The first detection of Cytauxzoon felis *in a wild cat (*Felis silvestris*) in Iran*

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BRIEF COMMUNICATION

The first detection of *Cytauxzoon felis* in a wild cat (*Felis silvestris*) in Iran

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Abstract A free-ranging, adult, male Arabian wild cat (*Felis silvestris*) was found in a protected zone at the province of Khorasan, Iran and transported to the Ferdowsi University of Mashhad Veterinary Teaching Hospital. The cat had normal temperature and respiratory and cardiac frequency, but was significantly dehydrated and had hindlimb lameness. The animal also was cachectic, with pale mucus membranes, third eyelid protrusion, and bilaterally enlarged submandibular lymph nodes. The cat was stabilized by intensive fluid and electrolyte therapy and hospitalized. In radiographic evaluations, comminuted and multiple fracture of the right femoral bone in midshaft with fissure fracture was seen. Hematologic analysis revealed parasitemia (0.5 %) and a mild normocytic normochromic anemia, neutrophilia, eosinopenia, lymphopenia, and parasite consistent with *Cytauxzoon felis*. In addition, biochemical changes included increased liver enzyme serum activity and increased serum concentration of cholesterol, bilirubin, glucose, protein, and fibrinogen. The results of molecular analyses confirmed the presence of *C. felis* piroplasm in the blood of the cat. The cat was treated with Tazocin and clindamycin for 4 days. This is the first detection of a *C. felis* in wild Felidae in Iran. Because most Iranian wild felids are endangered, knowing whether *Cytauxzoon* infection represents a threat for these animals is important.

Keywords *Cytauxzoon felis* · *Felis silvestris* · Iran

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Introduction

Cytauxzoonosis is a tick-borne disease of domestic and wild felids caused by *Cytauxzoon felis* (Karaca et al. 2007). Seasonal incidence of this disease correlated with tick activity (Meinkoth and Kocan 2005). *C. felis* is a genus of phylum Apicomplexa, order Piroplasmida, and family Theileridae (Brown 2010). The life cycle of *C. felis* is composed of two stages including intraerythrocytic phase known as a piroplasm and intraleukocytic phase termed a schizont (Meinkoth and Kocan 2005). Piroplasms are observed in red blood cells (RBCs) as single signet-shaped, bipolar oval form, tetrads and dots (Meinkoth and Kocan 2005; Kier et al. 1987) and commonly cause features of hemolytic anemia (Meier and Moore 2000). In the leukocyte phase, large macrophages containing *C. felis* schizonts are responsible for vessel blood occlusion in various organs such as the liver, lung, lymph node, bone marrow, and spleen (Shock et al. 2011; Snider et al. 2010).

Bobcats are natural reservoirs of *C. felis* and rarely display clinical signs (Haber et al. 2007). Rapid diagnosis is based on the microscopic identification of piroplasms within RBCs. In addition, diagnosis may be made by identifying the tissue phase in stained biopsy or aspiration specimens of affected organs. There is currently no serological test for detection of this parasite. There is a commercial molecular assay with high specificity and sensitivity for detection of *C. felis* that is recommended for use in epidemiological studies (Brown 2010). Although most infections have been described in wooded areas of south central, south eastern, and mild Atlantic state of the USA (Meier and Moore 2000; Carli et al. 2012), there are reports of clinical and subclinical *cytauxzoonosis* in other countries such as Italy, Spain, Turkey, and Brazil (Carli et al. 2012; André et al. 2009; Millan et al. 2007). Here we report the first detection of *C. felis* infection in a wild cat (*Felis silvestris*) from Iran.

Case history

A free-ranging, adult male Arabian wild cat (*F. silvestris*) was found in a protected zone in North Khorasan Province and transported to the Ferdowsi University of Mashhad Veterinary Teaching Hospital. The cat was restrained by ketamine (6–11 mg/kg, im). Upon physical examination, the cat had normal temperature and respiratory and heart rate, but was significantly dehydrated and had hindlimb lameness. The animal also was cachectic, with pale mucus membranes, third eyelid protrusion, and bilaterally enlarged submandibular lymph nodes. No tick was found on clinical examination. The cat was stabilized by intensive fluid and electrolyte therapy and hospitalized. Pelvic radiographs revealed comminuted and multiple fractures of the right femoral bone in midshaft with fissure fracture.

Microscopic examination of a Giemsa-stained blood smear revealed intraerythrocytic piroplasms, and ring form piroplasms (1–2 μm in diameter) of prominent shape and bipolar oval forms (1.5×2.5 μm) were also observed (Fig. 1). The degree of parasitemia was estimated about 5 %. *Mycoplasma haemofelis* was not observed in the peripheral blood smear and also the FeLV and FIV test that was carried out by a commercial kit (Pet Rapid Test, Quicking Biotech Co Ltd, China) was negative.

