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Comparison of hematological parameters of *Brucella*-seronegative naturally infected cattle with BVDV, BHV-1, and BHV-4

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Abstract: Abortion, neonatal deaths, and infertility cause important production losses in the livestock industry. Many viral infections are responsible for changes in hematological values of animals. This study aimed to find whether there is any difference in blood parameters of cattle that are naturally infected with BVDV, BHV-1, and BHV-4 and *Brucella*-seronegative. For this purpose, 401 whole-blood samples collected from cattle were analyzed by ELISA and PCR/RT-PCR to detect specific antibodies and antigens for BVDV, BHV-1, and BHV-4. Hematological analysis and results were compared between naturally infected BVDV, BHV-1, and BHV-4 groups and a control group. Results showed a statistically significant increase in hematological values of group 2 with BVDV compared to the corresponding parameters of group 1 (control) for mean corpuscular volume (MCV), hematocrit (Hct%), mean corpuscular hemoglobin concentration (MCHC), and mean platelet volume (MPV). The same was seen for hematological values of BHV-1 for MCV, Hct (%), and MPV and of BHV-4 for MPV ($P < 0.005$). However, it was determined that statistical differences in hematological values of naturally infected animals cannot be used as markers for clinical diagnosis of viral infections. Thus, we suggest that evaluations of hematological parameters with specific diagnostic tests are necessary to obtain more reliable results for the diagnosis of viral diseases.

Key words: BHV-1, BHV-4, BVDV, cattle, hematological parameters

1. Introduction

Abortions, neonatal deaths, and infertility lead to important production and economic losses in the livestock industry. The abortions and infertility are caused by viruses, bacteria, protozoa, fungal infections, and others (1). Bovine viral diarrhea virus (BVDV), bovine herpesvirus-1 (BHV-1), and bovine herpesvirus-4 (BHV-4) have worldwide spread in cattle populations and are among the main reasons for abortion in cattle (2–4). Changes in blood parameters could play important roles in the diagnosis of viral diseases in cattle with abortion and infertility.

In acute cases, BVDV causes fever, leukopenia, lymphopenia, and thrombocytopenia in cattle (5). Reports indicated the vaccines used against BVDV that were prepared from either modified live virus or inactivated virus may trigger leukopenia and lymphopenia (6). In recent years, some hematological values like hemoglobin, red blood cells (RBCs), platelets, mean platelet volume (MPV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean platelet volume (MPV) were investigated to find the effects of BVDV infections (6,7). Depletion was detectable in all leukocyte types (neutrophil granulocytes, eosinophils, lymphocytes, monocytes) and RBC counts with different kinetics in acute cases of BHV-1 infection (8). Experimentally infected calves with both BVDV and BHV-1 showed leukopenia, lymphopenia, and neutropenia (9). There are limited studies about the effects on blood parameters in cattle infected with BHV-4 and there are no significant differences in leukocyte counts (10). This study aimed to investigate whether *Brucella*-seronegative naturally infected cattle with BVDV, BHV-1, and BHV-4 have any differences in blood parameters.

2. Materials and methods

2.1. Sample collection

Blood samples from 401 randomly selected cattle aged ≥15 months old were collected from Kırıkkale (n = 215), Çorum (n = 95), and Yozgat (n = 91) provinces in Turkey. All the animals had no other clinical signs except for a history of abortion and/or infertility. Whole-blood samples were collected using EDTA-coated vacutainer tubes and

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serum samples were collected by using vacutainer serum tubes from each animal. Samples were transferred on ice to the laboratory of the Department of Virology, Faculty of Veterinary Medicine, Kırıkkale University as quickly as possible and whole-blood samples were analyzed on the same day. All serum samples were first tested with the rose Bengal plate test (RBPT). All RBPT-positive samples were analyzed by serum tube agglutination test (STAT) for detection of *Brucella* infections, according to the procedure described by the manufacturer (Pendik Institute, İstanbul, Turkey). A titer of ≥1:80 was considered as positive with STAT (11).

