

## THE EFFECT OF INFECTION WITH BOVINE VIRAL DIARRHEA VIRUS ON THE FERTILITY OF COWS AND HEIFERS

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*In the current study, blood samples from 319 cows and heifers were studied. Antibodies against BVDV infections in serum samples and BVDV antigens in leukocytes were present in 203 (148 cows and 55 heifers) and 56 (28 cows and 28 heifers) samples, respectively.*

*Although no significant difference was detected between seropositive [BVDV (Ag-/Ab+)] and uninfected [BVDV (Ag-/Ab-)] cows and heifers at the time of the first insemination (FIT), first service age (FSA) or conception rate (CR) ( $p > 0.05$ ), the difference in age of seropositive and infection free cows differed significantly ( $p < 0.05$ ). Furthermore, differences in FIT, FSA or age of seropositive and uninfected pregnant cows and heifers did not differ significantly ( $p > 0.05$ ).*

*Differences between persistently infected [BVDV (Ag +/Ab -)] and uninfected cows for FIT, CR and age were statistically different ( $p < 0.001$ ). On the other hand, the difference between persistently infected and uninfected heifers for FSA or CR did not differ significantly ( $p > 0.05$ ). Even though, FSA of pregnant persistently infected or uninfected heifers was not different ( $p > 0.05$ ), there was a difference between the age and FIT of persistently infected and uninfected cows ( $p < 0.001$ ). However, pregnancy was not detected in any of the persistently infected cows.*

*In conclusion, seropositivity for BVDV did not affect fertility of cows or heifers. Although, differences in fertility between persistently infected and uninfected pregnant cows and between persistently infected and uninfected non-pregnant cows were present, fertility of heifers was not affected by BVDV persistency.*

*Key words: cow, heifer, bovine viral diarrhea virus, fertility, ELISA*

### INTRODUCTION

Bovine viral diarrhea virus (BVDV) infection is believed to cause important economic losses, mainly related to possible BVDV-associated reproductive

problems (Houe, 1999). The belief that BVDV causes reproductive problems has emerged from case reports (Roeder and Drew, 1984; Carman *et al.*, 1998) and experimental studies. These studies suggested that BVDV infection could interfere with conception (McGowan *et al.*, 1993, Grahn *et al.*, 1984) and cause embryonic (McGowan *et al.*, 1993) and fetal death (Virakul *et al.*, 1988, Larsson *et al.*, 1994). On the other hand, other studies failed to demonstrate any effects of BVDV on fertility (Whitmore *et al.*, 1981; Kirkland *et al.*, 1997).

Cattle persistently infected (PI) are the main reservoir of BVDV within herds and play the most important role in spreading the disease (Bolin, 1990). There are a number of studies reporting the negative effect of PI on fertility of cows (Fray *et al.*, 2000, Rüfenacht *et al.*, 2001). Most primary postnatal infections are subclinical but there are numerous reports that inter-current BVDV infection seems to enhance the virulence of other pathogens or change the nature of the resulting pathology. Based on these observations, it has been suggested that animals are transiently immunosuppressed after acute infection (Potgieter, 1995).

Acute postnatal infections are terminated by a potent and long lasting immune response of the host animal. The damage done by the infections is predominantly caused by intrauterine infections of susceptible pregnant cattle (Moennig, 2001). In non-immune pregnant animals, the virus infects the conceptus, irrespective of the time of conception, with effectively 100% probability (Duffell and Harkness, 1985). BVDV infection may show no clinical sign in infected animals because of subclinical infections. Thus, serologic assessments can find most animals as seropositive even though no clinical signs are present (Harkness *et al.*, 1978).

The aim of this study was to determine whether BVDV infections may affect to fertility of seropositive and persistently infected cows and heifers in Burdur, Turkey.

#### MATERIALS AND METHODS

*Farm:* The studied group comprised 319 Holstein-Friesian cows and heifers, kept at different dairy farms in Burdur, southwest Turkey. The main cattle-rearing activity is mixed (crop-livestock), small-scale (2-5 cattle on 1-3 ha) dairy production. Cattle are primarily kept for milk production. Average annual milk production on these farms was 6,000 L per cow. Animals in each farm were housed in the same free stall barn with free contact among animals. Data were randomly collected at individual cattle and farm levels.

