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Evaluation of hemogram parameters in neonatal diarrhoeic calves with and without gastrointestinal protozoa infections

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ABSTRACT

Objective: The aim of this study was to compare the hemogram analysis results of neonatal diarrheal calves with and without gastrointestinal protozoa infection.

Materials and Methods: A total of 21 neonatal calves with diarrhoea were examined within the scope of the study. Eleven of the cases were calves with gastrointestinal protozoa infection and 10 were calves without gastrointestinal protozoa infection. Demographic, clinical, and laboratory data of calves were evaluated.

Results: When demographic data and vital signs were evaluated between the two groups, no statistically significant difference was found between the two groups ($p>0.05$). However, when the hemogram values between the two groups were compared, it was determined that there was a significant difference in white blood cell ($p=0.003$) and neutrophil ($p=0.01$) numbers.

Conclusions: Evaluating hemogram parameters should be taken into account as it is an inexpensive and easy-to-apply analysis and offers important outputs in the control and follow-up of neonatal calf health especially in neonatal calf diarrhoea cases which is one of the common diseases.

Keywords: *Neonatal, Calf diarrhoea, Hemogram, Protozoa*

INTRODUCTION

Calf mortality is one of the major problems of animal husbandry worldwide. Factors such as low calf performance, genetic losses due to reduced stocks in the herd during the breeding process and therefore reduced selection probability, and the budget spent on the diagnosis and treatment of the disease negatively affects the breeder and the country's economy (Smith, 2012; Yimer et al., 2015).

Veterinarians are often requested to investigate animal death or illness, including those related to neonatal calf diarrhoea, and to prevent calf death as soon as possible. The most common agents causing neonatal calf diarrhoea have been reported as bacterial (*Escherichia coli, Salmonella* spp., etc.), viral (Rota virus, Corona virus, etc.), and protozoan (*Cryptosporidium* spp. and, *Eimeria spp*.) infections (Altug et al., 2013; Cho and Yoon, 2014). Studies conducted in different countries have shown that neonatal calf diarrhoea due to protozoan agents is very common. Studies conducted in our country show that *Cryptosporidium* spp. and, *Eimeria* spp. are found to be positive in newborn calves up to 63% and 90%, respectively (Çitil et al., 2004).

The etiology of neonatal calf diarrhoea is quite complex and multifactorial and also environmental factors and practice differences in farm management play an important role, as well as the vulnerability of calves to infectious agents due to their predisposition. Therefore, whether preventive medicine practices are applied in the herd, feeding of pregnant and post-partum animals and related

diseases and the seasonal variations are effective in calf mortality rates (Singh et al., 2009).

Many enteric pathogens can play a role in calf diarrhoea, having fecal-oral route for transmission (Cho and Yoon, 2014). This increases the possibility of the rapid spread of pathogens, especially in farms where hygiene rules are not followed. For this reason, it is important to isolate the sick animal in cases where signs such as watery diarrhoea and/or blood in the stool, dehydration, anorexia, and lethargy are detected (Cho and Yoon, 2014; Elitok, 2020). In these acute neonatal diarrhoea cases, information obtained from the clinical examination findings of the patient as well as from easy-to-apply and low-cost analysis methods such as evaluation of hemogram parameters gains even more importance (Smith, 2012; Song et al., 2020).

This study aims to compare the results of hemogram analysis in diarrheal calves with and without gastrointestinal protozoa infections, which are the most determined etiologic agents in neonatal calf diarrhoea, and to contribute to the evaluation of the patient according to these parameters in field applications.

MATERIALS and METHODS

Animal material

Cases of neonatal calf diarrhoea brought to Ankara University Faculty of Veterinary Medicine Animal Teaching Hospital between February 2018 and June 2022 were determined retrospectively from the hospital software database. A written owner consent form was obtained from all animal owners. This study was reviewed by the Local Animal Ethics Committee of Ankara University it was decided that the study was not subject to Ethics Committee approval (Decision number: 2022-18-163)

The keywords 'calf' for animal species and 'diarrhoea' for diagnosis were used to search for cases. Records of cases were reviewed, and eligible calves meeting the inclusion criteria for neonatal calf diarrhoea were selected. Neonatal calf diarrhoea was defined as diarrhoea in calves aged 1-month-old or younger that was triggered by protozoans or other causes. Only the animals have the clinical examinations details as well as the results of both the hemogram and faecal examination findings were included in the study.

The animal material was consisted of 21 calves. Among the cases 11 calves were included in the first group infected with gastrointestinal protozoa infections while 10 calves were in the second group without gastrointestinal protozoa infections.

