Serological Investigation of Q Fever in Anatolian Buffaloes

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Abstract: Buffaloes as in other animals have been demonstrated to play a role in certain diseases transmitted to susceptible animals and human populations. In this study, serum samples were collected from Anatolian Buffaloes in breeding Samsun and around were examined for Q Fever. For this purpose 184 sera were analyzed with commercial ELISA test kit. Totally 29 (15.8%) were determined positive for Q Fever from examined 184 serum samples. Serum samples obtained from Anatolian Buffaloes were examined first time in terms of serologically in our region respect to Q Fever. As a result, the data provided within the scope of the research indicate a Q Fever seropositivity level that could pose a risk for our indigenous buffalo population. We concluded that the data obtained from this study can constitute a resource to similar studies in our region. The epidemiology of the disease can be elaborated in the light of studies that will be carried out with more comprehensive researches in our region.

Keywords: Anatolian buffalo, ELISA, Q fever, sera.

Anadolu Mandalarında Q Fever Hastalığının Serolojik Olarak Araştırılması

ÖZ: Mandaların, diğer hayvanlarda olduğu gibi bazı hastalıkların duyurul hvordan populasyonlarına ve insanlara bulaştırılmasında rol oynamaktadır. Bu çalışmada, Samsun il ve ilçeleri içinde yetiştirilen Anadolu Mandalarına ait kan örnekleri Q Fever hastalığı yönünden incelendi. Bu amaçla 184 kan serumu ticari bir ELISA kit ile test edildi. İncelenen 184 serum örneklerinin 29 (15.8%)’si Q Fever hastalığı açısından seropozitif bulundu. Araştırma ile belgelimizde ilk kez Anadolu Mandalarına ait serum örnekleri Q Fever yönünden serolojik olarak incelendi. Sonuç olarak, proje kapsamında sağlanan veriler bölge popülasyonu için risk oluşturma ilecek düzeyde Q Fever seropozitifliğe işaret etmektedir. Yüritilen araştırmanın elde edilen verilerin, yörendede yapılacak benzer çalışmalarla doğrulan teşkil edebilеченin kanıtına varıldır. Belgelimizde daha kapsamlı projelerle gerçekleştirebileceğiz araştırmalar sırasında hastalığın epidemiolojisi detaylı bir şekilde ortaya konulabilecektir.

Anahtar sözcükler: Anadolu Mandası, ELISA, Q fever, sera.

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INTRODUCTION

Buffalo (*Bubalus bubalis*) is an animal that is rearing worldwide, particularly in certain countries, like meat, milk and pack animal. Originated from domestic and wild forms of buffaloes have approximately 74 breeds. These breeds are roughly divided into two groups as marsh and river (brook) buffaloes. As marsh buffaloes are used for pack animals, river buffaloes are rearing for meat and milk. The buffaloes in Turkey are originated from the subgroup of river buffaloes of Mediterranean and named as Anatolian buffaloes. According to the latest data, 194 million buffaloes exist worldwide. According to 2017 data, it is reported that 161.439 buffaloes are grown in our country while there are about 19.869 buffaloes in Samsun (Anonymous, 2018). In the light of these data, Samsun is placed on the top of our country’s Anatolian buffalo rearing (Figure 1).

Q fever is a widespread disease caused by the bacteria *Coxiella burnetii*, which is able to infect mammals, birds, reptiles, and arthropods. It causes a mild disease in ruminants but can cause abortions and stillbirths in cattle, sheep, and goats. It is also known as a zoonotic disease. Q fever is listed in the OIE Terrestrial Animal Health Code and Member Countries and Territories are obligated to report occurrences of the disease to the OIE according to the OIE Terrestrial Animal Health Code. First identified in Australia in 1935, Q fever has since been found throughout the world with the exception of New Zealand. Cattle, sheep, and goats are the primary reservoirs of *C. burnetii*. The infection has been noted in a wide variety of other domestic animals including dogs, cats, rabbits, horses, pigs, camels, buffalo, rodents, and some birds, that can transmit the infection to humans without showing signs of illness (OIE, 2018; De Rooij et al., 2019).

