

Studies on the Determination of Seroprevalance of Q Fever in Sheep in the Region of Van

¹Mehmet Karaca, ¹Hasan Altan Akkan, ²Yunus Cetin, ¹Ihsan Keles,

³Mehmet Tutuncu, ¹Cumali Ozkan and ²Ibrahim Tasal

¹Department of Internal Medicine,

²Department of Reproduction, Faculty of Veterinary Medicine,

³Ozalp Vocational High School, Yuzuncu Yil University, 65080, Van, Turkey

Abstract: In the present study, seroprevalance of Q fever in sheep in the region of Van was aimed to investigate. A total 465 serum samples obtained from the city of Van and its different provinces were investigated with regard to Q fever specific antibodies using ELISA technique. Out of 465 serum sample 98 of them (21.07%) were found to be positive and 11 of them (2.37%) were doubtful with regard to Q fever specific antibodies.

Key words: Q fever, sheep, serology, ELISA, *C. burnetii*, Van

INTRODUCTION

Q fever, an obligate intracellular bacterium is caused by *Coxiella burnetii* and is a zoonotic rickettsial disease. The disease is commonly shown in the world and is mainly observed in sheep and goat, but it can be observed in other animals and human being (Imren and Sahal, 1991; Ozdemir *et al.*, 1999; Woldehiwet, 2004; Masala *et al.*, 2004; Cutler *et al.*, 2007).

Transmission of the disease occurs through infected ticks, inhalation of infected ducts, direct contact with contaminated materials and through digestive system. In general; apart from abortion and chronic mastitis, no other obvious symptoms do not occur. In sheep, fever, pneumonia, general condition discrepancies and abortions and in human begins; endocardi its, pericarditis, flu and pneumonia like problems occur (Kalender, 2001; Imren and Sahal, 1991; Ozdemir *et al.*, 1999; Woldehiwet, 2004; Cutler *et al.*, 2007).

C. burnetii is resistant to physical and biochemical factors, therefore can survive for a long time in the environment (Woldehiwet, 2004; Cutler *et al.*, 2007). This characteristic of the culprit make its control difficult. Furthermore, clinical diagnosis of the disease is difficult. Isolation of the agent is difficult and also is time consuming, therefore, serological tests have been preferred in its diagnosis. For this purpose, Micro Agglutination (MA), Complement Fixation (CF), indirect Immuno Flourescence Antibody (IFA), Polymerase Chain Reaction (PCR) and ELISA tests have widely been used

(Peter *et al.*, 1987; Kalender, 2001; Ozdemir *et al.*, 1999; Woldehiwet, 2004; Cutler *et al.*, 2007). The province of Van has important sheep population. Therefore, clinical signs of Q fever in sheep raised in the region have been observed from time to time. The problem cause important economical loses, thus, seroprevalance of Q fever in the region using sufficient number of animals were aimed to investigate using ELISA.

In present study, seroprevalance of Q fever, which cause important economical loses in the country and in the world in sheep in the region of Van and its provinces were aimed to investigate using ELISA.

MATERIALS AND METHODS

Animal material: For the animal material; 145 sheeps obtained from the city of Van, 75 sheep from the province of Ozalp, 55 sheep from Gulpinar, 40 sheep from Gevas, 30 sheep from Catak, 45 sheep from Edremit, 35 sheep from Muradiye and 40 sheep obtained from Caldiran Province were used (Fig. 1). Their ages were between 1-4 years old and were from both sexes. Total 10 mL of blood samples were taken from jugular vein in a tube without anticoagulant, after clotting, the tubes were centrifuged. Obtained serum samples were kept at -20°C, until analysis.

