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INVESTIGATION OF Q FEVER SEROPREVALENCE IN CATTLE IN TURKEY

TÜRKİYE'DE SIĞIRLARDA Q ATEŞİ SEROPREVALANSININ ARAŞTIRILMASI

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Abstract

Objective: The aim of this study is to investigate Q fever seroprevalence in cattle in Turkey. Q fever is a highly infectious zoonotic disease which is caused by *Coxiella burnetii* and which has occurrence in many countries of the world. As it is a multiple species disease, many different domestic and wild animals could be the carrier of the pathogen. However, cattle, sheep and goats are the main reservoirs and the disease generally appears with an increase in the cases of abortion and stillbirth.
Methods: Enzyme linked immunosorbent assay (ELISA) is preferred and recommended for the diagnosis of Q fever because they are highly sensitive and easy to use. In this study, blood samples of cattle randomly collected from 22 provinces of Turkey between 2017-2018 were tested by ELISA.
Results: Among 1114 blood samples analyzed, the detected seropositivity level is 18% and provincial seropositivity is between 2.3%-35.2%. Regional results are close to each other changing between 15.1% and 22.3%.
Conclusion: The results gathered have revealed the *C. burnetii* exposure of cattle in different regions of Turkey.The findings of this study display the necessity of strategies against this disease which poses hazards for both public and animal health. Since this disease leads to serious loss in animal production, determination of the fighting strategies against Q fever and evaluation of the methods after practice is important. This study is believed to contribute to the fight against this disease with the leading and comparable data it presents.

Keywords: *Abortion, Coxiella burnetii, ELISA, Q fever, seroprevalance*

Öz

Amaç: Bu çalışmanın amacı Türkiye'de sığırlarda Q ateşi seroprevalansının araştırılmasıdır. Q ateşi, *Coxiella burnetii* bakterisinin neden olduğu birçok ülkede görülen oldukça bulaşıcı zoonoz bir hastalıktır. Birden fazla türü etkileyen bir hastalık olduğundan birçok evcil ve vahşi hayvan patojenin taşıyıcısı olabilmektedir. Fakat sığır, koyun ve keçiler başlıca rezervuarlardır ve hastalık genellikle yavru atma ve ölü doğum vakalarının artışı ile kendini göstermektedir.
Yöntem: Yüksek sensitivitesi ve kullanım kolaylığından ötürü ELISA testleri tercih edilmekte ve Q ateşi hastalığının serolojik teşhisinde önerilmektedir. Çalışma kapsamında 2017-2018 yıllarında 22 ilden rastlantısal olarak toplanan 1114 sığır kan serumu ELISA testi ile analiz edilmiştir.
Bulgular: Analiz edilen numuneler arasında %18,04 seropozitiflik tespit edilmiştir. İl düzeyindeki sonuçlar %2,3-%35,2 aralığında dağılım göstermektedir. Bölgesel sonuçlar ise birbirine daha yakın olup %15,1-%22,3 arasında değişmektedir.
Sonuç: Elde edilen seroprevalans sonuçları, Türkiye'nin farklı bölgelerindeki sığırların Q ateşi hastalığının etkeni olan *C. burnetii* ile karşı karşıya kaldığını göstermektedir. Gerek halk sağlığı gerekse de hayvan sağlığı açısından tehlike oluşturan hastalığa karşı oluşturulacak mücadele stratejilerinin gerekliliği çalışma sonuçları ile desteklenmektedir. Çalışmada ulaşılan sonuçlar, yönlendirici ve kıyaslanabilir veriler paylaşarak hastalık ile mücadeleye katkı sunmaktadır.

