Original Article

Brucellosis in a water buffalo (*Bubalus bubalis*) herd in Balikesir province of Turkey: a bacteriological and pathological investigation

Hasan Özen[1*] Ziya İlhan[2] Mustafa Usta[1] Musa Karaman[1] Fatma İlhan[1]

Abstract

Brucellosis, caused by a facultative intracellular bacterium, induces great economic loss in the livestock economy worldwide. In this study, the abortion problem in an Anatolian water buffalo herd was investigated by pathological, bacteriological and serological means. An aborted fetus as well as blood and milk samples from 4 buffalo cows with abortion history were studied. The thoracic and abdominal cavities of the aborted fetus were filled with serohemorrhagic fluid, and abomasum had yellowish brown fluid containing fibrous material in it. Bronchointerstitial pneumonia was recognized in the lung tissues. *Brucella (B.) abortus* biotype 3 was isolated from the aborted fetus. Blood samples collected from the water buffalo cows with a known history of abortion were found to be positive for brucellosis by the Rose Bengal Plate test and a serum agglutination test. Three out of 4 animals were also positive according to a Milk Ring Test. The isolated *B. abortus* biotype 3 was found to be highly resistant to the tested antibiotics. This investigation is the first study reporting brucellosis by pathological, bacteriological and serological means in Anatolian water buffalo in Turkey. Since brucellosis is an important zoonotic disease it is required that precautions must be taken when handling and consuming milk products of water buffaloes.

Keywords: Water buffalo, brucellosis, pathology, bacteriology, serology

[1]*Department of Pathology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey*
[2]*Department of Microbiology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey*
****Correspondence**: hasanozen@hotmail.com* (H. Özen)
Received April 30, 2020.
Accepted January 6, 2021.
doi: 10.14456/tjvm.2021.18

Introduction

Brucellosis, an infectious and contagious disease of many species including man, is known to cause great economic impact on the livestock industry. The disease is widespread around the world with a few exceptions where severe eradication policies and/or banning of animal movements have been successfully applied (FAO/WHO 1986). Brucellosis is caused by facultative intracellular Gram-negative bacteria of the genus *Brucella (B.)*. *Brucella* spp. are non-spore forming, non-motile, non-capsulated, aerobic or microaerophilic coccobacilli (FAO/WHO, 1986; Quinn *et al.*, 2011; Dağ *et al.*, 2012). There are many *Brucella* species namely *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis, B. neotomae* etc., that are known to cause infection in domestic and wild animals and *B. abortus* is primarily responsible for bovine brucellosis (Osterman and Moriyon, 2006).

The water buffalo (*Bubalus bubalis*) is an economically important animal in some areas of the world. It is estimated that there are more than 172 million water buffalo, the majority being located in Asia, mostly India, Pakistan and China (Borghese, 2011). The water buffalo population in Turkey was announced as 178.397 head in 2018, representing only 1% of the total bovine animals. However, the population showed 10.5% increase compared to the previous year (TUIK, 2018). Water buffalo in Turkey are reared mostly in Samsun province followed by the Eastern Anatolian and Marmara regions. They are raised mainly for milk production because of the high quality of milk fat that is best appreciated for the cream that accompanies the famous Turkish baklava. Less importantly, they are also used for meat production and as draught animals. Water buffalo in Turkey are known to be only of Anatolian breed, which is a river type.

Knowledge about diseases of water buffalo is limited mostly due to comparably fewer being raised as livestock animals in the world. As an infectious disease, brucellosis in water buffalo has been reported in Egypt (Holt *et al*., 2011), Italy (Guarino *et al*., 2001), Trinidad and Tobago (Fosgate *et al*., 2011), Pakistan (Nasır *et al*., 2014), Argentina (Konrad *et al*., 2013), Brazil (Santa Rosa *et al*., 1961), Iran (Nowroozi-Asl *et al*., 2007), and Mexico (Suazo-Cortez *et al*., 2012). In Turkey, there has been only one study in water buffaloes that has reported 2.4% (2/82) seropositivity for *B. abortus* (Albayrak *et al*., 2012). In this study, brucellosis in an aborted water buffalo fetus and the mother as well as other buffalo cows in a herd with a known history of abortion was investigated pathologically, bacteriologically and serologically.

Materials and Methods

Animals: In January 2019, a water buffalo fetus aborted in last trimester of gestation was presented to Balıkesir University Faculty of Veterinary Medicine for detection purposes of the cause of abortion. According to the owner the herd consisted of a total of 30 water buffalo, 1 male, 15 female and 14 calves. The herd was vaccinated with a vaccine prepared from *B. abortus* S99 strain. The herd was housed separately from other livestock animals but used the same field for grazing.

Pathology: Necropsy was performed on the aborted fetus and tissue samples were collected and fixed in 10% neutral buffered formalin for routine pathological examination. Tissue samples were routinely stained with hematoxylin and eosin (H&E) and the sections were investigated under a light microscope.

Bacteriology: For bacteriological analysis, swabs from the stomach contents, pharynx, and liver of the aborted fetus were taken carefully and inoculated immediately onto duplicate plates of blood agar base no:2 (Oxoid, Hampshire, England) containing 7% defibrinated sheep blood. All the plates were incubated at 37°C, both in air and microaerobically (5–10% CO_2) for 4-6 days. Identification and typing of *Brucella* strains were performed using standard classification tests, including growth characteristics, catalase, oxidase and urease activity, H_2S production, growth in the presence of thionin (40 µg/ml, 20 µg/ml and 10 µg/ml) and basic fuchsine (20 µg/ml and 10 µg/ml) and agglutination with monospecific A and M antisera (Alton *et al*., 1988; İlhan *et al*., 2008[a]; Quinn *et al*., 2011).

