Buffalo Bulletin (April-June 2023) Vol.42 No.2
https://doi.org/10.56825/bufbu.2023.4224146

DEVELOPMENT OF ELISA KIT FOR SEROLOGICAL DIAGNOSIS OF Q FEVER

Timur Gülhan, Merve Gizem Sezener, Şeyda Yaman, Arzu Fındık and Alper Çiftci*

Received: 31 January 2021
Accepted: 26 June 2023

ABSTRACT

In this study, it was aimed to develop an ELISA diagnostic kit that can be used in the serological investigation of Q fever disease caused by *C. burnetii*. For this purpose, bovine, sheep, goat and buffalo sera were tested with a commercial ELISA kit and home-made ELISA kit developed using two different conjugates. Of the 92 buffaloes blood serum samples tested, 22 (23.9%) were found positive by commercial ELISA kit, 7 (7.6%) and 29 (31.5%) were positive by protein-A conjugated ELISA and 29 anti-bovine IgG conjugated ELISA, respectively. In addition, Q fever positive and negative cattle, sheep, and goat blood sera that were found to be positive and negative for Q fever in a previous study conducted on animals with abortion history were also re-examined in our laboratory. In conclusion, within the scope of the project, an alternative homemade ELISA kit was developed for the serological diagnosis of Q fever and serum samples of the target species of buffalo, cattle, sheep, and goats were tested. Thus, the home-made ELISA kit, which can be used in research aimed at detecting seropositivity, especially in buffalo sera, was made ready for use. It was concluded that the data obtained would contribute to wider ranging epidemiological studies. The epidemiology of the disease can be revealed in detail in the light of research to be carried out with more comprehensive projects in our region.

Keywords: *Bubalus bubalis*, buffaloes, Anatolian buffaloes, cattle, ELISA, goat, Q-fever, sera, sheep

INTRODUCTION

Query (Q.) fever is a worldwide zoonotic bacterial infection caused by *Coxiella (C.) burnetii* which an obligate intracellular Gram-negative bacterium (OIE, 2018). Although the disease is generally asymptomatic/subclinical in animals, it can cause significant economic losses in many domestic animals, especially ruminants, which may result in abortion, weak offspring birth, metritismetritis, and infertility. Abortions due to the disease are enzootic in sheep and goats, and sporadic in cattle. Whether or not there is a history of abortion in animals, the agent can be detected in materials such as milk and feces (De Rooij *et al*., 2019).

Numerous studies have been conducted in different countries to detect Q fever disease in various animal speciesand seroprevalence levels

Department of Microbiology, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, Samsun, Turkey, *E-mail: aciftci@omu.edu.tr

were found to be 19 to -45% in cats, 20- to 22% in dogs, 9.2% in deer, 40.7- to 51.5% in camels, 20- to 82% in cattle, 15- to 73% in sheep and goats (Pradeep *et al.*, 2017; Changoluisa *et al*., 2019). Similarly, serologicalserological, and molecular studies have been carried out about Q fever in sheep, goats, cattle and humans in our country (Çetinkol *et al*., 2017; Çıkman *et al*., 2017).

In studies conducted in different countries to determine the serological prevalence of Q fever in buffaloes (El-Mahallawy *et al*., 2012; Nahed and Khaled, 2012; Vongxay *et al*., 2012; Horton *et al*., 2014; Douangngeun *et al*., 2016) it has been reported that the seroprevalence varied between 0- to 34.5%. Although there are studies (Gülhan *et al*., 2019; Gülhan *et al*., 2020) in our country to detect different diseases in water buffaloes, it is observed that the number of these studies is very limited (Gunaydın and Pekkaya, 2016; Gülhan *et al*., 2019). Among the reasons for this, there are fewer abortion cases in buffaloes compared to other animal species, and perhaps ignoring *C. burnetii* among the factors causing abortions. By combining first regional and then obtained data in our country, it will be possible to detect genital system diseases in buffaloes throughout the country and to eliminate the deficiencies in this issue with epidemiological studies.