2.2. ELISA

Commercial ELISA kits were used to detect antibodies against BVDV (BVDV Ab ELISA, IDEXX USA, Cat. #99-44000), BHV-1 (Herdchek IBR gE, IDEXX Laboratories, USA, Cat. #99-09537), and BHV-4 (BIO-X Diagnostics, Belgium, Cat. #BIO K 263/2) according to the manufacturer's instructions and were evaluated after measurement with an automated ELISA reader (SIRIO S ELISA Reader, Indonesia).

2.3. PCR and RT-PCR

Extraction of BHV-1 or BHV-4 DNA from the blood was carried out with a spin column system (High Pure PCR Template Preparation, Roche, Germany, Cat. #11796 828001) according to the manufacturer's instructions. Each PCR analysis for determination of BHV-1 gB or BHV-4 gB genes was done separately with a mixture consisting of 5 µL of DNA, 25 mM Tris-HCl (pH 8.9), 3 mM $MgCl_2$ (MBI Fermentas), 2 mM dNTP mix (MBI Fermentas), 10 pmol of each primer (BHV-1gB1/BHV-1gB2 for BHV-1 and BHV-4gB1/BHV-4gB2 for BHV-4, as given in Table 1) and 1 U of Taq DNA polymerase (MBI Fermentas). PCR and RT-PCR were performed according to previously described methods (12). Briefly, the reaction conditions for PCR were as follows: 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 58 °C for 30 s (BHV-1 gB) or 56 °C for 45 s (BHV-4 gB), and 72 °C for 30 s; and finally 72 °C for 10 min.

Following the RNA extraction from blood with a spin column system (High Pure Viral RNA Kit, Roche Diagnostics, Germany, Cat. #11858882001), cDNA synthesis was carried out with a random primer (1.25 mM random primer; MBI Fermentas) for detection of BVDV. The first round of PCR was run in a reaction mixture that contained 5 µL of cDNA, 25 mM Tris-HCl (pH 8.9), 3 mM $MgCl_2$ (MBI Fermentas), 2 mM dNTP mix (MBI Fermentas), 10 pmol of each pan-pesti primer (324 and 326, Alpha DNA) (Table 1), and 3 U of Taq DNA polymerase (MBI Fermentas).

All PCR reactions were carried out in a BOECO thermal cycler (Hamburg, Germany). The InGenius LHR (Syngene, Cambridge, UK) was used to visualize PCR amplicons by using ethidium bromide staining after 1.5% agarose gel electrophoresis.

2.4. Hematological analysis and blood parameters

All blood samples were analyzed with the MS9-3 blood counter (Melet Schloesing Laboratories, France) for detection of the levels of leukocytes (white blood cells, WBC), lymphocyte percentage (Lym%), monocyte percentage (Mon%), neutrophil/granulocyte ratio (N/Gr%), lymphocytes (Lym), monocytes (Mon), neutrophils/granulocytes (N/Gr), erythrocytes (RBC), mean corpuscular volume (MCV), hematocrit (Hct), MCHC, hemoglobin (Hb), platelets (PLT), MPV, and MCH (13).

2.5. Statistical analysis

Data analyses were carried out using a statistical software program (SPSS for Windows, Version 11.5, SPSS Inc., Chicago, IL, USA). The associations between the occurrence of BVDV, BHV-1, and BHV-4 and the hypothesized risk factors, and differences between the groups, were analyzed by Kruskal–Wallis test, Mann–Whitney test, and Student t-test; $P < 0.005$ was considered significant.

3. Results

Blood samples were collected from 401 cattle. *Brucella* spp. specific antibody was detected in 45 of 401 serum samples with RBPT. Positive serum samples were analyzed by STAT to confirm the results and detect sera titers, and 41 of the 45 serum samples showed titers of ≥1/80 and were considered as positive and excluded from the samples of subsequent tests.

