

# Bovine Viral Diarrhea Virus (BVDV) Infection in Relation to Fertility in Heifers

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**ABSTRACT.** In this study, blood serum and leukocyte samples were collected from 400 Holstein heifers, all of which appeared to be healthy. Antibodies (Ab) against bovine viral diarrhea virus (BVDV) were detected in 57 serum samples, and BVDV antigen (Ag) was detected in 38 leukocyte samples. There were statistically important differences between the average first insemination ages (FIT) of the BVDV (Ag-/Ab+) heifers ( $p<0.0001$ ) (pregnant  $p<0.05$ , nonpregnant  $p<0.0001$ ) and BVDV (Ag-/Ab-) heifers. The average conception rates (CR) of BVDV (Ag-/Ab+) heifers and BVDV (Ag-/Ab-) heifers were not significant statistically. There were statistically important differences in average FIT between persistent infected (PI) BVDV (Ag+/Ab-) heifers ( $p<0.0001$ ; PI pregnant  $p<0.05$ , PI nonpregnant  $p<0.0001$ ) and BVDV (Ag-/Ab-) heifers. No significant differences in average CR between PI BVDV (Ag+/Ab-) heifers and BVDV (Ag-/Ab-) heifers were found. The differences in average FIT between BVDV (Ag+/Ab+;  $p<0.0001$ ; nonpregnant  $p<0.0001$ ) and BVDV (Ag-/Ab-) heifers were important statistically. Although there were no BVDV (Ag+/Ab+) pregnant heifers, the differences in average CR between BVDV (Ag+/Ab+) pregnant heifers and BVDV (Ag-/Ab-) heifers were found to be statistically important ( $p<0.0001$ ). We conclude that fertility is affected in heifers with BVDV (Ag-/Ab+, Ag+/Ab- and Ag+/Ab+).

**KEY WORDS:** BVDV, ELISA, fertility, heifer, Holstein.

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Bovine viral diarrhea virus (BVDV) has a high affinity for replicating cells. The virus therefore attacks germ cells and fetuses, negatively affecting calf health and reproductive performance of the herd [28]. Infection with BVDV yields different outcomes depending on the stage of pregnancy [32]. Several reports have investigated the effect of BVDV infection on reproductive parameters such as conception rate (CR) [19, 34], and it has been concluded that BVDV has a negative effect on the fertility of cows and heifers [11], with field studies reporting up to a 44% decline in CR [23, 40]. However, some experimental and field studies have found that BVDV infection does not affect the CR of heifers [22, 26].

In this study, blood serum and leukocyte samples were collected from 400 heifers in tie-stall barns in the Burdur Province area of SW Turkey. All had been fertilized for the first time. Antibodies (Ab) in the blood serum samples were searched for with the BVDV-enzyme linked immunosorbent assay (ELISA) (Ab) test, while antigen (Ag) in the leukocyte samples was searched for with the BVDV-ELISA (Ag) test. The serological and virological test results were sorted into groups, and the relationships between the fertility parameters of each group were evaluated.

## MATERIALS AND METHODS

*Animals:* For the present study, blood serum and

leukocyte samples were collected from 400 heifers between 13 and 24 months of age at 155 dairy farms in 22 residential areas (city center and villages bound to the center) that were registered with the Holstein Breeding Association in Burdur Province. After 60 days, a second set of blood serum and leukocyte samples were taken from the same animals. Before insemination, the body condition scores of all 400 heifers were evaluated (out of 5 points) [9]. An experienced veterinary surgeon examined each animal, vaginally and per rectum, and declared them healthy and free of anatomical abnormalities of the reproductive tract. Anamnesis data were also collected, with each stall barn manager being asked if the sample animals were vaccinated against BVDV; none of the heifers had ever been vaccinated against the virus. The animals chosen for sampling appeared to be healthy, had a body condition score of 3.5, were unmated and were about to be artificially inseminated for the first time.

*Estrus determination, artificial insemination and conception control:* The behaviors of 400 heifers that showed symptoms of estrus were observed. The vulva and vagina were inspected macroscopically, and the ovaries checked by rectal palpation, with the clinical data obtained being recorded. Estrus was determined by its classic symptoms: changes in the vulva and vagina and rectal palpation of the ovary to confirm the existence of fluctuant Graafian follicles. The heifers were fertilized by the rectovaginal method using BVDV-free straws (Alsole Benchmark BIRBO, Consozio Semenza-Italy Via Masaccio, 11–42010 Mancasale, Italy), with each containing at least 10 million motile spermatozoa. In the process of insemination, sperm was deposited in the corpus uteri.

