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ORIGINAL ARTICLE

Effect of transportation stress on blood and bronchoalveolar lavage fluid components in calves

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Abstract The effect of transportation stress on the content of bronchoalveolar lavage (BAL) fluid of 20 male Holstein–Friesian calves, 4–10 months old (mean weight 160 kg) was studied. The calves were healthy and had no previous history of respiratory tract diseases. During a period of 42 days experiment, the calves were kept indoors and were fed alfalfa hay and corn silage ad libitum. After a period of adaptation, on day 21, BAL fluid, blood samples, and nasal swabs were taken from all calves; then, the calves were divided into three groups: experimental (ten calves), which were transported and were deprived of food and water during transportation; control 1 (five calves), which were not transported and had free access to food and water during the 12 h of transportation of the experimental group; and control 2 (five calves), which were not transported but were deprived of food and water for the same time as the experimental group. On day 26, BAL fluid samples and nasal swabs were taken from control group 1. Blood samples were collected simultaneously from all groups at 0, 1, 3, 6, and 12 h of transportation. On days 27, 31, and 42, all previous samplings (BAL fluid, blood, and nasal swabs) were conducted on the experimental group and control group 2. Cytological, biochemical, and bacteriologic examination of BAL fluid and hematological and biochemical examination of blood samples revealed that the number of red blood cells, white blood cells, neutrophils, and the levels of cortisol, packed cell volume, total protein, and fibrinogen significantly increased, but lymphocytes significantly decreased in the experimental group compared with control groups 1 and 2 on the day of transportation ($p<0.05$). In addition, regarding BAL fluid content, total cell count, macrophages, neutrophils, and total protein increased in the experimental group ($p<0.05$). *Pasteurella multocida* was isolated from BAL fluid of three calves in the experimental group after transportation. Alteration in BAL fluid components in this study may be due to a depressed efficiency of mucociliary system and/or decreased amount of alveolar spatial surfactant either or both of which may predispose affected livestock to show the presence of *P. multocida* in bronchoalveolar fluid.

Keywords Bronchoalveolar lavage · Transportation stress · Blood · Calves

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Introduction

Excretions of the lower respiratory tract and alveolar surface can be obtained using bronchoalveolar lavage (BAL). BAL is a useful method in diagnosis of respiratory system diseases (Smith 1996) and, specifically, is a helpful method in evaluation of normal and diseased respiratory system in ruminants (Roberts et al. 1982; MacDonald 1983; Sutherland et al. 1983; Boon et al. 1987). BAL provides a valuable sample for cytology; therefore, BAL is preferable to other media in evaluation of general noninflammatory lower respiratory tract diseases. Weiss et al. (1991) assessed changes in blood and BAL fluid components in calves with experimentally induced pneumonic pasteurellosis. Allen et al. (1992a,b) evaluated the changes in the bacterial flora of

the upper and lower respiratory tracts and BAL differential cell counts in feedlot calves treated for respiratory diseases. BAL fluid was investigated biochemically, cytologically, and bacteriologically in healthy and pneumonic calves (Reinhold et al. 1992). Goncalues et al. (2004) found a nasotracheal technique for tracheobronchial lavage, an efficient method to characterize the cytological population of the lungs in clinically healthy calves. Identification and phenotyping of leukocytes in bovine BAL fluid were carried out by Soethout et al. (2004). Stress occurs when mechanisms of physiological adaptation to exogenous stimulants are activated in excess of their normal capacity. Physiologic and balancing changes in response to normal environmental changes need least adaptation. Severe exogenous influences that induce severe adaptation are deemed stress factors (Radostits et al. 1994), which can cause general adaptation syndrome when in excess in intensity and duration (Howard 1988; Griffin 1989). The effect of road transportation on the blood constituents and behavior of calves was investigated by Kent and Ewbank (1986a,b). The major part of transportation stress apparently occurs early during the transportation period, and longer-term exposure may not increase indicative parameters (Cole et al. 1988). Stressed animals can show eosinopenia, lymphopenia, and neutrophilia (Radostits et al. 1994). Furthermore, in ruminants, transportation can cause changes in metabolism of rumen, serum biochemical parameters, and serum cortisol, which are in association with the length of transportation. Weight loss and N_2 excretion increase transportation stress, but no significant changes in serum enzymes are noticed (Cole et al. 1988). Anderson et al. (1991) evaluated the effects of stressful exercise on leukocytes in cattle with experimental pneumonic pasteurellosis. Ishizaki et al. (2005) reported that a marked elevation of plasma cortisol concentration was observed 4 h after transportation of calves, but this level was unchanged in controls. There is a paucity of information about the effects of truck transportation on the function of BAL fluid cells in cattle (Ishizaki et al. 2005). The present study was therefore conducted to evaluate the effect of transportation stress on the hematological and serum biochemical parameters and the content of BAL in calves, as no information is currently available as far as we are aware.

