



## A Serological Investigation of Some Aetiological Agents Associated with Abortion in Domestic Water Buffalo (*Bubalus bubalis* Linnaeus, 1758) in Samsun Province of Northern Turkey

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**Abstract:** In Turkey, there is a lack of information about the frequency and aetiology of abortions in water buffalo breeding. The water buffalo (*Bubalus bubalis*) occupies an economically important place in Samsun province. Reproductive disorders like infertility and abortions in water buffalo are important problems in the livestock industry. In this study, the presences of Bovine viral diarrhoea virus (BVDV), Bovine herpes virus-1 (BHV-1), *Neospora caninum* and *Brucella abortus* were investigated serologically in 82 domestic water buffaloes in Samsun province of northern Turkey. Seropositivity rates for BVDV, BHV-1, *N. caninum* and *B. abortus* were found to be 68.3% (56/82), 80.5% (66/82), 28% (23/82) and 2.4% (2/82), respectively. This preliminary study, therefore, indicates for the first time that neosporosis and brucellosis exist in domestic water buffaloes and may contribute, either individually or in association with other agents to abortions in water buffalo breeding.

**Key words:** BHV-1, Brucellosis, BVDV, Neosporosis, Water buffalo

### Türkiye Samsun Yöresi Mandalarında (*Bubalus bubalis* Linnaeus, 1758) Bazı Abortla İlişkili Etiyolojik Ajanların Serolojik Olarak Araştırılması

**Özet:** Türkiye’de mandalarda aborta neden olan etkenler hakkında yeterli bilgi bulunmamaktadır. Samsun ilinde, manda yetiştiriciliği ekonomik açıdan önemli bir yer tutmaktadır. Mandalarda görülen infertilite ve abort gibi üreme bozuklukları hayvancılık endüstrisinin önemli problemlerindendir. Bu çalışmada, Samsun ilinde bulunan 82 mandadan toplanan kan serumlarında bovine viral diarrhoeae virus (BVDV), bovine herpes virus-1 (BHV-1), *Neospora caninum* ve *Brucella abortus*’un varlığı serolojik olarak araştırıldı. Seroprelans oranı BVDV için %68,3 (56/82), BHV-1 için %80,5 (66/82), *N. caninum* için %28 (23/82), *B. abortus* için %2,4 (2/82) olarak tespit edildi. Türkiye’de mandalarda brusellosis ve neosporosis seroprevalansı ile ilgili olarak ilk olan bu çalışma, bireysel veya diğer atık etkenleriyle birlikte değerlendirildiğinde manda yetiştiriciliğine katkı sağlayacaktır.

**Anahtar kelimeler:** BHV-1, Brusellosis, BVDV, Manda, Neosporosis

## INTRODUCTION

The State Institute of Statistics of Prime Ministry of Turkey has reported the water buffalo population in Turkey as 84,705. The water buffalo (*Bubalus bubalis*) occupies an economically important place in the livestock industry in many parts of the world. One of these is the northern Turkey (Sariozkan, 2010). Abortion of infectious origin is considered a noticeable pathology given the considerable economic losses due to loss of production income (e.g. loss of calf and milk), in one hand, and loss of breeding stock due to compulsory slaughters imposed in cases of suspected brucellosis on the other. Many bacteria, viruses, protozoa and fungi cause infectious abortions and the diagnosis of the exact cause is not easy in all cases. According to Anderson (2007), the aetiology is identified in less than half of the cases submitted to laboratories. Serology does not allow a precise diagnosis although it does determine whether there has been exposure to an abortive agent or not.

Brucellosis, a disease caused by various species of the genus *Brucella*, is widely seen in most parts of world except for some countries such as Australia, Japan and northern Europe (Anonymous, 2009). Its cross-transmission can occur between cattle, sheep, goats, buffaloes, camels and other species. Brucellosis is still endemic in countries of the Mediterranean basin, the Middle East and central Asia (Dawood, 2008). Brucellosis causes serious economic losses and also is considered as a zoonosis. The causative agent is *B. abortus*, a facultative intracellular pathogen that infects host macrophages. Only a few of infected water buffaloes develop clinical signs of the disease (spontaneous abortion). However, many infected cows shed *B. abortus* by milk. Eradication programs involving the slaughter of infected animals have been carried out for more than 20 to 30 years. However, latent infections, prolonged incubation of the pathogen, incomplete protection provided by vaccines, and difficulties in distinguishing vaccinated

and naturally infected animals serologically have limited the efficacy of eradication programmes (Boschiroli et al., 2001).

*N. caninum*, the causative agent of neosporosis, is a cyst-forming coccidian protozoan parasite that belongs to the Sarcocystidae family. It causes neuromuscular disease in dogs and also high rates of abortion in cattle worldwide (Hemphill and Gottstein, 2000). Additionally, clinical neosporosis has been reported in sheep, goats, deer, a rhinoceros, and horses and antibodies to *N. caninum* have been found in the sera of water buffaloes, red and gray foxes, coyotes, camels and felids (Dubey, 2003).

