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RESEARCH ARTICLE

Investigation of Relationship Between Bovine Herpesvirus-1 (BHV-1) Infection and Fertility in Repeat Breeding Dairy Cows in Family-Type Small Dairy Farms

Ayhan ATA * Mesih KOCAMÜFTÜOĞLU ** Sibel HASIRCIOĞLU ***
Mehmet KALE *** Mehmet Şükrü GÜLAY ****

* Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Deparment of Theriogenology and Artificial Insemination, TR-15030 Burdur - TURKEY
** Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Deparment of Gynaecology and Reproduction, TR-15030 Burdur - TURKEY
*** Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Deparment of Virology, TR-15030 Burdur - TURKEY
**** Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Deparment of Physiology, TR-15030 Burdur - TURKEY

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Summary

The aim of this study was to determine the seroprevalence of Bovine Herpesvirus-1 (BHV-1) and investigate the relationship between BHV-1 infection and fertility in Repeat Breeding (RB) dairy cows in Burdur province, Turkey. In this study, blood serum was collected from 108 RB dairy cows and tested by enzyme linked immunosorbent assay (ELISA) against BHV-1 antibodies (Ab). The prevalence of BHV-1 among RB dairy cows was 9.25% (10/108). The average days open (DO) for BHV-1 seropositive RB dairy cows was significantly longer than that of BHV-1 seronegative RB dairy cows (P<0.01). However, neither the age (P=0.053) nor conception rate (CR) (P=0.361) of the animals were significantly affected due to BHV-1 infection. The average time intervals between calving and the fourth insemination in non-pregnant seropositive BHV-1 dairy cows were higher than that of non-pregnant BHV-1 seronegative dairy cows (P<0.01). In addition, the average ages of pregnant BHV-1 seropositive and seronegative RB dairy cows differed significantly (P<0.05). Thus, results of the current study concluded that there is a close negative relationship existed between BHV-1 infections and DO in family type small dairy farms in Burdur. BHV-1 infection increased the calving interval and possibly caused economic losses in RB dairy cows. Moreover, further prospective studies with more number of RB dairy cows are needed to evaluate the relationship between BHV-1 infections and fertility in family type small dairy farms.

Keywords: *BHV-1, Dairy cow, Repeat breeding, Fertility*

Küçük Ölçekli Aile İşletmelerindeki Repeat Breeding Sütçü İneklerde Bovine Herpesvirus-1 (BHV-1) Enfeksiyonunun Fertilite İle İlişkisinin Araştırılması

Özet

Bu çalışmanın amacı Burdur ilindeki Repeat Breeding (RB) sütçü ineklerde Bovine Herpesvirus-1 (BHV-1) enfeksiyonunun seroprevalansını tespit etmek ve fertilite ile ilişkisini araştırmaktır. Çalışmada 108 RB sütçü inekten kan serumu toplandı ve BHV-1'e karşı şekillenen antikorlar yönünden enzyme linked immunosorbent assay (ELISA) ile test yapıldı. BHV-1 in prevalansı RB sütçü ineklerde %9.25 (10/108) olarak tespit edildi. Ortalama servis periyodu (SP) BHV-1 seropositive RB ineklerde, BHV-1 seronegative RB ineklerden istatistiksel olarak daha uzun (P<0.01) bulundu. Bununla beraber RB ineklerde ne yaş (P=0.053) ne de gebelik oranı (P=0.361) BHV-1 enfeksiyonundan etkilenmedi. Gebe olmayan gruptaki BHV-1 seropositive RB ineklerin doğum dördüncü tohumlama tarihi arasındaki zaman aralığı, gebe olmayan gruptaki BHV-1 seronegative RB ineklerden daha yüksek (P<0.01) olarak bulundu. İlave olarak, gebe gruptaki BHV-1 seropositive RB ineklerin ortalama yaşı ile gebe gruptaki BHV-1 seronegative RB ineklerin yaşı arasındaki farklılık önemli (P<0.05) olarak bulundu. Bulgulara göre Burdur yöresindeki aile tipi işletmelerdeki RB ineklerde BHV-1 enfeksiyonu ile SP arasında önemli bir ilişki bulunmaktadır. Bu işletmelerde BHV-1 enfeksiyonun RB ineklerde iki buzağılama arası sürenin uzamasına yol açarak ekonomik kayba neden olmaktadır. Ayrıca aile tipi küçük sütçü işletmelerde BHV-1 enfeksiyonu ile fertilite arasındaki ilişkinin ayrıntılı değerlendirilebilmesi için daha fazla hayvanı kapsayan çalışmalara ihtiyaç bulunmaktadır.

