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## Seroprevalence of Q Fever in Cattle and Sheep in the East of Turkey

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**Abstract:** The present study was carried out to determine the seroprevalence of antibodies to *C. burnetii* in cattle and sheep in the east of Turkey. Serum samples collected randomly from 92 cattle and 92 sheep were examined by ELISA (Viracell-SL, Spain) to detect IgG antibodies against *C. burnetii* phase II antigen. Seropositivity was observed in 16.3% of the cattle and in 5.4% of the sheep. Coxiellosis has an important seropositivity in both cattle and sheep and it can cause serious health problem in humans living in Eastern Turkey.

**Key words:** *Coxiella burnetii*, cattle, sheep, serum, IgG antibody, prevalence

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### INTRODUCTION

Q fever (query fever), a zoonosis caused by obligate intracellular microorganism *Coxiella burnetii*, is endemic throughout the world with the exception of Antarctica and possibly New Zealand, occurring in diverse geographic regions and climatic zones and infects arthropods, birds, pets, domestic and wild mammals, as well as humans (Woldehiwet, 2004; Rodolakis, 2006).

Natural reservoirs are more than 40 species of ticks, which remain infected life-long and which transmit *C. burnetii* transovarially and free-living vertebrates. Tick feces may contain large amounts (up to  $10^{10}$  infectious units  $g^{-1}$ ) of *C. burnetii* (Norlander, 2000).

The main sources of environmental contamination and infection of humans are parturient ruminants, dogs and cats infected with *C. burnetii* (Woldehiwet, 2004). The main route of *C. burnetii* infection is by inhalation of contaminated aerosols or dusts containing the microorganism shed from infected animals. The source of human infection is often unidentified, although sheep and goats are more frequently involved in the disease cycle than other animal species. Infected animals shed highly stable bacteria in urine, feces, milk and through placental and birth fluids (Marrie, 2003).

*Coxiella burnetii* is very stable in the environment, resisting to elevated temperature, desiccation, osmotic shock, ultra-violet light and disinfectants (Arricau-Bouvery and Rodolakis, 2005). Oral transmission, by ingestion of contaminated raw milk or dairy products could lead to seroconversion in few cases to Q fever (Cutler *et al.*, 2002; McQuiston *et al.*, 2002; Rodolakis, 2006).

Until recently, the agent has been regarded as economically unimportant for domestic livestock. However, *C. burnetii* has been detected in a number of cases of abortion in different parts of the world (Waldham *et al.*, 1978; Palmer *et al.*, 1983; Raju *et al.*, 1988; Zeman *et al.*, 1989) including Turkey. As a result, the role of *C. burnetii* in reproductive disorders of livestock has been questioned (Çetinkaya *et al.*, 2000). Although, the disease may cause agalactia (Kelly *et al.*, 1993) and infertility in addition to sporadic abortions in heavily infected animals, it usually does not cause obvious clinical signs in ruminant populations.

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In animals, *C. burnetii* can induce pneumonia as well as abortion, stillbirth and delivery of weak lambs, calves or kids, which are the most frequent clinical signs of the disease. In the majority of the cases, abortion occurs at the end of gestation without specific clinical signs until abortion is imminent, as observed with brucellosis or chlamydiosis. Aborted fetuses appear normal but infected placentas exhibit intercotyledonary fibrous thickening and discolored exudates, which are not specific to Q fever. A severe inflammatory response is observed in the myometrium and the stroma adjacent to the placentomal area during gestation in goats (Abe *et al.*, 2001). From time to time clinical signs mentioned above have been seen in the farm animals in this region. Taken into consideration of these clinical signs, the possibility of Q fever incidence in the region were therefore investigated serologically.

Isolation of *C. burnetii* is not performed for routine diagnosis in veterinary medicine, because the cultivation of the agent is a laborious and hazardous process (Rodolakis, 2006).

The diagnosis of Q fever remains difficult and epidemiological studies are often based only on serological investigations. These include Complement Fixation Test (CFT), Indirect Fluorescent Antibody Test (IFAT), Capillary Agglutination (CA) test, indirect immunofluorescence assay (IFA), microagglutination test (MA), ELISA and PCR. Routine diagnosis of Q fever in aborted animals is usually established by examination of fixed impressions or smears prepared from the placenta stained by the Stamp, Gimenez or Machiavello methods, associated with serological tests. Because farm animals are the major source of the infection for people, it is important to determine the prevalence of coxiellosis in these species. The present study was carried out to determine the prevalence of coxiellosis in cattle and sheep in the eastern part of Turkey, using ELISA.

