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Evaluation of Red Blood Cell and Platelet Indices in Cattle Naturally Infected With Bovine Viral Diarrhea Virus (BVDV)

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Abstract: The aim of this study was to evaluate red blood cell (RBC) and platelet indices in addition to white blood cell counts in cattle naturally infected with bovine viral diarrhea virus (BVDV). A total of 32 cattle, unvaccinated against BVDV, aged from 4 months to 9 months, were evaluated. Twenty two of them had clinical disorders regarding respiratory and gastrointestinal symptoms in which BVDV infection was virologically confirmed. Remaining 10 (BVDV free-cattle) were clinically healthy and used as controls. RBC indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], MCH concentration [MCHC], and RBC distribution width [RDW]) and platelet indices (plateletcrit [PCT], mean platelet volume [MPV], and platelet size distribution width [PDW]) were determined on a Cell-Dyn hematology analyzer. Hematological findings included neutrophilic leukocytosis or neutropenic leukopenia, lymphopenia, and monocytosis in BVDV-infected cattle. RBC, haemoglobin, and RDW were higher ($P<.001$) in infected cattle, but MCV was lower ($P<.001$), than those of controls. Platelet count, MPV, and PDW were higher in infected cattle, compared with controls ($P<.05$). In conclusion, changes in RBC and platelet indices may reflect changes in RBC and platelet production and reactivity. These indices should be used routinely during the diagnostic workup for BVDV infection in cattle practice.

Key Words: Bovine viral diarrhea virus, cattle, platelet indices, red blood cell indices.

Bovine Viral Diarrhea Virus (BVDV) ile Doğal Enfekte Sığırlarda Eritrosit ve Trombosit İndekslerinin Değerlendirilmesi

Özet: Bu çalışmanın amacı bovine viral diarrhea virus (BVDV) ile doğal enfekte sığırlarda lökosit (WBC) sayılarıyla birlikte, eritrosit (RBC) ve trombosit indekslerini incelemekti. BVDV'ye karşı aşılanmamış, 4 – 9 aylık, toplam 32 sığır değerlendirildi. Virolojik olarak BVDV enfeksiyonu belirlenen 22 sığırda klinik olarak solunum ve gastrointestinal sistem bulguları vardı. BVDV saptanamayan 10 sığır klinik olarak sağlıklıydı ve kontrol grubu olarak kullanıldı. RBC indeksleri (ortalama RBC volumü [MCV], ortalama RBC hemoglobini [MCH], MCH konsantrasyonu [MCHC] ve RBC dağılım genişliği [RDW]) ve trombosit indeksleri (plateletkrit [PCT], ortalama trombosit volümü [MPV] ve trombosit hacimsel dağılım genişliği [PDW]) Cell-Dyn hematoloji analizörü ile belirlendi. BVDV ile enfekte sığırlarda hematolojik olarak nötrofilik lökositozis ya da nötropenik lökopeni, lenfopeni ve monositozis belirlendi. Kontrol grubu değerlerine göre, enfekte sığırlarda ki RBC, hemoglobin ve RDW düzeyleri daha yüksek ($P<.001$) ancak MCV daha düşüktü ($P<.001$). Kontrollere göre, enfekte sığırlarda trombosit sayısı, MPV ve PDW değerleri daha yüksekti ($P<.05$). Sonuç olarak RBC ve trombosit indekslerindeki değişimler RBC ve trombosit üretim ve reaktivitelerindeki değişimleri yansıtabilir. Bu indekslerin sığır pratiğinde BVDV enfeksiyonlarınının tanısal sürecinde rutin olarak kullanılabileceği kanısındayız.

Anahtar Kelimeler: Bovine viral diarrhea virus, eritrosit indeksleri, sığır, trombosit indeksleri.