ELISA test: To determine antibodies occurred against Q fever (*C. burnetii*), a commercial test kit (IDEXX Laboratories, USA) was used. Serum samples were prepared according to test procedure. All serum samples



Fig. 1: Location of the regions. a) Turkey and b) Van from which blood samples were collected

were analyzed using digital and analog system (Italy) ELISA reader at the University of Yuzuncu Yil, Faculty of Veterinary Science, Department of Internal Diseases. The serum samples were read at 450 nm as reported in the test procedure and below equation was used to obtain calculation as given in the test procedure:

$$\text{Value (\%)} = \left(\frac{\text{Samples OD} - \text{Negative control OD}}{\text{Positive control OD} - \text{Negative control OD}} \right) \times 100$$

Value (%) fewer than 30% considered negative, between 30-40% considered doubtful and above 40% considered positive as given in the commercial test protocol.

RESULTS AND DISCUSSION

According to anamnesis; some of the sheep had abortion and 5 of them had mastitis. In the clinical examination of the sheep used in the present study, some had coughing and some had general condition weaknesses and had no other clinical sings of Q fever.

In the serological examination of the serum samples; out of 465 samples, 98 (21.07%) were seropositive, 11 samples (2.37%) were doubtful and the rest of them were seronegative (Table 1).

Table 1: Number of animals obtained from, number of seropositive animals and its percentage, number of doubtful animals and its percentage at different provinces

Provinces	n	Positive	%	Doubtful	%
Van	145	29	20.00	3	2.07
Ozalp	75	27	36.00	4	5.33
Gurpinar	55	8	14.55	-	-
Gevas	40	11	27.50	-	-
Catak	30	3	10.00	1	3.33
Edremit	45	6	13.33	1	2.22
Muradiye	35	9	25.71	-	-
Caldiran	40	5	12.50	2	5.00
Total	465	98	21.07	11	2.37

The most important livelihood source in the rural area in the county is the production of animals. Because, the region is mountainous, sheep production in the region is widespread. In the sheep production, problems mainly occur as flock problem. Animals kept indoor for a long period, lived in crowded and nonhygienic pens cause increase in the occurrence and spreading of contagious disease. Such predisposing factors in this region have usually been the case.

In the sheep production, the main income is getting lamb and milk. Abortion and mastitis seen in sheep cause important harm to the income of the animal producers and also to the country's economy. Q fever infection does not show apparent clinical symptoms apart from abortion and chronic mastitis (Imren and Sahal, 1991; Woldehiwet, 2004; Cutler *et al.*, 2007). The disease does not only threat animals health, it also cause important health risks for human begins.

Several studies have been put forward with regard to the presence of the disease in human begins in the country and in the world (Woldehiwet, 2004; Cutler *et al.*, 2007; Karabay *et al.*, 2007; Berberoglu *et al.*, 2004; Richardus *et al.*, 1987; Buke *et al.*, 2006). In the country, studies with concern to the seroprevalance of Q fever were reported to be 6% in Bolu (Karabay *et al.*, 2007), 25% in Ovakent/Izmir (Buke *et al.*, 2006), 13.2% in Antalya (Berberoglu *et al.*, 2004), 6% in Diyarbakir (Berberoglu *et al.*, 2004) and 6% in Samsun (Berberoglu *et al.*, 2004).

Lang *et al.* (1991) carried out a study in Canada at 103 sheep flocks to determine seropositivity to Q fever and found seropositivity to Q fever and found seropositivity at least 1-2 case in 22 farms and >2 in 7 farms. In Holland, a study carried out by Houwers and Richardus (1987) to determine seroprevalance of Q fever in sheep and found 39% seropositivity in the flocks, which had abortion and as a country whole at 191 flocks in 52 flocks (27.2%) seropositivity to Q fever were determine. In Canada, Hatchette *et al.* (2002) investigated seroprevalance of Q fever between 1997 and 2000 and reported to increase seropositivity from 3.1-23.5% and speculated that if a

protection program is not put forward, its seroprevalance may even increase. In Japan, Htwe *et al.* (1992) found seropositivity to Q fever as 18.1% using IFA test. Reinthaler *et al.* (1988) used micro agglutination test to determine seropositivity of Q fever in sheep in Sudan and found 62.5% seropositivity.

