Prevalence of bovine viral diarrhoea, bovine herpesvirus type 1 and 4 infections in repeat breeding cows in Western Turkey

Prevalência de infecções por Vírus da Diarreia Viral Bovina e Herpesvírus Tipos 1 e 4 em fêmeas bovinas repetidoras do cio na região oeste da Turquia

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Abstract

Bovine Viral Diarrhoea Virus, Infectious Bovine Rhinotracheitis and Bovine Herpesvirus Type 4 infections are widely known viral infections as the reproductive system pathogen in cattle. The purpose of this study was to investigate the presence and prevalence of these infections in Repeat Breeding diagnosed cows. A total of 139 blood samples were collected from nine different dairy herds in Western Turkey. All sampled animals were clinically healthy but not become pregnant even though they were three to eleven times artificially inseminated. The samples were controlled serologically for BHV1 and BHV4 using ELISA test, 2% and 3.9% proportions were detected, respectively. These values were found to be very low in comparison to other studies in Turkey. Out of 139, 81 (58.2%) cows were found to be seropositive for BVDV, and the percentage was above the 70% in six herds. BVD antigen was detected in two animals in a herd with 71.4% antibody rates. Antigen positive animals were sampled and tested again four weeks later and persistent viremia were confirmed. In conclusion, BVDV infection may be responsible for repeat breeding in studied herds.

Keywords: Bovine viral diarrhea. Bovine herpesvirus type 1 and 4. Repeat breeding.

Resumo

Infecções por Vírus da Diarréia Viral Bovina (BVDV), Vírus da Rinotraqueíte Infecciosa Bovina (BHV-1) e Herpesvírus Tipo 4 (BHV-4) são amplamente reconhecidas como infecções do trato reprodutivo em bovinos. O objetivo deste estudo foi investigar a presença e a prevalência destas infecções em fêmeas bovinas repetidoras de cio. Um total de 139 amostras de sangue foi colhido de nove diferentes propriedades leiteiras na região Oeste da Turquia. Todos os animais amostrados estavam clinicamente saudáveis, embora não tenham sido enxertados por inseminação artificial após três a onze tentativas. As amostras de soro foram testadas por método ELISA para detecção de anticorpos anti-BHV-1 e BHV-4, e 2,0 e 3,9% das amostras, respectivamente, foram consideradas positivas. Estes valores foram considerados baixos quando comparados com os de outros estudos na Turquia. De 139 amostras, 81 (58,2%) foram positivas para a presença de anticorpos anti-BVDV e mais de 70% das propriedades foram reveladas positivas. O antígeno de BVDV foi detectado em dois animais em uma propriedade em que 71,4% dos animais eram soropositivos. Animais positivos para a presença de antígenos foram amostrados e testados novamente quatro semanas depois e viremia persistente foi detectada. Concluindo, infecção por BVDV pode ser responsável pelas perdas reprodutivas nas propriedades estudadas.

Palavras-chave: Diarreia viral bovina. Herpesvírus bovino tipo 1 e 4. Repetição de cio.

Introduction

Decline in fertility creates huge economical loss especially in dairy industries. Repeat breeding is one of the most important reproductive disorders in cattle. A "Repeat-breeder" generally defines as the failure for conception after three or more inseminations. Anatomical disorders in the female reproductive tract which are well known cause to repeat breeding syndrome. Genetic, physiological and hormonal disorders along with failure in oestrous detection, embryonic deaths and infectious diseases are also common causes for repeat breeding syndrome.

Infectious agents (viral, bacterial and fungi) can cause a decrease in the rates of ovulation, fertilization,

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embrionic-foetal and/or perinatal survival. Bovine Viral Diarrhoea/Mucosal Disease and Infectious Bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis (Bovine Herpesvirus type 1) infections are one of the important viral agents causing reproductive problems in cattle. In last decades, Bovine Herpesvirus Type 4 was also recognized as the pathogen for reproductive system.

BVD/MD complex disease[1,2] is caused by pestiviruses that are classified in the *Flaviviridae* family. The agents are enveloped and include a positive sense single stranded RNA genome with a length 12.3kbp[3].

