase vaccinal emulsion formulated as water-in-oil-in-water (WOW).

Material and methods: A total of 120 three-week-old White Leghorn chickens, hatched from specific pathogen-free (SPF) eggs, were randomly assigned to four groups of 30 birds and monitored for 4 weeks. The chickens were vaccinated intramuscularly with 0.2, 0.5 and 1.0 ml of oil-based vaccine formulated as WOW emulsion (Pestikal[®], VETERINA Inc., Croatia) containing inactivated Newcastle disease virus. The control group was injected with 0.5 ml of sterile saline. Injection site tissue reaction was evaluated at 2 and 7-day intervals post inoculation. The severity of histopathological lesions was evaluated using a 1 - 4 scoring scale.

Results and discussion: Histopathological lesions in the pectoral muscles were correlated with the dose of injection. Predominant findings, regardless of the inoculated dose, were composed of macrophages, epitheloid cells and small interstitial cysts surrounded by the proliferating fibroblasts, lymphocytes and plasma cells. Significant dose-dependent findings included degenerative and necrotizing myositis, as well as occasional vacuolar and cyst formation. Edema formation was dose-dependent but inconsistent. Lesions, characterized as oleogranuloma, were present in high-dose WOW-inoculated chickens and persisted throughout the 4 weeks of study. The manufacturer-recommended dose, based on the immunological response, is 0.5 ml. Our findings support a dose of 0.5 ml of the WOW-formulated vaccinal emulsion as optimal in stimulating a local cellular reaction needed for further humoral response.

Expression of inducible nitric oxide synthase (iNOS), nitrotyrosine (NT) and manganese superoxide dismutase (Mn-SOD) in lungs of calves experimentally infected with *Mycoplasma bovis*

Hermeyer, Kathrin¹, Björn Jacobsen¹, Joachim Spergser², Renate Rosengarten², Marion Hewicker-Trautwein¹

¹ Department of Pathology, University of Veterinary Medicine, Hannover, Germany

² Department of Bacteriology, Mycology and Animal Hygiene, University of Veterinary Sciences, Vienna, Austria

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Introduction: *Mycoplasma (M.) bovis* is known to cause different pneumonic alterations in calves. The pathogenetic mechanisms involved in the development of these lung lesions are still largely unknown. Inducible nitric oxide synthase (iNOS) is expressed by macrophages upon activation by bacterial constituents and/or cytokines *in vitro*. Nitric oxide (NO) produced by iNOS-expressing macrophages is generally known to contribute to tissue damage. The damaging effects of NO are mediated by peroxynitrite formed from NO and superoxide and can be demonstrated by the detection of nitrotyrosine (NT). The antioxidative enzyme manganese-superoxide-dismutase (Mn-SOD) prevents cells against the influence of oxidative stress. The purpose of this study was to investigate the expression of iNOS, NT and Mn-SOD in different types of lung lesions in calves with experimental *M. bovis* infection and to characterise the macrophage populations infiltrating the lung tissue.

Materials and methods: Formalin-fixed, paraffin-embedded lung sections from 20 control calves and 30 experimentally infected calves were examined for histopathological lesions. Expression of iNOS, NT, and Mn-SOD was examined immunohistochemically. For detection of macrophages, different phagocyte markers (CD68, S100A8, S100A9) were applied.

Results: Histopathology revealed the presence of different types of pneumonic lesions, i.e. interstitial pneumonia, suppurative bronchopneumonia and/or necrotising pneumonia. In 8 calves examined at 21 days p.i., obliterative bronchiolitis was found. All infected calves showed increased numbers of iNOS-, NT- and Mn-SOD-expressing cells in the inflamed lung tissue. Strong expression of these markers was particularly noticed in macrophages demarcating necrotic lesions, in altered bronchial epithelial cells and in macrophages infiltrating obliterated bronchioli. There was a positive correlation between the severity of inflammatory lung lesions and the number of infiltrating macrophages.

Discussion: The expression pattern of iNOS, NT and Mn-SOD found in *M. bovis* infected lungs with different histopathological lesions strongly suggests that these products, by generating reactive oxygen and nitrogen species, are especially involved in the development of severe chronic lung tissue damage, i.e. necrotic lesions and obliterative bronchiolitis. The latter type of lesion was not reported previously in lungs of *M. bovis* infected calves. Furthermore, the study revealed that, beside neutrophilic granulocytes, macrophages strongly expressing iNOS-, NT- and Mn-SOD are the dominating inflammatory cell type in *M. bovis* infected lungs.

Myointimal cell formation in canine pulmonary arteriosclerosis

Hetzel, Udo, Simon Swift, Sonja Fonfara, Julie Wayne

University of Liverpool, Faculty of Veterinary Science, Department of Veterinary Pathology, Liverpool, UK

P12

Introduction: Compared to human medicine, arteriosclerosis is a less common finding in domestic mammalian animals. A case of a four year old female neutered Yorkshire Terrier with cardiomegaly, membranoproliferative glomerulonephritis and secondary pulmonary and hepatic alterations is presented with focus of pulmonary arterial alterations.

Results: Smaller pulmonary arteries of all pulmonary lobes showed moderate hyperplasia of the Tunica media and endothelial hyperplasia, partly resulting in 90% occlusion of the arterial lumen. Multifocally, moderate amounts of subendothelial, Alcian blue positive, basophilic material (proteoglycans) are observed. These are interpreted as myointimal cell / musculo-elastic layer formation, seen in arteriosclerosis. The positive immune histological reaction for Factor VIII related antigen and myoglobin confirms the diagnosis of myointimal cell / musculo-elastic layer formation within these arterial vessels.

Discussion: This case of a Yorkshire Terrier, showed cardiomegaly, with hydropericardium, fibroplastic epi- and pericarditis, mild diffuse lympho-plasmacytic myocarditis and subepicardial haemorrhages due to right atrio-ventricular valvular verrucous endocarditis with mild chronic lympho-plasmacytic valvular endocarditis. As secondary to the cardiomegaly and cardiac failure, pulmonary arteriosclerosis, interstitial pulmonary fibrosis, chronic fibrosing hepatopathy and membranoproliferative glomerulonephritis was found. Debatable is, whether the cardiac lesions are primary or secondary to chronic renal alterations.

Histology of inflammatory bowel diseases (IBD) in Interleukin-10 deficient and TNFdARE mutant mice

Hözlwimmer, Gabriele, Irene Esposito, Dirk Haller, Leticia Quintanilla-Martinez

Institute of Pathology Helmholtz Center Munich, Institute of Pathology
Helmholtz Center Munich

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Introduction: Human Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD) which cause significant morbidity. IBD is the result of inappropriate activation of the mucosal immune system driven by the presence of normal luminal flora, but the etiology remains still unknown. A variety of mouse models have been developed to investigate disease pathogenesis and novel treatment modalities. Il-10 deficient mice develop chronic enterocolitis similar to human UC, and TNFdARE heterozygous mutant mice a Crohn's-like IBD. Using mouse models requires a high similarity to the corresponding human diseases. Therefore, it was our goal to evaluate the validity of these mouse models when comparing the histological features in human and in mice.

Material and methods: The histological examination of four intestinal segments (proximal and distal ileum, caecum, and distal colon) was performed in 130 TNFdARE mutants and 80 Il-10-deficient mice at different ages. The tissue was embedded in paraffin, sectioned at 4 µm and stained with H&E. Additional histochemistry (PAS, Masson Trichrome, Alcian blue) and immunohistochemistry (CD3, B220, CD79a, panCK, Ki67, and p53) was performed in some cases.

Results: Almost all TNFdARE mutants developed (distal) ileitis and colitis. Inflammatory cell infiltration (predominantly CD3 positive T-cells) started sub-/mucosal and showed a progression to transmural inflammation. Associated epithelial damage included villus blunting, crypt loss and abscesses. Additionally, cryptal hyperproliferation, goblet cell loss, and minimal fibrosis were observed. Ulcer or fissures were rarely, and granulomas were not found. Il-10 homozygous knockout mice were affected by colitis and typhlitis of variable severity. The mixed mucosal inflammatory cell infiltrates often extended into the submucosa, but rarely through the muscularis. Mostly, prominent cryptal abscesses were present - sometimes accompanied by a marked stromal reaction. Mild epithelial damage was limited to mucin depletion, and small epithelial erosions. Advanced cases showed ulcer of variable size. Characteristically, diffuse epithelial hyperplasia was present, and sometimes, focal areas of dysplasia.

Discussion: Many characteristics of human CD could be found in the TNFdARE mutant mice: Typical "skip" lesions in the (distal) ileum and colon with a dominance of T-cell immune response, and with common transmural extension. In contrast to human CD, fibrosis was minimal. The lack of non-caseating granulomas and the low frequency of fissuring and ulceration represent significant differences between human CD and murine CD-like IBD. Moreover, we observed a high similarity of the IBD in Il-10 knockout mice with human UC, which is characterized by predominantly (sub-) mucosal colitis and typhlitis, with marked mucosal atrophy and regeneration, many crypt abscesses, and the development of colorectal carcinoma in 5 to 10%. The development of dysplastic foci in the Il-10 deficient mice may additionally provide the possibility to study the IBD-associated carcinogenesis. In conclusion, both murine IBD models provide a model for studying the patho-mechanism of IBD and for the development of new therapeutic strategies.

Atrophic dermatosis in a coatimundi (*Nasua nasua*)

Huisinga, Maike, Christiane Herden, Manfred Reinacher

Institut für Veterinär-Pathologie, Giessen, Germany

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We report a case of atrophic dermatosis in an eight year old male captive coatimundi. Macroscopically, the animal showed bilateral symmetrical alopecia of the trunk, alopecic areas of the tail and an erythematous and crusting dermatitis in the face and flank region. Histopathologic examination from skin of lateral trunk, back, tail and abdomen revealed moderate basket wave orthokeratotic hyperkeratosis and dermal fibrosis. There were only telogenic hair follicles present which showed a marked perifollicular fibrosis, faded follicles and infundibular hyperkeratosis. Sebaceous glands were decreased in number and size (atrophy). In skin regions with erythematous and crusting dermatitis, a superficial perivascular neutrophilic infiltrate, superficial serocellular crusts and clusters of coccoid bacteria were additionally present.

In endocrine organs, a disorganization of azidophilic, basophilic and chromophobe cells in the adenohipophysis was found. Adrenal medulla exhibited marked fibrosis whereas adrenal cortex seemed to be normal. Follicles of the thyroid gland were bilaterally collapsed and contained no colloid.

Due to histopathologic characteristics of atrophic dermatosis in association with collapsed thyroid follicles and disorganized pituitary gland we hypothesize that this might present a case of hypothyroidism in a coatimundi.

Immunohistochemical examination of MHC class II expression in canine skin samples with particular consideration of keratinocytes

Huisinga, Maike, Manfred Reinacher

Institut fuer Veterinaer-Pathologie, Justus-Liebig-Universitaet, Giessen, Germany

P123

Introduction: The purpose of this study was to establish a method to improve characterization and differentiation of dermatitides in dogs. It was the aim to get new knowledge about the relation between different dermatitides and the pathogenesis of skin inflammation. Since MHC class II molecules play a crucial role in many immune responses, detection of these proteins seemed to be appropriate for this purpose.

Material and methods: Investigations were performed on 169 skin biopsies from dogs collected from the routine diagnostic material of the Institut für Veterinär-Pathologie, Justus-Liebig-Universität Giessen between 2003 and 2006.

Histopathological examination was performed on H&E-stained and PAS-stained sections. Detection of MHC class II antigens was performed with immunohistochemical techniques.

Results: The immunohistochemical assessment of MHC class II expression revealed MHC class II proteins on different cell types of infiltrating inflammatory cells, i.e. antigen-presenting cells, macrophages, T lymphocytes and B lymphocytes. The plasma cells, however, did not show expression in each case. Furthermore, in numerous samples MHC class II positive keratinocytes and endothelial cells were found. Concerning MHC class II expression by keratinocytes, either an expression by epidermal and follicular keratinocytes (mainly in interface dermatitis), or by epidermal keratinocytes only (dermatitides with epidermal involvement), or only by follicular keratinocytes (folliculitis) occurred. Especially biopsies with mural folliculitis due to infection with Demodex mites or dermatophytes showed MHC class II positive follicular keratinocytes. MHC class II positive endothelial cells were found in numerous biopsies with inflammation in which different vascular plexuses as well as arteries and veins, either with activated or with non activated endothelial cells, were positive.

Discussion: MHC class II positive keratinocytes were observed particularly in biopsies with lymphocytic inflammation. These inflammations belonged mainly to the groups of interface dermatitis and mural interface folliculitis. Since it is known that MHC class II expression increases due to the influence of interferon γ (IFN γ), it is hypothesized that lymphocytes which infiltrate epidermis or hair follicles secrete IFN γ . This in turn may induce MHC class II expression by keratinocytes. We hypothesize that this is in association with the development and the maintenance of such inflammations.

Histological examination of the rat mammary gland: impact of tissue sampling and estrous cycle

Hvid, Henning^{1,2}, Inger Thorup², Martin B. Oleksiewicz² and Henrik E. Jensen¹

¹Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Denmark and ²Novo Nordisk A/S, Denmark

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Introduction: The guidelines from Registry of Industrial Toxicology Animal-data (RITA) for histopathological examination of tissues prescribe transverse sections of the mammary glands. However, occasionally very limited amounts of mammary gland tissue are found in transverse sections. In some toxico-pathological investigations, evaluation of proliferation of the mammary gland is valuable. The aim of the present study was 1) to compare standard transverse sections of the rat mammary gland with sections cut parallel to the skin surface, and 2) to evaluate the influence of the estrous cycle on the glandular proliferation.

Material and methods: The six pairs of mammary glands were collected from 24 ten weeks old female Sprague-Dawley rats. Stage of the estrous cycle was determined for each animal by histological examination of ovaries, uterus and vagina. In each animal, transverse sections were prepared from the right-sided glands, and sections parallel to the skin surface were cut from the left-sided glands. The area of mammary epithelium and connective tissue in the mammary fat pad was measured in sections stained with hematoxylin and eosin. Morphological differences between the two types of sections were examined by comparing the densities of glandular tissue and connective tissue, respectively. Additionally, immunohistochemical staining of markers for myoepithelial cells (α -smooth muscle actin) and glandular epithelial cells (cytokeratin 18) was performed. Proliferation in the mammary glands was assessed by immunohistochemical staining of proliferating cell nuclear antigen (PCNA), Ki67 and 5-bromo-2-deoxyuridine (BrdU).

Results: Sections cut parallel to the skin surface contained a significantly larger area of glandular and connective tissue compared with transverse sections. The two types of mammary gland sections revealed no difference in tissue densities or immunohistochemical staining for α -smooth muscle actin and cytokeratin 18. Proliferation of the glandular epithelial cells varied between glandular compartments and during the estrous cycle. The three markers of proliferation labelled a different proportion of the epithelial lining cells in the glands. However, the proportions of PCNA-, Ki67- and BrdU-positive cells were mutually correlated and independent of sectioning direction.

Discussion: Sections of the rat mammary gland cut parallel to the skin surface provided a larger area of glandular and connective tissue compared to transverse sections cut according to RITA-guidelines. The tissue morphology was not affected of the sampling technique. The pattern of proliferation varied with respect to glandular compartments as well as the estrous cycle. These findings are relevant to optimal design of toxico-pathological studies where mammary proliferation is to be examined.

General overview in ocular lesions of dog

Ionascu, Iuliana, Manuella Militaru, Emilia Ciobotaru, Georgeta Dinescu, Teodoru Soare

Faculty of Veterinary Medicine, Bucharest, Romania

P78

Introduction: Great frequency of ocular lesions in dog and cat in the last decade, generate the necessity of development of veterinary ophthalmology. Clinical diagnosis is sometimes an easy job, but there are many situations when therapy failed because of lack of an etiological diagnosis. In this context, pathological investigation, correlated with other types of investigation represents a necessary step in diagnosis and therapy of ophthalmological problems.

Material and methods: This study included 48 dogs, with different age and breed, all diagnosed with lesions of eye-ball and ocular adnexa. Investigation considered clinical ophthalmological exam and pathological investigation (surgical removals and fine needle aspiration). Smears were May Grunwald Giemsa stained and surgical removals underwent routine histological technique (10% formaldehyde solution with 5% picric acid, paraffin embedding and Masson trichromic staining).

Results: The diagnosed lesions were as follows: corneal dermoid (4%), chronic keratitis and keratoconjunctivitis (15%), ocular trauma (2%), chalazion (6%), Meibomian adenoma (8%), adenocarcinomas with different locations (15%), melanoma/melanocytoma (17%), parasitic granulomas (8%), non-tumoral proliferative lesions (4%), palpebral fibroma (6%) and mesenchymal malignant tumors (15%).

Discussion: These results proved a high incidence of tumoral lesions, those being represented by 60% of all cases. Benign and malignant melanocyte tumors represented 28% from all ocular tumors. Melanocyte tumors of ciliary body were the most frequently encountered. Previous studies considered that the majority of melanocyte tumors of the eye-ball have a benign behavior and enucleation is curative. Grossly, our cases did not present infiltrative aspect of the tumors, all of them being confined in eye-ball. Histologically, two cases exhibited vascular invasion, the risk of metastasis being obvious. Parasitic granulomas were quite frequently encountered in dog and created problems of clinical diagnosis. Cytological investigation from conjunctival smears was not enough. Surgical removal of granulomas and further pathological investigation presented different stages of larvae. Cytological and histological features exhibited a great resemblance with ocular parasitic granulomas diagnosed in human.

Altered myelinogenesis and glial responses in the attractin-deficient *mv* rat

Izawa, Takeshi, Shigeo Takenaka, Takao Kotani, Jyoji Yamate, Mitsuru Kuwamura

Osaka Prefecture University, Osaka, Japan

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Introduction: The myelin vacuolation (*mv*) rat is an autosomal recessive mutant characterized by hypomyelination and vacuole formation in the myelin throughout the central nervous system (CNS). Previous genetic studies have revealed a null mutation in the attractin gene of the *mv* rat. It has been known that mutation at the attractin locus results in myelin alterations, but the detailed pathogenesis is still unclear.

Material and methods: First we examined glial changes in the spinal cord of *mv* rats at 2, 4, 6, 8 weeks of age by immunohistochemistry for 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase; oligodendrocyte marker), glial fibrillary acidic protein (astrocyte marker) and CD11b (microglial marker). Next we identified attractin-expressing cells in the rat spinal cord by in situ hybridization combined with immunohistochemistry. To study the biosynthesis of myelin proteins in the spinal cord of *mv* rats, we examined immunoreactivity for proteolipid protein (PLP) and myelin basic protein (MBP), and measured mRNA and protein expression levels of PLP, MBP and CNPase using real-time polymerase chain reaction and Western blot analysis, respectively.

Results: No abnormality was found in the number and morphology of oligodendrocytes in *mv* rats at any age examined. Coincident with the myelin abnormalities, there was progressive astrogliosis from 2 weeks. Marked microglial activation was observed exclusively in the gray matter of *mv* rats from 6 weeks, coincident with severe myelin disruption. A double-labeling study demonstrated that attractin-expressing cells were mostly oligodendrocytes in the white matter of the spinal cord of 2-week-old wild-type rats, whereas no attractin-positive cells were detected in *mv* rats. Expression levels of PLP mRNA and protein were severely reduced in *mv* rats from an early stage of hypomyelination.

Discussion: This study indicates that the attractin defect results in oligodendrocyte dysfunction, and is associated with astrogliosis and microglial activation in *mv* rats. The data suggest that attractin may be directly involved in the function of oligodendrocytes in CNS myelination.

Histological Findings Findings In the Adrenal Glands of Slaughtered Cattle

Jelinek, Frantisek, Roman Konecny

Department of Anatomy and Physiology, Faculty of Agriculture. University of South Bohemia in Ceske Budejovice, Ceske Budejovice, Czech Republic

P101

Introduction: It is well known that the adrenal glands are in close relation to metabolism of minerals, carbohydrates, proteins and lipids and by this way they are closely related to farm animals productivity. It is generally accepted, apart of other, that zona fasciculata consists of radially arranged straight cords of cuboidal or columnar cells, the cells situated at periphery of the medulla produce adrenalin and therefore they are called A cells, the cells in central part of medulla secret noradrenalin (norepinephrin) and they are denoted N. In frame of health state analysis of cattle, histological examination of adrenals was done.

Material and methods: Thirteen bulls, 10 heifers and 10 cows. All the animals were clinically healthy food animals, slaughtered in routine manner in an abattoir in Czech Republic. Both adrenals from each individua were collected for histological examination. Each gland was transversaly dissected at three or four levels, the samples were fixed in 10% buffered formalin, processed by the common paraffin technique and histological sections were stained with haematoxylin and eosine. In the samples of ten animals S100 protein, chromogranin and synaptophysin have been proved by means of common immunoperoxidase method on paraffin wax sections .

Results: Findings of histological examination are summarized in table.

Finding	Bulls 13	Heifers 10
Eosinophilic granules in ZG	12	9
Nodular arrangement of ZF	13	4
Interlacing bands of A and N cells	13	4
Proliferation of the cortex into the medulla	13	8
Proliferation of cells into the medullary vessel wall	13	6
Proliferation of the cells into the vessel lumen	4	0

Moderate to strong S100 protein positivity was proved in cortical cells and in the interstitial cells located in the superficial zone of the medulla. A cells in the medulla gave only weak cytoplasmic positivity or were S 100 protein negative, N cells were weakly or moderately positive. Reaction for chromogranin was mild in the A cells, moderate in the N cells and in majority of the cortical cells. Strong cytoplasmic positivity of synaptophysin was present both in the A and N cells and mild or negative in the cortex. Immunohistochemical examination contributed considerably to identification of cortical cells in the medulla.

Discussion: In our opinion the nodular arrangement of zona fasciculata, proliferation of the cortex into the medulla, mutually interlacing bands of A and N medullary cells, proliferation of medullary or cortical cells into the vessel wall or in lumen of the vessels, could reflect very intensive metabolic activity or even metabolism on the physiological limits.

Gastrointestinal stromal tumour in a guinea pig

Jelinek, Frantisek, Pavel Hron, Frantiska Hozmanova

Veterinary Histopathological Laboratory, Prague 9, Czech Republic

P29

Introduction: Interstitial cells of Cajal (ICC) located in the intestinal wall possess of pacemaker function. Tumour arising from ICC is named gastrointestinal stromal tumour (GIST), and it is considered as a low-grade sarcoma harboring mutation of c-kit. In the available literature GIST has been described in two Rhesus macaques in horses, and in dogs. To the best of our knowledge tumour of ICC histogenesis has not been described in a guinea pig until now. For this reason we publish our observation.

Material and methods: Sample of the tumour was fixed in 10% buffered formalin and processed by the common paraffin wax method and histological sections were stained with haematoxylin and eosine. Immunohistochemical examination was performed by means of common immunoperoxidase method on paraffin wax sections.

Results: Animal: Guinea pig, male, 3.5 year of age, tricolor, kept as a pet animal in a household. Approximately for five weeks before the death wasting, depression of motion activity, soft and malodorous faeces were observed by the owner. By means of palpation a globoid formation of walnut size in the abdominal cavity was detected. Some days later the animal spontaneously died.

Gross pathology: A tumor of grey-pink colour located in terminal segment of the ileum was observed. Inside of the neoplastic formation there was a cavity communicating directly with lumen of the bowel and containing a intestinal content. No other pathological changes were macroscopically obvious.

Histopathology: Solid tumour of mesenchymal appearance. One part of the sample consisted of tightly packed spindle cells arranged in interlacing fascicles. In the other part predominated epitheloid cells with slightly myxoid intercellular matrix. Marked anisokaryosis and numerous mitotic figures were in the neoplastic cells. Cytoplasm was lightly basophilic, reticular or finely vacuolated with indistinct cellular boundaries. Immunohistochemically positivity to actin, CD 117 (c-kit), GFAP, NSE and p53 protein was proved. Reaction for desmin, synaptophysin and S100 protein were negative.

Discussion: In humans GIST most commonly arises in the stomach while in domestic and farm animals it is most frequently located in the intestine. GISTs are usually comprised of spindle cells arranged as interlacing fascicles or of epitheloid tumor cells arranged in trabecule or solid sheets with a somewhat myxoid intercellular. In our case both types of cells were present. On the basis of histological and immunohistochemical examinations we diagnosed GIST in the terminal ileum in one guinea pig. Though morphological properties, marked mitotic activity and p53 positivity were indicators for malignancy, no metastases or generalization were recorded.

Glia cytoarchitecture in the brain and spinal cord of Bearded Dragon (*Pogona vitticeps*)

Jiménez, CR, R Zafra , MJ Ruiz, E Mozos

University of Córdoba , Department of Comparative Pathology. Faculty of Veterinary Medicine, Ed. Sanidad Animal. Campus de Rabanales.Ctra. Madrid-Cadiz km 396. Córdoba, Spain

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Introduction: Glia cells are strategically distributed throughout the Central Nervous System (CNS) and provide valuable information in both phylogenetic comparative studies and animal models. The aim of this work is to analyze the distribution of four intermediate filament molecular markers in the brain and spinal cord of bearded dragon (*Pogona vitticeps*).

Material and methods: Three complete brains and cervical spinal cords were formalin fixed and paraffin embedded. Serial samples (4-5 µm thick) were consecutively for glial fibrillary acidic protein (GFAP), S100 protein, vimentin and neurofilaments antibodies (source: Dako® and Eurodiagnostica®). The immunohistochemical study was carried out using the Avidin-Biotin complex (ABC) method.

Results: GFAP reacted with numerous fibers of periependimal radial glia throughout the CNS, mainly in the telencephalon, mesencephalon, and optic tract. Star-shaped astrocytes were found scarcely in the diencephalon and mesencephalon. In the spinal cord, GFAP reacted with glia in the white matter, as well as, star-shaped astrocytes in the grey matter. S100 protein strongly reacted with fibers of different length and thickness as well as, cell bodies throughout the CNS in both the grey and the white matters. The Purkinje cells were strongly immunoreactive, whereas the cerebellar molecular and granular layers show a variable pattern of stained fibers. In the spinal cord, immunoreactive fibers and small cell bodies were only found in the grey matter close to neurons. The neurofilament pattern was similar to that observed for GFAP, and we observed that many neuron bodies were nonreactive for this antibody. Positive glial structures were not found in the CNS with the antibody against vimentin.

Discussion: This immunohistochemical study point out the presence of different types and cytoarchitecture patterns of glial cells in the CNS of *Pogona vitticeps* and its appears similar to what is found in some lizard as *Anolis sagrei* and *Eublepharis macularis* less elaborate than in birds and mammals.

A gross and histopathological assessment of feline appendicular osteoarthritis

Johnston, Pamela, Matthew Keifer, David Bennett

University of Glasgow, Glasgow, United Kingdom

Introduction: Osteoarthritis is a poorly documented condition in the cat. Recent retrospective radiographic studies, Hardie *et al*⁽¹⁾, Clarke *et al*⁽²⁾ and Godfrey⁽³⁾ indicate that the clinical condition has been poorly recognised with an incidence ranging from 4% to 17.5%. Recent publications by Clarke and Bennett⁽⁴⁾ and Godfrey^(5,6) demonstrated that feline osteoarthritis is an important clinical entity but rather than causing overt lameness it most often results in lifestyle changes – cats no longer jump or climb. The role of this pilot study is to define the pathology and relate the pathological findings to the clinical condition.

Material and methods: A total of 20 cats submitted to the post-mortem room for routine post-mortem examination had gross and histopathological examination of all appendicular joints as well as routine examination of body tissues. Digital images of all affected and representative normal joints were collected. Histological examination of all affected joints with H&E, Safranin O and Alcian blue stains was carried out in parallel with normal joints. Additional samples were collected to evaluate specific lesions e.g. intrameniscal calcification within the stifle joint.

Results: Early gross findings suggest that osteophytes are the most common abnormality found primarily in the shoulder, also in the hock, elbow, hip and stifle. Cartilage degeneration, often with formation of distinct linear grooves was found bilaterally in the hock and elbow in a proportion of the affected cats. Histopathological assessment of the gross findings is currently underway.

Discussion: Preliminary results confirm that the pattern of osteoarthritis in the cat, both radiographically and on gross pathological examination, is significantly different to that in the dog. As our findings to date confirm previous findings *i.e.* that most cases of osteoarthritis in the cat are primary/idiopathic rather than secondary as is most often the case in the dog, the cat may be a more relevant species for future comparative studies. This presentation will cover the detailed histopathological findings in cats with osteoarthritis.

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Rhabdomyosarcoma in the urinary bladder of a dog

Jovanovic, Milijan, Zivka Eri, Aleksandar Spasojevic, Vladimir Kukolj, Milijana Knezevic

Faculty of Veterinary Medicine, Belgrade, Serbia

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Introduction: Rhabdomyosarcoma of canine urinary bladder is rare tumor that has been reported occasionally in young dog, less than 2 years old. Because often showed botryoid appearance this type of tumor is also known as botryoid sarcoma. This paper describes light microscopical and immunohistochemical findings on a canine rhabdomyosarcoma arising in the bladder of dog old 13 years.

Material and methods: A 12-year-old, male Scottish Terrier, weighting 8 kg, was presented to private veterinary hospital because of difficulty in urination. The mass approximately 5 cm by 3 cm at the dorsocaudal urinary bladder wall was detected from the radiography and ultrasonography. The mass was excised and submitted for histopathological evaluation. Tissue specimens were fixed in formalin, processed routinely, embedded in paraffin wax, sectioned 5 µm thick and stained with hematoxylin and eosin (HE). Immunohistochemistry was carried out by means of the avidin-biotin peroxidase technique with the following primary antibodies: monoclonal antibodies against human MyoD1, against Ki-67 and desmin and polyclonal antibody against cow keratin.

Results: The neoplasm elevated the mucosa and infiltrated into the smooth muscle layer of the urinary bladder. There was extreme variation in both cellular and nuclear size and shape, and cells frequently contained multiple nuclei of various size. Most nuclei had large prominent nucleolus and margination of chromatin was observed. The eosinophilic cytoplasm was scant to moderate in abundance. The mitotic rate was high, and many bizarre mitotic figures were present. Immunohistochemically, the tumor cells were negative for keratin. The nucleus of most neoplastic cells was positive for MyoD, and Ki-67 but cytoplasm of those cells was weakly positive or negative for desmin. Adjacent, the smooth muscle cells were negative for MyoD and positive for desmin.

Discussion: It has been speculated that the bladder rhabdomyosarcomas arise from stores of undifferentiated mesenchyme which has the potential for rhabdomyoblastic differentiation. Histological and immunohistochemical characteristic depends on the degree of differentiation. In our case the neoplastic cells were poorly differentiated and positive for Ki-67. The expression of MyoD, myogenic transcriptional protein, is closely related to the degree of differentiation and it is expressed early in skeletal muscle development. The absence of staining for desmin does not always rule out the possibility of a neoplasm of myogenic origin. The age and breed of our dog do not support the supposition that this neoplasm occurs most often in dogs of larger breeds under 2 years old.

External quality assurance [EQA] in veterinary histopathology technique

Kelly, Brian

Veterinary School, University of Edinburgh, Edinburgh, Scotland

P81

Veterinary pathologists will be aware of the importance of consistently well prepared histological sections as an essential element in the interpretation of changes in cells and tissues and the subsequent diagnosis of disease. Most of the histological techniques used today date from the early part of last century and are well documented in standard texts. This is both its strength and a weakness in that whilst detailed instructions are readily available, there can be an assumption that little effort is needed to produce good results.

As with all areas of activity where there is an end product, histology laboratories require control measures to be in place to maintain and improve output. Most laboratories employ internal quality control [IQC] procedures. Whilst important, they are dependant on the limits of acceptability set by the laboratories concerned. It is generally accepted that additional measures are needed to support IQC. External Quality Assurance [EQA] uses universally agreed assessment criteria to measure quality standards and is able to detect differences between like laboratories. EQA is defined as a system of retrospectively and objectively comparing results from different laboratories by means of an external agency. EQA is a good indicator of the efficacy of IQC, provides quality assurance to users, is a benchmark to laboratories as to how good they are and can provide a prompt for remedial action.

Within the field of general histopathology technique, EQA in the UK has been around since the 1970s and specifically in veterinary histopathology since 1992. The earliest form of the veterinary scheme involved only 6 UK based laboratories. This has evolved to where today there are over 36 participants worldwide.

The scheme is both selective and distributive in that archival material is assessed as well as unstained sections sent to participant laboratories to stain. Session quality is assessed using agreed criteria by trained, experienced histologists using a standardised marking sheet. Participants received a results package containing the original marking sheets, details of their marks in relation to other participants and colour images of the highest scoring slides. In addition, information on the details of the processing and staining methods obtaining the highest marks is made available.

Confidentiality is an important element of the scheme and is only broken at the request of the participating laboratory should they wish help and support with remedial action for poor performance.

A full account of how the scheme operates will be presented

More recently as immunocytochemical techniques have moved from being research tools to standard procedures used in a diagnostic setting, the requirement for their own specific EQA scheme has been recognised. Consequently an Immunocytochemistry EQA pilot scheme, operating along the same lines as the general scheme, has just been completed and results from this will be shown and discussed.

Examples of the “good, the bad and the ugly” will be shown but should be viewed in the light of an overall good performance by participants.

The role of *Bartonella henselae* in cats with immunocompromising conditions

Kershaw, Olivia, Alexandra U. Buchmann, Achim D. Gruber

Department of Veterinary Pathology, Berlin, Germany

P1

Introduction: *Bartonella henselae*, the agent of cat scratch disease, has previously been characterized in its reservoir host, the domestic cat. As fleas are the main vectors for transmission among cats, the prevalence of *B. henselae* infections is high in animal shelters and in populations of stray cats. In humans infected with *B. henselae*, the course of disease differs greatly between immunocompetent and immunocompromised patients: while the former develop the generally self-limiting cat scratch disease, the latter may develop serious conditions including septicaemia, bacillary angiomatosis and peliosis hepatis. Although peliosis hepatis is commonly found in cats, there are no studies yet linking the condition in cats to an infection with *B. henselae* or comparing the prevalence and pathogenicity of *B. henselae* infections in immunocompetent cats with that in cats suffering from immunocompromising conditions.

Material and methods: Blood samples and a wide range of tissues from 88 cats from animal shelters were examined for *B. henselae* using PCR and immunohistochemistry. In addition, liver specimens from nine cats with peliosis hepatis were retrospectively screened for the presence of *B. henselae*.

Results: 51 out of 88 cats were classified as suffering from potentially immunocompromising conditions, e.g. cachexia, panleukopenia or FeLV infection (group A). 37 cats showed no evidence of such conditions (group B). Of the immunocompromised group, five cats tested positive for *B. henselae* in at least one organ (9, 8 %), compared to three cats (8, 1 %) in the immunocompetent group. *B. henselae* was primarily detected in lymphatic tissues or bone marrow, with or without bacteraemia. The bacteria were not detected in any of the liver specimens with peliosis hepatis, neither by PCR nor by immunohistochemistry.

Discussion: In contrast to the situation in humans, this study failed to reveal significant differences between the prevalence, pathogenicity and organ lesions of *B. henselae* infections in cats with potentially immunocompromising conditions compared to cats without such conditions. However, the actual presence or degree of immunodeficiency in group A cats could not be unequivocally established. Finally, no causative link was established between *B. henselae* infection and feline peliosis hepatis, again contrary to the human disease.

Effect of growth hormone on recovery from testicular damage induced by Methotrexate in rat

Khaki,Arash¹, Marefat Ghaffari Novin ², Amir Afshin Khaki ³, Mohamad Nouri ⁴ Chelar c,ozanci⁵.

- 1- Department of Veterinary pathology, Islamic Azad University Tabriz branch-Iran
- 2-Reproductive Biotechnology Research Center, Avesina Research Institute, Tehran, Iran.
- 3- Department of Anatomical Science, Tabriz University of Medicine Science.
- 4- Department of Biochemistry Science, Tabriz University of Medicine Science.
- 5- Department of Histology, Acdeniz University, Turkey.

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Introduction: Methotrexate (MTX) is a chemotherapeutic agent that used for the treatment of a variety of tumors and inflammatory diseases. This study was conducted to evaluate the role of growth hormone (GH) on testicular function recovery induced by MTX in rat.

Methods: Fifty male Wistar rats were randomly divided into five groups (n=10 each), with one group serving as controls. In the GH group, GH was intra peritoneally (IP) administered at a daily doses of 0.3 mg/kg for 28 consecutive day. In the MTX group, MTX was IP administered at weekly doses of 1 mg/kg for 4 weeks. In the protective group, GH and MTX were IP administered together at above doses for 28 days. In the treatment group, MTX was administered at above doses for 4 weeks and GH administration was started 14 days after MTX administration (from 14 day upto 28 day). However, the control group received vehicle (IP). Five rats from each group were sacrificed at days 14 and 28. Spermatozoa were removed from cauda epididymis and analyzed for sperm motility, concentration and viability. Testis tissues were also removed and prepared for histological evaluation. In addition, serum testosterone level was determined by radioimmunoassay on day 14 and 28.

Results: This study was confirmed MTX had destructive effects on testis germinal cells. There was a significant decrease in sperm count, viability and motility in MTX group when compared with control group ($P < 0.05$). Testosterone level had significant decrease in MTX, protective and treatment groups when compare to control and GH groups in day 14 and 28 ($P < 0.05$). GH had recovery effects on testis histology and improve sperm parameters and serum testosterone level ($P < 0.05$) as compared with MTX group.

Conclusion: These results suggested that administration of GH improved testicular function damaged by MTX.

Keywords: GH, MTX, Spermatogenesis, Testis, Rat.

Ultrastructural study of naturally occurring ovine pulmonary adenocarcinoma in sheep

Khodakaram Tafti, Azizollah, Alireza Hematian

Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

P106

Introduction: Ovine pulmonary adenocarcinoma (OPA, Jaagsiekte, sheep pulmonary adenomatosis) is a worldwide, naturally occurring transmissible contagious pulmonary neoplasm of sheep and goats. It is a retrovirus-induced bronchioloalveolar carcinoma (BAC) of sheep with morphological features similar to the human BAC.

Materials and methods: Ovine pulmonary adenocarcinoma was studied in the lungs of 15 affected sheep (9 lungs with classical type and 6 lungs with atypical type lesions) by transmission electron microscopy. Selected tissues were fixed in 4% glutaraldehyde solution, postfixed with 1% osmium tetroxide. Semi-thin sections stained with toluidine blue and ultrathin sections stained with uranyl acetate and lead citrate. Stained sections were viewed with a transmission electron microscope.

Results: Ultrastructural characteristics of tumor cells showed three groups of cells including type II pneumocytes, clara cells and undifferentiated cells. Neoplastic type II pneumocytes contained numerous cytoplasmic lamellar bodies, well developed rough endoplasmic reticulum and glycogen particles. Neoplastic clara cells contained apical electron-dense granules and well-developed smooth endoplasmic reticulum. Undifferentiated tumor cells lacked characteristic lamellar bodies or electron-dense granules. Neither complete virions nor viral inclusions were seen in the neoplastic cells.

Discussion: This is the first report of ultrastructural characteristics of the neoplastic cells of OPA in sheep in Iran and also comparative evaluation of lesions in both morphological types of the disease. There were no differences between classical and atypical lesions, ultrastructurally. Undifferentiated tumor cells lacked characteristic lamellar bodies or electron-dense granules but ultrastructural features of these cells indicate in the alveoli they may be immature type II pneumocytes and in the bronchioles, immature clara cells.

NMDA inhibits kainate-induced arteritis in hearts of mice through activation of NMDA receptor

Kiyokazu, Ozaki, Tomoya Sano, Tetsuro Matuura, Isao Narama

Setsunan University, Hirakata, Japan

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Introduction: Kainate (KA) is one of the neurotoxic analogs of excitatory amino acids that induce acute neuronal death of hippocampal neurons in rodent animals. In addition, we have previously reported KA induces coronary arteritis in mouse heart. Activation of NMDA receptors has been shown to induce either neuronal cell death or protective effects on KA-induced neuronal damage. We have recently reported that activation of NMDA receptors also inhibits KA-induced coronary arteritis of heart. In this study, the effect of non-competitive NMDA receptor antagonist MK-801 on the protection of NMDA in KA-induced coronary arteritis of the heart was investigated.

Material and methods: Five-week-old male ddY mice were divided into five groups. Mice of each group were treated with MK-801 at 0, 0.5, 1 and 2 mg/kg intravenously. After 20 minutes, NMDA was administered additionally at 10 mg/kg intravenously. After 24 hours, 25 mg/kg of kainate dosed intravenously. Control group was treated only with kainate. A total of 92 surviving mice sacrificed at just 48 hours after dosing of kainate and brains and hearts were examined histopathologically.

Results: Within one hour after KA treatment, 43% of control rats died immediately after the appearance of seizure. NMDA pretreatment decreased in mortality to 9-25% with/without MK-810 treatment. KA-induced coronary arteritis of heart was induced 52% of control mice. Arteritis was prevented by NMDA pretreatment, but the protective effect of NMDA was disappeared by MK-801 treatment. Neuronal death of hippocampus was observed in 48% of control rats. NMDA pretreatment led to inhibit neuronal death in 0, 0.5 and 1 mg/kg of MK-801, but had no effect at high dose (2 mg/kg) group of MK-801.

Discussion: These data suggest that the protective effect of NMDA on KA-induced arterial damage as well as neuronal cells was mediated through NMDA receptor, and the sensitivity of NMDA receptor antagonist is different between coronal artery and hippocampus neuron.

Systemic coronavirus-associated disease resembling feline infectious peritonitis in the domestic ferret (*Mustela putorius*)

Kiupel, Matti, Michael M. Garner, Annabel Wise, Katrina D. Ramsell, Roger Maes

Michigan State University, Lansing, Michigan, USA

Introduction: Two coronaviral diseases have been described in ferrets: experimentally transmitted severe acute respiratory syndrome (SARS) and naturally occurring epizootic catarrhal enteritis (ECE). ECE was first described in 1993 causing diarrhea in young and adult ferrets and ferret enteric coronavirus (FECV) has been implicated as the causative agent. FECV is most closely related to FCoV (feline coronavirus) and lesions of ECE are limited to the gastrointestinal tract. A disease resembling feline infectious peritonitis (FIP) has been recognized recently in the domestic ferret in the US and Europe.

Material and methods: Since 2002, 11 ferrets from the US were diagnosed with FIP-like disease. All ferrets were neutered, 8 were male, and 3 were female. Ages at onset of disease ranged from 2 to 36 months (average 11 months). Histological evaluation and immunohistochemical staining for group 1 coronaviral antigen were performed on tissues with gross lesions from all ferrets. Total RNA extracted from affected tissues from 2 ferrets was tested with real time RT-PCR that broadly detects the group 1 animal coronaviruses FCoV, CCV (canine coronavirus), and TGEV (transmissible gastroenteritis virus of swine). Furthermore, tissues were tested with a FECV-specific RT-PCR and degenerate consensus primers that will amplify a portion of the spike gene of any coronavirus. PCR products were purified from agarose gels and amplicons were sequenced bidirectionally. Sequence data were subjected to BLAST analysis.

Results: The disease was progressive in all cases (average duration 69 days). Most consistent clinical signs included weight loss, intra-abdominal masses, lethargy, anorexia, emaciation and central nervous disease. Microscopically, pyogranulomatous inflammation involved especially the visceral peritoneum, mesenteric adipose tissue, liver, lungs, kidneys, lymph nodes, spleen, pancreas, adrenal glands, and blood vessels. Positive staining for coronavirus antigen was detected in all cases in foci of pyogranulomatous inflammation in the cytoplasm of macrophages. Samples from 2 ferrets were negative for FCoV, CCV, and TGEV. One of the 2 cases yielded faint bands with the FECV-specific RT-PCR. The generic coronavirus PCR amplified a unique 599-bp sequence, and BLAST analysis showed significant similarity between the ferret-derived sequence and group 1 coronavirus spike gene sequences. Alignment of the deduced partial spike amino acid sequence (199 residues) to corresponding sequences of known group 1 coronaviruses showed 71% to 73% sequence identities to FCoV, TGEV, and CCV, and 77% sequence similarity to FECV.

Discussion: Visceral disease caused by mutated coronaviruses that closely resemble host enteric coronaviruses has been recognized for some time in cats as FIP and pathogenic visceral coronavirus infections related to viral mutations also occur in other species. Based on the data presented we hypothesize the occurrence of a recent mutation or shift in FECV as the cause of this FIP-like disease in ferrets and suggest the name *ferret systemic coronavirus (FSCV) disease*.

Investigation upon the role of apoptosis in Theiler's murine encephalitis virus-infected SJL/J and C57BL/6 mice

Klein, Stephanie Kristin, Ingo Gerhauser, Reiner Ulrich, Suliman Elmarabet, Wolfgang Baumgärtner, Andreas Beineke

Department of Pathology, University of Veterinary Medicine Hannover, Hannover, Germany

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Introduction: Infection of SJL/J mice with the Theiler's murine encephalomyelitis virus (TMEV) leads to a biphasic disease, characterized by an initial polioencephalitis and chronic demyelinating leukomyelitis. Viral specific immune responses during the early disease phase lead to an elimination of TMEV in C57BL/6 mice. However, viral persistence contributes to prolonged inflammation and subsequent myelin loss in SJL/J mice, representing an important animal model for the chronic-progressive form of human multiple sclerosis (MS). Apoptosis of resident neural cells contributes to lesion development, while activation-induced cell death (AICD) of immune cells results in termination of inflammatory responses in several central nervous system disorders. The aim of the present study was to detect apoptotic changes in white matter lesions and perivascular infiltrates of the spinal cord of TMEV-infected susceptible SJL/J and resistant C57BL/6 mice.

Material and methods: 60 female SJL/J and C57BL/6 mice were sham-infected or infected with the TMEV BeAn strain and necropsied at 7, 14, 56, 98, and 196 days post infection (dpi). Spinal cord was removed, formalin-fixed and paraffin-embedded for histology. The amount of T cells (CD3), B cells (CD45R/B220) and microglia/macrophages (Mac-1) were determined by immunohistochemistry (IHC). Apoptotic cells were detected by an caspase-3 specific antibody using IHC and TdT-mediated dUTP-biotin nick end labelling (TUNEL).

