

BOVINE HERPESVIRUS TYPE 4 (BHV-4) INFECTION IN RELATION TO FERTILITY IN REPEAT BREEDER DAIRY COWS

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(Received 1st May 2010)

In this study, blood serum was collected from 162 repeat breeder (RB) dairy cows and tested by enzyme linked immunosorbent assay (ELISA). Of these samples, 87 were bovine herpesvirus type-4 (BHV-4) seropositive and 75 were BHV-4 seronegative. The prevalence of BHV-4 was determined as 53.70% amongst RB dairy cows. In the current study the seroprevalence was high. The average days open (DO) of pregnant BHV-4 seropositive RB cows was significantly longer ($p < 0.01$) than that of pregnant BHV-4 seronegative RB cows, but was not significant for age of animals ($p = 0.186$) or conception rate (CR) ($p = 0.14$). The averages calving to the fourth insemination date (CFIDI) and age of non-pregnant BHV-4 seropositive RB cows was significantly higher ($p < 0.05$) than non-pregnant BHV-4 seronegative RB cows. In conclusion, a close fertility negative relationship existed between BHV-4 infections and fertility in RB dairy cows.

Key words: BHV-4, dairy cow, repeat breeder, fertility

INTRODUCTION

The Repeat Breeder (RB) syndrome is a major source of economic loss in dairy herds in the world (Bartlett *et al.*, 1986). RB cows are those that require three or more services to conceive (Enkhia *et al.*, 1983). All members in a population of RBs share an almost equal increase in fertilization failure, for various reasons, and an increase in embryonic loss (Casida, 1961). Moss *et al.* (2002) reported that days to first recorded heat (DFH) were associated negatively with RB syndrome in multiparous cows.

A higher prevalence of bovine herpesvirus type-4 (BHV-4) antibodies can be found in herds with reproductive problems when compared with healthy cattle (Naeem *et al.*, 1989). In cattle from Belgium, where BHV-4 seroprevalence is high, BHV-4 infection has been associated with postpartum metritis, abortion and chronic infertility (Czaplicki and Thiry, 1998). Other studies have also suggested that BHV-4 is a risk factor for abortion, metritis, pustular vulvovaginitis, stillbirth and reproductive deficiencies (Thiry *et al.*, 1992; Frazier *et al.*, 2001).

The aim of the present study was to determine the seroprevalence of BHV-4 in RB dairy cows and to investigate the relationship between BHV-4 and infertility in RB dairy cows.

MATERIAL AND METHODS

Animals: A total of 162 (3 to 9 year old) Holstein-Freisian RB dairy cows were selected from different dairy farms in the province of Burdur, Turkey. The cows were classified as RB if they had normal estrus cycles but remained subfertile after at least three inseminations (Ata, 1997). Body condition scores (BCS) were taken prior to artificial insemination (AI) (1 to 5) (Edmonson *et al.*, 1989) and animals with BCS lower than 2.5 were not included in the study. Blood samples were collected from all animals. All RB dairy cows were examined *per rectum* by an experienced veterinarian and were determined to be healthy and free of anatomical abnormalities of the reproductive tract. No animals in the trial had been vaccinated against bovine herpesvirus type 1 (BHV-1) or BHV-4 prior to the sampling period. The selected animals were free from bovine viral diarrhoea virus (BVDV) and bovine leucosis virus (BLV).

Oestrus detection, AI, and pregnancy checks: AI dates were recorded by the inseminator and pregnancy diagnosis was performed by rectal palpation and ultrasonography 6-8 weeks after AI. The AI was performed for the fourth time after postpartum in all cows. AI was performed using frozen-thawed semen samples containing at least ten million motile spermatozoa (Alsole Benchmark Birbo, Consorzio Semenza-Italy via Masaccio, 11- 42010 Mancasale/Italy) obtained from a single bull with proven fertility. The semen was placed in the corpus uteri. Breeding day (day 0) was accepted as equal to the day of onset of clear estrus signs. When AI led to a positive pregnancy check, it was defined as successful. If an animal was declared non-pregnant by rectal palpation or if it returned to heat, AI was coded as unsuccessful.