Anahtar sözcükler: *BHV-1, Sütçü inek, Repeat breeding, Fertilite*

İletişim (Correspondence)
+90 248 2132000
ayhana@mehmetakif.edu.tr

INTRODUCTION

Repeat Breeding (RB) has been described as a major concern in dairy cattle throughout the world [1]. There are many diseases and disturbances held responsible for RB. The causes can be management, nutritional, genetic origin, and hormonal as well as infectious agents. All cows with RB share an almost equal increase in fertilization failure and an increase in embryonic loss [2]. Moss et al (2002) [3] reported that days to first recorded heat were associated negatively with RB syndrome in multiparous cows. In Sweden about 10% of the dairy cows are culled annually because of reproductive failure, the main reason for this being RB [4].

Bovine Herpesvirus-1 (BHV-1) is a member of the genus *Varicellovirus* within the subfamily *Alphaherpesvirinae*, which belongs to the family *Herpesviridae* [5]. This virus causes respiratory disease (Infectious Bovine Rhinotracheitis, IBR) in cattle, genital diseases (Infectious Pustular Vulvovaginitis, IPV) in females or in males (Infectious Pustular Balanoposthitis, IPB) and other clinical syndromes such as conjunctivitis, metritis, mastitis, encephalitis, abortion, enteritis, loss in milk production and generalized systemic infections. Many infections run with a subclinical course [6]. Bovine Herpesvirus-1, like other alpha-herpesviruses, can cause a latent infection in the neurons of the sensory ganglia [7]. Latent BHV-1 can be reactivated and re-excreted at irregular intervals due to stressful conditions or corticosteroid treatment. Therefore, cattle latently infected with BHV–1 can also be a source of infection [8].

Bovine Herpesvirus-1 infections cause substantial economic losses such as weight loss, decrease in milk production, abortion, neonatal and embryonic death, stillbirth and fertility disorders such as endometritis and RB cow [9,10]. The most important reproductive disorders that the genital form of disease causes in infected cows are necrotizing endometritis and necrotizing oophoritis. Also, these disorders can lead to the problems of RB and infertility in infected cows [11]. Researchers reported that infertility problems have been associated with BHV-1 infections [10,12]. The virus may be spread within cattle populations via contact, aerosol, fomites and via infected semen, ova or embryos [13]. Miller [10] reported that the use of semen contaminated with BHV-1 for natural/artificial insemination would probably cause temporary infertility, lasting one to two weeks. Bovine Herpesvirus-1 may result in various clinical consequences, including severe respiratory disease, venereal disease with reduced reproductive performance and abortion [14].

There are about 19.000 small family operated dairy farms and 150.000 Holstein dairy cows were present in Burdur provenance. Number of dairy cows per dairy farms is between 2 and 200. Although, there are number of studies describing the relationship between BHV-1 infection and fertility in large dairy operations, limited data is available the effect of BHV-1 infection in small family dairy farms. Thus, the aim of this study was to determine the serological prevalence of BHV-1, and describe whether natural subclinical infections would lead to fertility losses BHV-1 infections on RB dairy cows in small family operated dairy farms of Burdur province.

MATERIAL and METHODS

Animals

A total of 108 (2 to 10 year old) Holstein-Freisian RB dairy cows were selected from 30 different dairy farms in the province of Burdur, Turkey. Numbers of animals in these dairy farms were between 2 and 10. The current definition of the RB animal is despite being inseminated three times or more they did not become pregnant (but returning to oestrus within normal intervals); they had no apparent pathologies explaining the RB, neither at palpation per rectum or at ultrasound examination of the genital tract [15,16]. Annual average milk yields and mean body condition score (BCS) RB dairy cows were 5.210±180.2 kg, 3.10±0.15, respectively. Body Condition Score of the dairy cattle was evaluated following the guidelines for grading the cattle on a 5 point basis [17] 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 BHV-1 prior to the sampling period. All serum samples were tested by enzyme linked immunosorbent assay (ELISA; Institut Pourquier, France) for the presence of BHV-4 antibodies (Ab) (Bio-X Diagnostics, Belgium), Bovine Viral Diarrhea Virus (BVDV) Ab (IDEXX BVDV Total Ab Test, USA), BVDV antigen (Ag) (IDEXX BVDV Ag Test, USA), Bovine Leukosis Virus (BLV) Ab (IDEXX Leukosis Serum Screening Ab Test) and all samples were free from BHV-4, BVDV and BLV.