Results: Immunophenotyping revealed a prominent infiltration of CD3-positive T cells at 7 and 14 dpi in perivascular spaces of both SJL/J and C57BL/6 mice. However, T cell-dominated immune responses at 56 dpi and prominent microglia/macrophages infiltrates at 196 dpi were only observed in SJL/J mice. In perivascular cuffs a significant increase of TUNEL-positive cells was observed at 7 and 98 dpi, while caspase-3 specific IHC revealed a significantly increased number of apoptotic cells at 7 dpi followed by a decline of perivascular apoptotic cells. In white matter lesions TUNEL- and caspase-3-positive density was most abundant at 14 dpi. In C57BL/6 mice only very few apoptotic cells were observed during the investigated time period.

Discussion: Apoptosis represents a frequent finding in TMEV-infected mice associated with white matter lesion development in the early disease phase. Further, apoptosis of perivascular inflammatory cells is suggestive of AICD in SJL/J mice. However, reduced induction of inflammatory cell death during the late chronic phase of TMEV-infection might lead to prolonged inflammation and demyelination as described for human MS.

Quantitative expression analyses of key molecular targets in microdissected canine mammary tumors and their metastases

Klopfleisch, Robert, Achim D. Gruber

Freie Universität Berlin, Department of Veterinary Pathology, Berlin, Germany

Introduction: Mammary tumors are among the most prevalent neoplasms in the dog. A fatal outcome of the disease is virtually always due to metastasis of the primary tumor to distant organs. Studies on mRNA expression of well established tumor marker genes in canine mammary tumors are numerous but lead to contradictory results. This might be due to the use of whole mount tissue samples with a mixture of different neoplastic and non-neoplastic cells. In this explorative study mRNA expression of tumor marker genes was quantified in simple canine mammary adenomas, adenocarcinomas, their lymph node metastases and non-neoplastic mammary epithelium using laser microdissection followed by quantitative polymerase chain reaction (qRT-PCR).

Material and methods: Six dogs with simple mammary adenocarcinomas metastatic to the inguinal lymph node and six dogs with morphologically benign adenomas were analyzed. Tissue samples of tumors, lymph nodes and adjacent non-neoplastic mammary gland of each patient were snap frozen within 15 minutes after surgical removal. Cryosections were used for microdissection of neoplastic, metastatic and non-neoplastic cells. Total RNA was extracted and reverse transcribed. Primers for ErbB2, 3, 4, BRCA1, 2, RAD51, p21, p27, p53, Derlin-1, stanniocalcin and beta-actin were designed using the Primer 3 algorithm. qRT-PCR was used to compare gene expression levels of neoplastic and non-neoplastic cells in relation to the housekeeper gene beta-actin.

Results: Expression analysis of EGF receptor family members revealed a substantial downregulation of erbB4 but no regulation of erbB2 and erbB3 in neoplastic mammary tissue when compared to non-neoplastic glandular epithelium. The human breast cancer susceptibility gene BRCA2 and its DNA-repair gene RAD51 were upregulated in metastatic tumor cells but not in the primary tumor. The cell cycle regulators p21 and p27 were found to be upregulated in the primary tumors but not in their metastases. p53 was up- or downregulated in most of the primary tumors but not regulated in the metastatic tumor cells. Derlin-1, a protein transporter was downregulated in the metastasis but not in the primary tumor. Stanniocalcin, a hypoxia marker was downregulated in most of the primary tumors and metastases.

Discussion: The molecular pathogenesis of canine mammary tumor metastasis is unclear. This study used microdissected tissue samples to quantify expression of key tumor markers for human breast cancer in canine mammary adenocarcinomas and their metastases. Regulation of p21, p27, Derlin-1 expression was found to be similar in human and canine mammary tumors. An opposed regulation was found for erbB4, BRCA1, RAD51 and stanniocalcin. The results indicate that canine mammary tumors are an acceptable model for some molecular aspects of human cancer but differ in many aspects from human malignancies.

Spontaneous tumors in domestic hamsters

Kondo, Hiroataka, Mamoru Onuma, Hisashi Shibuya, and Tsuneo Sato

Laboratory of Veterinary Pathology, College of Bioresource Sciences, Nihon University, Kanagawa, Japan

P75

Introduction: Hamsters are popular as pets in Japan. The majority of reports and studies on neoplastic diseases in these species have been related to spontaneous and induced tumors in laboratory hamsters. We performed a comprehensive study on spontaneous tumors occurring in domestic hamsters, particularly Syrian (S; *Mesocricetus auratus*) and Djungarian (D; *Phodopus sungorus*) hamsters.

Material and methods: A total of 90 biopsy specimens from 85 domestic hamsters submitted to the Laboratory of Veterinary Pathology, Nihon University between 1994 and 2007 were studied. Diagnosis were made using HE-stained sections and supplemental sections with special stains. Tumor type was classified according to organ. Mammary glands were included in the integumental system, and integumental tumors other than mammary tumors were subdivided into epithelial and mesenchymal tumors based on the World Health Organization classification. Data on species, age and sex of affected hamsters were also obtained.

Results: A total of 75 tumors, including neoplastic and non-neoplastic lesions, were seen in the 70 D hamsters, and 15 tumors were observed in the 15 S hamsters. Mean age of affected hamsters was 19.8 months (range, 5 to 36 months). Of the 75 tumors from D hamsters, 64 were neoplasms, and 11 were non-neoplastic lesions. Integumental neoplasms were the most frequent, and various types were present. The most common integumental neoplasms were mammary tumors (n = 15; including benign and malignant), atypical fibroma (13), papilloma (10), and squamous cell carcinoma (6). In S hamsters, 14 neoplasms and one non-neoplastic lesion were observed. Hematopoietic tumors, including plasmacytomas (4) and lymphomas (2), were the most common.

Discussion: The total number of spontaneous tumors in D hamsters was markedly greater than in S hamsters. The results of our study support the previously reported prevalence of spontaneous tumors in laboratory D and S hamsters. These species have different numbers of chromosomes, that may be related in differences in the prevalence and frequency of spontaneous tumors. The spectrum of affected sites and tumor types differed markedly between D and S hamsters. Domestic hamsters developed primarily integumental tumors rather than the multi-organ tumors reported in laboratory hamsters. Although it is difficult to clarify the origins and total populations of domestic hamsters, regional differences in tumor prevalence and spectrum may be present. Epidemiological studies on tumor prevalence in domestic S and D hamsters in different regions are thus necessary.

Differentiation of canine lymphatic and blood vascular endothelial tumours by co-expression of LYVE-1 and CD31

Krudewig, Christiane, Sheena Warman, Jan Rybnicek and Michael J. Day

Veterinary Pathology, Division of Veterinary Pathology, Infection and Immunity
School of Clinical Veterinary Science, University of Bristol, Langford House, Langford, United Kingdom

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Introduction: Canine lymphangiomas/lymphangiosarcomas are rare cutaneous tumours that arise most commonly in regions where there has been primary or secondary lymphoedema. Diagnosis is usually late in disease and made by histopathological examination which demonstrates vascular channels devoid of blood cells and lined by neoplastic endothelial cells. The major differential diagnosis is haemangiosarcoma. The aim of the present study was to investigate whether expression of the marker LYVE-1 would aid in the diagnosis of canine angiosarcomas. LYVE-1 is a member of the Link protein superfamily and has been shown to be specifically expressed by human cutaneous lymphatic endothelium, but not by blood vascular endothelium.

Materials and Methods: Eight canine skin/subcutaneous endothelial tumours previously diagnosed as “angioma/angiosarcoma suggestive of lymphatic origin” were investigated. The dogs were aged 5 months – 11 years. Serial sections from formalin fixed and paraffin wax embedded tissue were used for immunohistochemistry with the avidin-biotin complex method. Primary antibodies included polyclonal goat anti-human LYVE-1 antibody (10µg/mL) and monoclonal mouse anti-human CD 31 (1:25), the latter used to identify vascular endothelium of either blood or lymphatic origin. Normal goat/mouse IgG in appropriate concentration was used as negative control. A section from a case of cutaneous haemangiosarcoma served as positive control for CD 31.

Results: In all cases the skin lesions comprised endothelial cells forming vascular channels mostly devoid of blood cells with intervening collagenous stroma. In all eight tumours the neoplastic endothelia had strong cell membrane and weaker cytoplasmic expression of CD31. LYVE-1 was expressed by neoplastic endothelia in five cases. Normal blood vessels in the same section were negative for this marker. One tumour, from a 5-month-old rottweiler had metastasized to the lung and the para-aortic region. The metastases also expressed LYVE-1. The three tumours that expressed CD31 but not LYVE-1 may therefore have been of blood vascular origin. LYVE-1 was expressed by cells lining normal lymphatic vessels of a lymph node and skin. In the investigated haemangiosarcoma endothelial cells did not express LYVE-1.

Discussion: This is the first report of the expression of the marker LYVE-1 in fixed tissue samples from the dog. LYVE-1 was specifically expressed on normal and neoplastic canine lymphatic vessels, but not by vascular endothelium. Therefore LYVE-1 is a useful aid in the differentiation between lymphangiosarcoma and haemangiosarcoma. LYVE-1 was also useful for the assessment of metastasis in one case reported here.

Estrogen receptors (ER) dependent breast cancer in mice and cellular model

Kumar, Kishor S., Mahesh Kumar M.J.

Centre FOR Cellular and Molecular Biology, Hyderabad, India

P58

Introduction: Breast cancer is the leading malignancy in women, accounting for more than 3,50,000 deaths per year worldwide. Estrogen and estrogen receptors (ER) are playing major role in breast cancer progression and developments. Recently, we developed novel spontaneously mutated ER positive mammary adenocarcinoma animal model from heterozygous NIH nude mice in our animal house.

Material and methods: Using brother-sister mating with pedigree expansion system, we derived a colony of heterozygous breeding females showing ER-Positive tumor around the age of 6 months. Complete blood picture, differential leukocyte count, and serum levels of Estrogen, Alanine amino transferase (SGPT), Aspartate amino transferase (SGOT), total protein and albumin were estimated. Aspiration biopsies and microbiology were carried out. Gross pathology of the tumours and their metastatic potential were assessed. The tumors were excised and further characterized using histopathology, cytology, electron microscopy (EM), molecular markers and Mouse mammary Tumor Virus – Long Terminal Repeats (MMTV LTR) specific RT-PCR. Estrogen receptor expression was confirmed by real time PCR, western blotting and confocal immunofluorescence assay.

Results: Fifteen fold increase in serum estrogen levels was observed in this animals compared with normal mice. Histopathologically, invasive nodular masses of pleomorphic tubular neoplastic epithelial cells invaded into adjacent dermis, subcutaneous and muscular region.. Metastatic spread through hematogenous and regional lymph nodes, into multiple organs was observed. From this tumor, we developed few cell lines and this cell lines showing higher expression pattern of ER alpha receptor. ER alpha over expression was confirmed in cell lines and tumors in protein level by western blot analysis with mouse monoclonal antibodies specific to ER alpha. Higher expression ER alpha was confirmed in transcription level by real time PCR. Moderate to high expression of proliferating cell nuclear antigen (PCNA), moderate expression of vimentin and cytokeratin 19 (k19) and low expression of p53 were observed in tumor sections, when compared with that of the normal mammary gland. We checked in vivo tumorigenicity assay of this cell line (3×10^5) in the nude mice showing palpable tumour on 7th day.

Discussion: Since 75% of human breast cancers were classified ER positive and as our model mimic the ER- positive luminal epithelial -like human breast cancer, this model and cell lines will be an attractive tool to understand the biology of estrogen and estrogen receptor dependant breast cancer in women.

Related Publication

A mouse model for lumina epithelial like ER positive subtype of human breast cancer
M.J.Mahesh Kumar, K.S. Ponvijay, R.S.Nagaraj, R.Nandhini, J.Jose, G.srinivas, P.

Venkatesan

Kishor kumar S and shingh. BMC Cancer 2007, 7:180

Distribution of Theiler's murine encephalomyelitis virus in acute and chronic brain lesions of SJL/J and C57BL/6 mice

Kummerfeld, Maren, Reiner Ulrich, Ingo Gerhauser, Stephanie Klein, Wolfgang Baumgärtner, Andreas Beineke

Department of Pathology; University of Veterinary Medicine Hannover, Hannover, Germany

Introduction: Theiler's murine encephalomyelitis virus (TMEV) belongs to the family *Picornaviridae*. Intracerebral (i.c.) infection of mice with the BeAn strain causes an acute polioencephalitis. While viral elimination leads to clinical improvement in resistant C57BL/6 mice, viral persistence is associated with demyelination in the spinal cord during the late phase in susceptible SJL/J mice, representing an important animal model for the chronic-progressive form of human multiple sclerosis (MS). However, so far, information about viral spread and alterations within the brain of TMEV-infected mice is sparse. Therefore, the aim of the present study was to investigate the distribution of TMEV antigen and viral RNA as well as associated lesions in the brain of susceptible and resistant mouse strains during acute and chronic infection.

Material and methods: 108 female SJL/J and C57BL/6 mice were infected i.c. with the TMEV BeAn strain. Necropsies were performed 1 hour (hpi) as well as 4, 7, 14, 28, 42, 56, 98, and 196 days post infection (dpi). Samples of the cerebrum, cerebellum, and brain stem were formalin-fixed and paraffin-embedded. Perivascular mononuclear cuffing and parenchymal inflammatory infiltrates were evaluated using hematoxylin-eosin-stained sections and demyelination was graded using luxol fast blue-cresyl violet-stained tissue sections by a semiquantitative scoring system. Viral protein and RNA of infected cells were detected by immunohistochemistry and *in situ* hybridization, respectively.

Results: At 1 hpi, viral antigen and RNA were detected in the brain of SJL/J and C57BL/6 mice at the apical cell membrane of the ependyma and choroid plexus throughout the ventricular system, while periventricular regions of the third, fourth, and lateral ventricles exhibited virus at 4 dpi in both mouse strains. Infection of the cerebral cortex, hippocampus, thalamus, and hypothalamus was most intense at 4 and 7 dpi, followed by a significant decrease of viral antigen and RNA, starting at 14 dpi. The number of infected cells was significantly increased in the periventricular gray and white matter around the fourth ventricle at 28 dpi in both investigated mouse strains. However, viral persistence in the late chronic disease phase (196 dpi) was only present in the white matter of the brain stem of susceptible SJL/J mice. Generally, presence of virus was associated with lymphoplasmahistiocytic inflammation and gliosis in all investigated brain areas of SJL/J and C57BL/6 mice. Demyelination of cerebellar periventricular areas and brain stem with myelinophagia was predominately observed in SJL/J mice with a maximum at 28 to 98 dpi.

Discussion: Results of the present study are indicative of a liquorogenic spread of TMEV during the acute disease phase regardless of the mouse strain. However, appropriate anti-viral inflammatory responses during the acute phase result in elimination of TMEV in C57BL/6 mice, while viral persistence leads to demyelination within the cerebellum and brain stem of SJL/J mice. Distribution of myelin loss around the ventricular system shows similarities to lesion development within the brain of MS patients.

Late onset cerebellar cortical abiotrophy in a koala

Kuwamura, Mitsuru, Fumi Murai, Shin Nishioka, Mica Aoki, Fumihito Ohashi, Jyoji Yamate, Takao Kotani, Brian A. Summers

Osaka Prefecture University, Osaka, Japan

P86

Introduction: The term “abiotrophy” implies premature neuronal degeneration that does not result from an acquired insult, but rather is the consequence of an intrinsic metabolic disorder. Cerebellar cortical abiotrophies have been reported in many animal species and canine cases have most frequently been recorded. To our knowledge, no report of cerebellar cortical abiotrophy has been described in the koala. We report the clinical and pathological findings of cerebellar abiotrophy in a koala.

Material and methods: A 13-year-old male koala was the subject of this study. The koala was born at Tennoji Zoo in Osaka Prefecture in Japan. At 10 years of age, the koala started to fall from the tree while sleeping. Subsequently, the koala often fell down when walking and showed a gait abnormality, abnormal nystagmus, and hypersalivation. At 12 years of age, the koala’s gait became staggering, and it seemed to be blind. At 13 years of age, the koala exhibited signs of dysstasia and was euthanized. Necropsy was conducted.

Results: Necropsy revealed marked symmetrical atrophy of the cerebellum, with atrophy of the cerebellar vermis being especially prominent. Histopathologically, a severe loss of Purkinje and granule cells was evident in the cerebellum while the molecular layer was more cellular than normal with cells resembling small neurons. Reactive Bergmann glial cells (astrocytes) were present adjacent to the depleted Purkinje cell zone. The many small round cells with scant to moderate amounts of eosinophilic cytoplasm in the molecular layer were positively stained with parvalbumin immunohistochemistry. The cerebellar nuclei were remarkably gliotic, probably secondary to Purkinje neuron loss.

Additional findings were scattered and discrete degenerative lesions with pallor and vacuolated neuropil, neuron loss, reactively proliferated capillaries, mild perivascular cuffing and gliosis. These changes were found in the neocortex, tectum and medulla and likely related to traumatic insults caused by the falls.

Discussion: The present koala is a quite intriguing case of cerebellar cortical abiotrophy with very late onset and slow progression. Veterinarians should consider cerebellar cortical abiotrophy if koalas show cerebellar signs, even at advanced ages.

Feline Oral Squamous Cell Carcinoma: an 'osteogenic' type?

Lepri, Elvio, Chiara Brachelente, Leonardo Leonardi, Monica Sforza, Giovanni Vitellozzi

Dep. Biopathological Sciences and Hygiene of Food and Animal Products – Faculty of Veterinary Medicine, Perugia, Italy

P26

Introduction: Oral squamous cell carcinoma (SCC) is a common tumor in the cat, arising most frequently from tongue or gums, rarely from other structures. They are considered a model for human head and neck SCC for their pathogenetic and biological features. Gingival SCC are locally infiltrative and can invade bone inducing both osteolysis or reactive bone formation. In this latter scenario they must be differentiated from clinically similar osteosarcomas.

Material and methods: feline biopsic samples with a diagnosis of SCC have been reevaluated to verify the presence of bone tissue and characterize it as pre-existing infiltrated or newly formed bone. All samples were incisional biopsies, with additional 3 post-mortem cases.

Results: 169 SCC have been evaluated; 88 were cutaneous, including ear pinnae, 30 were from nasal planum, 44 were oral and 9 from other sites (mammary gland, larynx, etc.). Among the 44 oral tumors, 10 were from tongue, 12 from gums, and the majority from non specified sites. Bone tissue were identified in 15/44 samples (34%); in all cases the bone were interpreted as newly formed, based on topographic site of biopsy and histological features of bone tissue. Six tumors with bone tissue were gingival, while in the other 9 cases primary site was non specified. Bone tissue was characterized by scattered spicules of irregular woven bone with irregular cementing lines and multifocal mineralization, occasionally coalescing into larger trabeculae lined by osteoblast-like cells with osteocytes entrapped into lacunae. Multifocally more mature trabeculae underwent resorption by multinucleated osteoclast-like cells in Howship's lacunae. In most cases bone spicules were embedded into the desmoplastic reaction to infiltrative SCC and seems to originate from fibrous connective tissue for osseous metaplasia, while in other they were closer to neoplastic cells with abrupt transition from carcinomatous trabeculae to bone. In 2 cases osteogenic activity was indicated by the presence of an amorphous, finely fibrillar, extracellular eosinophilic material consistent with osteoid matrix. Grossly the 3 cases submitted for necropsy had enlargements of the mandibular branch with smooth to botrioid surface, hard consistency and white gritty appearance on cut surface. Histologically, trabeculae of woven bone extended radially at acute angle from the lamellar bone of mandibular cortical contour and merged with neoplastic lobules. No metastases were found in regional lymph nodes or other viscera.

Discussion: osteogenic tumors are most commonly of osteoblastic or mesenchymal origin. Occasionally, epithelial tumors are also associated with bone production, both from metaplasia of fibrous stroma and periosteal reaction of invaded bone. The mechanisms of this osteogenic impulse are poorly understood, but can include neoplastic-cell derived growth factors of the Bone Morphogenetic Protein (BMP) family, hormone-like substances such as PTHrP, or other proteins such as Osteoprotegerin. Bone formation seems to represent a common feature of feline oral SCC, suggesting the definition of an 'osteogenic' tumor. These osteogenic properties have not been investigated, but could represent an animal model for human osteogenic carcinomas (particularly prostate metastatic osteogenic carcinoma) to understand molecular pathways and controlling strategies.

Pathological findings in 11 dogs with naturally occurring fatal heatstroke

Loeb, Emmanuel, Itamar Aroch, Joseph Saragusty and Yaron Bruchim

Bactochem Ltd, 18 Hacharash St. Nes-Ziona, Israel

P7

Introduction: The objective of this study was to characterize the gross- and microscopic post mortal changes in naturally occurring fatal canine heatstroke and their association with clinical parameters.

Materials and methods: A retrospective case-series of 11 naturally occurring fatal canine heatstroke. All dogs underwent a full necropsy. Gross abnormalities were recorded. Samples of skin, lungs, heart, intestine, liver, spleen, brain and bone marrow were formalin-fixed, stained (hematoxylin & eosin) and examined microscopically. The signalment, heatstroke type, time lags from clinical signs onset to admission and death, and clinical as well as clinicopathological findings were recorded.

Results: The median time-lags from onset of clinical signs to admission and to death were 4 (range 1-7) and 13 h (range 4.4-30 h), respectively. Upon admission, 5 dogs were diagnosed with DIC. Post mortally, all dogs presented multiorgan hemorrhagic diathesis with coagulative necrosis and microthromboses consistent with DIC.

Hyperemia and diffuse edema were observed in the skin (8/11), lungs (11/11), brain (11/11) and bone marrow (1/11). Splenic red pulp and hepatic sinusoid congestion were common (10/11 and 9/10, respectively). Microscopically, small and large intestinal mucosa (7/9 and 8/10 respectively), renal tubuli (9/10), liver (7/10) and brain neural tissue (4/11) showed necrosis.

Discussion: Naturally occurring fatal canine heatstroke induced acute multiple organ lesions involving most body systems. Our results suggest that the more prevalent lesions in canine heatstroke, including hemorrhagic diathesis, microthromboses and coagulative necrosis, were sequels of hyperthermia-induced DIC, which led to multiorgan dysfunction and death. DIC should be suspected in most canine heatstroke patients although its antemortem diagnosis may be difficult.

A case of a complex mammary carcinoma in a male cat

Lopes, Célia, Irina Amorim, Patrícia Dias Pereira, Augusto Faustino

ICBAS-UP, Oporto, Portugal

P52

Introduction: Mammary tumours are the most common neoplasms in female cats but rare in males, representing approximately 1 to 5% of diagnosed mammary carcinomas in the species. Mammary gland carcinomas in cats are considered very aggressive due to their rapid growth and early metastasis to the regional lymph nodes, lungs, liver and spleen. Most feline mammary tumours are simple carcinomas, composed of neoplastic epithelial luminal cells, and the complex carcinomas (with both epithelial and myoepithelial cells) are rare. The lack of studies in carcinomas in male cats does not allow their classification according to predisposing factors, prognostic histological features and clinical course. This report describes a case of a complex mammary carcinoma in a 14-year-old male European Domestic Shorthair cat with a story of a short sexual hormonal treatment at one year of age.

Material and methods: The mammary sample was surgically obtained by mastectomy and submitted for histological examination. A lymph node was also isolated. Macroscopically, the mammary nodule was well circumscribed, firm, greyish-white solid mass measuring 5,0 cm of diameter. The lymph node presented no macroscopic changes. Three µm thick serial sections were used for haematoxylin-eosin staining and for immunohistochemistry following an avidin-biotin peroxidase complex method, employing the monoclonal antisera pan-cytokeratin, cytokeratin-14, vimentin, muscular α -actin and caveolin.

Results: The histological examination revealed a well-circumscribed nodular lesion composed of two distinct cell populations: cuboid to oval luminal epithelial cells forming tubules, surrounded by fusiform or spindle-shaped myoepithelial cells underneath areas of secretion accumulation and calcification foci. The lesion presented low mitotic index and no vascular invasion was observed. Isolated neoplastic epithelial cells were detected on the lymph node. Neoplastic luminal epithelial cells showed strong positive immunostaining for AE1/AE3 although they were negative for all other antibodies. The myoepithelial counterpart was only immunoreactive for CK14 and caveolin.

Discussion: The participation of myoepithelial cells in feline mammary tumours is an unusual event and its occurrence is even rarer in a male cat. To the best of our knowledge this is the first report of such lesion. The fact that the cat had been treated with progestin therapy, even if for a short period of time and in early age, suggests that the hormonal administration may have trigger the neoplastic process.

Primitive neuroectodermal tumor (PNET) in the left eye of a bulldog

Ludwig, Eva¹, ²Ekkehard Schaeffer, ³Dirk Emmelmann, ³Albrecht Fengler, ¹Walter Hermanns

¹Institute of Veterinary Pathology, Ludwig-Maximilians-University, Munich, Germany

²Veterinary Pathologist, Munich, Germany, ³Veterinarian Practice, Berlin, Germany

P122

Introduction: Common primary ocular tumours seen in the dog include melanocytoma, malignant melanoma, iridociliary epithelial tumour, and medulloepitheloma. The presented case describes an intraocular tumour in the left eye of a bulldog.

Material and methods: In the ophthalmologic examination of a 10-year-old female bulldog with clinical signs of blindness and retinal detachment, an intraocular mass was detected in the fundus. The eye was enucleated, fixed in formalin, processed and routinely embedded in paraffin. Sections were stained with haematoxylin-eosin (HE), Giemsa, Turnbull, periodic acid-Schiff (PAS), and a variety of markers was examined, using immunohistochemistry (i.e. CD3, CD79a, NSE, GFAP, S 100, neurofilament). In addition, formalin-fixed tissue was used for electronmicroscopic examination.

Results: The parasagittal section of the bulbus revealed a white irregular mass in the papillar region, measuring about 0.6x0.6x0.3cm. The histologic examination showed severe infiltration of the retina with small, round, basophilic tumour cells, with high mitotic index and a solid growth pattern; only a few rosulate structures were detectable. The adjacent retina was atrophic and detached with reactive hypertrophy of the retinal pigment epithelium. In the optic nerve, no tumour cell infiltration could be found. Immunohistochemically, the tumour cells stained positive for S100, partly positive for NSE and GFAP, and negative für CD3, CD79a, and neurofilament. By means of the histological and immunohistochemical results, the tumour was diagnosed as primitive neuroectodermal tumour (PNET).

Discussion: Primitive neuroectodermal tumours (PNET) are characterised as small cell tumours, arising from progenitor cells present in the nervous system. The WHO classification of nervous system tumours in domestic animals distinguishes between medulloblastoma (cerebellar PNET) and PNETs, excluding cerebellar origin. We have classified the present tumour as extracerebellar PNET. PNETs are rare in animals, were they predominantly occur in calves and young dogs. The presented case shows an intraocular form in an adult dog.

Ectopic paragonimiasis in a dog

Madarame, Hiroo, Haruhiko Suzuki, Masahiro Tachibana, Yasuhide Saitoh, Shigehisa Habe, Akihiko Uchida, Hiromu Sugiyama

Azabu university, Sagamihara, Japan

P37

Introduction: Paragonimiasis is a parasitic disease caused by infection with trematodes of the genus *Paragonimus*. Adult worms can be parasitic in the definitive hosts (man and many wild and domestic animals, including dogs), residing in their lungs. In man, ectopic infection with larva(e) and/or adult(s) is well recognized, however, few ectopic paragonimiasis in dogs have been reported. We describe here a canine case of ectopic paragonimiasis with an inguinal subcutaneous mass and systemic lymphadenopathy.

Material and methods: A 15-month-old male Hunting Setter was presented to the animal hospital with palpable inguinal mass and systemic lymphadenopathy. Clinically, lymphoma was suspected and needle core biopsy of the inguinal mass was performed. The tentative diagnosis on the biopsy specimen was helminth infection. Surgical removal was performed as the first choice of therapy and the formalin-fixed excisional specimen was submitted for histopathology.

Results: Histopathologically, the subcutaneous mass consisted of eggs with granulomatous response, cysts containing single or paired adult parasites, eggs, inflammatory cells, and necrotic debris, and lymphadenitis with eggs occupying the lymphatic sinuses. The light microscopic findings of the transverse section of the adult parasites and the stereoscopic microscopic findings of the eggs which showed the characteristic morphology of *Paragonimus* sp. led to a histopathological diagnosis of ectopic paragonimiasis. After about 2 week's interval, the affected area swelled again to approximately two thirds of the size prior to surgery. Oral praziquantel treatment (10mg/kg, orally, SID, for ten days) was administered and subsequently the patient has been free of the disease without any further treatment for 2 years. Further clinical examinations, including chest radiography, were not carried out. The formalin fixed specimen was further examined genetically and the *Paragonimus* worm was identified as *P. miyazakii*.

Discussion: In human medicine, not only pulmonary and cerebral paragonimiasis, but also cutaneous and lymphatic manifestations are well known. However, to the author's knowledge, this is the first canine case of ectopic paragonimiasis, involving inguinal lymph nodes and surrounding subcutaneous tissue with mature *P. miyazakii* as well as systemic lymphadenopathy. Our present case may provoke systemic lymphadenitis. Morphological analysis is one of the most reliable methods for confirming the causative parasite species, but the information obtained is sometimes limited, especially in cases where submitted samples are not fresh or fixed in formalin. Recent molecular studies have introduced new methodologies into the parasitology. In the present case, the genetic examination was applied and identified the causative species as *P. miyazakii*.

PTEN, PI3K and NF- κ B expression in feline post-vaccinal fibrosarcoma

Magi, Gian Enrico, Giacomo Rossi, Chiara Bertani, Giacomo Renzoni

Department of Veterinary Science – Camerino, Matelica, Italy

Introduction: During tissue repair, excess fibroblasts are eliminated by apoptosis in response to collagen matrix contraction. This physiological process seems to be mediated by the activation of the tumour suppressor phosphatase and tensin homolog (PTEN). PTEN antagonizes phosphatidylinositol 3-kinase (PI3K) action and negatively regulates cell proliferation and survival signals. Deletions or inactivation of PTEN contribute to tumorigenesis in several human tumours. A recent study on colon carcinogenesis pointed out that NF- κ B overexpression reduced PTEN expression. It was also ascertained that mitogen-activated protein kinase kinase-4 (MKK4) promotes cell survival by decreasing PTEN expression through an NF- κ B dependent pathway. Noteworthy, NF- κ B is a key mediator of the inflammatory process and its activity has also been recognized as a regulator of tumour cell growth and sensitivity to apoptosis. Few studies have assessed inflammatory mediators that may play a critical role on feline post-vaccinal fibrosarcoma (FPVF). In addition PTEN and PI3K expression has not been investigated until today in FPVF. Therefore, the aim of this study was to evaluate the expression of small molecule pathways upregulation (PTEN, PI3K and NF- κ B) in FPVF and their relationship with the histological grade.

Material and methods: In total, thirty FPVF with typical histopathological features were analysed in this study. The samples were classified as grade I, II or III using a scheme described by Couto *et al.* (2002) based on cellular differentiation, mitotic activity and presence of necrosis. Ten cases were classified as grade I, fourteen as grade II and six as grade III. The expressions of PTEN, PI3K and NF- κ B were analysed immunohistochemically.

Results: In FPVF, PTEN was positive in 9/30 (30%), PI3K was positive in 23/30 (76,6%) and NF- κ B was positive in 25/30 (83,3%). Regarding the grade, in grade I PTEN was expressed in 5/10 (50%), PI3K was expressed in 7/10 (70%) and NF- κ B in 10/10 (100%); in grade II PTEN was expressed in 3/14 (21,4%), PI3K was expressed in 10/14 (71,4%) and NF- κ B was expressed in 14/14 (100%); in grade III PTEN was positive in 1/6 (16,6%), PI3K was positive in 6/6 (100%) and NF- κ B was positive in 1/6 (16,6%).

Discussion: Immunohistochemical loss of PTEN and positive staining of PI3K were frequently seen, whereas in all cases of grade I and grade II loss of PTEN and positive cytoplasmic staining of NF- κ B was observed. In the more aggressive grade III, NF- κ B was positive only in one case. Our preliminary findings suggest that the downregulation of PTEN expression is positively related to PI3K upregulation, as it is postulated in early wound repair. Overexpression of NF- κ B and loss of PTEN in the first steps of tumorigenesis could indicate a possible role for an inflammatory process in early events of FPVF. However, this results support a significant role for PTEN/PI3K dysregulation in the development of FPVF.

Panniculitis in Dogs and Cats; A Retrospective Study of 115 Cases

Majzoub, Monir, Andriy Synilo, Daniela Denk, Walter Hermanns

Institute of Veterinary Pathology, Ludwig-Maximilians-University Munich, Germany

P131

Introduction: Panniculitis is a disease whose hallmark is inflammation of subcutaneous fatty tissue. Various aetiological causes are discussed especially in human medical literature. The classification of this condition into lobular and septal panniculitis also derives from human medicine. However, in the author's opinion, this classification is only partly applicable for skin samples of dogs and cats. Therefore, the aim of the present study was to define morphological criteria by which panniculitis in dogs and cats can be histologically characterised.

Material and methods: Specimens of 59 dogs and 56 cats were submitted for histological examination. Tissue samples were fixed in 7% buffered formaldehyde, embedded in paraffin (Paraplast®), cut into 4 to 6 µm-thick sections and routinely stained with haematoxylin and eosin (HE). Additionally, Giemsa and Ziehl-Neelsen stains, as well as a Periodic acid Schiff's reaction (PAS) were performed.

Results: In a total of 115 cases where panniculitis was diagnosed, no significant age, breed and sex predilection could be detected. Lesions were most commonly found to occur in the areas of the ventro-lateral thorax and abdomen.

The following criteria can be used to further describe the inflammation of subcutaneous fatty tissue of dogs and cats: Localisation of the inflammatory infiltrates: occurring either only in the subcutaneous fatty tissue or also in the dermis; type of inflammation: no inflammatory changes or purulent, lymphoplasmacellular or granulomatous inflammation; changes in the inter- or intra-lobular connective tissue and/or in the fat cells themselves; vascular changes; occurrence of bacteria; development of microcysts or large central cavities.

Discussion: Panniculitis is defined as an inflammation of the skin, in which the major focus is located in the subcutis. In cases with widespread inflammation including the adjacent tissue (dermis and fascia) the origin of the lesion cannot be determined. Furthermore, various kinds of inflammation can be found simultaneously within tissue samples, thus complicating the diagnosis. Only in a few cases, bacteria were identified as a possible aetiological agent. In most cases the aetiology and pathogenesis remained undetermined.

Retroperitoneal epithelioid leiomyosarcoma with disseminated metastasis in a donkey

Malatesta, Daniela, Mariarita Romanucci, Chiara Palmieri, Augusto Carluccio, Laura Bongiovanni, Leonardo Della Salda.

Department of Comparative Biomedical Science, Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy

P115

Introduction: A 13-year-old Martina Franca jack donkey was died suddenly and was submitted for necropsy. The animal was regularly vaccinated.

Material and methods: Different tissues were collected and routinely processed for histopathology. Sections were stained with haematoxylin and eosin, periodic acid-Schiff, Gomori's stain, Masson's trichrome. Some representative slides were evaluated for α -smooth muscle actin, vimentin, desmin, neuron-specific enolase, cytokeratin (AE1/AE3), von Willebrand factor, muscle-specific actin, melan-A, HMB45, α -1-fetoprotein and lysozyme using streptavidin-biotin-peroxidase method.

Results: At post mortem examination the animal showed abdominal distention by approximately 3 liters of fibrinous-haemorrhagic effusion admixed with blood clots. In the retroperitoneal region, a large, hemorrhagic, mottled mass with diffuse and irregular surface was found. The right kidney was incarcerated in this mass with a diffuse volume reduction. On cut section, an irregular surface with multifocal cysts containing clear yellow fluid was evident. Well-circumscribed red nodules were also present in mesentery, subcapsular region of the spleen and lung. Histologically, an unencapsulated, poorly circumscribed, expansile neoplastic mass was primarily constituted by a proliferation of pleomorphic polygonal cells with epithelioid features admixed to spindle-shaped tumour cells characterized by scant to moderate eosinophilic cytoplasm with ovoid to elongated vesicular nuclei. Scattered small capillaries surrounded by neoplastic cells were identified giving the lesion an angiomatous appearance. Cellular and nuclear atypia was high with a moderate number of bizarre mitotic figure and scattered multinucleated giant cells. Multifocal haemorrhagic and necrotic foci were also present. The right kidney capsule was infiltrated by neoplastic cells and focally the same cells were also found in the renal parenchyma. Neoplastic cell aggregates with the same pattern were observed in spleen, mesentery and lung. The majority of tumor cells revealed positive immunoreaction for vimentin, α -smooth muscle actin and muscle specific actin. Based on these findings, the neoplasm was classified as a primary retroperitoneal epithelioid leiomyosarcoma with diffuse metastasis.

Discussion: Tumors of the retroperitoneal space are rare in animals and there are few reports in horses. In human beings epithelioid leiomyosarcoma is recognized as a distinct morphologic variant of primary retroperitoneal smooth muscle neoplasm and must be considered in the differential diagnosis of epithelioid neoplasms of retroperitoneal space. Spontaneous rupture of such neoplasms is a possible cause of massive intraperitoneal bleeding. We hypothesized that the previously described neoplasm arose from the smooth muscle fibers of renal capsule. To the best of our knowledge, this is the first case of primary retroperitoneal epithelioid leiomyosarcoma in a donkey.

Feline endometrial carcinoma: evaluation of cytokeratins, vimentin and Ki 67 expression

Maria dos Anjos Pires, João Ribas, Carlos Augusto, Rita Payan-Carreira

Univ. Trás-os-Montes and Alto Douro, Vila Real, Portugal

P46

Introduction: Feline tumours of the uterus represents only 0,29% of all feline neoplasm, and includes endometrial carcinoma, adenosarcoma and smooth muscle tumors, the later being the most frequent. This is a preliminary study that aims to contribute to the advancement of the knowledge in feline endometrial carcinoma immunophenotype.

Material and methods: In this study, a panel of immunomarkers (large spectrum cytokeratins, vimentin, and Ki-67) was applied to normal uterus in the proliferative and secretory oestrous phases (the controls) and in uterus evidencing endometrial carcinoma, in order to establish the staining patterns indicative of the tumour aggressiveness and cellular differentiation. 10% formalin fixed uterine samples, embedded in paraffin wax were sectioned at 2 µm and routinely stained with haematoxylin and eosin to morphological characterization. Immunohistochemical analysis was performed using the avidin-biotin peroxidase method. Two independent observers performed a blind assessment of the degree of staining. The results for the cytokeratins (CK) and vimentin were evaluated as negative (0) and positive immunolabelling with different intensity as (+) to (+++). The number of immunopositive cells to Ki67 in 10 representative areas at the tumour was expressed as percentage.

Results: According to their morphological characteristics, the tumours were group in two types: a well differentiated group, showing cribriform or pappilar pattern, and those evidencing microglandular variants. CK expression was detected in the luminal neoplastic cells with irregular pattern. Positive staining to vimentin was only observed in stromal and in scarce epithelial cells. The tumours showed higher values of Ki67 than the normal uteri (1,42% and 0,4% in the proliferative and the secretory phases respectively), varying between 2,4% and 23,9%, with the microglandular pattern type showing higher Ki67 index value.

Discussion: The immunohistochemical pattern of CK suggested the surface epithelium as the origin for the neoplastic superficial epithelial cells, sharing similar staining patterns with the normal uterus. Vimentin expression was showed in the stroma and sporadic epithelial cells. The Ki67 proliferation rate was low in the normal uterus and differences were detected between the proliferative and the secretory phases, as expected. The two types of tumours showed quite different Ki67 rates when compared with normal uteri, with the tumours with microglandular variant pattern revealing the highest Ki67 index possibly correlated with its more aggressive behaviour.

MMP-9 and TIMP-1 expression in feline endometrial carcinoma

Maria dos Anjos, Pires, Carlos Augusto, João Ribas, Rita Payan-Carreira

Univ. Trás-os-Montes and Alto Douro, Vila Real, Portugal

P53

Introduction: Uterine tumours in the cat represents 0,29% of all feline neoplasms. Tumours of the uterus are classified in endometrial carcinomas, adenosarcomas and smooth muscle tumours. Leiomyomas and leiomyosarcomas are the most common type of tumours in the uterus of the cat. This preliminary study aims to study the immunolabelling of metalloproteinase 9 (MMP-9) and its inhibitor the TIMP-1 in feline endometrial carcinoma.

Material and methods: In this study, a panel of immunomarkers (large spectrum cytokeratins, MMP-9 and TIMP-1) was applied to normal uteri in proliferative and secretory oestrous stage and in two different groups of endometrial carcinoma. 10% formalin fixed uterine samples, embedded in paraffin wax, were sectioned at 2 µm and routinely stained with haematoxylin and eosin to morphological characterization. Immunohistochemical analysis was performed using the avidin-biotin peroxidase method. Two independent observers performed a blind assessment of the degree of staining. The results for cytokeratins (CK), MMP-9 and TIMP-1 were evaluated as negative and positive immunolabelling with different intensity as (+) to (+++). For the MMP-9 and TIMP-1 was also evaluated the predominance of the labelling in the cytoplasm, cellular membrane and nucleus.

Results: The tumours were divided in two morphological patterns: those evidencing well differentiated tumour and a cribriform or pappilar pattern, and the other one with microglandular variants. CK expression was detected in the luminal neoplastic cells with irregular pattern. In normal uteri, MMP-9 showed it the strongest expression in epithelial cells during the proliferative phase. In endometrial carcinomas, the pattern of immunolabelling was irregular between the two tumour types, with the epithelial cell of microglandular tumours showing the most intense membranar staining. The TIMP-1 labelling was strongest in the cytoplasm than in the cellular membrane of the epithelial cells during all the oestrus cycle, and most intense in the epithelial cells of the microglandular tumours.

Discussion: The immunohistochemical pattern of CK suggested that the endometrial surface epithelium was in the origin of the neoplastic superficial epithelial cells, as demonstrated by the immunostaining in the normal uterus. The expression of MMP-9 and TIMP-1 was relatively constant during the oestrous cycle and over-expressed in the microglandular tumours when compared with well-differentiated pattern tumours. This feature needs to be correlated with other factors, such as clinical and follow-up, before being use as predictive of the behaviour of this type of tumour.

Early events of jaagsiekte sheep retrovirus infection in the ovine lung

Martineau, Henny, Chris Cousens, Stuart Imlach, Massimo Palmarini, David J.Griffiths

Moredun Research Institute, Midlothian, United Kingdom

Introduction: Ovine pulmonary adenocarcinoma (OPA) is a fatal disease of sheep which follows infection with a contagious betaretrovirus called jaagsiekte sheep retrovirus (JSRV). The virus infects specific cells types within the lung initiating oncogenesis and bronchioloalveolar carcinoma (BAC) growth. To date it has not been possible to detect an antibody response to viral infection, and this has hampered development of a vaccine or sensitive test for individual animals. This project has been designed to examine the initial host response to viral infection and oncogenesis, to better understand the pathogenesis of OPA in sheep.

Materials and Methods: Twelve specific pathogen free (SPF) lambs were inoculated intratracheally with JSRV₂₁ shortly after birth. Sequential groups were euthanased at specific time points following infection, and a full range of samples were preserved in neutral buffered formalin, zinc salts, 4% paraformaldehyde and snap frozen. Tissues from age matched control animals were also taken. Immunohistochemistry and in situ hybridisation techniques were then used to analyse the samples.

Results: JSRV viral protein expression was identified in three out of four SPF lambs, 10 days post inoculation with JSRV₂₁. Positive labelling was seen in single/groups of cells within the region of the terminal bronchioles. Virus-positive cells were further characterised using antibodies specific for type II pneumocytes, Clara cells and tissue stem cell markers.

Discussion: The identification of cell types observed to express JSRV proteins within the terminal bronchioles will be considered and compared to mice models of lung adenocarcinoma and lung cancer in humans. The potential significance of these findings in relation to lung gene therapy and early identification of tumour growth will be discussed.

Immunocytochemical evaluation of the cell-cycle regulatory proteins (p53, p21, p16) in a case of multiple primary tumors in a Boxer dog

Martínez, Carlos M., Alberto Benito, Juan Manuel Corpa

Universidad CEU-Cardenal Herrera, Moncada (Valencia)/Spain

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Introduction: Carcinogenesis is characterized by the combination of several causes that might initiate or promote the risk of neoplastic transformations. Both genetic and environmental factors are related, and are likely to determine the growth of a tumor. Several epidemiologic studies establish different incidences of tumors between different dog breeds. Specifically, the boxer seems a high-risk breed for several types of tumors, and frequently, multiple primary tumors are described in a same animal. The exact reason of this high-risk is not completely understood and previous reports suggest certain genomic instability. The aim of this work is study the expression of several cell-cycle regulatory proteins (p53, p21 and p16) in a case of multiple primary tumors in a boxer dog. Alterations in the expression of these proteins would suggest fails in cell cycle regulation that might explain the reason of the high incidence of tumors in our case.

Material and methods: Formalin-fixed and paraffin embedded samples from a case of simultaneous squamous cell carcinoma, thyroid carcinoma, solid mammary carcinoma and chemodectoma (aortic body tumor) in an 11-years old female boxer dog were used in this study. Sections of the samples (4 μm thick) were stained with the ABC technique using antibodies against p53, p21 and p16. An antibody against Ki-67 antigen was also used in order to calculate the proliferation index.

Results: Analysis of stained samples revealed a high proliferation index (ki-67 positive cells) in squamous cell and mammary carcinomas, a moderate proliferation index in the thyroid carcinoma and low in the chemodectoma. Interestingly, high alterations in p53, p21 and p16 expression have been detected in high-proliferation index neoplasias.

Discussion: Our results suggest that fails in the cell-cycle regulation due to alterations in expression of cell-cycle regulation proteins (p53, p16 and p21) might increase the grade of tumoral malignancy. Previous reports (Stone et al., 1991a) points to a congenital genomic instability in boxer dogs. Our results points to several fail in cell cycle regulation. Further studies are necessary to establish the exact role of cell-cycle regulatory proteins in carcinogenesis in the Boxer breed, and would explain why the Boxer has a higher incidence of cancer.