Table 1. Primers used for RT-PCR and PCR in this study.

Virus	Primer	5' → 3'	bp	Reference
Pan-Pestivirus	324	ATGCCCWTAGTAGGACTAGCA	288 bp	(25)
	326	TCAACTCCATGTGCCATGTAC		
BHV-1	BHV-1gB1	AAGCGCAAAAACGTGTG	323 bp	(26)
	BHV-1gB2	TGCAGGTACAGCTTGGC		
BHV-4	BHV-4gB1	CCCTTCTTTACCACCACCTACA	615 bp	(27)
	BHV-4gB2	TGCCATAGCAGAGAAACAATGA		

Table 2. Mean ± SD of the hematological values of whole-blood samples collected from 360 cattle.

Parameters	n	Mean ± SD	Reference values
WBC ($\times10^3$/mm^3)	360	15.44 ± 9.85	4–12*
RBC ($\times10^6$/mm^3)	360	6.06 ± 0.99	5–10*
PLT ($\times10^3$/mm^3)	360	161.91 ± 90.95	100–800*
Lym (%)	360	46.05 ± 19.48	45–80**
Mon (%)	360	13.76 ± 6.85	0–8*
N/Gr (%)	360	39.62 ± 14.73	10–30**
Hct (%)	360	24.67 ± 3.64	21–30***
Lym# ($\times10^3$/mm^3)	360	8.13 ± 7.81	1.8–8.1***
Mon# ($\times10^3$/mm^3)	360	1.76 ± 0.75	0.1–0.7***
N/Gr# ($\times10^3$/mm^3)	360	5.48 ± 2.70	0.6–4.1*
MCV (fL)	360	41.10 ± 4.57	40–60****
MPV (fL)	360	4.65 ± 0.52	4.6–7.4*
MCHC (g/dL)	360	53.78 ± 182.06	30–40**
Hb (g/dL)	360	13.45 ± 49.94	8–15****
MCH (pg)	360	22.34 ± 78.25	11–17****

Statistical analysis performed using SPSS 11.5.
*Merck Veterinary Manual 2012 (http://www.merckvetmanual.com/mvm/index.html).
**Başoğlu and Aydoğdu, 2013 (28).
***Wood and Quiroz-Rocha, 2010 (22).
****Feldman et al., 2000 (19).

The mean values with standard deviations (±SD) of blood parameters of 360 cattle are shown in Table 2.

BVDV specific antibodies (Ab) were detected in 262 of 360 cattle samples by using ELISA. RT-PCR was used to detect BVDV RNA in 360 blood samples; 18 of 360 samples were found positive and marked as Ag-positive. The samples were divided into 4 groups [group 1 (Ab-/Ag-, n = 95); group 2 (Ab+/Ag-, n = 247); group 3 (Ab+/Ag+, n = 15); group 4 (Ab-/Ag+, n = 3)] to determine the statistical changes in hematological parameters according to ELISA and PCR results. Group 1 was selected as the control group. A statistically significant increase was found in MCV, Hct (%), MCHC, and MPV parameters between groups 1 and 2 ($P \leq 0.005$) (Table 3).

BHV-1 seropositivity by ELISA was found for 158 of 360 cattle. All samples were examined for detection of viral DNA for BHV-1 by using PCR. BHV-1 DNA was not detected in any of the tested samples. The samples were divided into 2 groups [group 1 (Ab-/Ag-, n = 202); group 2 (Ab+/Ag-, n = 158)] to determine the statistical changes in hematological parameters according to ELISA and PCR results. Group 1 was selected as the control group. A statistically significant increase was found in the MCV, Hct (%), and MPV parameters between groups 1 and 2 ($P \leq 0.005$) (Table 4).