Serology: Approximately 15 ml of whole blood (without anticoagulant) was aseptically taken from the jugular vein in sterile tubes. Sera samples were tested by the Rose Bengal plate test (RBPT) and a serum agglutination test (SAT). Antigen prepared from *B. abortus* S99 strain for RBPT and SAT was supplied by the İstanbul Pendik Veterinary Control Institute. The tests were performed as described previously (Alton *et al*., 1988). RBPT was scored according to the supplier's procedure. Serum samples testing positive in both RBPT and SAT were accepted as true positive serologically.

Milk samples were also collected under hygienic conditions from four udders. Approximately 15 ml of the milk were sampled and used in milk ring test (MRT). MRT antigen, which was prepared from *B. abortus* S99 strain and stained with hematoxylin, was supplied by the İstanbul Pendik Veterinary Control Institute. The test was performed in sterile tubes and the milk samples were tested within 2-5 h after collection. The milk samples were thoroughly shaken and MRT was performed as described; a: 1000 µl milk + 30 µl antigen, b: 500 µl milk + 500 µl PBS (pH: 7.2) + 30 µl antigen, c: 500 µl milk + 500 µl sterile distilled water + 30 µl antigen. The tubes were mixed thoroughly and incubated at 37°C for 1-4 h. When the antigen precipitated at the bottom of the tubes and/or a purple band occurred at the top of milk, those samples were then regarded as positive (İlhan *et al*., 2008[b]). MRT test results were scored according to the supplier's procedure.

Antimicrobial susceptibility testing: *B. abortus* biotype 3 strain isolated in the current study was tested against selected antibiotics (Bauer *et al.*, 1966). Mueller-Hinton agar (CM337, Oxoid) supplemented with 7% defibrinated sheep blood was used. A suspension of the culture was prepared in sterile saline solution (NaCl 0.85%, pH: 7.2) and turbidity was visually adjusted to the 0.5 McFarland Standard (1.5 x 10^8 cells/ml). The suspension was surface plated within 30 mins after preparation and the following antimicrobial

discs were applied to the surface of the plates: streptomycin (S) 10 µg (Bioanalyse), trimethoprim/sulphamethoxazole (STX) 25 µg (BBL), tetracycline (TE) 30 µg (Oxoid), ampicillin/sulbactam (SAM) 10/10 µg (Bioanalyse), enrofloxacin (ENR) 5 µg (Bioanalyse), amoxicillin/clavulanicacid (AMC) 30 µg (Oxoid), oxytetracycline*(T) 30* µg (Bioanalyse), clocsacin (OB) 5 µg (Oxoid), penicillin/novobiocin (PNV) 40 µg and neomycin/bacitracin/tetracycline (NBT70C) 30 µg/10 U/30 µg (MastDiagnostic). The National Committee for Clinical Laboratory Standards (NCCLS 2007) breakpoints for STX and TE that were for *Heamophilus (H.) influenza* were accepted for *B. abortus* biotype 3 in this study. Breakpoints used for S were recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie (Members of the SFM Antibiogram Committee, 2003). The following bacteria were considered: *Streptococcus pneumoniae* for PNV 40; *H. influenza* for SAM and AMC (NCCLS 2007). For the activity of the other antimicrobials (ENR; T, OB and NBT70C), the interpretations were based on inhibition zone diameter tables for aerobic microorganisms provided by disc suppliers for antibiogram analysis in human and veterinary medicines.

Results

In gross inspection of the aborted fetus at necropsy, the subcutaneous tissues were edematous and an excess amount of serohemorrhagic fluid filled the thoracic and abdominal cavities (Fig. 1). The lungs were diffusely enlarged and edematous. The bronchial and mediastineal lymph nodes were slightly enlarged. In the abomasum, a viscous yellowish-brown fluid containing white flecks reminiscent of fibrous material was observed (Fig. 2). No other prominent changes were observed in the other organs. In histopathological investigation of the hematoxylin and eosin stained sections of lung tissues, bronchointerstitial pneumonia was noted (Fig. 3). Bronchial lumens were filled with lymphocytes and alveolar macrophages. The bronchial and alveolar epithelia had degenerated and occasional desquamation was noted. Interalveolar septa were markedly broadened with edema, fibrin and mononuclear cellular infiltrations. Thrombosis of lymphatic and some blood vessels was also noted in lung tissue.