*Animals:* Reproductive status of a cow was determined by rectal palpation and the condition of their calves was recorded. All the dairy cows in this study were examined vaginally, and were healthy and free of anatomical abnormalities of the reproductive tract. All the cows had calved at least 50 days prior, and they were not pregnant at sampling time. To exclude the possible effects of reproduction problems related to nutritional deficiencies, cows and heifers with a lower than 2.5 body condition score were not included in the study. Body condition scores were allocated on a scale of 0-5 (in increments of 0.25), with a score of 0 representing extremely thin or emaciated cows and 5 representing

extremely fat or obese cows (Chiang *et al.*, 1990, Loeffler *et al.*, 1999). During the study period none of the cows and heifers exhibited any overt clinical signs of BVDV or any other disease. None of the animals had ever been vaccinated against BVDV.

*Data collection and artificial insemination (AI)*: Interviews were performed in order to gather information about farms. Information regarding the herd and each sampled animal were recorded through a personal interview with the farmer or farm manager. AI dates and presence of pregnancy following 6-8 weeks insemination by rectal palpation records were recorded by the inseminator. All inseminations were performed on the day of spontaneous estrus by the same experienced veterinarian using BVDV free frozen semen from a single bull (Boschini Lauda) with proven fertility. Semen contained at least ten million of motile spermatozoa (Consorzio Semenza-Italy Via Masaccio, 11- 42010 Mancasale, Italy). The stage of estrus cycle was determined by rectal palpation *per rectum* and observation of secondary signs of estrus. The insemination coincided with the middle of estrus, as evidenced by cervical mucous discharge (CMD) and high myometrial tone and contractility. Semen was placed into the *corpus uteri* in all cows and heifers. The AI was the first postpartum in all cows, and the first insemination for all unmated heifers in the study. They were subjected to AI according to the routine a.m.-p.m. scheme and used for fertility assessment. Breeding day (day 0) equals the day of onset of strong estrus signs. Calving dates were obtained from farmer's records.

*Pregnancy control and calculations of conception rate*: Eight weeks post-insemination, a rectal examination was made. The same inseminator performed AI checked and recorded the pregnancy diagnosis. When an insemination led to a positive pregnancy check, it was defined as successful. If the outcome of an insemination was not known (e.g. due to slaughter before pregnancy diagnosis) this observation was omitted from the calculations. An animal was declared non-pregnant by rectal examination, or if returned to heat, and was inseminated again; the insemination was coded as an unsuccessful. CR was calculated as the percentage of inseminations resulting in pregnancy lasting 8 weeks.

*Serology and antigen detection*: Blood samples from cows and heifers were always collected from the jugular vein. Blood samples for sera were collected into tubes containing no anticoagulant. Blood samples then were centrifuged at 2000 rpm for 20 min. for sera collection. Leucocytes were prepared from blood samples collected into tubes with EDTA by a standard method. Sera and leucocytes were kept at -20 °C for subsequent analysis.

BVDV antibodies were determined using a commercial available ELISA kit (Institut Pourquire BVDV-ELISA). The sera were diluted to 1/2 and, % of competition  $\leq 40\%$  was defined as seropositive to BVDV (Anonymous 2001). BVDV antigens were detected by ELISA kit (BVDV Antigen Immunocapture, LSI Kit). The cut-off of the leucocytes has been established as positive OD<sub>c</sub> (optical density) sample  $>0.40$  (Anonymous 2001a). A second sample was collected from the antigen positive animals, after a period of at least 3 weeks. Animals which retested positive were classified as persistently infected.

*Statistics analyses:* The differences in the time period for the first insemination (FIT) for cows and first service age (FSA) for heifers in BVDV (Ag -/Ab +) / BVDV (Ag -/Ab -) and BVDV (Ag +/Ab -) / BVDV (Ag -/Ab -) and pregnant v.s. non pregnant groups were compared by Proc Mixed procedure of SAS. Conception rate (CR) was compared by FREQ and LOGISTIC procedure of SAS.

## RESULTS

Pregnancy rates and test results of animals used in the current study are presented in Table 1. Out of 319 animals (210 cows and 109 heifers), 203 (148 cows and 55 heifers) were seropositive by ELISA. The presence of BVDV antigens was determined on the leukocytes of 56 animals (28 cows and 28 heifers). A total of 60 animals (36 cows and 24 heifers) was found to be uninfected with BVDV. Overall, no differences were detected between seropositive [BVDV (Ag -/Ab +)] and infection free [BVDV (Ag -/Ab -)] cows and heifers for FIT, FSA or CR ( $p > 0.05$ ). However, the age of cows differed ( $p < 0.05$ ) at the time of AI (Table 2). Differences in FIT, FSA or age of seropositive and uninfected pregnant cows and heifers were not significant ( $p > 0.05$ ; Table 3).