Two molecular analyses were performed to confirm the microscopic data. DNA extraction of whole blood sample was done by using a molecular biological system transfer kit (MBST Iran), based on the manufacturer's instructions. In the first step, the hypervariable region V4 of 18S rRNA was amplified as previously described by (Schnittger et al. 2004), showing the existence of a hemoparasite belonging to the Theileridae family (Fig. 2a). In the second step, the *C. felis* internal transcribed spacer region 2 (ITS2) plus 5.8S and 28S partial flanking regions was amplified as previously described by Brown et al. (2008), confirming the presence of *C. felis* piroplasm in the blood of the cat (Fig. 2b).

Hematologic and biochemical analyses were done on EDTA-whole blood and serum samples, respectively. Hematologic data that included RBC, PCV, Hb, MCV, MCH, and MCHC were measured by using an automated veterinary hematology analyzer (Nihon Kohden, Cell Tac a, MEK 6108, Tokyo, Japan). Some serum biochemical factors such as total protein, fibrinogen, albumin, glucose, cholesterol, total bilirubin, and serum activity of aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP) were measured by an automated analyzer (Biotechnica TARGA 3000, Italy). Clinical pathologic results are listed in Table 1.

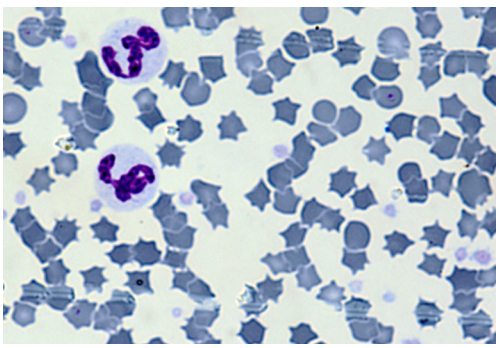

Fig. 1 Peripheral blood smear from a wild cat with *C. felis* infection. Ring-shaped piroplasms in erythrocyte (*arrow*)

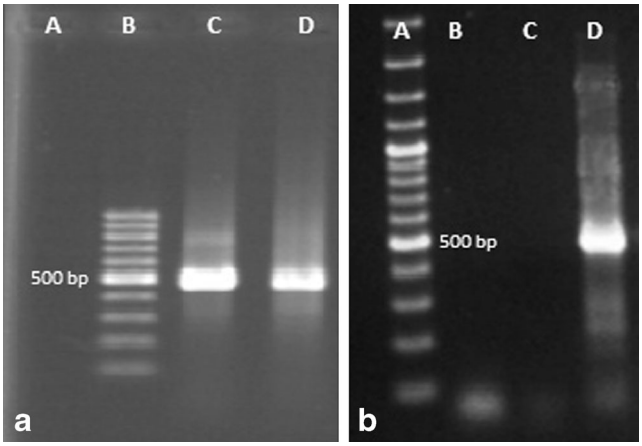

Fig. 2 Result of PCR assay from a wild cat with *C. felis* infection (**a** *lane A*: negative control, *lane B*: ladder, *lane C*: positive control (*Theileria* sp.), *lane D*: sample; **b** *lane A*: ladder, *lanes B and C* negative control, *lane D*: sample)

Hematologic findings revealed a mild normocytic normochromic anemia, neutrophilia, eosinopenia, and lymphopenia. Erythrocyte sedimentation rate was not measured, but red blood cells seemed to settle down faster than expected and rouleaux formation was observed in the blood smear. Biochemical analysis showed increased serum concentration of bilirubin, cholesterol, total protein, globulins, fibrinogen, and glucose and mild increased serum ALP, GGT, ALT, and AST activity.

The cat was treated with Tazocin EFH (piperacillin/tazobactam, iv infusion) and clindamycin (11 mg/kg per os every 12 h). The cat died after 4 days, and unfortunately, there is no information about postmortem lesions. This is the first detection of *C. felis* in a wild cat (*F. silvestris*) from Iran.

Discussion

According to Meinkoth and Kocan (2005), *cytauxzoonosis* in domestic cats is characterized by different clinical features such as depression, dehydration, icterus, reluctance to move, and fever that exceeds 106 °F (41.1 °C). Infection of wild felids is often asymptomatic (Meinkoth and Kocan 2005), but there are few reports of fatal *cytauxzoonosis* in cougar (Harvey et al. 2007), white tiger (Garner et al. 1996), and free-ranging bobcats (Nietfeld and Pollock 2002; Kocan et al. 1985).

Table 1 Hematological and Biochemical results. MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, ND: not determined (Fibrinogen: 1.4-3.3 g/l and ALP: 19-103 U/l)[a]