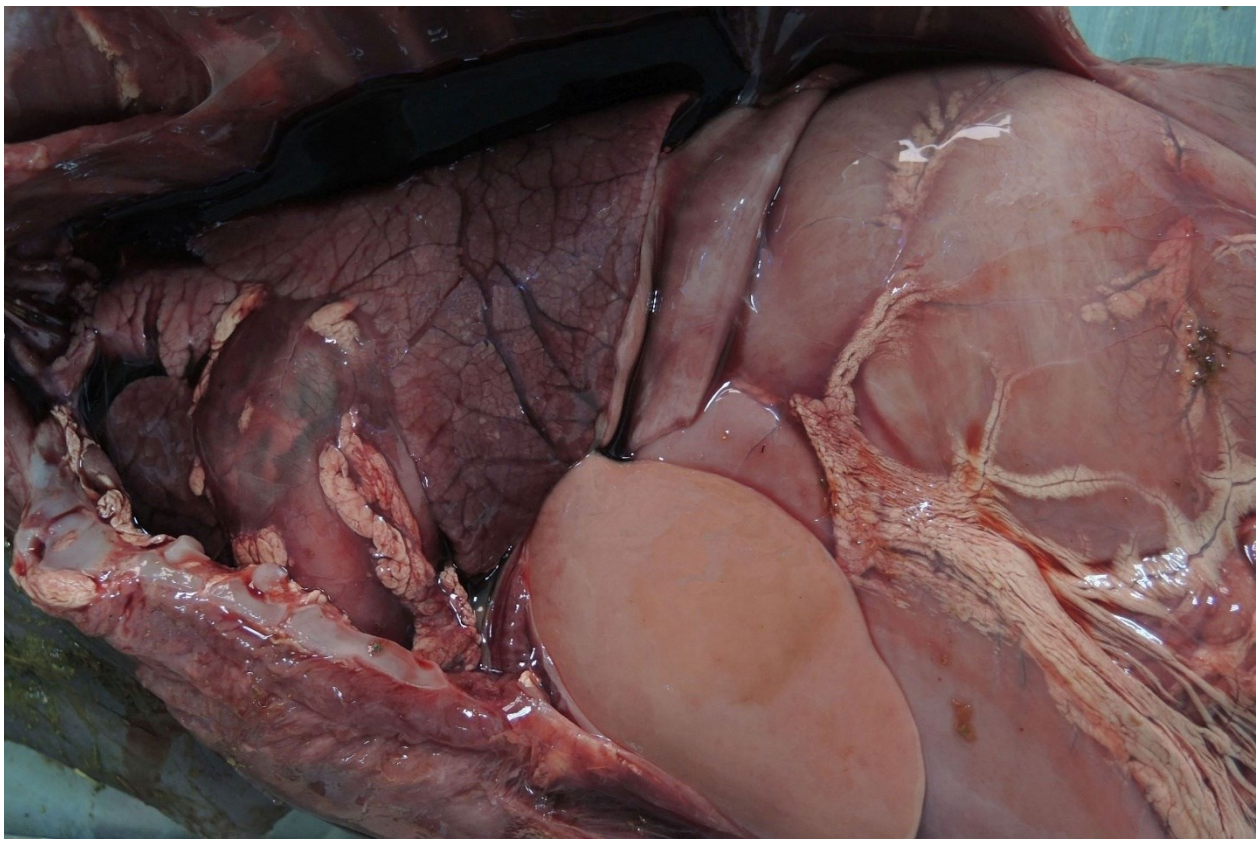

Figure 1 Sero-hemorrhagic fluid in the thoracic and abdominal cavities of the aborted water buffalo calf