Materials and methods

Twenty male Holstein–Friesian calves, 4–10 months old with a mean weight of 160 kg were selected for this study. Rectal temperature, respiratory rate, nasal discharge, coughing, and pulmonary sounds showed the calves to be healthy, and there were no previous histories of respiratory tract diseases. Calves were kept indoors and were fed alfalfa hay and corn silage ad libitum. The first 21 days of observation was designated as adaptation time, and during this period, all calves were vaccinated against foot-and-mouth disease and anthrax, and ivermectin was used for mass prophylactic treatment. On day 21, BAL, blood samples, and nasal swabs were taken from all calves to establish baseline control values. Blood samples were collected from the jugular vein using aseptic procedures. Sterile swabs were used to obtain samples for determination of upper respiratory system flora, and BAL fluid was taken to evaluate microbiological, cytological, and biochemical parameters of the lower respiratory system. After first sampling, at day 26, calves were randomly allocated to three groups; experimental (ten calves), which were transported for 12 h (approximately 300 km) and were deprived of food and water during that time; control 1 (five calves), who remained at their normal accommodation and had free access to food and water during the 12 h of transportation period of the experimental group; and control group 2 (five calves), who remained at their normal accommodation but were deprived of food and water during the transportation period of the experimental group. Blood samples were collected simultaneously from all groups at 0, 1, 3, 6, and 12 h of transportation. On days 27, 31, and 42, all previous parameters (BAL, blood and nasal swabs) were again evaluated in the experimental group and control group 2. A hemocytometer was used to establish the total cell counts in BAL fluid. For differential cell counts, after centrifugation of BAL fluid at $400\times g$, the sediment was resuspended in 0.5 ml normal saline and smears were prepared and stained by Giemsa stain. To evaluate the changes in microbial flora, nasal swabs and BAL fluid were cultured on selective media for special bacterial and fungal pathogens of the respiratory system, including blood agar, MacConkey agar, selective media for *Hemophilus somnus*, Sabouraud dextrose agar, and mycoplasma agar. After observation of colony growth, colony recognition was carried out by specific staining and biochemical tests. For hematological parameters, blood samples were taken into vacutainers containing ethylenediamine tetraacetic acid as an anticoagulant. Hematological parameters were measured using standard routine hematological techniques (Jain 1986). For the analysis of serum biochemical parameters, blood samples were collected into plain vacutainers, and the serum was separated after centrifugation for 15 min at $750\times g$ at room temperature. Any hemolyzed samples were discarded. Serum samples were stored at −20°C until analyzed. Biochemical analysis including serum total protein was carried out using the Biuret method, albumin using bromcresol green method, and fibrinogen using refractometry–precipitation method. BAL fluid total protein was measured using Lowry method (Burtis and Ashwood

1994). Cortisol was measured using the Gamma Coat TM [125] cortisol radioimmunoassay kit (Orion Diagnostica, Finland). The data were expressed in SI units and analyzed by one-way analysis of variance and independent *T* test using SPSS/PC software, and Duncan's multiple range tests was used to detect significant differences between means. All values were expressed as mean and standard error, and $p<0.05$ was deemed statistically significant.

Results

The mean±standard error of hematological, biochemical, and hormonal parameters of all calves and time points are presented in Tables 1 and 2, respectively.

Packed cell volume (PCV) increased significantly in the experimental group 3, 6, and 12 h after transportation compared with control group 1 ($p<0.05$) but not compared to control group 2 ($p>0.05$). The number of red blood cells (RBCs) increased significantly after 12 h of transportation in the experimental group and control group 2 in comparison with control group 1 ($p<0.05$). There was a significant increase in the number of white blood cells (WBCs) after 12 h of transportation in the experimental group in comparison with both control groups 1 and 2 ($p<0.05$). The number of neutrophils increased significantly after 1, 3, 6, and 12 h of transportation in the experimental group compared with both control groups 1 and 2 ($p<0.05$). A significant decrease in the number of lymphocytes in the experimental group was observed after 1, 3, 6, and 12 h of transportation compared with control group 1 ($p<0.05$) and after 3, 6, and 12 h of transportation in comparison with control group 2 ($p<0.05$). Total protein in the experimental group increased significantly after 6 and 12 h of transportation in comparison with control group 1 ($p<0.05$). There was no significant change in total protein between the experimental group and control group 2 ($p>0.05$). Fibrinogen showed significant change after 12 h of transportation in the experimental group compared with both control groups 1 and 2 ($p<0.05$). Significant changes in cortisol were observed after 3, 6, and 12 h of transportation in the experimental group in comparison with control group 1 ($p<0.05$) and after 1, 6, and 12 h of transportation compared with control group 2 ($p<0.05$). The results of nasal swab and BAL fluid culture are presented in Table 3. *Escherichia coli* and *Actinomyces* were isolated from samples of the upper respiratory system in control group 1. In the second sampling, there was an increase in the number of *Pasteurella haemolytica* isolated from nasal swabs. Isolation of *Pseudomonas* was simultaneous with the decrease in the number of *P. haemolytica* isolated in the third sampling. *Aspergillus*, *Alternaria*, and *Mucor* were isolated from the upper respiratory systems of all three groups. *Bacillus* (one case) and *Aspergillus* (one case) were isolated from the BAL fluid of control group 1 in the first sampling. Organisms isolated from BAL fluid of the experimental group were *P. multocida* (four cases), *Aspergillus* (two cases), *Alternaria* (one case) and *Mucor* (one case). No correlation was found between organisms isolated from BAL fluid and nasal swabs and clinical signs.