## MATERIALS AND METHODS

### Sample Collection

A total of 184 blood samples (92 samples each of cattle and sheep) were collected by a simple random sampling method from 16 herds and flocks as well, representing 16 locations (Van, Baskale, Caldıran, Catak, Edremit, Erciř, Gevař, Grpinar, Muradiye, Ozalp, Bitlis, Adilcevaz, Ahlat, Tatvan, Muř, Dođubayezit) in the eastern part of Turkey (Fig. 1) during 2006-2008 years. The herds and

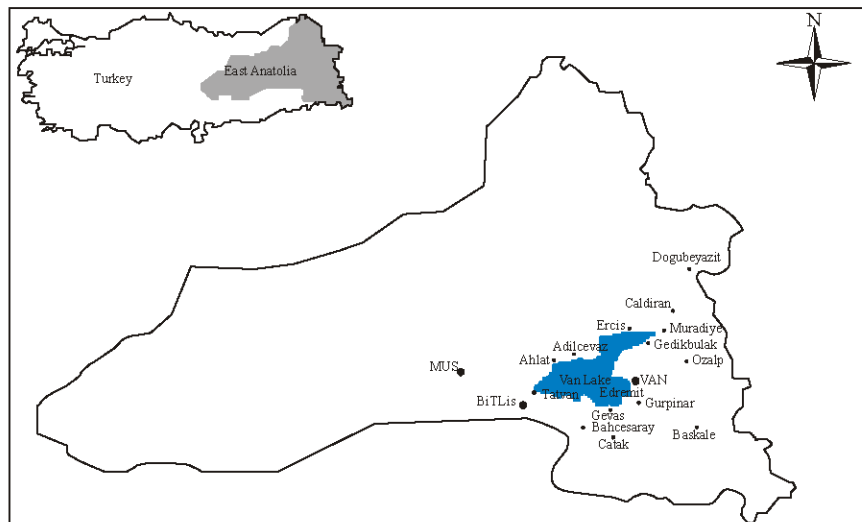


Fig. 1: The sites of blood samples collection at the Eastern part of Turkey

flocks were selected randomly. No criteria such as size of herd or flock were used in the selection of the herds and flocks. All animals were adult females and males. The 5-7 mL blood were collected from flocks were selected randomly. No criteria such as size of herd or flock were used in the selection of the jugular vein aseptically using Vacutainer® tubes containing separator gel by qualified personnel and kept on ice while being transferred to the Laboratory of Microbiology and Clinical Microbiology, Medical Faculty at Yüzüncü Yil University, where serum was separated by centrifugation (3000 rpm for 10 min) and stored frozen at -20°C until required.

### Test Procedure

Serum samples were tested for *C. burnetii* antibodies using ELISA test and Labsystems iEMS Reader MicroELISA system (Fiuland) was used. Commercial ELISA kit (Vircell SL®, Spain) was used to detect IgG antibodies against *C. burnetii* phase II antigen. The test was carried out according to the instructions of the manufacturer. Four wells (two for the cut off serum and one each for the negative and positive sera) in each ELISA plate were used as control. The mean OD for the cutoff serum and antibody index was calculated with the following formula:

$$\text{Antibody Index (AI)} = \frac{\text{Sample OD}}{\text{Cutoff serum mean OD}} \times 10$$

Samples were interpreted as: samples with indexes <9.0 were considered as not having IgG antibodies; samples with equovocal results (AI = 9.0-11.0) being retested for confirmation and samples with indexes >11 were considered as having IgG antibodies against *C. burnetii*.

## RESULTS AND DISCUSSION

*Coxiella burnetii* seropositivity reactions were obtained 10.9% of the sera tested from all animals (20 of 184). The highest percentage of positive animals was for cattle (%16.3) (Table 1).

The interest for Q fever is increasing worldwide as indicated by the rising number of reviews published (Arricau Bouvery and Rodolakis, 2005) even in countries where its incidence is supposed to be very low. Indeed, the disease is considered as a re-emerging zoonosis in many countries. This could be due to the evolution of its epidemiology, or of the agent, which could become more virulent, to modifications of its clinical signs, to an improvement of the sensitivity of diagnostic tests, or because practitioners are better informed and look for it more often (Arricau Bouvery and Rodolakis, 2005).