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Introduction

Bovine viral diarrhea virus (BVDV), a *Pestivirus* of the family *Flaviviridae* is classified into 2 biotypes, cytopatogenic (cp) and noncytopatogenic (ncp), and 2 genotypes, type I and type II[12]. BVDV is a widely distributed pathogen in cattle which often causes subclinical infections or only mild symptoms[15]. The disease occurs in veal calves and older animals and causes morbidity and mortality significant enough to result in alarming production losses[8]. BVDV infection may result in a number of distinct clinical and hematological syndromes, depending on the host, the virus and epidemiologic conditions[3,8]. Subclinical BVDV infections are associated with a mild fever and transient leukopenia of a few days duration. Nonregenerative anemia and thrombocytopenia have also been observed with ncp type II BVDV infections. Blood cytopenias due to direct cytotoxic effects, increased utilization, or bone marrow suppression were reported to be related to BVDV type II infections[4,6,7,15].

Recent advances in automated blood cell analyzers have made it possible to obtain new information about red blood cell (RBC) and platelets. These parameters which also called as RBC and platelet indices are used as an indirect indicator of bone marrow activation in clinical setting. RBC indices include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC), and RBC distribution width (RDW) while platelet indices include mean platelet volume (MPV), plateletcrit (PCT) and platelet size distribution width (PDW)[9,22]. MCV and MPV are measures of RBC and platelet sizes, and RDW and PDW are indicators of the variation in RBC and platelet sizes, respectively[9,22]. Plateletcrit, a parameter of platelet number and size, is constant, lower platelet number being associated with higher MPV. MCV and MPV have also been reported as indirect signs for disturbances of RBC and platelet productions as well as their activities[4,10,16]. Limited works have been published on routine utility of RBC[7,22] and platelet indices during diagnostic works in cattle practice[17,19,22]. A recently published study showed that a viral infection, bovine immunodeficiency virus, could not change significantly the RBC and platelet indices in cattle[22]. It is not fully elucidated yet whether these indices change in response to BVDV infection. Presence of BVDV-I and -II infections in Turkey was previously demonstrated[21].

This study was performed to evaluate the changes in RBC and platelet indices in cattle naturally infected with BVDV. The changes in white blood cell counts were evaluated, as well.

Materials and Methods

Animals

A total of 32 dairy cattle, Holstein-Fresian (4-9 months), unvaccinated against BVDV were used in this study. Twenty two cattle suffered from watery diarrhea and pneumonia were detected to be infected with BVDV (test group), whereas the remaining 10 were clinically healthy and were free from BVDV infection (control group). Both test and control groups were age (6.4 ± 0.2 months *vs* 6.2 ± 0.3 months; P = 0.738) and breed-matched. These cases were not further analyzed for other infections.

Virologic assays

For virological analysis blood and faecal samples from clinically affected animals as well as blood samples from non-affected animals were evaluated by a commercially available ELISA kit (BVDV antigen mix, Inst. Pourquier, France) for detection of BVDV antigens. From the samples generating positive results virus isolation was performed according to suggested methods including long-term cultivation followed by immunoperoxidase monolayer assay (IPMA)[20]. Obtained BVDV isolates were preliminary characterized for detection of their genotype by indirect IPMA[11] using a monoclonal antibody (SCR-4) discriminating BVDV type II strains[5].

Measurements

Blood samples were collected, within 2 days after virologic tests were completed, from jugular vein into vacutainer tubes (Becton Dickinson, NJ) with EDTA for leukogram [a five-part differential white blood cell (WBC) counts], erythrogram [red blood cells (RBCs), haemoglobin (Hgb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC), and RBC distribution width (RDW)], and thrombogram [platelet count, mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW)], by use of a fully automated blood analyzer [Abbott Cell Dyn

3500, Germany]. Peripheral blood smears were air-dried and stained according to the May-Grünwald-Giemsa method, to check if degenerative blood cells were present and to estimate platelet count by accepted formula: average number of platelet per field x 15.000[26]. Samples for CBC were processed within 4 h of collection.

