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RESEARCH ARTICLE

An Investigation of A Possible Involvement of BVDV, BHV-1 and BHV-4 Infections in Abortion of Dairy Cattle in Kars District of Turkey

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Summary

In this study, the possible effects of BHV-1, BHV-4 and BVDV involving abortions in the selected dairy cattle herds in Kars province of Turkey were serologically investigated. Serum samples obtained from 140 aborted cattle where frequent abortion cases frequently occurred during first and second trimesters of gestation last a few years were analyzed for the presence of specific antibodies for BHV-1 and BHV-4 using ELISA and BVDV using neutralization test, respectively. The results showed that 61.4% (86/140) and 29.3% (41/140) of samples were positive for BHV-1 and BHV-4 specific antibodies by ELISA respectively and 74 (52.9%) of tested samples had neutralizing antibodies to BVDV in virus neutralization test. Furthermore, the results also showed that 11.4% of the samples (16/140) had antibody against all of BHV-4, BHV-1 and BVDV. From our results, BHV-1, BHV-4 and BVDV may be responsible for causing abortions and BVDV seroprevalece in tested animals may be indicative for the presence of the persistently infected animals in Kars pasture. The control of abortions caused by viral infections vaccinations should be practiced before the gestation time of cattle.

Keywords: *Abortion, Bovine herpes virus type 1, Bovine herpes virus type 4, Bovine viral diarrhea virus, Seroprevalance, Cattle*

Kars Yöresindeki Süt Sığırlarında Görülen Abort Vakalarında BVDV, BHV-1 ve BHV-4 Enfeksiyonlarının Olası Etkilerinin Araştırılması

Özet

Bu araştırmada, Kars yöresinde süt sığırı sürülerinde görülen abort vakalarında BVDV, BHV-1 ve BHV-4 enfeksiyonlarının olası etkileri serolojik olarak araştırıldı. Kan serumu örnekleri son yıllarda abort vakalarının sıklıkla görüldüğü gebeliğin ilk ve ikinci üç aylık dönemlerinde, atık yapmış 140 sığırdan toplandı. Kan serumu örnekleri BHV-1 ve BHV-4 spesifik antikorların varlığı ELISA, BVDV spesifik antikorların varlığı ise nötralizasyon testleri ile analiz edildi. ELISA tekniği ile örneklerin %61.4'ünde (86/140) BHV-1 ve %29.3 (41/140)'ünde BHV-4 spesifik antikorlar yönünden pozitiflik saptandı. Virus nötralizasyon testi ile de örneklerin %52.9'unun (74/140) BVDV'üne spesifik nötralizan antikor taşıdığı belirlendi. Örneklerin %11.4'ünün ise üç enfeksiyona da spesifik antikorlara sahip olduğu tespit edildi. Sonuçlar değerlendirildiğinde, Kars yöresinde meydana gelen abort vakalarından diğer enfeksiyöz ajanların yanı sıra BHV-4, BHV-1 ve BVDV enfeksiyonlarının da sorumlu olabileceği anlaşılmaktadır. Ayrıca örneklenen sürülerde yüksek BVDV seroprevalansı'nın varlığı, sürü içerisinde persiste enfekte hayvanların varlığını akla getirmektedir. Bu çalışma'nın sonuçları ışığında viral enfeksiyonların neden olabileceği abort vakalarının kontrolü için aşılama çalışmalarının, gebelik döneminden önce yapılmasının faydalı olacağı kanaatine varılmıştır.

Anahtar sözcükler: *Abort, Bovine herpes virus type 1, Bovine herpes virus type 4, Bovine viral diarrhea virus, Seroprevalans, Sığır*

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INTRODUCTION

Abortion in dairy cattle is usually defined as a loss of the fetus between the age of 42 and approximately 260 days. Pregnancies lost before 42 days are generally called as early embryonic deaths, whereas a calf that is born dead between 260 days and full term is called as a stillbirth. Abortions can represent a significant loss of for the breeder or producer and thus appropriate precautions should be taken to prevent abortions and to search their cause that may occur [1].