Past investigation confirmed that some infections occur frequently in farmers, where the contact with cattle and sheep is associated with Q fever (Coleman, 2000). Salinas-Melendez *et al.* (2002) showed that exposure to *C. burnetii* is common in animals in the state of Nuevo Leon Mx. The risk of Q fever on people who work with domestic animals is related to contact with farm environment rather than any specific animal exposure (Thomas *et al.*, 1995).

Infection of livestock with *C. burnetii* often goes unnoticed. Indeed, sporadic abortion of up to 5% is considered normal and does not prompt investigation. Furthermore, the lack of overt clinical signs exhibited among infected livestock make diagnosis a challenge (Cutler *et al.*, 2007). The zoonosis must be considered as a truly global problem, both in terms of their distribution and the measures

Table 1: Serological status of cattle and sheep against *C. burnetii*

Animal species	No. of animals	No. of positives	Positives (%)
Cattle	92	15	16.3
Sheep	92	5	5.4
Total	184	20	10.9

required for their control. Q fever is a zoonosis that may occur worldwide, in all geographic and climatic zones. *Coxiella burnetii* is a highly infectious agent that is widespread among livestock around the world (Arricau Bouvery and Rodolakis, 2005). Its resistance to chemical and physical agents is exceptional and enables the organism to maintain itself in nature without an arthropod vector and makes air-borne dissemination of infection possible (Little, 1983).

The distribution of *Boophilus* ticks and a high seroprevalence in animals suggest that these ticks play a role in the transmission and maintenance of *C. burnetii* infection in cattle (Schutt *et al.*, 1976). The feces of ticks infected are heavily contaminated with the microorganisms, which remain viable for long period of time and therefore may be a potential source of infection for man and animals (Peacock *et al.*, 1983). Such infected feces may become powdered and windborne, thereby infecting the upper respiratory tract of man and animals (Salmon *et al.*, 1982). *Coxiella burnetii* was detected in 5 of 10 dust samples from a barn housing dairy cattle by the PCR (Yanase, 1998).

Because of the polymorphism of the clinical picture and because the diagnosis is based exclusively on serology, the prevalence of *C. burnetii* infection among animals is largely unknown (Rey *et al.*, 2000). The studies were mostly depend on serological investigation, worldwide and in Turkey as well (Kalender, 2001).

The ELISA test is more sensitive than the CF test and allows for testing a greater number of animals and flocks (Rodolakis, 2006). ELISA and microimmunofluorescence (MIF) tests give similar results with cow sera, but the ELISA test is more sensitive than the MIF test with goat and sheep sera (Arricau-Bouvery *et al.*, 2003).

Similar study was performed in Turkey and in Elazığ region. Çetinkaya *et al.* (2000) examined 416 cattle and 411 sheep sera by indirect fluorescent antibody test (IFAT) to determine the prevalence of Q fever. Seropositivity was observed 5.8% in cattle and 10.5% in sheep. In an another study (Ozdemir *et al.*, 1999) reported *C. burnetii* seropositivity in 33.8% of pregnant and 26.75% of aborted animals in Elazığ and its vicinity using indirect fluorescence antibody test. On the other hand, Kalender (2001) reported seropositivity in 38.59% of aborted sheep and 11% in sheep given healthy birth.

Furthermore, in a study carried out on 1593 cattle in the Marmara Region, Turkey, using ELISA, 8.04% was found to be positive to *C. burnetii* (Yurtalan, 2003). In addition, Kiliç *et al.* (2005) reported seroprevalence of *C. burnetii* in 3% of sheep in Aydın and its vicinity.

In the region of Eastern Anatolia, Seyitoğlu *et al.* (2006) detected seropositivity in 22.6% of cattle with a history of abortion and 5.6% in cattle given healthy birth, using ELISA test.