Statistical analysis

Hematological data in BVDV-positive and negative groups were compared using the *Student's t-test* (Sigma Stat 2.0, GmbH, Germany). Descriptive statistics were also performed to obtain maximum, minimum, median, 25 % and 75 % values for each parameter. The graphs were created in Sigma Plot 2000 (Chicago, IL). P value less than 0.05 was considered significant. Results were expressed as mean ± SD.

Results

All the clinically affected animals were positive by antigen detecting ELISA while the animals in control group were negative. BVDV isolates were obtained by VI-IPMA. All the isolates were non-cytopathogenic (ncp) and no positive result was recorded in IIPMA using BVDV type II specific monoclonal antibody.

Mean neutrophil, monocyte and basophile rates in BVDV-infected cattle was higher (P=0.007), but lymphocyte and eosinophil rates were lower (P=0.02), compared to controls (Table-I). Maximum monocyte rate of 44% was higher in BVDV-infected cattle than that (14%) of controls. Mean RBC, Hct, and Hgb values were higher (P=0.001-0.007) in BVDV-infected cattle, compared to controls (Table-II). There were significant differences (P<0.001) in mean MCV, MCH, MCHC, and RDW between infected cattle and controls. There was statistically significant difference (P=0.019) in mean platelet count between BVDV-infected cattle and controls (Table-III). Observed MPV, PCT, and PDW in infected cattle were higher (P<0.01-0.001) than those of controls. Platelet clumps were detected by peripheral blood smear in two out of BVDV infected animals. Platelet counts in infected and control cattle were estimated as 1.580±180 $x10^3$/µL and 495±10.5 $x10^3$/µL, respectively. There were not statistically differences on automatic and manual platelet counting within groups.

Table I. Total and differential white blood cell (WBC) count in healthy and BVDV-infected cattle

Tablo I. Sağlıklı ve BVDV ile enfekte sığırlarda total ve diferensiyal lökosit sayıları

Parameter	Maximum		Minimum		Median		25 %		75 %	
	Control	BVDV	Control	BVDV	Control	BVDV	Control	BVDV	Control	BVDV
WBC $x10^3$/µL	8.25	26.5	4.34	2.78	5.61	7.76	4.87	4.09	7.11	16.65
Neutrophil %	60	91	32	11	46	53	38	24	51	75
Lymphocyte %	50	54	34	4	44	22	37	9	49	38
Eosinophil %	4.9	0.7	0.2	0.0	1.5	0.8	0.9	0.0	2.6	2.9
Monocyte %	14	44	8	5	12	18	9	9	12	32
Basophile %	1.5	8.1	0.1	0.2	0.5	1.5	0.3	0.9	0.8	2.6

Table II. RBC indices in BVDV infected cattle and healthy controls

Tablo II. Sağlıklı ve BVDV ile enfekte sığırlarda eritrosit indeksleri

Parameter	Healthy controls	BVDV-infected cattle	P value
RBC count $x10^6$/µL	6.088 ± 0.894	11.335 ± 1.687	<0.001
Hemoglobin mg/dL	9.9 ± 1.4	13.0 ± 1.9	<0.01
Hematocrit %	25.1 ± 3.3	36.0 ± 5.6	<0.001
MCV fL	41.3 ± 2.7	31.9 ± 3.9	<0.001
MCH pg/dL	16.3 ± 1.1	11.5 ± 0.6	<0.001
MCHC g/dL	39.6 ± 0.5	36.6 ± 3.9	=0.09
RDW %	21.7 ± 2.3	30.4 ± 4.1	<0.001

RBC: Red blood cell
MCV: Mean corpuscular volume
MCH: Mean corpuscular haemoglobin
MCHC: MCH concentration
RDW: Red blood cell (RBC) distribution width

Table III. Platelet indices in BVDV-infected cattle and healthy controls

Tablo III. Sağlıklı ve BVDV ile enfekte sığırlarda trombosit indeksleri

Parameter	Healthy controls	BVDV-infected cattle	P value
Platelet count $x10^3$/µL	477 ± 201	1716 ± 1350	=0.195
MPV fL	9 ± 0	18 ± 2	<0.001
PCT %	0.4 ± 0.1	0.8 ± 0.2	<0.0.5
PDW %	18 ± 0	30 ± 2	<0.01