Antibody detection in serum samples: Presence of antibodies to BHV-4 in serum samples was detected by a BHV-4 enzyme linked immunosorbent assay (ELISA) kit (Bio-X Diagnostics, Belgium). All serum samples were tested by ELISA (Institut Pourquier, France) for the presence of BHV-1 antibodies and all samples were found to be seronegative.

Statistical data analysis: The differences between BHV-4 seropositive / BHV-4 seronegative cows with respect to average number of days open (DO) and age were compared using the *Proc Mixed* procedure of SAS. The conception rate (CR) for cows was compared by using the *FREQ* and *LOGISTIC* procedures of SAS. Risk estimation among BHV-4 and conception was evaluated using odds ratio (OR).

RESULTS

Out of 162 samples, 87 were BHV-4 seropositive and 75 were BHV-4 seronegative. After 21 days, a second set of samples from these 87 animals were

again found to be positive. The prevalence of BHV-4 was determined as 53.70% amongst the RB cows. The seroprevalence was high in the current study.

The average DO of pregnant BHV-4 seropositive RB cows was significantly longer ($p < 0.01$) than that of pregnant BHV-4 seronegative RB cows (Table 1). No significant difference was observed for age between animals ($p = 0.186$) or for CR ($p = 0.14$) (Table 1); the OR value was 0.8045 (95% CI = 0.333-1.162). The averages calving to the fourth insemination date (CFIDI) and age of non-pregnant BHV-4 seropositive RB cows was significantly higher ($p < 0.05$) than non-pregnant BHV-4 seronegative RB cows (Table 1).

Table 1. Reproductive parameters of pregnant and non-pregnant RB with BHV-4 seropositive and seronegative cows

Parameters	BHV-4 seropositive n=42	BHV-4 seronegative n=45	p<
PREGNANT COWS			
DO ^a	289.21 ± 12.59	231.06 ± 12.73	p = 0.002**
Age (days)	1446.93 ± 65.17	1584.67 ± 79.98	p = 0.186 NS
CR ^b	48.28	60	p = 0.14 NS
Parameters	BHV-4 seropositive n=45	BHV-4 seronegative n=30	p<
NON-PREGNANT COWS			
CFIDI ^c	263.67 ± 8.53	235.90 ± 6.42	p = 0.011*
Age (days)	2147.20 ± 141.91	1726.90 ± 115.34	p = 0.024*

^a Days open (DO), ^b Conception rate (CR), ^c Calving to fourth insemination date interval (CFIDI), NS* = not significant, * $p < 0.05$, ** $p < 0.01$. Values are mean ± SE.

DISCUSSION

The definition of bovine species as the natural host of BHV-4 and its consequent nomenclature as a bovine herpesvirus relied on the finding of a broad distribution of the virus in cattle populations (Thiry *et al.*, 1992). Naeem *et al.* (1989) investigated 4 herds with a high incidence of reproductive tract disorders and found a BHV-4 seroprevalence rate to be between 36% and 88%. Bilge-Dagalp *et al.* (2007) tested serum samples from 877 cattle from 6 dairy herds located in three different regions of Turkey for the presence of antibodies against BHV-4 and found a seroprevalence of 54.3%. Luini and Foni (1986) determined the prevalence of BHV-4 seropositivity in Bergamo, Brescia and Pavia provinces to be 57.1, 52.6 and 53.8%, respectively. Bilge-Dagalp (2007) detected prevalence of BHV-4 in infertile and healthy cattle as 52.3% and 40.5%, respectively. Higher prevalence of BHV-4 antibodies have been recorded in herds with reproductive problems than in normal cattle (Naeem *et al.*, 1989, Gür and Dogan, 2010). In the

current study, the prevalence of BHV-4 was determined as 53.70% amongst RB cows, which was a similar result to previous studies.