Therefore, whether or not there is a history of abortion, animals with suspicion of disease can be diagnosed early by serological screening. ELISA has been shown to be the most widely used technique in serological screening. However, the high cost of commercial ELISA kits necessitates the development of home-made ELISA kits in laboratories. With this study, an alternative home-made ELISA kit was developed for the serological diagnosis of Q fever disease, and the sera of target species of buffalo, cattle, sheepsheep, and goat were tested. As a result, a home-made ELISA kit that can be used in researchesresearch aimed at detecting seropositivity especially in buffalo serums was made ready for use. It was concluded that the data obtained will contribute to wider regional epidemiological studies.

MATERIALS AND METHODS

Serum samples

In the study, serum samples collected from various animals (buffalo, bovine, sheep, goat) when and stored in the laboratory of our department and sera that previously evaluated in a research processesresearch process were used as materials. For this purpose, 92 buffaloeso serum samples were used. In addition, blood serum samples from cattle (4), sheep (4) and goat (4), known to be Q fever positive (2) and negative (2), were analyzed.

Commercial ELISA

A commercial ELISA kit (IDEXX Q-fever test, Idexx Laboratories, USA) was used to examine blood sera for IgG antibodies formed against *C. burnetii* phase I and phase II antigens.

The test was carried out manufacturer's recommendations. Briefly, 100 µl of serum samples diluted 1: 400 was added to the plate wells coated with *C. burnetii* antigen and the plates were incubated at 37° C for 60 minutes. At the end of the incubation period, the wells were washed 3 times, 100 µl anti-ruminant IgG conjugate was added to the wells and kept under the same conditions for 60 minutes. The wells were washed 3 times, 100 µl of TMB substrate was added to each well, and the plates were incubated at room temperature for 15 minutes. At the end of the incubation period, the reaction was terminated by adding stop solution

to the wells. Plates were placed in an ELISA reader device at 450 nm and the results were read. EvaluationThe evaluation was made with the positive and negative controls provided in the test content. Two positive and negative controls were included inon each plate. OD of the positive control is 2.000 and the OD of negative control does not exceed 0.500; the difference between positive and negative controls was set to be ≥0.300. Optimal density (OD) value was calculated by the following formula.

% OD = OD sample-OD negative / OD positive-OD negative x 100

The results obtained were considered positive if the OD%> 40, and negative if the OD% <30. If the OD value of% was between 30 to -40, the results were considered suspicioussuspicious, and the test was repeated.

Home-made ELISA procedure and optimization

Lysis buffer, coating buffer, blocking buffer, washing buffer and stop solution were prepared to be used in all ELISA studies. Lysis buffer; 125 mMTris-HCl + 1% SDS pH: 6.8. Coating Buffer: 1.59 g Na_2CO_3 + 2.93 g $NaHCO_3$ + 0.2 g NaN_3 in 1 L of distilled water was formulated to be pH: 9.6. Blocking Solution; 1% Skimmed Milk Powder + 0.5% Tween 20, Wash Buffer; TBS containing 0.1% Tween 20 and Stop Solution were prepared as 2N NaOH.

In the home-made ELISA studies, U-bottomed 96-well polystyrene ELISA pellets were used. The *C. burnetii* strain isolated and characterized in previous studies was used as an antigen in the coating of ELISA plates. After the isolate was cultured in embryonic chicken eggs, the egg yolk collected was inactivated with 1% formaldehyde and the supernatant obtained was used as an antigen for coating the plates (OIE, 2018).

100 µl of different concentrations of the diluted antigen was added to the wells and incubated at room temperature for 24 h. Then 200 µl of blocking solution was added to each well and kept at 37°C for 24 h. The blocking plate was washed once with a washing buffer before use (OIE, 2018). Positive and negative control sera included in the commercial kit were also added to the wells and the plates were left for incubation at 37°C. After washing, different dilutions of the conjugate were added to the wells. It was kept under the same conditions for 60 minutes for incubation. The wells were washed 3 times. 100 µl of TMB substrate was added to each well and the plates were incubated at room temperature for 15 minutes. At the end of the incubation period, the reaction was terminated by adding stop solution to the wells. Plates were placed in an ELISA reader device at 450 nm and the results were read.

