Praca oryginalna *Original paper*

Effects of subclinical bovine leukemia virus infection on fertility of Holstein cows and heifers

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Summary

The purpose of this study was to determine the serological prevalence of bovine leukemia virus (BLV), and describe whether there are effects of BLV infection on the fertility of dairy cattle in Burdur, Turkey. The study population was 182 clinically healthy Holstein cows (>2-years-old) and 81 heifers (17- to 20-months-old) from a commercial dairy herd in Burdur. BLV prevalence was 66.48% (121/182) and 62.96% (51/81) in cows and heifers, respectively. There were no significant differences between BLV antibody-negative and antibody--positive cows for days open (DO; P = 0.2567) or between BLV antibody-negative and antibody-positive heifers for first service age (FSA; P = 0.65). Moreover, differences in conception rates (CR) between BLV antibody-negative and antibody-positive cows or heifers were insignificant (P > 0.1). In conclusion, even though the prevalence of BLV infection in Burdur region was found to be high, no effect of BLV infection was detected for fertility.

Keywords: bovine leukemia, dairy cows

Bovine leukemia virus (BLV), an exogenous C-type retrovirus with worldwide distribution, is causative agent of enzootic bovine leucosis (EBL) (21). The frequency of BLV infection was higher in dairy cows than beef cattle (20) and epidemiologic studies of BLV infection in individual dairy cattle herds have yielded prevalence rates as high as 95% (14, 16).

Bovine leucosis virus causes significant losses to the dairy cattle industry. More and more data are emerging to indicate that BLV infection by itself can cause reduction in productivity and shorter life span among highly qualified milk cows (3). Considering animal-health aspects and economical loss due to infection, elimination of the infection from the herds is very crucial. Clinical manifestations of BLV infection may result in economic losses due to decreased milk production, poorer reproductive efficiency, increased morbidity, reduced weight, deaths, and condemnations at slaughter (9). Although the relationship of BLV infection with specific subclinical and clinical disorders is well established, several studies have found no substantial differences in various measures of production or reproduction between BLV infected and uninfected dairy cows (3, 17).

Therefore, the purpose of the present study was to determine the serological prevalence of bovine leukemia virus infection in Burdur region and whether it may have any effect on fertility of seropositive dairy cows or heifers.

Material and methods

Farm. The samples of the current study comprised 263 Holstein-Fresian cows and heifers, kept in a dairy farm in Burdur (southwest Turkey) with average annual milk production of 6,000 L per cow. Animals were in free stall barns with intensive contact among them.

Animals. Cows over 2 years old were included in the current study. Heifers were between 17-20 months old. Prior to study, reproductive status of any given cow was determined by rectal palpation and calf condition was recorded. All the cows had calved at least 50 days prior, and they were not pregnant at sampling time. All the animals in this study were examined vaginally, and were healthy and free of anatomical abnormalities of the reproductive tract. Body condition scores (BCS) were obtained according to Loeffler et al. (19). To exclude the possible effects of reproduction problems related to nutrition deficiency, cows and heifers lower than 2.5 BCS were not included in the study. During the study period none of the cows and the heifers exhibited any overt clinical signs of BLV or any other disease. None of the animals had ever been vaccinated against BLV.

Data collection and artificial insemination (AI). Information regarding the herd and each animal sampled were recorded through a personal interview with the farm managers. AI was the first time after postpartum in all cows and first insemination for all heifers. AI dates and presence of conception following 6-8 weeks insemination by rectal palpation records were recorded by the inseminator. All inseminations were performed on the day spontaneous estrus by the same experienced veterinarian using BLV free (1) frozen-thawed semen from a single bull (Raul 242 GP 82) with proven fertility. Semen contained at least ten million of motile spermatozoa (Consorzio Semenzoo, Italy Via Masaccio, 11-42010 Reggio Emilia, Italy). The stage of estrus cycle was determined by rectal palpation and observation of secondary signs of estrus. The insemination coincided with middle of estrus, as evidenced by cervical mucous discharge (CMD) and high myometrial tone and contractility. Semen was placed into the corpus uteri for all cows and heifers.

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

Serology. Blood samples from cows and heifers were collected from the jugular vein. Blood samples were collected into tubes containing no anticoagulant. Blood samples, then, centrifuged at 2000 rpm for 20 min. for sera collection. Sera were kept at –70°C until use. BLV antibodies were determined using a commercial available ELISA kit (BLV-ELISA Antibody Test Kit, VMRD Inc., USA). Test was carried out as described by manufacturer.

Statistical analysis. The differences in days open (DO) for cows and first service age (FSA) for heifers in BLV seropositive and BLV seronegative and pregnant-non pregnant groups were compared by Proc Mixed procedure of SAS. Conception rate (CR) was compared by FREQ and LOGISTIC procedure of SAS.