Oestrus Detection, AI, and Pregnancy Checks

The RB dairy cows were checked for the signs of the oestrus: excitement, vocalization, licking, lordosis, oedema and redness of the vulva, and the presence of cervical mucus discharge (CMD). Artificial insemination (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 postpartum in all cows. All inseminations were performed by the same experienced veterinarian using BHV-1 free frozen-thawed semen containing at least ten million of motile spermatozoa (Vanzetti Valent Raul Et, Consorzio Semenzo-Italy via Masaccio, 11- 42010 Mancasale/Italy) obtained from a single bull with proven fertility. The stage of the estrus was determined by rectal palpation and observation of the secondary signs of estrus. The

insemination coincided with mid-estrus, as evidenced by CMD and high myometrial tone and contractility. 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. Days open (DO) was calculated as the time period between calving and conception in a given cow.

Antibody Detection in Serum Samples

The presence of antibodies to BHV-1 in serum samples was detected by a BHV-1 ELISA kit (Institut Pourquier, France).

Statistical Data Analysis

The differences between BHV-1 seropositive and BHV-1 seronegative cows with respect to the average number of DO and ages were compared using the *Proc Mixed* procedure of SAS. The conception rate (CR) for cows was compared using the FREQ and LOGISTIC procedures of SAS. Risk estimation among BHV-1 and conception was evaluated using odds ratio (OR).

RESULTS

Bovine Herpesvirus-1 seroprevalence was 9.25% (10/108) in RB dairy cows in Burdur province. Bovine Herpesvirus-1 positivity was detected only in four out of 30 herds and antibody values in those farms were 40.00% (2/5), 100.00% (2/2), 25.00% (2/8), and 57.14% (4/7). Conception rate was higher in BHV-1 serologically negative cows (55.10%) than BHV-1 serologically positive RB dairy cows (40.00%) but was not statistically significant (P>0.05). The average for DO of BHV-1 serologically positive RB dairy cows (344.00±35.11 d) was different from those of BHV-1 serologically negative cows (184.37±5.96 d) and was statistically significant (P<0.01). The difference for the averages calving to fourth insemination date interval between seropositive and seronegative non-pregnant RB dairy cows was significant (P<0.01). Days open of seropositive and seronegative pregnant RB dairy cows was not statistically different (P=0.114). Moreover, no significant difference was detected between ages of seropositive and seronegative RB dairy cows. However, this difference between seropositive and seronegative pregnant RB dairy cows was significant (P<0.001). The difference between the ages of seropositive and seronegative non-pregnant animals was statistically insignificant (P=0.091). Reproductive parameters of BHV-1 seropositive and seronegative RB dairy cows are presented in *Table 1*.

DISCUSSION

Bovine Herpesvirus-1 is a threatening disease affecting the genital tract and causing infertility in cows. Consequently, it causes substantial production and economic loss in the livestock/dairy cattle industry. Up to now, several studies have been conducted detecting the relationship between BHV-1 infections and infertility problems in cows (especially RB cows). Çabalar and Akça [18] collected 624 serum samples from cows with infertility problems (Repeat Breeding, metritis, abortion) and tested for the presence of BHV-1 Ab by microneutralization assay. They reported that 425 (68.1%) of them positive for antibodies to BHV-1, and BHV-1 virus

Table 1. *Reproductive parameters of RB dairy cows with BHV-1 (seropositive and seronegative)*
Tablo 1. *BHV-1 (seropozitif ve seronegatif) RB sütçü ineklerin üreme parametreleri*

Parameters	BHV-1 (seropositive) n=10	BHV-1 (seronegative) n=98	P
COWS			
DO [a]	344.00±35.11	184.37±5.96	P<0.001
Age (day)	2448.00±316.58	1738.78±58.57	P=0.053 NS*
CR [b]	40.0	55.10	P=0.361 NS
Parameters	n=4	n=54	
PREGNANT COWS			
DO [a]	319.25±60.00	187.03±8.61	P=0.114 NS
Age (day)	1800.00±48.05	1586.67±55.91	P<0.001
Parameters	n=6	n=44	
NON-PREGNANT COWS			
CFIDI [c]	373.28±40.97	182.14±9.00	P<0.01
Age (day)	2880.00±455.37	1925.45±105.05	P=0.091 NS