BHV-4 seropositivity by ELISA was found for 114 of 360 cattle. All samples were examined to detect viral DNA of BHV-4 by using PCR. BHV-4 DNA was not detected in any of the tested samples. The samples were divided into 2 groups [group 1 (Ab-/Ag-, n = 246); group 2 (Ab+/Ag-, n = 114)] to determine the statistical changes in hematological parameters. Group 1 was selected as the control group according to ELISA and PCR results. A statistically significant increase was found only in MPV between groups 1 and 2 ($P \leq 0.005$) (Table 5).

The hematological parameters of double and triple seropositive samples were compared with seronegative samples for BVDV, BHV-1, and BHV-4. The samples were divided into 5 groups [group 1 (BVDV Ab+/BHV-1 Ab+, n = 72); group 2 (BVDV Ab+/BHV-4 Ab+, n = 28; group 3 (BHV-1 Ab+/BHV-4 Ab+, n = 1); group 4 (BVDV Ab+/BHV-1 Ab+/BHV-4 Ab+, n = 73); group 5 (BVDV Ab-/BHV-1 Ab-/BHV-4 Ab-, n = 89)] to determine the statistical changes in hematological parameters. Group

Table 3. Mean ± SD of the hematological values for BVDV in group 1 (n = 95), group 2 (n = 247), group 3 (n = 15), and group 4 (n = 3).

Parameters	BVDV Ab-/Ag- (group 1)	BVDV Ab+/Ag- (group 2)	BVDV Ab+/Ag+ (group 3)	BVDV Ab-/Ag+ (group 4)
WBC (n/10^3)	13.29 ± 7.76	16.04 ± 10.36	18.99 ± 11.96	16.20 ± 6.20
Lym%	42.43 ± 18.13	47.38 ± 20.02	44.74 ± 17.73	57.76 ± 9.38
Mon%	15.43 ± 5.84	13.17 ± 7.16	13.66 ± 6.75	9.96 ± 0.75
N/Gr%	41.93 ± 13.61	38.71 ± 15.28	41.58 ± 11.73	32.23 ± 9.50
Lym#	6.36 ± 6.04	8.67 ± 8.25	10.04 ± 9.45	9.53 ± 4.50
Mon#	1.78 ± 0.70	1.73 ± 0.78	2 ± 0.56	1.60 ± 0.45
N/Gr#	5.12 ± 2.31	5.54 ± 2.82	6.96 ± 2.91	5.03 ± 1.65
RBC (n/10^9)	5.99 ± 0.83	6.10 ± 1.01	5.96 ± 1.54	5.86 ± 0.95
MCV (fL)	39.42 ± 4.41^{ab}	41.74 ± 4.53^{b}	41.33 ± 3.86	40.40 ± 4.01
Hct (%)	23.36 ± 2.65^{cd}	25.21 ± 3.81^{d}	24.15 ± 4.34	23.46 ± 1.88
MCH (pg)	16.95 ± 5.02	24.78 ± 94.38	17.32 ± 2.69	17.70 ± 2.43
MCHC (g/dL)	42.82 ± 6.05^{ef}	58.83 ± 219.71^{f}	42.18 ± 4.86	43.70 ± 1.83
Hb (g/dL)	9.94 ± 0.99	15.05 ± 60.26	10.02 ± 1.27	10.20 ± 0.45
PLT (n/mm^3)	141.66 ± 68.77	171.20 ± 99.17	137.73 ± 29.75	159 ± 110.53
MPV (fL)	4.49 ± 0.20^{gh}	4.72 ± 0.60^{h}	4.53 ± 0.21	4.53 ± 0.11

Kruskal–Wallis and Mann–Whitney U statistical methods were used; P < 0.005 is significant.
$^{a–h}$: Within a row, means with different superscripts are significantly different (P = 0).

Table 4. Mean ± SD of the hematological values for BHV-1 in group 1 (n = 202) and group 2 (n = 158).