Pathological features of Bovine Viral Diarrhoea are complicated with the interactions of two biotypes, cyto-pathogenic (cp) and non-cytopathogenic (ncp) at the stage of pregnancy. The virus can penetrate the placenta and infect with cp strain in the first trimester. The cp infection generally causes to abort or foetal resorption while foetal development continues in the ncp strain BVDV infections. These animals may be born clinically normal but they will be persistently infected (PI) for the rest of their lives[4]. PI individuals may be born with low birth weight, and they have growth retardation and low level of immunity. Therefore, they are vulnerable to other infectious diseases[5,6,7].

Infectious Bovine Rhinotracheitis is caused by bovine herpesvirus type 1 (BHV1) in *Alphaherpesvrinae* subfamily, *Varicellovirus* genus. Based on genome analysis and viral polypeptide patterns, five subtypes have been determined until now. Type 1 and 2a are responsible for respiratory form of the infection, where as 2b mainly causes many infections such as infectious pustular vulvovaginitis (IPV) and infectious pustular balanopostitis (IPB). Subtype 3a and 3b cause encephalitis[8]. Abortion mostly occurs in the second half of the gestation. Fetal death and absorption in the early pregnancy may be evaluted as infertility.

Bovine Herpesvirus type 4 (BHV4) was classified as a Gamaherpesvirus. Restriction endonuclease analysis showed that the virus was divided into two groups: Movar 33/63-like viruses were isolated from Europe, and DN 599-like viruses were isolated from North America[9,10,11]. The main host for the BHV-4 is cattle, but several ruminant species are susceptible to BHV-4. The virus was isolated from both healthy and sick animals[12,13] with respiratory and ocular symptoms[14]. Other prominent clinical findings are pneumonia, respiratory disorders, diarrhoea, mastitis, mammary pustuler dermatitis, abortion, metritis, vulvovaginitis, encephalitis, skin lesions, and tumors in the urinary bladder and the rumen[15,16].

The clinical diagnosis for herpesviral infections is generally not possible due to latent character of the virus but it can be reactivated using dexametasone treatment[17]. Virus neutralisation test have been routinely used for the detection of BHV-1 and BVDV specific antibodies but BHV-4 infection cause a lower level antibody titer[15]. Moreover, this test was not reliable. Therefore a more sensitive test is required for BHV-4 such as ELISA[18].

The prevalence of BVD and IBR infections is very high in Turkey. The virus isolations as well as serologic studies showed that the incidence may reach up to 96.8% for BVD[19] and 100% for IBR in some herds[20]. However there is quite limited data for BHV4[21]. The objective of this study was to investigate the role of BVDV, BHV1 and BHV4 infections in repeat breeding diagnosed cows in the herds with high rate infertility problems in Western Turkey.

Material and Method

Sample Collection

In this study, total of 139 cows were sampled from nine organized dairy herds in Aydin and Afyonkarahisar provinces in Western Turkey (Table 1). The numbers of breeding cattle in surveyed herds were around 46 and 240 and all herds were found negative for *Brucella spp.* and *Tuberculosis*. The herds 1-4 and 6

Table 1 - The test results for BHV 1, 4 and BVDV infections

Herd No	Herd size	No of Sample	Average AI No*	BHV1		BHV4		BVDV		BVDV	
				Ab**	%	Ab	%	Ab	%	Ag***	%
1	240	24	5.2	-	-	4	16.6	9	37.5	-	-
2	86	5	6	-	-	1	20	5	100	-	-
3	131	21	3.9	5	23.8	-	-	15	71.4	2	11.1
4	98	18	4.3	-	-	-	-	18	100	-	-
5	46	7	5	-	-	-	-	6	85.7	-	-
6	91	4	5.2	-	-	-	-	3	75	-	-
7	154	13	5	3	23	-	-	12	92.3	-	-
8	79	15	3.2	-	-	-	-	2	13.3	-	-
9	57	32	5.8	2	6.25	-	-	11	34.3	-	-
Total	**982**	**139**	**4.8**	**10**	**7.2**	**5**	**3.9**	**81**	**58.2**	**2**	**1.4**

AI No: Artificial insemination, **:Antibody, ***:Antigen

were kept under investigation for two years since the selected animals had been bought from different places in a province. The previous health records for investigated animals except for the last two years could not obtained for the most of the cows in these herds. The herds 5, 7-9 were also organized enterprises and all the health records were obtained. No vaccination was performed for BVDV, BHV 1 and 4 during in the last two years period in any of the studied herds.