All sera were tested for Q fever by performing the home-made ELISA procedure at the optimal concentrations determined by optimization. Data obtained with commercial and home-made ELISA were compared. The optimization of the kit was performed by the checkerboard titration method, using the positive and negative control sera included in the commercial kit.

RESULTS

The positive serum numbers obtained by commercial ELISA and two different conjugates (anti-bovine IgG and Protein-A) of 92 serum samples examined and the sensitivity / specificity values of the tested home-made ELISA techniques

are shown in Table 1.

DISCUSSION

It has been revealed that water buffaloes carry many pathogenic factors like other animals (El-Mahallawy *et al.,* 2012) and play a role in the transmission of some diseases to susceptible animal populations and humans. As in other animal species, Brucella, Chylamydia and Coxiella species are blamed for isolation, molecular and serological reasons for abortion / weak offspring occasionally seen in buffalo populations (De Rooij *et al*., 2019).

Isolation of the agent, allergic skin test, molecular and serological techniques are used in the diagnosis of Q fever disease caused by *C. burnetii*. However, for diagnosing zoonotic disease, laboratories with high safety levels are needed. Allergic skin test is used as a pre-vaccination screening test. However, it has been reported that the skin test has disadvantages such as requiring experience, not well-defined cut-off value, and variable outcome. Various techniques such as PCR, dot immunoblotting, Western blotting, indirect hemolysis test is used in the molecular diagnosis of the agent from ticks and especially from various materials belonging to aborted animals (Karami *et al*., 2017). Different methods such as indirect immunofluorescence (IFA), complement fixation test (CFT), microagglutination test, gamma interferon (IFN-γ) test, capillary agglutination test (KAT) and ELISA are preferred for serological diagnosis in animals at various stages of the disease. Serological diagnosis has been reported to be more easily applicable and cost-effective than other diagnostic methods. Among these techniques, it has been revealed that ELISA is superior to other techniques in terms of sensitivity and specificity. It is preferred especially in epidemiological studies conducted for field screening (Changoluisa *et al*., 2019).

In order to screen Q fever disease with serological techniques in different animal species, many studies have been conducted in various countries, as in our country. On the other hand, it is seen that epidemiological studies that can reveal the status of Q fever disease in buffaloes

Table 1. ELISA results for tested sera.

Test kit	Positive serum number (%)	Negative serum number (%)	Sensitivity	Specificity
Anti-bovine IgG	29 (31.5)	63 (68.5)	0.85	0.89
Protein-A	7 (7.6)	85 (92.4)	0.73	0.98
Commercial ELISA	22 (23.9)	70 (76.1)		
Single anti-bovine IgG	10			
Single protein A	0			
Single commercial ELISA	4			
Anti-bovine IgG + protein A	1			
Anti-bovine IgG + commercial ELISA	12			
Protein-A + commercial ELISA	0			

are limited. According to current literature data, it has been revealed that the prevalence of Q fever in water buffaloes varies between 0% and 34.5%.

Yadav and Sethi (1979) have reported seropositivity at 24.3% (119/490) in cattle, 16.8% (90/536) in sheep, 16% (162/1011) in buffaloes, 15.9% (307/1937) in goats, 14.7% (27/184) in pigs, 14.3% (7/49) in stray dogs, and 15.2% (249/1636) in humans.

In a study conducted in Pakistan to determine the seroprevalence of Q fever disease in different species (Ahmed, 1987), seropositivity rates were found as 26.8% (15/56) in humans, 4.6% (3/65) in goats, 18.3% (11/60) in sheep, 10.4% in cattle (4/35), 34.5% (19/55) in buffaloes and 18% (54/300) in rodents by complement fixation (CFT) technique.

In a similar study (Adesiyun and Cazabon, 1996), 3 (1.1%) of 266 chicken blood sera, 11 (4.3%) of 256 bovine sera, 17 (11.1%) of 153 pig sera, 5 (11.1%) of 53 buffalo sera were found to be positive for *C. burnetii* agglutination by capillary agglutination test (CAT), while 16 sheep and 7 goat blood serum samples were negative.