Parameters	BHV-1 Ab-/Ag- (group 1)	BHV-1 Ab+/Ag- (group 2)
WBC (n/10^3)	15.85 ± 9.58	14.92 ± 10.20
Lym%	46.95 ± 18.80	44.90 ± 20.31
Mon%	13.66 ± 6.68	13.88 ± 7.08
N/Gr%	39.11 ± 13.81	40.28 ± 15.85
Lym#	8.44 ± 7.55	7.73 ± 8.14
Mon#	1.80 ± 0.78	1.70 ± 0.70
N/Gr#	5.56 ± 2.65	5.38 ± 2.78
RBC (n/10^9)	6.03 ± 1.07	6.10 ± 0.88
MCV (fL)	40.29 ± 4.71^{ab}	42.13 ± 4.17^{b}
Hct (%)	23.98 ± 3.70^{cd}	25.54 ± 3.38^{d}
MCH (pg)	17.51 ± 7.92^{ef}	28.52 ± 117.70^{f}
MCHC (g/dL)	42.96 ± 11.59	67.61 ± 274.36
Hb (g/dL)	10.14 ± 2.44	17.68 ± 75.26
PLT (n/mm^3)	150.85 ± 81.15	176.05 ± 100.63
MPV (fL)	4.56 ± 0.42	4.77 ± 0.60

Student t-test was used; P < 0.005 is significant.
$^{a–f}$: Within a row, means with different superscripts are significantly different (P = 0).

Table 5. Mean ± SD of the hematological values for BHV-4 in group 1 (n = 246) and group 2 (n = 114).

Parameters	BHV-4 Ab-/Ag- (group 1)	BHV-4 Ab+/Ag- (group 2)
WBC (n/10^3)	14.66 ± 9.13	17.12 ± 11.13
Lym%	46.25 ± 17.79	45.64 ± 22.77
Mon%	14.18 ± 6.56	12.85 ± 7.40
N/Gr%	39.16 ± 13.25	40.62 ± 17.53
Lym#	7.68 ± 7.17	9.11 ± 8.99
Mon#	1.75 ± 0.68	1.77 ± 0.89
N/Gr#	5.18 ± 2.47	6.12 ± 3.07
RBC (n/10^9)	5.99 ± 0.98	6.21 ± 0.99
MCV (fL)	41.03 ± 4.81	41.24 ± 4.01
Hct (%)	24.31 ± 3.59	25.44 ± 3.63
MCH (pg)	23.89 ± 93.32	19.01 ± 23.55
MCHC (g/dL)	57.20 ± 216.29	46.40 ± 61.60
Hb (g/dL)	14.17 ± 59.24	11.90 ± 17.70
PLT (n/mm^3)	156.59 ± 88.76	173.37 ± 94.90
MPV (fL)	4.57 ± 0.42^{ab}	4.83 ± 0.64^{b}

Student t-test was used; $P < 0.005$ is significant.
$^{a–b}$: Within a row, means with different superscripts are significantly different ($P = 0$).

5 was selected as the control group according to ELISA results. A statistically significant increase was found in MCV, Hct (%), PLT, and MPV parameters when each group was compared to the control group ($P \leq 0.005$) (Table 6).

4. Discussion

Changes in hematologic parameters in cattle could be due to infectious, mechanical (transportation, calving types, storage conditions of blood samples, and different blood analysis devices), physiological (age, feeding, pregnancy, breed of livestock), and chemical factors. In our study, we investigated the effect of infectious agents on hematological parameters, excluding the effects of feeding types, breed, character and age of cattle, pregnancy, season, and transport on parameters reported in different studies. In this study, a statistically significant difference was determined in MCV, Hct (%), MCHC, and MPV values of BVDV; MCV, Hct (%), and MPV values of BHV-1; and MPV values of BHV-4 between groups 1 and 2 ($P < 0.005$). The changes of hematologic values in calves during the first 6 months of life were compared with those of healthy adult cows. It was found that MCV was lower and RBC count was higher in calves throughout the investigation period (14). In our study, we collected all blood samples from cattle over 15 months of age, and that could be a reason for the difference between the groups for the hematologic parameters according to age.