Hematological parameter	Patient	Reference value[b]
Hematocrit (%)	22.6	31–46
Hemoglobin (g/dl)	7.4	9.6–14.9
Red blood cell ($\times 10^6$)	5.22	7.85–11.41
MCV (fl)	43.3	34.7–47.7
M CH (pg)	14.2	10.8–14.6
MCHC (g/dl)	32.7	30–34.9
Platelet ($\times 10^3$)	446	ND
White blood cell ($\times 10^3$)	22.1	9.20–26.10
Matured neutrophils ($\times 10^3$)	21.658	3.68–14.88
Band neutrophil	0.0	0–0.52
Lymphocytes ($\times 10^3$)	0.442	1.82–7.35
Monocyte ($\times 10^3$)	0.0	0.11–0.99
Eosinophil ($\times 10^3$)	0.0	0.29–3.68
Biochemical parameters		
Total protein (g/dl)	9.5	7.0–8.8
Albumin (g/dl)	3.5	2.12–4.27
Globulins (g/dl)	6	ND
Fibrinogen (mg/dl)	1000	ND
Glucose (mg/dl)	340	101–316
Bilirubin total (mg/dl)	2.97	0–0.33
Cholesterol (mg/dl)	238	43.0–193.0
ALT (U/l)	216	22.0–94.2
AST (U/)	165.9	23–74.0
ALP (U/l)	209	ND
GGT (U/l)	5.3	0.50–5.0

[a] Macdonald et al. (1987)

[b] Marco et al. (2000)

Parasitemia level is varied between different repots (less than 0.5 to 5 %), but usually it is 1–3 % (Brown 2010). Morphologically, piroplasms were similar to those reported by Kier et al. (1987).

Unlike bobcats, acute cytauxoonosis results in hemolytic anemia in domestic cats that is nonregenerative because of bone marrow suppression by a hemoparasite or lack of time for bone marrow response (Meinkoth and Kocan 2005). It is noteworthy that investigators have recorded the occurrence of mild nonregenerative anemia in Texas cougar, white tiger, and bobcat *cytauxzoonosis* (Harvey et al. 2007; Garner et al. 1996; Kocan et al. 1985). Severe anemia was seen in a free-ranging bobcat suffering from *C. felis* (Nietfeld and Pollock 2002).

Stress leukogram, neutrophilia without left shift, lymphopenia, and eosinopenia were found in differential cell count that indicates endogenous glucocorticoid release resulting to bone fractures and possibly parasitemia. Leukopenia with toxic change was reported as a common feature in cytauxoonosis (Meinkoth and Kocan 2005). Also clinical *cytauxzoonosis* in wild felids is accompanied by pancytopenia (Harvey et al. 2007; Garner et al. 1996).

Biochemical findings showed hyperbilirubinemia, hypercholesterolemia, and mild increased serum ALP, GGT, ALT, and AST activity that are in agreement with those obtained by Harvey et al. (2007). These changes represent a mild hepatocellular damage and cholestasis. Elevated serum liver enzyme activity may be seen in febrile or comatose domestic cats suffering from *cytauxzoonosis* (Brown 2010). In the leukocytic phase, clinical signs and pathology of *C. felis* are mainly attributed to the vascular occlusion in many tissues such as the liver by schizont-laden macrophages (Meinkoth and Kocan 2005; Snider et al. 2010). In addition, anemia results in hypoxia condition and, consequently, cell injury especially in the centrilobular region of the liver (Brown et al. 2008). Hyperbilirubinemia and bilirubinuria are known as the common signs of acute *cytauxzoonosis* due to immune-mediated hemolytic anemia and also liver injury (Brown 2010). Besides hepatocytes, osteoblasts are also one of the major sources of ALP. Increased osteoblastic activity in bone disorders such as fractures causes mild increases [<4× upper reference limit (URL)] of ALP activity (Stockham and Scott 2002). So, increased ALP activity in this cat may consist of both bone and liver isoenzymes.

Serum protein findings include hyperproteinemia and hyperfibrinogenemia. Hyperproteinemia along with the normal level of serum albumin may be a sign of acute phase response (APR) because of increased serum acute phase proteins (APPs) especially serum amyloid A and haptoglobin or likely γ globulins. Although fibrinogen is not a major positive APP in dogs and cats, it is raised in inflammation condition due to femur bone fracture and acute *cytauxzoonosis*. Hyperfibrinogenemia and hyperglobulinemia result in rouleaux formation and then increased erythrocyte sedimentation rate (Duncan et al. 2003). Albumin is a negative APP, but due to its life span, hypoalbuminemia may not be detected in the first week after the onset of inflammation (Stockham and Scott 2002).

Several physiologic and pathologic conditions may change serum glucose concentration as well. Catecholamines and corticosteroids result in hyperglycemia by stimulation of glycogenolysis and gluconeogenesis, respectively, in an excited and stressed cat. In addition, ketamine used for restraint has a hyperglycemic effect for induction of epinephrine release (Duncan et al. 2003).

Conclusion

Wild cats are known as a natural reservoir of *C. felis*. After a transient leukocytic phase, bobcats undergo persistent intraerythrocytic phase, because of self-limiting *C. felis*

schizonts. There are few reports in the literature describing fatal *cytauxzoonosis* in wild felids. It seems that finding wild cats suffering from clinical *cytauxzoonosis* would be difficult, and epidemiologic studies have been limited to healthy cats that are persistently infected with *C. felis*. The cat of this study had clinical and laboratory signs of *cytauxzoonosis*. The cat probably had a persistent parasitemia that because of immune suppression due to bone fracture developed to a fatal stage. Because most Iranian wild felids are endangered, knowing whether *Cytauxzoon* infection represents a threat for these animals is important.

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