Inactivated Bovine Viral Diarrhea Virus Vaccine Trigger Leucopenia and Lymphopenia on Calves*

Baki Sarıkaya1, Ahmet Kursat Azkur1 & Serkal Gazyagci2

ABSTRACT

Background: Bovine viral diarrhea virus (BVDV) in cattle is a very common viral infection that causes economic losses. In acute infection fever, leucopenia and thrombocytopenia may be observed. BVDV, an enveloped, single-stranded positive RNA virus, is a member of the genus pestiviruses within the family of flaviviridae. Vaccination and eradication programs should be applied against BVDV in herds with high prevalence of BVDV that includes removal of persistently infected (PI) animals from the herds. The vaccines used against BVDV are either modified live virus (MLV) or inactivated-virus vaccines. These commercially produced vaccines are being tested before introduced to the market, although afterwards some have been withdrawn regardless of preliminary tests. For example in Germany in 2010, inactive vaccines were withdrawn from the market when 80% of the newborn calves from vaccinated cattle were hemophilia. This phenomenon indicates the side effects of vaccine were needed by independent laboratories. For these reason in this study, in a dairy farm in 23 calves were investigated the effect of vaccination on the blood values.

Materials, Methods & Results: In this study it were used 23 healthy heifers aged 6-12-months old, held in a dairy farm in Kirikkale. All of the heifers were vaccinated subcutaneously with one vial of commercial PregSure BVD inactive vaccine as recommended by the manufacturer. Whole blood samples collected before one week and three weeks after one dose commercial inactivated BVDV vaccination, blood values analyzed and compared. Before and after one week from vaccination, the blood values of hematocrit, hemoglobin, leucocytes, red blood cell, lymphocyte, neutrophil/granulocyte and mean corpuscular hemoglobin concentration were decreased and this decrease was statistically significant (P < 0.05). Before and after three weeks from vaccination mean corpuscular hemoglobin concentration increase was significant (P < 0.05). One and three weeks after vaccination were compared, hematocrit, hemoglobin and red blood cell values were decreased and white blood cell, lymphocyte and neutrophil/granulocyte values increased found significant (P < 0.05). Divided into three groups against to BVDV antigen and antibodies in the serum of samples could not be found.

Discussion: In the present study we compared to effect of inactivated BVDV vaccination on blood values analyzed and compared with kinetics. Before study it was confirmed that all animal did not have BDVD specific antibodies by Porquier ELISA. When Before and after one week from vaccination, the blood values of hematocrit, leucocytes, lymphocyte, and mean corpuscular hemoglobin concentration were decreased and this decrease was statistically significant (P < 0.05). According to these results we found that a single-dose of vaccination causes a partial leucopenia and lymphopenia. To investigate whether vaccinations suppress immune system in calves, number of Treg cell population might be more detail observed after vaccination. As a result, though one dose of inactive BVDV vaccine cause lymphopenia and leucopenia it was unable to achieve high titers of antibodies. However veterinarian and animal owner prefer to perform widespread usage of one dose inactive vaccination in Turkey in order to cheaper than multiple dose vaccination.

Keywords: Bovine, BVDV, vaccine, ELISA, leucopenia, lymphopenia, blood parameters.
INTRODUCTION

Bovine viral diarrhea virus (BVDV) in cattle is a very common viral infection [9]. In acute cases, BVDV causes fever, leukopenia, thrombocytopenia [1,3]. BVDV, a enveloped, single-stranded positive RNA virus, is a member of the genus pestiviruses in of Flaviviridae [2,4]. Vaccination should be applied against BVDV in herds with high prevalence of BVDV that includes removal of persistently infected (PI) animals from the herds [12,13]. The vaccines used against BVDV are either modified live virus (MLV) or inactivated-virus vaccines. These commercially produced vaccines are being tested before introduced to the market, although afterwards some have been withdrawn regardless of preliminary tests. For example in Germany in 2010, inactive vaccines were withdrawn from the market when 80% of the newborn calves from vaccinated cattle were hemophilia. This phenomenon indicates the side effects of vaccine were needed by independent laboratories. Several tests are used for diagnosis of BVDV infection [9]. Among these tests, ELISA either with whole virus or the P80 monoclonal antibody is being widely used. It is reported that, following vaccination thrombocyte and leucocytes values may vary different range [13]. However no other study that reports other blood parameters was conducted with BVDV. Additionally studies that determine the effects of either commercial BVDV vaccines or natural infections on blood parameters are needed. Present study investigates the kinetics of inactive vaccine on various blood parameters. The aim of this study was to evaluate the effects of inactivated vaccine on various blood parameters and immunity levels in a local dairy herd in Kirikkale.