Galiero *et al.* (1996) have reported that 12 (1.2%) of 1012 blood sera they received from buffaloes were positive for Q fever.

In a study conducted in Uganda (Kalema-Zikusoka *et al*., 2005), all 42 blood serum samples taken from free-living African buffaloes were found negative in Q fever with IFA.

In a study (Vaidya *et al*., 2010) conducted for the purpose of studying the occurrence of *C. burnetti* in cattle, sheep, goats, and buffaloes with genital system disorders, it has been reported that the seropositivity rates were 18.2% (6/33) in buffaloes, 11.4% in cattle (10/88), 9.3% (4/43) in sheep and 5.7% (3/53) in goats according to ELISA results. In a study conducted in Egypt (El-Mahallawy *et al*., 2012), 9 (9.8%) of 92 bovine blood serum samples and 2 (2.2%) of 92 buffaloes serum samples were found positive by ELISA. In another study conducted in the same country (Nahed and Khaled, 2012), 18 (32.7%) of 55 sheep sera, 7 (23.3%) of 30 goat sera, and 7 (13%) of 54 bovine sera were obtained by ELISA, while all 45 buffaloes blood serum tested were negative.

Vongxay *et al.* (2012) reported that the seropositivity rates were 3.3% (10/301) in cattle and 4.3% (26/604) in buffaloes in their study for the regional screening of Q fever disease with ELISA.

Horton *et al.* (2014) found the Q fever seropositivity rates with ELISA as 4% (6/153) in buffaloes, 8% (14/174) in sheep and 70% (7/10) in camels.

In a similar study (Douangngeun *et al*., 2016), Q fever seropositivity was determined in only 13 (1.2%) of 526 bovine blood sera collected for serological screening of abortion agents in different animal species, while all of 426 pigs, 130 buffalo and 6 goat blood serums were found negative.

In a study in which Q fever disease was investigated serologically in sheep and buffaloes in Iran (Karami *et al*., 2017), 47 (34.3%) of 137 goat sera were found to be positive, while all 135 buffaloes blood serum samples tested were found to be seronegative.

Although there are studies performed on a regional basis in animal species such as cattle, sheep and goats and humans for Q fever in Turkey, the number of detailed studies for the detection of the disease seems to be very limited in buffaloes.

In one of the first studies conducted for the serological diagnosis of Q fever disease in animals in our country (Payzın and Golem, 1948), all 13 buffales blood sera examined were found to be negative for Q fever with CFT. In a study conducted

by the same researcher in the following years (Payzın, 1953), seropositivity rates were detected as 16.5% (59/356) in sheep, 13% (36/278) in goats, 16% (58/362) in cattle and 4% (2/49) in buffaloes. In another report (Kaplan and Bertagna, 1955), 2 (4.1%) of 49 buffaloes blood serum samples tested were found positive.

Günaydın and Pekkaya (2016) found positivity in 8 (8.7%) of the 92 buffaloes blood serum samples they tested in their study to determine the seropositivity of Q fever disease in the buffalo population in Afyon.

In a recent study conducted in our region (Gülhan *et al*., 2019), 29 (15.8%) of 184 blood serum samples taken from Anatolian buffaloes with an abortion history were found to be seropositive in terms of Q fever by ELISA.

The following may be among the most important reasons for the limited studies in buffaloes. The water buffaloes are localized in specific geographical regions and their population varies from country to country. For these reasons, the cutting and consumption of buffaloes is less. On the other hand, the abortion cases occurring in buffaloes are less than other ruminants, the events seen are investigated especially in terms of Brucella and Campylobacter, and not evaluated in terms of Q fever.

It has been demonstrated that *C. burnetii*, which causes Q fever disease, is an important abortive factor. Commercial ELISA kits are widely used in the serological diagnosis of the disease in question. However, commercial ELISA kits cannot be used in extensive epidemiological studies due to their species specificity, high cost and limited sample analysis. In this research, a home-made ELISA kit was developed that allows the examination of different animal species and allows more samples to be examined at a lower cost than commercial ELISA kitsIn this study, a home-made ELISA and commercial ELISA using two different conjugates (anti-bovine IgG and protein A) were used for serological diagnosis of Q fever disease in buffaloes. The results obtained were compared. On the other hand, within the scope of previous studies, cattle, sheep, and goat blood sera found to be positive and negative with the commercial ELISA kit were also analyzed.