The breeds, ages and genders of the calves were noted for the analysis in both groups. The dates when patients were first brought to our clinic were also analysed to understand whether there was a distribution difference in the seasons in which the disease occurred between the groups.

Clinical examination and laboratory analysis

Clinical examination records including temperature, pulse and respiratory rate (TPR), mucous membrane color and dehydration status were noted. The hydration status was classified as normal <5%, mild 6-8%, moderate 8-10%, and severe >10 (Smith, 2009).

The fecal examination was performed within half an hour after fresh sample collection. The protozoan infections were diagnosed by fecal flotation and Ziehl–Neelsen staining under a light microscope (Cho and Yoon, 2014).

The hemogram (complete blood cell count) analysis measured in the Central Diagnostic Laboratory (by Exigo H400 Veterinary Hematology Analyzer, Boule Medical AB, Sweden) of Ankara University Animal Hospital including white blood cell (WBC), lymphocyte (LYM), monocyte (MONO), lymphocyte % (LYM%), monocyte % (MONO%), neutrophil % (NEUT%), red blood cell (RBC), hemoglobin (HGB), packed-cell volume (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MHC), red cell distribution width (RDW) and platelet (PLT) were also studied within the scope of this paper.

Statistical analysis

To analyze any differences in the parameters between the 1st and 2nd groups, we applied the student's t-Test for parametric values while Mann-Whitney U-Test was performed for non-parametric values. The gender differences between the groups were analyzed with Chi-Square test. The analyses were performed in SPSS 22.0 for Windows, SPSS Inc, Chicago, IL, USA. Significance was defined as $p<0.05$.

RESULTS

There were 9 Simental (81.8%) and 2 Holstein (18.2%) calves in the first group while the second group consisted of 7 (70%) Simental and 3 Holstein (30%) calves. While male calves were in the majority in the first group (54.54%), the number of female calves was higher (80%) in the second group. The

mean age at the presentation was 10.9 and 10.12 days in the first and second groups, respectively. When the demographic data of the calves were examined, no statistical difference was found between the two groups in terms of breed, age, and gender (p>0.05).

The dates of the first admission of the patients to our clinic were examined and the seasonal distribution was evaluated. While 7 out of 11 calves in the first group (63.63%) were first presented in the spring months (March, April, or May), the remaining 4 were (36.36%) presented during winter (December, January, or February). In the second group, while the majority of calves were presented in the winter season (n=6, 60%), 3 were presented in the spring and 1 was in the summer (August).

When the temperature, pulse, and respiratory rate data were examined to evaluate clinical examination findings, no statistically significant difference was found between the two groups (p>0.05). However, it was observed that the body temperature of the neonatal calves with protozoan infection (37.83°C±0.89) was slightly lower than the calves in the 2nd group (38.58°C±1.01). Moreover, the pulse and respiratory rates were also found to be higher in the second group compared to those in the first group. The pulse rate was 95±11.36 per/min in the first and 100.5±9.06 per/min in the second group while the respiratory rate was 40.1±6.42/min and 41.6±5.31/min in the first and second groups, respectively.

When the hydration status of the calves in both groups was evaluated, the average dehydration level of 8-10% was determined in both groups (p>0.05). Although the mucous membranes were noted as "pale" in 73.7% of the calves in the first group, this rate was found to be %60 in the second group.

Fecal examinations revealed 7 calves diagnosed with *Cryptosporidium* spp. (63.6%) while 4 with *Eimeria* spp. (36.3%) in the first group. We did not determine any gastrointestinal parasites from the samples of the second group.

The hemogram data of the calves in both groups were compared. While WBC (p=0.003) and NEUT (p=0.016) counts significantly differed between the two groups, we did not find any significant differences in the other parameters. The mean±std value of each parameter in the groups has shown in Table 1.

Table 1. The findings of hematological parameters in the first and second groups.

Parameters	First group (Mean±std)	Second group (Mean±std)	p value
White Blood Cell (×10⁹/l)	10.60±5.17	17.63±5.45	0.003
Lymphocyte (×10⁹/l)	6.45±3.61	5.21±3.71	0.30
Monocyte (×10⁹/l)	1±0.6	1.63±1.1	0.07
Neutrophil (×10⁹/l)	4.56±3.65	9.54±5.23	0.016
Lymphocyte %	48.59±21.48	36.81±21.89	0.36
Monocyte %	8.96±3.83	8.09±4.50	0.89
Neutrophil %	41.58±24.13	49.16±23.46	0.88
Red Blood Cell (x10¹²/l)	6.81±2.16	7.81±1.34	0.34
Hemoglobin (g/dl)	9.45±1.92	10.63±1.74	0.17
Packed-cell volume (%)	26.45±6.45	26.90±6.22	0.26
Mean Corpuscular Volume (fl)	39.18±3.51	38±2.57	0.48
Mean Corpuscular Hemoglobin (pg)	14.09±1.67	13.90±0.83	0.83
Red Cell Distribution Width(fl)	21.36±2.26	20.54±2.74	0.58
Platelet (10⁹/l)	402.27±213.31	527.45±226.40	0.16

DISCUSSION

Calf diarrhoea is a disease that highly affects the livestock economy worldwide. Although the overall liveborn calf mortality due to gastrointestinal system diseases including neonatal calf diarrhoea should be around 1% on a farm, previous studies conducted in different European countries reported the mortality rates varying between 1.5% to 17 while it is around 15% in Turkey (Norberg, 2008; Naylor, 2009; Raboisson et al., 2013; Elitok and Elitok, 2016; Motus et al., 2017). In the United States, these rates are between 6 to 8%, and the annual economic damage resulting from the

loss of calves is calculated to be about $125 million (Meyer et al., 2001; Jorgensen et al., 2017). The survival rate of calves due to the presence of protozoan-induced diarrhoea is reported to be significantly different compared to those without diarrhoea (Glombowski et al., 2017).

Due to the multifactorial and multi-etiological nature of the disease, the change in the severity of the disease also affects the chance of success in field practice (Cho and Yoon, 2014). In the presented study, demographic factors that may be effective in the development of the disease were evaluated among the calf groups with and without diarrhoea associated with gastrointestinal protozoa. According to our analysis results, no significant difference was determined between the groups included in the study in terms of breed, presentation age, and gender parallel to the findings of Lanz Uhde et al. (2008). Although the number of females in the second group was higher in percentage compared to the first group in our study, there was no statistical difference between the two groups, which may be due to the low number of animals in the groups, which is a limitation of our study.

In the present study, seasonal distribution was found to be associated with diarrheal pathogens. This finding is consistent with previous studies conducted in our country as well as in the world (Brenner et al., 1993; Raboisson et al., 2013; Chao et al., 2019; Berber et al., 2021). In our study, it was determined that neonatal calf diarrhoea cases intensified in both groups in the winter and spring months. While the majority of the cases are seen in the spring months of protozoan-induced diarrhoea, it is noteworthy that the first incidence of the disease in the calves in the second group increases in the winter months. This increase in cases in winter and spring may be due to the fact that cold weather is more optimal for the infectious effects of pathogens, as well as the fact that the herds are mostly kept indoors during these months and thus the spread of infections becomes more difficult to control (Berber et al., 2021).

Calf mortality during the first month of life is one of the biggest problems of farms. The morbidity and mortality rates in neonatal calf diarrhoea and sepsis lead to failure of colostral transfer and absorption of antibodies. Bacterial, viral, and parasitic infections can cause neonatal diarrhoea and sepsis, and in this case, morbidity and mortality rates increase considerably due to the failure of colostral transfer and absorption of antibodies (Naylor, 2009; Abuelo, 2017). Abnormal changes in temperature, heart and respiratory rate, and increased total leucocyte count are considered to be compatible with systemic inflammatory response syndrome (SIRS) findings (Singer et al., 2016) and any changes in these parameters should be a warning alarm for veterinarians in the field.

In this study, we have found a significant increase in the WBC and NEUT counts in the second group compared to those in the first group. The increase in these parameters is thought to be related to bacterial or viral aetiology of the diarrhoea in this group leading to more noticeable inflammatory response signs. Although there is no significant difference between etiological factors in terms of disease development, identification of pathogens is important in the treatment of patients (Cho and Yoon, 2014). Therefore, laboratory findings are also of great importance in terms of rational antibiotic use, since it is easier and cheaper to detect the presence of protozoa in stool samples from calves with diarrhoea than viral or bacterial agents, and since it is possible to evaluate the variability in the inflammatory response based on blood parameters.

Eimeria spp. is known to cause to malabsorption and enteritis with epithelial cell destruction in the intestines and can lead to enteric bleeding and eventually anemia in patients with an excessive protozoan load and severe disease (Elitok, 2020). However, this agent was diagnosed in one-third of the first group in this study and probably that is why there was not a significant difference in the RBC or the other RBC indexes. However, when the overall results of the groups are considered, the absence of a statistical difference in erythrocyte levels between the groups is in line with the previous study of Atcalı and Yıldız (2020).

CONCLUSION

In conclusion, since hematological parameters including WBC and Neutrophils show significant increase in non-protozoa induced diarrhoea cases, performing complete blood count analysis, which is an inexpensive and easy-to-apply analysis may help for preventing the losses due to neonatal calf mortality.