MATERIALS AND METHODS

Vaccination and sampling

In this study it were used 23 healthy heifers aged 6-12-months old, held in a dairy farm in Kirikkale. All of the heifers were vaccinated subcutaneously with one dose of commercial PregSure BVD inactive vaccine as recommended by the manufacturer. Two blood samples were collected from all 23 heifers in a serum tube either with (Green Vac Tube, Korea) or without (Vacutest, Italy) anticoagulant in days 0, 7 and 21 following vaccination. All sera samples were stored in -20°C until study. Blood samples with anticoagulant were studied immediately. This study was conducted under the permission of Kirikkale University Local Ethical Commision of Animal Experiments.

ELISA

The determination of NSP 2-3(P80) and E0(gp48) antigens and specific antibodies against P80 proteins of BVDV were analyzed with commercial kits2. Samples were analyzed and calculated with an automated ELISA Reader3 at 450 nm. The sum of standard deviations of both negative and 3X negative serum controls were taken as cut-off point between positive and negative results.

Data analysis

Blood parameters of three groups (0, 7, 21) were analyzed with ANNOVA test in SPSS v.15.0 and categorical variables with LSD test. Differences were accepted significant when \( P < 0.05 \).

RESULTS

Negative antigen and antibody ELISA results from day 0 samples ensured that BVDV infection was not seen in the sampled herd prior to present study. Leucocytes, lymphocytes and neutrophil/granulocyte numbers were given in Figures 1, 2, 3, respectively. Mean WBC, Lym %, Mon %, N/Gr %, number of lymphocytes and monocytes, neutrophiles/granulocytes (N/Gr), RBC, MCV, Hct, McHC, Hgb, PLT, MPV, MCH were given in Table 1. There was a significant effect of vaccination on leucocyte, lymphocyte and neutrophil/granulocyte numbers (\( P < 0.005 \)). However no significant effects were determined for values such as RBC, PLT, lymphocytes, Mon%, N/Gr% and Hct (\( P > 0.005 \)).

DISCUSSION

Besides separation of persisted infected animals, vaccination is important for prevention of fetal
infections in BVDV. It was reported that the usage of commercially available alive vaccines in pregnant cattle may cause fetal infections [11] and elevated mortality in calves due to immunosuppression [14]. Though inactive vaccines do not always give enough immunity for protection, their usage eliminates disease’s symptoms. Vaccinations were unable to supply protective immunity when they used commercially available active and inactive vaccines in Belgium [15].

Lately ELISA has become one of the most preferred tools in control and eradication programs due to its advantages such as; practical application, sensitivity, reliability and fastness [8]. It was reported that applied commercially available inactive BVDV vaccines, PregSure BVDV, Bovilis, Bovidec and Mucobovin-Vacoviron, twice to 4 groups of animals besides the negative control group, evaluated stimulated antibody levels by using commercially available total virus and P80 monoclonal antibody ELISA (Porquier) and compared these results. They were able to determine the antibodies in 28th day, the highest antibodies in 33rd day. Additionally they also reported that, total virus and P80 monoclonal ELISA kits were unable to determine NS3 proteins following first dose of vaccination. Accordantly, in our study we were also unable to determine antibodies by Porquier ELISA in either 1st or 3rd week following one dose of inactive Pregsure BVDV in our study [13]. These results are in compatible with our ELISA findings.

It was reported that BVDV vaccination did not able to have any effect of vaccination on leucocytes levels but showed a decline followed by an increase in leucocytes levels of naturally BVDV infected cows while another researchers found an increase in leucocytes levels when BVDV DNA vaccine was administered [7,10]. It was announced that there was a decline in leucocytes numbers between 7-12th days of neonatal calves infected with BVDV-2 [16]. In our the study a decline in leucocytes numbers was observed (Figure 1 and Table 1) most probably as a