Although it is known that *C. burnetii* infection in farm animals is enzootic in Turkey, there is not enough information about the herd prevalence, the frequency of infections that pose a risk to humans and different animal species. The seropositivity/seroprevalence values detected in various animals in different studies vary according to the years studied, the animal population examined, the region and the screening test applied. It is noteworthy that high seropositivity rates against Q fever disease are detected in occupational groups under the risk group in different countries and in neighboring provinces in our region. For this reason, close monitoring of Q fever disease in animals that constitute a risk group, determination of the status of vector carriers, especially ticks, and determination of the agent by molecular and serological methods, especially in abortive animals, will provide important epidemiological contributions.

It is understood that the disease should be regarded as important as the factors that cause other genital system disorders, especially in those who have aborted or weak offspring and in buffalo populations with infertility problems. The epidemiology of the disease can be revealed in detail in the light of research to be carried out with more comprehensive projects in our region.

As a result, home-made ELISA that can be used for serological detection of Q fever

disease in buffaloes, sheep, goat, and cattle was developed. Among the ELISA procedures tested, it was observed that the sensitivity and specificity of ELISA using anti-bovine IgG conjugate were found to be moderate. Although the sensitivity of the ELISA procedure, which was tested using the protein-A conjugate, was low, it was found that its specificity was quite high. The ELISA procedures tested were considered to have potential as an alternative to commercial kits for the serological detection of Q fever disease.

ACKNOWLEDGMENT

This study was supported by the Scientific Research Projects Commission of Ondokuz Mayis University (Project No: PYO.VET.1901.17.003).

REFERENCES

Adesiyun, A.A. and E.P. Cazabon. 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. *J. Pak. Med. Assoc*., **37**(5): 126-129. Available on: https://www.jpma.org.pk/PdfDownload/5897.pdf

Changoluisa, D., I.A. Rivera-Olivero, G. Echeverria, M.A. Garcia-Bereguiain, and J.H. de Waard. 2019. Serology for Neosporosis, Q fever and Brucellosis to assess the cause of abortion in two dairy cattle herds in Ecuador. *BMC Veterinary Research*, **15**.

Çetinkol, Y., Ö. Enginyurt, B. Çelebi, A.A. Yıldırım, S. Çankaya and O.C. Aktepe. 2017. Investigation of zoonotic infections in risk groups in Ordu University Hospital, Turkey. *Niger. J. Clin. Pract*., **20**(1): 6-11. DOI: 10.4103/1119-3077.181395

Çıkman, A., M. Aydin, B. Gulhan, F. Karakecili, A. Ozcicek, O.A. Kesik, M. Parlak, F. Ozcelik and B. Gültepe. 2017. The seroprevalence of *Coxiella burnetii* in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical features. *J. Vector Dis*., **54**(2): 157-163.

De Rooij, M.M.T., J.P.G. Van Leuken, A. Swart, M.E.E. Kretzschmar, M. Nielen, A.A. De Koeijer, I. Janse, I.M. Wouters and D.J.J. Heederik. 2019. A systematic knowledge synthesis on the spatial dimensions of Q fever epidemics. *Zoonoses Public Hlth*., **66**(1): 14-25. DOI: 10.1111/zph.12534

Douangngeun, B., W. Theppangna, V. Soukvilay, C. Senaphanh, K. Phithacthep, S. Phomhaksa, S. Yingst, E. Lombardini, E. Hansson, P.W. Selleck and S.D. Blacksell. 2016. Seroprevalence of Q fever, brucellosis, and bluetongue in selected provinces in Lao People's Democratic Republic. *Am. J. Trop. Med. Hyg*., **95**(3): 558-561. DOI: 10.4269/ajtmh.15-0913

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

Galiero, G., C.G. Goffredi and A. D'Orazi. 1996. Epidemiology of Q fever: Seroprevalence in buffalo dairies of Salerno province. *Selezione Veterinaria*, **6**: 407-412.