Table 1. Distributions of cows and heifers according to their pregnancies and test results

	Pregnant animals				Non-pregnant animals			
	Ag <sup>+</sup>		Ag <sup>-</sup>		Ag <sup>+</sup>		Ag <sup>-</sup>	
	Ab <sup>+</sup>	Ab <sup>-</sup>	Ab <sup>+</sup>	Ab <sup>-</sup>	Ab <sup>+</sup>	Ab <sup>-</sup>	Ab <sup>+</sup>	Ab <sup>-</sup>
Cow	-	-	63	9	-	28	85	25
Heifer	-	-	37	12	-	28	18	14
Subtotal	-	-	100	21	-	56	103	39
Total	319							

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

Parameters	BVDV (Ag - /Ab +)	BVDV (Ag - /Ab -)	P <
	n=148	n=34	
COWS			
FIT <sup>a</sup>	116.13 ± 5.53	101.58 ± 12.14	NS*
Age (day)	1804.47 ± 51.71	1564 ± 112.33	P < 0.05
CR <sup>b</sup>	42.57	26.47	NS*
Parameters	BVDV (Ag - /Ab +) n=55	BVDV (Ag - /Ab -) n=26	P <
HEIFERS			
FSA <sup>c</sup>	535.07 ± 9.92	540.67 ± 14.86	NS*
CR	67.27	46.15	NS*

<sup>a</sup> Time period for the first insemination (day), <sup>b</sup> Conception rate (%), <sup>c</sup> Average of first service age (day), NS\* = not significant, Values are mean ± SE.

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

Parameters	BVDV (Ag - /Ab +)	BVDV (Ag - /Ab -)	P<
	n=63	n=9	
PREGNANT COWS			
FIT <sup>a</sup>	124.37 ± 9.81	106.00 ± 25.96	NS*
Age (day)	1776.35 ± 83.94	1409.33 ± 222.1	P=0.1
Parameters	BVDV (Ag - /Ab +) n=85	BVDV (Ag - /Ab -) n=25	P<
NON-PREGNANT COWS			
FIT	110.74 ± 6.56	98.68 ± 12.11	NS*
Age (day)	1827.85 ± 65.27	1665.36 ± 120	NS*
Parameters	BVDV (Ag - /Ab +) n=37	BVDV (Ag - /Ab -) n=12	P<
PREGNANT HEIFERS			
FSA <sup>b</sup>	538.38 ± 12.26	542.5 ± 21.54	NS*
Parameters	BVDV (Ag - /Ab +) n=18	BVDV (Ag - /Ab -) n=14	P<
NON-PREGNANT HEIFERS			
FSA	530 ± 16.47	537.80 ± 18.68	NS*

<sup>a</sup> Time period for the first insemination (day), <sup>b</sup> Average of first service age (day), NS\* = not significant, Values are mean ± SE.

Table 4. Reproductive parameters of cows and heifers with BVDV (Ag + /Ab -) and BVDV (Ag - /Ab -)

Parameters	BVDV (Ag + /Ab -)	BVDV (Ag - /Ab -)	P<
	n=28	n=34	
COWS			
FIT <sup>a</sup>	133.41 ± 6.87	101.58 ± 12.14	P<0.001
Age (day)	2168.19 ± 10.93	1564 ± 112.33	P<0.001
CR <sup>b</sup>	0	26.47	P<0.01
Parameters	BVDV (Ag + /Ab -) n=28	BVDV (Ag - /Ab -) n=26	P<
HEIFERS			
FSA <sup>c</sup>	559.89 ± 13	540.67 ± 14.86	NS*
CR	57.14	46.15	NS*

<sup>a</sup> Time period for the first insemination (day), <sup>b</sup> Conception rate (%), <sup>c</sup> Average of first service age (day), NS\* = not significant, Values are mean ± SE.

In the current study BVDV antigen was present in 56 animals (28 cows and 28 heifers). The same 56 animals were negative for antibodies. There was a statistical difference between persistently infected [BVDV (Ag + /Ab -)] and uninfected cows in FIT, CR and age ( $p < 0.001$ ). On the other hand, FSA or CR did

not differ significantly between persistently infected and uninfected heifers ( $p > 0.05$ ; Table 4). FSA of pregnant persistently infected or uninfected heifers was not different ( $p > 0.05$ ). There was a difference between the age and FIT of persistently infected and infected cows ( $p < 0.001$ ). However, pregnancy was not detected in any of persistently infected cows (Table 5).