Inflammatory response to bovine and avian tuberculin and to phytohaemagglutinin skin testing in wild Iberian red deer

Martín-Hernando, M^a Paz, M^a Isabel G. Fernández de Mera, Joaquín Vicente, Raquel Jaroso, Christian Gortazar

IREC, Ciudad Real, Ronda de Toledo, s/n, Spain

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Introduction: The tuberculin skin reaction signals the presence of a delayed hypersensitivity induced by mycobacterial antigens. This test is widely used in domestic and wild ruminants. However, little is known on the characteristics of the local immune response, particularly in wild animals. The objective of this study was to describe the local inflammatory response to skin test antigens in wild deer.

Material and methods: Sixteen adult red deer (*Cervus elaphus hispanicus*) were euthanased after testing positive to bovine TB (skinfold increase larger than 2 mm, 72h post-injection). In addition to the two commonly used antigens, bovine purified protein derivative (bPPD) and avian PPD (aPPD), we also used the mytogen phytohaemagglutinin (PHA) as positive control, and phosphate-buffered saline (PBS) as negative control. All substances were injected in the side of the neck. A complete necropsy was carried out, and tissue samples from all organs and skin from the four antigen injection sites were fixed in 10% neutral buffered formalin and routinely processed. In the skin sections, we analyzed the superficial and deep dermis, and quantified the area with presence of mononuclear cells, macrophages and fibrocytes, and the numbers of eosinophiles and neutrophiles.

Results: A positive correlation between skinfold increase and the level of inflammatory infiltrate was evidenced both in the bPPD and PHA injection sites. The presence of neutrophiles was slightly higher in these two sites, too. Reaction was more severe in the deep dermis than in the superficial dermis, and this was again more evident in the bPPD and PHA injection sites as compared to the aPPD and PBS sites. In the PHA injection site the number of eosinophiles was larger than the number of neutrophiles (1.24:1). A slight but visible inflammatory response was evident in the PBS injection site.

Discussion: Responses to skin testing with different antigens were largely similar regarding the cell populations, but differed regarding intensity. Results from this study provide support for the use of both a positive control such as PHA and a negative control such as PBS in skin testing of wild deer.

Diagnosis of bovine tuberculosis in wild ungulates from Spain: culture and pathology

Martín-Hernando, M^a Paz, M^a José Torres, Joaquín Vicente, Manuel Reglero, Jose de la Fuente, Javier Aznar-Martín, Juan José Negro, Christian Gortazar

IREC, Ciudad Real, Spain

Introduction: Bovine tuberculosis (bTB) due to *Mycobacterium bovis* and closely related bacterial strains of the *Mycobacterium tuberculosis* complex is a worldwide disease that affects a wide range of domestic and wildlife animals and humans. In southern Spain, an increase in bTB prevalence in wild ungulates has been reported. Recently, the detailed study of wildlife from one single locality (Doñana National Park, DNP, southern Spain) showed a very high bTB prevalence in three sympatric wild ungulate species: European wild boar (*Sus scrofa*), 52.4%; red deer (*Cervus elaphus*), 27.4%; and fallow deer (*Dama dama*), 18.5%. This detailed survey allowed us to test whether the data obtained by macroscopic carcass inspection, or by histopathology, agree with prevalence figures obtained using the gold standard test microbiology. Furthermore, it allowed testing which samples is the best choice for detecting *M. bovis* in these species.

Material and methods: In this study, 124 European wild boar, 95 red deer, and 100 fallow deer were sampled in DNP from April 2006 to April 2007 and analyzed for bTB. Detailed necropsies were carried out in all deer, while only the head was available for inspection in wild boar. Head lymph node and tonsil samples from all animals were taken for microbiological culture, and samples of all organs (deer) or only from the head lymphoid tissues (wild boar) were fixed in 10% neutral buffered formalin and routinely processed.

Results: TB compatible macroscopic lesions were detected in 63.6% wild boar, 32.3% red deer, and 20.3% fallow deer. Microscopic but not macroscopic lesions were observed in one third of the culture-positive deer. Generalized TB was frequent in both deer species (5 of 17 red deer and 4 of 9 fallow deer). In deer, bTB granulomas were typically composed of a slightly calcified necrotic center, that was more clearly delimited in red than in fallow deer. Six of 43 wild boar showed no macroscopic lesions, but had microscopic ones. In wild boar, granulomas showed a more intense calcification and often had surrounding satellite lesions, mostly with macrophages. The kappa agreement coefficient between the detection of macroscopic TB-compatible lesions and the actual isolation of *M. bovis* was 0.3 for wild boar, 0.53 for red deer, and 0.78 for fallow deer. Kappa agreement in wild boar was slightly higher if adults were excluded ($k=0.44$), but did not improve considering histopathology instead of macroscopic lesions ($k=0.31$). The largest disagreement between lesions and microbiology occurred in the group of "lesion positive – culture negative" animals.

Discussion: Pathology and microbiology need to be combined in detailed studies on wildlife bTB. Sampling head lymph nodes and not the tonsils may lead to an underestimation of true bTB prevalence. These results are useful in the context of bTB epidemiology and control in Spain.

Evaluation of the hepatic damage in goats immunized with recombinant cathepsin L1 and challenged with *Fasciola hepatica*

Mendes, Ricardo, Alejandro Pérez-Écija, Rafael Zafra, Leandro Buffoni, Alvaro Martinez-Moreno, José Pérez

University of Córdoba, Córdoba/ Spain

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Introduction: Current control is based on the prophylactic/therapeutic use of anthelmintic drugs. However the increasing anthelmintic resistance and the risk of drug metabolites in foodstuff have raised an international increasing interest in the development of vaccines to control this economically important disease of ruminants. The aim of present study was to evaluate the hepatic damage in goats immunized with recombinant cathepsin L1 (CL1) and challenged with *F. hepatica*.

Material and methods: Twenty one Florida-breed goats were used for the study: group 1 (n =7) was used as uninfected control; group 2 (n=7) was immunized with two doses of recombinant CL1 on week 0 and 3; group 3 (n= 7) was inoculated with the adjuvant (Quil A) on the same weeks. Goats of both groups were orally infected with 200 mc on week 10, and sacrificed on week 27. Hepatic damage was evaluated by histopathology and by a gross and microscopical morphometric study and results were compared with fluke burdens.

Results: Gross hepatic lesions of infected animals consisted of fibrous perihepatitis, irregular scars in the hepatic surface, and thickening of the bile ducts and gallbladder. The gross morphometric study revealed that the percentage of hepatic surface damaged was 50.02 ± 17.5 and 30.63 ± 19.9 in the infected control and in the immunized group, respectively. Fluke burdens revealed a 38.7% decrease in the immunized group respect to the infected control group, although no significant differences were found for both gross lesions and fluke burdens. Microscopical hepatic changes consisted of portal fibrosis, bile duct hyperplasia, chronic tracts with fibrosis and infiltration of lymphocytes, plasma cells, eosinophils and macrophages, often containing brown pigment. Granulomas showing necrotic centre surrounded by multinucleate giant cells were also found. The percentage of hepatic parenchyma damaged was 14.62 ± 5.79 and 35.58 ± 24.46 , in the immunized and in the infected control group, respectively. The area occupied by bile ducts was 1.32 ± 0.51 and 4.61 ± 4.56 , in the immunized and infected control group, respectively.

Discussion: The percentage of hepatic parenchyma damaged in the immunized group was 58.9 % lower than in the infected control ($P < 0.01$). Bile duct hyperplasia was reduced 71.4% in the immunized group respect to the infected control ($P < 0.05$). These results suggest that CL1 in Quil A induced certain protection in goats indicated by worm burden and hepatic damage. Further studies using different formulations are required to confirm/improve the results of the present work.

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Occurrence of bacterial pathogens and porcine circovirus type II in colitis of post-weaning pigs in Switzerland

Miclard, Julien, Mamani Javier, Titus Sydler, Goy Naomi, Speiser Stephanie, Zeeh Friederike, Albini Sarah, Posthaus Horst

Institute for animal pathology; Bern, Switzerland

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Introduction: Diarrhea due to colitis in post-weaning pigs is a complex problem resulting from a combination of infectious agents, host immunity and management procedures. It causes considerable economic loss. The diagnosis and preventive measures are difficult because of the variety of enteric pathogens that can be isolated in pigs of this age. The most common causes of diarrhea in post-weaning pigs are *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*, *Escherichia coli* and the porcine circovirus type II. Many studies have concentrated on a single pathogen and there is inadequate information concerning infections with multiple enteric pathogens. The main objective of our study is to determine the relative importance of enteric pathogens causing post-weaning pigs diarrhea in Switzerland.

Material and methods: Both a retrospective and a prospective study on case material from the Institute of Animal Pathology and the porcine clinics were conducted. Retrospectively, 135 cases of porcine colitis were categorized histopathologically. Additionally, PCR analyses for the detection of *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* were performed on formalin fixed intestinal tissue samples. Detection of the porcine Circovirus 2 was performed by immunohistochemistry on paraffin-embedded tissues. Prospectively 21 animals with clinical signs of colitis were necropsied and evaluated by pathological and bacteriological analysis of the intestinal tract. Histopathological lesions were reviewed and semi-quantitatively quantified.

Results: Preliminary results show that porcine circovirus type 2 (38%) and *Lawsonia intracellularis* (28%) are the most often demonstrated pathogenic agents in postweaning pigs with diarrhea, followed by *Escherichia coli*, *Brachyspira pilosicoli* and *Brachyspira hyodysenteriae* (10% each). Parasitic infections are the less frequently observed and are often associated with other bacterial pathogenic agents. *Salmonella sp.* and *Clostridium difficile* were not isolated from the intestinal tract of pigs with diarrhea. Interestingly, in one third of the cases, multiple pathogenic agents were observed. In 20% of the cases, no pathogenic agents were demonstrated.

Discussion: The results demonstrate that diarrhea in post-weaning pigs is often a multifactorial disease with frequent association between bacteria, virus and parasites. *Lawsonia intracellularis* and Circovirus appeared to be the two main pathogenic agents in this class of age, followed by *Brachyspira sp.* and *Escherichia coli*. Parasites, surprisingly, are also frequently observed, often in association with other pathogenic agents. Around 20% of the cases remain unclear (no pathogenic agent demonstrated), and raise the hypothesis of possible dysbacteriosis as primary cause of the diarrhea.

Cyclooxygenase-2, EP2 receptor and microsomal prostaglandin E₂ synthase-1 expression in canine healthy, hyperplastic, and neoplastic mammary tissues

Millanta, Francesca, Canale Alberto, Ressel Lorenzo, Lorenzi Davide, Citi Simona, Poli Alessandro

Department of Animal Pathology, University of Pisa, Pisa, Italy

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Introduction: Cyclooxygenase (COX)-1- and COX-2-derived prostaglandins (PG) are implicated in the development and progression of many human malignancies including mammary tumours. The PGE₂ is a major metabolite of the COX pathway and has been shown to have tumour-promoting activity. Its synthesis is catalysed by the microsomal PGE₂ synthase-1 (mPGES-1), an enzyme downstream to COX-2. PGE₂ cellular effects are mediated through a family of receptors designated EP1, 2, 3, and 4. The aim of this study was to investigate the expression of COX-2, EP2, and of mPGES-1 in a series of healthy, hyperplastic, and neoplastic canine mammary tissues.

Material and methods: The expression of these markers was investigated by immunohistochemistry in 69 mammary tissue samples (6 healthy mammary glands, 22 hyperplastic tissues, 5 benign, and 36 malignant tumors) using the streptavidin-biotin peroxidase method. The immunolabelling was scored according to previously published criteria in veterinary (COX-2) and human literature (EP2 and mPGES-1).

Results: The percentage of COX-2 expressing malignant tumours (83%) was significantly higher than that of healthy and hyperplastic tissues and benign tumours. None of the healthy tissues were found to express EP2, while thirty-two hyperplastic tissues (32%) scored positive. The percentage of EP-positive benign (40%) and malignant tumors (64%) was not significantly different. The expression of mPGES-1 was observed in one sample of healthy tissue and absent in all the hyperplasia and benign tumors examined. Sixty-five percent of the malignant tumours were strongly immunoreactive

Discussion: COX-2, EP2 and mPGES-1 were overexpressed in a high percentage of malignant tumors and EP2 receptor was frequently expressed also in hyperplastic lesions and benign tumours. Since the aberrant upregulation of COX-2 enzyme and the consequent expression of mPGES-1, resulting in accumulation of PGE₂ in a cancer cell environment, is a marker for progression of human breast cancer, our data suggest the need of further studies on the mechanism of PGE₂ regulation also in veterinary oncology. Further investigations may in fact have broad implications for the prevention and treatment of canine mammary tumors by antagonizing COX/PG signaling.

Hepatopathies, assessment criteria of meat integrity in abattoir slaughtered lambs

Militaru, Manuella, Dumitru Militaru, Georgeta Dinescu, Teodoru Soare, Emilia Ciobotaru, Beatrice Stirbu Teofanescu

Faculty of Veterinary Medicine, Bucharest, Romania

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Introduction: One of the most important causes of hepatopathies diagnosed in abattoir slaughtered young sheep is represented by hepatic parasites or those which have migratory phases into the liver. Legislation stipulates partial or total rejection of the liver. Most of the time, liver rejection is not always connected with restrictive measures on the carcass. According with this idea this present study was focused to find any correlation between liver injuries and integrity of meat, metabolic profile and identification of anti-*Toxoplasma gondii* antibodies in abattoir slaughtered lambs.

Material and methods: Animals: 80 lambs for pathological investigation of liver and biochemical investigation of sera; 727 samples of sera for serological investigation; 30 samples of meat for chemical investigation. The methods of study are represented by pathological investigation of liver (gross, cytological – imprints May Grunwald Giemsa stained and histological investigation – fixation in 10% formaldehyde solution, paraffin embedding, Masson trichromic stain); serological investigation for detecting anti-*T. gondii* antibodies using ELISA, indirect method; biochemical investigation of sera for establishing metabolic profile (total protein, TGO, TGP, alkaline phosphatase) and chemical investigation to determine meat integrity (protein, humidity, easy hydrolyzed nitrogen, fat and ashes).

Results: Grossly, 40% of examined lambs presented a normal liver, the rest of the cases exhibiting dominantly cholangiohepatitis produced by *Dicrocoelium lanceolatum* and parasitic migratory hepatitis induces by *Cysticercus tenuicollis* (tenuicollis tracts). Cytologically, 90% of cases presented a polymorphous inflammatory population. Histopatologically, cholangiohepatitis were dominant associated with bile duct hyperplasia, multifocal inflammatory reaction in portal area and parasitic compact granulomas. Histological investigation considered also an injury score concerning general architectural aspect, injuries of hepatocytes, reaction of Kupffer cells, morphology of sinusoids and Disse space, involvement and lesions of portal area. According to the corresponding score, there were minor hepatopathy (4-5 points), medium hepatopathy (6-10 points) and severe hepatopathy (11-18 points). Considering this score, 50% of cases presented medium hepatopathy and 20% severe hepatopathy. Serological investigation proved that 24% of cases were positive to IgG anti-*T. gondii*. There were no major deviation concerning metabolic profile and chemical investigation of meat presented normally ranged values.

Discussion: Analyzing the finding, a direct correlation occurred between gross, cytological and histological features in lambs diagnosed with severe hepatopathies. Parasitic hepatopathies in abattoir slaughter lambs did not influence significantly metabolic profile of lamb, chemical profile of meat being normal. According to the results of serological investigation, we consider lambs as a natural reservoir of *T. gondii*.

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Beta-catenin expression in canine normal skin and epithelial skin tumours

Miragliotta, Vincenzo, Michela Gregori, Saverio Meini, Fabrizio Fabbrini, Maria Paola Ricciardi, Francesca Abramo

Department of Animal Pathology – University of Pisa, Pisa, Italy

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Introduction: β -catenin is the central mediator in the canonical wingless (Wnt) signaling pathway, which exerts remarkable control over cellular phenotype and behaviour. Activation of the Wnt pathway leads to cytosolic stabilization of β -catenin that subsequently translocates to the nucleus where it functions as a transcriptional co-activator of T cell factor/lymphoid enhancer factor (Tcf/Lef) target genes. Strong nuclear or cytoplasmic β -catenin staining has been confirmed to be frequent cause of Wnt signalling pathway activation in several human tumours including pilomatricoma.

Material and methods: Samples were obtained from the dermato-histopathology archive of the Department of Animal Pathology of the University of Pisa. The study included 5 samples of normal skin and 5 for each of the tumour types originating from epidermis and follicles classified according to the WHO criteria (1998). Immunohistochemistry was performed by the streptavidin-peroxidase method using a mouse monoclonal anti- β -catenin antibody.

Results: *Normal skin:* basal and supra-basal keratinocytes showed a diffuse membrane staining, with focal weakly positive nuclei in the basal layer. Follicular matrix cells and dermal papilla showed nuclear reactivity while membrane staining characterized hair root sheaths, sebaceous and sweat glands. A weak reactivity of endothelial cells was detected in the dermis. *Viral papillomas:* nuclear, cytoplasmic and membrane staining was detected. Stained nuclei belonged to spinous layer keratinocytes. Granular and corneal keratinocytes progressively lost the nuclear staining while they maintained the cytoplasmic and membrane ones. *Squamous cell carcinomas:* neoplastic keratinocytes showed strong membrane reactivity and lack of nuclear positivity. *Basal cell carcinomas:* only membrane and occasional nuclear staining was seen. *Infundibular keratinizing acanthomas:* weakly reactive nuclei were visible in the cells organized in rows while cells forming the corneal pearls were negative. *Trichoblastomas:* a prevalent membrane pattern with complete lack of nuclear staining was visible. *Trichoepitheliomas:* diffuse cytoplasmic staining was detected and neoplastic cells facing the cysts showed nuclear reactivity. *Pilomatricomas:* basaloid cells of the cyst walls showed nuclear staining whose intensity increased towards the cyst lumen. Ghost cells showed lack of staining. *Matrical carcinomas:* a strong nuclear reactivity was detected. Focal cytoplasmic and weak membrane staining were also observed.

Discussion: as in humans, beta-catenin nuclear expression identifies both normal and neoplastic follicular matrix cells. While nuclear immunoreactivity was detected in all trichoepitheliomas and pilomatricomas it was particularly evident in their malignant counterparts (matrical carcinomas) thus representing a useful as a marker of malignancy. Despite a moderate β -catenin nuclear immunoreactivity was occasionally seen in other epithelial tumour types, its expression always involved a limited number of nuclei.

Pathological effects of quinazolinones on the brain of newborn Balb/C mice

Moayer Fariborz*, Maryam Shams Lahijani**, Elham S. Hossein Pour**

*Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Iran

**Department of Zoology (Developmental Biology), Faculty of Biological Sciences, University of Shahid-Beheshti, Tehran, Iran

Introduction: Quinazolinones are heterocyclic components with various biological activities and pharmacological consumptions such as anti-inflammation, anti-blood pressure and sedative. In this survey, the teratogenic effects of two new synthetic derivatives of Quinazolinone including *4(3H) quinazolinone-2-propyl-2-phenyl ethyl* (QPPE) and *4(3H) quinazolinone-2-ethyl-2-phenyl ethyl* (QEPE) were performed on CNS of newborn Balb/C mice as an animal model.

Material and methods: 100 pregnant Balb/C mice (pregnancy detected by observation of vaginal plaque) were randomly divided into 4 groups of control, sham and experimental 1 & 2 which received intraperitoneally (IP), 10 ml/kg distilled water (control group), 10 ml/kg methyl cellulose 0.05% (sham group) and 100 mg/kg QPPE, QEPE (experimental groups 1 & 2). After euthanasia, newborn brains were removed (4 days after birth) and weighted. Brain samples (cortex, midbrain, medulla and cerebellum) were fixed in neutral buffered 10% formalin, routinely embedded in paraffin and stained with H&E.

Results: Histopathological examination indicated an increase in the number of astrocyte and microglial cells (gliosis) in cortex and demyelination in medulla, of cerebrum in experimental groups 1 & 2 which treated with (QPPE), (QEPE). Statistical analysis showed no significant difference between average weight of brain in all of groups, but there was significant in number of astrocyte, microglial cells and demyelination in experimental groups.

Discussion: Quinazolinones are heterocyclic and aromatic components, which pass through placenta barrier by simple diffusion, along a chemical gradient. Central Nervous System is one of the main organs that received QPPE, QEPE. Investigation of the effects of Quinazolinones on fetus because of their consumptions during pregnancy can be useful to detect congenital abnormalities due to teratogens. This study showed that Quinazolinones could be the cause of pathological changes in brain of newborn Balb/C mice.

Common astrocytic reactions in CNS injury are increase in size and number. The responses of microglia to injury include hypertrophy, hyperplasia, phagocytosis of cellular and myelin debris and neurophagia. Demyelination, may be occurred due to circulatory and physical disturbances. Some possible mechanisms include compression myelin, CNS ischemia, disease processes resulting in the accumulation of extracellular fluid and congenital defects. The mechanism of the effects of Quinazolinones on the embryonic cells is not clear yet, but there are quite a few reports showing its toxic characteristics. They inhibit polymerization of tubulin and pass through placental barriers, so there is a possibility that it has some sort of toxic and teratogenic effects on embryos. It is well known that quinazolinones are lipophilic agents and pass through the biological membranes quite easily. Exogenous aromatic hydrocarbon, such as methaqualone, a member of the quinazolinone drug family is bound to the aromatic hydrocarbon receptor (AHR). The complex of AHR and aromatic hydrocarbon is transferred into nucleus and activates several genes, such as: Cyp1A2 and Cyp1A1. It appears that QPPE, QEPE as an exogenous aromatic hydrocarbon, acts in this manner. Jeffery et al. suggested that AHR is coded before implantation and interacts with cell proliferation and differentiation in mice embryos.

Investigation on histopathological findings and serological methods for evidence of leptospiral abortion in Tehran dairy herds

Moayer, Fariborz*, Ourang Ataee**, Farhad Moosakhani

*Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Iran

**Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Iran

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Introduction: Leptospirosis is an economically important zoonotic bacterial infection of dairy herds that causes abortions, stillbirths, infertility and loss of milk production. In this survey, Leptospirosis was detected in 17 cases of aborted fetuses from dairy herds of Tehran submitted from Summer 2006 to Spring 2008 on the basis of histopathological findings, microscopic agglutination test (MAT) and enzyme-linked immunosorbant assay (ELISA) for antibody detection.

Material and methods: To detect the cause of abortion in Tehran dairy herds, 180 aborted fetuses were submitted for necropsy, microscopical examination, MAT and ELISA test. Tissue samples were obtained from the brain, heart, liver, spleen, kidney and lung and were fixed in 10% neutral buffered formaldehyde routinely embedded in paraffin and stained with H&E. To detect antibodies in dams, sera samples obtained from cows, were examined by MAT and determined by ELISA test.

Results: Gross findings during necropsy were non-specific, but in a few aborted fetuses placental lesions, which was usually limited to edema, were seen. Fetal lesions often were mild and obscured by autolysis. Some aborted fetuses expelled near term without extensive autolysis have gross lesions of ascites and fibrinous peritonitis. Microscopic lesions were including subacute interstitial nephritis and subacute necrotizing hepatitis.

Leptospirosis was diagnosed in 17 out of 180 aborted fetuses based on gross findings and microscopic examination. In MAT, thirty-one of the 180 dam's sera had agglutinating antibody titers >400 and leptospirosis was diagnosed in them but leptospiral infection was found to be positive of two serovars: *pomona* & *hardjo* in 17 sera of dams in ELISA test.

Discussion: Leptospirosis occurs worldwide and is caused by various species of *Leptospira*, a spirochete in the family Leptospiraceae, order Spirochaetales. The pathogenic leptospirae were formerly classified as members of the species *Leptospira interrogans*. The predominant serovars vary by geographic region and most common serovars are *L.canicola*, *gripotyphosa*, *hardjo* and *pomona*. Chronic infection is manifested as abortion, stillbirth, and the birth of premature and weak infected calves. *Hardjo* infection is associated with infertility (early embryonic death) and abortion (4 month to term), while *pomona* infection is associated with abortion during the last trimester. The most commonly used serologic tests to Leptospirosis diagnosis, are MAT & ELISA. The MAT test is serogroup but not serovar specific, and can be complicated by cross-reactions. In this study, the cause of abortion in 17 out of 180 cases (9.4%) was diagnosed by gross and histopathological findings and the involved serovars were detected *L.pomona* (2 cases = 11.8%) and *hardjo* (15 cases = 88.2%) in ELISA test.

A number of factors complicate interpretation of leptospiral serologic results. These factors include cross-reactivity of antibodies, antibody titers induced by vaccination, and lack of consensus about what antibody titers are indicative of active infection.

Preliminary findings in a case of Onchocercosis in Italian wild red deer (*Cervus elaphus*)

Morandi, Federico, Roberta Galuppi, Sandro Nicoloso, Barbara Brunetti, Barbara Bacci, Giuseppe Sarli, Renato Giulio Zanoni, Maria Paola Tampieri, Cinzia Benazzi

School of Veterinary Medicine, Bologna University, Ozzano Emilia, Bologna, Italy

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Introduction: Onchocercosis is a vector-transmitted (simuliids) parasitic disease which can be caused by 28 species of Nematoda belonging to the Genus *Onchocerca*. It involves wild and domestic ungulates, humans (*O. volvulus*), and dogs (*O. lupi*). Red deer (*Cervus elaphus*) host numerous *Onchocerca* sp. and only few of them have been reported in European countries but never in Italy. The most important species described in European deer are *O. flexuosa*, *O. jakutensis* or *tubingensis* and *O. tarsicola*. They have definite locations in the host and only two of them, *O. flexuosa* and *O. jakutensis*, live inside subcutaneous nodules.

Material and methods: During the early hunting season 2007-08, between September and November, in the Pistoia Province (Tuscany, 44°00'N, 11°00'E), subcutaneous nodules were observed on both thighs of red deer. During necropsy of four subjects, four nodules were collected for histopathological study, fixed in 4% buffered formalin, paraffin-wax embedded, sectioned at 4 micron and routinely stained with haematoxylin and eosin. Ten were sampled for microbiological and parasitological observations.

Results: The nodules were 8 or more per thigh, about 1.5-3.5 cm in diameter and the cut section showed a yellowish mucinous material with some long, thin, friable firmly intricated, white filamentous worms. No bacteria were isolated from the nodules. Histological examination evidenced cystic structures surrounded by a fibrous capsule, containing nematodes. Many eosinophilic and few neutrophilic granulocytes were present among the parasites, and macrophages infiltrated the fibrous capsule. In the uterus of nematodes some larval structures were evident. In the nodules females and males were irregularly interwoven. Females were longer than 19 cm. The cuticle showed superficial transversal relieves (range 44 to 57 micron) among which, in a deeper layer, 3-4 stripes were present. The tail (about 161 micron in length and 78 in thickness) showed a clublike shape with two phasmides. Males had many cuticular stripes and ended coiled up and two spiculae were present. These structures were measured in one male: the longest spicula was 310 micron and the shortest 110 micron. Moreover, the length of five microfilariae was within a range of 250-270 micron.

Discussion: In red deer, back and flank nodules can be caused only by two *Onchocerca* species, namely *O. flexuosa* and *jakutensis*. A third, *O. tarsicola* presents some similarities regarding cuticular aspects and shape of the tail of females (clublike) and males (coiled up) (Bain and Schulz-Key, 1974), but differ for type of lesions (*O. tarsicola* does not cause nodules) and location (this species is described free in the subcutaneous tibio-tarsal and radio-carpal articular tissue). Besides, the length of the spiculae of the male and the length of the microfilariae would exclude *O. tarsicola*. In fact the spiculae of *O. tarsicola* are 200 and 130 micron long, and microfilariae are 400 to 430 micron. In the nodules described in the present study the measurements were respectively 310 and 110 micron for the longest and shortest spiculae, and 250-270 micron for the microfilariae, the same reported in the literature for *O. flexuosa* and *jakutensis* (Bain and Schulz-Key, 1974). In the progress of our study, which is the first report of deer Onchocercosis in Italy, we intend to carry on a genetic study for a molecular diagnosis and a taxonomic location of this species.

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Ultrastructural study of cerebellum with methylmercury chlorid poisoning in newborn rat

Mortazavi, Pejman, Taki-Altirahi

Department of pathology, Faculty of specialized veterinary sciences, Islamic azad university, Science & research branch, Tehran, Iran

P87

Introduction: Methylmercury (MeHg) is a well- known environment pollutant toxic to the nervous tissue, particularly during development. At a low concentration Methylmercury chloride (MMC) can be transferred to the fetus through the placenta and to newborn offspring through breast milk. The purpose of this study were to evaluate ultrastructural alteration in the cerebellum of newborn rat on the 25th postnatal days.

Material and methods: Adult Female wistar rats were inoculated with MMC (0.5,4.5, 10mg/ kg /Hg/day) on the 12th, 13th and 14th gestational days. The newborn offspring were placed with the mothers until postnatal day 25. Newborn rats were observed for clinical signs and motor behavior daily. No changes were observed in the clinical signs and motor behavior of these animals. cerebellum were fixed with 2.5% gluteraldehyde in 0.1 M phosphate buffer and routinely processed for electron microscopy.

Results: Granular cells layer were decreased and purkinje cells were larger than normal condition. The component cells of the cerebellum male offspring in the 0.5 , 4.5 , 10 mg /kg groups showed several ultrastructural alteration, including: chromatin migration to the edge of nuclei ,dilation of the smooth endoplasmic reticulum, ribosomes to shed from the surface of RER. Mitochondria were rounded with condensed matrical spaces and expanded intercrystal space. and the matrix was condensed. With TUNEL staining, apoptosis is confirm in granular cells layer.

Discussion: Our observation confirm that MMC induces chromatin migration to the edge of nuclei in granular cells layer and this alteration relation to apoptosis.

A rare case of septicemia due to *Bacteroides melaninogenicus* in a Holstein cow

Movasseghi, Ahmad Reza, Mehrnaz Rad

Excelence Center for Ruminant Abortion and Neonatal Mortality, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

P39

Introduction: Black-pigmented Gram-negative anaerobic rods are found on mucosal surfaces as indigenous flora. With mucosal damage due to disease, trauma or surgery, these organisms may invade tissues and set up infection.

Material and methods: A 6-year-old Holstein cow was referred for necropsy due to sudden death. Systemic post mortem examination was conducted and tissue specimens were fixed in 10 percent neutral buffered formalin for histological examination. Specimens of blood and liver tissue were taken for bacteriological analysis.

Results: At necropsy, there were hydroperitoneum, hydropericardium, petechial hemorrhage on serosal surfaces, pulmonary edema, multiple pale foci on the kidneys and severe hepatic necrosis associated with multiple abscesses. Histopathological examination revealed extensive hepatocellular necrosis with neutrophilic infiltrates and abscess formation, epicardial hemorrhage and multiple renal infarcts. Cultures of the blood and liver lesions grew *Bacteroides melaninogenicus* exclusively.

Discussion: *Bacteroides* are generally resistant to a wide variety of antibiotics, so this high level of antibiotic resistance has prompted concerns that *Bacteroides* species may become a reservoir for resistance in other, more highly pathogenic bacteria.

Pathological study of experimental lead poisoning in sheep

Movasseghi, Ahmad Reza, Mohammad Reza Aslani, Hadi Mohebalian

Excelence Center for Ruminant Abortion and Neonatal Mortality, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad / Iran

P82

Introduction: Lead is a common cause of poisoning of domestic animals throughout the world. Livestock may find lead in rubbish dumps and around farm buildings and machinery. Stock may find sump oil and other sources of lead attractive as lead compounds can have a sweet taste. Among farm animals, ruminants are affected most often by lead toxicity, followed by horses, poultry, and swine.

Material and methods: For pathomorphological investigations of experimental lead poisoning, six sheep were taken 1 gm/kg of lead acetate orally and the animals were euthanized after two weeks. Tissue specimens from brain, liver, kidneys, heart and gastrointestinal tract were taken for histopathological examination.

Results: The clinical signs included muscle spasms, excessive response to external stimuli, dullness, loss of appetite, abdominal pain and mild diarrhea. At necropsy, there were just hyperemia and edema of the central nervous system with hemorrhagic foci in thalamus. The main histological changes were characterized by perivascular and perineuronal edema, laminar neuronal necrosis, status spongiosus and perivascular hemorrhage in cerebrum; granular degeneration in renal tubules and glomerular endothelial proliferation in kidneys; vacuolar degeneration and single cell necrosis of hepatocytes associated with acid-fast intranuclear inclusion bodies and activation of Kupffer cells in liver and severe hemorrhage between cardiac muscle fibers.

Discussion: It is concluded that one high dose exposure of lead can cause acute toxicosis in sheep and lead poisoning should be noted in every central nervous related signs in this species, and its possible cardiac lesions must be considered as well.

Involvement of Matrix Metalloproteinases and Anti-Apoptotic Factors for malignant characters in Canine Spontaneous Hemangiosarcoma

Mami Murakami, Hiroki Sakai, Atsushi Kodama, Takashi Mori, Tokuma Yanai, Kouji Maruo, Toshiaki Masegi

Gifu University, Gifu, Japan

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Introduction: Hemangiosarcoma (HSA) is a highly invasive malignant tumour of vascular endothelial cells (ECs). Its microenvironment, such as the expression of vascular endothelial growth factors, basic fibroblast growth factors, and their receptors, is similar to that in angiogenesis. Angiogenesis involves programmed dedifferentiation of sprouting ECs, allowing proliferation and extracellular matrix (ECM) invasion, which is caused by the production of degenerative enzymes such as matrix metalloproteinases (MMPs). Apoptotic stimuli-sensitive sprouted ECs escape apoptotic mechanisms by binding to the provisional ECM. We hypothesized that the infiltration and proliferation mechanisms in HSAs and angiogenesis are similar and investigated the structure of the basement membrane (BM), expression of MMPs, and activities of anti-apoptotic factors.

Material and methods: Eighty three canine HSAs, 24 hemangiomas, and 11 granulation tissues (GTs) containing active ECs were examined immunohistochemically for the expressions of type IV collagen and laminin to analyse the BM components; expressions of MMP-2, MMP-9, and membrane type 1 MMP (MT1-MMP) to study the association between MMPs and BM degradation; and for bcl-2 and survivin expressions (anti-apoptotic factors). We performed gelatin zymography to analyse MMP activities, using 25 HSAs, 8 hemangiomas, and 2 GTs. Real time RT-PCR was performed to examine the expression of the bcl-2 and survivin mRNAs, using 19 HSAs and 8 hemangiomas.

Results: For type IV collagen and laminin, many HSAs showed discontinuous linear or negative immunoreactivity in the BM (type IV collagen, 49.4% or 14.5%; laminin, 60.3% or 10.8%) and positive cytoplasmic immunoreactivity (type IV collagen, 97.6%; laminin, 91.6%). In contrast, almost all hemangiomas and mature vessels in GTs showed continuous linear staining in the BM. Although MMP-9 immunoreactivity was not detected in neoplastic as well as active angiogenic ECs, MMP-2 immunoreactivity was detected in all ECs in GTs and in neoplastic ECs of both HSAs and hemangiomas. A strong MT1-MMP immunoreactivity was observed in active angiogenic ECs in GTs and in neoplastic ECs in HSAs (65.1%); however, almost all hemangiomas showed weak or negative MT1-MMP immunoreactivity. Gelatin zymography revealed significantly strong activity of active MMP-2 in HSAs, which was similar to that in active angiogenesis in GTs; however, weak or no activity of active MMP-2 was detected in hemangiomas ($P < 0.01$). Immunoreactivity for bcl-2 was observed in 50 of the 83 HSAs (60.2%) but not in hemangiomas. The average survivin-positive index was 24.7% in the HSAs and 0.64% in the hemangiomas. In contrast to the upregulated average survivin mRNA expression, which was approximately 6 times that for hemangiomas, no significant difference was observed in the average bcl-2 mRNA expression between HSAs and hemangiomas.

Discussion: It was suggested that the BM surrounding neoplastic ECs in HSAs was degraded by MT1-MMP-activated MMP-2, and the increased production of BM proteins was due to a positive cytoplasmic reaction, which was associated with the high HSA infiltration. The bcl-2 and survivin expression may also prolong the survival of malignant ECs in HSAs. These

findings indicated that the anti-apoptotic mechanisms were regulated by ECM alterations and influence of growth

An unusual squamous cell carcinoma in a sheep

Namjo, A.¹, Nourani, H.² and Farid, M.³

¹Department of Pathobiology, School of Veterinary Medicine, Islamic Azad University of Shahrekord, Shahrekord, Iran

²Department of Pathobiology, School of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran

³Department of Clinical Sciences, Islamic Azad University of Shahrekord, Shahrekord, Iran

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Squamous cell carcinoma is a malignant tumor of epidermal cells in which the cells show differentiation to keratinocytes. The tumor is common in the horse, cow, cat, and dog, but relatively uncommon in the sheep, and rare in the goat and pig. Oral squamous cell carcinomas occur particularly in old cats and dogs. They may involve the epithelium of the tonsillar crypts, the gingiva, or the lateral margins and ventral surface of the tongue. A 3-year-old ewe due to presence of an oral haemorrhagic mass with the history of reduced appetite, weight loss and halitosis was referred to the Veterinary clinic of Islamic Azad University of Shahrekord. Grossly, the tumor mass was located in the floor of mouth posterior to the incisors and caused displacement of the left second intermediate and corner incisors. The ulcerated mass, measuring approximately 4.5×2.5 cm, was irregular, cauliflower like, red-black, friable, and did not show metastatic spread to the regional lymph nodes. Mild bone destruction was seen in the radiogram of mandible adjacent to the tumor. For histopathological examination, biopsy was performed, processed routinely and stained with haematoxylin and eosin. Histopathological examination of the excisional biopsy revealed irregular cords and clusters of pleomorphic neoplastic cells. These cells had vesicular nuclei, prominent nucleoli, and abundant cytoplasm. There was no evidence of keratin pearl formation and only a few individual cells keratinization were observed. Nuclear hyperchromatism, karyomegaly, and numerous mitotic figures were seen in the some sections. Based on gross and histopathological characteristics, the tumor was diagnosed as poorly differentiated oral squamous cell carcinoma. To the best of our knowledge, only one squamous cell carcinoma of the gum has been reported in the sheep and this case is unusual due to the site of development.

Key words: oral squamous cell carcinoma, pathology, sheep

Malignant lymphoma with intrahepatocellular invasion of neoplastic cells in the cats

Narana, Isao, Naoko Ano, Kohji Nomura and Kiyokazu Ozaki

Setsunan University, Hirakata, Japan

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Introduction: Intracellular invasion of the neoplastic cells is a characteristic feature of some special types of malignant lymphoma such as epitheliotropic cutaneous lymphoma and primary intestinal lymphoma in dogs and cats. However, intrahepatocellular invasion of neoplastic cells had been rarely reported in malignant lymphoma of any species of animals. We encountered 6 cases of feline malignant lymphoma showing intrahepatocellular invasion of neoplastic cells and examined histopathologically and immunohistochemically.

Material and methods: Six affected cats were consisted of one male and five castrated females. The age ranged 6 to 15 at the onset of disease. Only one case was necropsied and examined systemically. Small pieces of liver tissue were biopsied surgically from remaining 5 cats in animal hospitals and sent to our laboratory for histopathological examination. Blood films from two cases, and lymph nodes or small neoplastic nodules in peritoneal cavity from each one case were also examined.

Results: In all 6 cases, neoplastic lymphocytes were observed in the cytoplasm of hepatocytes other than sinusoid, interlobular vein and interstitium of Glisson's sheath. The nuclear size of the tumor cells was smaller than those of hepatocytes in 5 cases but comparable or much larger in one case. The nuclei of neoplastic cells had much amount of deeply stained chromatin and easily distinguishable from those of hepatocytes. The neoplastic cells had scanty eosinophilic cytoplasm. The size and shape of neoplastic lymphocytes varied from case to case and cell by cell within a case. A large number of neoplastic lymphocytes had round or ovoid nuclei but elongated or cleaved in some cells. The number of invaded cells was one to several per one hepatocyte. Mitotic figures are also observed among the invading neoplastic cells. Neutrophils and other segmented nuclear granulocytes increased in the sinusoid and emigrated into some hepatocytes and bile ducts. Some hepatocytes engulfing the neoplastic cells had lipofuscin pigment or clear vacuoles in the cytoplasm. However apparent morphological changes suggesting the cell death were not detected in these hepatocytes. Neoplastic cells invaded also into the epithelium and lumen of bile duct in two cases. Proliferation of bile ducts and perlobular hepatocytes were observed in two cases. Varying amounts of yellow pigments engulfed in Kupffer cells in many cases.

Immunohistochemically, neoplastic lymphocytes were positive for CD3 in 4 cases but negative in 2 cases. Apparent positive reaction for CD79, CD56, CD20, was not observed in tumor cells in all 6 cats. Tumor cells were detected in the blood films from two cases.

Discussion: The morphological characters of present cases were identical to previously reported two cases of feline malignant lymphoma (Ossent P et al. 1989, Vet. Pathol. 36:279-280). Immunohistochemical examination revealed that neoplastic cells in some cases of this type of malignant lymphoma are derived from T-cell.

Gene expression profiling of spleens of SJL/J mice experimentally infected with Theiler's murine encephalomyelitis virus

Navarrete-Talloni, María José, Arno Kalkuhl, Reiner Ulrich, Ulrich Deschl, Wolfgang Baumgärtner, [Andreas Beineke](#)

Institute of Pathology, University of Veterinary Medicine, Hannover, Germany

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Introduction: Theiler's murine encephalomyelitis (TME) is a chronic inflammatory demyelinating disease, induced in SJL/J mice via intracerebral injection of the Theiler's encephalomyelitis virus (TMEV) BeAn strain. TME shares many clinical and pathological features as well as immunological aspects with multiple sclerosis (MS) and canine distemper virus, therefore serving as an infectious model for animal and human demyelinating diseases. The aim of the present study was to detect genes and pathways involved in peripheral immune responses and immunoregulation, associated with induction and progression of this disease by gene expression profiling.

Material and methods: 48 female SJL/J mice were sham-inoculated or infected with the BeAn strain of TMEV. Animals were euthanized at 14, 42, 98 and 196 days post infection (dpi). Spinal cord was removed for histological examination and spleen was further processed for microarray analysis. From spleen, ribonucleic acid (RNA) was isolated, amplified, labelled and hybridized to Affymetrix GeneChip® Mouse Genome 430 2.0 arrays. Primary microarray data were normalized with RMA normalization. Filtering of these data included t-tests ($p \leq 0.05$) and fold change at 1.5/-1.5. After an explorative first approach, a detailed analysis was performed, where 34 signalling pathways involved in inflammation and immunoregulation were selected using Ingenuity Pathway Analysis and DAVID ontology annotation tool. In addition, selected pathways were compared among different time points.

Results: Pathohistological examination of the spinal cord revealed an increasing inflammation and demyelination from 14 until 196 dpi in TMEV-infected mice. Filtering of the 45101 gene chip annotations resulted in 1039 differentially expressed genes in the spleen of TMEV-infected mice at 14 dpi. A total of 1050, 781 and 677 genes were differentially expressed in these animals at 42, 98 and 196 dpi, respectively. From the selected pathways, cAMP pathway was significantly enriched at all time points. IL-6 pathway was found to be significantly upregulated at 14 dpi, and downregulated at 42 and 196 dpi. Leukocyte extravasation pathway was upregulated at 14, 98 and 196 dpi. Among other pathways, chemokine, Natural Killer (NK) cell, T cell receptor and complement system pathways were significantly enriched at 14 and 196 dpi. Apoptosis pathway was downregulated at 42 dpi and upregulated at 196 dpi. B cell receptor pathway was downregulated at 42 and 196 dpi.

Conclusions: This study revealed for the first time several significantly changed genes and pathways of the peripheral immune response related to an altered immunological status following TMEV infection. The data provide an overview of candidate gene expression at different phases of TME. Further analyses will provide a more comprehensive understanding of the prevailing processes in this infectious model for demyelinating diseases.

Changes in systemic and placental cytokine expression during infection with *Chlamydomydia abortus* in two murine strains

Navarro, Jose A, Antonio J Buendía, Carlos M Martínez, Joaquin Sánchez, Maria R Caro, Nieves Ortega, Jesus Salinas

Universidad de Murcia, Facultad de Veterinaria. Campus de Espinardo, Murcia, Spain

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Introduction: A Th1 response is necessary for the control of the *C. abortus* infection. IFN- γ have a key role in the response against these bacteria; the main anti-chlamydial action of this cytokine is based on the depletion of the aminoacid tryptophane by the activation of IDO. In the response to *C. abortus* Th1-cytokine activity is controlled by other immunosuppressor cytokines. Because these cytokines are produced in the placenta the aim of this study is to know the their role using pregnant mice from two strain with different susceptibility to *C. abortus*.

Material and methods: A mouse model of intragastric infection with *C. abortus* was established, being pregnant mice intragastrically inoculated with a feeding needle at day 7 of gestation. Two different strain of mice were used: a relatively resistant strain, C57BL/6 (C57) and a highly susceptible strain, CBA. Mice were killed at days 3, 6 and 9 post-infection, and then, two days after abortion or parturition. *C. abortus* shedding was confirmed by McCoy cells culture from vaginal swabs after abortion or parturition. Samples from liver, spleen and placenta or uterus were collected and fixed with zinc fixative. Immunohistochemical techniques (ABC or TSA) were used to demonstrate chlamydial antigen and the expression of pro-inflammatory and anti-inflammatory cytokines in the organs studied.

Results: The CBA mice showed a low percentage of abortion (2/10) while no abortions were observed in the C57 mice. When the vaginal shedding of *C. abortus* after abortion or parturition was analyzed, the 100% of CBA mice showed positive isolation in the vaginal swabs at days 1 and 2 post abortion or parturition. No *C. abortus* vaginal shedding could be detected in the C57 mice after parturition. *C. abortus* antigen was observed in liver and spleen associated with granulomatous hepatitis and splenic hyperplasia at day 9 pi in both mouse strains. The inflammatory foci in the C57 mice showed an increased number of neutrophils but a lower chlamydial antigen in relation with the CBA mice. Immunohistochemical analysis of the cytokine expression showed a higher presence of IFN- γ in the liver and spleen of the C57, while that CBA mice had a higher expresion of TNF- α and IL-10 in the liver and spleen. Presence of chlamydial antigen and inflammatory infiltrate in the placenta were observed at day 9 pi in all CBA mice and in the 75% of C57 mice, although C57 mice showed lower antigen and histopatohological changes. In both mouse strains a clear decrease in the detection of IDO, IFN- γ , IL-6 and IL-10 was observed from day 13 to day 16 of pregnancy (6 to 9 pi), coinciding with the onset of the placentitis. These changes seem to be unrelated with the infection, since were also observed in control non-infected mice.