The means±standard errors of parameters in BAL fluid are presented in Table 4. There was significant increase in the number of total cells, macrophages (Figs. 1 and 2), and neutrophils of the experimental group in the second sampling of BAL ($p<0.05$). This increase was observed between the experimental group and control group 2 in the third sampling ($p<0.05$). No significant increase was found between the experimental group and control groups 1 or 2 in the number of lymphocytes and epithelial cells ($p>0.05$). The number of total cells, macrophages, and lymphocytes showed significant changes between all three groups between the first and second samplings of BAL ($p<0.05$). There were significant changes in the number of total cells, macrophages, neutrophils, and epithelial cells in the second and third samplings in the experimental group and control group 2 in comparison with the first and fourth samplings ($p<0.05$).

In the first and second samplings, no significant change was observed in the volume of BAL fluid between the three groups ($p>0.05$) and in the experimental group and control group 2 in the third and fourth samplings ($p>0.05$).

Total protein in BAL fluid showed significant change in the experimental group compared with control groups 1 and 2 in the second sampling ($p<0.05$). This change was observed in the third sampling between the experimental group and control group 2 ($p<0.05$). Changes of total protein in the experimental group were statistically significant between second and third samplings in comparison with first and fourth samplings ($p<0.05$).

Discussion

Serum cortisol concentration in the experimental group increased after 3 h of transportation and then decreased at 12 h of transportation. However, serum cortisol concentration decreased in both control groups 1 and 2. Changes in serum cortisol concentration in control group 1 correlated with the circadian rhythm of glucocorticoid secretion, as they received no stress (Friend 1980; Barnett et al. 1981; Dantzer and Mormede 1983; Becker et al. 1985; Cole et al. 1988). This rhythm was observed in control group 2 with some changes. In the experimental group, the end of stressful conditions can be the cause of decrease in serum cortisol concentration at 12 h of transportation. Furthermore, circadian rhythm of cortisol secretion is able to effect

Table 1 Hematological, biochemical, and hormonal parameters of calves in the experimental group and control groups 1 and 2 on transportation day (mean±standard error)

Cortisol (nmol/l)	Fib (g/l)	Alb (g/l)	TP (g/l)	Eos ($\times10^9$/l)	Mon ($\times10^9$/l)	Lymph ($\times10^9$/l)	Neut ($\times10^9$/l)	WBC ($\times10^9$/l)	RBC ($\times10^{12}$/l)	Hb (g/l)	PCV (l/l)	Time of sampling (hour)	Number	Group
70.91±19.31	2.90±0.30	30.80±2.30	65.60±2.20	0.05±0.03	0.04±0.02	4.93±0.27	1.50±0.26	6.52±1.23	8.38±0.52	92.80±6.10	0.30±0.01	0	5	Control 1
60.42±20.97	3.80±0.40	31.20±3.20	68.20±3.20	0.02±0.02	0.07±0.03	5.29±0.14	1.33±0.17	6.70±0.43	8.33±0.9	96.60±9.90	0.31±0.03	1	5	
33.93±14.07	2.80±0.29	30.20±3.20	67.20±2.40	0.01±0.01	0.06±0.03	5.34±0.34	1.75±0.34	7.16±1.51	8.07±0.4	95.20±6.30	0.31±0.02	3	5	
36.14±7.17	3.50±0.11	30.00±4.10	66.40±1.70	0.01±0.01	0.04±0.03	5.65±0.39	2.01±0.38	7.72±0.56	8.06±0.57	96.20±2.40	0.31±0.01	6	5	
40.83±11.03	4.50±0.28	30.10±3.90	67.20±2.90	0.06±0.03	0.09±0.03	5.96±0.27	1.70±0.26	7.82±1.06	8.05±0.37	93.20±7.50	0.31±0.02	12	5	
56.01±13.24	4.10±0.22	30.60±3.00	64.20±2.30	0.02±0.02	0.07±0.02	5.02±0.49	1.20±0.52	6.32±0.88	8.40±0.32	98.40±4.50	0.31±0.01	0	5	Control 2
36.42±3.03	4.60±0.02	32.80±2.30	67.80±2.20	0.07±0.03	0.09±0.02	5.02±0.47	1.67±0.47	6.86±1.11	8.36±0.79	101.00±4.70	0.32±0.01	1	5	
63.18±9.65	4.60±0.05	33.80±2.30	68.60±2.00	0.03±0.01	0.03±0.02	5.10±0.14	1.73±0.14	6.93±1.42	8.37±0.79	99.00±3.50	0.32±0.02	3	5	
62.35±4.13	6.60±0.18	35.00±2.30	69.00±2.60	0.02±0.02	0.04±0.02	5.67±0.51	2.02±0.47	7.77±2.15	8.70±0.12	98.00±12.90	0.32±0.03	6	5	
33.11±6.89	6.80±0.09	36.00±7.90	73.90±3.40	0.01±0.01	0.01±0.01	5.88±0.28	2.03±0.27	7.95±0.56	8.75[c]±0.45	101.20±7.20	0.32[c]±0.01	12	5	
77.80±6.89	3.20±0.18	33.70±2.00	66.30±4.20	0.04±0.02	0.07±0.02	4.43±0.18	1.62±0.17	6.16±0.47	8.34±0.64	97.00±3.90	0.31±0.01	0	10	Experiment
80.01[b]±12.69	3.35±0.28	33.80±2.70	67.00±2.10	0.05±0.02	0.03±0.02	4.29[a]±0.14	2.38[a,b]±0.14	6.77±0.40	8.40±0.89	100.80±4.30	0.32±0.01	1	10	
108.98[a]±20.41	5.00±0.05	35.00±2.70	71.00±1.50	0.03±0.01	0.06±0.02	4.26[a,b]±0.22	2.80[a,b]±0.22	7.15±0.92	8.44±0.43	96.10±2.50	0.33[a]±0.01	3	10	
96.84[a,b]±9.65	6.70±0.18	36.00±2.70	72.00[a]±1.90	0.03±0.01	0.10±0.02	4.74[a,b]±0.37	3.67[a,b]±0.39	8.47±0.76	8.80±0.87	102.90±5.40	0.33[a]±0.01	6	10	
67.59[a,b]±6.34	8.00[a,b]±0.03	37.00±2.40	79.00[a]±4.60	0.02±0.01	0.03[a]±0.02	4.22[a,b]±0.39	4.94[a,b]±0.39	9.30[a,b]±1.06	8.90[a]±0.35	108.40±3.40	0.34[a]±0.01	12	10	