Table 5. Reproductive parameters of pregnant and non-pregnant cows and heifers with BVDV (Ag + /Ab +) and BVDV (Ag - /Ab -)

Parameters	BVDV (Ag + /Ab -)	BVDV (Ag - /Ab -)	P <
	n=28	n=25	
NON-PREGNANT COWS			
FIT	132.35 ± 5.99	98.68 ± 12.11	P < 0.001
Age (day)	2203.35 ± 105.70	1665.36 ± 120	P < 0.001
Parameters	BVDV (Ag + /Ab -)	BVDV (Ag - /Ab -)	P <
	n=16	n=12	
PREGNANT HEIFERS			
FSA <sup>b</sup>	544.25 ± 17.9	542.5 ± 21.54	NS*
Parameters	BVDV (Ag + /Ab -)	BVDV (Ag - /Ab -)	P <
	n=12	n=14	
NON-PREGNANT HEIFERS			
FSA	578.83 ± 19.77	537.80 ± 18.68	NS*

<sup>a</sup> Time period for the first insemination (day), <sup>b</sup> Average of first service age (day), NS\* = not significant, Values are mean ± SE.

## DISCUSSION

Mockeliūniene *et al.* (2004) concluded that the number of seropositive animals increases with age. The increase in antibody prevalence by increasing age is probably because once BVDV antibodies are present. In most cases BVDV antibodies persist in the body. Thus, older animals have a higher probability to be infected with BVDV during their lives. Mockeli *et al.* (2003) analyzed the age influence on disease distribution. They divided 439 animals in 8 groups by age. The smallest number of seropositive animals was determined in the age group < 1 year. In the third and consecutive years of life the number of seropositive animals increases reaching its maximum in the group of animals aged 5 years and older. Thus, they reported that the age of an animal is positively correlated with the number of seropositive animals. In our study, the average ages for seropositive and persistently infected cows were high; 1804.47 ± 51.71 days and 2168.19 ± 10.93 days, respectively (Table 2 and 4). The percent of seropositive cows was higher in the group of animals that were 5 years old (29.7%). In addition, 61.5% of all seropositive cows were aged 5 years or older. Similarly, there were no persistently infected cows younger than 3 years of age. Moreover, 85.7% of all persistently infected cows were older than 5 years.



The adverse effect of this virus on conception appears to be attributable to failure of fertilization (Grahn *et al.*, 1984). On the other hand, BVDV does not appear to inhibit conception of either seropositive or seronegative cattle when inoculated by an oral or intranasal route or infused into the uterus of a seropositive cow at the time of breeding (Whitemore *et al.*, 1981). Rüfenacht *et al.* (2001) reported that there was no significant difference in the incidence of conception failures between animals that were already seropositive before and animals that remained seronegative during the conception period. After investigation of fertility parameters of seropositive animals, Rüfenacht *et al.* (2001) concluded that BVDV infections during the first 45 days of conception did not affect early embryonic death but caused abortions between 46 to 120 days of gestation. In our study the conception rates of BVDV seropositive and seronegative cows (42.57% and 26.47%) were similar to the study where pregnancy rates of seropositive and seronegative cows were 48.1% and 20.4%, respectively (Grahn *et al.*, 1984). Many researchers (Wentink *et al.*, 1991; Brock and Stringfellow, 1993; Tsuboi and Imada, 1996) reported that non-cytopathic (Ncp) BVDV replication in cells around embryos has no effect on bovine embryonic development. Other researchers (Singh *et al.*, 1982; Potter *et al.*, 1984; Bielanski and Hare, 1988) reported that *in vitro* inoculation of zona pellucida-intact *in vivo* embryos with Ncp BVDV had no adverse effect on survival and embryonic development. An experimental study by McGowan *et al.* (1993) reported that conception rates after AI were high in a herd consisting of both uninfected and seropositive heifers. Moreover, in a herd infected with BVDV, conception rate was 78.6% in seropositive cows (Virakul *et al.*, 1988). McGowan *et al.* (1993) also demonstrated poorer conception rates in cows that were infected with BVDV at the time of insemination compared to the cows that were seropositive.