A large number of studies have been conducted in different countries to determine the Q Fever disease in various animal species (Horton et al., 2014; Douangneon et al., 2016; Karami et al., 2017; Pradeep et al., 2017). Likewise, there are serological and molecular studies related to Q Fever in sheep, goats, cattle and humans in our country (Karaca et al., 2009; Gunaydın et al., 2014; Can et al., 2015; Cetinkol et al., 2017; Cıkman et al., 2017). It has been reported in the studies (Nahed & Khaled, 2012; Vongxay et al., 2012; Horton et al., 2014; Douangneun et al., 2016) to determine the serological prevalence of Q Fever in buffaloes in different countries, the seroprevalence of Q Fever vary between 0-34.5%. Although there are studies in our country about the detection of different diseases in buffaloes (Gulhan et al., 2016; Nuhay & Gulhan, 2017; Ak & Gulhan, 2018), the number of studies conducted in order to research of Q Fever disease (Payzin, 1953; Gunaydın & Pekkaya, 2016) are limited. Among the reasons for this, it is possible to consider that compared to other animal species abortus cases are less in buffaloes and perhaps ignoring *C. burnetii* in aborting factors. In our country, by combining first the regional and then the country-wide data, detecting genital system diseases in buffaloes throughout the country will be able to determine by the epidemiological studies and the deficiencies can be eliminated in this subject. In this study, seropositivities of Q Fever disease in Anatolian buffaloes which are intensively reared in our region was revealed.

MATERIALS AND METHODS

Ethical approval: The ethical approval was taken from the Animal Experiments-Local Ethical Committee of the Samsun Veterinary Control Institute (Date: 28/02/2017, No.: 2017/2).

Study area and collection of blood samples: The materials of the study consisted of 184 blood serum samples obtained from Anatolian buffaloes reared in Samsun districts (Figure 2).

![Figure 1. The distribution of Anatolian buffaloes by the provinces in Turkey.](image1)

![Figure 2. Centers collected Anatolian buffalo serum samples.](image2)
tubes as 10 ml of each animal under aseptic and sterile conditions and were delivered to the Department of Veterinary Microbiology of the University of Ondokuz Mayis. The sera that separated and centrifuged from blood samples were collected in sterile Eppendorf tubes and stored at -36 °C until used in ELISA tests.

Table 1. The centers and the numbers of collected serum samples of the Anatolian buffaloes.

| Center        | Numbers of Anatolian buffaloes | Numbers of collected serum |
|---------------|-------------------------------|----------------------------|
| Bafra         | 6972                          | 100                        |
| Alacam        | 1936                          | 42                         |
| Ondokuz Mayis | 1116                          | 42                         |
| Total         | 184                           | 184                        |

**ELISA:** A commercial ELISA kit (IDEXX Q-Fever test, Idexx Laboratories, USA) was used in the examination of blood sera collected from Anatolian buffaloes in terms of IgG antibodies against C. burnetii phase I and phase II antigens. The test was carried out according to the manufacturer's recommendations. Briefly, 100 µl of 1:1400 diluted serum samples were added to plate wells which were coated with C. burnetii antigen and the plates were incubated at 37 °C for 60 minutes. At the end of the incubation time, the wells were washed for 3 times, 100 µl of antiruminant IgG conjugate added into the wells and were waited 60 minutes under the same conditions. The wells were washed for 3 times, 100 µl of TBM substrate was added to each well and the plates were incubated at room temperature for 15 minutes. At the end of the incubation time, stop solution was added to the wells and the reaction was terminated. The plates were placed on the ELISA reader at 450 nm and the results were obtained from ELISA reader. The evaluation was performed with positive and negative controls provided in the test content. Two positive and negative controls were included in each plate. The optical density (OD) of positive control and negative control were set not to exceed 2000 and 0.500, respectively and the difference between positive and negative controls was set not to exceed 2000 and 0.300 respectively. The OD value was calculated by the following formula.

\[
\text{OD\%} = \frac{\text{OD sample} - \text{OD negative}}{\text{OD positive} - \text{OD negative}} \times 100
\]

The results were considered positive if the OD > 40% and negative if the OD < 30%. If the OD was between 30-40%, the results were considered suspicious and the test was repeated.

**RESULTS**

In 184 blood serum samples which were examined within the scope of the research, 29 (15.8%) were Q fever seropositive and 155 (84.2%) were seronegative. The distribution of ELISA test results according to the centers where serum samples were provided is presented in Table 2.