[a] Indicates statistical significance ($p<0.05$) between experimental group and control group 1
[b] Indicates statistical significance ($p<0.05$) between experimental group and control group 2
[c] Indicates statistical significance ($p<0.05$) between control groups 1 and 2

Table 2 Hematological, biochemical, and hormonal parameters of calves in experimental group and control groups 1 and 2 (mean±standard error)

Cortisol (nmol/l)	Fib (g/l)	Alb (g/l)	TP (g/l)	Eos ($\times10^9$/l)	Mon ($\times10^9$/l)	Lymph ($\times10^9$/l)	Neut ($\times10^9$/l)	WBC ($\times10^9$/l)	RBC ($\times10^{12}$/l)	Hb (g/l)	PCV (l/l)	Time (days after transportation)	Number	Group
71.46±9.38	1.68±0.51	27.20±2.50	66.30±4.50	0.04±0.02	0.23±0.04	4.59±0.21	1.48±0.19	6.34±1.02	8.54±0.75	92.80±4.50	0.31±0.01	−5	5	Control 1
70.74±9.87	1.71±0.58	28.16±2.73	65.76±4.72	0.09±0.03	0.24±0.05	4.52±0.19	1.43±0.23	6.28±0.97	8.43±0.81	93.62±3.94	0.30±0.01	0		
56.01±19.59	2.30±0.47	36.40±2.20	61.20±5.80	0.02±0.01	0.17±0.08	4.60±0.24	1.34±0.16	6.14±0.76	8.39±0.76	98.80±1.60	0.32±0.01	−5	5	Control 2
99.32±8.82	6.80±0.50	32.60±2.90	64.20±5.00	0.06±0.02	0.12±0.05	4.38±0.56	1.71±0.52	6.28±2.40	8.50±0.83	100.50±5.70	0.31±0.01	1	5	
72.84±13.24	7.20±0.13	31.60±2.30	69.60±2.50	0.00±0.00	0.04±0.02	5.05±0.45	1.66±1.14	6.76±0.58	8.82±0.45	101.80±6.00	0.30±0.01	5	5	
57.66±6.34	4.00±0.10	37.00±2.40	67.60±2.00	0.16±0.01	0.11±0.03	5.38±0.29	1.04±0.30	6.70±0.32	8.23±0.98	97.60±3.10	0.30±0.01	17	5	
75.04±8.27	1.82±0.27	36.00±2.60	62.20±1.40	0.02±0.01	0.18±0.05	4.46±0.33	2.02±0.29	6.70±0.32	8.25±0.65	101.20±3.00	0.32±0.01	−5	10	Experiment
73.39±12.41	11.00[a]±0.04	33.10±1.20	70.10±3.40	0.02±0.02	0.05±0.02	4.14±0.16	1.96±0.18	6.18±0.63	8.52±0.71	98.40±7.50	0.32±0.01	1	10	
89.94±8.55	9.20±0.05	38.70±2.40	68.90±1.20	0.00±0.00	0.22±0.09	5.02±0.65	1.63±0.66	6.88±1.33	8.82±0.71	96.60±3.70	0.31±0.01	5	10	
72.28±9.93	4.10±0.04	37.60±1.50	64.00±2.20	0.08±0.05	0.11±0.03	5.42±0.01	1.25 ±0.08	6.82±1.40	8.49±0.76	91.10±3.50	0.30±0.01	17	10	