Results and discussion

Conception rates and test results of animals were represented in tab. 1. Out of 263 animals (182 cows and 81 heifers), prevalence of BLV infection for cows and heifers was 66.48% (121/182) and 62.96% (51/81), respectively. Prevalence of BLV was greater in cows older than 4 years old (26.4%) and heifers older than 18 months old (23.5%). Difference in CR between BLV antibody-negative and antibody-positive cows or heifers was not significant. In addition, no difference was detected between seropositive and infection free cows or heifers for DO (P = 0.2567) or FSA (P = 0.6523) (tab. 2).

Risk factors associated with the natural transmission of BLV infection such as herd size, management procedures, type of production, breed, age, parity, population density and seasonal influences have been studied (25). In the current study, the prevalence of BLV seropositive cows (66.48%) and heifers (62.96%) was higher than previous studies in different cities of Turkey (33.08% in Burgu et al. (4); 5.91% in Batmaz et al. (2); and 18.84% in Yavru et al. (27)). Prevalence rates of this high probably occur infrequently. Although Johnson and Kaneene (15) concluded that the prevalence of BLV infections in cattle under 17-24 months of age is much lower than in adult cattle, in our study the seropositive heifers for BLV infection was higher and 62.96% (51/81). Owing to use of BLV-free semen in the current study, the risk of BLV contamination due to artificial insemination is doubtful. The reason for higher incidence could be due to different reasons: animals in this farm were housed in free stall barns with intensive contact among animals, cows and heifers were kept together, the hygiene for the barn was mediocre, newborns were fed by milk from mixed cows, and the location of the herd was very close to Lake Burdur.

Prevalence of BLV is also influenced by age and seropositivity increases in older cows (18). In current study, the percent of seropositive cows were higher in group of animals that were 4 year old (26.4%). In addition, 89.2% of all seropositive cows were aged 4 years or older. Similarly, the percent of seropositive heifers were higher in group of animals that were 18 months of age (23.5%) and 67.2% of all seropositive heifers were 18 months of age or older. Similarly, occurrence of BLV infection was found to be higher for cows 4 year old or older (24) and for heifers 18 months or older (5). Increasing age may increase the prevalence

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

Animals	BLV seropositive (n = 172)	BLV seronegative (n = 91)	
Pregnant cows	49 (40.5%)	23 (37.7%)	ns
Non-pregnant cows	72 (59.5%)	38 (62.3%)	ns
Pregnant heifers	33 (64.7%)	16 (53.3%)	ns
Non-pregnant heifers	18 (35.3%)	14 (46.7%)	ns

Tab. 2. Reproductive parameters of cows and heifers with BLV seropositive and seronegative ($\bar{x}$ ± SE)

Parameters	BLV seropositive	BLV seronegative	P=
DO for Cows*	111.68 ± 6.18 (n = 121)	116.73 ± 8.72 (n = 61)	0.2567
FSA for Heifers**	534.84 ± 10.23 (n = 51)	540.0 ± 13.42 (n = 30)	0.6523

Explanations: * – days open (day), ** – verage of first service age (day)

of BLV infection. The longer an individual remains in a herd with BLV-infected herdmates, the greater the likelihood of occurrence of sufficient contact to result in transmission and infection (11). These findings were significant in that they formed the basis for the hypothesis that the initiation of close physical contact and intensive management practices associated with the increased human intervention in cattle beginning at 1.5 to 2.0 years are important risk factors in transmission of BLV infection (26).

Even though the prevalence of BLV was high in the current study, general averages for DO for serologically BLV positive cows did not differ from those of BLV negative cows. Similar to our findings, Huber et al. (10) reported no significant difference ($p > 0.05$) in DO between BLV positive (91.7-134.7) and negative (91.7-130.3) cows. In another study with 2079 dairy cattle, the mean DO for BLV seropositive and seronegative dairy cows were 101 and 114.3 days, respectively (12). Although, Pollari et al. (23) reported that BLV seropositive cows were bred more times and had longer calving than BLV seronegative cows, there was no statistical difference in DO between two groups ($p > 0.05$). Several studies (6, 10) have found no significant differences between BLV antibody positive and antibody negative cows with regard to ages at first calving, length of most recent calving interval, mean length of calving interval, number of DO and number of times bred, although a tendency to a greater number of days between calving and conception has been reported in BLV positive cows (7). Recently, Jamrozik et al. (13) reported that mean FSA was 499.7 ± 53.6 day in Holstein heifers ($n = 53{,}158$). General averages of FSA for Heifers in the current study were similar to the previous report (13) and FSA of BLV serologically positive Holstein heifers (534.84 ± 10.23) were not different from those of BLV negative Holstein heifers (540.0 ± 13.42).

Similar to the findings previously reported (3, 8, 12), in the current study no difference in CR were detected neither in cows nor in heifers due to BLV. Conception rates for BLV seropositive cows and heifers were 40.5% ($n = 49$) and 64.7% ($n = 33$), respectively. It is suggested that although subclinical BLV infection has little or not effect on reproductive efficiency, these animals must often culled from the herd sooner than their negative counterparts (22). However, the culling rates are very low in Burdur and high producing cows tend to stay in the herds very long time periods. Thus, according to the results of our study, the BLV infection in this dairy herd did not adversely influence fertility parameters in Burdur.

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