**DISCUSSION**

Buffaloes can carry many pathogenic agents as in other animals (El-Mahallawy et al., 2012), and these agents play a role in the transmission of some diseases to susceptible animal populations and humans (Pradeep et al., 2017). *Brucella, Chlamydia* and *Coxiella* species are considered to be responsible for cases of abort/weak offspring seen in buffalo populations (Didugu et al., 2016). Isolations of agents, allergic skin tests, molecular and serological techniques are used for diagnosis of Q fever disease. For the isolation of the agent of zoonotic diseases, high-security level laboratories are needed (Natale et al., 2012). The allergic skin test is used as a pre-vaccination screening test. It has been reported to have disadvantages like the skin test requires experience, the cut-off value is not well defined and can give various results (Schoffelen et al., 2013). A variety of techniques are used in the molecular identification of the agent as a vector in ticks (Ghashghaie et al., 2017), and especially from various materials of aborted animals, such as PCR, dot immunoblotting, Western blotting, indirect haemolysis test (Khalifa et al., 2016; Abdel-Moein & Hamza, 2017).

Different methods are preferred for serological diagnosis in animals at various stages of the disease, such as indirect immunofluorescence (IFA), complement fixation (CFT), microagglutination, gamma interferon (IFN-γ), capillary agglutination test (CAT) and ELISA. It is reported that serological diagnosis is easier to apply and lower cost than other diagnostic methods (Luccese et al., 2016). It has been shown that, in these techniques, ELISA is superior to other techniques in terms of sensitivity and specificity (Rizzo et al., 2016). It is especially preferred in epidemiological studies aimed at field screening (Lyoo et al., 2017). The epidemiological studies that can reveal the state of Q fever disease in buffaloes are limited. According to recent literature, the prevalence of Q fever in the buffaloes ranged from 0-34.5%. In a study conducted in Pakistan in order to determine the seroprevalence of different species of Q fever disease (Ahmed, 1987), 26.8% (15/56) in humans, 4.6% in goats (3/65), 18.3% in sheep (11/60), in cattle 10.4% (4 / 35), 34.5% (19/55) in buffaloes and 18% (54/300) in rodents were detected as positive with complement fixation (CFT) technique. In a similar study (Adesiyun & Cazabon, 1996), 3 of 266 (1.1 %) chicken blood serum, 11 of 256 (4.3 %) cattle serum, 17 of 153 (11.1 %) of pig blood serum, 5 of 53 (9.4 %) large ruminants were detected as positive with CFT technique.
In one of the studies on the serological diagnosis of Q fever disease in animals in our country, 16.5% (59/356) in sheep, 19.3% (162/840) in cattle, 11.2% of buffaloes (215/1955) in goats, 1.9% (4/216) in sheeps, 1.1% in buffaloes (2/188) and 1% (2/206) in cattle were detected positive for Q fever by ELISA. In a recent study in which Q fever disease was searched serologically in sheep and buffaloes in Iran (Karami et al., 2012), 9 of 45 buffalo blood serum tested was negative. Vongxay et al. (2012) reported in their study that aims regional scanning of Q fever disease by ELISA, seropositivity values were 3.3% (10/301) in cattle and 4.3% (26/604) in buffaloes. Horton et al. (2014) found that seropositivity rates of Q fever by ELISA were 4% (6/153) in buffaloes, 8% (14/174) in sheep and 70% (7/10) in camels. In another study (Douangneun et al., 2016) found that in 526 cattle blood serum samples that were found seropositive for Q fever by ELISA while all of 45 buffalo blood serum tested was negative. The presence, prevalence and carriage rates in the target animal populations of Q fever disease in serum samples of Anatolian buffaloes in Samsun province and its districts were examined for the first time in this study. Within the scope of the study, positivity were not detected in some serum samples obtained from Anatolian buffalo populations. However, the rate of seropositivity determined in this study was found to be lower in buffaloes of some countries (Ahmed, 1987; Vaidya et al., 2010), but higher in others. Different seropositivity values obtained from the researches can be due to factors such as differences in the method used, the geographical distribution of sample population and individual differences in population. This situation can be seen in the study findings in the same country in previous years, and also in the reported values between different countries.

In conclusion, the data provided in this study indicate the presence of Q fever seropositivity level in our region which could pose a risk for the buffalo population.
is understood that, especially in buffalo populations, which have abort cases or weak offspring and infertility problems, the disease should be considered as an important factor as causing other genital system disorders. Although this study has been conducted on a limited number of animals, the detection of C. burnetii antibodies in abortive buffaloes shows that there is contact with the agent. Therefore, the owners of the buffaloes which collected samples as part of the study were informed in detail about the disease. The epidemiology of the disease can be elaborated in the light of the more comprehensive research that will be carried out in our region.

