

Full Length Research Paper

Seroprevalance of Coxiellosis in cows, sheep, goats and humans in Diyarbakir region of Turkey

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This study aims at determining the seroprevalance of *Coxiella burnetii* in cows, sheep, goats and staff, working in the stock breeding sector in Diyarbakir region. Therefore, *C. burnetii* antibodies were investigated in sera samples of 612 sheep, 700 goats, 584 cows and 90 staff by enzyme-linked immunosorbent assays (ELISA). In the study, seropositivity was obtained as 25.4, 38.6, 20.0 and 6.6% in sheep, goats, cows and stockbreeding staff, respectively. Consequently, *C. burnetii* seropositivity, whether in people or in animals, had a ratio that should not be ignored in Diyarbakir region. Abort cases in ruminant should be assessed from the viewpoint of *Coxiellosis*. Also, people, especially those who are in risk group, should be made to be conscious of *Coxiellosis* infection, and measures for preventing this illness should be taken.

Key words: Cow, *Coxiella burnetii*, goat, human, seroprevalance, sheep.

INTRODUCTION

The disease, which has been known as Coxiellosis, or Q. fever, caused by *Coxiella burnetii*, is a zoonotic infection seen all over the world as endemic. Its agent is a obligate intracellular Gram negative bacterium which constitutes spore like formations and causes infections in arthropods, birds, domestic and wild mammals besides human (Woldehiwet, 2004; Kennerman et al., 2010; McCaughey et al., 2008).

Infected animals are important reservoirs of illness for humans because of the fact that Q fever proceeds subclinically (Woldehiwet, 2004; Rodolakis, 2006; McCaughey et al., 2008). People, associated with domestic animals, can be infected by the different ways as consuming unpasteurized milk (rarely pasteurized milk), contacting with carcass, and inhalation of infected

aerosol specimens (Woldehiwet, 2004; Cekani et al., 2008; McCaughey et al., 2008). Also, ticks are the vectors of *C. burnetii*, which play important role in the transmission of the infection (Rodolakis, 2006). Coxiellosis causes spontaneous abortions, ill and birth of weak offspring in mammals whereas it causes different symptoms such as acute fever, pneumonia, hepatitis, endocarditis, and abortions in human (Levesque et al., 2007; Rey et al., 2000). Also it can commonly proceeds asymptomatic in human.

Isolation and identification of Coxiellosis agent for the aim of diagnosing it is a procedure that is dangerous, needs a long time interval, expensive, and applicable only in reference laboratories. In addition, serologic methods, such as: microagglutination, radioimmunoassay, indirect immunofloresan (IFA), complement fixation (CFT) and Enzyme-linked immunosorbent assay (ELISA) for determining anti-*C. burnetii* antibodies are used safely. During acute infection, antibodies used against

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Table 1. Positive results of blood samples, tested by Elisa for *C. burnetii* antibodies.

Species	Number of tested serums	Number of positive serums	Ratio of positive serums (%)	Number of negative serums	Ratio of negative serums (%)
Sheep	612	156	25.4	456	74.6
Goat	700	270	38.6	430	61.4
Cow	584	117	20.0	467	80
Human	90	6	6.6	84	93.4
Total	1986	549	27.6	1437	72.4

phase II antigens are observed in higher rates, while during chronic infection, antibodies used against both phase I and phase II antigens were in higher rates because of the fact that *C. burnetii* is a phase variability bacterium (Rodolakis, 2006).

Q fever, although it is known that this infection is very common in Turkey, there is no enough study from the assessment of society health. In this study it is aimed to determine seroprevalance of Coxiellosis in cows, goats, sheep, and stockbreeding stuff, who are under risk (farmers, veterinarians, butchers, laborants, and abattoir workers). Also, it is targeted to carry out the seroprevalance of the disease in Diyarbakir region and assess the results from the viewpoint of society health.

MATERIALS AND METHODS

Serums

The blood samples of 612 sheep, 700 goats, and 584 cows from different herds were collected from ten different locations of Diyarbakir, and its districts between 2007 to 2009 years and serums of those blood samples were obtained. In addition, serums of 90 blood samples, taken from staff working in stockbreeding, were acquired. All humans were between 18 and 45 years of age. None of the human, or animal of which blood samples were taken showed clinical findings. The obtained serums had been preserved at -80°C till they were analysed by serologically.

ELISA

Ruminant serum samples were tested using the kit LSI Fièvre Q ruminants Serum®, (Laboratoire Service International, Lissieu, France). Phases II antigen was used in this assay to detect total immunoglobulins G (IgG) anti-*C. burnetii*. Serum samples were analysed according to the manufacturer's instructions. The absorbance was read using an ELISA reader (DSX, Dynex technologies) at a wavelength of 450 nm. The criterion for considering a sample as positive was based on ELISA value calculated as follows: ELISA value = $100 \times [\text{Absorbance of test sample} - \text{Absorbance value of negative control}] / (\text{Absorbance value of positive control} - \text{Absorbance value of negative control})$. Samples with antibody titer equal or above 40 were considered positive.