Anahtar Kelimeler: *Düşük, Coxiella burnetii, ELISA, Q fever, Seroprevalans*

Introduction

Q fever (for query fever) or Coxiellosis is a zoonotic disease caused by *Coxiella burnetii* with a worldwide occurrence except New Zeeland.[1-3] The prevalence of Q fever is higher than the reported cases particularly in endemic countries.[3] Q fever was first recognized in Australia and the letter 'Q' stands for Query. This term was used because of the unknown cause of the infection at that time. By the contribution of Harold Cox and Frank MacFarlane Burnet in the identification of the causative agent, it is named as C.*burnetti* in these researchers' honour.[1,4]

C.*burnetii* is classified as a potential bioterror agent belonging to group B because of its air-bone transmission route, resistance in the environment, low infective dose and capability to cause debilitating disease in great number of people.[3-6] The range of susceptible hosts for C.*burnetii* is quite wide including domestic and wild animals like livestock, pets and even non-mammalian (reptiles, birds) species.[2,3,7,8] Therefore, the pathogen *C. burnetii* is indexed in multiple species diseases in World Organisation for Animal Health (OIE) list.[2] The main reservoirs are such livestock as cattle, sheep and goat.[2,3,6,7] Coxiellosis leads to abortions, stillbirth, weak offspring and reproductive failures in livestock.[2,3,6,9] Similar to brucellosis and chlamydiosis, abortion developed in the late phase of gestation and any specific manifestation is not observed until abortion.[7]

Infertility or metritis associated with Q fever in cattle is also a common consequence of the disease.[3,7,10,11] Abortion storms in naive herds after the *C. burnetii* exposure depend on the herd population and immune response.[4,6]

Infected animals can shed *C. burnetii* via different secretions and excreta particularly birth products involving huge bacterial load and milk, feaces and urine with lower concentration of bacteria.[3,6,9,12] An infected female whose gestation results in abortion or even normal parturition is capable of shedding bacteria for different time periods without any noticeable clinic signs. These animals maintain posing risk to humans and other animals.[4,7,10] Particularly shedding the pathogen via milk is more common in cows and lasts several months, which is longer than in sheep and goats.[7,10,11]The common transmission route of the disease is the inhalation of infectious aerosol particles or dusts.[3,6,8,9,13] Ingestion of dairy products obtained from contaminated raw milk is not considered to be a serious transmission route in the spread of the disease to human.[1,3,4,7] There could be both sexual and vertical transmission in animals, but the significance of these transmission ways is unidentified.[3]

In humans, infection can induce acute, chronic or subclinical form with different clinical signs.[2,3,7,9] The acute form can be mildly progressive and confused with other flu-like syndromes.[3,7,8,12] However, the chronic form may lead to fatal consequences due to endocarditis without appropriate medication.[2,3,7,8] Q fever leads to miscarriage, premature birth or fetal death in pregnant women.[3,7,12]

Serological analysis is an appropriate way to assess the prevalence of the disease.[3,11] Determination of specific antibodies against C.*burnetii* demonstrates the recent or previous infections.[3,11,14] Because of the difficulties in the diagnosis of Q fever and risks posed by the pathogen, serological surveys become a suitable choice for epidemiological studies.[7] Complement Fixation Test (CFT) has got a poor sensitivity[15] and Indirect Immunofluorescence Assay (IFA) is not acceptable for working with large scale samples.[7]

Among other serological tests, ELISA which is interpreted objectively as an automated, simple method,[3,6] is considered to be a recommended test especially for large scale screening of livestock.[3,7,11] A significant portion of carrier animals may show seronegative results even if they continue shedding the bacteria via feces, vaginal mucus or milk.[3,10,11,14]

Q fever is known to have existed in Turkey since 1948.[16] Several studies which investigated cattle seroprevalence led to results varying between 5.8%-20%.[17-23] Regarding these results, farm animals' exposure to *C. burnetii* is considerably high in different part of Turkey. These domestic ruminants are also considered to be the major sources of human infection.[3,10,11,13,14] New prevalence data are always necessary to have better strategies against zoonosis in the control and eradication programs. In this sense, the aim of this study is to determine the seroprevalence of Q fever in cattle in different provinces of Turkey in order to supply new data for the prospective control measures.

Methods

The study was carried out in Pendik Veterinary Control Institute (PVCI), Istanbul, Turkey. In this study, a total of 1114 blood samples of cattle were tested by ELISA for the detection of Ig antibodies against C.*burnetii*. The samples were obtained randomly from cattle herds in 22 provinces which are coloured with grey in Figure 1.