In Europe, there are several studies reporting the seroprevalence of Q fever using different diagnostic methods. In Italy, Capuano *et al.* (2001) detected seropositivity in 14.4% of cattle using IFAT. Masala *et al.* (2004) found that 38% of sheep and 47% of goats was seropositive using ELISA. Parisi *et al.* (2006) identified *C. burnetii* in 11.6% of cattle and 21.5% of sheep and goats using PCR. Cabassi (2006) detected seroprevalence of 44.9% in aborted cattle and 22% in healthy cattle using ELISA. In France, Rousset *et al.* (2007) found 88% seropositivity in aborted group and 60% in healthy group using ELISA in 8 goat flocks. Berri *et al.* (2007) also examined a goat flock and found that 30% seropositivity in aborted animals and 80% 6 weeks later after abortion in the same flock by ELISA. Tellez *et al.* (1989) used indirect immunofluorescence test, reported the seroprevalence of Q fever as 76.6% in goat and 17.7% in cattle in Madrid, Spain. Christoffersen (2007) investigated seroprevalence of Q fever in Denmark between 2004 and 2006 in cattle using ELISA and found seropositivity as 35 and 25%, respectively.

Furthermore, Wagner *et al.* (2005) tested a total of 744 blood samples (70 Styrian sheep and 674 goats) by CFT for antibodies to *C. Burnetii* in Vieuna, Austria and found 1.5% seropositivity. Also, Lausevic (2001) investigated seroprevalence of Q fever in sheep in Yugoslavia using microagglutination and microimmunofluorescence and found 5.03% seropositivity.

In USA, studies with concern to Q fever are summarized by McQuiston and Childs (2002) and reported as 41.6% in goat, 16.5% in sheep and 3.4% in cattle. In addition, Deforge and Cone (2006) in California, seroprevalence of Q fever in 268 bighorn sheep reported to be 10% using complement fixation test.

In Canada, Hatchette *et al.* (2002) examined Q fever seroprevalence between 1997 and 2000 and found an important increase in seropositivity from 3.1 to 23.5% using microimmunofluorescence test (MIF).

In Mexico, seroprevalence of Q fever in dairy cattle found to be 28%, beef cattle 10%, goat 35% and 40% in sheep (Salinas-Meléndez *et al.*, 2002).

In Africa, Reinthaler *et al.* (1988) found that 62.5% of sheep, 53% of goat and 40.4% of cattle using MAT in Southern Sudan, were seropositive. In Zimbabwe, Kelly *et al.* (1993) detected seropositivity to *C. burnetii* in 39% of cattle, 10% of goats using IFAT. Furthermore, in Chad, Schelling *et al.* (2003) found that 80% of camel, 13% goats, 11% sheep and 4% cattle using ELISA method, were seropositive. Nakoune *et al.* (2004) found 14.3% in cattle using IFAT in Central African Republic. In addition, Çekani *et al.* (2008) investigated Q fever seroprevalence in 1656 serum sample and found 9.8% in sheep and goat and 7.9% in cattle in Algeria using ELISA technique.

In other countries, seroprevalence of Q fever were; 46.6% in cattle, 28.1% in sheep and 23.5% in goats were observed in Japan using IFAT (Htwe *et al.*, 1992). In Cyprus, Psaroulaki *et al.* (2006) found 48.2% in goats, 24% in cattle, 18.9% in sheep using IFAT. On the other hand, Loukaides *et al.* (2006) found 13.8% using same IFAT in Cyprus.

It is shown that seroprevalence of Q fever is very much changeable with regard to country and even provinces. These alterations is also changeable with regard to test and kit used. Studies regarding to Q fever seroprevalence in Turkey indicated that it varied between 5.6-22.6% in cattle and 3-38.59% in sheep.

The present study indicated that animal population in this region is determined to be infected with Q fever at 10.9%. Infection rate in cattle was higher (16.3%) In the seroprevalance studies with concern to cattle and sheep, some found seroprevalance higher in cattle (Htwe *et al.*, 1992; Psaroulaki *et al.*, 2006) some in sheep (Schelling *et al.*, 2003; Çekani *et al.*, 2008). In the present study, several reasons could play role for the high seroprevalence observed in cattle. One of the reason is the tick density in the farm which could be more compared to field and cattle are mainly kept in indoor conditions in comparison to sheep therefore, seroprevalence were higher in cattle. The disease morbidity is very high and pathognomonic symptoms of the disease do not occur. Therefore, challenge against this disease is quite difficult. Thus, required precautions with regard to animal discharges and vector ticks to be taken into consideration and effective treatment should be implied.

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