The presence of IgG antibodies against phase II *C. burnetii* in human sera were measured using a commercially available indirect enzyme-linked immunosorbent assay (ELISA) test kit (Virion or Serion, Wurzburg, Germany) according to the manufacturer's recommendations. The plates were read at 405 nm by an ELISA

reader (DSX, Dynex technologies). Optical density (OD) cut-off values and control sera were checked. For phase II, antibody activities in U/ml were calculated by a standard curve which was incorporated in the kit using the manufacturer's guidelines as follows: < 20 U/ml, negative; 20 to 30 U/ml, doubt; > 30 U/ml, positive.

RESULTS

In the study the results of serums, which were tested for *C. burnetii* antibodies by ELISA and belongs to 612 sheep, 700 goats, 584 cows and 90 people, are given in Table 1.

DISCUSSION

Q fever is a zoonotic infection, seen in a lot of countries and caused by *C. burnetii*. *C. burnetii* infection was detected in animals and humans for the first time in 1946 to 1947, and 1948 in Turkey, respectively (Kalender, 2001). Diagnosis serologic tests are very important because of the fact that isolation of the agent is a procedure that is dangerous, needs a long time interval, expensive, and applicable only in reference laboratories. For this reason, techniques such as IFA, CFT, and ELISA can be used in diagnosis process. ELISA is reported as a suitable technique used in reference test that has lower cost. It is simply used than IFA, which is accepted as a reference method, and also, it has higher specificity and sensitivity when compared with CFT (Heinzen et al., 1999).

In this study 156 (25.4%) of 612 sheep serums, 270 (38.6%) of 700 goat serums, 117 (20.0%) of 584 cow serums, 6 (6.6%) of 90 human serums were determined as positive by ELISA. In the studies for detecting the seroprevalance of Coxiellosis in animals by ELISA, Vaidya et al. (2008) reported seroprevalance as 11.36% in cows, which had reproductive orders, 9.3% in sheep, and 5.6% in goats. In addition Cekani et al. (2008) reported *C. burnetii* seropositivity as 8.8% in sheep and goats and 10.9% in cows whereas Van den Brom and Vellema (2009) reported the seropositivity ratios as 2.4 and 7.8% for sheep, and goats in Holland, respectively. Banazis et al. (2009) obtained the seropositivity in only 2 of 329 cow serums by ELISA in West Australia while they

did not obtained any seropositivity in sheep serums. Chang et al. (2009) reported seropositivity as 42.6% in goats, 28.6% in cows, and 26.3% in human by using IFAT. In Turkey, Kennerman et al. (2010) obtained 20% seropositivity in sheep in South Marmara region. Also by using IFAT, Kalender (2001) determined *C. burnetii* in sheep as averagely 23.5% in Elazi and its neighborhood cities. It showed that Coxiellosis seropositivity in sheep, goats, and cows is higher when the results of this study are compared with the other researches done. The reason of this situation can be explained such that geographical diversity, breeding conditions, and tick control can be the factors, increasing the infection risk ratio.

In the studies, where tests were done for people who are under risk, by ELISA, Levesque et al. (2007) and McCoughey et al. (2008) obtained 9 and 12.8% seropositivity, respectively. In the researches, done in Turkey, Özgür et al. (1996) obtained seropositivity as 51% in risk group, and as 25% in the individuals who do not any contact with animals. Eyiğör et al. (2006) reported seropositivity by ELISA as 13% with respect to Immunoglobulin M (IgM) antibodies, and as 34.8% with respect to IgG antibodies in a total of 92 people. In a study that was done in people, with IFAT technique, Berbero lu et al. (2004) obtained seropositivity as 13.2% (a total of 111 samples), 6.0% (a total of 116 samples), and 1.8% (a total of 109 samples) in Antalya, Diyarbakir, and Samsun cities, respectively. In addition, Karabay et al. (2009) reported 20.8% seropositivity in Bolu. In this study, 6.6% seropositivity ratio was obtained in people by using ELISA. This is assessed as low when compared with the studies commonly done by others, who used ELISA, except the study that was done by Berbero lu et al. (2004).

The lower seropositivity ratio of humans obtained in this study, according to that of animals, is compatible with the findings of various studies (Dolcé et al., 2003; Scrimgeour et al., 2003) that were done before. It is well known that, there are many transmission patterns of *C. burnetii* including inhalation of contaminated aerosols, contact with the infected animals depending on duration, and consumption of raw milk products (Casolin, 1999). Thomas et al. (1995) reported a correlation between seroprevalence and the extent of total contact with the farm animals due to the fact that full-time employees were more likely to be seropositive to *C. burnetii* than part-time employees. It is considered that the reason for the lower seropositivity ratio in humans, obtained in this study according to animals, can be resulted from short time contacts humans had with animals.

Consequently, *C. burnetii* seropositivity whether in people or in animals has a ratio that shouldn't be ignored in Diyarbakir region. In addition abort cases in ruminant should be assessed from the viewpoint of Coxiellosis. Also, people especially those who are in risk group, should be made conscious of Coxiellosis infection and measures should be taken to prevent this illness.

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