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# Serologic and virologic investigation of BHV-1, BVDV and BHV-4 in cattle with metritis

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**Abstract:** The purpose of the present study was to evaluate the possible effects of Bovine Herpes virus-1 (BHV-1), Bovine Viral Diarrhea Virus (BVDV), and Bovine Herpes virus-4 (BHV-4) involving metritis in the selected unvaccinated dairy cattle herds in Afyon province of Turkey by serologically and virologically methods. A total of 63 dairy cattle with metritis were sampled in order to investigate the presence of BHV-1, BVDV and BHV-4 infections. The sera samples were tested for presence of antibodies to BHV-1, BVDV and BHV-4 using a commercially available indirect Enzyme Linked Immunosorbent Assay. Leukocyte samples were tested for presence of BVDV viral genome using Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Polymerase Chain Reaction (PCR) for BHV-1 and BHV-4 viral genome. Detectable antibodies were detected in 6 (9.52%) of 63 against BVDV and 51 (80.95%) of 63 against BHV-4. Detectable antibodies were detected against BVDV from 6 (9.52%) of 63 sera samples and BHV-4 from 51 (80.95%) of 63. No antibodies against BHV-1 were detected as well as the results of RT-PCR for BVDV, and PCR for BHV-1 were all negative. Positive PCR results found BHV-4 genome from 8 (12.69%) of 63 leukocyte samples. Presence of BVDV and BHV-4 antibodies in unvaccinated animals indicates that these cattle had contracted infection. In conclusion, BHV-4 infections may play a direct or indirect role in causing bovine metritis; therefore their importance in the etiology of metritis and their economic impact needs further attention.

**Keywords:** BHV-1, BVDV, BHV-4, ELISA, PCR

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## 1. Introduction

Bovine herpes virus type 1 (BHV-1), Bovine viral diarrhea virus (BVDV) and Bovine herpes virus type 4 (BHV-4) are very important viral pathogens and worldwide distribution. These viruses have a worldwide distribution and tend to be endemic in most domestic and wild cattle populations [1,2].

BHV-1 is a member of subfamily Alphaherpesvirinae, genus *Varicellovirus*. BHV-1 infects the respiratory and genital tracts of cattle. It is a double stranded DNA (dsDNA) virus and is causes different symptoms including conjunctivitis (red eye), infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB) and infectious bovine rhinotracheitis (IBR) [3]. BHV-1 is one of the most common causes of reproductive and respiratory disease and in the world cattle industry, however major economic losses occur due to by this virus [4].

BVDV is a member of the family Flaviviridae, and genus *Pestivirus* [5]. It is the causative agent of bovine viral diarrhea mucosal disease (BVD-MD). BVDV is one of the most important viral pathogen agents of cattle and it is distributed worldwide [6]. It has been associated with mucosal disease [7], respiratory disease [8], reproductive failure [9] and fetal infections [10] in cattle populations. The reproductive problems of BVDV infection include abortion, early embryo loss and congenital anomalies [11].

BHV-4 is a member of the family Herpesviridae, subfamily Gammaherpesviridae and genus *Rhadinovirus* [12]. It has no close relationship to other herpes viruses of the family Bovidae [13]. BHV-4 is a risk factor for some clinical symptoms such as abortion, conjunctivitis, metritis, stillbirth, reproductive deficiencies, pustular vulvovaginitis, ocular discharge and respiratoric changes [14-16]. Like other herpes viruses animals infected with BHV-4 develop a latent infection as a lifelong, asymptomatic infection [14] and virus reactivation and reexcretion occurs in these

animals following any applications like glucocorticoid treatment [4].

The aim of this study was to evaluate the possible effects of BHV-1, BVDV and BHV-4 infections involving metritis in the selected unvaccinated dairy cattle herds in Afyon by serologically and virologically tests.

## 2. Materials and Methods

### 2.1. Animals and Samples

A total of 63 dairy cattle with metritis were sampled in order to investigate the presence of BHV-1, BVDV and BHV-4 infections in Afyon. The sera samples were tested for presence of antibodies to BHV-1, BVDV and BHV-4 using a commercially available indirect Enzyme Linked Immunosorbent Assay (ELISA, Biox, USA). Leukocyte samples were tested for presence of BVDV viral genome using Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Polymerase Chain Reaction (PCR) for BHV-1 and BHV-4 viral genome.

### 2.2. Preparation of Serum

Blood was collected into serum tubes, the samples packed in ice were brought to the laboratory, and centrifuged at  $3000 \times g$  for 10 min at 4°C. Approximately 1 mL of serum were then collected into sterile microfuge tubes (Eppendorf, Germany) and stored at -20°C until analysis.

### 2.3. Preparation of Leukocyte

Leukocyte samples were prepared from blood samples taken into tubes with EDTA by a standard method. The leukocyte samples were kept in deepfreeze under -20°C until used.

### 2.4. Serological Testing and Interpretation

Commercial indirect ELISA kits with antigen-coated micro titer plates were used for detection of antibodies to BHV-1, BVDV and BHV-4 in serum, according to the instructions of the manufacturer. The corrected optical density (COD) level was calculated before interpretation of the results by subtracting the optical density (OD) for the control antigen from sample OD (OD sample – OD control = COD).

### 2.5. Virological Testing

Leukocyte samples were used for detection of BHV-1 and BHV-4 viral genome by PCR, and BVDV viral genome by RT-PCR.

### 2.6. Statistical Analysis

Differences between antibody and viral genome statuses were calculated by using chi-square test (Minitab 14.0 Inc., State College, PA, USA). Difference were considered significant when  $P < 0.05$ .

## 3. Results

**Table 1.** Antibodies and viral genome results

Infection	ELISA/Ab	PCR and RT-PCR/viral genome
BHV-1	Not detected	Not detected
BVDV	6/63 <sup>b</sup> (9.52%)	Not detected
BHV-4	51/63 <sup>a</sup> (80.95%)	8/63 (12.69%)

a, b: Values marked with different letters in the same column are statistically significant ( $P < 0.05$ ).

Detectable antibodies were detected against BVDV from 6 (9.52%) of 63 sera samples and BHV-4 from 51 (80.95%) of 63 ( $P < 0.05$ , Table 1). No antibodies against BHV-1 were detected as well as the results of RT-PCR for BVDV, and PCR for BHV-1 were all negative. Positive PCR results found BHV-4 genome from 8 (12.69%) of 63 leukocyte samples (Table 1).

## 4. Discussion

Reproductive disorders and abortion problems in cattle have caused serious economic losses in worldwide. High or low results of the prevalence of abortion-causing diseases may be illustrated with a result of different reasons such as vaccination (killed or attenuate), herd management, insemination (natural or artificial), types of cattle, feed, climatic condition, transport, number of pregnancies [17]. In the current study, 9.52% BVDV and 80.95% BHV-4 seropositivities were detected (Table 1).

BVDV usually causes reproductive problems, and birth of persistently infected carrier calves [18]. BVDV and BHV-1 are most important and costly viral pathogens of cattle worldwide [19] that mainly associated with reproductive problems and increased abortion [3,10]. There were no antibody and antigen positive animals for BHV-1 infection (Table 1). The reason that couldn't obtain any positive cattle in this study can be explained by mix infections. In cattle industry, it is known that animals being infected by herpes virus are never able to eliminate the infection and all herpes viruses infections are leading to a latent infection [20].

It is considered that most metritis problem is originated by bacteria. Viral pathogens are rarely evaluated even though abortion may follow infection with various herpes viruses. 8 (12.69%) leukocyte samples were detected positive for BHV-4 genome by PCR (Table 1). Association between reproductive disorders and viral infections of cattle reported in Turkey by different studies [21,22]. Clinically postpartum metritis has also been associated with BHV-4 demonstrated with various studies in different countries such as USA, Spain, Serbia, Italy, and Turkey [15,16,23-25]. In this study, detection of antibodies against BVDV and BHV-4 in unvaccinated animals demonstrates that these cattle had contracted with these viruses. Determination of above mentioned infections in cattle with metritis could pose a risk to other animals in herd. Although determination of BHV-4 viral genome indicated that this

herd must be controlled periodically for latent infection. It is reported that BHV-4 has been isolated from dairy cattle with different genital problems as well as healthy ones [25]. The reason of the highest rates of seropositive animals to BHV-4 infection can be explained by circulated subclinically infected cattle in the herds. Although in the current study dairy cattle with metritis were sampled it is considered that other cattle in the herd should be investigated for mentioned infections as soon as possible.

## 5. Conclusion

In conclusion, BHV-4 infections may play an important role in metritis. Viruses (BHV-1, BVDV, BHV-4) importance in the etiology of reproductive problems and their economic losses needs further attention.

## Competing Interests

The authors declare that they have no competing interests.

## Acknowledgement

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