[a] Indicates statistical significance ($p<0.05$)

Table 3 Organisms isolated from nasal swab and bronchoalveolar fluid in calves before and after transportation

Groups	Control 1				Control 2								Experiment							
Time of sampling (days after transportation)	−5		0		−5		1		5		17		−5		1		5		17	
Organis	NS	BA	NS	BA	NS	BA	NS	BA	NS	BA	NS	BA	NS	BA	NS	BA	NS	BA	NS	BA
Actinomycess spp.	1	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0
Bacillus spp.	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
E. coli	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0
Pasteurella haemolytica	0	0	1	0	1	0	2	0	1	0	1	0	2	0	5	0	3	0	3	0
Pasteurella multocida	3	0	4	0	3	0	4	0	5	3	2	0	2	0	4	2	8	2	6	0
Pseudomonas aeruginosa	0	0	1	0	2	0	1	0	1	0	1	0	1	0	1	0	4	0	2	0
Streptococcus spp.	1	0	2	0	1	0	2	0	3	0	1	0	1	0	2	0	3	0	1	0
Aspergillus spp.	2	1	1	0	1	0	1	0	1	1	1	0	2	0	2	1	2	1	1	0
Alternaria spp.	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0
Mucor spp.	0	0	1	0	1	0	0	0	1	1	0	0	0	0	1	0	1	1	0	0

NS Nasal swab *BA* bronchoalveolar fluid

Table 4 Parameters in BAL fluid of calves in experimental group and control groups 1 and 2

Total protein (mg/l)	Epithelial		Neutrophil		Lymphocyte		Macrophage		Number of cells (/µl)	Volume of fluid	Time (days after transportation)	Group
	(/µl)	(%)	(/µl)	(%)	(/µl)	(%)	(/µl)	(%)				
36.60±5.40	3.71±1.26	0.50±0.17	10.62±5.27	1.43±0.71	56.83±19.16	7.65±2.58	662.75±27.71	89.20±3.73	743.00±23.97	46.20±3.10	−5	Control 1 (n=5)
36.60±5.40	3.11±1.94	0.40±0.25	12.43±3.96	1.60±0.51	51.28[b]±16.78	6.60±2.16	710.18[b]±20.04	91.40±2.58	777.00[b]±33.97	47.60±3.60	0	
30.60±5.80	4.60[d]±1.91	0.60±0.25	7.20[d]±2.11	1.00±0.33	73.60±30.88	9.60±3.70	645[d]±27.45	88.80±4.04	710.40[d]±48.99	48.50±4.33	−5	Control 2 (n=5)
51.40±9.10	25.00[e]±7.04	3.40±0.75	15.40[e]±3.64	2.20±0.58	53.60[b]±20.22	7.40±2.73	631.8[b,e]±40.66	87.00±3.74	715.80[b,e]±44.01	50.54±3.83	1	
36.80±4.70	31.80[e]±4.85	4.40±0.51	9.80[e]±1.74	1.40±0.15	51.40±17.29	7.20±2.38	621.8[e]±31.2	87.00±2.53	714.60[e]±32.14	49.36±1.46	5	
35.20±4.80	17.80[d]±2.54	2.40±0.25	10.00[d]±3.49	1.20±0.49	48.40±12.5	6.80±1.99	662[d]±49.01	89.60±1.69	737.00[d]±47.26	50.02±1.43	17	
28.10[g]±2.80	3.90[d]±1.33	0.50±0.17	8.90[d]±4.55	1.10±0.5	62.70±10.48	8.50±1.92	649.5[d]±30.29	89.90±2.18	726.10[d]±37.47	47.64±2.30	−5	Experiment (n=10)
174.00[a,c,f]±23.10	50.40[e]±10.4	3.60±0.45	107.40*[e]±24.09	19.30±1.99	83.90[b]±25.03	5.50±0.89	980.54[b*,e]±120.18	71.60±2.19	1379.40[b,*,e]±183.06	47.30±1.61	1	
150.60[a,f]±22.90	61.10[e]±12.52	4.40±0.4	102.10[a,e]±23.63	13.10±2.33	74.40±8.67	5.90±0.80	1035.7[a,e]±141.34	76.60±8.13	1337.30[a,e]±177.81	48.40±1.25	5	
81.90[g]±19.00	52.10[d]±29.94	2.40±0.58	22.50[d]±6.12	5.00±2.11	58.00±0.77	6.60±0.73	762.4[d]±46.72	86.00±2.51	881.40[d]±45.41	49.26±1.04	17	

*Indicates statistical increase

[a] Indicates statistical significance between experimental group and control group 2

[b] Indicates statistical changes between the first and second samplings

[c] Indicates statistical significance between experimental group and control group 1

[d,e] There were significant changes in the number of total cells, macrophages, neutrophils, and epithelial cells in second and third samplings in experimental group and control group 2 in comparison with first and fourth one ($p<0.05$).