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REFERENCES

Abuelo A. Investigation of an outbreak of neonatal calf diarrhoea in a dairy herd. Vet Rec. 2017; 4(2):e000372.

Altug N, Yuksek N, Ozkan C, *et al.* Neonatal buzağı ishallerinin immunokromotografik test kitleri ile hızlı etiyolojik teşhisi. YYU Vet Fak Derg., 2013; 24(3):123-128.

Atcali T, Yıldız R. Neonatal buzağı ishallerinde farkli etiyolojik faktörlerin hemogram parametreleri üzerine etkisi. MAKÜ Sag Bil Enst Derg. 2020; 8(3):119-27.

Berber E, Çanakoğlu N, Sözdutmaz İ, *et al.* Seasonal and age-associated pathogen distribution in newborn calves with diarrhea admitted to ICU. Vet Sci. 20219; 8(7):128.

Brenner J, Elad E, Markovics A, Grinberg A, Trainin Z. Epidemiological study of neonatal calf diarrhoea in Israel-A one-year survey of faecal samples. Isr J Vet Med. 1993; 48:113.

Chao DL, Roose A, Roh M, Kotloff KL, Proctor JL. The seasonality of diarrheal pathogens: A retrospective study of seven sites over three years. PLOS Negl Trop Dis. 2019; 13(8):e0007211.

Cho YI, Yoon KJ. An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. J Vet Sci. 2014; 15(1):1-7.

Çitil M, Arslan MÖ, Güneş V, Erdoğan HM. Neonatal buzağı ishallerinde Cryptosporidium ve Eimeria enfeksiyonlarının rolü. Kafkas Univ Vet Fak Derg. 2004; 10(1):59-64.

Elitok B. Epidemiology of coccidiosis and effects of the infection on some clinical and hematological examination findings in calves. JIVS. 2020; 4(1):21-25.

Elitok ÖM, Elitok B. Parenteral applications of colostrum serums in treatment and prevention of neonatal calf diarrhea. Kocatepe Vet J. 2016; 9(3):211-214.

Glombowsky P, Da Silva AS, Volpato A, *et al.* Relation between diarrhea and infection by protozoans in dairy calves. Comp Clin Path. 2017; 26:929-33.

Jorgensen MW, Adams-Progar A, de Passillé AM, Rushen J, Salfer JA, Endres MI. Mortality and health treatment rates of dairy calves in automated milk feeding systems in the Upper Midwest of the United States. Journal of dairy science. 2017; 100(11):9186-9193.

Lanz Uhde F, Kaufmann T, Sager H, *et al*. Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. Vet Rec. 2008; 163(12):362-366.

Meyer CL, Berger PJ, Koehler KJ, Thompson JR, Sattler CG. Phenotypic trends in incidence of stillbirth for Holsteins in the United States. JDS. 2001; 84(2):515-523.

Mõtus K, Reimus K, Orro T, Viltrop A, Emanuelson U. On-farm mortality, causes and risk factors in Estonian beef cow-calf herds. Prev Vet Med. 2017; 139:10-19.

Naylor JM. Neonatal calf diarrhea. Vet Clin North Am Food Anim Pract. 2009:70.

Norberg E. 2008. A genetic study of postnatal mortality in Danish Jersey heifer calves. Page 117 in Book of Abstracts of the 59th Annual Meeting of the EAAP, Vilnius, Lithuania. Wageningen Academic Publishers, Wageningen, the Netherland

Raboisson D, Delor F, Cahuzac E, Gendre C, Sans P, Allaire G. Perinatal, neonatal, and rearing period mortality of dairy calves and replacement heifers in France. JDS. 2013; 96(5):2913-2924.

Singer M, Deutschman CS, Seymour CW, *et al*. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016; 315(8):801-810.

Singh DD, Kumar M, Choudhary PK, Singh HN. Neonatal calf mortality–an overview. Intas Polivet. 2009; 10(2):165-169.

Smith DR. Field disease diagnostic investigation of neonatal calf diarrhea. Vet Clin North Am Food Anim Pract. 2012; 28(3):465-481.

Smith GW. Treatment of calf diarrhea: oral fluid therapy. Vet Clin North Am Food Anim Pract. 2009; 25(1):55-72.

Song RH, Kang JH, Park KM, Youm JH, Park JH. Analysis of hematological changes in normal and diarrhea calves. Korean J Vet Service. 2020; 43(3):161-165.

Yimer M, Gezhagne M, Biruk T, Dinaol B. A review on major bacterial causes of calf diarrhea and its diagnostic method. JVMAH. 2015; 7(5):173-185.