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REFERENCES

Abdel-Moein, K.A. & Hamza, A. (2017). The burden of Coxiella burnetii among aborted dairy animals in Egypt and its public health importance. Acta Tropica, 166, 92-95.

Adesiyun, A.A. & Cazabon, E.P.I. (1996). Seroprevalences of brucellosis, Q-fever and toxoplasmosis in slaughter livestock in Trinidad. Revue d'élevage et de Médecine Vétérinaire des pays Tropicaux, 49(1), 28-30.

Ahmed, I.P. (1987). A serological investigation of Q fever in Pakistan. Journal of Pakistan Medical Association, 37(5), 126-129.

Ak, S. & Gulhan, T. (2018). Isolation of enterococci species from Anatolian buffaloes and determination of antibiotic resistance. Journal of Etilik Veterinary Microbiology, 29, 40-45.

Anonymous (2018). The distribution of Anatolian buffalo numbers in Turkey by province. http://rapory.tuik.gov.tr/20-02-2018-11:47:01-24737139611988075961700884630.pdf Date of access: 10.06.2019.

Can, H.Y., Elmali, M. & Karagöz, A. (2015). Detection of Coxiella burnetii in cows’, goats’, and ewes’ bulk milk samples using polymerase chain reaction. Mljekarstvo, 65, 26-31.

Cetinkol, Y., Enginyurt, O., Celebi, B., Yıldırım, A.A., Cankaya, S. & Aktepe, O.C. (2017). Investigation of zoonotic infections in risk groups in Ordu University Hospital, Turkey. Niger Journal of Clinical Practice, 20, 6-11.

Çıkman, A., Aydin, M., Gulhan, B., Karakeçili, F., Özciçek, A. & Kesik, O.A. (2017). The seroprevalence of Coxiella burnetii in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical features. Journal of Vector Diseases, 54, 157-163.

De Rooij, M.M.T., Van Leuken, J.P.G., Swart, A., Kretzschmar, M.E.E., Nielen, M., De Koeijer, A.A., Janse, I., Wouters, L.M. & Heederik, D.J.J. (2019). A systematic knowledge synthesis on the spatial dimensions of Q fever epidemics. Zoonoses and Public Health, 66, 14-25.

Didugu, H., Narasimha, R.C.E., Ramanipushpa, R.N., Ramaraju, S.S.B., Reddy, M.V. & Satyanarayana, M. (2016). Serological investigation of chlamydial infection among ruminants in Krishna district of Andhra Pradesh, India. Journal of Livestock Science, 7, 187-191.

Douangngeun, B., Theppangna, W., Soukvilay, V., Senaphanh, C., Phithaethep, K. & Phomhaksa, S. (2016). Seroprevalence of Q fever, brucellosis, and bluetongue in selected provinces in Lao People’s Democratic Republic. American Journal of Tropical Medicine and Hygiene, 95(3), 558-561.

El-Mahallawy, H.S., Abou-Eisha, A.M. & Fadel, H.M. (2012). C. burnetii infections in cattle and buffaloes and its public health significance. Suez Canal Veterinary Medicine Journal, 17(2), 51-64.

Ghashghaei, O., Nourollahi-Fard, S.R., Khalili, M. & Sharifi, H. (2017). A survey of ixodid ticks feeding on cattle and molecular detection of Coxiella burnetii from ticks in Southeast Iran. Turkish Journal of Veterinary and Animal Sciences, 41, 46-50.

Guhan, T., Ciftci, A., Onuk, E.E. & Boyunkara, B. (2016). Detection of Escherichia coli O157:H7 from Anatolian Buffaloes in Samsun, Turkey, p. 136. In Proceedings of 3rd International Congress VETIstanbul Group Bosnia and Herzegovina-2016, 17-20 May, Sarajevo, Bosnia and Herzegovina.

Gunaydin, E., Pekkaya, S., Mustak, K.M. & Dalkılıc, B. (2014). Investigation of Q fever in Kilis and Shamal goats by ELISA and touchdown-PCR. Journal of Ankara University Veterinary Faculty, 61, 161-165.

Gunaydin, E & Pekkaya, S. (2016). Serologic and molecular investigation of Q fever on water buffalo in Afyon. Van Veterinary Journal, 27(1), 17-19.

Horton, K.C., Wasfy, M., Samaha, H., Abdel-Rahman, B., Safwat, S. & Fadeel, M.A. (2014). Serosurvey for zoonotic viral and bacterial pathogens among slaughtered livestock in Egypt. Vector-Borne Zoonotic Diseases, 14(9), 633-639.