The sampled herds were selected for their high reproductive problems especially for the higher rate of repeat breeding syndrome. The all "repeat breeding" diagnosed cows were examined rectally for ovarian disturbances. The animals had reproductive system disorders such as metritis and ovarian cyst were not used in this study. The selected cows for sampling appeared healthy but they did not become pregnant although they were artificially inseminated for 3 to 11 times. The mean numbers of insemination for each herd was given in table 1. According to the obtained previous health records, the numbers of birth for animals varied between 1 to 4, and no reproductive problems had been observed before in sampled animals.

The blood samples were collected by venopuncture from vena jugularis into two tubes containing EDTA and silicone, and transported to the laboratory at + 4 °C, and clotted blood samples were freshly tested on the day of sampling. The sera samples were centrifuged at 3000xg for 10 min. and separated into the stock tubes, and stored at - 20 °C until testing. The animals that were found to be BVD antigen positive were sampled again four week later to confirm persistent viremia.

Antibody and antigen detection

An indirect ELISA kit [BIO-X Abortion ELISA Kit (BIO K 072) Belgium] was used for sero-diagnosis of IBR\IPV, BVDV, and BHV-4 infections in cattle.

In the screening for the presence of the pestiviruses, a direct ELISA test (Institute Pourquier-France) was employed. The kit detects NSP 2-3 (P80) and E0 (= E^{ms} or gP48) of bovine pestiviruses in blood clots etc. Both of the tests were performed as the producers describe. The plates were assessed using an ELISA reader at 450nm and obtained results were calculated.

Results

The total samples of 139 cows diagnosed as "repeat breeding" in nine herds in two provinces (Afyonkarahisar and Aydin) were kept under control for IBR/IPV, BHV4 and BVD specific antibodies. IBR/IPV positivity was detected only in herds, 3, 7 and 9, and antibody values were found to be 23.8%, 23% and 6.25%, respectively. Of the 139 samples, 10 (7.2%) animals were found to be IBR positive. Proportions for BHV-4 was lower, positivity was detected only in five animals (3.9%) in herds 1 and 2.

The sampled cows in herds 4-6 and 8 were found to be negative for both IBR and BHV4.

The most prevalent infection was found to be BVDV. Specific antibodies were observed in all of the herds between 13.3% and 100% percentages, and six herds had above 70% antibody presence. In total, 81 (58.2) cows were found to be positive for BVD.

The BVDV antigen presence was determined in two animals in herd 2 and antibody value was 71.4% in this herd. Antigen positive animals were sampled and tested second time after four weeks of sampling to discriminate the persistent and acute infection and were found to be positive.

Discussion

IBR/IPV infection was reported in 1971 for the first time in Turkey, subsequently many serologic and virologic studies were performed all over Turkey. Çabalar[22] reported that there was 68.1% positivity in 624 cows with fertility problems, the prevalence of infection was between 6.6% and 100% on a herd basis and all of the herds were positive. Likewise Bilge[20] reported 74% positivity in 486 cows.

In the present study, the presence of specific antibody for IBR was detected only in 3 herds, out of 9, and 23% positivity was observed in two herds (3, 7). Minimum value was determined as 6.25% in herd 9. In total, 7.2% (10/139) were found positive for IBR. This is a surprising value since it is one of the lowest rates in comparison to the other studies in Turkey.

There are not many studies for the BHV-4 in Turkey. Bilge-Dagalp et al.[21] investigated the BHV-4 infection in dairy cows and 476 out of 877 animals, (54.3%) were reported as antibody positive. In another study[23], repeat breeding diagnosed and healthy cows were comparatively assessed in the animals that have been breeding in the same enterprises. The differences were found statistically significant between two groups. The positivity was found to be 69% in repeat breeder animals while 44% in reproductively normal individuals.