In an examination of different age categories, Nikolin *et al.* (2008) showed a rapid increase in the number of seropositive animals older than 2 years. Bilge-Dagalp (2007) reported that 46.6% of 1368 serum samples taken from dairy cows (2 years of age and over) were found BHV-4 seropositive between infertile and healthy animals. They determined that the BHV-4 seroprevalences observed in all animals, as well as in cows of herds with reproductive disorders, or in the herds without reproductive disorders, significantly differed according to the age of cows ($p < 0.01$ and $p < 0.05$). They reported that adult and older cows were more often infected than younger animals (<2 year old). Guo *et al.* (1988) also reported a considerable correlation with age. Wellenberg *et al.* (1999) recorded an increase in BHV-4-seropositive animals in a group of 2 to 3 year old cattle. Hence, it is likely that most cattle become infected after introduction to the dairy herd. In the current study, all pregnant-non pregnant and BHV-4 seropositive and seronegative cattle were older than 3 years of age. Presumably, this situation results from the selection of animals that had RB. Furthermore, in the present study, although the differences in age between BHV-4 seropositive and seronegative RB cows was statistically significant ($p < 0.05$), no significant difference for age was noted between pregnant animals ($p = 0.186$).

Like other herpesviruses, BHV-4 can establish latent infections in cattle, particularly in macrophages (Monge *et al.*, 2006) and the viral infection is often identified concurrently with bacteria that cause uterine diseases (Frazier *et al.*, 2001). The virus is highly tropic for endometrial cells, rapidly replicating and killing epithelial or stromal cells (Donofrio *et al.*, 2007). Although Fabian *et al.* (2008) reported no clear association between BHV-4 infection and endometritis/infertility problems, the virus was detected by immunofluorescence in the endometrium of cows suffering from endometritis (Frazier *et al.*, 2001). Stringfellow *et al.* (1990) also demonstrated adherence of BHV-4 to the zona pellucida and intact bovine embryos for the first time. By destroying placental cells and by the effect of local antiviral immune responses, the virus might disturb the physiological role of the placenta, endangering gestation (Deim *et al.*, 2006).

Animals with the subclinical form of the disease have more DO, take longer to conceive, and have CRs about half of those of normal animals (Kasimanickam *et al.*, 2004). The overall herd CR affects the percentage of RBs. In herds with low CRs, the incidence of RBs is high; with high CRs, the incidence of RBs is low (Garverick and Youngquist, 1993). The CR of animals with RB was determined as 39% by Stevenson *et al.* (1988) and 47% by Ghanem *et al.* (2006). A duration of DO greater than 145 days indicates a severe problem for all herds (Varner *et al.*, 2009). The average DO of cattle with RB was determined as 183.2 and 199.0 by Al-Hassan (2003) and Ata (1997), respectively. For a CR at first insemination of 49 ± 50 (%), DO of Holstein dairy cows that were observed to be healthy was determined as 109.2 ± 38.4 by Fonseca *et al.* (1983), while for a CR at first insemination of 60% DO of healthy dairy cows was reported as <120 by Diskin and Sreenan (1980). However, in the present study, CR (48.28%) of BHV-4 seropositive pregnant cattle was lower than that of non-infected cattle (60%).

Furthermore, the differences between pregnant BHV-4 seropositive and seronegative RB cows for DO and the differences for CFIDI and between BHV-4 seropositive and seronegative RB cows were statistically significant ($p < 0.01$ and $p < 0.05$ respectively).

In conclusion, BHV-4 infection was observed at a high level in RB dairy cows. It was accompanied by a decrease in CR and a prolongation of DO and CFIDI. Thus, BHV-4 infection had a negative effect on RB dairy cows.

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INFEKCIJA GOVEĐIM HERPES VIRUSOM TIPA 4 I PLODNOST KRAVA KOJE POVAĐAJU

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SADRŽAJ

U ovom istraživanju su ELISA testom ispitivani uzorci krvnog seruma do-
bijeni od 162 krave koje povađaju, na prisustvo antitela protiv bovinog herpes vi-

rusa tipa - 4 (BHV-4). Ukupno je otkriveno 87 seropozitivnih i 75 seronegativnih jedinki. Seroprevalenca na BHV-4 je, među mlečnim kravama koje povadaju, iznosila 53,70% što se smatra visokom vrednošću. Srednja vrednost dužine servis perioda je kod gravidnih BHV-4 seropozitivnih krava bila statistički značajno veća nego kod gravidnih BHV-4 seronegativnih jedinki ($p < 0.01$). Nisu utvrđene statistički značajne razlike u starosti jedinki ($p = 0.186$) i stepenu koncepcije ($p = 0.14$). Na osnovu dobijenih rezultata se može zaključiti da infekcija sa BHV-4 negativno utiče na plodnost krava.

