Avci, Oguzhan; Yavru, Sibel; Bulut, Oya
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Universidade Federal do Rio Grande do Sul
Porto Alegre, Brasil

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Changes in Hematological Parameters in Cattle Infected with Bovine Viral Diarrhea Virus

Oguzhan Avci, Sibel Yavru & Oya Bulut

ABSTRACT

Background: The bovine viral diarrhea virus (BVDV) causes mucosal lesions, respiratory disorders, spontaneous abortion, congenital abnormalities, and stillbirth in cattle and wild ruminant populations worldwide. Clinical categories of BVDV infection include persistent subclinical infection, acute transient infection, and mucosal disease. Virus neutralization, enzyme-linked immunosorbent assay (ELISA), and reverse transcription and polymerase chain reaction have been used for the detection of BVDV-infected cattle, but are time-consuming and costly methods, especially when screening large herds for persistent subclinical infections. In the current research, it was hypothesized that hemogram and blood gas values can be valuable indicator in the diagnosis and prognosis of infectious disease like metabolic disorders. The aim of this current study was to determine whether changes in the hematological parameters of BVDV-infected cattle represent potentially useful diagnostic factors.

Materials, Methods & Results: Blood samples were collected from the jugular vein of 15 BVDV-antigen-positive (sick group) and 15 BVDV-antigen-negative (control group) Holstein cattle on a dairy farm in Konya Province in the Central Anatolia region of Turkey between January 2012 and September 2012. The presence of the BVDV antigen in the blood samples was determined with commercially available ELISA kit by using ELISA reader. Hemogram parameters [white blood cell counts (WBC), lymphocytes, monocytes, granulocytes, red blood cell counts (RBC), hematocrit (Hct), hemoglobin (Hb) and thrombocyte counts (THR)] obtained from anticoagulated bloods were measured with automatic cell counter. Blood gas values [power of hydrogen (pH), partial pressure of carbon dioxide (pCO2), partial pressure of oxygen (pO2), sodium (Na+), potassium (K+), calcium (Ca+), glucose (Glu), lactate (Lac), actual bicarbonate (HCO3act), standard bicarbonate (HCO3std), total carbon dioxide (tCO2), base excess in vivo (BEvv), base excess in vitro (BEvt), oxygen content (sO2)] were measured by blood gas analyzer. The data for the antigen-positive cattle were compared to those of the control cattle using independent-sample t-tests (SPSS 19.0 for windows). The results were expressed as Mean ± Standard Deviation. Difference were considered significant when \( P < 0.05 \). The white blood cell count, the relative proportions of lymphocytes and monocytes; the pO2 and sO2 values; and the serum levels of sodium and potassium in the BVDV-antigen-positive cattle were significantly lower \( (P < 0.05) \) than those of the control cattle. By contrast, the relative proportion of granulocytes, the total CO2 value, and the serum levels of actual bicarbonate and standard bicarbonate in the BVDV-antigen-positive cattle were significantly higher \( (P < 0.05) \) than those of the control cattle.

Discussion: Bovine viral diarrhea virus infection is a common viral disease in cattle and wild ruminant populations in the world. BVDV may result in mucosal, reproductive and respiratory disorders, abortions, mummification, congenital anomalies, and still-births. Changes in the numbers of the various WBCs, blood gas values, and biochemical parameters might be useful diagnostic factors for the preliminary identification of BVDV infection in cattle. Leukopenia and lymphopenia might also represent important prognosticators for BVDV-infected cattle because of the increased risk of secondary infections.

Keywords: BVDV, viral disease, cattle, hemogram, biochemical parameters, blood gas values.
INTRODUCTION

The bovine viral diarrhea virus (BVDV) causes mucosal lesions, respiratory disorders, spontaneous abortion, fetal mummification, congenital abnormalities, and stillbirth in cattle and wild ruminant populations worldwide [9, 23]. The BVDV is a single-stranded, positive-sense RNA virus [15, 22]. Two distinct biotypes of the BVDV, non-cytopathic and cytopathic, have been reported [19].

