

## THE EFFECT OF SUBCLINICAL BOVINE HERPESVIRUS 1 INFECTION ON FERTILITY OF COWS AND HEIFERS

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*The aim of this study was to determine the serological prevalence of bovine herpesvirus 1 (BHV-1), and describe whether natural subclinical infections will lead to fertility losses in dairy cows and heifers in Burdur, Turkey. BHV-1 prevalence was 11.94% (201/24) and 14.60% (89/13) in cows and heifers, respectively. Conception rate (CR) was higher in BHV-1 serologically negative cows (38.98%) than BHV-1 positive cows (33.33%) but the difference was statistically insignificant ( $P > 0.05$ ). However, CR was higher ( $P > 0.05$ ) in BHV-1 serologically positive heifers (84.61%) than BHV-1 negative heifers (56.57%). Average open days period (OD) of BHV-1 serologically positive cows ( $99.3 \pm 16$  d) was different from BHV-1 negative cows ( $82.0 \pm 3.8$  d) ( $P < 0.05$ ). Average for the first service (FSA) of BHV-1 serologically positive heifers ( $18.01 \pm 1.1$  mo) did not differ from BHV-1 negative heifers ( $18.0 \pm 0.3$  mo) ( $P > 0.05$ ). Results of the current study showed that BHV-1 serologically positive cows have required for successful conception about 17 days more than negative cows.*

*Key words: cow, heifer, bovine herpesvirus 1, fertility, ELISA*

### INTRODUCTION

Bovine herpesvirus 1 (BHV-1) is the aetiological agent of a number of diseases not only of infectious bovine rhinotracheitis (IBR), but of as well as infectious pustular vulvovaginitis (IPV), infectious balanoposthitis (IBP), conjunctivitis, encephalomyelitis, mastitis, abortion, enteritis, lesions of the interdigital space, and fatal multisystemic infection in newborns (Gibbs and Rweyemamu, 1977; Kahrs, 1977; Higgings and Edwards, 1986; Miller and Van der Maaten, 1987). The virus causes significant economic losses to livestock around the world. BHV-1 infection can also occur as a subclinical infection (Van Oirschot *et al.*, 1993; Hage *et al.*, 1998). However, little is known about the economic impact of natural subclinical BHV-1 infection on reproductive performance in a dairy herd. A decrease in the conception rate (CR) of initially-seronegative animals after experimental BHV-1 infection has been described (Miller and Van der Maaten,

1987; Chiang *et al.*, 1990). Experimental BHV-1 infection has been described to cause embryonic death *in vitro* (Bowen *et al.*, 1985) and in early pregnancy in seronegative cattle (Miller *et al.*, 1988). However, Miller and Van der Maaten (1987) showed that initially, seronegative heifers carried their fetuses to term when inoculated with BHV-1 14 days after mating. Infectious bovine rhinotracheitis (IBR) caused by BHV-1 has been known to exist in Turkey since 1971 (Erhan *et al.*, 1971).

The aim of this study was to determine the serological prevalence of bovine herpesvirus 1 (BHV-1), and describe whether natural subclinical infections will lead to fertility losses BHV-1 infections on dairy cows and heifers in Burdur.

#### MATERIAL AND METHODS

*Farm:* The study period started on May 26, 2003 and ended on June 15, 2004. The samples of this study comprised 290 Holstein-Friesian cows and heifers, (aged 2 to 10 yrs for cows and 13 to 24 mo for heifers) kept at 107 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. Farms and cattle were randomly sampled. All animals on each farm were housed in the same free stall barn with intensive contact between animals. Data were collected at individual cattle and farm levels.

*Animals:* Cow reproductive status was determined by palpation and condition of the fetus 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 earlier and they were not pregnant at sampling time.

To exclude the possible effects of reproduction problems related to nutrition deficiency cows and heifers with body condition lower than 2.5 score were not included in this 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 (Domecq *et al.* 1997, Loeffler *et al.* 1999). During the study none of the cows and the heifers exhibited any overt clinical signs of BHV-1 or any other disease. None of the animals had ever been vaccinated against BHV-1.