Bovine herpesvirus type-1 (BHV-1) is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*. It is an important pathogen of cattle worldwide [2]. Infection with BHV-1 causes a variety of clinical diseases including infectious bovine rhinotracheitis (IBR) (BHV-1 subtypes 1 and 2a), infectious pustular vulvovaginitis, infectious pustular balanoposthitis (BHV-1 subtype 2b) and encephalitis (BHV-1 subtype 3) [3]. IBR may result in various clinical consequences, including severe respiratory disease, venereal disease with reduced reproductive performance and abortion. Like other herpes viruses, BHV-1 also results in lifelong latent infections. The virus may be spread through aerosols, fomites, infected semen and embryos within cattle populations [4]. There are substantial economic consequences associated with respiratory disease as a result of BHV-1. In addition, BHV-1-free status is an important issue in the international trade of live animals and some animal products [5].

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Bovine herpes virus type 4 (BHV-4) belongs to the family *Herpesviridae,* subfamily *Gammaherpesviridae* and species *Rhadinovirus*. BHV-4 has no close biological and virological relationship to other known herpes viruses of the family Bovidae [6,7]. The virus has been identified in the respiratory tracts of infected animals in cases of vulvovaginitis, endometritis, mastitis, abortion and also from apparently healthy cattle [8-13].

Bovine viral diarrhea virus (BVDV) is a member of the genus *Pestivirus*, a group of small-enveloped RNA viruses in the family *Flaviviridae* [14]. Two genotypes of BVDV (BVDV-1 and BVDV-2) have been recognised based on serological and genetic relatedness and they can exhibit two different biotypes namely, non-cytopathic (ncp) and cytopathic (cp), according to their lytic effect in infected cells [15,16]. BVDV can cause repeat breeding, embryonic death, abortion, stillbirths and congenital defect in infected pregnant cattle [17]. Generally, in non-vaccinated herds, the seroprevalence differs among areas or countries, ranging between 20% and 90% [18,19]. It was also estimated that 1-2% of persistently infected animals were found in countries having no BVDV control program [19,20].

Several studies have been indicated that BVDV infections are common in Turkey [21,22]. For instance, Burgu et al.[23] reported a study based on the examination of BVDV status of nonvaccinated cattle showed that antibody prevalence was ranged from 0.6% to 70%.

The aim of this study was to investigate the prevalence of antibodies to BoHV-1, BoHV-4 and BVDV in selected herds where frequent abortions occurring in dairy cattle within Kars district.

MATERIAL and METHODS

Herd History and Clinical Materials

Ten selected cattle herds located in willages of central Kars district where frequent abortion cases were reported last a few years occurred. The time of abortion cases were estimated to be occurring mainly during first and second trimesters of the gestation by interviewing farmers in the herds subjected for the present study. A total of 140 blood samples were collected from aborted dairy cattle from September to February in 2009. Cattle did not receive vaccinations against BHV-1, BHV-4 and BVDV prior to this study. The blood samples were collected from coccygeal vein in to sterile tubes and taken to the laboratory. The samples were centrifuged at 1.500 g for 10 min to separate the serum and then stored at -20ºC until they are tested. Before testing the serum in virus neutralisation test, serum samples were heated at 56°C for 30 min.

ELISA to Test the Antibodies to BHV-1 and BHV-4

Sera were tested for the detection of IgG antibodies to BHV-1 and BHV-4 by using commercial ELISA kit (Bio X, Belgium, Bio K 027 for BoHV-1; Bio K 066 for BoHV-4) according to manufacturers' instruction. Briefly, 100 µl of test sera diluted at 1:100 in dilution buffer were added to wells and incubated for 1 h at room temperature. The plates

were washed and 100 µl of anti-bovine immunoglobulin-peroxidase conjugate (horseradish peroxidase conjugated anti-bovine IgG1 monoclonal antibody) diluted at 1/50 were added to each well. Following incubation for 1 h at room temperature, unbound conjugate was remeoved by washing and 100 µl of enzyme substrate (hydrogen peroxide) and chromogen tetramethylbenzidine (TMB) were added to wells. After incubation at room temeperature for 10 min, the enzymatic reaction was stopped by the addition of 50 µl of 1M phosphoric acid stop solution. The optical density (OD) was measured at 450 nm filter (PowerWaveXS, Biotek, USA). The intensity of resulting blue colour development is proportionate to the titre of the specific antibody in the sample. The OD values obtained for negative control wells were subtracted from the OD values of the corresponding positive wells and the resulting values were divided by the value of the corresponding positive control and multiply the results by 100. The results were expressed as a percentage and the degree of positivity was assessed from 0 to +++++ according to manufacturer's quality control procedure. The result greater than or equal to one plus sign (+) was considered positive.