MPV: Mean platelet volume; PCT: Plateletcrit; PDW: Platelet size distribution width

Discussion

Different WBC responses were detected in the present study; neutropenic leukopenia may be related with the early phase of the infection, whereas neutrophilic leukocytosis was likely due to complications by a secondary infection. Leukopenia may be resulted from de-

struction, redistribution and gastrointestinal loss of circulating cells[1,19]. Lymphopenia may also be related to BVDV-induced lymphoid atrophy[18], increased apoptosis, and endogenous glucocorticoid production increased by disease stress[1]. Monocytosis, eosinopenia and neutrophilia observed in BVD-infected animals may be caused by the chronic inflammatory leukogram. In acute BVDV infections, however, a significant drop in the number of circulating monocytes was reported[2]. The initial mechanism causing leukopenia may be regardless of virulence, but more virulent isolates may cause leukopenia to persist longer[1,3,8,13]. Viral isolates obtained in this study were characterized not to belong to BVDV type II. Since animals were either necropsied or died short after sampling, it was not possible to investigate persistent viremia. Moreover detection of only ncp biotype in all cases elicits the chance of mucosal disease. Although further analyses of the isolates are continuing results obtained in this study mainly represent ncp BVDV type I infections.

Microcytic normochromic RBCs without anemia in infected cattle was detected in the present study, but microcytic normochromic anemia was reported in calves[7]. This difference on RBC responses between two studies may be attributed to the severity of BVDV infection, since anemia is resulted from hypoplasia and necrosis of bone marrow and hemorrhagic syndrome in response to more virulent BVDV isolates[7]. Higher RBC populations in infected animals in this study may be related with activated bone marrow[1] and, in part, dehydration and hemoconcentration due to watery diarrhea. Observed increase in the RDW indicated the amount of variation (anisocytosis) in RBC size in the circulation, as a sign of an active bone marrow[9,10,22].

The current literature is confusing with respect to the causes of thrombocytopenia in BVDV infection. Megakaryocyte hyperplasia, normal megakaryocyte populations, necrosis and dysmegakaryopoiesis have been reported as possible reasons[1]. Thrombocytopenia associated with type II BVDV infection has been reported in adult cattle and calves under natural and experimental conditions[6,16,17]. However, in the present study, platelet count along with PCT value was not lower in infected cattle than controls. This may be related to pathogenicity and genotype of the virus. In a study[14], calves experimentally inoculated with a strain of BVDV showed a marked drop in platelet count 4-8 days post-inoculation, followed by a rebound thrombocytosis. Observed platelet count in infected cattle of this study is in consistence with the study of Bezek et al.[4] reporting that infection of calves with a cp isolate did not result in thrombocytopenia.

In response to changes in the number of circulating platelets, MPV shows considerable variation; a high MPV in the face of thrombocytopenia generally means that megakaryocytes are attempting to respond to the low platelet number by releasing larger platelets into the circulation, but a low MPV may indicate the presence of insufficient number of megakaryocytes or that they are failing to respond. Walz et al[16,17] demonstrated a significant decrease in MPV and platelet count in BVDV-infected *versus* control animals. In this study, increased MPV and PDW without thrombocytopenia indicating increased heterogeneity of platelet volume and size, probably due to BVDV-induced megakaryocytic hyperplasia[14]. These differences in platelet count and MPV may be attributed to differing degrees of viremia induced during infection or may have been related to differing infection kinetics for BVDV isolates[16,19]. Experimental BVDV infection showed that platelet count and bone marrow responses varied with time; acute infection may cause thrombocytopenia, whereas rebound thrombocytosis and megakaryocyte hyperplasia were seen later[14].

In conclusion, these results showed that RBC and platelet indices as well as differential WBC count changed in cattle naturally infected with BVDV. Further researches attempting to monitor such animals during infection period would be valuable. These results can be important in hematological observations in cattle practice.

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