*Data collection and artificial insemination (AI):* Interviews were performed in order to gather information about the farms. Information regarding the herd and each animal sampled were recorded through a personal interview with the farmer or farm manager. AI dates, presence of pregnancy following 6-8 weeks insemination by rectal palpation records were recorded by the inseminator. All inseminations were performed by the same experienced veterinarian using BHV-1 free frozen-thawed semen containing at least ten million of motile spermatozoa (Consorzio Semezo-Italy Via Masaccio, 11- 42010 Mancasale, Italy) of proven fertility from a single bull (Alsole Benchmark BIRBO). Insemination was carried out on the day of spontaneous estrus. The stage of the estrus cycle was determined by rectal palpation and observation of the secondary signs of estrus. The

insemination coincided with mid-estrus, as evidenced by cervical mucous discharge (CMD) and high myometrial tone and contractility. The semen was placed into the corpus uteri. The AI was the first after postpartum in all cows. Also, AI was performed for the first time on 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 the farmer's records.

*Pregnancy control and calculations of conception rate:* Eight weeks post-insemination, rectal examinations to determine pregnancy were carried out. 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. If two inseminations had occurred less than 10 days apart, they were recorded as only one insemination. CR was calculated as the percentage of inseminations resulting in pregnancy lasting at least 8 weeks.

*Serology:* Blood samples (10 ml) were collected from the tail vein of each animal at estrus period before AI, using disposable needles (21x1.5 mm) and vacutainer tubes. After centrifugation at 1700 g for 15 min, separated sera were stored at -20°C until analysis. BHV-1 antibodies were determined using a commercial available ELISA kit (Institute Pourquire IBR-ELISA). The sera were diluted 20 fold and, S/P% $\geq$ 55% was defined as seropositive to BHV-1 (Anonymous, 2001).

*Statistics analyses:* The mean values and standard errors of means were calculated for both BHV-1 serologically-positive and BHV-1 serologically-negative groups. The data were determined by  $\chi^2$  test and student's *t*-test, and values of  $P < 0.05$  were regarded as significant (SPSS software, version 11.0).

## RESULTS

BHV-1 prevalence was 11.94% (24/201) in cows and 14.60% (13/89) in heifers Burdur province. Conception rate was higher in BHV-1 serologically negative cows (38.98%) than BHV-1 serologically positive cows (33.33%) and was not significant ( $P > 0.05$ ). However, CR was higher in BHV-1 serologically positive heifers (84.61%) than BHV-1 serologically negative heifers (56.57%), but the difference was not significant ( $P > 0.05$ ). Average for OD (open days) of BHV-1 serologically-positive cows ( $99.3 \pm 16$  d) being pregnant were different from those of BHV-1 serologically negative cows ( $82.0 \pm 3.8$  d) and was statistically significant ( $P < 0.05$ ). The average for the age of first service (FSA) of BHV-1 serologically positive heifers at which became pregnant ( $18.01 \pm 1.1$  mo) was not different from those of BHV-1 serologically negative heifers ( $18.0 \pm 0.3$  mo). Reproductive parameters of BHV-1 seropositive and seronegative cows and heifers are presented in Table 1 and 2.

Table 1. Reproductive parameters of cows BHV-1 seropositive and seronegative

Cow n=201	Age (year)	OD <sup>a</sup>	CR <sup>b</sup>	BHV-1 Ab					
				Seropositive			Seronegative		
				n	OD <sup>a</sup>	CR <sup>b</sup>	n	OD <sup>a</sup>	CR <sup>b</sup>
Pregnant n=77	4.4 ± 0.2	100.2 ± 5.8	38.30	8	99.3 ± 16	33.33	69	82.0 ± 3.8**	38.98
Non pregnant n=124	4.9 ± 0.1	79.8 ± 23.8*	61.70	16	88.3 ± 6.9	66.67	108	78.7 ± 2.9**	61.02

<sup>a</sup>Average open days (day), <sup>b</sup>Conception rate (%), \*Values differ significantly between the pregnant and non-pregnant groups (P<0.05), \*\*Values differ significantly within the pregnant and non-pregnant groups (P<0.5), Values are mean ± SE.

Table 2. Reproductive parameters of heifers BHV1 seropositive and seronegative

Heifer n=89	Age (month)	CR <sup>a</sup>	BHV-1 Ab					
			Seropositive			Seronegative		
			n	FSA <sup>b</sup>	CR <sup>a</sup>	n	FSA <sup>b</sup>	CR <sup>a</sup>
Pregnant n=54	17.9 ± 0.3	60.67	11	18.01 ± 1.1	84.61	43	18.0 ± 0.3	56.57
Non pregnant n=35	17.9 ± 0.4	39.33	2	17.0 ± 0.0	15.39	33	17.8 ± 0.4	43.43

<sup>a</sup>Conception rate (%), <sup>b</sup>Average of first service age (month), \*Values differ significantly between the pregnant and non-pregnant groups (P<0.05), \*\*Values differ significantly within the pregnant and non-pregnant groups (P<0.5), Values are mean ± SE.