Differences in storage conditions for blood samples and blood analysis devices may cause variations in blood parameters of cattle. It was reported that at room and refrigerator temperatures, blood samples from cattle and goats can be stored for up to 12 h. Blood samples from pigs can be stored for up to 8 h without any significant changes in Hb concentration, and for packed cell volume, RBC count, and total WBC count blood from all 3 species can be stored for more than 24 h without significant changes (15). In our study all blood samples were stored at 4 °C and analyzed within 4 h after transport to the laboratory. It was reported that in canine, feline, equine, and bovine blood samples analyzed with different hematology analyzers (Sysmex pocH-100iV Diff, Cell-Dyn 3500, Vet ABC, Coulter Counter, and Coulter LH 750), there were some significant differences in comparative results of the same blood parameters of dog, horse, and cattle according to the hematology analyzers (16,17). In our study all blood

Table 6. Mean ± SD of the hematological values of double and triple seropositive samples for group 1 (n = 72), group 2 (n = 28), group 3 (n = 1), group 4 (n = 73), and group 5 (n = 89).

Parameters	BVDV Ab+ BHV-1 Ab+ (group 1)	BVDV Ab+ BHV-4 Ab+ (group 2)	BHV-1 Ab+ BHV-4 Ab+ (group 3)	BVDV Ab+ BHV-1 Ab+ BHV-4 Ab+ (group 4)	BVDV Ab- BHV-1 Ab- BHV-4 Ab- (group 5)
WBC (n/10^3)	14.23 ± 9.66	19.36 ± 10.37	10.03 ± 0.00	15.91 ± 11.01	13.25 ± 7.43
Lym%	45.60 ± 16.74	48.35 ± 21.33	66.70 ± 0.00	44.41 ± 23.27	41.30 ± 16.67
Mon%	14.34 ± 7.08	11.65 ± 7.56	9.60 ± 0.00	13.27 ± 7.29	15.74 ± 5.63
N/Gr%	38.99 ± 12.87	38.89 ± 16.09	14.60 ± 0.00	41.55 ± 18.18	42.84 ± 12.30
Lym#	7.38 ± 7.33	10.67 ± 8.39	6.69 ± 0.00	8.39 ± 9.18	6.33 ± 6.08
Mon#	1.68 ± 0.71	1.80 ± 1.16	0.96 ± 0.00	1.71 ± 0.69	1.79 ± 0.57
N/Gr#	5.07 ± 2.82	6.74 ± 3.16	1.47 ± 0.00	5.71 ± 2.75	5.11 ± 1.95
RBC (n/10^9)	5.93 ± 1.00	6.08 ± 1.16	6.93 ± 0.00	6.33 ± 0.73	6.05 ± 0.88
MCV (fL)	43.07 ± 4.51^{ab}	41.32 ± 4.33	34.00 ± 0.00	41.41 ± 3.79^{op}	39.19 ± 4.29^{bp}
Hct (%)	25.30 ± 3.64^{cd}	24.81 ± 4.25	23.79 ± 0.00	26.12 ± 3.12^{rs}	23.42 ± 2.80^{ds}
MCH (pg)	39.13 ± 171.9	17.35 ± 4.07	11.50 ± 0.00	20.06 ± 29.32	16.96 ± 5.14
MCHC (g/dL)	90.95 ± 399.29	41.72 ± 6.06	33.70 ± 0.00	49.11 ± 76.92	43.07 ± 6.14
Hb (g/dL)	23.84 ± 109.36	10.15 ± 0.90	8.00 ± 0.00	13.02 ± 22.09	10.03 ± 1.03
Plt (n/mm^3)	189.59 ± 108.80^{ef}	198.75 ± 99.98^{ij}	458.00 ± 0.00^{kl}	164.97 ± 92.80	135.89 ± 61.52^{fjl}
MPV (fL)	4.74 ± 0.67^{gh}	4.95 ± 0.88	6.00 ± 0.00^{mn}	4.82 ± 0.56^{tu}	4.47 ± 0.13^{hnu}

Student t-test was used; $P < 0.005$ is significant.
$^{a–u}$: Within a row, means with different superscripts are significantly different ($P = 0$).

samples collected from cattle were analyzed by the MS9-3 blood counter (Melet Schloesing Laboratories, France). We suggest that flow cytometry is an important method for diagnosis of infections and analyses of the hematological parameters, rather than hematology analyzers. In human medicine, flow cytometry is more common than in veterinary medicine in Turkey, and therefore it was speculated that the flow cytometry method will be a necessary method for diagnosis of experimental and wild-type cattle infections in Turkey in the future.

There are different studies conducted in different years that showed variabilities in reference blood values. Changes over time were reported by investigating the comparisons of bovine hematology reference intervals from 1957 to 2006 (18). Since the first volume of *Veterinary Hematology* was written by Schalm in 1957, bovine hematology reference intervals have changed over time and within all further editions to date (19–22).

Acute BVDV infection causes leukopenia, lymphopenia, and thrombocytopenia in cattle (5). It was reported that there were no significant differences in RBC and Hb values. However, MPV was significantly higher in BVDV-infected calves than in noninfected controls (7). In our work, MCV, HCT, MCHC, and MPV values were found to be statistically different in BVDV antibody-positive samples ($P \leq 0.005$) (Table 3). We did not find any difference in RBC and Hb values and also the statistical difference in MPV is compatible with previous results. Additionally, leukopenia, lymphopenia, and thrombocytopenia signs were not determined according to differences in WBC, Lym, Mon, and N/Gr values in cattle infected with BVDV. A prior study investigated the inactive BVDV vaccine effects on blood parameters and reported that inactive BVDV vaccines trigger leukopenia and lymphopenia in calves according to statistically significant decreases in WBC and Lym values. It was also reported that there was no significant effect of vaccination on RBC, Mon, N/Gr, PLT, MPV, or MCH values (6). Although we detected statistical differences in MCV and MPV values, compatible with previous studies, the hematological parameters were within normal reference intervals (Table 2).

It was reported that leukopenia was consistently detected within 24 h and on day 3 after BHV-1 infection. The depletion was detectable in all leukocyte types (neutrophil granulocytes, eosinophils, lymphocytes, monocytes) and RBCs in acute BHV-1 cases (9). A strong decrease in leukocytes, neutrophils, and lymphocytes was detected in healthy calves, calves with subclinical BVDV, and calves inoculated with BHV-1 (10). In our study MCV, Hct (%), and MPV were found to be statistically different for BHV-1 infection ($P \leq 0.005$) (Table 4). It was reported that only partial leukopenia was seen after BHV-1 infections in cattle in some studies, and it was not useful for differential clinical diagnosis (23).

Severe leukopenia cases were seen in cattle infected with BHV-4 with metritis and infertility (24). In our study it was found that natural infection of BHV-4 did not influence the hematological parameters, except for MPV; however, these values were also within normal reference value limits (Table 2). We compared the hematological parameters of double and triple seropositive samples with seronegative samples. Statistically significant changes were determined in values of MCV, Hct (%), PLT, and MPV in the compared groups, similar to the single positive sample comparisons of BVDV, BHV-1, and BHV-4 (Table 6).

In conclusion, according to the present results, evaluation of hematological parameters could give some idea of cattle infected with BVDV, BHV-1, and BHV-4, although not sufficient for differential clinical diagnosis. This is the first study for investigating the effects of natural BHV-1 and BHV-4 infections on hematological parameters in Kırıkkale, Çorum, and Yozgat provinces in Turkey. Further research attempting to monitor such animals during the infection period would be valuable. Although virological tests are necessary for both precise and differential diagnoses, these results can be important in hematological observations in cattle practice and for veterinarians and scientists studying in this area.

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