In the country, the presence of Q fever Ege (Sertpolat and Karakartal, 2005), Marmara regions (Kennerman *et al.*, 2008) and Elazig (Ozdemir *et al.*, 1999; Kalender, 2001), Aydin (Kilic *et al.*, 2005) were reported. In Elazig and in its neighboring cities, seroprevalance of Q fever were determined to be 20%, 20% in Malatya, 27.95% Bingol and 27.27% in Mus (Kalender, 2001). Abortion, which occur very often, may occur trough infection by several pathogens (Imren and Sahal, 1991; Zeman *et al.*, 1989; Parisi *et al.*, 2006). In these pathogens, *C. burnetii* take important place. As a matter of fact in a study, seropositivity to Q fever reported to be 78.6% in sheep, which had abortion (Kalender, 2001).

Ozdemir *et al.* (1999) investigated Q fever seroprevalance in sheep in the region of Elazig using IFA. They found seropositivity in 22 flocks out of 27, which having abortion or not. Furthermore, they also found seropositivity in 69 sheep out of 208 pregnant sheep and 65 had seropositivity out of 243 sheep, which had abortion. In addition, out of 5 goats 3 of them were also seropositive to Q fever. In this study Ozdemir *et al.* (1999), a total of 451 sheep investigated and 137 (30.39%) of them were seropositive to Q fever.

In a study carried out in Van (Ceylan *et al.*, 2009) with concern to Q fever seroprevalance in cattle and sheep. They examined only 92 serum samples and found only 5.4% seropositivity to Q fever. In contrast, the present study 450 samples were obtained and seropositivity was very high. The number of animals in seroprevalance studies important therefore, the present study is more reliable with concern to exact seroprevalance.

Kennerman *et al.* (2008) investigated seropositivity to Q fever in South Marmara region (Bursa, Balikesir and Canakkale) and found 20% (151 sheeps) seropositivity to Q fever in 743 sheep. On the other hand, Kilic *et al.* (2005) studied Q fever seroprevalance in the region of Aydin and found only 3% seropositivity. In addition, Cetinkaya *et al.* (2000) studied seroprevalance of Q fever in the region of Eastern Anatolia and found 10.5% seropositivity. Cetinkaya *et al.* (2000) also reviewed that seroprevalance of Q fever since, 1947 up to 1990 in sheep are between 2.7-22%.

In the present study, seropositivity of Q fever found to be 21.07%. When, the results obtained in the present study compared with the literature values, it was similar to the findings reported by Kennerman *et al.* (2008), which

investigated South Marmara region. But, these results were well above the country's mean values. This situation could be attributed to the hygienic condition take place in the region.

CONCLUSION

As a result, Q fever seroprevalance in the region of Van, which sheep production widespread, is quite high. This situation is not important for only animal health, it is also, important for men and women living in the region. Therefore, veterinary surgeons, butchers and animals producers should be aware of the danger and required precautions need to be taken immediately.

ACKNOWLEDGEMENTS

This study is supported by Scientific Research Project Management of the Yuzuncu Yil University (a grant number 2007-VF-B10).