Discussion: The increased susceptibility of CBA mice seems to be related with a lower systemic production of IFN- γ . Changes in cytokine expression are not related with the infection, but could favour the multiplication of *C. abortus* in this organ in the last days of pregnancy.

Descriptive epidemiology of canine neoplasia: a retrospective study of a histopathology laboratory database (2000-2005, France, 67087 dogs)

Nguyen, Frédérique, Dessimoulie Anne-Sophie, Lagadic Marie, Albaric Olivier, Poujade Agnès, Abadie Jérôme

National Veterinary School of Nantes, NANTES cedex 3, France

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Introduction: The most recent epidemiologic data regarding canine oncology in France were performed in the 1990s. Since then, the canine population (ageing, represented breeds) as well as veterinary practice (improvement of medical imaging, diagnostic and therapeutics) have evolved, and the tumour prevalence has increased. The objective of our study was to update epidemiologic knowledge of canine cancer in France.

Material and methods: Retrospective study based upon the database of the Veterinary Histopathology IDEXX laboratory located in Alfortville, France, which receives samples from the whole country.

Results: During the 01-jan-2000 to 31-dec-2005 period, 92198 samples from 67087 dogs were examined microscopically. The tumours (40500 cases) accounted for 43.9% of the samples and comprised 18563 benign (45.8%) and 21937 malignant (54.2%) tumours. There is a gender predilection of females for tumours (odd ratio OR=1.77; $P<0.001$), benign tumours (OR=1.43; $P<0.001$) and malignant ones (OR=1.41; $P<0.001$), an unsurprising result linked to the high prevalence of mammary tumours. The age of tumour-bearing dogs (mean 9.3 ± 3.2 years; median 9.7) is higher than the age of tumour-free dogs (mean 7.6 ± 3.7 years; median 7.9). The relative risk to bear a tumour progressively increases up to 13 years, then reaches a plateau at relative risk= 1.2 (reference= 1 for 6-8 years old dogs). Among the 201 canine breeds represented in the database, 12 show a predilection for tumours, including the Poodle (OR=1.58), Fox Terrier (OR=1.37), Siberian Husky (OR=1.31), Cocker Spaniel (OR=1.25) and Yorkshire Terrier (OR=1.19) breeds. On the contrary, 33 breeds show a significantly lower risk to bear a tumour, such as the Bichon (OR=0.85), Shih Tzu (OR=0.74), Golden Retriever (OR=0.71), Rottweiler (OR=0.67) and Cavalier King Charles (OR=0.45) breeds [$P<0.01$ for all]. The 10 most commonly diagnosed tumour types in the database are: 1. Mammary adenocarcinoma (6057 cases). 2. Mammary adenoma (4539 cases). 3. Testicular interstitial cell tumour (1609 cases). 4. Cutaneous sebaceous adenoma (1481 cases). 5. Oral malignant melanoma (1476 cases). 6. Testicular seminoma (1437 cases). 7. Cutaneous histiocytoma (1357 cases). 8. Cutaneous mast cell tumour (1233 cases). 9. Nodal malignant lymphoma (1210 cases). 10. Cutaneous or subcutaneous lipoma (940 cases).

Discussion: One of the advantages of a histopathology-based epidemiologic survey of tumours is that all the diagnoses are confirmed histologically. However, a bias exists due to an over-representation of surgically accessible tumours (mammary gland, skin, oral cavity) as well as malignant tumours (more commonly sampled for analysis than benign ones).

Compared to previous data collected in France, there is an increase in the relative proportion of benign tumours (now more prevalent than malignant ones in a histopathology laboratory), and new (popular) breeds appear in the dogs at high or low risk for tumour onset.

Diskospondylitis in mink (*Mustela vison*): A preliminary study in Spanish mink farms

Nieto, J.M., López-Peña, M, Faílde, LD(1), Astorga, J(2), Barreiro, A., Espino, L, Fernández-Antonio R.

Departamento de Ciencias Clínicas Veterinarias. Universidad de Santiago de Compostela. Lugo. (2) Clínica Veterinaria Santa Cruz, La Coruña. (3) NUPE S.L., La Coruña. Spain.

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Diskospondilitis in mink is a disease with nonspecific to severe neurological signs. When the disease is advanced in time posterior paresis/paralysis of the animals is the most common symptom observed. Diskospondylitis in mink farmed has been associated with bacterial infections, mainly *Streptococcus sp.* The aim of this study was to describe the lesions observed in minks which developed diskospondylitis by means pathological, radiographic and mielographic methods.

Paresis/paralysis of the extremities was found in mink from 2 to 5 months old -mainly since june until september-, though some new cases were detect in adults mink along breeding season and after whelping. In association with this signs urinary and fecal incontinence was observed when the animals developed posterior paralysis. Frecuently these animals developed skin lesions.

Seven animals were necropsied for this study. Macroscopically the degree of the lesions was variable and consists in deviation of the spine in different levels. Some of them had a pale exudate around the site of the lesion. Lisis of the intervertebral disk and bony proliferation of the adjacent vertebral bodies were observed too in association with subacute suppurative inflammation.

Radiographic lesions were localized in the cervical and/or thoracic region and they were characterized by lytic and proliferative bony changes with collapse of the intervertebral disk space. The number and severity of lesions may vary but usually is centered in one intervertebral disk affecting the two adjacent vertebral bodies.

Myelography and CT studies evidence the compression of the spinal cord in some of these animals.

Pododermatitis in farmed mink (*Mustela vison*) in Spain

Nieto, JM, L D Fraílde; AP Losada; M López-Peña; S Vázquez; R Bermúdez; MI Quiroga; R Fernández-Antonio

¹Departamento de Ciencias Clínicas Veterinaria.²Departamento de Anatomía y Producción Animal. Universidad de Santiago de Compostela. ³NUPE, S.L. FACULTAD DE VETERINARIA, LUGO, Spain

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During 2006, 2007 and 2008 several mink were submitted to our Department for necropsy with history of superficial to deep ulcers on the foot pads, often covered by scabs and debris. The aim of this study is to describe the main macroscopic and microscopic lesions found in mink, to describe some epidemiological data and to establish some criteria of differential diagnosis with previous cases reported since the 90's in Canada and the US with an etiology probably related with marine Calicivirus.

Foot pad lesions were found mainly in adult females mink (more than 2 years old) and less frequently in young females and males (young females became the more affected group after whelping). Lesions consist on hyperkeratosis, necrosis, crusting and abscesses. Until sporadic cases are seen in most farms, the highest prevalence (between 1 and 5%) is observed in a cluster of genetically related farms; but the distribution of the condition in these farms doesn't follow an strict pattern linked with a particular genetic group.

Lessions were observed on the plantar and palmar surfaces of the metatarsal and metacarpal regions. Less often we saw similar lesions in the face, mainly in the nose and eyes. Microscopically follicular plugging, hyperkeratosis and folliculitis were common findings in the first stage of the lesion which evolved to severe ulcerations with a marked suppurative inflammatory response – abscesses-.

Selected samples for bacteriology revealed the presence of *Staphylococcus intermedius*.

Our study discuss the role of *S. intermedius* in the pathogenesis of the pododermatitis in mink and its relation with several predisposing factors such as alterations in keratinization, frictional damage, dirt, trauma, parasites or hipersensibility reactions. Other diseases causing skin lesions in mink such as distemper, tirosinemia, zinc and cadmium deficiency or endocrine disorders were eliminated as primary cause of pododermatitis by our histopathological study.

Histological and immunohistochemical development of methods to evaluate endometrial biopsies in mares with infertility

Nilsson, Ebba DVM, Westergren Ewa lab. Technician, Gavier-Widen Dolores DVM PhD, Depart of Pathology and Wildlife Diseases, Nyman Gorel DVM PhD, Depart of Animal health and Antimicrobial Strategies

National Veterinary Institute, Depart of Pathology and Wildlife Diseases, Uppsala, Sweden

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Introduction: Endometrial disease is an important contributor to reduced fertility in mares. Hereby we describe the initial phases of a study that has as a goal to develop a standardized histopathologic evaluation of endometrial biopsies incorporating immunohistochemical characterization of the hormonal status of the endometrium and the proliferative activity of the epithelium. The final goal is to provide a more accurate prognosis about reproductive fitness of the mares.

Material and methods: Archived and newly collected endometrial biopsy samples from mares with history of infertility will be analysed. A panel of histochemical and immunohistochemical methods will be applied. The following antibodies will be used: Monoclonal Rabbit Anti-Human Ki-67 Antigen, Monoclonal Mouse Anti-Human Estrogen Receptor (clone 1D5) and Monoclonal Mouse Anti-Human Progesterone Receptor Cocktail (clone PR002+PR003).

Results: The sections were evaluated and classified into 4 categories according to the system developed by Kenney (ref 1) taking into consideration the nature and severity of inflammation and fibrosis of the endometrium. In the next steps, the morphological findings and the results of the immunohistochemistry will be correlated and evaluated. Our results will be put into relation to earlier studies conducted on normal mares at various stages of the reproductive cycle (ref 2).

Discussion: The examination of endometrial biopsies allows assessments of the pathological changes of the endometrium and provided estimations of the mares reproductive potential. The study further describes the expression of estrogen and progesteron at various stages of the oestrus cycle, the proliferation of the epithelium and their correlation to the histopathological grading. This correlation can be an important tool in investigation of infertility in mares.

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Mechanistic study on fenofibrate-induced hepatocarcinogenesis of rats: Involvement of oxidative stress

Nishimura, Jihei, Yasuaki Dewa, Toshiya Okamura, Meilan Jin, Yukie Saegusa, Masaomi Kawai, Takashi Umemura, Makoto Shibutani and Kunitoshi Mitsumori

Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo, Japan

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Introduction: Despite the numerous reports on hepatocarcinogenesis induced by peroxisome proliferator activated receptor α (PPAR α) agonists, the molecular mechanism of hepatocarcinogenesis induced by them is not completely understood. In the present study, to clarify whether oxidative stress, which is considered to be one of its mechanism, is involved in the development of hepatocellular tumors induced by PPAR α agonist, fenofibrate (FF), we conducted molecular pathological analyses using a medium-term 2-stage hepato carcinogenesis model of rats.

Material and methods: Male F344/N rats were fed diet containing 6,000 (only Experiment 1; Exp. 1), 3,000 or 0 ppm of FF for 13 (Exp. 1) or 28 weeks (Exp. 2) after DEN initiation. The obtained livers in the Exps.1 and 2 were subjected to molecular pathological analyses. In addition, we used laser microdissection technique and performed the region-specific mRNA expression analysis in Exp. 2.

Results: In Exp. 1, the numbers of hepatocellular foci, which mainly consists of GST-P negative foci, and cell proliferations increased in DEN-FF groups. In gene expression analyses using whole liver, significant up-regulations of Aco and Cyp4a1 related to lipid metabolism, Gpx2, Cat, Cyp2b15 and Ugt1a6 related to metabolic oxidative stress, Apex1, Mgmt, Xrcc5 and Gadd45a related to DNA repair, and CyclinD1 related to cell cycle were observed in FF-treated groups, while the expression levels of Cyp1a2, Gsta2, Gstm2 and Gstm3 related to phase I or II metabolism, Mlh1 and Top1 related to DNA repair, and p21, p27, chek2 and Gadd45b related to cell cycle / apoptosis significantly decreased in these rats. In addition, microsomal ROS formation in the liver, 8-OHdG in the liver DNA and lipofuscin deposition in the liver significantly increased in DEN-FF groups. Base on these results, in Exp. 2, we investigated the region-specific mRNA expression analyses of the genes involved in oxidative stress in the preneoplastic and/or neoplastic lesions. Our data revealed that GST-P positive foci were devoid of the expression of Nrf2 and GST P-negative foci expressed higher levels of Nrf2 the increase of number GST-P negative foci and tumors. However, the expression of Nrf2-inducible enzymes such as Gpx2 and HO-1 was identified in GST-P positive foci and/or tumors. The mRNA expression of TGF- β related enzymes such as plasminogen activator inhibitor type-1 and α_2 -Macroglobulin increased in GST-P negative foci in the liver of rats given FF. In the evaluation using the whole liver, an elevation of 8-OHdG in the liver DNA and a decrease in total GST activity were observed in the liver of rats treated with FF.

Discussion: These results suggest the possibility that oxidative stress is involved in the development of hepatocellular foci and tumors induced by FF. However, Nrf2 is not responsible for GST-P expression in rat hepatic lesions, and this finding indicates that Nrf2 is not related to the tumor formation in rats treated with FF. Up-regulation of TGF- β related enzymes in the GST-P negative foci may indicate a possible involvement of the signal pathway that is different from that involved in the formation of GST-P positive foci.

Histopathological and immunohistochemical findings in lungs and lymph nodes of pigs naturally affected with post-weaning multisystemic wasting syndrome and porcine reproductive and respiratory syndrome

Novosel, Dinko, Lipej Zoran, Mihaljević Željko, Deždek Danko

P150

Introduction: Porcine circovirus type 2 (PCV2) has been identified as the causal agent of post-weaning multisystemic wasting syndrome (PMWS). Porcine reproductive and respiratory syndrome virus (PRRSV) can cause reproductive disorders in breeding sows or respiratory disease. PRRSV can be common but not essential component in pathogenesis of PMWS. The co-infection of PRRSV in PMWS diseased animals in previous reports range from 42% to 84%. There is evidence that many cells types support PCV2 replication. PRRSV replicated in alveolar macrophages and pulmonary epithelial cells. Both viruses were reported as causative agent of proliferative and necrotizing pneumonia (PNP). The aim of this study was to investigate distribution of PCV2 and PRRSV in lungs and lymph nodes in naturally affected pigs with both PMWS and PRRS.

Material and methods: Study is performed on 7 animals submitted to the Croatian Veterinary Institute during 2007/ 08 in which PRRS and PMWS were diagnosed. Animals were necropsied and after complete post-mortem examination lungs and mediastinal and mesenterial lymph nodes were collected in 10% buffered formalin. For immunohistochemical (IHC) detection of PCV2 and PRRSV anti-PCV and anti-PRRSV monoclonal antibodies were used. IHC detection of viral antigens was performed on a serial cuts from same tissue.

Results and discussion: Alveolar septa of lungs were markedly thickened or there is completely absence of alveolar spaces. In alveolar walls was found strong proliferation of pneumocytes type 2 and infiltration of macrophages and lymphocytes. In remaining alveolar spaces were present large number of necrotic cells and macrophages. In peribronchial area accumulations of macrophages were clearly seen. Lymph nodes had depleted lymphoid follicles and hystiocytic proliferation. Depleted follicles often contained macrophages with basophilic grape-like intracytoplasmatic inclusions, which are specific findings for PMWS diseased animals or had centro-follicular necrosis as seen in PRRS diseased pigs. PCV2 positive signal in lungs were found mainly in necrotic cells and macrophages in necrotic debris in alveoli lumen. The positive signal was rarely observed in cytoplasm and nuclei of epithelial cells and cytoplasm of macrophages in proliferated area. Similarly, PRRSV positive signal was found in macrophage cytoplasm in alveolar spaces, and necrotic debris, but also in proliferated area. There were few positive pneumocytes with signal in cytoplasm found in alveolar walls. The moderate presence of PCV-2 antigen was found in macrophages-like cells mainly in lymphoid follicles of mesenterial and mediastinal lymph nodes. Large number of PRRSV positive cells was observed in hystiocytes in stroma of mediastinal lymph nodes and just few of them in mesenterial lymph nodes. Positive PRRSV hystiocytic cells were rarely found in lymphoid follicles. Pulmonary lesions in all animals were considered as PNP. In all examined animals was detected large amount PCV2 and PRRSV antigen in pulmonary lesions. Observed distribution of both viruses founded in same area in large amount strongly support etiological hypothesis that both viruses were causative agents of this condition.

Comparative pathology and viral distribution of the experimental infection with highly pathogenic avian influenza H5N1 A/turkey/Turkey/05 (Asian lineage) in chicken, turkey and Pekin duck

Núñez, Alejandro, Angela Richard, Brandon Londt, Yvonne I. Spencer, Ian H. Brown and Jill Banks

VLA – Weybridge, New Haw, Addletone, Surrey, United Kingdom

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Introduction: Highly pathogenic avian influenza (HPAI) is caused by infection with influenza A viruses of the family Orthomyxoviridae, with devastating consequences for poultry and zoonotic potential. The highly publicised, currently circulating, Asian-like H5N1 HPAI has, uncharacteristically for previously isolated AI, caused large numbers of deaths in wild birds. Knowing the dissemination of this H5N1 within the organs and cause of death in infected hosts is vital for disease prevention in commercial and domestic poultry populations. This study investigates the pathological changes and virus distribution of HPAI H5N1 A/turkey/Turkey/1/05 in chicken, turkey and Pekin ducks.

Material and methods: SPF derived chickens and turkeys and commercial Pekin ducks of 3 weeks of age were inoculated via intranasal and intraoral route with 106 EID₅₀ HPAI H5N1 A/turkey/Turkey/05. Postmortems were conducted on the animals dying of the disease and tissue samples from multiple organs, including respiratory, alimentary, integumentary, endocrine and nervous system, were collected, fixed in buffered formalin and routinely processed for histopathology. Immunohistochemical detection of Influenza A nucleoprotein on tissue sections was used to determine viral distribution.

Results: All inoculated chickens and turkeys died without significant clinical changes 24-48 hours post infection. Pekin ducks displayed nervous clinical signs from 4 dpi showing rapid deterioration and death by day 5. Histopathological lesions were observed in most organs in both chicken and turkey, with a similar distribution although severity and extension of lesions were more prominent in turkey. These changes included interstitial pneumonia and oedema in the lung and necrosis in multiple organs, very remarkable in spleen and pancreas, followed in severity in lymphoid tissues, liver, kidney and adrenals, with scarce inflammatory changes. Degenerative changes and occasional necrosis of myocardiocytes was observed in the heart. In duck, the distribution of the lesions was more limited, no major lesions being observed in the lungs, liver, kidney and only of mild lymphocytic depletion in lymphoid tissues. Necrotising lesions in pancreas and adrenal glands were less extensive. Lesions were more prominent in heart, with a severe necrotising myocarditis, and in brain, showing severe meningoencephalitis. Distribution of viral antigen was also widespread in the three species, especially in chicken and turkey in which most endothelial cells appeared immunolabelled. Prominent immunolabelling was observed in lungs, spleen, heart, brain, pancreas, liver and kidney in chicken and turkey. Brain, heart and pancreas were the organs where viral immunolabelling was more prominent in ducks, with few positive cells observed in lung and spleen, and no virus detected in kidney or liver.

Discussion: Infection of chicken, turkey and Pekin duck with HPAI H5N1 A/turkey/Turkey/1/05 resulted in 100% mortality in the three species, with a longer incubation period and clinical course in Pekin ducks. Severity of lesions and viral distribution was milder in ducks and similar to those described in recently emerged H5N1 of Asian lineage.

Phenotypic spectrum of listeric encephalitis in ruminants: a comprehensive study

Oevermann, Anna, Carlos Abril, Andreas Zurbriggen, Marc Vandeveld

Neurocenter, DCR-VPH, Vetsuisse Faculty Bern, Switzerland

P89

Introduction: Listeriosis is a reportable food-borne infection of humans and animals caused by *Listeria monocytogenes* (LM). The disease occurs in different clinical presentations of which fatal CNS disease and abortions are the most common. Recently, in the frame of a large active TSE survey, an unexpectedly high prevalence of listeric encephalitis was identified in small ruminants in Switzerland, demonstrating that passive surveillance has dramatically underestimated the true incidence of this disease. The present study focused on the detailed histologic characterisation of the disease in order to gain insight into its pathogenesis and to identify possible interspecies differences.

Material and methods: Brains from more than 150 cattle, sheep and goats with natural listeric encephalitis were retrospectively evaluated by histopathology. Lesions were classified into acute or subacute encephalitis based on the cellular composition of microabscesses. Furthermore the extension of lesions, the size of microabscesses and perivascular cuffs were assessed. The involvement of specific neuroanatomic regions, particularly cranial nerve nuclei, was recorded. Additionally, immunohistochemistry for LM was performed on brainstem sections with a LSAB-AEC method in order to assess the bacterial load and location.

Results: Histopathological lesions were most severe in the brainstem and tapered continuously into the more rostral regions. Isolated lesions in the diencephalon and telencephalon without involvement of brainstem were not observed. In few animals, ependymitis with subependymal microabscesses were observed in the entire ventricular system. Most animals had microabscesses of different age within their brain, and in those cases, acute microabscesses tended to occur more in the rostral regions. Small ruminants were often affected by massive lesions with complete replacement of parenchyma by massive accumulations of neutrophils and/or macrophages. In contrast, listeric encephalitis of cattle was more frequently characterized by the presence of small-sized subacute microabscesses containing frequently multinucleated giant cells. In most animals, elongated microabscesses could be observed following the course of axonal fibers. Cranial nerve nuclei and their roots were regularly involved, particularly the trigeminal motor nucleus, spinal tract nucleus of the trigeminal nerve, hypoglossal nucleus and facial nucleus. LM were observed most frequently in microabscesses with a high variation of bacterial load, which was generally lower in cattle than in small ruminants. Furthermore, LM were observed in degenerating and intact axons as well as in intact neurons, within ependymal cells and in the ventricular system.

Discussion: Results of the present study are compatible with local spread into and within the brain by intraaxonal migration of bacteria. However, LM may be capable to spread within the CSF as well. Species differences were observed in the extent and composition of microabscesses and in the bacterial load.

Expression of eosinophil chemotactic factors eotaxin and IL-5 in mast cell tumors: relation with stromal eosinophilia and prognosis

Oliveira, Justina P., Anabela Alves, Laura Peña

Dpto. Medicina y Cirugía Animal (Anatomía Patológica). Fac. Veterinaria. Carretera de la Coruña s/n. Ciudad Universitaria., Madrid, Spain

P4

Introduction: Tumor associated eosinophilia is well recognized in canine mast cell tumors (MCTs), although little is known about its cause, extension and meaning. In several human malignancies eosinophilic infiltrates have been related with poor or better prognosis. In dogs, variable numbers of eosinophils are present in almost all mastocytomas, either dispersed throughout the neoplastic tissue or forming aggregates. It is supposed that the eosinophilotactic effect of the mast cells decreases with increasing anaplasia in MCT, but there is a lack of descriptions that confirm this assumption. IL-5 and eotaxin are chemokines that play a major role as eosinophils chemoattractants, enhancing tissue eosinophilic infiltration. Nevertheless, their presence and relation with pathological and clinical features has not been assessed in mast cell tumors. The aim of this study was to analyze the eosinophilic infiltrate and the presence of IL-5 and eotaxin in a series of MCTs, considering their pathological and clinical characteristics.

Material and methods: Surgical samples of 90 MCTs from 90 dogs (aged 2 to 17 years) with a known clinical history and follow-up were histopathologically evaluated and graded (grades I, II, III), according to WHO's histopathologic diagnostic criteria. Tumor associated eosinophilia was categorized in 3 classes: sparse (<50 eosinophils/HPF), moderate (50-100 cels/HPF), and abundant (>100 cells/HPF). Immunohistochemical detection of eotaxin and IL-5 (monoclonal, R&D Systems), was performed and semiquantitatively evaluated in 3 categories. Statistical univariate and multivariate analyses were performed with SPSS program.

Results: Eosinophilic infiltrate was not related to histological grade nor prognosis. In grade I tumors the infiltrate was sparse in 38,7% (12/31) moderate in 35,5% (11/31) and abundant in 25,6% (8/31). Grade II tumors presented sparse infiltrate in 16,7% (5/30), moderate in 50% (15/30), and abundant in 33,3% (10/30). Grade III tumors showed sparse infiltrate in 48,3% (14/29), moderate in 31% (9/29) and abundant in 34,5% (6/29). Chemokines expression was demonstrated in the cytoplasm of mast cells and eosinophils. There was no statistical association between the expression of the chemokines and the extent of the infiltrate. A high expression of IL-5 revealed prognostic significance in univariate survival analysis for tumor related death and it was an independent and significant unfavorable prognostic factor for recurrence/metastases on multivariate analysis, in animals with grade I and grade II tumors.

Discussion: Eosinophilic infiltrate is almost a constant in mast cell tumors, not related with the degree of tumor differentiation, nor with prognosis.

Our data indicate that neoplastic mast cells express eotaxin and IL-5, but their expression doesn't correlate with eosinophilia, suggesting that other mechanisms are involved in the recruitment of eosinophils into the tumor. The production of IL-5 by mast cells leads to an unfavorable prognosis, but little is known about the role of IL-5 in the malignant behavior of the disease, maybe related to a shift in the inflammatory balance in favor of a Th2 response.

Prognostic significance of matrix metalloproteinases MMP-2 and MMP-9 immunoexpression in canine mast cell tumors

Oliveira, Justina P., Anabela Alves, Laura Peña

Dpto. Medicina y Cirugía Animal (Anatomía Patológica). Fac. Veterinaria. Carretera de la Coruña s/n. Ciudad Universitaria., Madrid, Spain

P5

Introduction: Mast cell tumors (MCTs) are among the most common cutaneous neoplasms in dogs, accounting for up to 21% of cutaneous neoplasms, having a highly variable biological behavior. The presence of matrix metalloproteinases, a zinc-dependent family of proteins have been studied in a variety of tumors, including MCTs, for prognostic purposes, using mainly zymography techniques. This study demonstrates the presence, by immunohistochemistry, of MMP-2 and MMP-9 in mast cell tumors and evaluate their validity as prognostic factors in comparison with other known prognostic factor for MCTs: the Ki-67 proliferation index, and c-Kit.

Material and methods: Surgical samples of 90 MCTs from 90 dogs were histopathologically evaluated and graded (grades I, II, III), according to WHO's histopathologic criteria. Immunohistochemical detection of MMP-2 and MMP-9 expression was performed using polyclonal antibodies (Neomarkers®). Ki-67 expression was studied immunohistochemically using a monoclonal antibody (MIB-1, Dako) and c-Kit with a polyclonal antibody (Dako). MMP-9 positive neoplastic cells were evaluated semiquantitatively in 3 classes (<25%; 25%-50% ; >50%) and MMP-2 overexpression was categorized according to an immunoreactivity index, combining staining intensity and % of positive cells. Three c-Kit staining patterns were identified: membrane-associated, focal cytoplasmic, and diffuse cytoplasmic staining, according to previous references. Statistical univariate and multivariate analyses were performed with SSPS program.

Results: Immunoreactivity to both MMP-2 and MMP-9 were observed in all tumors. Mast cells and eosinophils showed cytoplasmic immunostaining. MMP-2 overexpression was observed in 21/90 tumors (23,3%), and MMP-9 overexpression was found in 6 tumors (6,7%). Highly significant univariate statistical associations ($p < 0,0001$) were found between MMP-9 expression and histological grade, Ki-67 and c-Kit. MMP-9 showed significant statistical association with recurrence/metastases ($p = 0,001$) and with tumor related death ($p < 0,0001$) in univariate analyses. Skin ulceration, histological grade III, Ki-67 index, c-Kit and MMP-9 expression were selected as independent prognostic factors in multivariate analyses. MMP-9 overexpression was demonstrated as an efficient tool of poor prognosis in grade I tumors.

Discussion: The results suggest that gelatinases expression occur in spontaneous canine mast cell tumors, assessed by immunohistochemistry. MMP-9 seems to play a major role in the invasive and metastatic process; the immunohistochemical detection of MMP-9 is useful for accurate prognosis in MCTs, specially in grade I tumors.

Microscopic screening pulmonary lesions in European bison from Białowieża Primeval forest

Osirńska, Barbara, Magdalena Matuszewska, Wojciech Bielecki, Magdalena Skrzypczak, Krzysztof Anusz

Devison Pathomorphology, Department of Clinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

P108

Introduction: There are very few data concerning pathology of the lung of European bison (*Bison bonasus*). The free-living in Białowieża Primeval Forest bison weak or injured were culled according to the selection program between 2000 and 2007.

Material and methods: Post mortem examination of 70 both sexes, from 3 months to 27 years old European bison was carried out. The lungs were collected for histopathological examination. Suspected specimens of the lungs were fixed in buffered 10% formalin and embedded in paraffin. The microtome sections were stained with H&E and in some cases PAS, Gomori - histochemical method and immunohistochemical method – vimentin, cytokeratin and chromogranin A.

Results: Histopathological examination revealed focal infiltrations of inflammatory cells in pulmonary tissue. The dominant cells were eosinophiles but macrophages, neutrophils, and some other mononuclear cells were observed too. In some cases the parasitic nodules were observed. There were also pulmonary parasites of various numbers observed. In some cases focal atypical adenomatous hyperplasia was seen. In one case – female two years old - the neoplastic cells were also seen. They recognised as Bronchogenic non-small cell carcinoma.

Discussion: The pulmonary parasites, bronchointerstitial or purulent pneumonia were observed in European bison. Earlier investigation bison of Białowieża-Caucasian line in Bieszczady (Poland) revealed the same lesions and some cases of tuberculosis. In our investigation of European bison in Białowieża Forest we had not observed pulmonary tuberculosis. Tumors are very rare in European bison observed. It is the first report concerning pulmonary neoplasia in European bison.

Ovarian neoplasia in European bison (*Bison bonasus*). Case report

Osińska, Barbara, Maria Katkiewicz

Devison Pathomorphology, Department of Clinical Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

P55

Introduction: There are very few data concerning pathology of the female reproductive organs of European bison (*Bison bonasus*). There is no information concerning ovarian neoplasia in the European bison.

Material and methods: Post mortem examination of three female European bison from the free-living in Białowieża Primeval Forest was performed and ovaries were collected for histopathological examination. These bison were culled according to the selection program. Ovaries were fixed in buffered 10% formalin and embedded in paraffin. The microtome sections were stained with H&E and PAS/AB histochemical method.

Results: Histopathological examination confirmed ovarian epithelial cyst in all three females. They were numerous and of various dimension. The biggest one of 12mm diameter in the female no 1 was found. In the same female and in the female no 2 the focus of granulosa cell tumor and disseminated small nest of granulosa-theca cells proliferation were observed. The pathological lesions which may illustrate primary dysplastic lesions were simultaneously in ovarian follicles observed. In bison no 1, in spite of the observed pathological lesions also corpus luteum was present, what means the passed ovulation process. In two bison (no 1 and no 3) the neoplastic lesions originated from ovarian interstitial cells were found. The tumor cells were also seen in the lymphatic vessels lumen. Probably dissemination of ovarian interstitium took place via lymphatic vessels lumen infiltration.

Discussion: It is the first report concerning ovarian neoplasia in European bison. The observed neoplastic lesions showed complex origin. The transformed cells were of follicular and interstitial origin. The endocrine disturbances may be incriminated in the etiology of these lesions. In spite of epithelial cysts present in all bison and also previously described – other pathological lesion were visible only in microscopic examination. The described ovarian neoplasia demonstrated the age-related lesions. The pathological stimuli responsible for the disturbances in ovarian cells homeostasis were of the complex nature, as it was reflected in the character of the observed structural ovarian lesions.

Myostatin is overexpressed and binds Decorin in canine masticatory muscle myositis

Paciello, Orlando, Francesca Trapani, Serenella Papparella

University of Naples Federico II – Dept. of Pathology and animal health, Naples, Italy

P116

Introduction: Myostatin (MSTN), a member of TGF- β superfamily of growth factors, acts as a negative regulator of skeletal muscle mass. The mechanism whereby myostatin controls the proliferation and differentiation of myogenic cells is mostly clarified. Furthermore, recent studies have shown that myostatin, may also be involved in fibrosis formation within skeletal muscle (Wagner, K. R. Et al. 2002), although a direct link between MSTN and fibrosis has yet to be identified. Decorin (DCN), a small leucine-rich proteoglycan, binds TGF- β and regulates its activity in the ECM. DCN has been shown to strongly inhibit fibrosis formation in various tissues via blocking of TGF- β activity. Muscle atrophy and fibrosis are pathological features often observed as a consequence of inflammatory myopathies. Canine Masticatory Muscles Myositis (CMMM), is a chronic immune-mediate inflammatory process, characterized by atrophy of masticatory muscles and their replacement with fibrous tissue. Fibrosis and muscle atrophy lead to inability to jaw movement therefore the dog feeding often becomes impossible.

Material and methods: We studied Myostatin and Decorin in 10 CMMM compared to 3 normal muscle by: 1) confocal laser scanning microscopy (CLSM) in parallel with conventional immunohistology; 2) immunoblots; and 3) immunoprecipitation, seeking a physical association of Myostatin with Decorin.

Results: By immunofluorescence atrophic muscle fibres were strongly and diffusely immunostained with the antibody against-Mstn/MstnPP as compared to the control. Immunoblots of muscle biopsies obtained from control and CMMM revealed two specific bands of 28 and 55 kDa, corresponding to the Mstn dimer and the upper to MstnPP. Both of those bands were increased in CMMM. Densitometric analysis revealed that amount of Mstn protein, in CMMM, was increased 2.4-fold ($p < 0.05$), while the MstnPP was increased 2.6-fold ($p < 0.05$) in comparison with normal control. Decorin was located prominently in the perimysium and endomysium and in association with fibre surfaces. Decorin apparently increased in muscle biopsies with high amount of inflammatory cells and less fibrosis. In several fibers, decorin co-localized with myostatin. Myostatin co-immunoprecipitated with 43kDa –Decorin.

Discussion: Our findings suggest that the inhibition of MSTN by Decorin, might be a new therapeutic approach for CMMM improving skeletal muscle healing through enhancement of regeneration and reduction of fibrosis.

Cholesterol, *Chlamydophila pneumoniae*, Alzheimer's disease: expanding the horizons of pathogenesis

Papaioannou, Nikolaos G.,¹ Dimitra Psalla¹, Victoria I. Siarkou², Paschalis Fortomaris³, Michael Patsikas⁴ and Ioannis Vlemmas¹

¹Department of Pathology, ²Department of Microbiology, ³Department of Animal Production, ⁴Section of Diagnostic Imaging, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54 124 Thessaloniki, Greece

Introduction. Recent epidemiological, clinical, pathological and experimental data suggest that cholesterol and intranasal infection with *Chlamydophila pneumoniae* may involve in pathogenesis of Alzheimer's disease (AD). To further evaluate a possible relationship between critical coronary disease (cCAD) and amyloidogenesis, we investigated the effect of hypercholesterolemia and *C. pneumoniae*, infection, two of the major risk factors for cCAD, on deposition of β -amyloid material in the brain of rabbits.

Materials and Methods. Four groups of one-month-old New Zealand White rabbits were used in this study: (i) ten rabbits were fed cholesterol-free chow, (ii) ten rabbits were fed 2g/100g cholesterol-supplemented chow, (iii) ten rabbits were fed 2g/100g cholesterol-supplemented chow and were inoculated three times within 8 weeks with 1×10^6 IFU of two strains of *C. pneumoniae* via the posterior nasopharynx, (iv) ten rabbits were inoculated three times within 8 weeks with 1×10^6 IFU of *C. pneumoniae* strains and (v) ten rabbits (controls) were fed normal food and were inoculated with sterile carrier broth. All the rabbits were euthanized after three months. Biochemical parameters, ultrasound examination of the aorta and pulmonary artery, gross lesions, histopathological findings (brain, heart, aorta and pulmonary artery), immunohistochemical evaluation (brain, heart, aorta and pulmonary artery) and molecular techniques (lung, heart, vessels, aorta and brain) were performed. Single-labeling immunohistochemistry by avidin-biotin complex (ABC) with several primary antibodies was performed in serial sections of the brains in order to detect the presence of β -amyloid depositions in the brain of experimental animals.

Results. The total cholesterol in the serum was measured enzymatically and the serum cholesterol concentration at the end of the experimental period was significantly high in the animals' serum of the second experiment. Grossly, in the animal brains of the second and third experiment, light brain atrophy, thickening of leptomeninges, narrowing gyri and widening of sulci, hemorrhages were revealed. Atheromatic plaques obvious in the aortic and pulmonary trunks were detected. Histopathologically degenerative lesions were detected in the animals' brains of the second and third experiment while the typical atheromatic plaques were revealed in the aortic and pulmonary trucks. Immunohistochemically amyloid deposited in the wall of cerebro-meningeal vessels while A β positive-material (for A β -40 and A β -42) was distributed throughout the cortical layers.

Discussion. According to our results we could assume that hypercholesterlemic rabbits and rabbits infected with *C. pneumoniae* may provide a useful animal model for further understanding of mechanisms involved in the initiation of pathogenesis of sporadic AD. The premature presence of senile plaques in coronary disease (CAD) suggests a neuropathologic link between cCAD and AD.

Immunohistochemical profile of one case of canine endometrial carcinoma

Payan-Carreira, Rita, Fernanda Seixas, Maria dos Anjos Pires

Univ. Trás-os-Montes and Alto Douro, Vila Real, Portugal

P45

Introduction: Uterine tumours other than leiomyoma and leiomyosarcoma are rare in dogs, with only sporadic reports on endometrial carcinomas that mostly occur in geriatric bitches. This is a preliminary study that aims to contribute to the advancement of the knowledge in canine endometrial carcinoma immunophenotype.

Material and methods: In this study, a panel of immunomarkers (cytokeratins AE1/AE3 and 14, vimentin, CD10 and Ki-67) was applied to the uterus of a ten-years old female Boxer evidencing an endometrial carcinoma on the uterine body, to establish the staining patterns indicative of the tumour aggressiveness and cellular differentiation. Uteri in different stages of the oestrous cycle were used as controls. 10% formalin fixed uterine samples, embedded in paraffin wax, were sectioned at 2 μ m and routinely stained with haematoxylin and eosin. Immunohistochemical analysis was performed using the avidin-biotin peroxidase method. Two independent observers performed a blind assessment of the degree of staining. The results for the cytokeratins (CK), vimentin and CD10 were evaluated as positive and negative immunolabelling. The number of immunopositive cells to Ki67 per 1000 examined cells in 10 representative areas at the periphery of the tumour was expressed as percentage.

Results: In the case described here, the tumor showed papillar pattern, with large pleomorphic, anaplastic cells and also some aberrant multinucleated and giant cells. In some areas of the tumor, it was also observed cytotrophoblastic-like cells outlining the papillae. CK AE1/AE3 expression was detected in the luminal neoplastic cells. CK 14 positivity was sporadic and irregular, and was observed mainly in the luminal epithelium. Only stromal and aberrant cells showed a positive staining to vimentin. Positive membranous staining to CD10 was evidenced by clear epithelial, cytotrophoblastic-like cells at the tumor surface but not by the stromal cells. The multinucleated and giant cells evidenced a positive immunostaining to CK AE1/AE3, and CD 10; its positivity to vimentin was sporadic. The Ki-67 index was low.

Discussion: In this carcinoma, CK AE1/AE3 staining pattern suggested an endometrial surface epithelium origin for the neoplastic superficial epithelial cells, while the existence of sporadic CK 14 positive cells pointed to a low invasive phenotype of the tumour. Vimentin expression was showed in the stroma and in aberrant epithelial cells and could be associated with undifferentiation of the neoplastic cells. In normal uteri, CD10 positivity is only observed in the stroma; however, in this tumor, membranous immunoreactivity to CD10 was scarce and only found in the epithelial cells of the papillae surface, in a staining pattern similar to that observed in the human trophoblast or chorion tumours. This feature clearly suggests a radical change in the cellular behaviour of the neoplastic epithelial cells. Although, the Ki67 proliferation rate was low, in accordance to CK immuno-pattern, and suggestive of a weak aggressiveness of the tumour. This feature could be responsible the absence of metastasis on this Boxer and for its long post-surgery survival (3 years).

The bovine brachyspina syndrome: a congenital malformation in Holstein calves

Peperkamp, Klaas, Stefania Testoni, Jørgen S. Agerholm

Animal Health Service Deventer, Deventer The Netherlands

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Introduction: The brachyspina syndrome is a recently reported malformation in the Holstein breed. Until May 2008, 4 cases have been diagnosed in Denmark, 4 cases in The Netherlands and 2 cases in Italy. The familial occurrence of these cases may indicate that it is an autosomal recessively inherited disorder.

Material and methods: 10 stillborn affected calves, 6 males and 4 females, were necropsied. In all cases both parents were related to a common ancestor in generation VI.

Results: The affected calves were delivered stillborn at term or after a slightly prolonged gestation period. Growth retardation resulted in a body weight of around 10 kg. The entire vertebral column was significantly shortened due to severe disorganisation and malformation of almost all vertebrae. The anterior part of the thoracic spine showed prominent spinous processes with synostosis and increased height. The number of ribs was reduced in some cases and several ribs were synostotic proximally. Most calves also showed inferior brachygnatism and appeared to have relatively long and slender limbs. Additionally, several internal organs were affected. Cardial lesions consisted of interventricular septal defects, myocardial hypertrophy and displacement of the aorta and/or truncus pulmonalis. Severe bilateral renal dysplasia was a remarkable finding in all cases. Testicular hypoplasia, hypoplasia of the uterine horns and atresia of the colon were common lesions.

Discussion: The brachyspina syndrome and the Complex Vertebral Malformation syndrome (CVM) are malformations occurring in the Holstein breed. Differentiation between these defects can be done morphologically. Additionally, CVM can be diagnosed by genotyping. The inherited aetiology of the brachyspina syndrome still needs to be proved. The common ancestor of the Danish, Dutch and Italian cases is a widely used sire of US Holstein origin. Therefore, this disorder is suspected to occur widely. Veterinary pathologists are urged to be aware of this disorder and report cases to the authors. Skeletal muscle, spleen and pleural effusions should be stored at -20 °C, and kidney, liver, spine and metacarpus/metatarsus should be preserved in formalin for further examination.

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Evaluation of the local immune response in goats immunized with a synthetic peptide of the Sm14 antigen and challenged with *Fasciola hepatica*

Pérez, José, Alejandro Pérez-Écija, Leandro Buffoni, Alvaro Martinez-Moreno, Rafael Zafra

University of Córdoba, Córdoba, Spain

Introduction: Fasciolosis is an economically important disease of ruminants. Current control is based on the use of anthelmintic drugs. However the increasing anthelmintic resistance and the risk of drug metabolites in foodstuff have raised an international increasing interest in the development of vaccines. The aim of present study was to evaluate local immune response in goats immunized with a synthetic peptide of the Sm14 antigen of *S. mansoni* and challenged with *F. hepatica*.

Material and methods: Eighteen Florida-breed goats were used for the study, group 1 (n=6) was unimmunized uninfected control; group 2 (n=6) was immunized with three doses of a peptide of the Sm14 (pSm14) antigen of *Schistosoma mansoni* on days 0, 10 and 90; group 3 (n= 6) was used as unimmunized and infected control. Group 2 and 3 were orally infected with 200 mc on day 90, and all goats were killed on week 30. One goat of group 2 died during the experiment due to a traumatism. The distribution of CD2, CD4, CD8, $\gamma\delta$ T lymphocytes, IgM, IgG, IL-4 and IFN- γ was assessed by immunohistochemistry in snap frozen tissue samples of the liver and hepatic lymph nodes (HLN). A morphometric study of HLN was also carried out to evaluate the hyperplasia of lymphoid follicles.

Results: Fluke burdens revealed a 45.9% decrease in the immunized group respect to the infected control group, although a high individual variability was found in both groups. A marked increase of the hepatic infiltration of CD2, CD4 and CD8 T lymphocytes IgM⁺ and IgG⁺, B cells was found in the immunized and in the infected control group respect to the uninfected group. This infiltrate was less severe in the immunized group than in the infected control group. HLN showed a marked enlargement due to the hyperplasia of lymphoid follicles in both the immunized and the infected control group respect to the uninfected control group. In HLN only CD4 and CD8 T lymphocytes were significantly lower in the immunized group compared with the infected control group. IL-4 and INF- were not expressed in the hepatic inflammatory infiltration, whereas in the HLN the number of IL-4⁺ lymphoid cells was higher than the number of INF- γ .

Discussion: Results of the present study suggest that the pSm14 induced a limited protection against *F. hepatica* in goats. Local cellular and humoral immune responses were lower in the immunized group than in the infected control, particularly in animals showing lower number of parasites. The local immune response showed a polarized Th2 response in both immunized and in infected control groups.

Acknowledgment: Work funded by projects FOOD-CT-2005-023025-DELIVER and AGL2002-02777.

Pathological and morphometric study of the liver of goats immunized with recombinant thioredoxin peroxidase and challenged with *Fasciola hepatica*.

Pérez-Écija, Alejandro, Rafael Zafra, Leandro Buffoni, Ricardo Mendes, Francisco-Javier Martínez-Moreno, Alvaro Martínez-Moreno, José Pérez

University of Córdoba, Córdoba, Spain

Introduction: The increasing anthelmintic resistance and the risk of drug metabolites in foodstuff have raised an international increasing interest in the development of immunisation strategies for the control of *F. hepatica* in ruminants (Dalton et al., 2003). The aim of present study was to evaluate hepatic damage of goats immunized with recombinant thioredoxin peroxidase (Trx) and challenged with *F. hepatica*.

Material and methods: Fourteen Florida-breed goats were used for the study, group 1 (n=7) was immunized with two doses of Trx on week 0 and 3; group 2 (n= 7) was inoculated with the adjuvant (Quil A) on the same weeks. A non-parasited, non-immunized control group (n=2) was also included. Goats of both groups were orally infected with 200 mc on week 10, and sacrificed on week 27.

Results: Hepatic fluke burdens revealed a non-statistically significant reduction of 33.1% in the immunized group. Serum levels of glutamate dehydrogenase (GLDH) were similar in both groups during pre- and post-infection stages. Gross and microscopical hepatic changes (fibrous perihepatitis, fibrous tracts containing pigment laden macrophages, portal fibrosis, hyperplasia of bile ducts and infiltration of lymphocytes and plasma cells) showed high individual variability, but in general were more severe in group 2. Morphometric studies showed a reduction in lesions (bile ducts hyperplasia and portal fibrosis) in group 1 in comparison with group 2. However, these differences were not significant. Macroscopic morphometric revealed that the damaged hepatic surface was significantly diminished ($P<0,05$) in group 1. Hepatic lymph nodes showed severe hyperplasia of lymphoid follicles and medullary cords, except in some goats of the immunized group with a very low number of flukes.