BVDVs are enveloped, single-stranded, positive sense RNA viruses. BVDV belongs to the pestivirus genus of the Flaviviridae that contains many viruses affecting the livestock industries of cattle, sheep, goat and swine (Heinz et al., 2000). BVDV is one of the most important viral pathogens of cattle and its control and prevention are of worldwide concern. Moreover, BVDV infection has been associated with enteric disease, mucosal disease and reproductive failure (Booth et al., 1995; Carman et al., 1998). Several studies have shown that pestiviruses are not highly host-specific. It has been reported that BVDV can infect not only cattle but also sheep, swine, goat, deer, buffalo and giraffe (Paton, 1995).

BHV-1 is a member of the *Herpesviridae* family and has been placed within the subfamily *Alphaherpesvirinae*. The herpesviruses are characterised by large double-stranded DNA genomes ranging in size from 80 to 250 kbp (Roizman, 1996). It is a major pathogen of cattle throughout the world. BHV-1 is responsible for severe respiratory, reproductive, neonatal and dermal disease in cattle (Yates, 1982).

Therefore, the objective of this study was to investigate the BHV-1, BVDV, *N. caninum* and *B.*

*abortus* infections serologically in domestic water buffaloes in Samsun province of northern Turkey.

## MATERIALS and METHODS

Blood samples were collected from 82 domestic water buffaloes in Samsun province and its towns between 2008 and 2010. The age of animals varied from 2 to 7 years-old. Blood tubes were centrifuged at 3,000 g for 10 min, and the samples were transferred into sterile tubes and stored at -20°C until being used. The commercial enzyme-linked immunosorbent assay (ELISA) kits were obtained from Institut Pourquier, Montpellier, France for BHV-1 and BVDV, and from VMRD Inc., WA, USA for *N. caninum* and the tests were performed according to the producer's description. Plates were read with an ELISA reader at 450-nm and 650-nm for Institut Pourquier and VMRD Inc. kits, respectively. OD values determined were calculated. The serological tests for brucellosis were carried out by Rose Bengal plate test (RBPT) and complement fixation test (CFT). Both tests were performed following the instructions of the method reported by Alton et al. (1988). *B. abortus* antigen used in the study for RBPT was obtained from Pendik Veterinary Control and Research Institute, Istanbul, Turkey.

## RESULTS

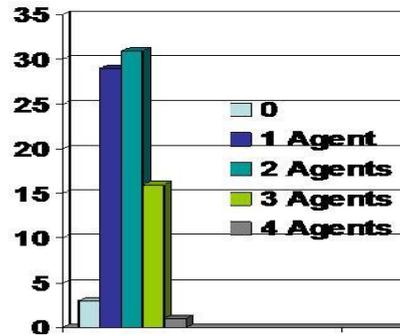
The serum samples were analysed for antibodies to BVDV, BHV-1 and *N. caninum* by ELISA, and for antibodies to *B. abortus* by the RBPT and CFT tests. Positivity rates for the aetiological agents varied and were as follows: BHV-1 80.5% (66/82), BVDV 68.3% (56/82), *N. caninum* 28% (23/82), and *B. abortus* 2.4% (2/82) (Table 1).

Among the 82 buffaloes, one animal was detected to have antibodies against all the agents investigated while only 3 animals were free of antibodies to those agents concerned. Most commonly, anti-agent antibodies were detected against 2 agents in the same animal (n=31) that was followed by triplet detection of anti-agent antibodies in 16 animals. Only 29 animals were found to be seropositive against one agent. Multiple (double, triple and quartet agents) infections seropositivity obtained at end of the study were 52.4% (43/82) (BHV-1+BVDV), 24.4% (20/82) (BHV-1+N. caninum), 23.1% (19/82) (BVDV+N. caninum), 20.7% (17/82) (BHV-1+BVDV+N. caninum), and 1.2% (1/82) (BHV-1+BVDV+N. caninum+ *B. abortus*) (Figure 1). However, no antibodies were detected in 3.7% (3/82) of the 82 sera samples. CFT showed antibody titers from 1/40 to 1/320 for *B. abortus*.