EpiTools epidemiological calculators (http://epitools.ausvet.com.au/content.php?page=home) was utilized to be able to calculate the sample size. The following values were taken into consideration during calculation: assumed prevalence 50%, desired precision 4% and confidence level 99%. The distribution of the samples is shown in Table 1.

The blood sera were stored at −20ºC until the time they were analyzed. An ELISA test kit which includes positive and negative control sera (Q fever antibody ELISA test kit, IDEXX Laboratories, USA) was used to identify the presence of specific C.*burnetii* antibodies. The method was employed in accordance with the manufacturer's instructions. Predilutions of each sample and control sera at 1:400 were prepared as recommended. Peroxidase-labeled anti-ruminant immunoglobulin conjugate, which binds to antibody-antigen complex was used in order to measure specific antibodies. The absorbance of the test sample, negative and positive control was measured at a wavelength of 450 nm by an ELISA reader. The results were shown as a percentage of the optical density (OD) reading of the test sample (OD%). Interpretation of the results was done according to the manufacturer's recommendation in that S/P $\leq$ 30% were negative, 30%$\leq$ S/P $\leq$40% were suspect, and S/P $\geq$ 40% were evaluated as positive.

Results

A total of 201 samples giving positive results demonstrated the total seroprevalence as 18% (n: 201). However, seroprevalence among the provinces is between 2.3%-35.2% as shown in Table 2. The lowest and highest values were obtained in Hakkari province (Eastern Anatolian Region) and in Konya province (Central Anatolia region), respectively.

In contrast to provincial seroprevalence, regional seroprevalence results were close to each other varying between 15.1% and 22.3% as shown in Table 2.

Mediterranean region has the lowest and Central Anatolian region has the highest seroprevalence results.

Table 1. The distribution of the samples regarding the province and regions

Region	Provinces	Number of Samples	Total number of Samples
Aegean	AFYON	52	205
	AYDIN	52	
	IZMIR	51	
	USAK	50	
Black Sea	KASTAMONU	51	51
Central Anatolia	KONYA	51	152
	NIGDE	51	
	YOZGAT	50	
East Anatolia	AGRI	52	299
	ARDAHAN	52	
	HAKKARI	42	
	IGDIR	51	
	KARS	51	
	VAN	51	
Marmara	BALIKESIR	51	152
	CANAKKALE	50	
	TEKIRDAG	51	
Mediterranean	ADANA	52	153
	ISPARTA	51	
South EastAnatolia	BATMAN	50	152
	MARDIN	51	
	SANLIURFA	51	

Table 2. Seroprevalence results

Region	Provinces	Provincial Seropositivity %	Regional Seropositiviy %
Aegean	AFYON	9.6	16.5
	AYDIN	11.5	
	IZMIR	29.4	
	USAK	18.0	
Black Sea	KASTAMONU	19.6	19.6
Central Anatolia	KONYA	35.2	22.3
	NIGDE	17.6	
	YOZGAT	14.0	
East Anatolia	AGRI	25.0	19.4
	ARDAHAN	32.6	
	HAKKARI	2.3	
	IGDIR	23.5	
	KARS	21.5	
	VAN	7.8	
Marmara	BALIKESIR	17.6	15.7
	CANAKKALE	16.0	
	TEKİRDAG	13.7	
Mediterranean	ADANA	15.3	16.5
	ISPARTA	17.6	
South EastAnatolia	BATMAN	22.0	15.1
	MARDIN	17.6	
	SANLIURFA	5.8	

Discussion

Q fever is mentioned as a re-emerging disease in many countries.[7,11] Interest due to the biorisks posed by Q fever has increased in Europe and therefore, a risk analysis procedure was demanded for humans and animals by the European Commission.[24] The characteristics of C.*burnetii* make it possible to be called a biological weapon.[4,5,12] Q fever is identified as a reportable disease in many countries with the help of the CDC's classification of *C. burnetii* as a bioterrorism agent candidate.[6] If the pathogen is used for bioterrorism attacks, it may not induce excessive amount of mortality but debilitating disease is an expected damage of the pathogen.[5,6]

Figure 1. The provinces investigated (coloured with grey)