Gülhan, T., M. Tütüncü, M. Güzel, M.G. Sezener,

A. Çiftci, Y. Kılıçoğlu, V.E. Ergüden, S. Akgöz and B. Boynukara. 2019. Serological investigation of Q fever in Anatolian buffaloes. *Journal of Anatolian Environmental and Animal Sciences*, **4**(3): 338-343. DOI: 10.35229/jaes.579953

Gülhan, T., M.G. Sezener, S. Akgöz, V.E. Ergüden, A. Fındık and A. Çiftci. 2020. Molecular characterization of thermophilic Campylobacter species isolated from Anatolian buffalo feces. *Journal of Anatolian Environmental and Animal Sciences*, **5**(1): 86-92. DOI:10.35229/jaes.688263

Günaydın, E. and S. Pekkaya. 2016. Serologic and molecular investigation of Q fever on water buffalo in Afyon. *Van Veterinary Journal*, **27**(1): 17-19. Available on: https://dergipark.org.tr/en/download/article-file/492867

Horton, K.C., M. Wasfy, H. Samaha, B. Abdel-Rahman, S. Safwat, M.A. Fadeel, E. Mohare and E. Dueger. 2014. Serosurvey for zoonotic viral and bacterial pathogens among slaughtered livestock in Egypt. *Vector-Borne Zoonot*., **14**(9): 633-639. DOI: 10.1089/vbz.2013.1525

Kaplan, M.M. and P. Bertagna. 1955. The geographical distribution of Q Fever. *B. World Health Organ*., **13**(5): 829-860.

Karami, M.H., B.M. Pourmahdi, D. Garibi and H.M.R. Haji. 2017. Serological survey of Q fever in goats and buffaloes in Ahvaz region using the ELISA method. *Vet. Clin. Path*., **11**(4): 25-35.

Kalema-Zikusoka, G., R.G. Bengis, A.L. Michel and M.H. Woodford. 2005. A preliminary investigation of tuberculosis and other diseases in African buffalo (*Syncerus caffer*) in Queen Elizabeth National Park, Uganda. *Onderstepoort J. Vet.*, **72**(2): 145-151. DOI: 10.4102/ojvr.v72i2.210

Nahed, H.G. and A.A. Khaled. 2012. Seroprevalence of *Coxiella burnetii* antibodies among farm animals and human contacts in Egypt. *Journal of American Science*, **8**(3): 619-621. Available on: https://www.jofamericanscience.org/journals/am-sci/am0803/082_8392am0803_619_621.pdf

Office International Epizootica, (OIE). 2018. Q-Fever. Office International Epizootica, Available on: http://www.oie.int/animal-health-in-the-world/animal-diseases/q-fever/

Payzın, S. 1953. Epidemiological investigations on Q fever in Turkey. *B. World Health Organ*., **9**: 553-558.

Payzın, S. and S.B. Golem. 2007. Turkey's Q Fever (Report 1). *Turk. Hyg. Exper. Biol*., **64**(3): 45-59.

Pradeep, J., S. Stephen, P. Pooja, A. Akshayavardhini, B. Sangeetha and P.X. Antony. 2017. Coxiellosis in domestic livestock of Puducherry and Tamil Nadu: Detection of *Coxiella burnetii* DNA by polymerase chain reaction in slaughtered ruminants. *Vet. World*, **10**(6): 667-671. DOI: 10.14202/vetworld.2017.667-671

Vaidya, V.M., S.V.S. Malik, K.N. Bhilegaonkar, R.S. Rathore, S. Kaur and S.B. Barbuddhe. 2010. Prevalence of Q fever in domestic animals with reproductive disorders. *Comp. Immunol. Microb*., **33**(4): 307-321. DOI: 10.1016/j.cimid.2008.10.006

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

10.1089/vbz.2011.0850

Yadav, M.P. and M.S. Sethi. 1979. Sero-epidemiological studies on coxiellosis in animals and man in the state of Uttar Pradesh and Delhi (India). *Int. J. Zoonoses*, **6**(2): 67-74.