Table 1. Heifers (n = 23) were vaccinated subcutaneously with one dose of commercial PregSure BVD inactive vaccine. After that whole blood samples collected before one week and three weeks after vaccination, blood values analyzed and compared. Statistically comparison of the effect inactivated BVDV vaccine on blood parameters of calves.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean Blood Parameters</th>
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<tbody>
<tr>
<td></td>
<td>Before vaccination</td>
</tr>
<tr>
<td>Leucocytes (m/mm³)</td>
<td>28.14±10.16</td>
</tr>
<tr>
<td>Lym %</td>
<td>64.23±14.27</td>
</tr>
<tr>
<td>Mon %</td>
<td>10.35±3.69</td>
</tr>
<tr>
<td>N/Gr %</td>
<td>25.41±11.03</td>
</tr>
<tr>
<td>Lym (m/mm³)</td>
<td>19.30±9.61</td>
</tr>
<tr>
<td>Mon (m/mm³)</td>
<td>2.61±0.61</td>
</tr>
<tr>
<td>N/Gr (m/mm³)</td>
<td>6.21±1.16</td>
</tr>
<tr>
<td>RBC (m/mm³)</td>
<td>9.86±0.87</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>38±3.16</td>
</tr>
<tr>
<td>Hct %</td>
<td>37.34±2.82</td>
</tr>
<tr>
<td>McHC (g/dl)</td>
<td>28.76±1.49</td>
</tr>
<tr>
<td>Hgb (g/dl)</td>
<td>10.73±0.77</td>
</tr>
<tr>
<td>PLT (m/mm³)</td>
<td>204.34±57.08</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>4.80±0.17</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>10.96±1.26</td>
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result of single dose vaccination inconsistent from others [5,16]. Though it was reported that an increase in monocyte numbers following administration of E1 and E2 coding DNA vaccine, no statistically significant difference was observed in monocyte numbers in days 0, 7 and 21 [5,16]. It was showed an increase in platelet levels till 24th day of BVDV infection in calves [5] while no change between groups was observed in our study (Table 1). Lymphopenia was reported with infections of either calves with BVDV [5] or neonatal calves with BVDV-2 [5,16]. However, lymphocyte numbers declined following first 7 days of vaccination and increased afterwards in our study (Figure 2). These results are in compatible with other researchers findings. It was reported declined levels of neutrophil/granulocyte for 21 days in calves infected with ncp BVDV [3,5,16]. We also found declined levels of neutrophil/granulocyte in 7th day but increased numbers on 21st day (Figure 3 and Table 1) indicating that one dose inactive vaccine administration affects neutrophil/granulocytes such as natural infections. It was reported MPV levels was decreased following BVDV vaccination [16] while in our study no significant decrease was observed. This may be resulted from administration of inactive BVDV1 vaccine. It was observed that an increase followed by a decrease in erythrocyte levels, increases in Hgb concentration and MCV while no change in McHC and MCH levels of cattle that were infected with strains of Clostridium chauvoei [6]. In the present study, no significant differences were observed in erythrocyte, Hgb and MCH following vaccination. Similar results were also obtained in study in which established protection immunity were generated injection of BVDV

![Figure 1](image1.png)

Figure 1. Heifers (n = 23) were vaccinated subcutaneously with one dose of commercial PregSure BVD inactive vaccine. After that whole blood samples collected before one week and three weeks after vaccination. The determination of leucocytes values in calves on day 0, 7, 21 following BVDV vaccination.

![Figure 2](image2.png)

Figure 2. Heifers (n = 23) were vaccinated subcutaneously with one dose of commercial PregSure BVD inactive vaccine. After that whole blood samples collected before one week and three weeks after vaccination. The determination of lymphocytes values in calves on day 0, 7, 21 following BVDV vaccination.
We and others have reported that although one dose of inactive BVDV vaccine causes lymphopenia and leucopenia it was unable to achieve high titers of antibodies [13]. However, in Turkey, veterinarian and animal owner, prefer to perform widespread usage of a single dose inactive vaccine, since it is less expensive than multiple dose scheme of vaccination.

![Figure 3. Heifers (n = 23) were vaccinated subcutaneously with one dose of commercial PregSure BVD inactive vaccine. After that whole blood samples collected before one week and three weeks after vaccination. The determination of neutrophiles values in calves on day 0, 7, 21 following BVDV vaccination.](image)

SOURCES AND MANUFACTURES
1 Pfizer, Animal Health, Dusseldorf, Germany.
2 PourQuier ELISA BVD/MD, Montpellier, France.
3 SIRIO S Elisa Redaer, Jakarta Raya, Indonésia.

Ethical approval. This research was approved Kirikkale University Animal Ethical Committee.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES