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Conception monitoring of the subject heifers was carried out by means of rectal palpation and ultrasonography 8 weeks after insemination. Heifers determined as pregnant at the end of rectal palpation were recorded as pregnant. Those that were determined as nonpregnant had received another insemination between the insemination date and rectal palpation date or were again in estrus and were registered as nonpregnant. Multiple inseminations carried out within 10 days of each other were accepted as a single insemination. Any animal whose insemination results were not determined (for reasons such as death or wounding) were eliminated from the study group.

**Ab-ELISA:** A BVD/MD/BD P80-ELISA test kit (Institut Pourquier, Montpellier, France) was used to determine the existence of Ab against BVDV in the blood serum of heifers. The same kit was used when a second test was needed. The tests were carried out according to the kit procedure.

**Ag-ELISA:** A BVD/MD Ag Mix Screening ELISA kit (Institut Pourquier, Montpellier, France) was used to determine the existence of BVDV Ag in the leukocyte samples of heifers. The same kit was used when a second test was needed. The tests were carried out according to the kit procedure.

**Statistical analysis:** In this study, the "PROC MIXED" method in the Statistical Analysis System [35] (SAS) statistics program was used to determine the significance of difference between the first insemination ages (FIT) of BVDV (Ag-/Ab+) / BVDV (Ag-/Ab-), BVDV (Ag+/Ab-) / BVDV (Ag-/Ab-) and BVDV (Ag+/Ab+) / BVDV (Ag-/Ab-) heifers. The SAS [35] "FREQ and LOGISTIC" method was used for the significance of differences between the CRs of BVDV (Ag-/Ab+) / BVDV (Ag-/Ab-), BVDV (Ag+/Ab-) / BVDV (Ag-/Ab-) and BVDV (Ag+/Ab+) / BVDV (Ag-/Ab-) heifers. For conception evaluations, the SAS [35] "PROC MIXED" method was used for the significance of differences between the FITs of BVDV (Ag-/Ab+) / BVDV (Ag-/Ab-), BVDV (Ag+/Ab-) / BVDV (Ag-/Ab-) and BVDV (Ag+/Ab+) / BVDV (Ag-/Ab-) heifers. Risk estimation among BVDV and conception was evaluated using the odds ratio (OR).

## RESULTS

**Distribution of heifers according to conception status and test results:** For pregnant heifers, ELISA (Ag-/Ab+) determined the highest Ab+ rate, while ELISA (Ag+/Ab-) deter-

mined the lowest Ag+ rate. For nonpregnant heifers, ELISA (Ag+/Ab+) determined the highest rates for both Ag+ and Ab+, while the lowest Ag+ rate was determined with ELISA (Ag+/Ab-). The distribution of heifers according to conception status and test results is shown in Table 1.

**Reproductive parameters of BVDV (Ag-/Ab+) and BVDV (Ag-/Ab-) heifers (pregnant and nonpregnant):** The average FIT of the BVDV (Ag-/Ab+) heifers was significantly longer ( $P<0.0001$ ) than that of the BVDV (Ag-/Ab-) heifers. However, there was no statistical significance between the groups with respect to their average CRs (Table 2); the OR value was 0.0364 (95% CI = 0.237–1.025). There was no difference between the average FIT values of the pregnant BVDV (Ag-/Ab+) and BVDV (Ag-/Ab-) heifers. However, the average FIT values of the nonpregnant BVDV (Ag-/Ab+) heifers were significantly longer ( $P<0.0001$ ) than those of the nonpregnant BVDV (Ag-/Ab-) heifers (Table 2).

**Reproductive parameters of BVDV (Ag+/Ab-)/BVDV (Ag-/Ab-) heifers (pregnant and nonpregnant):** The average FIT of the BVDV (Ag+/Ab-) heifers was significantly longer ( $P<0.0001$ ) than that of the BVDV (Ag-/Ab-) heifers. However, there was no statistically significant difference between the two groups with respect to their average CRs (Table 3), and the OR value was 0.879 (95% CI = 0.243–2.249). There was no difference between the average FIT values of the pregnant BVDV (Ag+/Ab-) and BVDV (Ag-/Ab-) heifers (Table 3). The average FIT values of the nonpregnant BVDV (Ag+/Ab-) heifers were significantly longer ( $P<0.0001$ ) than those of the nonpregnant BVDV (Ag-/Ab-) heifers (Table 3). The same test results were obtained in the second sampling of the blood serum and leukocytes of the (Ag+/Ab-) heifers carried out after 60 days.

**Reproductive parameters of BVDV (Ag+/Ab+)/BVDV (Ag-/Ab-) heifers (nonpregnant):** The average FIT of the BVDV (Ag+/Ab+) heifers was significantly longer ( $P<0.0001$ ) than that of the BVDV (Ag-/Ab-) heifers. However, although none of the heifers was BVDV (Ag+/Ab+), the average CR values of the BVDV (Ag-/Ab-) heifers were significantly higher ( $P<0.0001$ ) than those of the BVDV (Ag+/Ab+) heifers (Table 4), and the OR value was 0 (95% CI=2.251–2.952). The average FIT values of the nonpregnant BVDV (Ag+/Ab+) heifers were significantly longer ( $P<0.0001$ ) than those of the nonpregnant BVDV (Ag-/Ab-) heifers (Table 4). The same test results were obtained in the second sampling of the blood serum and leukocytes of the (Ag+/Ab+) heifers carried out after 60 days.

Table 1. Distribution of heifers according to conception status and test results

| Samples  | Pregnant Animals |              |              |                | Nonpregnant animals |             |              |              |
|----------|------------------|--------------|--------------|----------------|---------------------|-------------|--------------|--------------|
|          | Ag+              |              | Ag-          |                | Ag+                 |             | Ag-          |              |
|          | Ab +             | Ab-          | Ab+          | Ab-            | Ab +                | Ab-         | Ab+          | Ab-          |
| Heifer   | –                | 7<br>(1.75%) | 14<br>(3.5%) | 202<br>(50.5%) | 25<br>(6.25%)       | 6<br>(1.5%) | 18<br>(4.5%) | 128<br>(32%) |
| Subtotal |                  |              | 223          |                |                     |             | 177          |              |
| Total    |                  |              |              | 400            |                     |             |              |              |

Table 2. Reproductive parameters of BVDV (Ag- /Ab +) and BVDV (Ag- /Ab-) heifers (pregnant and nonpregnant)

| HEIFERS             |                      |                       |                   |
|---------------------|----------------------|-----------------------|-------------------|
| Parameters          | BVDV (Ag- /Ab+) n=32 | BVDV (Ag- /Ab-) n=330 | P=                |
| FIT <sup>a)</sup>   | 626.19               | 535.27                | P<0.0001          |
| CR <sup>b)</sup>    | 43.75                | 61.25                 | P= 0.0545 (NS)    |
| PREGNANT HEIFERS    |                      |                       |                   |
| Parameters          | BVDV (Ag- /Ab+) n=14 | BVDV (Ag- /Ab-) n=202 | P=                |
| FIT <sup>a)</sup>   | 583.21               | 538.61                | P=0.0251 (P<0.05) |
| NONPREGNANT HEIFERS |                      |                       |                   |
| Parameters          | BVDV (Ag- /Ab+) n=18 | BVDV (Ag- /Ab-) n=128 | P=                |
| FIT <sup>a)</sup>   | 689.77               | 530.32                | P<0.0001          |

a) First insemination age (days). b) Conception rate (%). NS= Insignificant.

Table 3. Reproductive parameters of BVDV (Ag + /Ab-) / BVDV (Ag- /Ab-) heifers (pregnant and nonpregnant)

| HEIFERS             |                      |                       |                  |
|---------------------|----------------------|-----------------------|------------------|
| Parameters          | BVDV (Ag+ /Ab-) n=13 | BVDV (Ag- /Ab-) n=330 | P=               |
| FIT <sup>a)</sup>   | 693.21               | 535.37                | P<0.0001         |
| CR <sup>b)</sup>    | 53.85                | 61.21                 | P=0.5934 (NS)    |
| PREGNANT HEIFERS    |                      |                       |                  |
| Parameters          | BVDV (Ag+ /Ab-) n=7  | BVDV (Ag- /Ab-) n=202 | P=               |
| FIT <sup>a)</sup>   | 596.61               | 538.61                | P=0.039 (P<0.05) |
| NONPREGNANT HEIFERS |                      |                       |                  |
| Parameters          | BVDV (Ag+ /Ab-) n=6  | BVDV (Ag- /Ab-) n=128 | P=               |
| FIT <sup>a)</sup>   | 844.16               | 530.32                | P<0.0001         |

a) First insemination age (days). b) Conception rate (%). NS= Insignificant.