**Table 1.** Distribution of the seropositivities for abortion-associated agents

**Tablo 1.** Seropozitifliklerin abort etkenlerine göre dağılımı

Agents	Total number of serum samples tested	Number of positive (%)
BVDV	82	56 (68.3)
IBR	82	66 (80.5)
<i>N. caninum</i>	82	23 (28)
<i>B. abortus</i>	82	2 (2.4)



**Figure 1.** Multiple seropositivity numbers against abortion agents among the 82 water buffaloes.

**Şekil 1.** Abort etkenlerine karşı 82 mandada çoklu pozitiflik sayıları.

## DISCUSSION

An indigenous breed of water buffalo (*Bubalus bubalis*) is mainly reared in the northern part of Turkey and is particularly appreciated for the production of Turkish delight. One-fifth of all water buffalo population is located in Samsun province of Turkey (19,391) (Sariozkan, 2010). Abortion is one of the major causes of economic losses in livestock industry and great majority of abortion cases originate from infectious agents (Givens, 2006). Brucellosis is the most important abortifacient agent among the livestock; however, nowadays *N. caninum* infection has been steadily reported from livestock farms, especially from those with history of high abortion rates (Hall et al., 2005; Simsek et al., 2008). Positivity rates for *N. caninum* and *B. abortus* were found to be 28% and 2.4%, respectively. Seroprevalence of cattle neosporosis ranged from 3.1% to 35% and from 12.1% to 77% in different parts of Turkey (Aktas et al., 2005; Simsek et al., 2008; Paksin and Utuk, 2009) and in the world (Morales et al., 2001; Moore et al., 2002), respectively. Seroprevalance of *N. caninum* in water buffaloes varies between 1.5% and 64% worldwide (Huong et al., 1998; Fujii et al., 2001). The seroprevalence of cattle brucellosis varies between 1.4% and 35.3% in different geographical regions of Turkey (İyisan et al., 2000; Sahin et al., 2008). Seroprevalance of brucellosis in water buffaloes ranged from 0.3% to 20.5% worldwide (Refai, 2002; Nowroozi-Asl et al., 2007). There exists no study on the prevalence of brucellosis and neosporosis in water buffalo in Turkey. This is the first study about both infections in water buffalo in Turkey. The positivities for *N. caninum* and *B. abortus* determined in water buffalo herein were found to be lower compared to the value reported previously. We know that the seroprevalance values of similar infections may be affected by various factors such as the number of animals sampled, population size, age of the animals, time of sampling, managerial conditions, climatic and

geographical features of the territories, individual differences, and so on. *N. caninum* may cause an increased susceptibility to other infectious agents (Bjorkman et al., 2000; Mineo et al., 2006). Bjorkman et al. (2000) found that the seroprevalence of *N. caninum* in dairy cattle was as low as 2%, and 17 out of 26 *N. caninum* seropositive cattle were also the carrier (vector) of BVDV. Consequently, they pointed out that there is an important relationship between *N. caninum* and BVDV infections. Similarly, Mineo et al. (2006) reported that *N. caninum* co-existed with BVDV and BHV-1 among dairy cattle in Brasil.

Serological studies carried out worldwide show that the seroprevalances of BVDV and BHV-1 ranged from 30.6% to 40% and from 16.7% to 80.6% in water buffalo, respectively (Akhtar and Asif, 1996; Gur and Akca, 2008). In Turkey, the herd seroprevalance varies from region to region, but the average percentage ranged from 31.7% to 96.8% and from 54% to 74% for BVDV and BHV-1, respectively. Only a single serological study has been performed for BHV-1 and BVDV in water buffalo in Turkey. Gur and Akca (2008) carried out a serosurvey in water buffalo in different parts of Turkey, and the seroprevalances ranged from 47.1% to 90% and from 27.5% to 70% for BVDV and BHV-1, respectively. They also reported that the average percentages for BVDV and BHV-1 were found to be 53% and 48.6% in Samsun province, respectively. In the current study, the seroprevalances of BVDV and BHV-1 were determined to be 68.3% and 80.5%, respectively. The seroprevalances detected herein are higher than those reported by Gur and Akca (2008) for Samsun province. It is well-known that the result of seroprevalance studies are influenced by many factors; such as the number of animals sampled, age of animals, time of sampling, conditions of care and feeding, individual differences, and so on. In this respect, when the results of these two studies were evaluated extensively, there were similar findings about the existence/prevalance of

infection in this area. The present study shows that infections caused by abortion agents are very common in Samsun province of northern Turkey, as the most popular water buffalo production area. Multiple detection rates of different agent antibodies were also analysed in this study. Double detections of infective agent antibodies were very common, as was followed by triple and quadruple combinations. Only 3 animals were negative for antibodies to all the viruses analysed, while 29 animals had antibody to one virus.

In conclusion, this study demonstrates that viral (BVDV and BHV-1) and parasite (*N. caninum*) abortion agents are more common than bacterial agent (*B. abortus*) in water buffalo herds in Samsun province of northern Turkey. Evaluations of BHV-1, BVDV and *N. caninum* infections together or separately, showed the existence of high seropositivity in water buffaloes. As a result, the clinical presence of infections causing high level of abortion in water buffaloes was observed in the present study. The current study is the first serological attempt for the detection of antibodies against different reproductive diseases in domestic water buffaloes of Samsun. Thus, the results are very important not only for diseases studied, their localisation and the local breeds but also beneficial to discuss the future aspects in later studies.

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