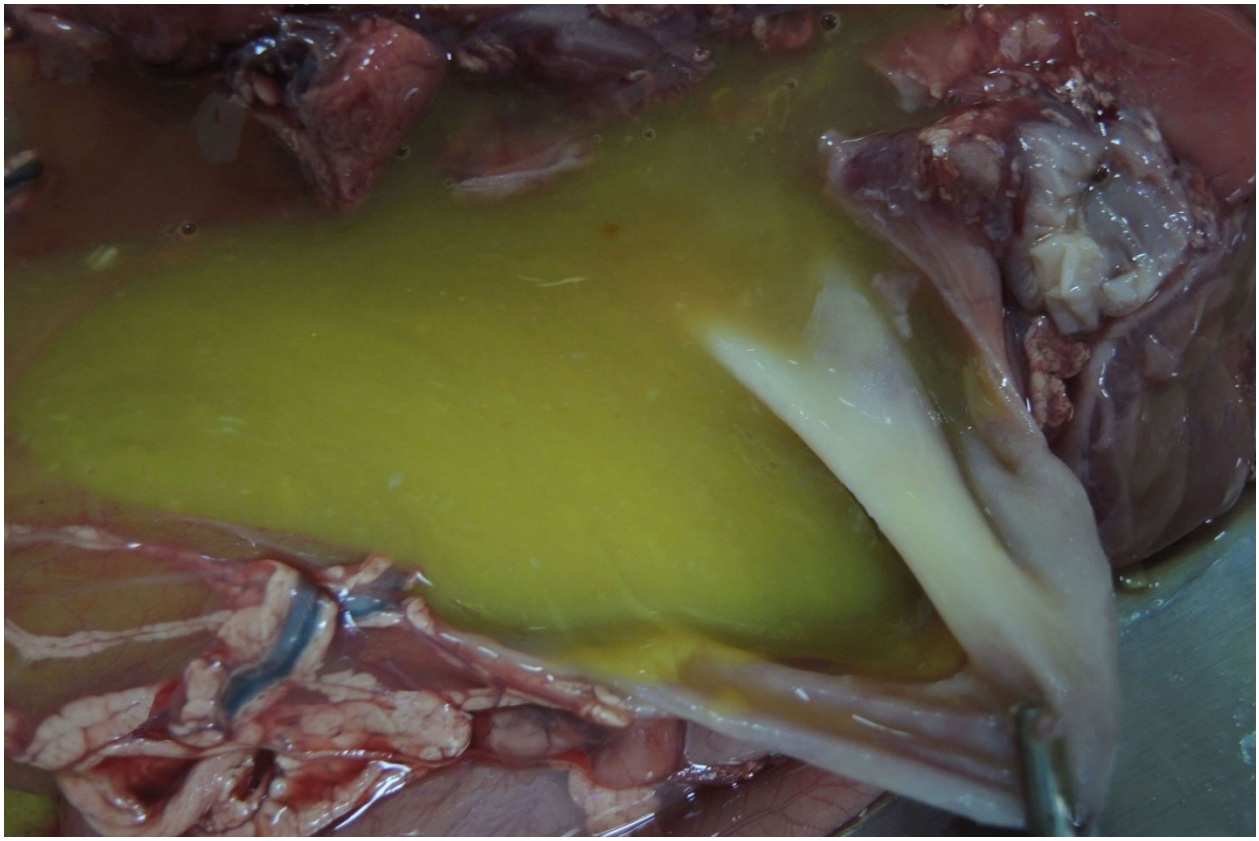

Figure 2 Viscous yellowish-brown fluid containing fibrins in abomasum of the aborted water buffalo calf

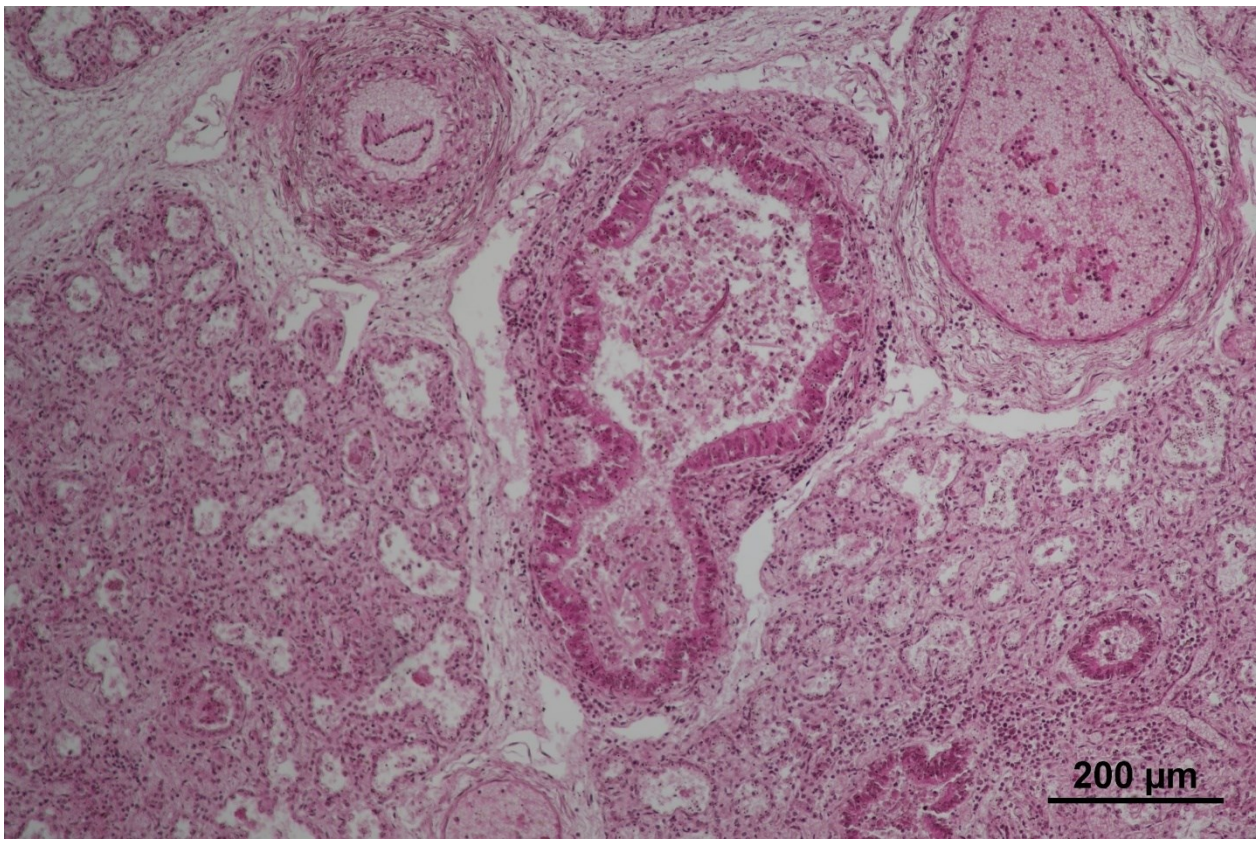

Figure 3 Histopathological view of lung of the aborted water buffalo fetus showing bronchointerstitial pneumonia. H&E

In microbiological analysis, *B. abortus* biotype 3 was isolated from the aborted fetus as pure culture. All sera samples collected from the mother buffaloes with a known history of abortion including the current aborted fetus were found positive for brucellosis by RBPT and SAT. For milk samples, 3 were also positive by MRT (Fig. 4). Serological test results were presented in Table 1.