One of the disadvantages of fighting against this disease is the difficulty in determining the real prevalence in humans and animal because of the different forms of the disease that complicates clinical diagnosis.[7] The incidence is obviously higher in herds including subclinical carriers.[3] In the last two decades, several epidemiological studies on Q fever have been conducted in ruminant herds in Turkey.[20-23,25-27] Detection of specific antibodies is a useful indication of recent infections and previous exposure. ELISA is recommended to detect these disease indications as a practicable and highly sensitive test.[3]The superiority of highly specific ELISA is that it reduces the possibility of cross-reaction with other pathogens.[17]

In a previous study, the seroprevalence of Q fever in dairy cattle was investigated in Konya in central Turkey and the seroprevalence was found to be 12.4%, which is lower than both Konya provincial (35.2%) and central Anatolian regional prevalence result (22.3%) in our study. These differences might stem from different climates, geographic location, sample size, year, species screened, and the cut-off value.[20,25]

Previous studies[17,18,23] carried out in different provinces in eastern Anatolian region of Turkey showed that there has been an increase up to 14.8% in Q fever seroprevalence in cattle herds during the last decade. This increase may continue according to our result (19.4%) in the same region as well. The seroprevalence result (20%) of a previous study carried out in Aydın province in Aegean Region[22] is parallel to regional seroprevalance result (17%) of our study in Aegean region.

In another study investigating the seroprevalence of Q fever in humans in a district of Black Sea region revealed the prevalence at the level of 13.5 % in the area of investigation.[28]In our study, the seroprevalence of Q fever in cattle in another district of Black Sea Region was found to be 19.6%.When considering the cattle both as a susceptible host and a source of infection for humans, the results ofthe two studies might indicate the possible relationship between cattle and human cases. Controlling the zoonotic disease in animals always creates a positive aftereffect on public health.[8,9] Even in the same region, some of the provincial results are relatively higher or lower than others as in this study; therefore, it should be taken into consideration that regional seroprevalence percentages may differ depending on the investigated province.

It is possible to label individual animals as free of disease on condition that the herd or flock is free and there is no serological and clinical data.[3] Another challenge in the fight with this diseases is that aerosol contamination could be carried to neighboring areas, which are 30 km or farther away from the main disease area by winds.[6] In this context, strict control measures should be taken both in the regions with low seroprevalence and in the regions having outbreaks. It should also be kept in mind that low percentages might not guarantee that these regions are safer for breeding. In the assessment of control measures, cumulative and integrated control and eradication strategies should be established.

In this study, seroprevalence of Q fever in cattle in South Eastern Anatolia was found to be 15.1%. A previous study carried out in the same region particularly in Diyarbakir presented 20% seroprevalence level in cattle,[21] which is partially higher than the present study. In the western part of Turkey, particularly in Marmara region, this study confirms the significant C. *burnetti* exposure to ruminant herds with a 15.7% seroprevalence. This result is in line with the result of a recent study of Q fever seroprevalence in small ruminants in the entire Marmara Region.[26]

Vaccination can be organized as outbreak vaccination or more effective preventive vaccination that aims to weaken the risk of possible outbreaks.[2] Serological results like the ones in this study and previous ones could guide and support the vaccination programs for the determination of the most risky regions. In addition to preventive vaccination, combination of other measures such as management of manure, wool-shearing, redesigning the farm quality, discharging of risk material, separate kidding area, visitor restriction and control of ticks and reservoir animals could be used to achieve the expected efficiency.[3] It should be kept in mind that management of useful control measures depends on the adequate awareness of possible risk factors.[9] Serological surveys could be used to understand and estimate these risk factors.

Conclusion

Significant seropositivity rates in cattle gathered through this study displayed the risk created by the pathogen particularly in the research area. The seroprevalence level of the provinces should be taken into account both collectively and individually while evaluating the abortion cases and redesigning the regional or territorial control strategies against this disease.

Conflict of Interest

The authors declare that there is no conflict of interest.

Compliance with Ethical Statement

Research was approved by the Local Ethics Committee for Animal Experiments, Istanbul, Turkey (Ethics Committee Decision No: 09-3/2018).

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