Kalema-Zikusoka, G., Bengis, R.G., Michel, A.L. & Woodford, M.H. (2005). A preliminary investigation of tuberculosis and other diseases in
African buffalo (Syncerus caffer) in Queen Elizabeth National Park, Uganda. *Onderstepoort Journal of Veterinary*, 72(2), 145-151.

Karaca, M., Akkan, H.A., Çetin, Y., Keles, I., Tutuncu, M., Özkın, C. & Tasal, I. (2009). Studies on the determination of seroprevalence of Q fever in sheep in the Region of Van. *Journal of Animal and Veterinary Advances*, 8(10), 1925-1928.

Karami, M.H., Pourmahdi, B.M., Garibi, D. & Haji, H.M.R. (2017). Serological survey of Q fever in goats and buffaloes in Ahvaz region using the ELISA method. *Veterinary Clinical Pathology*, 11(41), 25-35.

Khalifa, N.O., Elhofy, F.I., Fahmy, H.A., Sobhy, M.M. & Agag, M.A. (2016). Seroprevalence and molecular detection of *Coxiella burnetii* infection in sheep, goats, and human in Egypt. *Journal of Microbiology, Biotechnology, and Food Sciences*, 2(1), 1-7.

Klemmer, J., Njeru, J., Emam, A., El-Sayed, A., Moawad, A.A., Henning, K., Elbeskawy, M.A., Sauter-Louis, C., Straubinger, R.K., Neubauer, H. & Diasty, M.M. (2018). Q fever in Egypt: Epidemiological survey of *Coxiella burnetii* DNA by polymerase chain reaction in slaughtered ruminants. *Vernary World*, 10(6), 667-671.

Kriz, F., Vitale, N., Ballardinia, M., Borromeo, V., Luzzago, C. & Chiavecci, L. (2016). Q fever seroprevalence and risk factors in sheep and goats in northwest Italy. *Preventive Veterinary Medicine*, 130, 10-17.

Schoffelen, T., Joosten, L.A.B., Herreman, T., de Haan, A.F.J., Ammerdorffer, A. & Rumke, H.C. (2013). Specific interferon γ detection for the diagnosis of previous Q fever. *Clinical Infectious Diseases*, 56(12), 1742-1751.

Vaidya, V.M., Malik, S.V.S., Bhilegaonkar, K.N., Rathore, R.S., Kaur, S. & Barbu, S.B. (2010). Prevalence of Q fever in domestic animals with reproductive disorders. *Comparative Immunology, Microbiology & Infectious Diseases*, 33, 307-321.

Vongxay, K., Conlan, J.V., Khounsy, S., Dorny, P., Fenwick, S. & Thompson, R.C.A. (2012). Seroprevalence of major bovine-associated zoonotic infectious diseases in the Lao People’s Democratic Republic. *Vector-Borne and Zoonotic Diseases*, 12(10), 861–866.

**Office International Epizootica (OIE) (2018).** http://www.oie.int/animal-health-in-the-World/animal-diseases/q-fever/. Date of access: 06.03.2019.

**Payzın, S. (1953).** Epidemiological investigations on Q fever in Turkey, *Bulletin of the World Health Organization*, 9, 553-558.

**Pradeep, J., Stephen, S., Pooja, P., Akshayavardhini, A., Sangheetha, B. & Antony, P.X. (2017).** Coxiellosis in domestic livestock of Puducherry and Tamil Nadu: Detection of *Coxiella burnetii* DNA by polymerase chain reaction in slaughtered ruminants. *Vernary World*, 10(6), 667-671.

**Nahed, H.G & Khaled, A.A. (2012).** Seroprevalence of *Coxiella burnetii* antibodies among farm animals and human contacts in Egypt. *Journal of American Science*, 8, 619–621.

**Natale, A., Bucci, G., Capello, K., Barberio, A., Tavella, A. & Nardelli, S. (2012).** Old and new diagnostic approaches for Q fever diagnosis: Correlation among serological (CFT, ELISA) and molecular analyses, *Comparative Immunology, Microbiology & Infectious Diseases*, 35, 375-379.

**Nuhay, C. & Gülhan, T. (2017).** Determination of *Escherichia coli* O157:H17 in Anatolian buffaloes’ feces in and around Samsun. *Journal of Edik Veterinary Microbiology*, 28(1), 39-45.