Virus Neutralization (VN) Test to Detect BVDV Antibodies

Virus neutralization (VN) test were performed essentially as described by Howard et al.[23]. Briefly, serial two fold dilutions of serum samples were made in maintenance medium in 96-well flat-bottom tissue culture plates. Duplicate wells were used for each assay and 50 µL of each dilution of serum (1/5) were retained in the wells. An equal volume of BVDV (NADL) that was added 100 TCID/0.05 mL was added to each well. Virus and cell controls were included to test. The plates were incubated for 1 h at 37°C and 5% CO_2. Finally, 50 µL of a suspension of cells at 3×10^5 cells mL suspended in maintenance medium was added and the plates were incubated up to 6 day at 37°C and 5% CO_2 until complete cytopathic effect was observed in virus control under inverted microscope (Olympus, CKX31). The neutralising titer of BVDV antibodies was assessed as the highest antibody dilution that inhibits 50% of cytopathic effect in cells.

RESULTS

In order to investigate the presence of the antibodies to BHV-4, BHV-1 and BVDV and assessment of their possible association in abortions, ELISA for BHV-1 and BHV-4 and virus neutralization test for BVDV were used to test a total of 140 sera from aborted dairy cattle. ELISA kits were available for testing antibodies to BHV-1 and BHV-4 antibodies in the present study. Since Neutralisation test was considered to be sensitive and commonly used for detecting BVDV antibibodies and assessment of vaccine, efficacies [24] it was also applied in the present study to detect the presence of antibodies in aborted cattle sera.

ELISA for testing the presence of antibodies to BHV-4 and BHV-1 in 140 aborted cattle sera revealed that 41 (29.3%) and 86 (61.4%) had antibodies to BHV-4 and BHV-1, respectively, whereas 74 (52.9%) had neutralizing antibodies to BVDV in virus neutralization test. It was observed that 11.4% of the samples (16/140) had antibodies to three viruses of BHV-4, BHV-1 and BVDV. In addition, 11.4% of cattle sera had antibodies to three viruses and 23.6% cattle had antibodies to BHV-1 and BVDV and 8.6% of cattle had antibodies to BHV-1 and BHV-4. The results were detailed in *Table 1*.

Table 1. *The seropositivity rates of BHV-1, BVDV and BHV-4 infections in aborted cattle sera*

Tablo 1. *Abort yapan sığır serumlarında BHV-1, BVDV ve BHV-4 enfeksiyonlarının seropozitiflik oranları*

Viral Antibodies	Seropositivity Rates (%)
Triple Seropositivity	
BHV-1 + BVDV + BHV-4 +	16 (11.4%)
Double Seropositivity	
BHV-1 + / BVDV + / BHV-4 - BHV-1 + / BVDV - / BHV-4 + BHV-1 - / BVDV + / BHV-4 +	33 (23.6%) 12 (8.6%) 6 (6.4%)
Single Seropositivity	
BHV-1 + / BVDV - / BHV-4 - BHV-1 - / BVDV + / BHV-4 - BHV-1 - / BVDV - / BHV-4 +	86 (61.4%) 74 (52.9%) 41 (29.3%)

DISCUSSION

Despite the complex etiology of abortion cases in cattle, the present study was the first trial for evaluation of a possible involvement of BHV-1, BHV-4 and BVDV. Serum samples obtained from 140 dairy cattle from selected herds were tested for the presence of the antibodies to BHV-1 and BHV-4 by using ELISA and to BVDV by using neutralization assay. The dairy cattle subjected for this study were selected from the herds where frequent abortion cases were reported last a few years in Kars district.