REFERENCES

- Berberoglu, U., A., Gozalan, S. Kilic, D. Kurtoglu and B. Esen, 2004. A seroprevalance study of *C. Burnetii* in Antalya, Diyarbakir and Samsun Provinces Mikrobiyol Bult, 38: 385-391. PMID: 15700664. http://209.85.129.132/search?q=cache:HHprBlRwHlHJ:www.vircell.com/fileadmin/bibliografia/IFI/Berberoglu_U_et_al_2004_A_seroprevalance_study_of_Coxiella.pdf+U.+Berbero%C4%9Flu,+A.+G%C3%B6zalan,+S.+K%C4%B1l%C4%B1%C3%A7,+D.+Kurto%C4%9Flu,+B.+Esen&cd=2&hl=tr&ct=clnk&gl=tr.
- Buke, C., S. Atalay, M. Tuncel, G. Arsu, M. Cicekoglu and M. Turk, 2006. The seroprevalance of Q fever in ovacik, Izmir: A cross-sectional study. Turk. J. Infect., 20 (3): 155-158. <http://209.85.129.132/search?q=cache:kxI-z3mGq60J:infeksiyon.dergisi.org/pdf.php3%3Fid%3D84+U.+Berbero%C4%9Flu,+A.+G%C3%B6zalan,+S.+K%C4%B1l%C4%B1%C3%A7,+D.+Kurto%C4%9Flu,+B.+Esen&cd=1&hl=tr&ct=clnk&gl=tr>.
- Cetinkaya, B., H.B. Ertas, A. Muz, H. Ongor, H. Kalender, N. Arslan and M. Gurcay, 2000. Seroprevalance of coxiellosis in cattle, sheep and people in the East of Turkey. Vet. Record., 146 (5): 131-136. <http://veterinaryrecord.bvapublications.com/cgi/content/abstract/146/5/131>.
- Ceylan, E., M. Berktaş, I. Keles and Z. Agaoglu, 2009. Seroprevalance of Q fever in cattle and sheep in the East of Turkey. Asian J. Anim. Vet. Adv., 4 (3): 114-121. DOI: 10.3923/ajava.2009.114.121.

