

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 duyarlı hayvan popülasyonlarına ve insanlara bulaştırılmasında rol oynadıkları ortaya konulmuştur. Bu çalışmada, Samsun ili ve ilçelerinde yetiştiriciliği yapılan Anadolu Mandalarına ait kan serum örnekleri Q fever hastalığı yönünden incelendi. Bu amaçla, 184 kan serumu ticari bir ELISA kiti ile test edildi. İncelenen 184 serum örneğinin 29 (%15.8)'u Q fever hastalığı açısından seropozitif bulundu. Bu araştırma ile bölgemizde ilk kez Anadolu Mandalarına ait serum örnekleri Q fever yönünden serolojik olarak incelendi. Sonuç olarak, proje kapsamında sağlanan veriler bölgemiz manda popülasyonu için risk oluşturabilecek düzeyde Q fever seropozitifliğine işaret etmektedir. Yürütülen araştırmadan elde edilen verilerin, yöremizde yapılacak benzer çalışmalara kaynak teşkil edebileceği kanısına varıldı. Bölgemizde daha kapsamlı projelerle gerçekleştirilecek araştırmalar ışığında hastalığın epidemiyolojisi detaylı bir şekilde ortaya konulabilecektir.

Anahtar sözcükler: Anadolu mandası, ELISA, Q fever, serum.

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).

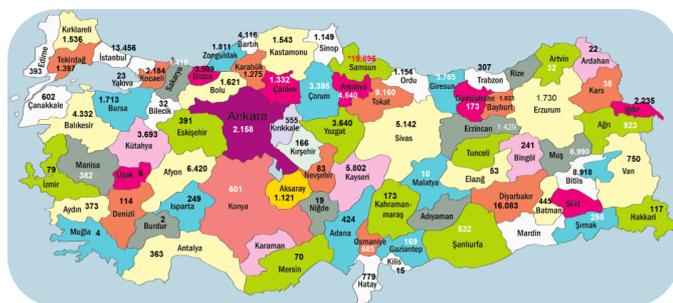


Figure 1. The distribution of Anatolian buffaloes by the provinces in Turkey.

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; Douangneun 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; Gunaydin 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 (Payzın, 1953; Gunaydin & 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).

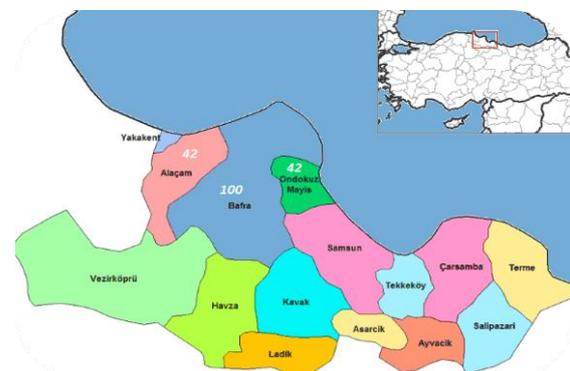


Figure 2. Centers collected Anatolian buffalo serum samples.

Buffalo populations planned to be sampled within the scope of the study were selected from herds which's population was previously known and from aborted animals. Blood samples were collected from Bafra (n=100), Alacam (n=42) and Ondokuz Mayıs (n=42) which are the most buffalo reared districts from January to December 2017 (Table 1). Blood samples were collected to anticoagulant-free 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 Mayıs. The serums 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 Mayıs	1116	42
Total	10 024	184

ELISA: A commercial ELISA kit (IDEXX Q-Fever test, IDEXX Laboratories, USA) was used in the examination of blood serums 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 to $0.300 \geq$. 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.

Table 2. Distribution of ELISA test results by centers of the collected sera.