The antibody prevalence's of BHV-1, BHV-4 by using ELISA and BVDV by using Virus neutralization tests in 140 aborted cattle sera were found to be 61.4%, 29.3%, and 52.9% respectively. The results were indicated that these viruses were circulating among aborted cattle herds subjected to the present study. It can be observed from our results that the prevalence's of BHV-1 and BVDV antibodies are nearly two fold higher than BHV-4 antibodies in aborted cattle. It is interesting to see that cattle came in to contact with each of three viruses in dairy cattle herds subjected for this study *(Table 1)*.

In Turkey, several studies showed the seroprevalences of BHV-1 antibodies varying from 59.7% to 74% [22,25-27]. A study carried out by Yıldırım et al.[28] reported that the antibody prevalences of BHV-1 and BVDV were 63.54%, 56.25%, respectively.

Abortions due to BoHV-1 infections have been reported to occur throughout the gestation being more frequently observed in the final stage. In Turkey, the previous studies carried out by several investigators indicated the seroprevalence rates of BHV-1 antibodies varying from 59.70% to 74% [22,25-27]. Bulut et al.[29] compared the presence of antibodies to BHV-1 in cattle the antibodies in cattle with repeat breeding and in cattle without repeat breeding problem Elazig province of Turkey. The use of ELISA for testing cattle sera revealed that cattle with repeat breeding problem and those without repeat breeding problem had antibody prevalence's 85% and 74%, respectively.

The BoHV-4 virus is distributed worldwide and several species are susceptible to infection [30]. Czaplicki and Thiry [31] reported that BoHV-4 associated with abortion could be suspected when abortions occur between 5 and 9 months of gestation. It should be noted that BoHV-4 associated abortions have been rarely reported. For instance, Wellemans and Van Opdenbosch [32] reported 14.1% antibody prevalence against BHV-4 in a total of 205 abortion cases. Naeem et al.[33] also showed BHV-4 specific antibodies in the thoracic fluid in five out of 420 aborted fetuses. A recent study carried out by Dagalp et al.[34] reported that the seroprevalence of BHV-4 was estimated as 69.6%. In Dagalp's study, and the samples were obtained from clinically healthy cattle housed together with the cows with post-partum metritis having antibody to BHV-4.

Bovine viral diarrhea virus (BVDV) is a worldwide pathogen with great economical impact mainly related to the immunosuppressive effects of primary infections and to associated reproductive problems [19,35]. An important factor for the spread of disease is the birth of persistently infected (PI) animals, which remains infected throughout their life. There are indications that 0.5 to 2% of the cattle in endemically infected countries are PI and 60-85% of adult cattle are antibody positive [19]. The prevalence's of BVDV antibodies appears to be higher and varying from 14.3-100% in different parts in Turkey [25,36]. Azkur et al.[37] recently investigated the seroprevalence of antibodies to Border disease virus (BDV), a closely related pestivirus to BVDV, in sheep from Kirikkale province of Turkey. Azkur's study revealed that the seroprevalence of BDV was 75.51% in tested 1075 blood sera by ELISA.

In conclusion, our results are in accordance with above-mentioned similar studies describing the seroprevalences of BHV-1, BHV-4 and BVDV antibodies in cattle in Turkey. In particular, the prevalences of BVDV and BHV-1 antibodies were found to be higher than BHV-4 antibodies as indicated in our present study. The most important outcome of our study was to highlight the possible involvement of viral infections in particular BVDV and BHV-1 for abortion cases in dairy cattle in Kars province. In particular, the high seroprevalence of BVDV infections is indicative for persistently infected animals in Kars pasture. Although the identification and elimination of PI animals is the most ideal for controlling BVDV associated infections including abortions, vaccination remains the most essential control strategy for most countries including Turkey. Vaccinations should be extensively practiced for also controlling BHV-1 and BoHV-4 infections. In addition to the viruses (BHV-1, BHV-4 and BVDV) subjected for the present study, bacteria, fungi, parasite and matabolic reasons for involving abortions in Kars province should also be taken in to account or considered.

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