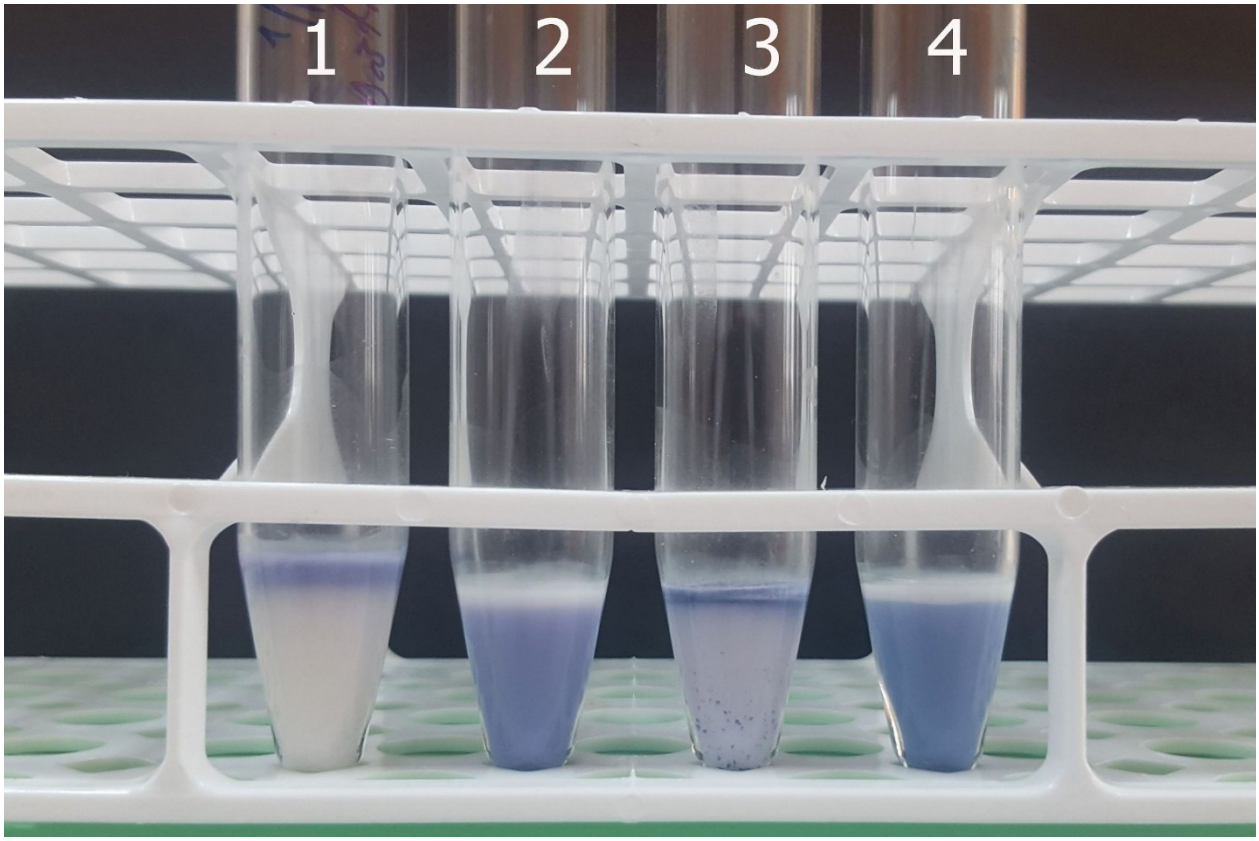

Figure 4 MRT test results in milk samples of the water buffalo cows with known history of abortion from the same herd. Tube 1 and 3 are MRT positive samples, and tube 2 and 4 are MRT negative at 37°C in 2 hours

Table 1 Serological test results of blood and milk samples of water buffalo mothers with known history of abortion.

Animals	Gestation Month of Abortion	RBPT	SAT	MRT
1	6th	+	1/160	-
2*	9th	++	1/320	++++
3	9th	++	1/320	+++
4	10th	++	1/320	++++

RBPT: Rose Bengal plate test, SAT: Serum agglutination test and MRT: Milk ring test
*The mother of the fetus *B. abortus* biotype 3 strain was isolated

A total of 10 different antibiotic discs were tested in the current investigation and the *B. abortus* biotype 3 strain isolated from the aborted Anatolian water buffalo was found to be highly resistant to all tested antibiotics.

Discussion

Brucellosis has worldwide distribution and causes great economic loss in the livestock industry in the Mediterranean basin, the Middle East and Asia where it is endemic. A wide variety of animals such as cattle, sheep, goats, buffalo and camels may suffer from brucellosis (FAO/WHO, 1986). Brucellosis has also been reported in the water buffalo, which in comparison to the other livestock animals is a less economically important animal. However, in some regions it bears great importance because of the high milk quality preference and the animal power it provides. since water buffalo may better appreciate watery or muddy areas where other livestock animals do not like, it is a choice as an alternative to cattle. In those locations where water buffalo are raised, knowledge of water buffalo diseases is essential to prevent and/or treat the conditions that hamper the profitability.

Brucellosis is a highly contiguous disease. While the venereal route is not a major concern for disease transmission contaminated semen can easily transmit the bacteria through artificial insemination (Rankin, 1965). On the other hand, aborted fetuses and contaminated materials are the main sources of bacterial transmission (Büyük and Şahin, 2011). Although *Brucella* agents do not cause clinical mastitis, they can be shed heavily in the milk providing a source of bacteria that may spread. Following exposure, the bacteria enter the host through oropharynx, upper respiratory tract and conjunctiva, and then are phagocytosed mainly by macrophages. The agents can

survive within the mononuclear cells and be transported to regional lymph nodes. Hematogenous and lymphatic transmission to selected organs such as mammary glands, the pregnant uterus, osteoarticular tissues and male reproductive organs then takes place. Decrease in milk production and an increase in somatic cell numbers are other important outcomes of the disease (Quinn *et al*., 2011). A dramatic decrease in milk production has also been reported by the owner in this study.