[f,g] Changes of total protein in experimental group were statistically significant between second and third sampling in comparison with first and fourth one ($p<0.05$).

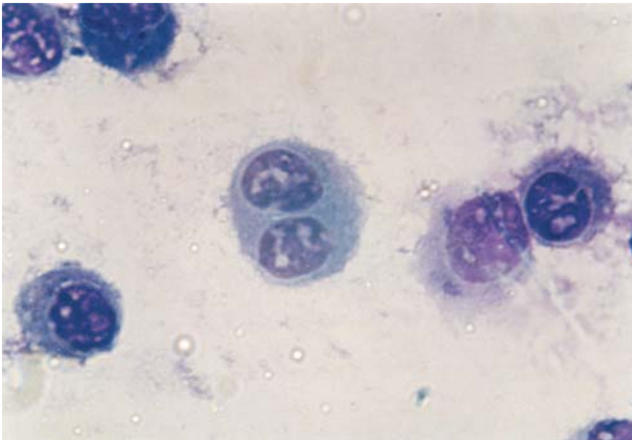

Fig. 1 Alveolar macrophages and one binucleated alveolar macrophage in bronchoalveolar fluid of the calf (Giemsa stain ×900)

cortisol concentration decrease (Crookshank et al. 1979; McCaughan and Malecki 1981; Morrison 1983; Rushen 1986; Howard 1988; Barnett et al. 1989; Agnes et al. 1990). Ishizaki et al. (2005) reported that a marked elevation of plasma cortisol concentration was observed 4 h after transportation of calves but this level was unchanged in controls. Hematocrit, the number of RBCs, and serum total protein increased in the experimental group on the transportation day, which was almost certainly due to dehydration (Friend et al. 1998). These parameters showed no significant changes in control group 1 but increased in control group 2. Other parameters showed no significant change in control group 2 compared with control group 1 except PCV at 12 h of transportation. Calves of the experimental group were more dehydrated in comparison with ones in control group 2 due to sympathetic stimulation and catecholamine secretion as a result of transportation, which caused increase in digestive system motility, defecation, looseness in feces consistency, and urination (Cole et al. 1988; Lay et al. 1992). The number of WBCs and percentage of neutrophils increased significantly in the experimental group on transportation day. In contrast, there was a significant decrease in the percentages of lymphocytes in the experimental group on transportation day. The significant increase in WBCs could be due to decreased emigration of neutrophils from the circulation into the tissues and an increased blood transient time, increased bone marrow release of neutrophils, decreased stickiness of neutrophils, and a shift of cells from the marginal neutrophil pool to the circulating neutrophil pool. Decrease in lymphocytes could be due to redistribution of recirculating lymphocytes; they remain transiently sequestered in the lymphoid tissues or bone marrow rather than entering efferent lymph and blood (Cole et al. 1988; Murata 1989, 1997; Buechner-Maxwell 1993; Katoh and Ito 1995; Latimer et al. 2003). There was an increase in fibrinogen in the experimental group after transportation, which indicates inflammatory reactions in this group, as fibrinogen is an acute phase protein and a valuable factor in evaluation of inflammatory response (Latimer et al. 2003).

Seven kinds of bacteria and three kinds of saprophyte fungi were the isolated organisms from the upper and lower respiratory systems from which *P. haemolytica* and *P. multocida* are known as bovine respiratory system pathogens. Experimental fibrinous bronchopneumonia can be induced by *P. multocida*, which was the only isolated organism from some lungs with pneumonia. Although Allen et al. (1990) reported that the number of isolated *P. multocida* was statistically more in diseased animals than controls, the role of this organism in the pathogenesis of complicated bovine respiratory disease is not clear. In this study, the number of isolated *P. haemolytica* in control groups before transportation was very few. The dominant organism isolated from the upper respiratory system of all three groups was *P. multocida*. There was no significant change in the number of isolated organisms in control groups after the transportation period. In contrast, an increase was observed in the number of isolated *P. haemolytica* and *P. multocida* in the experimental group after transportation. No change was observed in other bacteria except *Pseudomonas* that increased in the third sampling of the experimental group. *Alternaria*, *Aspergillus*, and *Mucor* were isolated from BAL fluid after transportation. *P. haemolytica* and *P. multocida* are considered as normal flora of bovine upper respiratory system and have a commensal role in the nasopharynx of ruminants. *P. multocida* can be isolated easily from nasal swabs of healthy cattle. In contrast, *P. haemolytica* is isolated rarely and sporadically from nasal swabs in spite of its apparent commensal existence. The number of isolated *P. haemolytica* increases when calves are transported stressfully. It is thought that *P. haemolytica* is carried to the pharynx and proliferates rapidly; then, a largely increased number of this bacteria is transported toward nasal cavities and lungs in