Discussion: Results of the present study suggest that Trx induced a limited protection against *F. hepatica* in goats. New vaccine formulations and/or antigens combinations should be evaluated in further studies.

Acknowledgement: Work funded by EU project (FOOD-CT-2005-023025-DELIVER).

New acquisitions about the diagnosis of proventricular dilatation disease (PDD) in parrots

Pesaro, Stefano, Renato Ceccherelli, Raffaello Gambi, Giacomo Rossi

Department of Veterinary Science – Camerino, Matelica, Italy

Introduction: Proventricular Dilatation Disease (PDD) is a progressive often fatal pathology that occurs in different species of parrots. Until now the etiology and the pathogenesis are unclear. The ill birds develop, both or alone, gastrointestinal and neurological signs. The clinical signs are related to a progressive non-suppurative encephalomyelitis and ganglionitis accompanied by inconsistent clinical laboratory findings. To date, the only specific diagnostic test is the crop biopsy for histological ganglia examination. Until now, no serological tests were available to screen the parrots. This study describes the most common histological lesions in crop sections of affected animals and shows the correlation between results obtained by the histology with preliminary results of a new serological essay, performed on 300 parrots.

Material and Methods: 300 parrots belonging to different species were screened for PDD in 12 large aviaries and private collections of Italy. Briefly, animals were anesthetized and two crop's biopsies, sampled as reported in a previous work, and plasma samples were taken from each parrot. Crop serial histological sections were stained with H/E to morphological evaluations, and additional stains as LFB for myelin, DHTS for epithelium and mucins characterization were also performed. To test mitotic activity of epithelium basal layer and to immunophenotyping the perigangliar infiltrates, PCNA (pAb, Santacruz), anti-CD3 (mAb, Serotec), anti macrophages (mAb Serotec), and anti-CD8 (mAb, VMRD) antibodies were used. Histological results were correlate with serological data, obtained by ELISA and DotBlot tests using a mix of three new selected PDD antigens.

Results: perigangliar infiltrates were characterized by an high percentage of CD3+ lymphocytes, with a variable percentage of plasma cells and macrophages. Precocious infiltrates of clinically health animal showed occasional CD8+ cells and macrophages admixed with scattered CD3+ T cells whereas a large percentage of CD8+ lymphocytes and macrophages were associated with moderate to severe periganglia infiltrates. In these latter, LFB stain revealed a demyelization. Crop basal layer epithelium of all affected birds showed highest percentage of PCNA+ cells, independently to the severity of the pathology. Specimens stained with DHTS showed a different thickness in pre-keratinized epithelial layer of the crop between infected and uninfected groups, with thinness of this stratum in affected birds. Finally we found a significant correlation between all histological data and serology in PDD positive parrots.

Discussion: histology confirm the nature of non suppurative ganglioneuritis, accompanied by gliosis, demyelization and ganglia swelling, of PDD. These findings, according to the immunophenotype of infiltrates, suggest an autoimmune mechanism for PDD, induced by a still indeterminate aetiology. Our preliminary results, showed a possible important employ of a new serological test to screen the parrots for PDD. In attempt to control the epidemiology of PDD, serological tests are fundamental considering the high number of nondiagnostic or false-negative crop biopsies.

Dynamic relationship between Sialyl-Lewis x and E-cadherin expression in canine malignant/invasive mammary tumour phenotype

Pinho, Salomé S, Augusto J F Matos, Célia Lopes, Nuno T. Marcos, Júlio Carvalheira, Mary L Alpaugh, Celso A Reis, Fátima Gärtner

IPATIMUP, Porto, Portugal

Introduction: Spontaneous mammary tumours represent the most common neoplasia in female dogs, accounting for 25 to 50% of all neoplasias. In general canine malignant tumours metastasize via the lymphatics to the regional lymph nodes or hematogenously to the lungs. This phenomenon depends mainly on the coordinated expression of adhesion molecules to remodel cell-cell and/or cell-matrix adhesion. Both canine and human breast cancer progression, including invasive and/or metastatic properties, is associated with abnormal glycosylation, resulting in expression of altered carbohydrate determinants, such as the Sialyl Lewis x (sLe^x) antigen. sLe^x antigen is a carbohydrate antigen that is considered not only a marker for cancer but also implicated functionally in the malignant behaviour of cancer cells. Overexpression of sLe^x is associated with enhanced tumour progression and metastases of many types of cancer including those of the mammary gland. E-Cadherin is specifically involved in epithelial cell-to-cell adhesion. In cancer, E-Cadherin underexpression is one of the alterations that characterizes the invasive phenotype and is considered a potent invasion/tumour suppressor gene. The aim of this study was to analyse the sLe^x expression in canine malignant mammary tumours and to evaluate the relationship between sLe^x expression and clinicopathological features. We also investigated the coordinated expression of both adhesion molecules (sLe^x and E-cadherin) in canine malignant mammary tumours.

Material and Methods: Fifty-three cases of canine mammary carcinomas were analysed immunohistochemically using monoclonal antibodies against sLe^x and E-Cadherin. Evaluation of sLe^x was analysed according to the clinicopathological characteristics of the cases. Double labelled immunofluorescence staining was performed to evaluate the combined expression of sLe^x and E-Cadherin. E-cadherin immunoprecipitation was also performed followed by Western-blot analysis using sLe^x monoclonal antibody.

Results: sLe^x expression was consistently demonstrated in all cases of canine mammary carcinomas. We found a significant relationship between sLe^x expression and the presence of lymph node metastases. We also demonstrated that when E-Cadherin expression was increased sLe^x was reduced and vice-versa. The combined analysis of both adhesion molecules revealed an inverse relationship (1). We also demonstrated that Sialyl Lewis x was not detected in the 120 kDa range that correspond to the molecular weight of E-cadherin

Discussion: In the present study we demonstrate the importance of sLe^x as a marker of malignant phenotype which supports the role of sLe^x in the process of lymphatic invasion and metastases in canine mammary carcinomas. Our results also showed that sLe^x and E-Cadherin were inversely correlated which could suggest the presence of a dynamic and coordinated mechanism of gene expression that could contribute to the malignant and invasive phenotype.

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Immunohistochemical detection of E2 protein (gp55) of classical swine fever virus in brain and ileocecal valve of experimentally infected pigs

Polaček, Vladimir, Nikola Vasković, Jasna Prodanov, Milijana Knežević, Sanja Aleksić Kovačević

Veterinary Specialized Institute "Kraljevo", Kraljevo/ Serbia

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Introduction: Classical swine fever (CSF) is a highly contagious viral disease of domestic pigs and wild boars with severe consequences on animal welfare, livestock production, trade and national economy. The aim of this investigation was immunohistochemical detection of E2 (gp55) glycoprotein of CSF virus in paraffin embedded formalin fixed tissue samples, in conditions of experimentally infection.

Material and methods: The trial was carried out on 32 piglets aged 21 to 60 days, with different immunological status (with presence/absence of specific colostral antibodies for CSF virus), and different way of artificial infection (intramuscular/oronasal). Experimentally infection was performed by Baker strain of CSF virus. We used LSAB immunohistochemical method with monoclonal antibodies WH303 for E2 glycoprotein of CSF virus.

Results: Immunohistochemical examination of brain samples detected presence of glycoprotein E2 of CSF virus in endothelial cells of blood vessels, macrophages and cell infiltrate of leptomeninges, as well as in cerebellum. The largest expression of E2 glycoprotein in ileocecal valve was determined in endothelial cells of blood vessels, monocytes, macrophages, plasma cells, lymphocytes and epithelial cells.

Discussion: Immunohistochemical detection of E2 glycoprotein of CSF virus in paraffin embedded formalin fixed tissue samples with monoclonal antibodies WH303 is very reliable and precise method that may find its place in routine laboratory diagnosing of CSF and further studies of CSF pathogenesis. CSF is also very useful animal model for biomedical research into the pathogenesis of human virus infections prompting immunosuppression and hemorrhage.

Epidemiological investigation and characterization of *Chlamydia*-like organisms in brown trout (*Salmo trutta*) over a two year period

Polkinghorne, Adam,¹ Heike Schmidt-Posthaus², Angelika Lehner³, Helmut Segner², Lloyd Vaughan¹

¹Institute of Veterinary Pathology, University of Zurich, ²Centre for Fish and Wildlife Health, University of Berne and ³Institute for Food Hygiene, University of Zurich, Switzerland

Introduction: *Chlamydia*-like organisms are emerging as a cause of disease in many different animal species, including humans. Here we investigate epitheliocystis, a disease of the gills and skin, in a variety freshwater and marine fish species. In 1996-1998, a study was performed to determine the effects of poor water quality on the health of brown trout (*Salmo trutta*) (1). A number of fish exposed to polluted river water were diagnosed to have epitheliocystis, based on histological examination of gill preparations. The aims of the present study were to use molecular methods to confirm and characterize these putative epitheliocystis agents of chlamydial origin and to examine their prevalence amongst brown trout examined as a part of this environmental study.

Materials and Methods: A total of 65 samples from brown trout were available for retrospective analysis, including 46 river-water exposed samples and 19 tap-water kept control fish. 11 river-exposed brown trout showed signs of epitheliocystis. DNA was extracted from formalin-fixed and paraffin-embedded samples of trout gills using a Qiagen DNeasy Tissue Kit. The presence of chlamydial DNA was detected by a 16S rRNA *Chlamydiales* order-specific PCR, as previously described (2). The identity of PCR products was determined by direct sequencing and comparison of the sequences obtained against available sequences in GenBank using the BLAST server from the National Centre for Biotechnology website.

Results: 16S rRNA PCR screening of trout samples revealed a number of unexpected results. All 11 epitheliocystis positive trout samples were PCR positive for chlamydial DNA, confirming the earlier identification (1). Interestingly, 23/35 epitheliocystis-free trout exposed to river water were also PCR positive as were similar the majority of tap-water kept control trout (11/19). Direct sequencing of three PCR positive epitheliocystis samples revealed sequences sharing closest similarity to two uncultured *Neochlamydia*-like 16S rRNA sequences ([AY225596.1](#), 88%; [AY225594.1](#), 91%), which we had previously detected in the eyes of cats with ocular disease (3). Curiously, Arctic charr (*Salvelinus alpinus*) epitheliocystis agents share strongest 16S rRNA sequence similarity to the latter (4). The presence of chlamydial DNA in the gill lesions is currently being confirmed by *in situ* hybridization and the identity of other PCR positive samples is being examined.

Discussion: Novel *Chlamydia*-like organisms appear to be associated with epitheliocystis in brown trout. This work provides the first opportunity to determine the prevalence of these organisms in a large population of brown trout sampled over an extended period and provides an important insight into the biodiversity and host range of these *Chlamydia*-like organisms. Given the propensity for *Chlamydia* to flourish in hosts subject to crowding and stress, raises the question of zoonotic transfer, especially in aquaculture and in aquaria.

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Localisation of *Pasteurella multocida* in experimentally infected immunosuppressed pigs

Pors, Susanne E., Jens Nielsen*, Magne Bisgaard and Henrik E. Jensen.

Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Denmark *National Veterinary Institute, Copenhagen, Denmark

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Introduction: Bacterial pneumonia is an important cause of morbidity and mortality in humans and animals. *Pasteurella multocida* is one of the most important causes of bronchopneumonia in Danish pigs. Porcine bronchopneumonia associated with *P. multocida* may result in systemic spread and septicaemia resulting in foci of infection in more organs. The pathogenesis of dissemination to other organs from the lungs is almost unknown. The aim of the present study was to evaluate the impact of leukocyte depletion on bacterial localization in pigs experimentally infected with *P. multocida* through intratracheal inoculation.

Material and methods: Twelve female pigs were housed in animal isolation facilities. Groups A (4 animals) and B (2 animals) were depleted of leukocytes four days prior to infection/mock-infection using treatment with a cytostatic drug, cyclophosphamide (CP) ("A-Pharma", 20 mg/ml). A dose of 2.5 ml/kg was injected i.v. The remaining pigs (groups C and D) received isotonic saline i.v. Blood parameters were examined by FACS analysis after infusion of CP/placebo. At the day of infection (4 days after treatment), all pigs in groups A and C, were infected intratracheally with 6 ml of an overnight culture of *P. multocida* at a concentration of 10^9 CFU/ml. The strain of *P. multocida* originated from a natural case of porcine bronchopneumonia. Infection of the animals was done through an endotracheal tubus placed under light sedation. Blood parameters were examined by FACS analysis after infection. The animals were euthanized 24 hours after infection and subjected to a complete necropsy. Lesions in the lungs were evaluated macroscopically, bacteriologically, and histologically. Presence of *P. multocida* was determined by fluorescent in situ hybridisation (ISH).

Results: At the time of infection, treatment with CP had reduced the total number of leukocytes significantly. Especially the neutrophils had been depleted constituting less than 5% of the total number of leukocytes. In all infected pigs, acute lobular bronchopneumonia was present in one or more of the cranial lung lobes. Significantly higher counts of *P. multocida* was obtained from group A compared to group C. The lungs of 3 out of 4 control animals (groups B and D) were free from lesions and sterile. Histologically, the presence of necrosis in all pigs of group A showed a clear contrast to the animals with a normal immune status (group C), in which no necrosis was found. The presence of *P. multocida* demonstrated by ISH confirmed the results of cultivation. In group A, the bacteria were diffusely spread and often present at intravascular sites. In group C, the bacteria were found primarily contained in cells resembling neutrophils localized in lumen of alveoli.

Discussion: In conclusion, the present study indicates that absence of a well-functioning leukocyte population and in particular neutrophils paves the way for the development of severe lung lesions in pigs infected with *P. multocida*. Therefore, the different types of lesions and spread of bacteria reported to exist under natural infections could be a result of differences in immune status rather than being the result of related to the virulence factors of the bacterium.

Inflammatory reactions in lung tissue of pigs infected with *Pasteurella multocida*: An immunohistochemical study

Pors, Susanne E., Tine Iburg, Mette S. Hansen, Ole L. Nielsen, Magne Bisgaard and Henrik E. Jensen

Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Denmark.

Introduction: *Pasteurella multocida* is an important cause of pneumonia in pigs and also takes part of the porcine respiratory disease complex. Infection with *P. multocida* may induce a variety of pulmonary lesions, i.e. from mild suppurative to extensive necrotising lesions. Various immunoreactive cells and cytokines are of importance for the development of lesions in the respiratory tract. Information about the inflammatory cells in the different types of *P. multocida*-induced lung lesions is, however, lacking. The aim of the present study was to characterise different aspects of the inflammatory response in two different types of chronic bronchopneumonia in pigs naturally infected with *P. multocida*. In the study we focused on the distribution pattern of the T-lymfocyte population, neutrophils and active macrophages.

Material and methods: From a cross-sectional study of bronchopneumonia in Danish slaughter pigs, lung tissue samples from 20 animals with cranioventral bronchopneumonic lesions were selected. *P. multocida* had been recovered from all lungs and the pigs were grouped according to the type of lesion in their lungs (Group A, necrotic pneumonia (n = 9); group B, suppurative pneumonia (n = 11)). Lung tissue from five animals without lesions and sampling sterile pon culture served as controls. Lung tissues from all animals were fixed in formalin and by freezing. Formalin fixed tissue was used for histochemical staining and immunohistochemistry for lysozyme and L-1, respectively. Serial frozen tissue sections were used for immunohistochemistry for CD3⁺, CD4⁺ and CD8⁺ T-lymphocytes. Presence of *P. multocida* in lesions was verified by in situ hybridisation.

Results: The infected groups (groups A and B) showed a significant increase of all types of inflammatory cells compared to the control animals. Immunohistochemistry for lysozyme showed numerous active macrophages in different parts of the lungs. They were especially prominent in the zone surrounding the necrotic parts in group A, and within the exudate in the alveoli. Neutrophiles were identified by expression of L-1 antigen and were most prominent within areas of necrosis in group A, whereas in group B they were predominately located in the lumen of the alveoli. T-lymphocytes of both the CD4⁺ and CD8⁺ types were evenly distributed and generally found in bronchiolar lymphoid tissue and in the alveolar septa. Also the T-lymphocytes often aggregated in areas adjacent to the interlobular interstitium and pleura. In the necrotic areas in group A the T-lymphocytes were primarily identified as CD8⁺ cells. In group B, perivascular cuffings consisting of T-lymphocytes (both CD8⁺ and CD4⁺ cells) were present. Occasional CD8⁺-CD4⁺ double-positive T-lymphocytes were identified in both groups.

Discussion: In the present study it was found, that in natural infection the pattern of distribution of some main immunological cells differed according to the pulmonary lesion groups associated with *P. multocida*. The fundamental cause for the difference in type of lesions caused by *P. multocida* might relate to host differences of the inflammatory reaction from the host and/or differences of the bacterial strain.

Primary and metastatic spinal cord tumours in dogs – a study of 26 cases

Priestnall, Simon, Emma Rayner, Nanny Wenzlow, Brian Smyth, Brian Summers and Sandra Schöniger

The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, United Kingdom

Introduction: The aim of this study was to provide an overview of the spectrum of spinal cord tumours in dogs, since previously descriptions are mostly individual cases or small case series.

Materials and methods: We summarize the pathological findings of 26 primary and metastatic canine spinal cord tumours. Cases were from the archives of the Royal Veterinary College or the personal collection of Dr. Summers.

Results: Of the submitted tumours, 20 were in pure-bred and 4 in mixed breed dogs. In two cases, the breed of the dogs was unknown. The age of affected dogs ranged from 2 to 13 years with a mean age of 7.7 years. In three cases the age of the dog was not provided. Tumours were present in all levels of the spinal cord; however they most frequently involved the thoracic segments 52.4% (11/21). Primary tumours accounted for 88.5% (23/26) with 11.5% (3/26) metastatic. 52.0% (13/25) of tumours were intramedullary, 48.0% (12/25) extramedullary and a single case was in an extra- and intramedullary location. Primary intramedullary tumours included haemangioma (4), oligodendroglioma (1), high grade glioma (1), histiocytic sarcoma (1) and spinal cord gliomatosis (3). Extramedullary tumours were meningeal sarcomatosis (4), meningeal histiocytic sarcoma (2), meningioma (5), paraganglioma (1) and neuroblastoma (1). Haemangiosarcoma (2) and leiomyosarcoma (1) represented the metastatic tumours.

Discussion: A wide array of neuroepithelial and mesodermal neoplasms affect the canine spinal cord. The most frequently diagnosed intramedullary tumours were haemangioma (28.6%) and spinal cord gliomatosis (21.4%). The most frequently diagnosed extramedullary tumours were meningioma (41.6%) and meningeal sarcomatosis (33.3%).

Pathogenesis of a highly pathogenic avian influenza virus (H7N1) in chickens

Ramis, Antonio, N, Busquets, F.X. Abad, R. Dolz, R. Valle, A. Chaves, A. Darji and N. Majó

Universitat Autònoma de Barcelona, Barcelona, Spain

Introduction: Recent outbreaks of Highly Pathogenic Avian Influenza Virus (HPAIV) caused by H₅N₁ subtype in birds and also in humans, have demonstrated the requirement of a deep understanding of this viral infection. Despite considerable experimental knowledge, current understanding of in vivo influenza pathogenesis in its natural host (chicken) is limited. The aim of our study is to evaluate the dynamics of HPAIV infection in chicken inoculated with different viral doses and to investigate the viral entry in the Central Nervous System (CNS)

Material and methods: The A/Chicken/Italy/5093/99 H7N1 HPAIV strain (IVPI: 2,8), provided by Dr. Ana Moreno (Istituto Zooprofilattico Sperimentale, Brescia, Italy), was used. Four groups of 15 day-old SPF chickens were housed in negative-pressure isolator units. Three groups of chickens were infected intranasally with 10^{5.5} (G₁), 10^{3.5} (G₂) or 10^{1.5} (G₃) ELD₅₀, respectively. A fourth group was used as sham-inoculated control group (G₄). Clinical signs, as well as mortality, were recorded up to 16 dpi. After inoculation, three birds of each group were euthanized and necropsied on days 1, 3, 5, 7, 10 and 16; and tissue samples for histopathology and immunohistochemistry, using a commercial monoclonal antibody against Avian Influenza Virus (AIV) nucleoprotein, were taken. A Taq-Man-based real-time one step RT-PCR for quantification of M gene of AIV was used to estimate the number of viral RNA copies from: several tissues (lung kidney, intestines, and CNS), cloacal and oropharyngeal swabs, cerebrospinal (CNS) fluid; and also from the different blood compartments: plasma, erythrocytes and peripheral blood leukocytes (PBL).

Results: In the G₁ group, specific clinical signs and lesions were seen at 3 dpi.; and mortality started on 3 dp.i. until 6 dpi. In G₂ group, only a slight depression from day 1 to 5 dpi were seen and no mortality was recorded. G₃ and G₄ didn't show neither clinical signs, nor mortality. Microscopic lesions and presence of viral antigen were evident in G1 and some G2 animals and they consisted in vascular changes and parenchymal necrosis and there was a high correlation between lesions and quantity of viral antigen; most affected organs were central nervous system, pancreas, adrenal glands and myocardium. Regarding viral quantification by real time RT-PCR in selected tissues, a high amount of viral genome in all of the animals from G1 was detected, but it was also detected in some tissues of animals in G2 and G3. Viral excretion was also detected and quantified in the three groups (G₁, G₂ and G₃). Lastly viremia, as the demonstration of viral genome by means of RT-PCR in the different compartments of blood (plasma, erythrocytes and PBL), and also the presence of viral genome in CSF as soon as 1 day p.i. were showed.

Discussion: We can conclude that the inoculation of 10^{5.5} ELD₅₀ dose (G₁) of H7N1 reproduce the clinical signs, gross and microscopic lesions of a classical HPAI. H7N1 strain has a pantropic potential with an early dissemination to different tissues but replication may only occur in some parenchymal cells. Regarding the shedding routes of the virus, oronasal route is more relevant than cloacal and it occurs even at the lowest doses. Lastly, viremia and early dissemination to the CNS has been demonstrated and the detection of viral genome in the CSF at 1 d.p.i. may indicate the importance of the blood route for entrance and dissemination of the virus into the CNS. Moreover CSF infection may contribute to the early viral dissemination throughout the CNS and spinal cord.

Retrospective assessment of veterinary cytopathology and histopathology correlation studies of various organs

Reppas, George, Paul Canfield

VETNOSTICS, 60 Waterloo road, North Ryde. NSW, Australia

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Introduction: Retrospective assessment of veterinary cytopathology and histopathology correlation studies of various organs

Material and methods: Literature review and metanalysis

Results: There appears to be good correlation between the results of cytological and histopathological diagnoses of pathological processes - particularly neoplastic and inflammatory processes across a wide range of body systems.

Discussion: Cytology was first implemented in human medicine in the 1930s. The usefulness of cytology in investigating disease processes in various organs has steadily increased since the first report of its use in veterinary medicine in the late 1960's. During the 1980's and 1990's a greater interest was shown in the use of cytology, particularly in relation to easily aspirated lesions such as those of a cutaneous nature, resulting in the first correlative studies between cytopathological and histopathological diagnoses. The results of these limited studies generally found the correlation between cytology and histopathology to be favourable especially with regards to the diagnosis of neoplasia and inflammation, although there were obvious limitations. Over the last decade, especially in conjunction with the increased affordability and routine use of ultrasound guided examinations and also CT guided imaging in veterinary practice, the usefulness of cytopathology as an ancillary laboratory investigative technique has been extended. Samples for cytopathological examination are now often presented in the initial workup of intra-cavitary pathological processes because of the ease with which such samples can be procured, the rapid turnaround in result reporting of such specimens and reduced patient morbidity and post-collection complications following the fine needle aspiration biopsy procedure. Correlative studies are beginning to flourish which generally support the usefulness of cytopathology in such investigations across the various body systems.

Expression of leptin and leptin receptor (OB-R) in normal, hyperplastic, and neoplastic canine mammary tissues

Ressel, Lorenzo, Finotello Riccardo, Vannozzi Iacopo, Innocenti Maria Viola, Poli Alessandro

University of Pisa, Pisa, Italy

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Introduction: Recently, increased body weight has been shown to be associated with increased risk of and mortality from various cancers. In particular, a strong association was found between adiposity and increased risk of breast cancer in postmenopausal women. Leptin, a fat cell-derived peptide hormone, seems to elicit a growth-stimulating effect in breast cancer cells with leptin receptor (OB-R) expression. Previous studies, in human breast cancer, demonstrated that this hormone may have a promoting effect on the carcinogenesis and metastasis of breast cancer, possibly in an autocrine manner. The purpose of our study is to evaluate leptin and OB-R expression normal, hyperplastic and neoplastic mammary tissues.

Material and methods: Thirty-six cases of canine mammary tumours (7 benign and 29 malignant tumours) were included in the study. Samples from healthy mammary gland and hyperplastic mammary tissue were also examined. The expression of these markers was investigated by immunohistochemistry using commercial anti-leptin and anti-OB-R antibodies with a streptavidin-biotin peroxidase method. The immunolabelling was scored using a semi-quantitative method. The relationship between the expression of leptin and OB-R and clinicopathological features was also analysed.

Results: Leptin expression was observed in normal mammary epithelial cells. Over-expression of leptin, as determined by the number of deeply stained cells, was observed in adenosis and benign mammary tumours, but not in lobular hyperplasia and in malignant tumours. Interestingly, OB-R staining was detected in epithelial cells in adenosis and in benign mammary tumours, while neoplastic cells, especially in carcinomas were OB-R-negative.

Discussion: In this study, we used polyclonal antibodies that specifically react with leptin and OB-R to perform immunostaining of canine mammary normal, hyperplastic, and neoplastic tissues. Our experiments allowed to recognise cytoplasmic expression of leptin and cytoplasmic as well as membranous expression of OB-R. Over-expression of the leptin and its receptors seems to be present in adenosis and in benign tumours, while mammary carcinoma seems to lose this expression pattern. Recent studies have shown positive expression of OB-R in human malignant breast epithelial cells lines and that leptin can stimulate the proliferation of both normal and malignant breast epithelial cells. In our study on canine mammary tissues, leptin and OB-R expression was evident in hyperplastic and well differentiated tumours, while epithelial cells in more aggressive carcinomas seem to present a reduced expression of both markers. These preliminary results seem to be inconsistent with previous studies carried out in human breast cancer and need a further investigation.

Expression of PTEN in mammary tumours of dog and cat

Ressel, Lorenzo, Millanta Francesca., Caleri Elvanessa., Poli Alessandro

Dipartimento di patologia animale profilassi e igiene degli alimenti, Pisa, Italy

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Introduction: Phosphatase and tensin homolog (PTEN) is the protein product of a gatekeeper tumor suppressor gene, involved in double mechanism of defense against tumor developing and progression. The protein is involved in reducing the amount of phosphoinositides available for AKT and in consequence in reducing all downstream signaling leading to cell proliferation. This protein seems to be involved also in maintainance of cell adhesion to surrounding tissues and inhibition of cell movement. Loss of PTEN expression was observed in human breast carcinoma and recently in dog breast cancer, but no data are available on cat mammary tumors. Aim of this work is to investigate the expression of PTEN in canine and feline healthy, hyperplastic and neoplastic mammary tissues and to investigate the significance of the loss of PTEN among the pathologic process of breast cancer in the two species.

Materials and methods: Twenty-three dog and 11 cat formalin fixed paraffin embedded hyperplastic and neoplastic mammary tissues were morphologically analysed and classified using the WHO classification. Sections from each tumour were tested by immunohistochemistry protein expression using commercial anti-PTEN antibody. The expression of PTEN in tumours was compared with normal and hyperplastic mammary tissues as well as with control tissues. Scoring of PTEN expression was assessed with a semiquantitative method previously used in human medicine literature. Statistical analysis was performed using Chi-square test.

Results: Cytoplasmic PTEN expression was detected in healthy and hyperplastic mammary tissues in both species, as well as in the cytoplasm of normal mesenchymal stromal cells. In the dog 100% of benign and 64% of malignant tumours expressed PTEN, as strong as the normal surrounding tissue. A reduced PTEN staining was observed in the 24% of malignant tumour, compared with normal tissue, and 12% expressed PTEN in none of the neoplastic cells. A positive PTEN staining was detected in the 20% of feline mammary carcinomas, while 80% expressed no staining. The eighty-three% of canine and 75% of feline mammary tumours with reduced or absent expression of PTEN showed a metastatic behaviour.

Discussion: In this preliminary study PTEN expression is lost or reduced in a conspicuous number of canine and feline mammary tumours. In the subpopulation identified by loss or reduced PTEN expression, the majority of tumours had morphological and biological aggressive features. The observation that the loss of this gatekeeper is related with metastatic behaviour is in agreement with the biological functions of the normal expressed protein. Canine and feline mammary carcinomas seems to share with human breast cancer this feature, these data are a further evidence that these animal models could be useful to study human breast cancer.

The localization of histochemical activity of 3 α , 3 β and 17 α -HSD in the adrenal cortex of cow (*Bos taurus L.*)

Rexhepaj, Daut

Faculty of Natural Sciences, Department of Biology, University of Prishtina, Kosovo

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Introduction: The adrenal cortex tissue is developed only at the vertebrates. This tissue is anatomically integrated with the chromaffin tissue in a pair of organs – adrenal cortex which has an endocrine function. These both tissues of this organ differ among each other by morphogenesis, structure and function. In the cell of adrenal cortex tissue between capsule and medulla, are synthesized mineral – corticoids, glucocorticoids and androgen hormones which are necessary for life. Histochemical demonstration of HSD at some mammals (Levy et al., 1959., Deane et al., 1962., Fowler et al., 1970., Gupta et al., 1974., Hullinger et al., 1984., Zeqiri et al., 1987) have shown different zonal order of adrenal cortex at mammals.

Material and Methods: By applying histochemical methods (Bailie, A. H., et al., 1966) we determined the distribution of enzymatic activity of 3 α , 3 β and 17 α -HSD in steroid tissue of adrenal cortex (AC) in the cow. This was achieved through the use of nitro-blue tetrazolium salt, that served as a last acceptor of hydrogen ions and other corresponding steroid substrates; 3 α -HSD with androsterone; 3 β -HSD with dehydroepiandrosterone, pregnenolone and 17 α -OH pregnenolone. The histochemical activity of these enzymes has been clearly defined in the granula shapes of formazane associated with a dark color localized in the cellular cytoplasm.

Results: According to the research, we concluded that the localization and the intensity of these enzymes are different in different zones of the adrenal cortex. The activity of the enzyme 3 α -HSD (androsterone) was negative in all zones of the gland while 3 β -HSD (DHA and pregnenolone) showed a high activity in the fasciculate zone and a lower activity in the glomerulosa and reticularis zone. 17 α -OH pregnenolone showed a very low activity in the glomerulosa and fasciculate zone and a negative reaction in the reticularis zone.

Discussion: The capsules, septa and medulla cells have reacted negatively which is in compliance with the results of Deane et al., (1965) for some mammals and Hullinger et al., (1984) at horse. Based on our results, 3 α -HSD has reacted negatively in the entire zones which is in correlation with data of Hullinger et al., (1984) at sheep and Zeqiri et al., (1986) at water buffalo. A very high activity of 3 β -HSD with DHA is located in the outer part of fasciculate zone which gradually was decreased in the glomerulosa zone, inner fasciculate and reticularis zone that agree with data of Hullinger (1984) and Zeqiri et al., (1984, 1985) at water buffalo. Between glomerulosa and fasciculate zone can be observed a narrow zone called intermediate zone or biproliferativa (Hullinger, 1978). Cells of this zone are not differentiated and multipotential characterized by a low activity of HSD which is not in compliance with Caffer et al., (1955) as according to them, the 3 β -HSD activity is present in the whole adrenal cortex of rat and is more present in the intermediate zone. 17 α -OH pregnenolone enzyme is located in the glomerulosa and fasciculate zone characterized with a very low activity. Reticularis zone reacted negatively which is not in accordance with data of Hullinger et al., (1984) for some domestic mammals and Zeqiri et al., (1987) at water buffalo.

Blackhead disease-like typhlohepatitis in two Red-breasted Mergansers (*Mergus serrator*) caused by tetratrachomonads

Richter, Barbara, Christoph Schulze, Jens Kämmerling, Herbert Weissenböck

Institute of Pathology and Forensic Veterinary Medicine, University of Veterinary Medicine Vienna, Austria

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Introduction: The Red-breasted Merganser (*Mergus serrator*) belongs to the family Anatidae, order Anseriformes, and lives on shores and banks of the Northern hemisphere. Histomonosis (caused by *Histomonas meleagridis*) and a very similar looking form of Trichomonosis (caused by *Tetratrachomonas gallinarum*) has been described in turkeys and other members of the order Galliformes. In ducks, Histomonosis is not known but *T. gallinarum* has been identified in various diseases with inconsistent pathological lesions, mainly limited to the intestine.

Material and methods: Necropsy and histopathology was performed on two Red-breasted Mergansers that had died at an interval of 6 weeks in a zoological garden. At the same time, young turkeys from the same collection died of typical Histomonosis and one of these was used as control during the following examinations. In-situ hybridizations (ISH) specific for the 18S ribosomal RNA (rRNA) of *H. meleagridis* and *T. gallinarum* / *Trichomonas gallinae* were carried out on samples from caeca and livers of the three birds. To identify the protozoal species, PCR and partial sequencing of 18S rRNA was performed on liver and caecum samples from the Mergansers.

Results: Necropsy and histopathology findings in the Mergansers were necrotizing typhlitis in both and necrotizing hepatitis in one bird with numerous large polymorphic protozoa, morphologically very similar to *H. meleagridis*. ISH revealed a double infection with *T. gallinarum* and *H. meleagridis* in the caeca and an infection with *H. meleagridis* in the liver of the turkey. Both Mergansers showed a monoinfection with *T. gallinarum* in the caeca and one bird in the liver. PCR and sequencing of the protozoa from tissue samples of the Mergansers resulted in a sequence with 98 % homology to *T. gallinarum*.

Discussion: This is the first report of blackhead disease-like typhlohepatitis caused by *T. gallinarum* in a duck species, the Red-breasted Merganser. A similar disease caused by *T. gallinarum* has been described only in turkeys. Experimental infections of ducks with this protozoan species resulted in various outcomes. Virulence factors or genotypes of this parasite have not clearly been determined, yet. Possibly the existing species of *T. gallinarum* is not homogenous and includes various cryptic species with differing pathogenicity. Because of the morphological similarity of the tetratrachomonads in these cases to *H. meleagridis*, the diagnosis could not be achieved based on histopathology alone. With molecular pathological methods, the etiologic agent could be identified.

Immunohistochemical diagnosis of porcine circovirus from pigs with postweaning multisystemic wasting syndrome

Ristoski, Trpe, Slavco Mrenoski, Iskra Cvetkovic

Faculty of veterinary medicine, Department of pathology, Skopje, Macedonia

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Introduction: Porcine circovirus type 2 (PCV 2) is a recently recognized agent that is consistently associated with postweaning multisystemic wasting syndrome (PMWS) in swine and this disease become an economical very important disease of pigs in many countries.

Material and methods: Samples of lung, liver, kidney, spleen and lymph node from pigs with postweaning multisystemic wasting syndrome and from similarly diseased pigs were examined. In order to determine if these virus (PCV 2) interact in natural acquired wasting diseases, affected tissues from field cases were examined by immunohistochemistry for PCV2 with F217 2C6-H9-A2 monoclonal antibody using LSAB method.

Results: We examined more than 400 pigs from different herds with signs of wasting disease. The clinicopathological manifestations were progressive weight loss, tachypnea, dyspnea and jaundice, accompanied by interstitial pneumonia and lung oedema, ascites, hydrothorax, lymphadenopathy, hepatitis and nephritis. The histopathological investigations showed lymphohistiocytic to granulomatous interstitial pneumonia and at the same time infiltration by histiocytes. In accordance with reports from other countries, we found the depletion of the lymphoid elements and the occurrence of giant cells in the lymphoid organs as the most characteristics of the syndrome. Immunohistochemical examination of tissues with PCV2 antibody showed positive staining on more than 200 affected pigs and the viral antigen was widespread in lesions of numerous organs. Low to moderate amounts of PCV2 antigen were detected in a wider range of tissues of lung, lymphoid organs, liver and lesser degree in kidney.

Discussion: These results demonstrate a high degree of association between the presence of the circovirus and PMWS in affected swine and PCV2 antigens were regularly detected in cells of monocyte/macrophage lineage in multiple organs.

Pathological findings of primary Xanthinuria in a family of wire-haired Dachshunds

Roccabianca, Paola, Paola Scarpa, Stefano Faverzani, Carolina Ramirez-Sanchez, Pietro Riccaboni, Giancarlo Avallone

Department of Pathology, Hygiene and Public Health- Veterinary Anatomic Pathology Section, School of Veterinary Medicine of Milano, Milano, Italy

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Introduction: Xanthinuria is a rare disorder deriving from defects in the metabolic pathway that converts purine into uric acid. Xanthinuria can be hereditary (autosomal recessive) or secondary to allopurinol administration. Human hereditary Xanthinuria is classified according to functional deficiencies of three enzymes: Xanthine Oxidase (XO), Aldehyde Oxidase (AO) and Sulphide Oxidase (SO). Xanthinuria type I derives from a loss of function mutation in the XO gene. Type II Xanthinuria lacks XO and AO activity due to a mutation in the molybdenum cofactor sulphurase necessary for their activity. These two diseases are associated with development of urinary xanthine calculi, urinary tract obstruction and acute to chronic renal failure in approximately 40% of human patients. Distinctively, neonatal Xanthinuria is a severe hereditary disease with growth retardation and nervous signs due to a mutation of the molybdenum cofactor synthetase with loss of function of all the three enzymes. Xanthine urolithiasis has been reported in humans, dogs, cats, sheep, pigs and cattle. In dogs the most common cause of xanthinuria is allopurinol administration but primary forms have been described in Cavalier King Charles Spaniels and Dachshunds.

Material and methods: Three, male, wirehaired Dachshunds from the same sire (2 were siblings) developed xanthine urolithiasis. Two siblings presented with ureteral obstruction at 70 days, associated with acute renal failure in one dog. The 2 siblings, the dam and the sire had severely reduced uric acid urinary concentration (0,0-0,6 mg/dl; normal range 4,7-8,7 mg/dl). The third dog developed ureteral obstruction associated with acute renal failure at 5 months of age. Urolith X ray-diffraction demonstrated a 100% xanthine composition in one dog. The dogs with acute renal failure were euthanized. One of the siblings eliminated xanthine calculi persistently but did not develop renal failure or urinary tract obstruction and died at 3,6 years due to ingestion of a perforating foreign body. All dogs underwent full necropsy and histopathologic examination.

Results: Major gross and histopathologic findings involved the urinary system. Hydronephrosis was bilateral (1/3) or monolateral (1/3) with associated renal atrophy, hydroureter (2/3) and cystitis (1/3). Xanthine uroliths were present in the pelvis (1/3), massively in the urinary bladder (3/3) and in the urethra (2/3). Uroliths were massively present in all dogs and were irregular with smooth surface, brown-red to yellow-green and varied in size but were smallest in the older dog with no ureteral obstruction. Cortico-medullary atrophy and fibrosis were evidenced in 2 dogs. Renal medulla was most severely involved. Distal and collector tubules had luminal xanthine uroliths (3/3) with secondary tubular necrosis (3/3). Severe and diffuse interstitial pyogranulomatous (3/3) to lymphoplasmacytic inflammation (1/3), fibroplasia and edema (2/3) or fibrosis (1/3) were observed. Renal pelvis was ulcerated with presence of crystals in 1 dog.

Discussion: Familial history, clinical and pathological findings, composition of uroliths, reduced uric acid excretion (purine catabolism stops at the oxidation of xanthine with absence of uric acid synthesis) were all consistent with hereditary xanthinuria. Reduced or absent uric acid in the urines indicated a decreased or absent function of XO that associated with lack of severe neurological signs and/or growth retardation were consistent with a type I/II hereditary

Xanthinuria. The unusual juvenile age and severity of signs of the siblings compared to the other cases described in the literature could derive from a possible inheritance of two mutated alleles of the XO gene from the sire and the dam.

Ultrastructural findings of lymphoid tissues from postweaning multisystemic wasting syndrome affected pigs

Rodríguez-Cariño, Carolina, Joaquim Segales

Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola), Spain

Introduction: Porcine circovirus type 2 is considered the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS). The disease is pathologically characterized by lymphocyte depletion and granulomatous inflammation in different tissues, but mainly lymphoid ones. There is limited information regarding ultrastructural observations of PMWS affected animals, which have shown the presence of virus-like particles (VLPs) of 12-23 nm in diameter. Based on the lack of extensive ultrastructural studies on PMWS lymphoid tissues, the aims of the present work were to evaluate the ultrastructural and subcellular lesions in lymph nodes of affected animals and to correlate these alterations with the viral presence.

Materials and Methods: Small blocks of mediastinal and inguinal lymph nodes taken from control (n=2) and PMWS affected (n=4) pigs were fixed by immersion in 2.5% paraformaldehyde (v/v) and 2% glutaraldehyde (v/v), and processed by standard methods for transmission electron microscopy studies.

Results: Significant ultrastructural alterations were only noted in PMWS affected pigs, mainly in histiocytes. This cell type, which was the most numerous in the evaluated lymphoid tissues, showed severe swelling and proliferation of mitochondria, proliferation and dilation of rough endoplasmic reticulum, lysosome proliferation and also proliferation and severe dilation of Golgi complex. Virus-infected histiocytes contained large numbers of electron-dense intracytoplasmatic inclusions (ICIs) bodies with viral-like particles (VLPs); some histiocytes also had intranuclear inclusions (INIs). ICIs had round, oval or irregular shapes, with variable electron-density and they were usually dispersed throughout the cytoplasm; their diameter varied from 0.1 μm to 4.5 μm . Large and small ICIs showed VLPs with 8 to 17 in diameter, in granular arrange or paracrystalline arrays with membrane fragments. ICIs were usually finding arrange about mitochondria. Rough endoplasmic reticulum dilation and mitochondrial swelling were also found in lymphocytes, endothelial and dendritic cells.

Discussion: Virions with different forms and sizes and subcellular changes have been found in cytoplasm of histiocytes, which are indicative of viral replication and viral factory formation. Membrane structures of cytoplasm might be used for viral replication and built viral factories.

Ultrastructural findings of PK-15 cells infected with porcine circovirus type 2

Rodriguez-Cariño, Carolina, Maria Fort, Joaquim Segales

Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola), Spain

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Introduction: Porcine circovirus type 2 (PCV2) is considered the essential infectious agent of postweaning multisystemic wasting syndrome. It is a small virus that belongs to the Circoviridae family, genus Circovirus. Ultrastructurally (in tissues and tissue homogenates), intracytoplasmic inclusions have been described with viral-like particles (VLPs) between 12 to 23 nm in diameter. Three-dimensional features of the virus have showed an icosahedral structure. However, there is no information regarding ultrastructural observations on PCV2 infected cells. The aim of this study was to evaluate the subcellular and viral findings in PK-15 cells at different times post-inoculation.

Materials and methods: PCV2 negative PK-15 cells were infected with PCV2 Stoon-1010 strain, moi 0.01, and fixed at 0, 6, 12, 24, 48, 60 and 72 hours post-inoculation (hpi) for conventional and immunogold-labelling electron microscopy. At the same time, an immunoperoxidase monolayer assay (IPMA) was performed to detect PCV2 in the infected PK15 culture cell monolayers.

Results: The presence of PCV2 in infected cells was confirmed by IPMA from 24 to 72 hpi. No significant ultrastructural alterations were noted in PK15 cells. However, large intracytoplasmic inclusions with VLPs were observed at 24, 48, 60 and 72 hpi, which were usually located in close proximity to mitochondria; VLPs had 17 nm in diameter. Immunogold labelling preliminary results showed colloidal gold in cytoplasm and mitochondria.

Discussion: No significant ultrastructural findings were found in PK-15 cells infected by PCV2 at any time post-inoculation, which is in accordance with the lack of known cytopathic effect of this virus. Further studies are needed to establish a detailed morphogenesis process of PCV2 in cell culture.

Head injury in New Zealand sea lion neonates: is there a shaken pup syndrome?

Roe, Wendi, Joe Mayhew, Christine Thomson and Robert Jolly

Massey University, Palmerston North, New Zealand

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Introduction: Subdural haematomas (SDHs) in human infants are believed to be a marker of inertial brain damage due to inflicted head injury ("shaken baby syndrome"). At necropsy, affected children may also have subarachnoid haemorrhages and cervical spinal cord damage. Similar lesions are seen at necropsy in New Zealand sea lion (NZSL) pups, and intra-specific trauma is considered to be a significant cause of neonatal mortality in this threatened species. Adult and subadult males have been observed to bite and vigorously shake pups, and we hypothesised that shaking may be the cause of subdural and spinal cord haemorrhages. We conducted gross necropsies and histological examinations on 36 recently dead NZ sea lion pups in the 2008 breeding season in order to quantitate and characterise deaths due to cranial trauma.

Material and methods: Thirty six recently dead New Zealand sea lion pups were necropsied at Sandy Bay in the Auckland Islands between 12 January and 10 February 2008. Brains and spinal cords were removed, and representative body tissues collected and fixed in 10% buffered formalin. Digital photographs were taken of gross lesions. All tissues were transported to Massey University, Palmerston North, New Zealand in March 2008. Formalin-fixed samples were embedded in paraffin, sectioned at 5µm, stained with haematoxylin and eosin, and examined under light microscopy.

Results: 24/36 (67%) of pups had gross lesions consistent with head trauma (skull fracture, head and neck bruising, SDH in the cranial vault, sub- or epidural spinal cord haemorrhage, or subarachnoid haemorrhage). 19 these 24 (79%) had lesions suggestive of shaking (spinal cord haemorrhage or SDH). Histological examination of brains revealed that 12/19 (63%) of the shaking lesion group had meningitis. Other lesions suggestive of a coagulopathy were seen in only one of these pups. Of the remaining 7 pups, 2 had concurrent disease (sepsis) that could have caused a coagulopathy, although only one had haemorrhages in other organs. Three pups had meningitis without SDH or spinal cord haemorrhage. An overall total of 15 pups (42%) had meningitis; *Klebsiella pneumoniae* was cultured from most of these pups.