- Cutler, S.J., M. Bouzid and R.R. Cutler, 2007. Q fever. *J. Infect.*, 54 (4): 313-318. DOI: 10.1016/j.jinf.2006.10.048.
- Hatchette, T., N. Campbell, H. Whitney, R. Hudson and T.J. Marrie, 2002. Seroprevalence of *Coxiella burnetii* in selected populations of domestic ruminants in New foundland. *Can. Vet. J.*, 43 (5): 363-364. PMID: 12001502. http://www.ncbi.nlm.nih.gov/pubmed/12001502?ordinalpos=3&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DefaultReportPanel.Pubmed_RVDocSum.
- Houwens, D.J. and J.H. Richardus, 1987. Infections with *Coxiella burnetii* in man and animals in the Netherlands. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene*, 267 (1): 30-36. <http://www.cababstractsplus.org/abstracts/Abstract.aspx?AcNo=19882204836>.
- Htwe, K.K., K. Amano, Y. Sugiyama, K. Yagami, N. Minamoto, A. Hashimoto, T. Yamaguchi, H. Fukushi and K. Hirai, 1992. Seroepidemiology of *Coxiella burnetii* in domestic and companion animals in Japan. *Vet. Rec.*, 131: 490. PMID: 1471330.
- Imren, H.Y. and M. Sahal, 1991. *Veterinary Internal Diseases*. 2nd Edn. Feryal Press. Ankara, pp: 323-324. ISBN: 975-7774-00-6.
- Kalender, H., 2001. The incidence of *Coxiella burnetii* infection in Ewes in Elazig and neighboring Provinces. *Turk. J. Vet. Anim. Sci.*, 25: 51-55. <http://journals.tubitak.gov.tr/veterinary/issues/vet-1-25-1/vet-25-1-8-9911-5.pdf>.
- Karabay, K., E. Kocoglu, G. Baysoy and S. Konyalioglu, 2007. *Coxiella burnetii* seroprevalence in the rural part of Bolu, a city located in Western black sea region of Turkey. Public health and community-acquired infections. 17th European Congress of Clinical Microbiology and Infectious Diseases. ICC, Munich, Germany. <http://www.blackwellpublishing.com/eccmid17/abstract.asp?id=58022>.
- Kennerman, E., E. Rousset, E. Golcu and P. Dufour, 2008. Seroprevalence of Q fever (coxiellosis) in sheep from the Southern Marmara Region, Turkey, pp: 9 (Press). CIMID-684. DOI: 10.1016/j.cimid.2008.07.007.
- Kilic, S., S. Pasa, C. Babur and M.B. Ozlem, 2005. Investigation of *Coxiella burnetii* antibodies in sheep in Aydin region, Turkey. *Revue. Med. Vet.*, 156 (6): 336-340. http://209.85.129.132/search?q=cache:zTRSMbOMFEgJ:revmedvet.com/2005/RMV156_336_340.pdf+revue+med.+vet.+2005,+156,+6,+336-340&cd=4&hl=tr&ct=clnk&gl=tr&client=firefox-a.
- Lang, G., D. Waltner-Toews and P. Menzies, 1991. The seroprevalence of coxiellosis (Q fever) in Ontario sheep flocks. *Can. J. Vet. Res.*, 55 (2): 139-142. PMID: 1884293.
- Masala, M., R. Porcu, G. Sanna, G. Chessa, G. Cillara, V. Chisu and S. Tola, 2004. Occurrence, distribution and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy. *Vet. Microbiol.*, 99(3-4): 301-305. DOI: 10.1016/j.vetmic.2004.01.006. PMID: 15066733.
- Ozdemir, H., R. Can, Y. Sezen, H.B. Gulcu, H. Kalender and O. Basbug, 1999. Determination of prevalence of Q fever in sheep, Elazig Province and its treatment. *Firat Univ. Saglik Bilimleri Derg. (Veteriner)*, 13 (2): 143-149. http://www.fusabil.org/summary_en.php3?id=288. <http://www.pubmedcentral.nih.gov/pagerender.fcgi?artid=1790252&pageindex=1>.
- Parisi, A., R. Fraccalvieri, M. Cafiero, A. Miccolupo, I. Padalino, C. Montagna, F. Capuano and R. Sottili, 2006. Diagnosis of *Coxiella burnetii*-related abortion in Italian domestic ruminants using single-tube nested PCR. *Vet. Microbiol.*, 118 (1-2): 101-106. DOI: 10.1016-j.vetmic.2006.06.023.
- Peter, O., G. Dupuis, G. Peacock and W. Burgdorfer, 1987. Comparison of enzyme linked immunosorbent assay and complement fixation and indirect fluorescent antibody tests for detection of *Coxiella burnetii* antibody. *J. Clin. Microbiol.*, 25: 1063-1067. <http://jcm.asm.org/cgi/content/abstract/25/6/1063>.
- Reinthal, F.F., F. Mascher, W. Sixi and C.H. Arbesser, 1988. Incidence of Q Fever among cattle, sheep and goats in the upper Nile Province in Southern Sudan. *Vet. Rec.*, 122: 137. PMID: 3369066.
- Richardus, J.H., A. Donkers, A.M. Dumas, G.J. Schaap, J.P. Akkermans, J. Huisman and H.A. Valkenburg, 1987. Q fever in the Netherlands: A sero-epidemiological survey among human population groups from 1968-1983. *Epidemiol. Infect.*, 98(2): 211-219. PMID: 3549343. <http://www.ncbi.nlm.nih.gov/sites/entrez>.
- Sertolat, M. and G. Karakartal, 2005. The investigation of *Coxiella burnetii* seroprevalence by indirect immunofluorescent antibody test in the healthy blood donors living in the Izmir region. *Turk. J. Infect.*, 19 (4): 419-423. http://infeksiyon.dergisi.org/pdf/pdf_INF_125.pdf.
- Woldehiwet, Z., 2004. Q fever (coxiellosis): Epidemiology and pathogenesis. *Res. Vet. Sci.*, 77: 93-100. DOI: 10.1016/j.rvsc.2003.09.001.
- Zeman, D.H., C.A. Kirkbride, P. Leslie-Steen and J.R. Duimstra, 1989. Ovine abortion due to *Coxiella burnetii* infection. *J. Vet. Diagn Invest.*, 1: 178-180. <http://jvdi.org/content/vol1/issue2/index.dtl>.