Bovine brucellosis causes osteoarticular and reproductive problems in animals, resulting in reduced yield. The disease significantly reduces fertility by causing orchitis and epididymitis in males and placentitis in females. It is also manifested in the birth of weak calves and abortion especially in the last trimester of gestation. However, abortions can happen earlier in gestation (Das *et al*., 1990). In this study, abortions were reported to occur between 6-10 months of gestation. Sero-positive herds with no known history of abortion can also be seen (Nicoletti, 1992). *Brucella* infected animals usually abort only once due to the development of cellular immunity (Sousa *et al*., 2017). However, in the following pregnancies bacterial shedding through the membranes and fluids can occur due to reinvasion.

In brucellosis associated aborted bovine fetuses, bronchopneumonia and interstitial pneumonia with lymphoid hypertrophy are the most commonly seen lesions. Abnormal abomosal content, fibrinous pleuritis, vasculitis and meningitis are some of the other changes seen in aborted fetuses (López *et al*., 1984). Mild lesions in the liver, spleen, kidneys and heart have also been occasionally described in aborted bovine fetuses (Sönmez *et al*., 2004). Although there is a lack of literature describing the histopathological changes in brucellosis associated aborted water buffalo fetuses it is feasible to speculate that similar lesions to cattle cases may occur. In the present study, bronchointerstitial pneumonia, abnormal abomosal content and serohemorrhagic fluid in the thoracic and abdominal cavities were noted.

Brucella species and biotypes may vary in animals from region to region. Bovine brucellosis is mainly caused by *B. abortus*. In buffalo, *B. abortus* biotype 1 has been reported in India (Renukaradhya *et al*., 2002), Italy (Borrillo *et al*., 2013) and Trinidad and Tobago (Fosgate *et al*., 2002). It is presumed that *B. abortus* biotype indigenous to the cattle population in one area may also be found in buffalo living in the same area. However, this might not be always true as in Italy *B. abortus* biotype 6 causes infection in cattle while biotype 1 is seen in buffalo (Di Giannatale *et al*., 2006). On the other hand, more research is certainly needed since biotype 6 has also been identified in buffalo in Italy, more dramatically in addition to *B. melitensis* (Di Giannatale *et al*., 2006). In Argentina, *B. abortus* biotype 5 was reported in an aborted water buffalo fetus as well (Martínez *et al*., 2014). In the Eastern Mediterranean region *B. abortus* biotype 3 is the predominant biotype in cattle (Refai, 2002; Büyük and Şahin, 2011). However, there is also a study in Turkey reporting higher presence of *B. abortus* biotype 1 in cattle (Sözmen *et al*., 2004). In the current study, *B. abortus* biotype 3 was isolated in an aborted water buffalo fetus in Turkey.

Diagnosis of brucellosis can be achieved by direct or indirect methods (FAO/WHO, 1986). Bacterial isolation and identification of the causative *Brucella* species is the golden standard for direct detection. For serological diagnosis of brucellosis in cattle and sheep RBPT, SAT, ELISA, dot-blot assay, complement fixation test, fluorescence polarization assay, 2 mercaptoethanol agglutination test and etc. are some other indirect methods frequently used (Ramnanan *et al*., 2014; Sousa *et al*., 2017). Since there are no specific serological tests for water buffalo currently, RBPT and SAT were used in the present study for detection of brucellosis. Positive results from both tests were accepted as true positive for brucellosis. In the current investigation, detection of 3 out of 4 animals tested by RBPT and SAT showing strong positivity against *B. abortus* biotype 3 was evaluated as water buffalo produces high titer of antibodies in natural infections.

MRT is a commonly used test for cattle and sheep milk samples for the detection of brucellosis and the milk in this test is used without any treatment (Göktürk *et al*., 1999; İlhan *et al*., 2008[b]). Biochemical features of water buffalo milk are quite different from other livestock animal's milk. Since the fat content of water buffalo milk is very high, milk samples in this study were also tested in different dilutions and diluents as well as not diluted. The best result for MRT was provided by 500 µl milk + 500 µl sterile distilled water + 30 µl antigen treatment with 37˚C incubation at 2 hours. This finding suggests that MRT can also be applicable for water buffalo in field investigations.

A wide variety of antibiotics were tested in this study. Since animal brucellosis is not treated by antibiotics (WHO/CDS/EPR, 2006) and the susceptibility testing of *Brucella* spp. is not routinely used in human medicine (Baykam *et al*., 2004), the presence of high resistance against *B. abortus* biotype 3 strain against the antibiotics tested in this study is a remarkable finding. Water buffalo milk is used in many products such as cheese, yogurt, cream etc. *Brucella* positive water buffalo milks that are not pasteurized properly can be a great risk for human health.

In the current study, *B. abortus* biotype 3 was isolated and identified from the aborted fetus. As an indirect detection system, serological tests can also be successfully used especially in surveillance studies. RBPT, SAT and MRT were performed to show the presence of *Brucella* antibodies in the blood and milk of the mother of the aborted fetus as well as another three mothers from the same herd with a known history of abortion in this study. Bacteriological culture, immunohistochemistry, PCR and qPCR are the direct detection methods that have been suggested for detection of *Brucella* agents (Sözmen *et al*., 2004; Caitano *et al*., 2014). Easy, cheap and fast applicability of most indirect tests are the interest of choice especially for surveillance purposes as compared to direct tests that are more costly and require equipped personal and laboratories. However, it must be kept in mind that the tests used for cattle have different sensitivity and specificity in water buffalo (Fosgate *et al*., 2014).