Table 4. Reproductive parameters of BVDV (Ag + /Ab +) / BVDV (Ag - /Ab -) heifers (nonpregnant)

| HEIFERS             |                      |                       |          |
|---------------------|----------------------|-----------------------|----------|
| Parameters          | BVDV (Ag+ /Ab+) n=25 | BVDV (Ag- /Ab-) n=330 | P=       |
| FIT <sup>a)</sup>   | 793.72               | 535.40                | P<0.0001 |
| CR <sup>b)</sup>    | 0                    | 61.21                 | P<0.0001 |
| NONPREGNANT HEIFERS |                      |                       |          |
| Parameters          | BVDV (Ag+ /Ab+) n=25 | BVDV (Ag- /Ab-) n=128 | P=       |
| FIT <sup>a)</sup>   | 793.72               | 530.32                | P<0.0001 |

a) First insemination age (days). b) Conception rate (%).

## DISCUSSION

In this study, as it was thought that BVDV (Ag+/Ab-)

heifers could be immune after acute infection, blood serum samples from these heifers were taken a second time, 60 days after the first, and the tests were repeated, again detect-

ing Ag+ and Ab-. It was therefore decided that the BVDV (Ag+/Ab-) heifers were persistent infected (PI). In addition, it was determined that the optical density (OD) obtained from the blood serum samples (in pregnant and nonpregnant heifers) was higher in the second sample than in the first. Different researchers [3, 8] have said that BVDV (Ag+/Ab+) animals can be found in herds and accepted as being PI.

Thompson *et al.* [39] researched the relationship between BVDV infection and age groups in 2343 cattle in Brazil. They determined that the Ab+ rate was at a particularly high level in heifers between months 19 and 30. In this study, the mean FITs of BVDV (Ag-/Ab+) heifers (626.19), BVDV (Ag-/Ab+) pregnant heifers (583.21) and BVDV (Ag-/Ab+) nonpregnant heifers (689.77) were high and statistically significant in comparison to the Ab- groups. The highest Ab+ rate was determined as 8% in 19- to 22-month-old heifers. This situation corresponds to the statements expressed by Thompson *et al.* [39] for 19- to 30-month-old heifers. However, the present research found that the efficiency of passive Ab resulting from BVDV infection was quite low compared with the results of Mockelinuniene *et al.* [27] and Kale *et al.* [20].

In the present study, statistically significant differences were determined between the FIT means of the PI BVDV (Ag+/Ab-), pregnant PI BVDV (Ag+/Ab-) and nonpregnant PI BVDV (Ag+/Ab-) heifers and the FIT means of the BVDV (Ag-/Ab-) heifers and were also determined between the FIT means of the BVDV (Ag+/Ab+) and nonpregnant BVDV (Ag+/Ab+) heifers. The existence of viral antigen was determined in the PI BVDV (Ag+/Ab-) heifers at a rate of 3.25% and in the BVDV (Ag+/Ab+) heifers at a rate of 6.25%. Houe *et al.* [19] found PI rates of between 3.75% and 6.12% in 28-month-old dairy cattle. Otachel-Hawranek [31] determined the rate of BVDV PI heifers in 17- to 24-month-old herds as 5%. The Animal and Plant Health Inspection Service [1] reported that the range for BVDV PI heifer in intermediate herds (100–499 head) was 6.7% and was 4.0% for entire dairy cattle herds. Estimates [17, 24] put PI prevalence among dairy cattle in New Zealand at between 1% and 5%. In this study, the results obtained for the PI BVDV (Ag+/Ab-) and BVDV (Ag+/Ab+) heifers broadly agree with the data obtained from the studies carried out in New Zealand and those carried out by Houe *et al.* [19] and the Animal and Plant Health Inspection Service [1]. However, many researchers [33, 42] think that maternal Abs in young animals originate since they cannot be determined by means of ELISA (Ag) techniques and some researchers [36] think that maternal Abs originate since the commercial ELISA (Ag) kits do not have enough sensitivity. It has been reported that BVDV affects fertilization in cattle, causing a decrease in conception rate [13].

Epidemiologic studies carried out on BVDV epidemics show that the virus decreases the rate of pregnancy and causes abortion in the early periods of pregnancy [23]. Houe *et al.* [19] demonstrated the appearance of a low pregnancy rate in cattle exposed to BVDV infection, while