In cattle, BVDV infections may be manifested as persistent subclinical infections or acute transient disease in cows [18, 21], and the BVDV can cause mucosal disease in calves. The BVDV is transmitted in nasal secretions, and has been found in the fetal fluids and membranes of aborted fetuses [8]. Persistently infected cattle function as reservoirs of the BVDV in a herd [10, 26]. Identifying and removing persistently infected animals are crucial to BVDV control efforts [14, 24]. Enzyme-linked immunosorbent assay (ELISA), immunohistochemistry, reverse transcription and polymerase chain reaction, and virus isolation have been used for detecting BVDV antigens [5, 27], but are relatively time-consuming and costly methods, especially for identifying persistent subclinical infections in large herds.

Differences in the hematological parameters of BVDV-infected cows, relative to those of healthy cattle, might provide a cost-effective means of preliminary screening [12]. However, information regarding such changes in cattle with natural persistent BVDV infections is scant. The aim of our study was to compare the hematological parameters of cattle with natural BVDV infections to those of healthy cattle.

MATERIALS AND METHODS

Animals and blood collection

Using a direct ELISA for a BVDV antigen, 15 BVDV-antigen-positive and 15 BVDV-antigen-negative (control group) Holstein cattle were identified on a dairy farm in Konya Province in the Central Anatolia region of Turkey between January 2012 and September 2012. None of the cattle used in our study had been previously vaccinated against BVDV. Blood samples were collected from the jugular vein using sterile vacuum tubes containing EDTA as an anticoagulant. The blood samples were placed on ice in a cooler, and analyzed within 30 min following collection.

BVDV antigen ELISA

The presence of BVDV antigen was determined for all of the 30 leukocyte samples using the direct ELISA, according to the manufacturer’s instructions. The absorbance of the contents of the wells was measured at 450 nm using an automatic plate reader, and the percentage inhibition was calculated from the absorbance measurement for each well.

Measurement of hematological parameters

The hematocrit, the hemoglobin level, and the number of white blood cells (WBC), lymphocytes, monocytes, granulocytes, and red blood cells were measured using an automatic cell counter. The pH, pCO2, pO2, total carbon dioxide (tCO2), oxygen saturation (sO2), base excess in vivo, base excess in vitro, and the serum levels of sodium, potassium, calcium, glucose, lactate, actual bicarbonate (HCO3act), and standard bicarbonate (HCO3std) were measured using a blood gas analyzer.

Statistical analyses

The data are expressed as the mean ± standard deviation. The differences between the study groups were evaluated using independent sample t-tests. The data analysis was performed using the SPSS, version 19.0, software package for Microsoft Windows. The results of comparisons with P < 0.05 were considered to represent statistically significant differences.

RESULTS

BVDV infection alters hematological parameters

The hemogram and blood gas values for both groups are provided in Table 1. The pO2 and sO2 values, the serum levels of sodium and potassium, the WBC count, and the relative proportions of lymphocytes and monocytes in the BVDV-antigen-positive cattle were significantly lower (P < 0.05) than those of the control cattle. By contrast, the serum levels of HCO3act and HCO3std, the tCO2 value, and the relative proportion of granulocytes in the BVDV-antigen-positive cattle were significantly higher (P < 0.05) than those of the control cattle.

### Table 1. Hemogram and blood gas values of BVDV-antigen positive and negative cattle (Mean ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BVDV-positive*</th>
<th>BVDV-negative#</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell x10^3/mL</td>
<td>7.48 ± 2.99</td>
<td>9.69 ± 1.35</td>
<td>P &lt; 0.014</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>25.1 ± 8.86</td>
<td>51.3 ± 4.71</td>
<td>P &lt; 0.024</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>3.85 ± 0.74</td>
<td>4.46 ± 0.25</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Granulocyte %</td>
<td>70.9 ± 8.63</td>
<td>61.4 ± 6.41</td>
<td>P &lt; 0.002</td>
</tr>
<tr>
<td>Red blood cell x10^6/mm³</td>
<td>7.71 ± 1.37</td>
<td>7.15 ± 0.83</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Thrombocyte 10^9/mm³</td>
<td>554 ± 589</td>
<td>430 ± 186</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Hematocrit %</td>
<td>28.6 ± 8.49</td>
<td>27.4 ± 1.46</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Hemoglobin g/dL</td>
<td>9.07 ± 2.21</td>
<td>9.72 ± 0.63</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.06</td>
<td>7.42 ± 0.04</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>pCO₂ mmHg</td>
<td>44.2 ± 5.70</td>
<td>37.0 ± 1.31</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>pO₂ mmHg</td>
<td>25.8 ± 6.19</td>
<td>32.2 ± 6.75</td>
<td>P &lt; 0.011</td>
</tr>
<tr>
<td>HCO₃act mmol/L</td>
<td>26.8 ± 2.80</td>
<td>23.5 ± 2.67</td>
<td>P &lt; 0.002</td>
</tr>
<tr>
<td>HCO₃std mmol/L</td>
<td>25.3 ± 2.05</td>
<td>23.4 ± 2.53</td>
<td>P &lt; 0.035</td>
</tr>
<tr>
<td>tCO₂ mmol/L</td>
<td>28.2 ± 2.95</td>
<td>24.2 ± 2.86</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Base excess in vitro mmol/L</td>
<td>2.47 ± 2.33</td>
<td>3.56 ± 1.76</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Base excess in vivo mmol/L</td>
<td>-2.27 ± 2.09</td>
<td>-3.24 ± 1.48</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>sO₂ %</td>
<td>42.5 ± 19.5</td>
<td>60.2 ± 15.1</td>
<td>P &lt; 0.010</td>
</tr>
<tr>
<td>Na⁺ mmol/L</td>
<td>137 ± 7.40</td>
<td>144 ± 3.23</td>
<td>P &lt; 0.004</td>
</tr>
<tr>
<td>K⁺ mmol/L</td>
<td>3.45 ± 0.48</td>
<td>3.98 ± 0.35</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Ca²⁺ mmol/L</td>
<td>0.83 ± 0.09</td>
<td>0.82 ± 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Glucose mg/dL</td>
<td>81.0 ± 24.6</td>
<td>77.0 ± 22.1</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Lactate mmol/L</td>
<td>1.61 ± 1.24</td>
<td>1.20 ± 0.11</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

* N = 15; # N = 15.

**DISCUSSION**

Epidemics of BVDV infection in cattle can result in substantial economic losses. Hematological tests can aid in the diagnosis of microbial, parasitic, and viral infections. In our current study, various hematological parameters were altered in cattle with naturally acquired BVDV infections. The WBC count and the relative proportions of lymphocytes and monocytes in the BVDV-antigen-positive cattle were lower (P < 0.05), whereas the relative proportion of granulocytes was higher (P < 0.05), compared with the BVDV-antigen-negative cattle (Table 1). Leukopenia occurs in various types of viral infections [16,17,25]. Our findings of lymphopenia and leukopenia in BVDV-infected cattle are consistent with those of previous studies [1,2], and are likely the results of lesions in lymphoid tissue [3].

The pO₂ and sO₂ values and the serum levels of sodium and potassium in the BVDV-antigen-positive cattle in our study were also significantly lower (P < 0.05), compared to the BVDV-antigen-negative cows, whereas the tCO₂ level and the serum levels of HCO₃act and HCO₃std were significantly higher (P < 0.05; Table 1). Similar changes in the hemodynamic parameters of BVDV-infected cattle were previously reported [1]. Such changes are likely the result of impaired fluid-electrolyte and acid-base homeostatic processes in the gastrointestinal tract caused by BVDV-induced diarrhea.

The etiological role of BVDV in various clinical manifestations, such as respiratory tract infection, birthing abnormalities, and infertility, and the seroprevalence BVDV have been reported in previous studies of cattle in Turkey [4,11,20,28]. It was already
reported that BVDV-antigens were consistently present in macrophage-like cells in the stroma and theca externa of the ovaries and the subepithelial stroma and vasculature-associated mucosa of the uterus [6]. In cases of mucosal disease, BVDV antigens have been detected in a wide range of cell types and organ tissues not often associated with lesion formation in viral infections [7,13]. In our current study, only leukocytes were examined for the presence of BVDV antigens.

**CONCLUSION**

In conclusion, our results showed that BVDV infection causes leukopenia, lymphopenia, impaired fluid-electrolyte and acid-base balance, and altered blood gas parameters. These hematological changes might provide useful information for the diagnosis of BVDV infections. The BVDV-induced leukopenia and lymphopenia may place animals at greater risk of secondary microbial infections, such as bacterial pneumonia. Thus, these hematological parameters should also be monitored during the treatment of BVDV-related diseases.

**REFERENCES**