Brucella species are fastidious and relatively slow growing organisms (Stack *et al*., 2002). The strain isolated in this study was harvested on the 6th day. In isolation, identification and typing of the current strain, *B. abortus* biotype 3 showed activity on approximately on the 5th day. This finding may be evaluated as the water buffalo originated strain adapting late to laboratory conditions.

Brucellosis in water buffalo has been previously reported in some countries including Turkey. Most of the brucellosis reports have been based on serological tests. In this study, bacteriological isolation and identification of the *B. abortus* biotype 3 from an aborted fetus was successfully performed and histopathological changes were described in addition to the seropositive diagnosis from the mother. This study is the first to show brucellosis associated abortion in a water buffalo in Turkey. *Brucella* agents are well known microorganisms that can cause abortion in livestock animals and therefore attention must be directed against them to prevent the occurrence of such losses in water buffalo where they are raised. Thus, regular serological surveillance can be suggested and may contribute to the control of the disease. Since brucellosis is a zoonotic disease, care must also be taken when handling and using associated animal materials and milk products of water buffalo.

References

Albayrak H, Özan E, Beyhan YE, Kurt M and Kılıçoğlu Y 2012. A serological investigation of some aetiological agents associated with abortion in domestic water buffalo (*Bubalus bubalis Linneaus*, 1758) in Samsun province of Northern Turkey. Atatürk Üniversitesi Vet Bil Derg. 7: 155-160.

Alton GG, Jones LM, Angus RD and Verger JM 1988. Bacteriological Methods. In: Techniques for the Brucellosis Laboratory Institute National de la Recherche Agronomique, Paris, France. 13-61.

Bauer AU, Kirby WM, Sherris JC and Tack M 1966. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 45: 493-496.

Baykam N, Esener H, Ergonul O, Eren S, Celikbas AK and Dokuzoguz B 2004. In vitro antimicrobial susceptibility of *Brucella* species. Int J Antimicrob. 23: 405-407.

Borghese A 2011. Situation and perspectives of buffalo in the World, Europe and Macedonia. Maced J Anim Sci. 1: 281–296.

Borriello G, Peletto S, Lucibelli MG, Acutis PL, Ercolini D and Galiero G 2013. Link between geographical origin and occurrence of *Brucella abortus* biovars in cow and water buffalo herds. Appl Environ Microbiol. 79: 1039-1043.

Büyük F and Şahin M 2011. Investigation of *Brucella* species from various samples of aborted cattle in Kars Province (Turkey) by cultural and molecular methods and epidemiological analysis of cases. Kafkas Univ Vet Fak Derg. 17: 809-816.

Caitano MAB, Soares CO, Ramos CAN, Ferraz ALJ, Sanches CC and Rosinha G 2014. Detecção de *Brucella abortus* em tecidos bovines utilizando ensaios de PCR e qPCR. Pesq Vet Bras. 34: 497-502.

Dağ S, Büyük F, Özen H, Çelebi Ö, Karaman M, Akça D and Şahin M 2012. Detection of *Brucella* spp. in vaginal swab samples of aborting cattle: Comparison of immunoperoxidase to bacteriological culture technique. Kafkas Univ Vet Fak Derg. 18: 617-622.

Das VM, Paranjape VL and Corbel MJ 1990. Investigation of brucellosis-associated abortion in dairy buffaloes and cows in Bombay India. Indian J Anim Sci. 60: 1193-1194.

Di Giannatale E, De Massis F, Ancora M, Zilli K and Alessianti A 2008. Typing of *Brucella* field strain isolated from livestock populations in Italy between 2001 and 2006. Vet Ital. 44: 383-388.

FAO/WHO 1986. "Joint Food and Agriculture Organization/World Health Organization. Expert Committee on Brucellosis. World Health Organization Technical Report Series 740. World Health Organization, Geneva." [Online]. Available: http://apps.who.int/iris/handle/10665/40202. Accessed April 1, 2020.

Fosgate GT, Adesiyun AA, Hird DW, Hietala SK and Ryan J 2002. Isolation of *Brucella abortus* biovar 1 from cattle and water buffaloes on Trinidad. Vet Record. 151: 272-273.

Fosgate GT, Diptee MD, Ramnanan A and Adesiyum AA 2011. Brucellosis in domestic water buffalo (*Bubalus bubalis*) of Trinidad and Tobago with comparative epidemiology to cattle. Trop Anim Health Pro. 43: 1479-1486.

Guarino A, Fusco G, Serpe L, Gallo P, Di Matteo A, Urbani G, Condeleo R, Tittarelli M and Di Ventura M 2001. Indirect ELISA for diagnosis of brucellosis in water buffaloes (*Bubalus bubalis*) in Italy. Vet Record. 149: 88-90.

Gürtürk K, Boynukara B, İlhan Z, Ekin İH and Gülhan T 1999. Comparison of the dot-immunobinding assay with the serum agglutination test, the rose Bengal plate test and the milk ring test for the detection of *Brucella* antibodies in bovine sera and milk. J Vet Med B. 46: 279-85.