Discussion: Lesions comparable with those believed to be due to shaking injury in human infants were also present in dead NZSL pups. Many of these pups however, had meningitis, which may either have directly caused haemorrhage into subdural and epidural tissues, or may have predisposed pups to being shaken (due to decreased ability to escape) or to developing these haemorrhages when exposed to trauma. It is also possible that meningitis was an unrelated syndrome present in shaken pups, although the prevalence of meningitis in the shaking lesion group (63%) was appreciably greater than that of the group as a whole (42%). Shaking alone may have been the cause of SDHs and spinal cord haemorrhages in 6 pups.

Isolation of *Streptococcus suis* from a cat with meningo-encephalitis

Roels, Stefan, Olivier Devroye, Herma Buys, Hilde Smith, Patrick Butaye

CODA/CERVA, Brussels, Belgium

P90

Introduction: *Streptococcus suis* is an important worldwide pathogen of swine and causes outbreaks of meningitis, arthritis, pneumonia, endocarditis, abortions, and abscessation in these animals. It does not only infect pigs, but also man and other animal species, such as horses, sheep, fallow deer and cats. It is also known to be a normal inhabitant of the tonsils of pigs, cats and dogs.

Material and methods: A 6-months-old male pure breed cat (Carthusian) was presented for autopsy. The animal had presented progressive nervous symptoms such as ataxia, weakness associated with anorexia and hyperthermia during 15 days. The animal did not react to therapy with corticoids and antibiotics. Due to continuous decrease of the health status of the animal it was decided to euthanize the cat.

Samples of the cerebrum, cerebellum and brainstem, as well as samples from the liver, kidneys and spleen were taken for further bacteriological and histopathological examination.

Results: Histological examination of the liver tissue revealed a granular degeneration of the hepatocytes without any infiltration of inflammatory cells. A similar absence of inflammatory cells, were noticed in both the kidneys and spleen. On the level of the brain there was a leptomeningitis with a fibrinous and necrotic exudates interlaced with a mixture of inflammatory cells (neutrophils, lymphocytes, plasma cells and macrophages). This inflammation was diffusely present but focally more pronounced. The adjacent underlying nervous tissue contained a linear infiltrate of neutrophils and/or mononuclear inflammatory cells with superficial perivasculitis and vasculitis. The severity of encephalitic lesions was related to those seen in the associated meninges. These lesions are comparable to those described in cases of *S. suis* meningitis following septicaemia in pigs and man

The *Streptococcus suis* isolate was non-typable (polyagglutinable) and negative for 2 of the 3 virulence-related proteins, namely MRP and EF.

Discussion: The presence of *Streptococcus suis* was already described in clinically normal cats and cats with pleuropneumonia and moist dermatitis. This report is the first time that it is associated with meningoencephalitis in this species.

Although *S. suis* as pathogen in different animal species still needs further research, this case documents the neurotropic pathogenic potential of this organism in cats and the need for considering this bacterial species in the differential diagnosis of meningoencephalitis in these animals. Additionally, cats could also be considered as potential danger for transmitting this agent to man.

Poorly differentiated carcinoma of the external ear canal with chondroid metaplasia at metastatic site in a dog

Romanucci, Mariarita, Daniela Malatesta, Alessia Marinelli, Pierluigi Di Lorenzo, Leonardo Della Salda

Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy

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Introduction: An 8-year-old, male Pug with a history of bilateral recurrent otitis externa was presented with coughing, dyspnoea, anorexia and weight loss. Necroscopic examination revealed diffuse enlargement of submandibular, parotid, retropharyngeal, cervical, prescapular lymph nodes of the right side. Submandibular and retropharyngeal lymph nodes of the left side were also enlarged to a lesser degree. Cut section of such lymph nodes appeared to be whitish in colour, with admixed hemorrhagic and necrotic areas. Both ear canals contained abundant brownish-yellow, crusty discharge; the skin of the right horizontal canal was also moderately thickened, causing partial obstruction. Both lungs showed diffuse oedema, as well as disseminated, firm, greyish miliary nodules.

Material and methods: Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Deparaffined sections were stained with haematoxylin and eosin, periodic acid-Schiff (PAS) with and without diastase, alcian blue (pH 1, pH 2.5) and toluidine blue. The immunohistochemical expression of cytokeratin (CK) AE1/AE3, CK7, CK8, vimentin, -smooth muscle actin (SMA), S100 were evaluated using a streptavidin-biotin-peroxidase technique.

Results: At histopathological examination the skin of the right ear canal appeared to be diffusely infiltrated by cords and nests of neoplastic epithelial cells showing multifocal contiguity with the overlying hyperplastic squamous epithelium. Most of carcinomatous cells were arranged in a glandular-like pattern with formation of pseudolumens containing epithelial cells attached to the peripheral cell layer by elongated intercellular bridges. Foci of keratinization with central accumulations of compact laminated keratin were also observed. Diffuse metastatic infiltration of all enlarged lymph nodes was revealed. Pulmonary metastatic nodules showed multifocal areas of cartilaginous metaplasia with evidence of a transition of carcinomatous cells to chondroid cells with nuclear atypia. Alcian blue pH 1, as well as PAS with diastase failed to detect mucinous secretory material in the glandular-like structures. The extracellular matrix in the chondroid areas strongly stained with alcian blue pH 2.5 and toluidine blue. Carcinomatous cells were intensely positive for CK AE1/AE3 and CK8, but not for CK7. Those cells surrounding chondroid areas also revealed focal vimentin immunoreactivity. Chondroid cells were diffusely positive for vimentin and S100 and focally for CK AE1/AE3. Numerous SMA-positive myofibroblasts were evidenced in the stroma of primary and metastatic tumour.

Discussion: To the best of our knowledge, this is the first report on a poorly differentiated carcinoma of the external ear canal showing chondroid metaplasia at metastatic site in dog. Histological, histochemical and immunohistochemical findings support a diagnosis of acantholytic squamous cell carcinoma. Chondroid differentiation at metastatic foci has been described in human esophageal squamous cell and undifferentiated carcinoma. CK8 expression correlates positively with malignancy in human head and neck cancer. Stroma remodeling associated with human invasive squamous cell carcinoma and characterized by gain of SMA-positive myofibroblasts has also been reported.

Possible role of oxidative stress in the pathogenesis of canine zinc-responsive dermatosis

Romanucci, Mariarita, Laura Bongiovanni, Anita Russo, Silvia Capuccini, Luca Mechelli, Laura Ordeix, Leonardo Della Salda

Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy

Introduction: Zinc deficiency causes skin diseases in humans and animals but the underlying pathogenic mechanisms still remain unclear. There is a growing body of evidence for the role of zinc in protecting skin against free radical-induced oxidative damage. Zinc ions may induce the synthesis of metallothioneins (MTs), sulfhydryl-rich proteins that store zinc and act as free radical scavengers. Cu/Zn superoxide dismutase (SOD) is also important for skin health as a result of its antioxidant activity. Skin represents a major target for various environmental stress agents able to induce synthesis of Heat Shock Proteins (HSPs) or Stress Proteins, which are one of the most evolutionarily conserved classes of molecules playing a crucial role in the maintenance of cellular homeostasis. A large number of studies have also demonstrated the anti-apoptotic activity of several HSPs, that usually show elevated levels in proliferating mammalian cells and cell cycle-dependent expression.

Material and methods: The study was carried out on biopsy samples of canine zinc-responsive dermatosis from 8 Siberian Huskies and necropsy samples of normal skin from different sites from 5 Siberian Huskies (excluding sites of frequent mechanical trauma but including those where zinc-responsive dermatosis commonly occurs). Samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. The immunohistochemical expression of Hsp27, Hsp72, Hsp73, Hsp90, Cu/Zn SOD, MT, Ki-67 and active caspase-3 were evaluated using a streptavidin-biotin-peroxidase technique. Ki-67 labelling index (KI) was calculated as the percentage of positive nuclei divided by the total number of keratinocytes examined. At least 1000 keratinocytes per specimen were examined in ten randomly selected fields using light microscopy (x400).

Results: All investigated HSPs showed intense cytoplasmic immunostaining in the affected epidermis. Focal nuclear positivity of Hsp72 was also detected in isolated keratinocytes. Although Cu/Zn SOD expression was similar to that observed in normal skin, MT immunoreactivity was revealed in both the cytoplasm and nucleus of basal cells in normal skin but lacked in the affected epidermis. Caspase 3 activation was also absent in the involved epidermis, which revealed a high Ki-67 index (a 3.5- to 9-fold increase compared to normal skin).

Discussion: The results of this study support the hypothesis that cellular stress response, in particular response to oxidative stress, is involved in the pathogenesis of skin lesions occurring in canine zinc-responsive dermatosis. The lack of MT immunoreactivity in the affected epidermis may be indicative of low zinc levels, thus resulting in vulnerability to oxidative damage. On the other hand, high levels of HSP expression in skin during zinc deficiency may confer protection against a variety of dangerous stimuli, contributing to inhibition of apoptosis, as well as to cell cycle regulation of proliferating keratinocytes. Detection of nuclear Hsp72 expression in affected keratinocytes is consistent with its involvement in protection of the nucleus and repair of DNA damage, particularly induced by UV light exposure.

New acquisitions about canine lymphangiectasia during IBD

Rossi, Giacomo, Gian Enrico Magi, Simona Cortese, Chiara Tarantino, Graziano Pengo, Giacomo Renzoni

Department of Veterinary Science – Camerino, Matelica, Italy

Introduction: The inflammatory bowel diseases (IBDs) that affect dogs there appears to be deregulation of normal mucosal immunity, characterised by polyclonal lymphocytic infiltrates which are presumably specific for luminal antigens. Lymphocytic-plasmacytic enteritis (LPE) is a most prevalent histopathological type of canine inflammatory bowel disease (IBD). In some cases the severe mononuclear cell infiltration of the intestinal chorion is related with the insurgence of secondary lymphangiectasia (SL) characterized by a block of lymphatic drainage. This complication clinically produces malabsorption, protein-losing enteropathy (PLE), hypogammaglobulinemia, and weight loss. The aim of this study was to evaluate in a three-dimensional *in vitro* model the role of TNF- α on modifications occurring to canine intestinal lymphatic endothelium and endothelial tight-junctions (TJs).

Material and Methods: thirty-six – 2 cm in diameter intestinal biopsies belonging to dog without enteric inflammation were collected and cultivated for 48h using increased doses of human-recombinant TNF- α . Immediately after resection, tissue samples were placed onto Petri's plates with culture medium (RPMI 1640 medium containing 10% v/v heat-inactivated fetal bovine serum added with 100 units/ml antibiotic-antimycotic solution - Sigma) and incubated at 37°C with 5% CO₂ until the examination. In treated group (27 plates) TNF- α was added to medium at three different concentrations (0.5; 1, and 10% v/v) whereas the remained 9 plates were maintained as controls. Samples were fixed at three different times (5hrs, 12hrs, and 48hrs after TNF- addition) and embedded for histological and ultrastructural examination. The experiment was repeated three times to confirm the results. For immunohistochemical and immunoelectronmicroscopy, a pAb anti VWF (Dako), Occludin and anti Zo-1 (Zymed) were used, employing a gold-labeled secondary antibody (Sigma) for TEM examination.

Results: an over expression of the TJ complexes associated-proteins (Occludin and Zo-1), and a closure of the TJs was observed by TEM after 5h of incubation only in treated samples, whereas morphological alterations of lymphatic vessels and villi were observed after 12h of incubation. Inconsistent differences were observed between treated groups. Substantial modifications were also observed in sub-endothelial basal lamina of treated samples.

Conclusions: Our results demonstrate that SL during canine IBD is an active process induced also by pro-inflammatory cytokines themselves. We can confirm that acute phase of phlogosis causes exudative process by the opening of TJs while chronic stimulus, as observed during LPE, increasing the tissue levels of TNF- α , may be determinant for the TJs closure and block of lymphatic drainage. The present data suggest that lymphangiogenesis and lymphangiectasia probably play an important role in the pathogenesis of LPE, in terms of maintenance and progression of the IBD. Moreover, a possible central role of TNF- α in the SL development, suggest news therapeutic strategies for the treatment of this long-standing disease.

In vivo and in vitro study of phagocytic function during ovine paratuberculosis

Rossi, Giacomo, Laura Bonfili, Mauro Angeletti, Gian Enrico Magi, Ennio Taccini, Giacomo Renzoni, and Anna Maria Eleuteri

Department of Veterinary Science – Camerino, Matelica, Italy

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Introduction. Recently, mutations in Nod2 gene, a general sensor of peptidoglycan by the recognition of muramyl dipeptide (MDP), the minimal bioactive peptidoglycan motif common to all bacteria, have been shown to be associated with Crohn's disease. Nod2 activates the NF-kb pathway following intracellular stimulation by bacterial products. We also demonstrated in ovine intestinal tissues the presence of conserved regions in the CARD1 and CARD2 domains by using designed primer pairs based on bovine Nod2 mRNA sequences. RNA from lymphocytes isolated from healthy and MAP infected ovine whole blood showed SNiPs in amplify sequences of the Nod2 region. In attempt to demonstrate that these SNiPs could be related with an altered macrophage response to the MAP infection, we evaluated the NF-kb expression and the apoptotic rate in macrophages of uninfected and infected lepromatous or tuberculoid sheep. Furthermore we estimated the ability of the NBT reduction by non- and PMA-stimulated monocytes isolated from peripheral blood of the same animals.

Materials and Methods. 18 sheep, (4 uninfected and 14 infected) were utilized for histological and immunohistochemical evaluations. Serial sections of tuberculoid and lepromatous forms were analyzed for macrophages stain into inflammatory infiltrates (anti-VPM32 mAb), for NF-kb expression (anti-NF-kb pAb, Zymed) and for macrophages apoptotic index evaluation (TUNEL - Promega). Frozen samples of the same tissues were utilized for RT-PCR analysis in attempt to amplify the transcripts of TNF- α and IL-1 β interleukins. The spontaneous and induced NBT tests were done in the same sheep. The abilities of non- and PMA-stimulated monocytes isolated from peripheral blood were estimated by measuring the reduction of NBT by superoxide anion (O₂⁻), according to the modified method of Secombes.

Results. High NF-kb activity in intestinal epithelial cells and macrophages was observed in lepromatous sheep, and an high percentage of these macrophages were apoptotic. Lepromatous sheep exhibited an NF-kb over activation as a response to MDP, a more efficient processing and secretion of IL-1 β and TNF- α . In tuberculoid sheep, the non- and PMA-stimulated monocytes capacity to reduce NBT was found to be significantly increased in comparison to lepromatous and very similar to control animals.

Conclusions. We believe that an increased susceptibility to bacterial-MDP observed in lepromatous form of paratuberculosis is related to an over expression in apoptosis rate of macrophages and to high cellular level of phosphorylate form of NF-kb expression. This different sensitivity is indicated by high level of TNF- α and IL-1 β expression in the same tissues. In according to this, spontaneous and induced NBT tests results reflected the status of the host's specific reactivity during MAP infection. Further studies will be necessary to identify disease-related polymorphisms in CARD1/CARD2 domains, which act as a positive regulators of NF-kb activation and IL-1 β secretion.

Acquired polycystic kidney disease in 4 African black rhinoceroses (*Diceros bicornis michaeli*) with renal haemochromatosis

Ruetten, Maja, Steinmetz Hanspeter, Claus Markus, Hatt Jean-Michel, Ossent Pete, Pospischil Andreas

Institute of veterinary pathology, University of Zürich, Zürich, Switzerland

P63

Introduction: Kidney cysts can arise during organogenesis, and may be associated with histological criteria of renal dysplasia as seen in human cystic kidneys. Many chemicals such as long-acting corticosteroids, polychlorinated biphenyls, alloxan or chronic renal disease can also cause cyst formation. This work describes polycystic lesions in the kidneys of 4 African black rhinoceroses, possibly caused by renal haemochromatosis.

Materials: Four African black rhinoceroses aged between 23 and 39 years old of the zoological garden of Zürich Switzerland were sent for necropsy to our institute. Two animals were born in the wild in Kenya, one was the daughter of a wild born and the last came from the zoological garden in Chester in England. The animals were euthanized due to poor body condition or old age or inability to stand.

Results: Serologically, the levels of transferrin saturation were in all animals close to 90% and mean ferritin levels were 6046 ng/ml (reference 133ng/ml). One animal had increased urea concentrations in serum, the others were within the normal range. Macroscopically, all animals were cachectic and showed bilateral multiple cysts in their kidneys, the livers were firm and pale and the intestines showed a diffuse black colouration of the mucosa. Histologically, multiple renal cortico-medullary cysts were found in all cases, filled with protein rich clear fluid and lined by a cuboidal to flattened unicellular epithelium. In addition, mild to moderate lymphoplasmacytic interstitial nephritis with mild to moderate fibrosis was seen. All livers had moderate to severe lymphoplasmacytic hepatitis, chronic with severe fibrosis, bile duct proliferations, multifocal to coalescing necrosis and massive deposition of haemosiderin in Kupffer's cells and hepatocytes (severe haemochromatosis). Besides the liver, haemosiderin-laden macrophages and haemosiderin deposits in cells were seen in nearly all organs, especially lung, kidney (glomeruli), intestine, myocardium and spleen. In one animal, epithelial lined cysts were also seen in the liver and interpreted as bile duct dilatations.

Discussion: The severity of the renal cysts correlated with the degree of the interstitial renal inflammation and the amount of haemosiderin deposits. This leads to the conclusion that the renal cysts are likely caused in this species by a chronic nephritis, enhanced by the frequently occurring hemochromatosis (acquired polycystic kidney disease) rather than a hereditary adult polycystic kidney disease caused by a mutation of the polycystic kidney disease gene 1 or 2.

Pathological findings associated with physalopterid larvae in *Cordylus tropidosternum* (Tropical Girdled Lizard)

Ruiz, MJ, CR Jiménez, R Zafra, E Mozos

Department of Comparative Pathology. Faculty of Veterinary Medicine, Ed. Sanidad Animal. Campus de Rabanales.Cordoba, Spain

P30

Introduction: References about the pathology associated with Phphysaloptera species (Nematoda: pirurida) are limited to australian and american reptiles¹ and little is known about transmission and parasite biological cycle of this parasite in the reptiles. The Tropical

Girdled Lizard is an viviparous, arboreal or rupicolous lizard very appreciated actually in Europe as pet and located in dry forests of East Africa.

Material and methods: A pregnant female of Tropical Girdled Lizard, imported from Africa, developed, during the quarantine, anorexia, lethargy, dehydration and abdominal dilatation; two weeks after, bore three specimens and death two days later. Other animals showed similar symptoms, but only this one was sent to postmortem analysis.

Results: At necropsy, the animal was thin and a severe acute hemorrhagic metritis was observed. Moreover, a pale and moderately thick stomach and small intestine were found. The reptile was fixed in 10% formalin and samples from the main tissue and organs were processed for histopathological study. Stomach contents were submitted to parasitological examination after collecting a nematode from the mucosa. Histopathological analysis showed a severe acute microbiological hemorrhagic and fibrinous endometritis that was considered the main cause of sickness and death of the animal. Numerous cysts, similar in size, containing nematode larvae were present along the stomach wall, from the cardia to the pyloric and duodenum; Most of the cysts were located in the submucosa but also within the musculature and beneath the serosa. The cysts were composed of thick fibrous walls and scarce cellular infiltrate of macrophages, giant cells and lymphocytes; occasionally, the infiltrate around or within the cysts was moderate. Parasitological analysis of the adult nematode found in the gastric lumen and the evaluation of larvae stages on serial sections of the stomach samples, as well as, the pattern of lesions were consistent with *Physaloptera spp* parasitism. A later study of different organs including the complete analysis of the gut showed two parasitic cysts in the subcutaneous tissue of the abdomen as well as an oesophageal and gastric (only cardiac) cryptosporidiosis.

Discussion: Reports of physalopterid parasitism and pathological findings in reptiles are very rare and, to the date, it has not been reported in African lizards yet. In this case, despite the severe parasitism, the inflammatory response was minimal as has been observed in other infections, but little is known about the biological cycle and the potential risk of this exotic host in relation with the dissemination of these parasites.

Reference

Jones H.I.1995. Pathology associated with Physalopterid larvae in the gastric tissues of Australian Reptiles. *Journal of Wildlife Disease*, 31 (3), 299-306.

Expression of BAG3 protein in the urothelial tumour in cattle

Russo, Valeria¹, Roberto Brun¹, Sante Roperto¹, Chiara Urraro¹, Alessandra Rosat²ⁱ, Maria Caterina Turco², Franco Roperto¹.

¹Department of Pathology and Animal Health, Faculty of Veterinary Medicine, Naples, Italy

²Department of Pharmaceutical Science (DiFarma), University of Salerno, Italy

Introduction: Urothelial tumours are very common in cattle grazing on bracken fern infested lands. There is little information about pathogenetic mechanisms by which biological (papillomavirus) and/or chemical carcinogen factors are responsible for cell transformation. We are investigating about the expression of some proteins which can be involved in bladder tumorigenesis in cattle. It is well known that during the process of cell transformation, cells acquire resistance to apoptosis through a number of different mechanisms. Several proteins have been identified to promote carcinogenesis by inhibiting apoptosis. These proteins include, among other, members of the Bcl-2 family, which regulate apoptotic cell death. BAG3, also known as CAIR-1 or Bis, is a member of the BAG (Bcl-2-Associated Athanogene) co-chaperone family (Lee et al., 1999; Antoku et al., 2001). This protein can potentially downmodulate cell apoptosis via its interaction with heat shock proteins 70 (Hsp70), a chaperone that influences the death process (Beere, 2005). In humans, BAG3 is expressed in myocytes and in a few other normal cell types, its expression has been also detected in a various type of tumours (leukemias, pancreatic and thyroid carcinomas) (Romano et al., 2003; Homma et al., 2006; Chiappetta et al., 2007; Rosati et al., 2007). The objective of this study is to verify the BAG3 expression and investigated its possible interaction with the transforming BPV-2 oncoproteins in urinary bladder tumour in cattle.

Material and methods: For immunohistochemical analysis, we used a polyclonal antibody raised against the recombinant BAG3 protein, followed by incubation with streptavidin-conjugated horseradish peroxidase. For western blot analysis, we used an anti-BAG3 polyclonal antibody raised against the full-length BAG3 protein, we used also a β -actin monoclonal antibody to determine equal loading conditions. Bands intensity was evaluated by densitometry as ratio of BAG3 levels in respect to β -actin protein. Significance between the two groups was calculated by t-test. In addition, the BAG3 mRNA levels was evaluated by quantitative real-time polymerase chain reaction (RT-PCR).

Results: By immunohistochemistry and western blot analyses, we demonstrate significantly increase ($p=0.00005$) of BAG3 expression in urothelial tumours compared with normal tissues. By quantitative RT-PCR analysis, on pathological bladder tissues, BAG3 mRNA levels are significantly ($p=0.039$) reduced compared to normal bladder tissues

Discussion: Our results show that BAG3 is overexpressed in urinary bladder tumours by immunohistochemistry and is increased to the level of the immunoblots. These data suggest that this protein display antiapoptotic properties in urinary bladder tumour. The findings reported here provide preliminary evidence that BAG-3 may be involved in the pathogenesis of urinary bladder tumour in cattle.

Pathology of alimentary B-cell lymphoma in juvenile Miniature Dachshunds

Sakai, Hiroki, Atsushi Kodama, Keiya Kobayashi, Tokuma Yanai, Toshiaki Masegi

Laboratory of Veterinary Pathology, Department of Veterinary Medicine, Gifu university, Gifu, Japan

Introduction: Miniature Dachshunds (MD) have not been reported to be prone to increased risk of lymphomas. However, Japanese clinicians have recently encountered many lymphoma types, especially alimentary lymphoma in juvenile MDs. We investigated the morphological features, immunophenotypes and rearrangement of antigen-receptor genes causing alimentary lymphoma in juvenile MDs.

Materials and methods: Alimentary lymphomas surgically excised from 13 juvenile MDs (age, 1.8–4.8 y; mean, 2.7 y) and 17 nonMDs (age, 3.4–15 y; mean, 9.3 y) were analysed histopathologically and immunohistochemically. The rearrangement of antigen-receptor genes was investigated by PCR, using paraffin sections. Intraperitoneal masses from 3 juvenile MDs were examined by cytology alone, and intestinal lymphoma in 1 MD was examined by EM.

Results: Cytological analysis of the 3 intraperitoneal masses and 1 impression smear of a surgically excised sample revealed numerous small- to medium-sized lymphoid cells—resembling Mott cells—containing multiple pale droplets. Histopathologically, neoplastic proliferations of 2 types of lymphoid cells were observed in 11 MD lymphomas. The majority comprised uniform medium-sized lymphoid cells containing hyperchromatic oval or round nuclei with inconspicuous nucleoli. The second type resembled the Mott cells and contained several periodic acid-Schiff-positive (PAS+) eosinophilic globules in the cytoplasm and pyknotic nuclei. Most neoplastic cells stained positive for vimentin, CD20, CD79a and Pax-5 but negative for CD3 and lysozyme. The Mott cell-like cells strongly stained positive for certain immunoglobulins but negative for Pax-5. The other MD lymphomas were lymphoblastic, and the immunophenotypes were the B-cell type (CD20+ and Pax-5+). The average Ki-67-positive index in the MD and nonMD lymphomas was 53.6% and 61.8%, respectively. Monoclonal rearrangement of *IgH* was detected in 8 MD lymphomas; clonal rearrangement of *TCR* γ was also detected in some. Immunohistochemical analysis of the nonMD lymphomas revealed 8 cases each of the T-cell and B-cell types. Clonal rearrangement of *IgH* (3 cases) and *TCR* γ (7 cases) was also detected. EM of juvenile MDs revealed that the neoplastic lymphoid cells possessed poorly developed organelles, except for the ribosomal rosettes and a small rER. The Mott cell-like cells frequently exhibited homogenous substances with intermediate electron density in the rER cisternae; however, they were rarely observed in the perinuclear space of lymphoid cells.

Discussion: In the alimentary lymphoma of the juvenile MDs, the morphological features, immunophenotypes and clonal rearrangement of *IgH* revealed neoplastic cells displaying the mature B-cell phenotype and some others producing immunoglobulins. However, both B-cell and T-cell lymphomas were observed among the conventional nonMD alimentary lymphomas. Alimentary lymphomas in the juvenile MDs exhibited a relatively high Ki-67-positive index despite a mature morphology. Therefore, the intestinal lymphoma observed in the MDs differed from typical mature B-cell lymphoma; the former is presumed to be a special B-cell lymphoma variant.

Studies on the coinfection with scrapie and visna-maedi virus in ovine natural cases

Salazar, Eider, Pérez Marta, Marta Monzón, Crsitina Acín, Rosa Bolea, Neila Álvarez, Eva Monleón, Beatriz Amorena, Juan José Badiola, Lluís Luján

Zaragoza University, Zaragoza, Spain

Introduction: In natural scrapie PrP^{sc} normally accumulate in nervous and lymphoid tissue but it is known that PrP^{sc} can also be found in “no target” organs suffering from chronic inflammation in both natural and experimental conditions. Recently, PrP^{sc} has also been found in “no target” organs without inflammatory evidence. In this study we describe the tissue distribution of PrP^{sc} in sheep naturally coinfecting with scrapie and visna-maedi virus (VMV).

Material and methods: We study four groups of animals, coinfecting with scrapie and VMV (n=4), infected with scrapie or VMV (n=4 in both) and non infected sheep. This study is specially focused on two VMV target organs: lung and mammary gland. VMV infection was determined by ELISA and histopathology whereas scrapie lesions were studied by histopathology and presence of PrP^{sc} by immunohistochemistry (mAb L42) and Western Blotting (mAb P4).

Results: Preliminary results indicate that PrP^{sc} is detected in lungs and mammary glands of VMV infected sheep if there is presence of VMV-associated lesions, namely hyperplasia of lymphoid follicles and interstitial inflammatory reaction. So far, PrP^{sc} has only been found in lymphoid follicles in both tissues and not all follicles showed presence of PrP^{sc}. Pathologic prion protein has not been found in “no target” organs in any of the other group of animals.

Discussion: In the scrapie and VMV natural coinfecting model, the development of chronic lesions associated to VMV, mostly lymphoid follicle formation, is a pre requisite for PrP^{sc} deposition in “no target” organs such as lungs and mammary glands. Therefore, the VMV associated lesions can modify the known pathogenesis of scrapie disease.

Immune response to cavitary pulmonar tuberculosis in goat

Sánchez, Joaquin, Antonio J Buendía, Laura Tomás, José A. Navarro

Universidad de Murcia, Facultad de Veterinaria. Campus de Espinardo, Murcia, Spain

P111

Introduction: Caprine tuberculosis is caused by bacteria of the *Mycobacterium tuberculosis*-complex (*M. bovis* and *M. caprae*). Although the typical granulomatous lesions are usually observed in the lungs and lymph nodes of infected goats, presence of cavitary lesions with exuberant mycobacterial growth is a common feature in this species. To deepen in the understanding of the immunological mechanisms that leads to liquefaction and cavity formation, a comparative study has been performed between typical granulomatous lesions and cavitary lesions

Material and methods: Samples fom animals positive to delayed-type hypersensitivity skin test were collected in zinc fixative. A histopathological study was carried out using hematoxylin-eosin and Ziehl-Neelsen stains. Adjacent sections were labelled using immunohistochemical amplification systems (ABC or TSA) to detect different leukocyte subsets (B, T, CD4, CD8, macrophages), proliferation markers (Ki-67) and macrophage products (arginase and iNOS).

Results: Results showed substantial differences between the immune response observed in both kind of lesions. In cavitary lesions an important population of neutrophils was found, some of them phagocytting bacteria. A high expression of iNOS could be detected in the macrophage of the cavitary lesions, while an increased number of B cells was found in the lymphocytic population surrounding this lesions. A high proliferation index was associated to the lymphocytic areas.

Discussion: All these findings point to that cavitary lesions are reactivation sites, where the environmental conditions are optimal for *Mycobacterium* proliferation and the innate and specific immunological mechanisms developed are not able to control the bacterial multiplication.

p63 expression in canine primary cutaneous adenocarcinomas

Saraiva, Ana Laura, Fátima Gärtner, Maria dos Anjos Pires

Univ. Trás-os-Montes and Alto Douro, Vila Real, Portugal

P132

Introduction: p63, a recently identified homologue of the p53 gene, is necessary to maintain a stem cell epithelial population and is consistently expressed in basal cells of several types of multilayered epithelia. Expression of p63 has been detected in basal and myoepithelial cells in normal human epidermis, cutaneous appendages (hair follicles, sebaceous glands and sweat glands) and cutaneous carcinomas, as in its counterparts in dogs.

Material and methods: We used 31 tissue samples of tumours that were classified histologically according to the World Health Organization's diagnostic criteria for epithelial tumours of the skin of domestic animals. The tumours included 7 sebaceous carcinomas, 10 hepatoid gland carcinomas, 8 apocrine carcinomas and 6 ceruminous carcinomas.

p63 was detected by immunohistochemistry using a specific monoclonal antibody against p63 (1:150; 4A4; Neomarkers). Staining for additional markers was undertaken to further assess the epithelial or myoepithelial phenotype of the cells: cytokeratin (CK) AE1/AE3 (1:50; CKAE1/AE3; DAKO Corp.), CK14 (1:20; NCL-LL 002, Novocastra), calponin (1:400; CALP; DAKO Corp.), and vimentin (1:100; NCL-V9, Novocastra). Immunohistochemistry was performed using the streptavidin-biotin-peroxidase technique and expression of p63 was assessed semi-quantitatively. Adjacent normal skin tissues were used as internal positive controls. Negative controls were carried out by replacing the primary antibody with phosphate-buffered saline

Results: In the sebaceous carcinomas the basaloid reserve cells were markedly positive for p63, whereas differentiated sebocytes were mainly negative.

Perianal gland carcinomas were characterised by differentiation to hepatoid gland epithelium, lacking a well-defined and organised pattern and showing peripheral invasion of adjacent tissues. More than 50% of the cells revealed p63 nuclear immunoreactivity.

In the apocrine carcinomas secretory epithelial cells were consistently negative for p63 expression. Resting myoepithelial cells variably stained with this antibody and proliferating myoepithelial cells were always positive

p63 expression in ceruminous gland carcinomas was very similar to that observed for apocrine carcinomas

Discussion: As expected, our results with apocrine and ceruminous tumours were very similar. p63 expression in these tumours revealed selective expression of this molecule in basal/myoepithelial cells, similar to the canine mammary gland. In sebaceous carcinomas, we detected p63 expression, not only in basal cells, but also in some sebocytes. In hepatoid gland carcinomas both basal cells and a high proportion of mature cells showed strong nuclear staining.

Our results suggest the participation of basal/myoepithelial cells in the oncogenesis of these tumours.

Ovine neosporosis and toxoplasmosis

Sasani, Farhang, Parvaneh Seifori, Gholamreza Habibi

Tehran University, Tehran, Iran

P91

Introduction: *Neospora caninum* is a coccidian parasite of animals. Lesions include placentitis, myocarditis, myositis and necrogranulomatous reaction in CNS tissues.

Material and methods: Histopathological studies and Nested-PCR examination of formalin-fixed, paraffin-embedded tissues used as a confirmatory test to identify the protozoal agents in brain sections of 109 aborted fetal sheep.

Results: *Toxoplasma gondii* was detected by Nested-PCR in the brains of 86.95% of sheep aborted fetuses. *N.caninum* was detected as aborting agent in one fetal brain of sheep by nested-PCR which had microscopic lesions indicative for cerebral protozoal infections. Histopathologic findings were focal gliosis in 30.27% of 109 sheep, multifocal necrosis (necrogranuloma) in 21.1% of 109 sheep. Three toxoplasma cysts in ovine fetal brains mainly in brain stems were observed.

Discussion: Histopathological lesions indicative of cerebral protozoal infections were observed mainly in cortex. Pathological feature of neosporosis in sheep closely resembled to those of bovine neosporosis and ovine toxoplasmosis. This is the first report of neosporosis (*N.caninum*) in sheep diagnosed in Iran.

Immunophenotypes of canine mammary carcinomas

Sassi F., Benazzi C., Sarli G.

Department of Veterinary Public Health and Animal Pathology, Bologna, Italy

Introduction: A new classification system of breast tumours is presently used in human pathology and is based on the immunohistochemical characterization of 4 tumour types, namely luminal-like (A and B type), basal-like, and ERB-B2 positive neoplasms. This approach reveals extremely useful in humans in terms of prognosis. In fact, luminal phenotype seems to have a better prognosis than basal and ERB-B2 respectively. In this preliminary study this classification method has been applied to a series of mammary tumours of the female dog with the aim to verify its prognostic value in veterinary medicine.

Material and methods: A series of 45 canine mammary carcinomas with a known two-year post-mastectomy follow-up were selected from our database. A panel of the following antibodies was applied: anti-cytokeratins 14, 5/6, oestrogen receptor (ER), progesterone receptor (PR), and ERB-B2. PR and ER nuclear staining and ERB-B2 membranous staining were considered positive when observed in >10% of tumor cells; CK5/6 and CK14 cytoplasmic staining was considered positive when observed in at least one tumor cell. According to the phenotypic expression, the cases were grouped as follows: luminal-like (ER+/-, PR+/-, CK14-, CK5/6-) type A (ERB-B2-), and B (ERB-B2+); basal-like (ER-, PR-, CK14+, CK5/6+, ERB-B2-); ERB-B2 (ER-, PR-, CK14-, CK5/6-, ERB-B2+). Kaplan-Meier survival curves were estimated and compared by survival analysis.

Results: Thirty-five cases showed a luminal pattern (ER+ and PR+) further subgrouped into 13 A type and 22 B type, according to ERB-B2 positive or negative expression. Ten cases revealed a basal phenotype, and no cases were classified as ERB-B2. Survival analysis produced no significant results, even though a clear prognostic distinction was apparent between basal (more favorable) and luminal (less favorable) after 24 months from mastectomy. As for the two luminal groups, A type showed a better prognosis than B type

Discussion: Luminal-like A and B types share the same phenotype but differ in ERB-B2 expression considered an important index for prognosis and tumour progression in canine as well as in human breast cancer. In fact, the survival curve indicates a worse prognostic index for luminal B tumours compared to A subtype. The basal group shows a more favorable behaviour than the others, unlike the human models. If the results are confirmed on a larger number of cases, the hypothesis that tumours originating from myoepithelial cells have a low malignant potential will be reinforced in the dog unlike the human.

Effects of unilateral nephrectomy and high-fat diet to diabetic nephropathy in alloxan-induced diabetic WBN/Kob rats

Sato, Mai, Kiyokazu Ozaki, Tomoya Sano, Tetsuro Matsuura, and Isao Narama

Setsunan University, Hirakata, Japan

P70

Introduction: Male WBN/Kob rats develop spontaneous diabetic conditions from 40 weeks of age. We have attempt to induce severe diabetic nephropathy by earlier onset and longer duration of diabetic condition by single dosing of alloxan, however, the diabetic nephropathy was still mild at terminal stage of male rats of this strain. In this study, the effects of unilateral nephrectomy and feeding of high-fat diet on diabetic nephropathy was examined in diabetic WBN/Kob rats induced by alloxan.

Materials and Methods: 10-week-old male WBN/Kob rats were received single intravenous dosing of alloxan (AL). Rats were divided for each group fed normal diets after AL treatment (AL), fed high-fat and high-sugar diets after AL treatment (AL+HF group) and fed the normal diets after unilateral nephrectomy after 3 weeks of AL dosing (AL+NX group). Together with non-treated group fed with normal diet (control), all rats were examined for blood glucose level, urinary glucose level, blood pressure, urinary protein and blood lipid level once every 10 weeks and were sacrificed for full histopathological examination, blood chemical analyses and urinalysis at 40 week after AL dosing.

Results: Blood and urinary glucose levels in all AL-treated groups were higher than those of control throughout the experimental period. Control rats showed high glucose levels in blood and urine just before the end of experimental period, so period under diabetic condition was much shorter than rats of other groups. Blood pressure was increased with age in all groups, but there was no significant difference among the groups. Urinary albumin and protein increased with lower creatinine concentration in AL+NX group at the end of experimental period. Urinary creatinine concentration was also decreased in AL+HF group, but the increase in urinary protein was mild. Triglyceride increased from 10 weeks after AL-dosing in AL+HF group and levels of total cholesterol and LDL cholesterol were also high at the end of examination period. Only cholesterol level was high in AL+NX group. Serum creatinine level was high in AL+NX group but normal level in AL+HF group at end of examination period.

Kidney weight was heavier in AL+NX group. Histopathologically, accumulation of glycogen in proximal tubular epithelium was detected in all AL-treated groups. Glomerular size increased with mild increase in mesangial matrix and hypertrophy and vacuolation of podocytes in AL+NX group. Dilatation of proximal tubule with deposition of hyaline droplets in tubular epithelium and hyaline casts in distal tubules were often observed in these kidneys. No significant glomerular changes were detected in AL+HF group.

Discussion: Induction of mild diabetic nephropathy similar to early stage of diabetic human was confirmed blood and urine chemically, and histopathologically in AL-induced diabetic rats under unilateral nephrectomy. It is evident that unilateral nephrectomy accelerates the glomerular lesion but high-fat diets show no apparent effects on glomerular changes in diabetic WBN/Kob rats.

The Immunohistological Detection of feline CD8⁺ T Cells in Paraffin-embedded Lymphoid Tissues Using the HOPE Fixation Technique

Schmiedeknecht, Gisa, Manfred Reinacher

Institut fuer Veterinaer-Pathologie, Justus-Liebig-Universitaet, Giessen, Germany

P18

Introduction: Providing excellent morphological detail, formalin fixation followed by paraffin-embedding is the commonly used method of tissue fixation in routine diagnostic work. However, the formalin-fixed material is subsequently not always suited for further immunohistological examinations, as the usage of cross-linking, aldehyde-based fixatives may result in decreased antigen survival. Especially cell surface antigens, e.g. the so called fixation- and processing-sensitive feline T-lymphocyte marker CD8, are extremely susceptible to the chemical influences during tissue fixation and can often be detected immunohistochemically only in frozen material. The HOPE fixation technique (Hepes Glutamic Acid Buffer Mediated Organic Solvent Protection Effect) is a patented, aldehyde-free method of tissue fixation, comprising a commercial, organic buffer-based protection solution as fixative, acetone as the only dehydrating agent and low temperature paraffin-embedding. According to the inventors, HOPE fixation provides an excellent antigen survival and permits the immunohistological demonstration of various human antigens in paraffin-embedded tissues without the need for antigen retrieval methods, while morphological detail is well preserved. Purpose of this study is the immunohistological detection of feline CD8⁺ T-lymphocytes in paraffin-embedded lymphoid tissue using the HOPE technique and evaluating the preservation of morphological detail compared to traditionally formalin-fixed material.

Material and methods: Spleen and mesenterial lymph nodes of cats were obtained at necropsy. Small tissue samples were placed in HOPE I solution for 42 h at 4 °C before dehydration in ice cold acetone for 8 h at 2° C and subsequent embedding in low melting paraffin at 54° C, according to the manufacturer's instructions. Parallel to this procedure, tissue samples of the respective animal were either formalin-fixed or snap frozen by routine methods for comparison. HOPE-fixed and frozen sections were immunostained in parallel for CD8, the latter serving as positive controls. Additionally, paraffin sections of HOPE-fixed and formalin-fixed material, respectively, were stained with haematoxylin and eosin (HE).

Results: The morphologic details of HOPE-fixed specimens are well preserved and almost comparable to formalin-fixed material. Furthermore, a precisely localized immunostaining for CD8 was achieved in HOPE-fixed paraffin-embedded material. Antigen retrieval techniques were not necessary. Although the number of reactive cells and the intensity of the reaction were slightly higher in frozen sections, the suboptimal morphology hampered a precise evaluation of individual cells.

Discussion: HOPE fixation permits the immunohistological demonstration of the usually processing- and fixation-sensitive CD8 antigen in feline paraffin-embedded tissue without the need for antigen retrieval techniques. Although frozen sections provide a higher sensitivity, HOPE fixation is a convenient technique, combining good morphological detail with improved antigen survival.

An unusual encephalomyelopathy in a litter of Middle White pigs

Schock, Alexandra, J. Paul Hutchinson

Veterinary Laboratory Agency, PSP, Bush Loan, Penicuik, United Kingdom

P92

Introduction: In veterinary medicine, familial and acquired motor neuron disease has been described in a large number of species. In pigs, hereditary motor neuron disease has previously been reported in Yorkshire and Hampshire breeds ^{1,2}. We describe the findings of a motor neuron disease in a litter of Middle white pigs.

Material and methods: A litter of Middle White piglets presented with acute onset, progressive hind limb ataxia at the age of 5-7 weeks of age. Gross and histopathology was carried out on 2 affected animals. No other pigs were affected.

Results: Gross post mortem examination of both pigs did not reveal any significant changes. The total brain weight (TBW) was 55.3g of pig 1 and 63.7g of pig 2. The cerebellar weights were 6.5g (11.8% TBW) and 7.9g (12.4% TBW) respectively. Liver copper levels were 163 mmol/kgDM in pig 1 and 250 mmol/kgDM in pig 2 (reference range 300-5000 mmol kg/DM). Histopathology revealed a severe, bilateral, symmetrical encephalomyelopathy characterised by widespread chromatolysis of motor neurons in the brain stem and spinal cord.

Discussion: Hereditary disease, mycotoxicosis and copper deficiency were considered as a diagnosis. Though copper values appeared to be marginal in both pigs, the pathology in porcine copper deficiency is characterised primarily by demyelination^{3,4}. No other litters were affected which militates against a diagnosis of copper deficiency or mycotoxicosis. Considering clinical history, pathology and epidemiology, a diagnosis of an intrinsic disease of motor neurons of genetic aetiology appears to be the most likely diagnosis.

¹Higgins et al, 1983 ²Montgomery et al, 1989 ³McGavin et al, 1962 ⁴Cancilla & Barlow, 1970

Tracheal rupture with subcutaneous emphysema and unilateral pneumothorax in an adult sheep

Schock, Alexandra, Jackie Willmington

Veterinary Laboratory Agency, PSP, Bush Loan, Penicuik, United Kingdom

P112

Introduction: In veterinary medicine, tracheal rupture is an unusual event due to the anatomical characteristics of the trachea such as mobility and elasticity. It is most commonly observed in small animals following intubation and in horses subsequent to trauma. We describe a case of cervical tracheal rupture in a ewe.

Material and methods: A ewe at grass with her two week old twins was found with subcutaneous emphysema and died during examination. The day before, she was seen well. The animal had been drenched 2 days after lambing. The case was investigated by gross pathology and histology (H&E, PPB, elastic van Gieson). In addition, immunohistochemistry for CD68 with antibody clone EBM11 at 1:100 was carried out using the Dakocytomation autostainer plus following protease digestion.

Results: Gross pathology revealed subcutaneous emphysema of the ventral and lateral surface of the neck, thorax and abdomen. There was bruising of the intermandibular space. A horizontal, slit-like, 1.5 cm long wound between two cartilaginous rings was detected in the ventral wall of the cervical trachea 9 cm from the base of the epiglottis. There was some displacement of the peritracheal tissue into the lumen of the trachea as well as haemorrhages in the connective and muscular tissue underlying the affected area. Unilateral right pneumothorax was present. No penetrating wound could be detected. Histopathology showed mucosal necrosis and severe recent haemorrhages of the trachea associated with some clumps of neutrophils, few gram positive bacterial, activation of fibroblasts and endothelial cells in the underlying tissue. PPB showed very small amounts of haemosiderin whereas elastic van Gieson failed to demonstrate any significant amounts of collagen. CD68 IHC showed a significant increase in numbers of macrophages in the area surrounding the lesion.