Center	Number of sera	ELISA positive (%)	ELISA negative (%)
Ondokuz Mayıs	42	10 (23.8)	32 (76.2)
Alacam	42	9 (21.4)	33 (78.6)
Bafra	100	10 (10)	90 (90)
Total	184	29 (15.8)	155 (84.2)

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 (Ghashghaei 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 (Lucchese 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 (Lyo 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 %) buffalo serum were detected as positive with capillary

agglutination test (CAT) for *C. burnetii* agglutination while 16 sheep and 7 goat blood serum were negative. In a study conducted in Uganda (Kalema-Zikusoka et al., 2005), 42 samples of blood serum from free-living African buffaloes were all found negative for Q fever with IFA. Vaidya et al. (2010) reported in their study that for the agent isolation, PCR and serological diagnosis in cattle, sheep, goats and buffaloes with genital system disorder, the compare of the percentage of positivity tested by ELISA were 18.2% (6/33) in buffaloes, 11.4% (10/88) in cattle, 9.3% (4/43) in sheep and 5.7% (3/53) in goats. In a study conducted in Egypt (El-Mahallawy et al., 2012), 9 of 92 (9.8%) cattle blood serum samples and 2 of 92 (2.2%) buffalo serums were positive for Q fever by ELISA. In another study carried out in the same country (Nahed & Khaled, 2012), 18 of 55 (32.7%) sheep blood serum, 7 of 30 (23.3%) goat serum and 7 of 54 (13%) cattle serum were found seropositive for Q fever by ELISA while all 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 taken for serological screening of abort agents in different animal species by ELISA, only 13 (1.2%) cattle blood serums were detected as seropositive for Q fever while 426 pigs, 130 buffaloes, and 6 goat blood serum were all negative. In a recent study in which Q fever disease was searched serologically in sheep and buffaloes in Iran (Karami Mirazizi et al., 2017), 47 of 137 (34.3%) goat serum were detected as positive while all of 135 buffalo blood serum samples tested were found to be seronegative. In a study conducted to determine the seropositivity of Q fever in different animal species in India (Pradeep et al., 2017), 5.6% (11/195) in goats, 1.9% (4/216) in sheeps, 1.1% in buffaloes (2/188) and 1% (2/206) in cattle were detected positive with ELISA. In a similar research (Klemmer et al., 2018) total of 2,699 blood samples investigated using ELISA and *Coxiella burnetii* specific antibodies were detected in 40.7% of camels (215/528), 19.3% of cattle (162/840), 11.2% of buffaloes (34/304), 8.9% of sheep (64/716) and 6.8% of goats (21/311), respectively.

In Turkey, there are studies conducted on a regional basis related to Q fever in humans and animal species such as cattle, sheep, and goat, the number of detailed studies for the detection of the disease seems to be very limited in buffaloes. In one of the studies on the serological diagnosis of Q fever disease in animals in our country 16.5% (59/356) in sheep, 13% (36/278) in goats, 16% in cattle (58/362) and 4% (2/49) in buffaloes seropositivity detected with CFT (Payzın, 1953). Gunaydın & Pekkaya (2016), conducted a study to determine

seropositivity of Q fever disease in buffalo population in Afyon and determined positivity in 8 (8.7%) of 92 buffalo blood serum samples they tested. The most important reasons for the limited work in buffaloes might be; localization of the buffaloes in specific geographic regions and the variation of the population from country to country. For these reasons, buffalo slaughter and consumption is less.

In this study, 29 (15.8%) of 184 buffalo blood sera collected from Bafra, Alacam and Ondokuz Mayıs districts of Samsun were found positive with commercial ELISA test kit. The total positivity rate determined in studied buffalo populations was higher than other studies with buffalo in our country (0,4, 4.1 and 8.7%). On the other hand, 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.

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 while in some populations were found to be high. Seropositivity was found to be 15.8% in total animal populations. It is remarkable that this ratio is higher than the values reported in previous years in our country. It is important that seropositivity of factor that has zoonotic importance is high in buffaloes.

Although it is known that *C. burnetii* infection in Turkey is enzootic in farm animals, there is not enough information about herd prevalence, frequency of infection and which animal species pose a risk to humans. Seropositivity/seroprevalence values determined in various animals in different studies vary according to years, animal population studied, region and applied screening test. Both in risk groups of occupational groups in different countries and in our region in neighboring provinces, detection of high rates of seropositivity against Q fever disease in humans is remarkable. Therefore, close monitoring of Q fever disease in animals of risk groups, assessment of the carriers in vector position, especially in ticks, and molecular and serological detection of the agent, especially in abortion animals, will make important contributions in epidemiological terms.

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. It 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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