The relationship between BHV-4 and abortion in cows has been reported before[24]. Mastitis and ulcerative endometritis caused by BHV-4 were also reported by Frazier et al.[25]. The indirect ELISA is a commonly used test method for serologic examination for BHV-4 because of its high sensitivity and specifity[18,24,26]. Similar advantage of ELISA was reported comparing to other indirect methods for BHV-1 and BVDV.

Despite the animals appeared healthy, BHV-4 has been detected[12]. It plays an important role with respect to reproductive disorders such as abortion, postpartum and chronic metritis[27] following an infection. In this study, the positivity was detected only in two herds (1-2) in Afyonkarahisar province a 16.6% and 20%. Totally, five cows (3.9%) were found as positive. This value is very low comparing to other two studies. The data indicated that it is difficult to infer BHV 1 and 4 infections having a role on repeat breeding in the studied herds due to surprisingly low values.

The presence of BVDV has been known since 1964 in Turkey[28]. Many virological and serological studies were reported in cattle and as high as 96.8% seropositivity was reported until now[19]. In another survey, of the 3360 cattle, 64.2% antibody and 0.25% BVDV antigen presence were reported by Burgu et al.[29].

As similarly with the previous reports in Turkey, the seropositivity proportion in this study was found as 58.2% (81/139). A low value was detected in herd 8 as 13.3%, but the BVDV specific antibody proportion were above 70% in six enterprises, and all of the sampled animals were found to be seropositive in herds 2 and 4. BVD antigen was detected in two cows in herd 3 which have 71.4% antibody presence. At the end of four weeks sampling, the antigen positive animals were sampled and tested again to confirm the persistent viremia and they were found as positive. Since all animals were not assessed using laboratory tests

for the studied herds, the detection of BVD antigen in only one herd does not mean that the other herds are negative. The test results show that BVD is very common nearly in all of the studied herds and it is possible to suggest that the virus circulation is continuing in herds 2-7. The numbers of average inseminations were between 3.9 and 6 in these enterprises.

In herd 8, BHV 1, 4 and BVDV were not detected. The BVD antibody proportion was found to be only 13.3%. Therefore, the mean number of artificial insemination was the lowest as 3.2. In addition, the highest insemination numbers (up to 11) were detected in herds 2-7. This data shows that there is a correlation with the infection rate and the repeat breeding in surveyed animals. BVD was found to be quite prevalent in most of the studied herds. The high antibody rates and virus detection shows that BVDV may have an effect in repeat breeding in studied animals.

Beside alimentary system disorders, reproductive problems such as failure of fertilization and conception rates[30,31], early embryonic deaths[31,32] and abortions have been reported to occur following BVDV infection[33]. Grooms, Brock and Ward[34] infected heifers with the ncp strain BVDV. Ovariectomies were then performed between 4 and 60 days of post infection. The presence of virus was detected in macrophage-like cells and stroma cells in ovum cortex. In addition to these findings, oophoritis was determined from six to 60 days of post infection and it has been stressed that the acute infection results with the ovarian dysfunctions and fertility disorders.

Repeat breeding can have many causes. The factors like other infections, semen quality, oestrus detection and moment of insemination, the skill of the inseminator, organic reproductive system disorders and nutritional problems were excluded in the sampled animals. The use of infected bulls for insemination may create these problems, but this is not a case in this study since artificial insemination was preferred in all herds. In the acute BVDV infection, interval between two oestrous may prolong due to early embryonic deaths[35]. According to the farm records, the period of returned to service were not normal in most of the herds, especially in herds 2 to 7. The interval between two oestrous may last for nine weeks in some animals.

In conclusion, BHV 1 and 4 infection rates were found to be very low than expected, but BVDV was epidemic in most of the studied herds. Based on the test results and farm records together, it was concluded that BVDV may be an important factor in the repeat breeding in the studied herds. IBR and BVDV infection were among prominent and well described reproductive system pathogens but pathogenetic features of BHV4 still need further investigation.

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