Discussion: Clinical history and pathology confirmed a diagnosis of cervical tracheal rupture with secondary subcutaneous emphysema and unilateral pneumothorax. The lack of laryngeal lesions and the localisation of the tracheal rupture are consistent with a diagnosis of blunt trauma rather than a drenching injury. Based on the presence of few neutrophils, many macrophages, activated endothelial cells and fibroblasts as well as the absence of easily detectable collagen, the lesion was estimated to be older than 24 hours. However, adequate scientific data of lesion development in sheep are not available and more research is needed to create a basis for the aging of lesions in farm animals.

Plexiform neurofibroma and diffuse neurofibroma as subtypes of canine peripheral nerve sheath tumours

Schöniger, Sandra, Brian A. Summers

The Royal Veterinary College, Hawkshead Lane, North Mymms, UK

Introduction: Canine peripheral nerve sheath tumours commonly differ from these tumours in man with regard to their histopathological features.

Material and methods: We describe seven dogs with tumours resembling human plexiform neurofibroma (6 cases) and diffuse neurofibroma with tactile structures (1 case).

Results: The dogs were of different breeds and 7 months to 15 years of age. Five dogs were male, one dog was female and the gender of one dog is unknown. Three plexiform neurofibromas were located in the subcutis, two in the large intestine and one in the tongue. Plexiform neurofibromas presented as multinodular masses composed of centrally located nerve bundles surrounded and expanded by proliferated nerve sheath tissue. Both colonic plexiform neurofibromas were observed in young dogs and occurred together with ganglioneuromas. The diffuse neurofibroma infiltrated the dermis and subcutis and was composed of neurofibromatous tissue containing pseudomeissnerian corpuscles.

Discussion: This study shows that although rare, plexiform neurofibroma and diffuse neurofibroma are subtypes of spontaneous canine peripheral nerve sheath tumours. Furthermore, lingual peripheral nerve sheath tumours and concurrent intestinal ganglioneuromas and peripheral nerve sheath tumours (as occurs in man) have not been previously reported in dogs.

An experimental study on early pathogenesis of a very virulent isolate of Infectious Bursal Disease Virus, using histopathology

Siavosh, Minoosh, Abbas Tavassoly, A. Shoshtry, M.A. Bahmani Nejad, S.H. Marjanmehr

University of Tehran, Faculty of veterinary medicine, Tehran, Iran

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Introduction: This study was conducted in order to reveal more details of the early pathogenesis -before clinical signs- of IBDV-IR499-strain, which was previously described as a very virulent virus

Material and methods: The virus (vvIBDV,IR499) was inoculated to 4 week-old SPF chickens (n= 20) and tissues from bursa of Fabricious, cecal tonsils, liver, spleen, thymus and thigh muscle were harvested at time-intervals : 3, 6, 12, 24, and 48 and 72 hours post inoculation.

Results: Typical histopathologic lesions , were first observed as early as 12 hrs.P.i. in the bursa of faricious, including necrosis(pyknosis) of cortical follicular lymphoid cells. At this time interval the other harvested lymphoid organs devoid of any histopathologic lesions.

Discussion: Gradually onward, the histopathological lesions intensified during the time intervals especially in the bursa and other lymphoid organs such as spleen , cecal tonsils and thymus. The lesions consist of follicular lymphoid cell depletion and necrosis (pyknosis and karyorrhexis), increasing reticular epithelial cell and macrophage population and cystic formation in follicles. Lymphoid cell necrosis and depletion in other lymphoid organs such as thymus and spleen were observed but less prominent and in a more delayed manner comparing to those of the bursa.

An experimental study on early pathogenesis of a very virulent isolate of Infectious Bursal Disease Virus, employing immunohistochemistry

Siavosh, Minoosh, Abbas Tavasoly, Abdolhamid Shoshtary, Mohammad Ali Bahmaninejad, S. Hossein Marjanmehr

University of Tehran, Faculty of veterinary medicine, Tehran, Iran.

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Introduction: Employing immunohistochemistry, this study was conducted to characterize the early pathogenesis -before clinical signs- of IBDV -IR499- strain, which was previously described as a very virulent virus.

Material and methods: The virus was inoculated to 4 week-old SPF chickens (n= 20) and tissues from bursa of Fabricious, cecal tonsils, liver, spleen, thymus and thigh muscle were harvested at time-intervals : 3, 6, 12, 24, and 48 hours post inoculation.

Results: Typical positive signals were first observed at, as early as 3 hours post inoculation, in the lymphoid cells of cecal tonsils (the organ of primary affinity) and Kupfer cells of liver. The first occurrence of viral antigen in the bursa, spleen and thymus were at 6, 12 and 12 hrs. p.i, respectively, which demonstrated the primary and secondary viremiae.

Discussion: According to the speed of kinetic and propagation of the virus in each step, IR499, showed a more rapid course and severe pathogenicity, comparing to previously investigated classical strains

Key words: Infectious bursal disease, very virulent infectious bursal disease virus, pathogenesis, immunohistochemistry

Quality and quantity assessment of potentially pathogenic bacteria in aquaculture

Skowrońska, Agnieszka, Karol Korzekwa, Ewelina Sosnowska and Izabella Zmysłowska

Department of Environmental Microbiology, Poland

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Introduction: The bacterial population in the gastrointestinal tract, water and mucus of fish has been studied qualitatively and quantitatively. The gastrointestinal microbiota of fish are peculiarly dependent on the external environment, due to the water flow passing through the digestive tract. Most bacterial cells are transient in the gut, with continuous intrusion of microbes coming from water and food. Rapid identification of microorganisms in digestive tract of fish is required to optimize empirical treatment at an early stage. Bacterial population in rainbow trout digestive tract was characterized by the fluorescent *in situ* hybridization (FISH) method. 21 *Aeromonas hydrophila* strains isolated from water, mucus and digestive tract were tentatively classified by three class of primers, including specific (BOX) and nonspecific by AP-PCR, RAPD. Genotype patterns were compared with *Escherichia coli*, *Pseudomonas* sp. and *Bacillus* sp.

Material and methods: Microbial density of rainbow trout intestine was estimate by direct microscopic counts (DAPI). A set of oligonucleotide probes was used to detect and enumerate the bacterial community structure by fluorescence *in situ* hybridization. *A. hydrophila* strains were isolated from 1 ml of water. Skin mucus were scraped from both surfaces of fish body, and diluted in ddH₂O water. Simultaneously, digestive tract content was prepared and diluted. One millilitre both dilutions were cultivated on Petri plates, biochemical properties were screened (catalase, peroxidase, O/F) and confirmed by amplification with 16S rDNA primers.

Results: The gamma-Proteobacteria were dominated by *Aeromonas* (AER66) and *Enterobacteriaceae*. About 88% of all bacteria were detected by FISH analysis by EUB338 probe. Thirty six loci for arbitrary and random primed markers were revealed as well as 12 complemented BOX sites. Genotypic diversity of *A. hydrophila* strains was $G=100\%$, all loci were polymorphic, mean genetic diversity was $h=0.28$ (SD 0.14). The most identity each other were strains isolated from digestive tract ($I=1$), then from mucus ($I=0.9$) and strains isolated from mucus and water.

Discussion: The combination of bacterial culture, molecular markers (AP-PCR, RAPD and BOX) genome scanning and subsequent FISH analysis with oligonucleotide probes proved to be a powerful tool for detailed insight into microbial diversity and to estimate potentially pathogenic bacteria in aquaculture.

Local muscular tolerance of titanium-nickel alloy implants coated with oxides and polymers – acute experiment in rabbit

Soare, Teodoru, Emilia Ciobotaru, Iuliana Ionascu, Manuella Militaru, Dan Batalu, Georgeta Dinescu, Georgeta Cosmeleata

Faculty of Veterinary Medicine, Bucharest, Romania

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Introduction: The researches focused on biomaterials and alloys are a very important subject of our days. The alloy of Titanium-Nickel is an important solution used in the medical industry of vascular stents. Our study is aimed on biocompatibility of Ti-Ni alloy coated with various types of substances, which will be used in the future in stent production.

Material and methods: 9 healthy rabbits were used in the experiment. The Ti-Ni implants were 15/4/0.2 mm medium sized. 9 different types of implants were used for testing: Sol-gel 0 (Ti-Ni), Sol-gel 1 (Ti-Ni coated with TiO_2), Sol-gel 2 (Ti-Ni coated with SiO_2), Sol-gel 3 (Ti-Ni coated with ZrO_2), P_1 (Ti-Ni coated with poly (DL-lactide-co-glycolide – DL(PLG)), P_3 (Ti-Ni coated poly (DL-lactide – DL(PLA)), P_4 (Ti-Ni coated first with TiO_2 and second with DL(PLG)), P_5 (Ti-Ni coated with ZrO_2 and DL(PLG)) and P_6 (Ti-Ni coated with SiO_2 and DL(PLG)). The rabbits were prepared for surgery, followed by incision in lumbar region of skin, subcutaneous connective tissue and fascia. UV sterile implants were introduced into the muscle, without surgical incision. The surgical wound was sutured, followed by postsurgical treatment. Body temperature was measured twice-a-day. Ten days after, the implants were recovered together with skin, subcutaneous connective tissue and muscle, 1.5 cm around the implant. X-ray investigations were performed after the first surgery and before second one. The implants were removed and samples of tissues were fixed in 10% formaldehyde solution, embedded in paraffin and Masson trichromic stained.

Results: Clinically, all the implants used in this experiment were well tolerated, wound healing and body temperature being recorded in normal limits. Histological results proved different types of local reaction in rabbits with implants coated with different oxides. The most powerful reaction in this group was recorded in Sol-gel 2, represented by obvious exudation, abundant granulation tissue and an inflammatory barrier strictly orientated around the implant. Sol-gel 3 induced excessive granulation tissue, associated with a discrete, focal inflammation. Sol-gel 1 induced a narrow area of granulation tissue and a focal, discrete inflammation. Sol-gel 0 exhibited classical aspects of local healing, without important inflammation around the implant. The implants coated with polymeric substances recorded different reaction the smallest being recorded in P_3 (orientated collagen fibers, few fibrocytes and heterophils). P_1 generated one of the most powerful reactions of the experiment, with abundant inflammatory cell population and hyperplastic granulation tissue. The implants coated both with oxides and polymeric substances generated moderated local response. P_4 induce a smaller reaction, comparatively with P_5 and P_6 .

Discussion: The implants coated with oxides are protective against inflammation, because of the minimal ion discharge which creates a better tolerance into the tissue. SiO_2 and ZrO_2 proved undesirable local effect, comparing TiO_2 . Polymeric coated implants are inert and biocompatible when are blood exposed, but it does not provide supplementary effect comparing oxide coated implants. Despite of this idea, poly (DL-lactide) exhibited the most encouraging results with the smallest local reaction. The implants coated both with oxides and polymeric substances did not exhibit better results, inflammatory reaction and granulation tissue being observed mostly in P_5 and P_6 .

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Re-emergence of Morbillivirus infection in the Mediterranean sea

Soto, Sara, Jorge Martínez, Iván José Galindo, Rocío González, Beatriz González, Toni Raga, Mariano Domingo

Veterinary Faculty, Universitat Autònoma de Barcelona (UAB), Bellaterra-Barcelona, Spain

Between 1990-1992, several thousands of striped dolphins (*Stenella coreuloalba*) stranded in the Mediterranean coasts due to an epidemic of Dolphin Morbillivirus (DMV). Except apparent chronic infections between 1992-1995, all the dolphins necropsied between the end of the epidemic until June 2007 did not show DMV-associated lesions, and when tested, were negative for the DMV immunohistochemistry. But in July 2007 the virus has been detected again in the Mediterranean associated to dead of striped dolphins, causing a much less severe process.

From July 2007 to May 2008, 30 out of 136 beached dolphins (24 in 2007 and 6 in 2008), considered suitable for necropsy, were transported to the necropsy room of the Veterinary Faculty of Barcelona. Samples from lung, lung associated lymph nodes, prescapular lymph node, laryngeal tonsil, liver, kidney, adrenal glands, gonads, tongue, stomachs, skin, skeletal muscle and brain were collected, fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut at 5µm, and stained with haematoxylin-eosin (H/E) for light microscopy. Similar sections from lung, lymph nodes and brain (including cortex (2 sections), thalamus, cerebellum and brain stem) were used for immunohistochemical demonstration of DMV, using a mouse monoclonal antibody against nucleoprotein of Canine Distemper Virus, which cross-react with DMV. Besides, the archived H-E sections of the dolphins autopsied between 1995 to 2007 were reevaluated. 60 animals were selected, based on the presence of lung inflammatory lesions (whatever the cause), and a DMV immunohistochemistry study of lung and brain tissues was done in those animals.

From July 2007 to May 2008, 10 striped dolphins out of the 30 dolphins necropsied were positive for DMV immunohistochemistry. Most of them showed the typical lesions associated to the virus, as bronchiolointerstitial pneumonia with cellular syncytia, lymphoid depletion with syncytia, or non-suppurative encephalitis. In 6 animals concurrent, probably lethal infections were observed, supposed to be consequence of the morbilliviral associated immunosuppression: toxoplasmosis (3/6), with necrotizing pneumonia, adrenalitis, miocarditis and encephalitis with intralesional parasites; aspergillosis (1/6), with haemorrhagic-necrotizing encephalitis and pneumonia, fibrinonecrotizing tracheobronchitis, with intralesional and intravascular fungal hyphae; herpesvirus-like infection (1/6), with large basophilic intranuclear inclusion bodies; and pyogranulomatous pneumonia (1/6), with intralesional gram positive bacteria compatible with *Nocardia spp.* All the positive animals corresponded to the second half of 2007. Neither compatible lesions nor positive cases were found in the reevaluated dolphins autopsied between 1995 and June 2007. From 9 dolphins found in 2008, 6 were necropsied and investigated for DMV, with negative results. Compared with the 1990-1992 epidemic, this second one has had milder consequences, both in deaths and duration. This could be probably due to residual immunity from the first epidemic.

Intersexuality in the dog – pathomorphological findings in true hermaphrodites and literature survey

Stassen, ¹Tanja , ¹Wolfram Breuer, ²Beate Kienzle, ³Werner Hecht, ¹Walter Hermanns

¹Institute of Veterinary Pathology, Ludwig-Maximilians-University, Munich, Germany; ²Clinic for Veterinary Surgery and Gynecology for Small Animals, Ludwig-Maximilians-Universität, Munich, Germany;

³Institute of Veterinary Pathology, Justus-Liebig-University, Giessen, Germany

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Introduction: Intersexuality is an uncommon congenital abnormality in domestic animals. The affected individuals have parts or all genital organs of both sexes resulting in a variety of phenotypes. The intersexual condition can be classified into true hermaphroditism (hermaphroditismus verus) and pseudohermaphroditism. True hermaphrodites are individuals having both, ovarian and testicular tissue in three possible combinations: either unilateral (testicular and ovarian tissue on one side, testicular or ovarian tissue on the other side), bilateral (testicular and ovarian tissue on both sides), or lateral (testicular tissue on one side, ovarian tissue on the other side). True hermaphroditism has been described in swine, goats, horses, cats, and dogs. In the latter species, numerous cases of true hermaphroditism among different breeds are reported in the literature. The etiology and pathogenesis of this malformation is poorly understood.

Material and methods: Two female dogs, aged six and ten months, were admitted into the Clinic for Veterinary Surgery and Gynecology for Small Animals of the LMU in Munich due to a penis-like enlargement of the clitoris. The uterus and gonads of both cases, as well as the enlarged clitoris of one of the dogs were subjected to pathological examination. Organ samples were fixed in 7% buffered formaldehyde, embedded in paraffin (Paraplast®), cut into 4 to 6 µm-thick sections and routinely stained with haematoxylin-eosin (HE). Heparinized whole blood samples were taken to evaluate the genotypical sex and the presence of the SRY-gene.

Results: Both dogs had bicornuated uteri with physiologically formed horns. The endometrium was in the stage of anoestrus. The gonads were positioned at each uterine horn tip and closed in the bursa. They had a testis-like appearance and an attached epididymis-like structure. Histologically, they were divided into a peripheral zone resembling ovarian tissue and in a medullar part consisting of testicular tissue. There was no evidence of spermatogenesis. Parts of hypoplastic epididymal tissue and sections of ductus deferens adjoined the mixed gonadal tissue. The considerably enlarged clitoris showed a knobby, shamrock-formed apical swelling and a large central os clitoridis.

Discussion: The diagnosis of bilateral true hermaphroditism in both cases is based on the presence of bilateral ovotestes in combination with female genitalia and an enlarged clitoris as a sign of masculinization. Most reports about true hermaphrodites in dogs describe animals with a female phenotype, a normal female karyotype (78, XX), and a lack of the sex-determining region of the Y-chromosome (SRY-gene), which is postulated to be responsible for masculine differentiation during ontogenesis. Therefore, SRY-independent mechanisms inducing the growth of testicular tissue in XX intersexes have to be discussed. The presentation gives a literature survey about the actual theories of sex determination, especially the development of intersex individuals in the dog.

Pathological findings and viral protein expression in the central nervous system of harbour seals (*Phoca vitulina*) infected with Phocine Distemper virus

Stimmer, Lev, Jean-Jaques Fontaine, Wolfgang Baumgärtner, Andreas Beineke

Department of Pathology, University of Veterinary Medicine Hannover, Germany;
Department of Pathology, University of Veterinary Medicine Alfort, Maisons-Alfort, France

Introduction: Mass mortalities in harbour seals during Phocine Distemper virus (PDV) epidemics have been observed in the North Sea in 1988 and 2002. Similarly to canine distemper virus (CDV) in dogs, the central nervous system (CNS) represents an important target organ of PDV. The aim of the present study was to characterise pathological changes in the CNS of seals suffering from natural PDV infection. Furthermore, viral distribution and virus protein expression as well as associated cellular immune responses within the brain and spinal cord were investigated.

Material and methods: Formalin-fixed and paraffin-embedded brain and spinal cord samples of 16 necropsied harbour seals naturally infected with PDV were investigated histologically. Morbillivirus nucleo- (N), phosphor- (P), matrix- (M), fusion- (F) and hemagglutinin (H) protein were detected by immunohistochemistry (IHC). Additionally, morbillivirus N and P mRNA were visualized by *in situ* hybridization (ISH). Phenotypical characterization of inflammatory responses in the CNS was performed by IHC using specific antibodies against CD3 (T cells), CD79 α (B cells), major histocompatibility complex class II (MHC-II) antigen (antigen presenting cells), and glial fibrillary acidic protein (GFAP, astrocytes). Furthermore, microglia/macrophages were detected by IHC (lysozym, MAC387) and lectin histochemistry (BS-1).

Results: Histological lesions were characterised by gliosis and perivascular mononuclear infiltrations of the grey matter and lymphohistiocytic meningitis. Phenotypically, inflammatory lesions were dominated by astrocytes (GFAP), microglia/macrophages (BS-1, lysozyme, MAC387) and T cells (CD3), while infiltration of B cells (CD79 α) was infrequently observed. MHC-II antigen was expressed on the majority of lymphocytes, microglia and endothelial cells in affected areas. Using IHC, morbillivirus N, P, M, F and H proteins were found in inflamed areas. Comparing IHC and ISH, viral protein and mRNA were detected in the same cell types and brain compartments. However, a significantly reduced translation of viral P protein was observed.

Discussion:

Described histological CNS lesions and inflammatory responses in PDV-infected seals are similar to CDV-induced polioencephalitis in dogs. In addition, restricted viral protein expression might represent a mechanism of viral persistence as observed in other morbillivirus infections, such as canine distemper and human measles.

Porcine necrotizing enteritis revisited: cellular targets for *Clostridium perfringens* beta-toxin

Sutter, Esther, Marianne Wyder, Julien Miclard, Horst Posthaus

Institut of Animal Pathology, Vetsuisse Faculty, University of Berne, Berne, Switzerland

Introduction: *Clostridium perfringens* type C is the causative agent of necrotizing enteritis in newborn piglets. The characteristic lesion is a segmental hemorrhagic necrosis of the small intestine. Beta-toxin, a member of the class of pore-forming toxins, is considered to be the major virulence factor leading to disease. However, the exact cellular targets of beta-toxin have not been investigated so far. Thus, the aim of our study was to evaluate target cells of beta-toxin in the porcine intestine.

Material and methods: *In situ* localisation of beta-toxin in affected intestinal segments from 15 affected animals was performed by Immunohistochemistry. Toxin containing supernatant of 2 different *Clostridium perfringens* type C strains, two type A strains and one type B strain were prepared. *In vitro* assays using these supernatants were performed on primary porcine aortic endothelial cell cultures. Cytopathic effects were monitored by light microscopy and measured by cell vitality tests. Additionally, cells were fixed and immunofluorescence stainings for beta-toxin and actin were performed. As control, supernatants containing active toxins were inactivated using a combination of trypsin incubation and heat inactivation or neutralisation with monoclonal anti-beta-toxin antibodies.

Results: Immunofluorescence on histological sections revealed binding of beta-toxin to endothelial cells of mucosal vessels, specifically in areas affected by necrotizing lesions. *In vitro* assays confirmed cytopathic effects on porcine endothelial cells only in the presence of beta-toxin. Furthermore immunolabeled beta-toxin was detected on the surface of cells showing cytopathic effects. Both effects were inhibited by a combination of trypsin and heat inactivation or antibody mediated beta-toxin-neutralisation of the supernatant.

Discussion: Our results demonstrate that *Clostridium perfringens* beta-toxin targets porcine endothelial cells. Due to the cytopathic effect seen in cell culture assays it can be hypothesised that the activity of beta-toxin, leads to primary vascular necrosis in the intestine of piglets and subsequent ischemic necrosis.

The pathomorphological pattern of the liver in black-striped field mice living near the pesticide tomb, in Iławskie Lake District, during its existence and three years after its liquidation

Szarek, Jozef, Andrzejewska Anna, Skibniewska Krystyna, Grzybowicz Mirosław, Guziur Janusz, Sawicka Kapusta Katarzyna, Babinska Izabella, Zakrzewska Marta, Zmysłowska Izabela

University of Warmia and Mazury, Faculty of Veterinary Medicine, Department of Forensic and Administration of Veterinary Medicine, Olsztyn, Poland

Introduction: Different plant protection chemicals, including DDT, were intensively eliminated in Poland in the 70's. 330 pesticide tombs with dangerous deposits were constructed then. Some of them have become dangerous for the environment. The aim of the study was to compare the effect of the pesticide tomb (PT) on pathomorphological pattern of the liver in black-striped field mouse (*Apodemus agrarius* Pallas 1771) living in this polluted environment during existence of PT and three years after its liquidation.

Material and methods: 80 black-striped field mice (n = 10) caught in four zones of increasing distance from the PT in the autumn 2004 and 2007 were examined. The area between the PT and the lake shore was divided into four consecutive zones: I - slope of the PT hill, south-east exposition, II - area between road and fish pond, being a flat ground among trees, III - continuation of zone II in the direction towards the lake, IV - control, a dam about 4 km of PT. During macroscopic examination the samples of the liver were taken for microscopic and ultrastructural examination. Material for histopathological evaluation was fixed in 10% neutralized formalin. Paraffin slides were stained with haematoxylin, eosin and Sudan III according to the method by Lillie Ashburn and according to PAS by McManus. The material for ultrastructural evaluation was fixed in 2.5% glutaraldehyde in a 0.2 mol/l phosphate buffer of pH 7.4 and embedded in Epon 812. The ultrathin sections were contrasted with uranyl acetate and lead citrate. Ultrastructural analysis was conducted using an Opton 900 PC TEM.

Results: Usually no lesions were noted macroscopically in the liver of the mice. Microscopic and ultrastructural lesions were relatively most numerous in the liver of the mice living near PT and in its previous location (zone I and II). The amount and intensity of the lesions diminished with an increase in the distance to the PT. The majority of lesions were damaging, and some of them showed adaptive features. Parenchymatous degeneration, necrotic microfocci, hyperemia and infiltration of lymphocytic cells were often observed. Hepatocytes with double nuclei were noted relatively often. The number of morphological lesions and the degree of their intensity noted in the liver of mice living in the area of PT in 2004 and in 2007 were sometimes similar and sometimes they showed growing tendency. The distribution and character of the lesions showed the participation of environmental pollutants in their creation.

Conclusions: Pathomorphological examination of the liver of black-striped field mice living nearby PT revealed the influence of xenobiotics on microscopic and ultrastructural pattern of the organ presented. The study showed that three years after liquidation of the pesticide tomb, its influence on the surrounding environment is still significant.

Effect of fish technology production on the pathomorphological pattern of the liver in carp (*Cyprinus carpio* L.)

Szarek, Jozef, Skibniewska Krystyna, Guziur Janusz, Mieszczynski Tomasz, Babinska Izabella, Gesek Michal

University of Warmia and Mazury, Faculty of Veterinary Medicine, Department of Forensic and Administration of Veterinary Medicine, Olsztyn, Poland

Introduction: The sort of diet and environmental features significantly influence health state and condition of carps. The aim of the study was to determine the influence of semi-extensive (based on natural feed) and highly intensive technology (based exclusively on feed of high protein content) fishery production on morphological pattern (macroscopic, histologic and ultrastructural) of the liver in carps.

Material and methods: The analyses were carried out on 120 carps caught for experiments in the autumn 2007 and divided into 4 groups (n = 30). Two control groups (K) – fish fed with natural feed (benthos), not fattened and coming from Knyszyn (KK) and Siemianówka (KS). Two other groups were fed according to highly intensive production with granulate (Aller-Aqua). They came from Ostroleka (O) and Mokre (M). Each of this group was divided into: A – carps 2+ years old and B – carps 3+ years old. Macroscopic, microscopic and ultrastructural evaluations were conducted. Liver samples were fixed in 5 % buffered formalin, stained with hematoxylin and eosine (HE) and with PAS method according to McManus. The samples of the liver for ultrastructural examination were taken from 4 carps from each group. The material was fixed in 2.5 % glutaraldehyde in 0.2 mol/l phosphate buffer of pH 7.4, embedded in Epon 812. The ultrathin sections were contrasted with uranyl acetate and lead citrate. Analysis was conducted using the Opton 900 PC TEM (Germany).

Results: Macroscopically all carps showed the regular pattern. Microscopically small regressive lesions and circulation disturbances were most frequent in the liver of carps from group K. Parenchymatous degeneration, steatosis simplex (especially in fish from group K2) and hyperemia were relatively often noted. Other lesions were observed sporadically. The lesions enumerated above were more numerous in fish from the groups O and M when compared to the group K. Moreover, these cases also showed lymphoid cell infiltration and sporadic necrotic lesions. Ultrastructural changes in the liver of carps from the group K were represented mainly by steatosis simplex that was especially clear in fish from the group KS. Ultrastructural pattern of the liver in fish from the groups O and M most frequently showed slight lesions in mitochondria, necrotic microfocci and disorganization in RER. Steatosis simplex was more rare than in fish from the group K. Carps from the groups K showed slightly less morphological lesions than carps from the groups O and M. Moreover, fish 2+ years old had less morphological lesions than one year older fish.

Glycogen content in hepatocytes in fish from the groups O and M was slightly higher than in carps from the group K.

Conclusions: Feeding technologies that were analyzed and environment of carp had small effect on morphological pattern of the liver in these fish. Highly intensive system of farming caused small intensification of morphological lesions. The degree of their intensity was slightly increased in carps that were 3+ years old in comparison with one year younger fish.

Occurrence of porcine circovirus type 2 (PCV2) in cases of antibiotic non-responsive diarrhoea in pigs

Szczotka, Anna, Katarzyna Podgórska, Jacek Żmudzki, Wojciech Kozaczyński, Zygmunt Pejsak, Tomasz Stajejko

National Veterinary Research Institute, Pulawy, Poland

Introduction: Porcine circovirus type 2 (PCV2) is a causative agent of postweaning multisystemic wasting syndrome (PMWS), characterized by wasting, dyspnea, enlarged lymph nodes, pallor and jaundice. Sometimes diarrhoea could also be observed and this clinical manifestation should be differentiated from other swine enteric diseases, like proliferative enteropathy (PE) and swine dysentery (SD). The etiological agents of these diseases are, respectively: *Lawsonia intracellularis* (*L. Intracellularis*) and *Brachyspira hyodysenteriae* (*B. hyodysenteriae*). The aim of the study was to analyze the presence of PCV2 in cases of antibiotic non-responsive diarrhoea and to evaluate the possible role of the virus in development of enteritis in pigs.

Material and methods: Internal organs and feces from 17 5-19 weeks old pigs from 17 large-scale farrow-to-finish Polish farms were used in the study. The animals suffered from clinical symptoms of PMWS, including diarrhoea. Sections of lymph nodes and intestines (ileum, caecum and colon) were analyzed for presence of PCV2 DNA by *in situ* hybridization test (ISH). They were also hematoxylin-eosin stained for standard histopathological examination. Additionally, fecal samples from the same pigs were tested for presence of *B. hyodysenteriae* and *L. Intracellularis* by multiplex PCR.

Results: Large amounts of PCV2 DNA in lymph nodes characteristic for PMWS were detected by ISH in samples from 10 pigs, 10 – 17 weeks old. PCV2 was also found in abundant amount in samples of ileum, mainly in villi and in Peyer's patches, from 10 pigs. From only 1 pig a sample of ileum contained marginal amount of PCV2 in submucosa. In samples from 2 animals PCV2 was present in little amount in submucosa of colon and from 1 animal in submucosa of caecum. These animals were 10, 14 and 12 weeks old, respectively. In 9 samples presence of PCV2 in lymph nodes was correlated with abundant amount of PCV2 in ileum and in 1 PMWS-positive case intestines were negative for PCV2. In samples from only 1 animal lymph nodes were negative for PCV2 in ISH, but virus was detected in considerable amount in submucosa of ileum. In the corresponding HE stained sections of lymph nodes lymphocyte depletion, histiocyte infiltration, multinucleated giant cells and inclusion bodies were identified. Similar lesions were observed in PCV2-positive samples of ileum. Sixteen samples of feces were negative in multiplex PCR for both *L. intracellularis* and *B. hyodysenteriae*. Only in one fecal sample from 17 weeks old pig DNA of *L. intracellularis* was detected, but in histopathological examination of intestinal sections from this animal no lesions typical for PE were detected. Also, lesions characteristic for PMWS were not observed in lymph nodes from this pig but PCV2 DNA was detected in ISH in ileum. Samples from 5 pigs were free from PCV2 and *B. hyodysenteriae* and *L. Intracellularis*.

Discussion: The results show that in PMWS affected pigs similar lesions could be observed both in lymph nodes and in ileum and they correlate with diarrhoea. Also, it was found that presence of PCV2 in ileum could be correlated with diarrhoea in PMWS-free animal. In the animals negative for *B. hyodysenteriae* and *L. Intracellularis* and PCV2 other causative agents of diarrhoea should be considered. Because the study was performed on small number of samples, further investigations need to be performed to confirm the role of PCV2 as an etiological agent of diarrhea in pigs.

Flow cytometric analysis of dendritic cells in peripheral blood of healthy and BLV infected cattle

Szczotka, Maria, Jacek Kuźmak

National Veterinary Research Institute, Pulawy, Poland

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Introduction: Dendritic cells (DCs) are professional antigen presenting cells (APC), which initiate primary immune responses and play an important role in the generation of peripheral tolerance. Following their encounter with antigen or danger signals, DC migrate to the lymph nodes, where they activate effector cells essential for tumour clearance. Although the DC system is highly heterogeneous, the differentiation and function of DC populations is largely regulated by exogenous factors. Malignancies and retroviruses appear to exploit this by producing immunosuppressive factors capable to affect DC, thus exerting systemic effects on immune response. The aim of this paper was to isolate and characterize dendritic cells from healthy cattle and after infection with BLV.

Material and methods: Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by density-gradient centrifugation (Histopaque, Sigma) and then monocytes were generated. CD14⁺ cells were isolated by negative selection, using super-paramagnetic particles labelled with antibodies to human CD14 (Miltenyi-Biotech). Then, cells were cultured in complete RPMI-1640 medium containing FCS, glutamax and gentamycine. To generate DCs, GM-CSF or rbGM-CSF and IL-4 were added to the cultures. The lymphocytes taken from BLV-infected cow were added to the cell culture and cultivation was performed in incubator with CO₂ flow. After 10 days cells were harvested. Phenotypic characterization of DCs was performed by flow cytometry using different fluorochrome-conjugated antibodies: CD14, CD1a, CD11b, CD11a, CD11c, MHC-I, MHC-II and CD13. Morphology of cells was determined after their staining with Giemsa stain.

Results: After immunomagnetic separation of CD14 cells we found that purity of selection was about 92%. In the cell cultures different morphological types of DCs were observed and they were on different levels of maturity. We found that dendritic cells generated from normal blood cells had different CD markers in comparison to cells infected with BLV.

Discussion: There is no reliable method established for the isolation of bovine peripheral blood DCs and the phenotypes and the functions of bovine DCs are still not completely clear. In the present study we have attempted to identify bovine peripheral blood DCs by special selection. Dendritic cells differentiated from monocytes in peripheral tissues by factors generated by inflammation or various factors. Immature myeloid DCs transmit this information to peripheral lymphoid tissues, where they relay it to T-lymphocytes. The results obtained during this experiment may be useful in the knowledge of the mechanism BLV infection.

Histopathological survey of cervicovaginal samples of Holstein dairy cows with or without reproductive tract disorders

Talebkhan Garoussi, Massoud, Farhang Sassani, Parviz Hovareshti

Section of Theriogenology, Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad-Iran

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Introduction: The increase of reproductive performance such as: first service/conception, conception rate, improvement of days open and calving interval in dairy cattle herds depend on several factors. Several complexes relationship among the effective factors influencing on the fertility are related to the health status of reproductive system organs of dairy cattle. The objective of this study was: to evaluate the cervicovaginal histopathological changes in Holstein dairy cows with or without clinical signs of reproduction system abnormalities.

Material and methods: In total, 144 lactating Holstein cows with (No. 103) or without (N. 41) recorded postpartum clinical symptoms of reproduction diseases were examined in treatment and control groups, respectively. The cervical and vaginal specimens of 99 and 103 cows in treatment group were obtained, respectively. The cervical and vaginal samples of 38 and 41 cows in control group were prepared, respectively. Biopsies of the internal wall of cervix and vagina were obtained using a sterile alligator-jawed (rounded) biopsy forceps. Cows were inseminated artificially (No.83; 58%) for three times after the calving or had not been recorded any estrus signs since three months after their last calving (No.61; 42%). They were at various stages of the estrus cycle. The samples were placed in 10% formalin. After the related processing of the samples, they were evaluated for microscopic histopathological changes.

Results: The results showed that 74 out of 99 (78%) dairy cows had no cervical pathological lesions. There was no vaginal pathological lesion for 97 (94%) cows. However, it was showed that 4 (11%) and 5 (12%) cows in control group had cervical and vaginal histopathological lesions, respectively. Chronic cervicitis (No. 8; 8%) and mild chronic vaginitis (No. 3; 3%) was the most common lesion in treatment group, respectively. However, 2 (5%) cows had chronic follicular cervicitis and 2 (5%) cows showed acute cervicitis in control group. In control group, 36 (88%) cows had no vaginal histopathological changes. Acute vaginitis was indicated in 5 (12%) cows of control group.

Discussion: It was shown that most of the cows with recorded postpartum genital system diseases had no histopathological lesions. The most cervicovaginal lesion was chronic in the cows. However, the healthy cows might have acute cervicovaginal lesions.

Detection of frequency rate of reactive amyloidosis in affected cows with Tuberculosis

Tavasoly, Abbas, Piman Mohammadi

Faculty of veterinary medicine, University of Tehran, Tehran, Iran

P146

Introduction: Amyloid is the generic term for a variety of proteinaceous materials that are abnormally deposited in tissue interstitium in a spectrum of clinical disorders. By light microscopy and standard H&E staining amyloid appears as an amorphous, eosinophilic, hyaline, extra cellular substance that with progressive accumulation result in pressure atrophy of adjacent cells and tissue. This study based on reactive systemic amyloidosis which has a systemic distribution and is also referred to as secondary amyloidosis because it is often secondary to chronic inflammation of the destructive tissue process. This research detected the frequency rate of renal and hepatic reactive amyloidosis which occurred in bovine tuberculosis. Tuberculosis is an important and famous chronic inflammatory disease of cattle.

Material and methods: 39 cows were chosen from abattoirs around Tehran area. Tuberculosis was diagnosed in these cattle with a skin screening tests (Tuberculin). 4 different tissue mass were removed from each cattle. Affected lymph nodes and lungs were chosen for confirmation of tuberculosis with microscopic lesions. Tissue samples from kidney and liver were chosen for diagnosis of amyloidosis. For microscopic study, tissue samples were fixed in a 10% buffer neutral formalin solution. 7 micron paraffin sections were stained with H&E.

Results: classic and non classic granulomatous inflammation was observed in 36 cows. Epithelioid and macrophages were the main cells which infiltrated in the affected tissue (lymph nodes and lungs). Reactive amyloidosis was observed in 5 cases equal (13.8%) of affected cattle with tuberculosis. Grossly, the renal amyloidosis appears as a large, pale, gray and firm kidney. Amyloidosis of the liver caused massive enlargement. Microscopically, glomerular tufts were enlarged and basement membrane of the capillaries. Infiltrated by eosinophilic amyloid a lot of hyaline casts were observed in the lumen of renal tubules which confirmed the nephrotic syndrome. In the liver amyloid was deposited in the walls of the sinusoids. There amorphous eosinophilic materials were seen around the hepatocytes.

Discussion: for confirmation of amyloidosis, tissue samples must be stain with special staining (Congo red). In this method amyloid stains an orange to red color when these slides viewed with polarized light amyloid have green birefringence. Congo red staining after treatment of a section of affected tissue such as kidney with potassium permanganate suggests the amyloid is in AA-amyloid. This research revealed that amyloidosis can occur after chronic inflammatory process includes infectious and non infectious disease. Reactive amyloidosis is a systemic and dangerous disease. In this reason we must pay attention to all inflammatory process which occur in the body and try to treat all of them.

A preliminary study on alternative therapy in Wistar rats RS1 hepatoma

Tirifon, Eugenia¹, Zenaïda Stoenescu² Mihai Vasilescu³, Iuliana Gruia¹, Monica Nistoroiu¹, Marieta Panait¹, Ana-Maria Burdusel¹, Monica Vasilescu¹, Madalina Chesnoiu¹, Crina Tirifon², Nicolae Manolescu¹, Sabin Cinca¹

1.Institute of Oncology 'prof. Dr. Al. Trestioreanu' Bucharest, Romania; 2.University of Medicine and Pharmacy 'Carol Davila' Bucharest, Romania; 3.Ministry of Agriculture and Rural Development, Bucharest, Romania

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Introduction: In diverse forms of cancer, the usual chemotherapy is limited due to the secondary effects of drugs – including significant reducing of immunity – that favour cancer evolution and metastasis. Despite significant efforts done in order to stimulate the host immunological response, the results are still not very favorable. To identify possible new immunostimulating and/or antitumor derivatives, extract from roots plants (*Acanthopanax senticosus*/*Eleutherococcus senticosus*, *Calendula officinalis*, *Chelidonium majus*) were tested in experimental tumors on animals.

Material and methods: Wistar rats were inoculated with RS1 hepatoma and the tested extracts were administrated in several modes: previously to tumor inoculation (2 months before); concomitently with tumour inoculation; 20-25 d after tumor inoculation. To all animals, extracts were administered both in food and in peritumoral applications. There were surveyed: whole body weight, tumor volumes, survival rates, and after sacrifices: tumor weight, macroscopic aspects, as well as some molecular markers: malondialdehyde, thiols, P450 cytochromes concs, ceruloplasmin and GST activities, S-phase cell fractions, proliferative index.

Results: Survival rates were increased by extracts administration; comparing to 31 d for tumor lot, values were: 42 d - lot (i), 38 d – lot (ii), and 37 d – lot (iii), respectively. A significant tumor volume decrease in extracts cases, (more than 50%) was observed, comparing to tumor case. Diminution of the oxidative markers indicated a protective effect over oxidative stress, mainly in 'preventive' administration (case i). In case (i) a significant decrease of DNA synthesis, and an accumulation of cells in G1 phase – comparing to only tumor case – were observed. Values of cytochromes P450 concs and GST activities – enzymes involved in detoxication pathways – revealed the efficiency of studied extracts. Significant increases of GST activities were noticed, both in healthy animals and in tumor cases, especially in (i) and (ii) cases.

Discussion: Taking into account the previous mentioned results: a higher survival rate, a higher weight, decreases of proliferative index, of S-phase cells, effects on enzymes and systems involved in oxidative stress or detoxication, we assume that plant extracts exhibit an immunostimulatory effect. We have to mention lack of toxicity and of secondary effects, too, that suggests that the investigated extracts could represent some possible adjuvants to normal chemotherapy.

Acanthocephalans in Southern California Pinnipeds

Tkalcic, Suzana, Vanessa Fravel, Jennifer Boardman, Lauren Palmer

Western University of Health Sciences, Pomona, CA, USA

P33

Introduction: Anoplocephalans (thorny-headed worms) are common intestinal parasites of pinnipeds and cetaceans. Their life cycle involves ichthyofauna, mostly fish of sculpin and sole species. In pinnipeds, these parasites are predominantly reported as incidental findings within the intestinal tract. The occurrence, morphology and histopathological lesions associated with Anoplocephalans in Southern California Pinnipeds were investigated.

Material and methods: During a 2 year period, a total of 20 stranded pinnipeds were necropsied due to the unsuccessful rehabilitation efforts at the Marine Mammal Care Center in San Pedro. This group included 16 California Sea Lions (*Zalophus californianus/ZC/*) and 4 Northern Elephant Seals (*Mirounga angustirostris/MA/*). During the necropsy, the intestinal tract was fully dissected, lesions were observed and described, parasitic status was evaluated and tissue and parasite samples were collected for histopathology and further processing.

Results: Anoplocephalan parasites were present in 5/16 California Sea Lions and 4/4 Northern Elephant Seals. The lesions ranged from a minimal subacute enteritis to severe acute diffuse necrohemorrhagic typhlocolitis. Most severe infestations were present in Northern Elephant Seals, and were associated with extensive histopathological lesions and melena.

Discussion: Most common Acanthocephalans in Southern California Pinnipeds are of genus *Corynosoma*. In most pinnipeds they rarely penetrate deep into the intestinal wall. We found that 31% of necropsied Sea Lions (ZC) and 100% of Elephant Seals (MA) were infested with this parasite. The most severe intestinal lesions were necrosis and hemorrhage present in the large intestine of MA. Unlike in some seals, no evidence of perforative lesions was found.

Current trends in veterinary pathology education: evidence based medicine and problem based learning

Tkalcic, Suzana

Western University of Health Sciences, POMONA, CA 91766, USA

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Evidence based medicine (EBM) is defined as a medical practice based on integration of knowledge acquired from the available research evidence, clinical expertise, and patients clinical values, individual factors and circumstances. This trend is not new for veterinary medicine and veterinary pathology, but it supports the need to integrate both areas of medicine into a one medicine concept, with a strong emphasis on comparative medicine and interdisciplinary approach. Problem based learning (PBL) is a teaching methodology based on the clinical scenarios and learner's collaborative problem solving initiative and active learning through identification of specific learning issues within several medical disciplines at the same time. The key for its effectiveness is a small group setting. Educator's role is facilitating, rather than lecturing, and the cult of coverage is replaced by clinical medical reasoning. EBM is a prerequisite for an effective PBL. With global initiatives and information explosion, technological advancement and availability of resources, there is a need for new approaches in teaching medicine and pathology. Founded on very principles of EBM, pathology as a basic science has a leading role in these efforts. These trends are already present in the postgraduate medical education and residency training throughout the world, however, following the successes in undergraduate human medical education they are gradually being accepted in the undergraduate veterinary education as well. Through this paradigm, learning is shifted from teacher-centered to student-centered and becomes a life-long habit of a new graduate that will provide him with good leadership skills and communication capabilities, as well with good clinical and ethical practices that will ensure his professionalism and success.

First report of acute toxic nephrosis by leaves of Boxelder or Manitoba Maple (*Acer negundo*) in Greek sheep and goats

Tontis, Dimitrios¹, Dimitrios Doukas¹, John Edwards², Fotios Lykotrafitis¹, Maria Kritsepi-Konstantinou³

¹ Laboratory of Veterinary Pathology, Veterinary Faculty, University of Thessaly, Karditsa, GREECE

² Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A & M University, USA

³ Diagnostic Laboratory, Veterinary Faculty, Aristotle University of Thessaloniki, Greece

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Introduction: Manitoba maple or boxelder (*Acer negundo*) are medium-sized trees of the family Aceraceae. They have been widely cultivated as ornamental plants in many urban areas of Greece for the last decade. The toxicity of leaves from the red maple (*Acer rubrum*) was first described in horses at Cornell, USA. Since then, many cases of maple poisoning were observed mainly in horses and ponies, but with reports in zebras and alpacas. The toxic principle in red maple leaves is believed to be gallic acid. It causes methemoglobinemia and methemoglobinuria in horses, and if left untreated, it can cause a fatal nephrosis. In a recent report in two alpacas, it was hypothesized that there may be 2 toxic components, a methemoglobin-causing compound (possibly gallic acid) and a hemolytic compound (possibly pyrogallol). Here we report an acute toxic nephrosis of sheep and goats ingesting leaves of Manitoba maple in Greece that shares a similar pathology with oak toxicosis of domestic animals.

Material and methods: Fourteen animals (10 sheep and 4 goats, ranging in age from 9 months to 4 years, 11 female and 3 male) from 2 flocks were affected. The farmers fed affected animals fresh trimmings from *Acer negundo* animals in the Autumn and late Spring.

Results: All the animals began demonstrating clinical signs of anorexia, dyspnea, muscular tremor and progressive depression within 1 to 2 days after ingestion of the leaves. Most deaths occurred 3 to 4 days after ingestion. The animals that were not found dead showed complete recovery during the next several days. Clinical pathology parameters were studied only in 4 of the affected sheep. Main findings were only mild anemia (may have been due to endoparasitism?) and increased CPK. Heinz bodies were seen only in 1 case and 2 sheep had a leucocytosis (1 with concurrent embolic pneumonia and 1 with pasteurellosis). Urinalysis showed marked proteinuria, mild elevation of bilirubin and creatinine. Granular casts were in the urine sediment. The gross pathologic findings were slightly swollen kidneys suggesting nephrosis, and the rumen contained identifiable fragments of the maple leaves. Additional findings included variable degrees of endoparasitism of the lungs, gastrointestinal tract and liver. At necropsy, organ samples from 10 sheep were fixed in 10% buffered formalin and routinely processed for light microscopy. The carcasses of 4 goats were too autolytic for histopathologic exam. Histopathology revealed acute, segmental nephrosis with tubular epithelial cell degeneration and necrosis and some proteinaceous and cellular casts. No tubular pigment or hemoglobin casts were noted.