McGowan *et al.* [25] showed that artificial insemination of infected *Bos indicus* cattle produced a lower pregnancy rate than that of immune cattle. It has been reported that this situation can result from the direct embryotoxic effect of BVDV or from ovaritis and follicular function disorder [15, 38]. Houe *et al.* [19] researched the effect of BVDV infection on the rates of pregnancy in dairy cattle herds. They determined the CR to be between 27% and 52% in groups carrying low-level BVDV Abs and between 41% and 61% in groups carrying high-level BVDV Abs. It was shown that the CR decreased temporarily in dairy herds carrying low-level BVDV Abs. In this study, the CRs of the BVDV (Ag-/Ab+) and BVDV (Ag-/Ab-) heifers were determined to be 43.75% and 61.25%, respectively. In addition, a statistically significant difference in CR ( $p < 0.05$ ) was determined between the BVDV (Ag-/Ab+) and BVDV (Ag-/Ab-) heifers. Kale *et al.* [21] showed that there was statistically significant difference in CR ( $p < 0.05$ ) between the BVDV (Ag-/Ab+) and BVDV (Ag-/Ab-) heifers. McGowan *et al.* [25] determined that the CRs of BVDV (Ag-/Ab+) and uninfected dairy heifers were higher after insemination than those of the cows in the same herd. Similarly, it has been reported that the CRs of heifers will be higher than those of cows until they are 2 years old [7]. Ola Refsdal [30] reported that the CR in heifers after first insemination in Norway was high (60.7%) and that the CR decreased in other lactation periods.

BVDV causes problems in various tissues [37]. The virus can be recovered from cells within the oviduct, endometrium, myometrium and placental membranes [4, 12]. Within the ovary, the virus has been located in interstitial, luteal, granulosa and thecal cells, as well as in follicular fluid [10, 14]. Ovaries have been shown to be one of the possible areas of BVDV replication, and this could lead to abnormal ovum development [10, 14]. In addition, the virus has been detected in oocytes recovered from PI animals [10]. Houe and Meyling [18] found that the CR and calf lifetime levels were low and that the early embryonic death event levels were high in a herd of 8 PI dairy cattle with BVDV.

It has been stated that the low level of CR in PI infected animals (compared with that of uninfected animals) can result from the existence of BVDV in circulating blood [23]. Similarly, it has been reported that the CR showed a significant decrease in a PI 5 herd with BVDV [19]. Archbald *et al.* [2] explained that BVDV (or its direct embryotoxic effect) at the uterus cornu can cause infertility by preventing preimplantation embryo development. The virus can cause ovaritis [38] and follicular function disorders [14]. Fray *et al.* [10] found BVDV Ag in the stroma segment of 6 ovaries taken from 3 PI heifers. Brownlie *et al.* [5] determined the existence of BVDV Ag in all ovaries of PI heifers. Some researchers [16] explained that the BVD virus in acutely infected animals can cause infertility by affecting ovarian function. In this study, the CR of PI BVDV (Ag+/Ab-) heifers was found to be 53.85%, and that of uninfected animals was found to be 61.21%; no statistical significance was

determined between the two groups. Kale *et al.* [20, 21] stated that they found no statistical significance between the CRs of PI BVDV (Ag+/Ab-) heifers and uninfected animals. Munoz-Zanzi *et al.* [29] found the CR for BVDV-infected heifers in a herd to be 67.3%. Kirkland *et al.* [22] stated that when they fertilized 73 heifers with sperm from a bull carrying BVDV, the average CR in the heifers was 65%, the same as the CR of the uninfected heifers. However, although there were no BVDV (Ag+/Ab+) pregnant animals in our study, a statistically significant difference ( $p < 0.0001$ ) was determined between the CRs of the BVDV (Ag+/Ab+) and uninfected heifers. Kale *et al.* [21] determined a statistically significant difference ( $p < 0.01$ ) between the CRs of BVDV (Ag+/Ab+) and uninfected heifers. It has been reported that the BVDV rarely infects the fetus and only replicates in trophoblasts during acute viremia before crossing into the fetus and spreading to placentomas [5]. Wentink *et al.* [41] concluded that PI BVDV infection could cause early-period reproductive problems in heifers.

In this study, the effect of BVDV infection on FIT was found to be statistically significant in all heifers, regardless of conception status. The reproductive performance parameters, milk yield and prevalence of illness, occur at a low or temporary level because of the slow progression of the BVDV in dairy cattle herds. This is particularly noticeable when these herds meet acutely infected or immune animals.

It has been suggested that reproductive performance decreases significantly when a new PI calf enters the herd or after the birth of an infected calf [6].

In this study, the effect of infection on fertility in BVDV (Ag-/Ab+), PI BVDV (Ag+/Ab-) and BVDV (Ag+/Ab+) dairy cattle was examined. It was determined that the fertility of BVDV (Ag-/Ab+), PI BVDV (Ag+/Ab-) and BVDV (Ag+/Ab+) heifers was affected. For this reason, it is necessary to investigate BVDV infections, serologically and virologically, in all new dairy cattle herds and during heifer purchase and sale. We recommend that these studies should include all heifers showing clinical signs in order to discriminate between BVDV (Ag-/Ab+), PI BVDV (Ag+/Ab-) and BVDV (Ag+/Ab+) and that different management conditions should be taken into account.

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