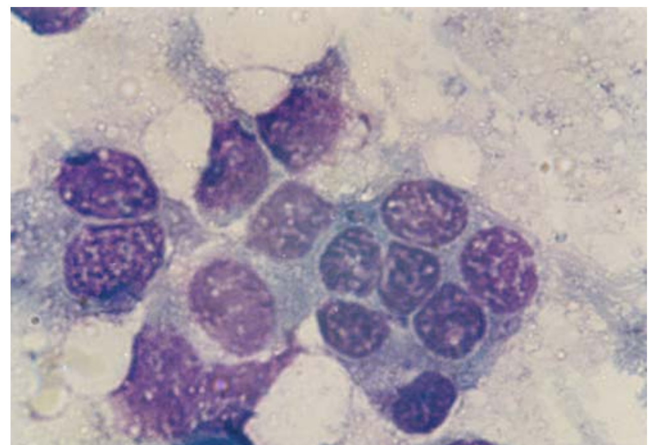

Fig. 2 Alveolar macrophages and one multinucleated giant alveolar macrophage in bronchoalveolar fluid of the calf (Giemsa stain ×900)

aerosol form; therefore, the number of isolations from nasal swabs will show an elevation. Isolation of *P. multocida* from BAL fluid indicates its colonization in the lower respiratory system, as the clearance mechanisms of the respiratory system such as mucocilliary system and phagocyte activity of alveolar macrophages decrease in stressful transportation (Wilkie and Markham 1979; Webster 1981, 1983; Heilmann and Muller 1987; Anderson et al. 1991; Wentink et al. 1994; Olchowy et al. 1997). The increase in the number of isolated *Pseudomonas aeroginosa* may be due to its opportunistic nature. After the changes in nasal cavity microbial flora as a result of stressful transportation and tissue damage, *Pseudomonas aeroginosa* proliferates and becomes dominant among nasal cavity flora; therefore, an increase in the number of isolations was observed. Spores of *Bacillus* spp. and saprophyte fungus are often isolated from respiratory system samples, as they usually exist endogenously in farms (Pringle et al. 1988; Allen et al. 1990). Their isolation from BAL fluid after transportation is due to weakened clearance activity of the respiratory system. Experimental aspergillosis is reported with administration of fungal spores or spores and fungal mycelium together (Pawaiya et al. 1995). Isolation of *Bacillus* (one case) and *Aspergillus* (one case) could be as a result of entrance of their spores to the lower respiratory system or contamination of samples taken from the upper respiratory system. The second theory is more reliable, as the cytological parameters in this calf were normal (Allen et al. 1990, 1992a,b). Allen et al. (1991) assessed the microbial flora of the respiratory tract in feedlot calves to find the association between nasopharyngeal and BAL cultures. The most prevalent organisms were *P. multocida* and *Mycoplasma bovis*. Allen et al. (1992a,b) evaluated the changes in the bacterial flora of the upper and lower respiratory tracts and BAL differential cell counts in feedlot calves treated for respiratory diseases. A variety of organisms, including *P. multocida*, *P. haemolytica*, *Haemophilus somnus*, *M. bovis*, and *M. bovirhinis*, were present in the upper and lower airways of both clinically normal and those received treatment for respiratory disease groups during the first month after arrival in the feedlot.