Houe and Meyling (1981) stated that low conception rates, high early embryonic deaths, and lower calf survivals were seen in 8 herds with a high incidence of animals that were persistently infected with BVDV. The conception rate was probably lower in cows exposed to a viral circulation (presence of PI-animal), compared with cows in herds defined as non-infected (without PI-animals) (Larsson *et al.*, 1994). The possible influence of BVDV on conception rate was studied in five herds having animals persistently infected with BVDV (Houe *et al.*, 1993). Their study showed that the BVDV infection circulating during the risk period seems to cause a temporary decrease of the conception rate in dairy herds. Archbald *et al.* (1979) reported that the presence BVDV in the uterus can inhibit the development of embryos prior to implantation and could cause infertility. BVD is probably directly embryotoxic (although studies of contaminated embryos do not necessarily demonstrate such an effect *in vitro*). It can cause ovaritis (Sentongo *et al.*, 1980) and impairment of follicular function (Grooms *et al.*, 1996). In our study the difference between persistently infected [BVDV (Ag +/Ab -)] and uninfected cows in CR was significant ( $p < 0.001$ ). On the other hand, the CR difference between persistently infected and uninfected heifers was not significant ( $p > 0.05$ ).

In this study, general averages for FIT of BVDV serologically positive cows and seropositive pregnant and open cows did not differ from those of BVDV

negative cows and seronegative pregnant and open cows. Furthermore, general averages for FSA of BVDV serologically positive heifers or seropositive pregnant or open heifers were not different from those of BVDV negative heifers and seronegative pregnant or open heifers. Valle *et al.* (2001) observed no effect of BVDV on the number of services for heifers or cows. In addition, there appeared to be no effect of BVDV on the herd's average calving interval. BVDV infection had no significant effect on the risk of return-to-service (Robert *et al.*, 2004). Whitehead (2002) reported that no difference between the three groups of BVDV seropositive heifers when the first service interval, conception interval and the number of services per conception were compared. Christiansen *et al.* (2001) had studied the effect of BVDV infection on the reproductive performance parameters of dairy cows throughout the year. In their study, results showed that there was no statistically significant difference between the BVDV breakdown herds and the control herds in any of the reproductive performance parameters. In the current study, our findings for FIT and FSA rates of BVDV seropositive or uninfected cows were similar to the findings of Christiansen *et al.* (2001).

Wallner (1995) used modified BVDV-antigen capture ELISA test to detect persistently infected cows. Out of 695 persistently infected cows, 78 of them showed fertility problems. In our study, FIT was significantly different between persistently infected and uninfected cows or between non-pregnant persistently infected and uninfected cows. However, FSA of heifers was not affected by BVDV infection regardless of pregnancy. When BVDV infection enters a herd, its adverse effects are not always immediately apparent. It may spread slowly through the dairy herd, and any effects on reproductive performance, milk yield and disease outcomes may be small or transient. This is especially likely if BVDV infection is introduced into the dairy herd through an acutely infected or immune animal rather than a persistently infected animal. Significant losses may not be seen until cows in the dairy herd give birth to persistently infected calves and these calves enter the dairy herd (Christiansen *et al.*, 2001).

Results of the present study offer insights into the effect of BVDV infection on seropositive and persistently infected dairy herd fertility. Seropositivity for BVDV did not affect fertility of cows or heifers. Although there was a statistically significant difference between the BVDV persistently infected cows and the control herds in the fertility, fertility of heifers was not affected by BVDV persistency.

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## UTICAJ BVD INFEKCIJE NA PLODNOST JUNICA I KRAVA

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### SADRŽAJ

U okviru ove studije bilo je ispitano 319 uzoraka seruma i leukocita poreklom od junica i krava. Antitetela protiv BVD virusa u uzorcima seruma i antigeni BVD virusa u leukocitima su dokazani u 203 uzoraka (148 krava i 55 junica) i 56 (28 krava i 28 junica) uzoraka respektivno.

Između seropozitivnih jedinki [BVDV (Ag-/Ab+)] i onih koje nisu bile inficirane [BVDV (Ag-/Ab-)] nisu utvrđene statistički značajne razlike u vremenu prve inseminacije (FIT), dužini servis perioda (FSA) i stepena koncepcije (CR) ( $p > 0.05$ ). Utvrđene su samo razlike u starosti seropozitivnih i seronegativnih jedinki ( $p < 0.05$ ). Osim toga nije bilo razlike u FIT, FSA ili starosti seropozitivnih i neinficiranih gravidnih krava i junica ( $p > 0.05$ ).

Razlike u FIT, CR i starosnom dobu između krava sa perzistentnom infekcijom [BVDV (Ag +/Ab -)] i neinficiranih krava bile su statistički značajne ( $p < 0.001$ ). Istovremeno, razlike između perzistentno inficiranih junica i neinficiranih junica za FSA i CR nisu bile statistički značajne ( $p > 0.05$ ). Može se zaključiti da jedinke pozitivne na BVD nisu imale poremećene reproduktivne parametre.