*[a] Day's open (DO) (day), [b] Conception rate (CR) (%), [c] Calving to fourth insemination date interval (CFIDI), * **NS**= Not significant. Mean values were expressed as mean ± SE*

may be an etiological agent in infertility problems of cows since high incidence against BHV-1 had been detected in the herds. Özkul et al.[19] collected 538 serum samples from cows with infertility problems (Repeat Breeding, abortion, metritis) which were housed in 19 closed dairy herds and tested by serum neutralisation (SN) for the presence of neutralizing antibodies against BHV-1. They found that 113 of the 528 (21%) serum samples tested for BHV-1 were seropositive. They reported that the BHV-1 virus might cause infertility in cows produced in closed dairy herds. Bulut et al.[20] collected serum samples from 120 RB cows and 85 healthy cows to investigate the frequency of BHV-1 infections in RB cows and test by ELISA. They found that 85 (70.8%) of 120 RB cows and 59 (73.7%) of 85 healthy cows were seropositive for BHV-1. In another study, 10 out of 139 cows (7.2%) were reported as BHV-1 antibody positive in RB dairy cows, and there was no significant relation between seropositivity of BHV-1 and RB [21]. Biuk-Rudan et al.[22] investigated the prevalence of antibodies to BHV-1 and BVDV in dairy cows (mostly RB cows) with reproductive disorders in four different farms. For this purpose, they collected serum samples from 73 cows with reproductive disorders and 47 cows without reproductive disorders and examined them for BHV-1 and BVDV by SN. They found that the seroprevalance of BHV-1 was 8.2% (6/73) in cows with reproductive disorders whereas seroprevalance of BHV-1 was 14.9 (7/47) in cows without reproductive disorders. In all cows tested, they found that seroprevalance of BHV-1 was 85.8%. In addition, they reported that 80.8% of cows with reproductive disorders and 46.8% cows without reproductive disorders had antibodies to both BHV-1 and BVDV. They stated that differences in antibody prevalance among groups with/without reproductive disorders was significant and suggested a connection between reproductive disorders and simultaneous infections with BHV-1 and BVDV in dairy cows. In the current study, the seroprevalence of BHV-1 in RB dairy cows was 9.25% (10/108). Although this rate agreed with another roport [22], it was lower than the other findings reported by Çabalar and Akça [18]. That is why the sensitivity of tests used as well as other factors such as the structure and size of herd sampled, individual and regional differences.

Magana-Urbina et al.[23] investigated the risk factors affecting serological prevalence such as age, flooring type, origin of replacement heifers (same location, different location), herd size, and abortion history. They reported that the highest risk of BHV-1 positive serology was in animals >4 years, in herds with >10 cows and in herds that replacement heifers come from other locations. However, they concluded that BHV-1 serological prevalence increased as animal age increased. The results of the current study did not agree with the findings of Magana-Urbina et al.[23]. We found that ages of seropositive and seronegative cows was insignificant (P=0.053). Similar to the reports of Magana-Urbina et al.[23] all seropositive cows were aged 4 years and older in our study. Another reason for lower BHV-1 seroprevalence in the current study could be attributed to the smaller herds with low population densities (generally herds with <10 cows) and very low cow transfers among the herds.

Miller et al.[24] and Chiang et al.[25] have described a decrease in the CR of initially seronegative animals after experimental BHV-1 infection. Elazhary et al.[12] reported that the reason of the low CR and the high number of services per pregnancy in a herd having abortion and infertility problems could be due to the virus infection of the embryo resulting in its death and absorption in the early stage of pregnancy. However, Hage et al.[9] reported that it was doubtful that early pregnancies were terminated by BHV-1 infection although they detected a significant decrease in the number of successful insemination in seronegative and seropositive dairy cows in their study. Allan et al.[26] concluded that BHV-1 infection had no effect on pregnancy. In another study, the difference for CR among these groups was statistically insignificant although CR was higher in BHV-1 serologically negative cows than BHV-1 positive cows in Burdur province [27]. They also concluded that BHV-1 seropositivity had no effect on CR of cows [27]. In the same study, there was a difference for DO between seropositive and seronegative cows and BHV-1 serologically positive cows required a longer time to conceive [27]. It has been reported that the Holstein cow is profitable if she has a calving interval of 12 to 13.5 months, if milk yield exceeds 13.500 kg milk per lactation and the ideal number of DO is 90 [28]. Among animals examined herein, the number of DO in seropositive and seronegative cows was longer than 90 days. Similarly, the average DO in RB cows reported by Al-Hassan [29] and Ata [16] were 183.2 and 199 days, respectively.

In conclusion, the results of this study showed that there was a close relation between BHV-1 infections and reproduction in RB cows. Seropositivity for BHV-1 in these animals affected the calving interval negatively. The DO period was longer in seropositive RB dairy cows than seronegative RB dairy cows. Seropositivity for BHV-1 also prolongs the age at pregnancies in seropositive RB dairy cows for more than 200 days. Thus, BHV-1 infections can cause substantial economic losses due to a prolonged calving interval. However, further studies with more number of RB dairy cows are merited to better understand the relationship between BHV-1 infection and reproduction in family operated small dairy farms.

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