The types of cells in the first sampling of BAL fluid were in agreement with the findings of others (Walker et al. 1980; Wilkie and Markham 1981; Allen et al. 1992a,b) where macrophages were the dominant cells. The number of cells, neutrophils, and macrophages increased significantly in the experimental group after transportation, which could be as a result of transportation stress on cytological population of BAL fluid. Transportation had no significant effect on the cytological composition of BAL fluid in horse (Hobo et al. 1997). The difference between cytology of the first and second samplings of BAL fluid may be due to tissue stimulation and host response in the first sampling. Inflammatory response due to transportation and sampling stress could be the cause of differences in the number of cells, macrophages, neutrophils, and epithelial cells between the experimental group and control groups (Weiss et al. 1991; Reinhold et al. 1992; Otto et al. 1995; Hobo et al. 1997; Rashid et al. 1997; Sato et al. 1998). Castleman et al. (1985) carried out a study on light microscopic lesions, microbiology, and lavaged lung cells in experimental bovine respiratory syncytial virus infection in conventional calves. Alterations were not detected in the numbers of cells recovered by BAL after inoculation with the virus. Pringle et al. (1988) evaluated cellular, microbiological, serological, and histological variables in BAL of cranial and caudal lung regions in selected normal calves. Microorganisms were present in small numbers in the lower respiratory tract of some normal calves. There were no differences in the above parameters between the cranial and caudal lobes. There were statistically significant changes in BAL cell proportions with age. Four-week-old calves had a lower percentage of macrophages and a higher percentage of epithelial cells than 2-week-old animals ($p<0.05$). There was also a trend toward an increased percentage of neutrophils in older calves, but this did not reach significance ($p>0.05$). Richards and Renshaw (1989) reported research on functional and metabolic activity of bovine pulmonary lavage cells phagocytically stimulated with pathogenic isolates of *P. haemolytica*. Anderson et al. (1991) evaluated the effects of stressful exercise on leukocytes in cattle with experimental pneumonic pasteurellosis. Neutrophils in BAL differed from control values 24 h after treadmill exercise and 1 and 4 h after *P. haemolytica* A1 infection. Decreased neutrophils in BAL fluid at 1 and 4 h after infection indicated that exercise transiently inhibited neutrophil egress from the blood to the alveoli; BAL neutrophils peaked at 1 and 4 h after infection but declined after 24 h. Allen et al. (1992a,b) evaluated pulmonary cytology in 59 feedlot calves with clinical signs of respiratory disease and 60 clinically normal cases. Many calves in both cases and control groups showed inflammatory changes in the lower respiratory tract as determined by changes in proportions in BAL differential cell count. A positive association was found between an increased proportion of neutrophils in BAL fluid and isolations of *P. multocida* and *M. bovis* from BAL fluid. Goncalues et al. (2004) found a nasotracheal technique for tracheobronchial lavage, an efficient method to characterize the cytological population of the lungs in clinically healthy calves. The mean number of total cells present in the samples was 133,750 cell/μl. Differential counting showed 77.2% macrophages, 14.9% cylindrical epithelial cells, 6.0% neutrophils, and 1.8% lymphocytes. Cylindrical epithelial cells were present in 79.0% of the samples and non-ciliated cells in 21.0% of the samples. The mean number of

macrophages per smear was 78.5 binucleated cells, 20.5 trinucleated cells, and 28.5 giant cells. Total protein in BAL fluid increased in the experimental group after transportation in contrast with control groups; therefore, this increase can be as a result of transportation (Weiss et al. 1991; Reinhold et al. 1992; Pawaiya et al. 1995; Hobo et al. 1997; Olchowy et al. 1997; Rashid et al. 1997). BAL fluid was investigated biochemically, cytologically, and bacteriologically. An increase in both protein content and number of cells and a marked influx of neutrophils were found to be the most important alterations caused by pneumonic lesions. The diagnostic potential of lactate dehydrogenase, alkaline phosphatase, and the size of alveolar macrophages in BAL fluid were validated (Reinhold et al. 1992). Weiss et al. (1991) assessed changes in blood and BAL fluid components in calves with experimentally induced pneumonic pasteurellosis. By postinoculation, significant increases were detected in total cell count, total protein and albumin concentrations, and alkaline phosphatase and lactic dehydrogenase activities in BAL fluid.

Differential evaluation of hormonal, hematological, and biochemical parameters in calves of three groups revealed that transportation stress causes significant changes in these parameters in transported calves. The first stage of transportation is the most stressful part, and prolonged transportation causes more severe changes in hormonal, hematological, and biochemical parameters. The changes in BAL fluid indicate an inflammatory process in the lower respiratory system after transportation (Olson and Brown 1986; O'sullivan et al. 1988). Disturbance in two defensive mechanisms of the respiratory system could be the cause of inflammatory process:

1. Mucocilliary system: Decrease in affectivity of mucocilliary system results in proliferation and invasion of bacteria located in the upper respiratory system to the lungs and inflammation occurs in the lower respiratory system as a defensive mechanism. Considering the microbiological findings, inflammation occurrence can be as a result of infection with *P. multocida*. Serial isolation and recognition of this organism in BAL fluid and cytological and biochemical findings strengthen this theory (Lopez et al. 1986; Heilmann and Muller 1987; Allen et al. 1990; Anderson et al. 1991; Weiss et al. 1991; Reinhold et al. 1992; Klucinski et al. 1994; Katoh and Ito 1995; Barbour et al. 1997; Belayat et al. 1998).
2. Disturbance in protective function of surfactant in alveolar region: Transportation may cause decreases in surfactant excretion from alveolar regions. Increase in permeability and exit of plasma exudates from capillaries of blood air barrier of alveolar walls may be the results of decrease in surfactant. Exudative process, accumulation of proteinous substances, and cellular remains can cause damage in alveolar construction and parenchymal gas exchange surfaces areas. Weakened defensive mechanisms of alveolar regions may result in inflammatory process and infection. The increase in total protein found in the present study, which was in agreement with the findings of others (Kostro and Ledwozyw 1991; Haddad et al. 1993; Hobo et al. 1997), strengthens this theory (Olson and Brown 1985, 1986; Engen and Brown 1991; Kostro and Ledwozyw 1991; Kuroki et al. 1992; Haddad et al. 1993; Mcintosh et al. 1996a,b; Hobo et al. 1997).

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