Discussion: Toxic nephrosis is well described in domestic animals, but little is known about maple nephrosis in small ruminants. Other differentials for nephrosis in sheep include hemoglobinuric, bilirubinuric, and myoglobinuric conditions, heavy metals, antibiotics, oxalosis, and toxic plants such as oak, yellowwood tree, lilies, pigweed, and *Isotropis sp.*. The nephrotoxicosis in our cases most resembled that of oak. To our knowledge, *Acer negundo* toxicosis has not

been reported in ruminants. This report describes toxic nephrosis in 14 small ruminants that consumed fresh leaves of *Acer negundo*. The haemolytic anemia with methemoglobinemia and methemoglobinuria reported with red maple toxicosis was not an important feature in our cases. Further study of erythrocytic disorders needs to be done in order to absolutely eliminate hemolysis from the pathogenic mechanism of the nephrosis. The diagnosis of *Acer negundo* toxicosis was based on history, clinical signs, and necropsy and histopathologic findings in these pastured, untreated animals.

Morphogenesis of ocular changes and microphthalmia in Royal College of Surgeons (RCS) rats

Tsuji, Naho, Tetsuro Matsuura, Kiyokazu Ozaki, Isao Narama

Setsunan University, Hirakata, Japan

P120

Introduction: Royal College of Surgeons (RCS) rat is an inbred strain with well-known inherited retinal degeneration, and widely used for the research in hereditary retinal dystrophies. Microphthalmia and other ophthalmic changes such as downward deviation of the pupil are frequently observed in RCS rats by clinical observation of anterior chamber. In the present study, we examined morphological character of ocular lesions including microphthalmia in adult RCS rats and attempted to clarify the morphogenesis.

Material and methods: A total of 978 male and female RCS rats aged 3-78 weeks were subjected to clinical observation of anterior chamber using a penlight and surgical microscope and histopathologic examination on both eyes. The length of the iris was measured under the stereoscopic microscope after the fixation. Embryos from pregnant dams on gestation day (GD) 11-19 of 46 RCS rats were also examined along with the age-matched embryos from 17 F344 female rats.

Results: Downward deviation of the pupil was observed in 87 rats (8.9%) with microphthalmia (laterally in 73 and bilaterally in 14 cases) out of 978 RCS rats. Stereoscopic examination revealed that brown area in ventral fundus fanned out from neighborhood of the optic disc, and shortening of the inferior iris with elongation of the superior iris after elimination of cornea and lens. In addition, characteristic mushroom-like structure protruded from the area. Histopathological changes consisted of complete or incomplete loss of retinal and choroidal layers, partial loss or hypoplasia of iris, heterotopic ciliary body, heterotopic pupillary sphincter muscle and persistence of hyaloid artery. Although the optic fissures in F344 embryos had fused completely by GD 14, the inner layer (presumptive retina) and the external layer (retinal pigment epithelium) of the optic cup in the affected RCS embryos did not show normal continuity at the optic fissure. The fissure margins were hypoplastic in many cases and did not sufficiently extended with reduced cell layers and numbers in the inner layer. Lens epithelial cells were disarranged and irregularly arrayed in many cases and the size of each cell was much smaller than normal ones. Anterior pole of lens adhered to the thinned cornea. In the most severe cases, no conformation of ocular tissue were observed anywhere except a primordial retinal tissue.

Discussion: The histopathologic changes of the eyes with downward deviation of the pupil or irregular structure of the fundus suggested that they are resulted from varying degree of defect and hypoplasia of some ocular components (coloboma) and persistent hyaloid artery. Hypoplastic changes seem to be main factor involved in colobomatous microphthalmia in RCS rats.

First Asian report of spontaneous Chytridiomycosis in captive frogs

Une, Yumi, Syou Kadekaru, Kenichi Tamukai, Kouichi Goka, and Toshiro Kuroki

Azabu University, Sagamihara, Kanagawa, Japan

P34

Introduction: The fungus *Batrachochytrium dendrobatidis* is an important pathogen implicated in the worldwide dramatic decline in frog populations except Asia. We confirmed the presence of chytridiomycosis for the first time in Japan in December 2006. The present report describes the nature of this outbreak in captive, exotic frogs, and warns of the presence of this highly pathogenic fungus in Japan.

History: A lethal outbreak of chytridiomycosis was discovered in a colony of 45 aquatic and terratic frogs from 18 species belonging to a private owner in Tokyo. All frogs were exotic and included both species bred in captivity and wild-caught frogs. Twenty-three frogs from 9 species were infected by chytrid fungus. One of 16 dead frogs reportedly died in September 2006 and another 15, from 7 species, were subsequently affected by the disease. Of the 15, 14 died successively during November and December 2006. The species of the infected frogs were *Lepidobatrachus laevis*, *Ceratophrys cornuta*, *Ceratophrys cranwelli*, *Ceratophrys ornata*, *Ceratophrys calcarata*, *Chacophrys pierotti*, *Occidozyga lima*, *Leptodactylus pentadactylus* and *Plethodontohyla tuberata*.

Material and methods: Three of the 16 dead frogs, one each from the species *L. laevis*, *C. cornuta*, and *L. pentadactylus*, were submitted for pathological examination and molecular biological examination. Swabs from the body surface of survivors were used to carry out PCR examination. Skin from one dead frog was observed with two kinds of electron microscope.

Results: Macroscopic Findings: A large quantity of mucus and sloughed skin were found stuck to the body surface of the *L. laevis* examined, but the *C. cornuta* and *L. pentadactylus* specimens showed no particular skin lesions. **Microscopic Findings:** Skin lesions showed similar characteristics in all frogs, but the degree varied by species of frog. The fungus grew in the superficial keratinizing epithelium, where various developmental stages of fungus were observed. Zoosporangia containing some zoospores, observed as round, flask- or pot-like forms with discharge tubes, and chytrid thalli, both empty and with faintly visible internal septation (colonial thallus) were observed. Thickening of the keratinized layer was severe in *C. cornuta*, and the degree of infection was also highest. **PCR Observation:** We extracted DNA from skin samples of the 3 dead frogs which had been histologically examined and from swab samples of the 7 survivors. A region of the 5.8S rRNA gene and the flanking internal transcribed spacer regions (ITS1 and ITS2) for *B. dendrobatidis* was amplified by PCR from the extracted DNA using the specific primers Bd1a and Bd2a cited by Annis et al.. We obtained consistently a single fragment of approximately 300bp from the PCR amplification. All of the DNA fragment sequences were entirely consistent with the ITS1-5.8S-ITS2 region sequences from *B. dendrobatidis* registered in the DDBJ databank (accession number AY997031). With scanning and transmission electron microscope, we confirmed the characteristic fungi in the keratinized layers.

Conclusions: This report is the first chytridiomycosis in Asia.

Pathological studies of host-parasite interactions: morpho-pathological and biochemical aspects (field cases)

Urdes, Laura, Cristiana Diaconescu, Hangan Marius, Cornila Nicolae, Vasile Petrica

Faculty of Animal Science, Bucharest, Romania

Introduction: According to the latest findings, there are some fish parasiting helminths (acanthocephalans and cestods) that can be used in toxicological studies, providing data on fish's and lakes' health. These helminths show a high heavy metal accumulation capacity in their hosts, acting as filters. The exposure of fish to aquatic heavy metal pollutants is believed to indirectly have increased host's susceptibility towards the parasites. This study examined a number of field cases in the Danubian Delta, in order to reveal the inter-relationships between freshwater fish, heavy metal concentrations and fish parasiting helminths.

Material and methods: Concentrations of some heavy metals (Cu, Zn, Hg, Cd, Pb) and the enzymatic activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined in hepatic tissue and musculature of some freshwater fish. It has been used atomic absorption spectrophotometry for heavy metals content and photocolometry for SOD and GPx. The determination of SOD enzymatic activity was based on the enzyme's capacity to inhibit the generation of superoxide anions. The method is based on the inhibition of spontaneous adrenaline degradation in adrenochrome by SOD. Histological changes in the liver and musculature following the natural exposure of fish to heavy metals were examined in light microscopy. The parasiting fish helminths and water samples were also examined in order to assess pollutants' concentrations.

Results: The biochemical results revealed that heavy metals concentrations in helminths were many times higher than in the host tissues and water samples. The carassius musculature had the highest zinc content. Mercury had an inhibitive action on the studied antioxidant enzymes.

Discussion: By comparison, the parasitized fish accumulated less metals than the unparasitized ones. Heavy metal concentrations in helminths were many times higher than in the host tissues and water samples. Even though exfoliative cytology and histology might be seen as biomarkers in the assessment of liver exposure to some pollutants, we consider that the fish parasiting helminths are far more consistent and reliable indicators for metal pollution than the host tissues (liver and musculature) and water.

Feline post-vaccinal sarcoma. An immunohistochemical study.

Watanabe, Tatiane, Rui M. Gil da Costa, Irina Amorim, Alexandra Rema, Célia Lopes, Eduarda Matos, Manuela Duarte, Fátima Gärtner

São Paulo State University, Brazil

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Introduction: Feline post-vaccinal sarcomas (FPS) are a group of locally aggressive soft tissue tumours that occur at vaccination sites in cats and the pathogenesis is as yet unknown. The purpose of this study was to describe the morfologic spectrium of a serial of previously classified post-vaccinal sarcomas and report their immunohistochemical feature. Additionally compare the proliferative activity determined by the mitotic index (MI) and the Ki-67% (MIB-1) and correlates them with several clinicopathological variables.

Material and methods: Hundred cases of FPS were retrived from the arquive of the Laboratory of Veterinary Pathology, ICBAS-Porto University. For microscopic examination, specimens were previously fixed in 10% phosphate buffered formalin solution, embedded in paraffin wax, 2µm-thick section were stained with HE. For immunohistochemical detection of vimentin, α -actin, desmin, S-100 protein and Ki-67, was used a modified avidin-biotin peroxidise complex method. Inflammatory infiltration was considered either lymphocytic or mixed cell type. Presence of multinucleated giant cells (GC) and apoptotic cells was also depicted. MI was assessed as the number of mitotic figures per 10 HPF. The immunostaining for α -actin, desmin, S-100 protein was assessed as positive or negative and vimentin was also according to staining intensity. For Ki-67 labelling index was calculated as the number of positive nuclei per 1000 cells. Chi-square test was used to study correlations between all categorical variables, and Student t-test was used to study differences between Ki67 and MI mean values, using SPSS 15,0 and considering a significance value of $p < 0,05$.

Results: Fifty eight animals were female and 42 were male. 61 animals were European and 39 had different breeds. The mean age of affected animals was 9 years; 38 cases showed GC, which correlated closely with increased age ($p=0,04$) and 53 had apoptotic cells/bodies observed in histology; 21 cases showed a lymphoid infiltrate. All tumours showed immunopositive for vimentin, 67 for α -actin, 28 for S-100 protein and 20 for desmin. The Ki-67 labelling index ranged between 4% and 91%. The MI ranged between 2 and 74 mitotic figures. Higher Ki-67 and mitotic indices significantly correlated with the absence of α -actin (Ki-67 $p=0,033$; MI $p=0,006$) and presence of S-100 protein (Ki-67 $p=0,010$; MI $p=0,001$). Higher MI indices significantly correlated with the presence of desmin ($p=0,040$). Mixed inflammatory cell infiltration significantly correlated with higher MI ($p=0,034$), higher numbers of apoptotic cells ($p=0,003$) and with the presence of α -actin.

Discussion: As reported in the literature, there was no sex or breed predilection. Immunohistochemical results demonstrate a significant phenotypic variation within these tumours, with up to 67% showing muscular differentiation and 28% neural differentiation markers. Proliferation activity varied widely and correlated positively with desmin and S-100 protein expression and lack of α -actin. The balance of apoptosis and cell proliferation was shown to be affected by the type of cell infiltration.

Differentiation among egg stages of *Angiostrongylus vasorum* in the lungs of experimentally infected foxes

Webster, Pia, Sigridur O. Magnúsdóttir, Pia Petersen, Jesper Monrad, Henrik E. Jensen

Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark

Introduction: The adult stage of the oviparous nematode *Angiostrongylus vasorum* is situated in the pulmonary arteries and female worms continually produce unembryonated eggs, in which larvae develop, hatch, and progress into the alveoli. The presence of undefined immature eggs in vessels and lung lesions has been reported several times and recently by Bourque et al. (2008). However, differentiation among the stages of eggs has not been attempted. In the present study we were able to discriminate between four developmental stages of the *A. vasorum* egg.

Material and methods: Twenty-eight foxes were experimentally infected with *A. vasorum* third-stage larvae. Ten weeks after infection the foxes were euthanized and tissue samples from the lungs were fixed in 10% buffered formalin and subsequently sectioned for histological examination. All samples were stained with haematoxylin and eosin, and in selected cases other histochemical and immunohistochemical methods were applied. Additionally, an immunohistochemical method for detection of parasitic antigens was developed. Antibodies from a seropositive dog were used as the primary reagent and labelled goat-anti-dog antibodies were applied secondarily.

Results: Parasitic eggs and larvae were found in granulomas and small vessels, from which the lesions expanded into the surrounding lung tissue. The granulomas often contained cell debris of parasitic origin. From the observation of eggs within vessels and lesions, four distinct stages of eggs were observed. The egg stages reflected the progressing cellular organisation into a completely developed larva. Eggs in all cases were ellipsoidal in shape and surrounded by a shell. The appearance of the four different stages of eggs was as follows:

- Stage I eggs: bulky content of eosinophilic granules and no defined cells. Dimension: $52.5\mu\text{m} \pm 13.9 \times 39.5\mu\text{m} \pm 7.5$.
- Stage II eggs: large well-defined cell borders and distinct basophilic nuclei and few eosinophilic granules (morula stage). Dimension: $56.0\mu\text{m} \pm 16.2 \times 40.3\mu\text{m} \pm 5.4$.
- Stage III eggs: cell mass organised in a larval shape with many small basophilic nuclei but without well-defined cell borders. Dimension: $58.9\mu\text{m} \pm 15.0 \times 40.0\mu\text{m} \pm 6.4$.
- Stage IV eggs: well defined first-stage larvae (embryonated egg). Dimension: $58.4\mu\text{m} \pm 14.1 \times 37.2 \mu\text{m} \pm 8.8$.

Discussion: The observation of four different developmental stages of parasitic eggs concurrently in most samples suggests that production of larvae is continuous in foxes in contrast to observations in dogs. The immunohistochemical staining method for *A. vasorum* antigen proved useful for localisation of eggs and disintegrated parasitic products. Moreover, in trouble cases the method allowed discrimination between parasitic material and ellipsoid-shaped fibrinous material, resembling stage I eggs, within small vessels.

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Endemic bovine tuberculosis by *Mycobacterium caprae* in a Tyrolian valley

Weikel, Joachim, Walter Glawischnig

AGES Institute for veterinary disease control, Innsbruck AUSTRIA

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Introduction: Starting 1999 endemic tuberculosis is present in the Lech Valley of Tyrol. Single cows slaughtered in Germany showed equivocal lesions. The infection was also demonstrated in red deer hunted in this county. 2008 in two different farms from one small village tuberculosis was detected and the official veterinarian started testing all the cattle in the region by intradermal testing. All the reagents were examined at the institute in Innsbruck and the results will be presented.

Material and methods: 21 cattle slaughtered or killed for diagnostics were examined for gross lesions of tuberculosis. Pathohistology and staining for acid fast bacilli was implemented as well as PCR for *Mycobacterium tuberculosis*-complex and bacteriological culture followed by sequencing.

Results: Most of the animals showed granulomas in lymphnodes and lungs with epitheloid and giant cells and partly central necrosis and calcification. Just one cow had generalised tuberculosis including lesions in the liver and the portal lymphnode. Cultural detection of mycobacteria succeeded in more than 50 percent and *Mycobacterium caprae* was ascertained by sequencing. The result of DNA fingerprinting was that the same clone of *M. caprae* was responsible for the tuberculosis in cattle and deer.

Discussion: Bovine tuberculosis caused by *M. caprae* was demonstrated in different farms in a small valley with shared pastures in the alps in summertime. Also calves were infected which were under one year of age and were sold within there first weeks of life. This was proofed by testing all animals at the contact farms. It is very likely that the reservoir of the infection is the overcrowded population of red deer living in close vicinity to the summer pastures. Further epidemiologic studies are planned together with the competent authorities.

First Description of sheep associated Malignant Catarrhal Fever in Reindeer in Austria

Weinberger, Hubert, Börge Schichl, Sandra Revilla-Fernandez

AGES IVET Graz, Graz, Austria

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Introduction: Malignant Catarrhal Fever (MCF) is a widespread disease complex in wild and farm ruminants, caused by different Gamma Herpes Viruses (AIHV-1; OvHV-2 CpHV-2, HipHV-1). Different breeds of wild and domestic sheep are the reservoir of OvHV-2 and show signs of a subclinical infection. Susceptible ruminants show the typical clinical and pathomorphological alterations, deriving from lymphoplasmocytic (peri)vasculitis and necrotizing vasculitis, as gastroenteritis, keratoconjunctivitis, dermatitis or, meningoencephalitis

Material and methods: 2 reindeers (*Rangifer tarandus*), 9 and 12 months old, held in a private zoo together with sheep and goats, died within 2 weeks, showing therapy resistant fever, inappetence, cramps, reddening and clouding of the cornea. A third one, 2 years old, with the same clinical signs recovered, but died half a year later under identical clinical symptoms. From the first two animals dissection, histological and PCR investigation was done. Only PCR was done from swabs and blood of the third reindeer.

Results: The one year old animal showed a poor body condition, putrid osteomyelitis of the left mandible, deriving from food particles in a dental alveole; the 9 month old reindeer was in a good body condition; edema of the conjunctiva, of subcutis and vulva, ascites, hypopyon, white spots in the kidneys and hyperemia of the leptomeninx were seen. Histological findings were lymphoplasmocytic and necrotizing vasculitis as well as perivascular edema in the brain, spinal cord and leptomeninx. Additionally, the younger animal showed putrid uveitis and iridocyclitis and fibrin masses in the anterior eye chamber, vasculitis and edema in subcutis and submucosa of the intestine; necrosis in the stratum basale of the skin. With PCR ovine Herpesvirus (OvHV-2) was detected in samples of the brain and organs of both animals; blood samples and conjunctival swab of the third reindeer were PCR positive for OvHV-2 during the acute phase of symptoms; two months later OvHV-2 could not be detected any more. From 4 sheep and 1 goat of the zoo, tested for OvHV-2 by PCR 6 months after the death of the first two reindeers, 2 sheep and the goat were positive, 2 sheep negative. Neither of them showed clinical symptoms.

Discussion: In populations of ruminants sheep (also wild sheep breedings) and goat frequently are the source of OvHV-2 infections in susceptible animals like cattle or exotic or wild ranging ruminants, as reindeer, moose, roe deer, red deer, bison. Especially in pet zoos when clinical signs are not typically or when affected animals recover, MCF might be not identified as cause and can lead to great losses. In conclusion we can say, that wild and farm sheep and goats should not be held together with susceptible ruminants without previous testing for OvHV-2. Recovered animals should be held in quarantine and tested several times, because from cattle it is known, that after recovering the animal may be virus carrier although it cannot be detected in every blood sample.

Immunohistochemical analysis of survivin and X-linked inhibitor of apoptosis protein (XIAP) expression in canine lymphoma

Wimmershoff, Julia, Paula Grest, Adam Polkinhorne, Franco Guscetti

Institute of Veterinary Pathology, University of Zurich, Switzerland

Introduction: Survivin and X-linked inhibitor of apoptosis protein (XIAP) belong to the Inhibitors of Apoptosis Proteins family. They exert their antiapoptotic function in the intrinsic apoptotic pathway downstream of the mitochondria. While XIAP has a direct inhibitory effect on the initiator Caspase-9 and downstream effector Caspases, survivin has been shown to exert its antiapoptotic effect through interactions with other proteins, including XIAP. The aim of this study was to establish a immunohistochemical detection method for these proteins in canine tissues and investigate their expression in canine lymphoma.

Material and methods: A polyclonal antibody against survivin (Novus Biologicals) and a monoclonal antibody against XIAP (MBL International Corporation) cross-reacting with the canine recombinant molecules were selected. A immunoperoxidase method was developed for immunohistochemical detection of these proteins using formalin-fixed paraffin-embedded cultured canine cells and a panel of normal canine tissues. The method was then used to assess expression of survivin and XIAP in a series comprising 85 canine lymphoma samples assembled in tissue arrays.

Results: The immunohistochemical labelling patterns of both antigens in canine normal tissues exhibited a high degree of similarity, with minor differences, to findings from studies on human tissues. In addition, all lymphoma samples exhibited cytoplasmic labelling for survivin and for XIAP. In most of the cases, the majority of tumour cells were labelled.

Discussion: Our data indicate that survivin and XIAP are commonly expressed by the majority of tumour cells in canine lymphoma. This suggests that the antiapoptotic mechanisms mediated by these molecules may be intact and might possibly contribute to the genesis of these tumours.

Histopathological classification and immunohistochemical features of canine mammary tumors

Woo, Gye-Hyeong, Ha-Young Kim, Jung-Won Park, You-Chan Bae, Yi-Seok Joo, Cheong-Up Choi

National Veterinary Research & Quarantine Service, Gyeonggi-do, Republic of Korea

P57

Introduction: Mammary gland tumors are the most common neoplasms in female dogs, with a 3-fold higher incidence comparing with breast tumors in women. These canine mammary gland tumors show great morphologic heterogeneity which is prominent proliferation of myoepithelial cells and formation of ectopic mesenchymal tissue such as cartilage or bone. In this study, canine mammary tumors collected in our laboratory were histopathologically classified and immunohistochemically evaluated.

Materials and Methods: One hundred forty two surgical specimens from local animal hospital were collected. The tissues were fixed in 10% buffered neutral formalin (pH7.4), processed routinely for embedding in paraffin, sectioned at 4 μm , and then stained with hematoxylin and eosin for histological examination. The diagnosis of each tumor was based on the World Health Organization (WHO) classification. Routine 4 μm sections were also subjected for immunohistochemical staining performed with antibodies against Ki-67, progesterone receptor, estrogen receptor, p53, phosphor-histone H3, HER2/*neu* or cytokeratin HMW, respectively.

Results and discussion: One hundred eighteen out of 142 cases were classified as benign tumors (83.1%) including hyperplasia and eighteen cases as malignant tumors (16.9%). Necrosis were more frequently found in malignant than in benign mammary tumors. In malignant mammary tumors, necrosis was more frequently observed in simple carcinoma or carcinosarcoma than in complex carcinoma. Mitotic figures were more highly present in malignant than in benign tumors. Tumor emboli were seen in one case (4.2%) of 18 malignant mammary tumors. Expression of Ki-67 or phosphor-histone H3 was higher in malignant than in benign tumors. Whereas progesterone or estrogen receptors were highly expressed in benign than in malignant tumors.

Conclusions: Ki-67 or phospho-histone H3 appears to be useful as a selective marker of malignant mammary tumors in dogs.

A canine spinal cord meningioma with two unusual features: amianthoid collagen fibres and secretory pattern

Woolford, Lucy, John J. Kepes, F. Clarke Berryman, Alexander de Lahunta, Sandra Schoeniger, Brian A. Summers

Royal Veterinary College, Hawkshead Lane North Mymms, Hatfield, Hertfordshire, United Kingdom

Meningiomas are common tumours in dogs with different subtypes recognized. We describe an unusual spinal cord meningioma in a 9-year-old male Husky dog which presented with pain at L5-6, faecal and urinary incontinence and tail paralysis. A myelogram revealed an intradural extramedullary mass at L5-6. Laminectomy showed the presence of a compressive meningeal tumour and segmental spinal cord malacia; euthanasia was elected. Microscopic examination of the mass revealed sheets of neoplastic meningothelial cells often arranged as rosettes around extracellular deposits of fibrillar eosinophilic material identified as collagen by a Masson's trichrome stain. Fewer neoplastic cells contained intracytoplasmic clear vacuoles, some with PAS-positive globular inclusions indicating a secretory pattern. Neoplastic cells were immunopositive for vimentin and negative for glial fibrillary acidic protein. On ultrastructural examination, the fibrillary material was composed of haphazardly arranged collagen fibres of increased widths (amianthoid collagen). Some neoplastic cells contained a small amount of intracellular collagen. The neoplastic cells formed long interdigitating processes connected by intercellular junctions, typical of meningioma. Secretory meningioma is a rare variant in man once previously reported in the dog and this may be the first report of a meningioma with amianthoid fibres in a non-human species, a previously described but rare meningioma variant in man.

Effects of natural transplacental infection of bluetongue virus serotype 8 in cattle: abortion and hydranencephaly

Wouda, Willem, Klaas Peperkamp, Thijs Roumen, Jan Vos, Evert van Garderen, Jan Muskens

Animal Health Service, Deventer, The Netherlands

P155

Introduction: A large proportion of the cattle population in the Netherlands was infected during a bluetongue virus serotype 8 (BTV-8) epidemic in 2007. An increase of bovine abortions was noticed during the last semester of 2007, followed by submissions of aborted and newborn calves with severe developmental defects of the brain.

Material and methods: 35 aborted fetuses and 20 liveborn calves were submitted to the Animal Health Service between October 5th 2007 and May 6th 2008, showing gross and/or histopathological lesions compatible with BTV infection. In all cases splenic tissue was investigated for the presence of BTV-8 at the Central Veterinary Institute, Lelystad, using an in-house reverse transcriptase-PCR (RT-PCR). An antigen ELISA for BVDV was used to investigate pooled samples of spleen and lung. Sera from 15 dams were screened for BTV-antibodies and BVDV-antibodies, using ELISA techniques. Maternal blood samples were also tested for BTV-8 by RT-PCR and for BVDV by antigen ELISA.

Results: Submitted fetuses were aborted or stillborn between 4 and 9 months gestation. In 21 of 35 fetuses BTV-8 was detected by PCR. All fetuses were negative for BVDV. In most cases gross lesions were confined to the cerebrum. Cerebral hemispheres consisted of fluctuant fluid-filled sacs bounded by a rim of parenchyma and leptomeninges. The thickness of the remaining rim of cortical parenchyma varied but was very thin and transparent in most cases. Histologically, the remnants of cerebral cortex consisted of loosely arranged glial cells with scattered calcium deposits and haemosiderin filled macrophages. The brainstem structures and the cerebellum were mostly not affected, except for five cases, in which the cerebellum was absent as well. This type of cerebral malformation, known as hydranencephaly, has been associated with infection of teratogenic viruses. In seven fetuses with a positive PCR for BTV-8 no gross lesions were found but histological lesions were present in the brain consisting of multiple areas of malacia and calcification. Twenty live born calves showed similar lesions of severe hydranencephaly. These calves had survived for four one day up to 14 weeks before they were euthanased. Clinical signs noticed were opisthotonus, blindness, abnormal posture, uncontrolled gait, circling and behavioural abnormalities (dummy calves). In one 1-day-old calf BTV-8 was detected by PCR, but all others were negative. Two calves were positive for BVDV. Fifteen dams of BTV-8 PCR negative calves or fetuses with hydranencephaly could be traced and were blood sampled. All fifteen dams had serum antibodies to BTV, but were negative for BTV-8 by PCR. Eight dams had serum antibodies to BVDV, but all maternal blood samples were negative for BVDV using antigen ELISA.

Discussion: We conclude that our findings provide evidence that infection of BTV-8 during pregnancy in cattle may lead to abortion and congenital brain defects in calves, particularly hydranencephaly. The fact that BTV could not be detected in all calves is consistent with experimental work of MacLachlan et al. (1985), who were not able to isolate BTV in calves that survived in utero infection. A role of BVDV in the pathogenesis of hydranencephaly is unlikely.

Accumulation of advanced glycation end products in canine atherosclerosis.

Vandenberge, Valerie, Sara Van der Heyden, Koen Chiers, Richard Ducatelle

Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Poultry Diseases, Merelbeke, Belgium

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Introduction: In humans, atherosclerosis is a major cause of morbidity and mortality in the western world. In dogs however, this is a rare lesion. Three major components are described in atherosclerotic plaques: cells, including smooth muscle cells, macrophages and other leucocytes; extra cellular matrix, including collagen, elastic fibers and proteoglycans and intracellular and extra cellular lipid. The aetiology is not clear but hyperlipidemia and endothelial dysfunction are regarded as important factors. Based on research in human medicine and animal experiments, Advanced Glycation End products (AGEs) are thought to play an important role in the pathogenesis of atherosclerosis. AGEs are a heterogeneous and complex group of components originating during oxidative and carbonyl stress.

Material and methods: In this study, atherosclerotic lesions found in 3 dogs were analyzed for the presence of AGEs. One of these dogs had died suddenly. Samples from atherosclerotic lesions and myocardium were taken. HE staining, Von Giesson, Von Kossa, immunohistochemistry for smooth muscle actine, CD3, CD20, MAC387, elastine and AGE were performed on the formalin fixed, paraffin embedded samples.

Results: Atherosclerotic lesions of the coronary and myocardial arteries were found in 3 dogs during necropsy. In 2 dogs atherosclerotic plaques in the abdominal aorta were present and a thrombus was found in one dog. The atherosclerotic lesions were localised in the tunica media with a subsequent thickening of the tunica media and narrowing of the vascular lumen. These lesions consisted of foamcells, cholesterol crystals and sometimes mineralization with the limited infiltration of T and B cells and macrophages. Immunohistochemistry for AGEs was positive in the atherosclerotic lesions and was mostly associated with foamcells.

Discussion: To our knowledge, what we report here is probably the first fatal case of atherosclerosis in dogs. The sudden death of one dog with atherosclerotic lesions and myocardial fibrosis may be caused by myocardial ischemia. In human medicine, six different types of atherosclerotic lesions are described. The lesions found in these dogs can be classified as type IV. The correlation of positive staining for AGE and the 'lipid core' may suggest a role of AGE in the macrophage transformation into foamcells.

Cancer registry of dog and cat, living in Venice and Vicenza provinces (Veneto region, North-eastern Italy)

Vascellari, Marta, Elisa Baioni, Antonio Carminato, Giuseppe Ru, Franco Mutinelli

Istituto Zooprofilattico Sperimentale delle Venezie, Italy

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Introduction: Domestic animals, particularly dogs and cats, share the environment intimately with owners. Furthermore, many forms of pet neoplasm resemble their human counterparts in biologic behaviour, pathologic expression, and recognised risk factors. Thus, pet may constitute a sentinel species for human disease. Exposure to passive smoking, magnetic fields and pesticides are some of the investigated risk factors for human and dog cancer. Often logistic issues have inhibited the development of animal cancer registries in the past. Poor census data currently exists for pets, and cancer is not a reportable disease in companion animals.

Material and methods: In April 2005, a pilot project aiming at establishing a cancer registry of dog and cat, living in Venice and Vicenza provinces (Veneto Region, north-eastern Italy), was activated. The project brings together veterinarians and epidemiologists, and the study works in cooperation with the human tumour registry already established in the area. In addition, the canine and feline populations of the provinces of Venice and Vicenza were calculated by means of three different statistical methods (mark recapture technique, phone survey, and regression model), in order to establish cancer incidence in dogs and cats.

Results: During the first three years overall, 2,509 canine and 494 feline cases of neoplasia were diagnosed. The phone survey appeared to be the most reliable method to estimate animal population. The estimate of canine and feline population turned out to be of 296,318 and 214,683 subjects, respectively, for a human population of 659,442 families. The crude annual malignant cancer incidence rate was estimated to be of 143 per 100,000 dogs and 63 per 100,000 cats. In particular the canine breed-specific incidence rate was of 89 per 100,000 mixed-breed dogs and 194 per 100,000 pure breed dogs. The same difference was estimated for the cat population: 40 malignant tumors per 100,000 mixed-breed cats and 168 per 100,000 pure-breed cats. We also noticed an increase of the age-specific incidence rate in the 10-12 years age group of cats and in the 12-14 years age group of dogs.

Discussion: Developing a population based cancer registry for animals is certainly not a new idea. The first companion animal tumour registry, sponsored by the National Cancer Institute, was created in the late 1960s. Since then, various registries have been developed for pets, but only few of them were able to provide incidence estimates as the main problem is in determining the appropriate denominators. Since centralized dog registries lack of a continuous update producing unreliable figures, we are developing a model to estimate size and structure of the canine population recruited in the study. Further activity is scheduled to extend the tumour data base and to refine the animal population estimate.

Induced hyperuricaemia results in severe nephropathy in Green Iguanas (*Iguana iguana*)

Veldhuis Kroeze, E.J.B., P.Zwart, S.J.Hernandez-Divers, D.Martinez-Jiminez, S.Bush, K.S.Latimer

Dep Pathobiology, faculty of veterinary medicine, Utrecht University, Utrecht, The Netherlands

Introduction: In a two-phase cross-over study including 19 green iguanas (*Iguana iguana*) hyperproteinaemia and hyperuricaemia was induced by feeding a high protein diet. This resulted in severe proliferative changes in the glomeruli and degenerative lesions of tubular epithelia. Less severe lesions were seen in the animals which were orally treated with allopurinol (4-hydroxypurinol) that significantly reduced blood uric acid levels.

Material and methods: 19 green 2-3 year old male iguanas (bw: 0.66-1.18 kg) were maintained on mixed green leafy vegetables supplemented with calcium carbonate and fresh water ad libitum. They were group housed in a dedicated outdoor animal room with environmental control. During phase 1, 19 iguanas were fed a vegetarian diet and medicated orally with either allopurinol (A, n=10, 1 ml syrupalta suspension of 20 mg/ml allopurinol; Qualitest Pharmaceuticals) or a placebo control (B, n=9, 1 ml syrupalta syrup; Huma Texarkana) once a day for 7 days. Their bodyweights, plasma uric acid and total protein and PCV were recorded before and after the treatments. In phase 2 the iguanas were fed a high protein diet (suppl. 10 ml Hills a/d; Hills pet nutrition) to induce hyperuricaemia and treated in the same way, however group B animals were now treated with allopurinol and group A received the placebo syrup. All animals were euthanased and postmortem examined, tissues including samples of liver and kidneys placed in 10 % neutral buffered formalin, routinely processed and examined histologically. 5 µm sections of five random animals of each group were stained with H&E, PAS, reticulin and periodic acid methanamine silver. All methods were evaluated and accepted by the University of Georgia's (USA) Institutional animal care and use committee.

Results: No postmortem macroscopic abnormalities were observed. In the hyperuricaemic placebo treated animals severe histological lesions were encountered in all kidneys. They consisted of: protein deposits in glomerular capillaries and thickening or splitting of the capillary basal membranes, proliferation of parietal and visceral epithelium of Bowman's capsules, proteinaceous deposits in Bowman's urinary space, irregular and broadened brush borders of the proximal tubules with cytoplasmic cloudy vacuolation and swelling, nuclear pycnosis and intraluminal proteinaceous material. The epithelium of distal tubules and collecting ducts showed lesions ranging from degenerative vacuolation to necrosis. In one animal proteinaceous remnants of epithelia and urate globules were observed in both proximal and distal tubules. The lesions seen in the allopurinol treated hyperuricaemic group were less severe. They included mild protein deposition in the glomerular capillaries with mild thickening of basement membranes and proximal tubular irregularity with broadening of the brush borders. Other organs, including livers, were histologically unremarkable. Mean plasma uric acid concentration (218.3 µmol/l) and total protein concentration (83 g/l) on a high protein were significantly higher ($P=0.0028$, $P=0.029$ respectively) than the vegetarian fed group (129.8 µmol/l and 76 g/l respectively). Allopurinol treatment in both normo- and hyperuricaemic iguanas significantly resulted in lower uric acid values (100.3 µmol/l) than the placebo-treated iguanas (159.3 µmol/l) ($P=0.020$). There were no detectable interactions between other recorded parameters and allopurinol treated and placebo treated animals.

Discussion: Given the fact that green iguanas often suffer from chronic nephropathies this study was performed to evaluate the effects of an induced hyperuricaemia. Hyperuricaemia and hyperproteinaemia can be reliably induced by providing green iguanas a high protein diet and can also be reliably reduced by oral allopurinol treatment. The data indicates that severe renal pathologic lesions can result from induced hyperuricaemia in green iguanas. The pathogenesis remains unclear; it maybe caused by high uric acid concentrations however the proteinaceous remnants of renal tubular epithelia and urate globules suggest urinary stasis.

Bluetongue virus serotype 8 (BTV-8) associated hydranencephaly in calves

Vercauteren, Griet, Cora Miry, Frank Vandebussche, Richard Ducatelle, Sara Van der Heyden, Elise Vandemeulebroucke, Ilse De Leeuw, Piet Deprez, Koen Chiers, Kris De Clercq

Laboratory of Pathology, Department of Pathology, Bacteriology and Poultry diseases, Ghent University, Belgium

Introduction: Bluetongue (BT) is an arthropod-borne viral disease of domestic and wild ruminants and emerged for the first time in North-Western Europe in 2006. During the 2007 BT epidemic in Belgium, a significant increase in bovine abortions and stillbirths, as well as the birth of weak or deformed calves and calves with 'dummy' syndrome and blindness was observed. So far, bovine foetal infections caused by BTV have only been reported in the United States and in South Africa in association with the use of modified live vaccines against serotypes BTV-10,-11,-13 and -17, leading to abortion, congenital deformities, cerebral abnormalities such as hydranencephaly, and the birth of viraemic calves. In this report we describe 29 cases of hydranencephaly in neonates, fetuses and calves associated with BTV-8 infection in an unvaccinated cattle population.

Material and Methods: Three aborted fetuses, four neonatal calves and twenty-two 8-67 days old mixed-breed calves, born normally but with increasing severity of nervous system disorders, were examined. Complete necropsy, histological and virological examination were performed. A BTV specific RT-qPCR was performed on 16 spleen samples, 13 EDTA blood samples and 9 cerebral samples. Serum samples of 17 calves and blood samples of two of the dams were also checked for the presence of anti-BTV specific antibodies. Virus isolation to detect the presence of live virus was performed and presence of bovine viral diarrhoea virus (BVDV) was tested by an antigen ELISA.

Results: At necropsy, 3 calves showed mild dilation of the lateral ventricles whereas the other calves, neonates and fetuses showed hydranencephaly with only meninges and remnants of cerebral parenchyma surrounding wide ventricles filled with cerebrospinal fluid.

Histological examination of the cerebral remnants showed atrophy of the neural tissue with vasculitis in several calves. There were no gross or histological lesions in cerebellum and brain stem, except in one calf, where multifocal severe vasculitis was found both in cerebellum and brain stem. In 15 animals the presence of BTV RNA was confirmed (8 out of 9 brain samples, 7 out of 16 spleen samples and 2 out of 13 blood samples). The two dams both had antibodies against BTV, while only one was BTV RT-qPCR positive. All except one calf had antibodies against BTV. One hydranencephaly-affected calf was positive in the BVD antigen ELISA. BTV could not be isolated in any of the affected calves.

Discussion: In the present study, BTV RNA was demonstrated in 15 affected calves, pointing to the involvement of BTV in the development of hydranencephaly. Other viruses such as Akabane virus and Rift Valley fever virus are unlikely to occur in Northern Europe. BVDV infection was excluded in all except one calves, although it is more likely for intra-uterine BVDV-infection to cause cerebellar hypoplasia. Since 8 out of 9 brain samples and none of the blood samples of these calves contained BTV RNA, the calves were no longer viraemic at the time of sampling and were probably infected early in gestation. In conclusion, hydranencephaly in calves can be associated with natural intra-uterine BTV-8 infections and sampling for the detection of BTV RNA should not be restricted to blood and/or spleens, but should also include cerebrum remnants.

Magnal adenocarcinoma in a budgerigar (*Melopsittacus undulates*)

Yarim, Murat, M.Yavuz Gulbahar, Ahmet Ozak, Tolga Guvenc, Ozlem Nisbet, Yonca B. Kabak, Onder Karayigit

University of Ondokuz Mayıs, Faculty of Veterinary Medicine, Department of Pathology, Kurupelit, Samsun, Turkey

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Introduction: Most adenocarcinomas of oviduct of birds originate in the magnal portion of the oviduct, with occasional cases occurring in the uterus and infundibulum. Adenoma and adenocarcinoma originated from the magnal region of the oviduct are commonly found and well documented in domestic fowls and turkeys, great tit, but not in other birds. To the best of our knowledge, there is no report of magnal adenocarcinoma in budgerigar. The present study describes a magnal adenocarcinoma of oviduct of a budgerigar.

Material and methods: A female 8-year-old budgerigar was presented with a six-month history of a slowly progressive growth at abdominal region. The bird died during the exploratory laparotomy and necropsied. The tumoral mass and tissue samples from the bird was fixed in neutral buffered 10% formalin, embedded in paraffin and stained with hematoxylin and eosin. Additional sections were stained by the streptavidin biotin peroxidase complex technique using monoclonal antibodies. Monoclonal antibodies included mouse anti-human pancytokeratin and mouse anti-human alpha smooth muscle actin.

Results: The abdominal cavity was completely filled by the tumoral mass composed of vesicular/cystic soap bubble-like polypoid masses. The metastatic foci were not seen in other organs. Microscopically, tumor masses consisted of multiple foci of cystic adenomatoid tissue contained closely packed columnar or cuboidal cells with pale nuclei, including secretory granules in the apical surface. Adenomatoid structures were surrounded by little fibrous tissue. Immunohistochemically, tumour cells were positive for pancytokeratin, Immunostaining for pan-cytokeratin were intense, whereas immunostaining for alfa smooth muscle actin was present in fusiform cells that were scattered between the tumour cells and around the blood vessels. Immunohistochemical stains confirmed the cell of origin as the epithelial cells of oviduct.

Discussion: The growth, which originated from the magnal region of the oviduct, was histopathologically confirmed as adenocarcinoma. Metastases were not seen in other organs. The magnal adenocarcinoma in budgerigar has not been reported earlier.

Key Words: Avian, Budgerigar, Magnal adenocarcinoma, Neoplasm, Oviduct

Histopathological effects of morphine on kidney in rat embryo

Zahedi, Afshin, Arash Khaki², Tahmineh Payrovi³.

1-Department of Veterinary pathology, Islamic Azad University Rasht branch-Iran,
2-Department of Veterinary pathology, Islamic Azad University Tabriz branch-Iran,
3-Department of Anatomical Science, Uromia University of Medicine Science.

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Introduction: Morphine is the prototype narcotic drug and is the standard against which all other opioids are tested. The withdrawal symptoms associated with morphine addiction are usually experienced shortly before the time of the next scheduled dose, sometimes within as early as a few hours (usually between 6–12 hours) after the last administration.

Method: 40 wistar rats (250 ±10 gr weight, 2 month age) randomly divided into two groups control (N=10) and experimental group (N=10). Experimental group received morphine (4 mg/kg, IM, daily for 30 days) and control groups received normal saline (1ml, IM, daily for 30 days) than in day of 30, after withdrawal symptoms One female rat of control and experimental groups were caged with one male rat overnight. Finding of vaginal plug on the following morning was regarded as a gestational day 0. after gestation in 21 day Newborns kidney tissues were also removed and prepared for histological evaluation in light microscope.

Results: This study was confirmed morphine had destructive effects on Newborns kidney tissues. There was a significant decrease in proximal tubules diameter in morphine group when compared with control group ($P < 0.05$).

Conclusion: These results suggested that administration of morphine in rat's parents can transmit by plasma and had harmful affect in newborns and improved kidney function damaged.

Keywords: morphine, Newborns, kidney, Rat.

Immunomarkers expression in feline mammary tumours

Zappulli, Valentina, Diego Caliari, Roberta Rasotto, Massimo Castagnaro

Faculty of Veterinary Medicine, Legnaro (Padova), Italy

Introduction: The use of companion animals as a cancer model for human oncology is leading to a better understanding of many cancerogenic aspects and to new therapeutic approaches. The high incidence, some morphological features and tumorigenic aspects and the estrogen-independency, make the feline mammary carcinoma a suitable model for the study of hormone-independent breast cancers.

Material and methods: In the present study, immunohistochemical evaluations of the expression of hormonal receptors, HER2 oncogene product, oncosuppressor protein p53, different cytoplasmic filaments (cytokeratins, vimentin, alpha-smooth muscle actin), calponin, and proliferation marker Ki-67, have been performed with an automated immunostainer on feline mammary tumours, hyperplastic/dysplastic lesions, and normal mammary glands in 80 queens. The results were statistically analysed and compared with morphological aspects of the tumours, anamnesis, and follow-up.

Results: More than 89% of the feline tumours were malignant. CK 8/18 were highly (>60%) expressed in more than 90% of the tumours, comparable to vimentin that was present in more than 60% of cells in 93% carcinomas. Multifocal areas of carcinomas were positive to CK5/6 and CK14 that showed statistical positive correlation. Luminal cells of differentiated terminal intralobular ducts were frequently intensely CK14+. Feline carcinomas were ER+, PR+ and HER2+ in less than 5% of the cases. ER significantly increased in benign and hyperplastic lesions, while HER2 expression did not show any significant differences between malignancies and benign/hyperplastic/normal tissues. Ki-67 presented a significant difference in expression between neoplastic and hyperplastic lesions, being positively correlated with grading and p53 overexpression (approx. 13% of carcinomas) and having a negative correlation with survival.

Discussion: The expression of positively correlated basal cytokeratins (cytokeratin 5/6 and 14) and the high incidence of vimentin expression in ER-/HER2- tumours suggest a pluripotent/basal cell or a terminal duct cell as progenitor of the mammary tumours in the cat. In addition, the analysis of α -SMA, calponin, and CK14 showed the presence of a myoepithelial component that was not always detected in routine histological sections, even in highly malignant tumours. HER2 expression was lower than those described in the literature, suggesting that this oncoprotein might not play a fundamental role in feline mammary tumours. Ki-67 confirmed its well-known role as an indicator of the biological behavior of a neoplastic population. Finally, evidence of a large group of ER-/PR- feline mammary tumours offer a potential model for hormone-independent human breast cancers.

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