## DISCUSSION

Our results showed that the prevalence of BHV-1 in dairy cows (11.94%), and heifers (14.60%) without any disorders was lower than reported by Bulut *et al.* (2003). Biuk-Rudan *et al.* (1999) reported the presence of BHV-1 antibodies to be higher in cows with reproductive disorders compared to the cows without reproductive disorders.

A decrease in the CR of initially seronegative animals after experimental BHV-1 infection has been described (Chiang *et al.* 1990, Miller and Van der Maaten 1987). Allan *et al.* (1975) reported no effects on pregnancy. Yet in the presence of BHV-1, oocytes can be fertilized in vitro and co-cultured to the blastocyst stage; there was almost no difference with the control group (Bielanski and Dubuc 1993). Hage *et al.* (1998) reported that BHV-1 infection did not seem to affect pregnancies that were older than 50 days or younger than 10 days.

A serological study by Magana-Urbina *et al.* (2005) in dairy herds in Michoacan, Mexico, did not show differences in open days (OD) and calving intervals (CI) between IBR seropositive and seronegative cows. Our results did not agree with the results of Magana-Urbina *et al.* (2005). Although the difference in conception rate in our study was close to the results published by Magana-Urbina *et al.* (2005), there are some differences in our findings, specially concerning the open day period. Our study showed that BHV-1 serologically positive cows required a longer time to conceive. It is reported that a Holstein cow is profitable if she has a CI of 12 to 13.5 months, and if milk yield exceeds 13.500 kg milk per lactation and the number of OD is lower than 90 (Jainudeen and Hafez 2000). Among the studied animals the average OD period in serologically positive cows was longer than 90 days.

During the study, no clinical signs related to the disease were detected. This is consistent with observations of Magana-Urbina *et al.* (2005). The lack of match between clinical BHV-1 manifestations in disease outbreaks and our findings might possibly be related to the fact that our study was conducted under conditions of natural disease evolution. This suggests the possibility that animals in contact with the virus might have developed immunity, resulting in no further abortions, since for abortions to occur an antibody free status would be necessary (Anonymous, 1986). Clinical manifestations will also depend on other factors such as the strain involved, and the virus dose size. The evolution history of the virus should also be considered, since it determines the transformation of pathogenic strains with different virulence characteristics, as well as the host's immune status, that determines both virus shedding time and magnitude.

The better relative reproductive performance of serologically-positive heifers could have been due to the fact that they possess antibodies against the BHV-1 strain that is already present in the herd, so that reinfections could be better controlled by these animals with improved cellular/humoral immunity as to prevent clinical signs (Van Donkersgoed and Babiuk 1991).

In this study, we have described the fertility losses associated with a natural subclinical infection of BHV-1 in commercial dairy cows, but not in heifers in Burdur. In our study we found that BHV-1 seropositive cows have required about

17 days longer time than seronegative cows to conceive. This difference causes both longer calving interval and economic losses.

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### UTICAJ SUBKLINIČKE INFEKCIJE BOVINIM HERPES VIRUSOM TIPA 1 NA PLODNOST JUNICA I KRAVA

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#### SADRŽAJ

Cilj ovih ispitivanja je bio da se serološki odredi zastupljenost infekcije goveđim herpes virusom 1 (BHV-1) i utvrdi da li prirodne subkliničke infekcije dovede do smanjene plodnosti kod mlečnih krava i junica u oblasti Burdur, Turska. Zastupljenost BHV-1 infekcija je bila 11,94% kod krava i 14,6% kod junica. Stepenn koncepcije je bio veći kod krava koje su bile serološki negativne na BHV-1 (38,98%) u odnosu na serološki pozitivna grla (33,33%), ali ova razlika nije bila statistički značajna. Međutim, na suprot ovome, stepenn koncepcije je bio veći kod serološki pozitivnih junica (84,61%) u odnosu na negativne jedinke (55,57%). Ova razlika takođe nije bila statistički značajna. Prosečna dužina servis perioda je kod serološki pozitivnih krava iznosila  $99,3 \pm 16$  dana, a kod negativnih je bila manja ( $82 \pm 3,8$ ). Naši rezultati ukazuju da je kravama koje su serološki pozitivne na BHV-1 virus potrebno 17 dana više do momenta uspešne koncepcije.