Holt HR, Eltholth MM, Hegazy YM, El-Tras WF, Tayel AA and Guitiyan J 2011. *Brucella* spp. infection in large ruminants in an endemic area of Egypt: Cross-sectional study investigating seroprevalence, risk factors and livestock owner's knowledge, attitudes and practices (KAPs). BMC Public Health. 11: 341.

İlhan Z, Aksakal A, Ekin İH, Gülhan T, Solmaz H and Erdenlig S 2008[a]. Comparison of culture and PCR for the detection of *Brucella melitensis* in blood and lymphoid tissues of serologically positive and negative slaughtered sheep. Lett Appl Microbiol. 46: 301-306.

İlhan Z, Solmaz H, Aksakal A, Gülhan T, Ekin İH and Boynukara B 2008[b]. Detection of *Brucella melitensis* DNA in the milk of sheep after abortion by PCR assay. Arch Med Vet. 40: 141-146.

Konrad JL, Campero LM, Caspe GS, Brithuega B, Draghi G and Moor DP 2013. Detection of antibodies against *Brucella abortus*, *Leptospira* spp., and *Apicomplexa* protozoa in water buffaloes in the Northeast of Argentina. Trop Anim Health Pro. 45: 1751-1756.

López A, Hitos FP and Navarro-Fierro RR 1984. Lung lesions in bovine fetuses aborted by *Brucella abortus*. Can J Comp Med. 48: 275-277.

Martínez D, Thompson C, Draghi G, Canavesio V, Jacobo R, Zimmer P, Elena S, Nicola AM and Echaide ST 2014. Pheno and genotyping of *Brucella abortus* biovar 5 isolated from a water buffalo (*Bubalus bubalis*) fetus: first case reported in the Americas. Vet Microbiol. 173: 172-176.

Members of the SFM Antibiogram Committee 2003. Comité de l'Antibiogramme de la Société Française de Microbiologie Report 2003. Int J Antimicrob Agents. 21: 364-391.

Nasır A, Ashraf M, Shakoor A, Adil M, Abbas T, Kashıf M, Rana MY and Reichel MP 2014. Co-infection of water buffaloes in Punjab, Pakistan, with *Neospora caninum* and *Brucella abortus*, Turk J Vet Anim Sci. 38: 572-576.

NCCLS 2007. Performance Standards for Antimicrobial Susceptibility Testing. Eighteenth Informational Supplement. Document M100-S18, PA: NCCLS, Wayne.

Nicoletti P 1992. An evaluation of serologic tests used to diagnose brucellosis in buffaloes (*Bubalus bubalis*). Trop Anim Health Pro. 24: 40-44.

Nowroozi-Asl A, Oliaei A and Poormahmood-Shalgahian M 2007. A serological survey of *Brucella* spp. in water buffalo in Khoozestan province, Iran. Ita J Anim Sci. 6: 825-827.

Osterman B and Moriyon I 2006. International committee on systematics of prokaryotes; subcommittee on the taxonomy of *Brucella*. Int J Syst Evol Micr. 56: 1173-1175.

Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S and Hartigan PJ 2011. Veterinary Microbiology and Microbial Disease. 2nd ed. West Sussex, UK: Wiley-Blackwell.

Ramnanan A, Diptee M, Asgarali Z, Campbell M and Adesiyum AA 2012. Serological and bacteriological responses of water buffalo *(Bubalus bubalis)* vaccinated with two doses of *Brucella abortus* strain RB51 vaccine. Trop Anim Health Pro. 44: 1451-1458.

Rankin JEF 1965. *Brucella abortus* in bull: A study of twelve naturally-infected cases. Vet Record. 77: 132-135.

Refai MK 2002. Incidence and control of brucellosis in the Near East region. Vet Microbiol. 90: 81-110.

Renukaradhya GJ, Isloor S and Rajasekhar M 2002. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Vet Microbiol. 90: 183-195.

Santa Rosa CA, Castro AFP and Troise C 1961. Títulos aglutinantes para "*Brucella*" embúfalos do Estado de São Paulo. Arq Inst Biol São Paulo. 28: 35-39.

Sousa MGS, Salvarani FM, Bomjardim HA, Brito MF and Barbosa JD 2017. Brucellosis in water buffaloes. Pesq Vet Bras. 37: 234-240.

Sözmen M, Erginsoy SD, Genç O, Beytut E and Özcan K 2004. Immunohistochemical and microbiological detection of *Brucella abortus* in aborted bovine fetuses. Acta Vet Brno. 73: 465-472.

Stack JA, Harrison M and Perrett LL 2002. Evaluation of a selective medium for *Brucella* isolation using natamycin. J Appl Microbiol. 92: 724-728.

Suazo-Cortez R, Romero-Salas D, Villagómez-Cortés JA and Martínez-Herrera DI 2012. First notification on the presence of brucellosis in water buffalo (*Bubalus bubalis*) in Mexico by serological tests. Afr J Microbiol Res. 6: 3242-3247.

TUIK 2018. "Hayvansal Üretim İstatistikleri" [Online]. Available: (http://www.setbir.org.tr/wp-content/uploads/Hayvansal-Üretim-İstatistikleri-2018-Haber-Bülteni.pdf. Accessed April 1, 2020.

WHO/CDS/EPR 2006. "Brucellosis in Humans and Animals" [Online]. Available: (https://www.who.int/csr/resources/publications/Brucellosis.pdf. Accessed April 1, 2020.