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The Relationship of *Coxiella burnetii* Seropositivity Between Farm Animals and Their Owners: A Pilot Study

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Abstract: Q fever is a zoonotic disease caused by *Coxiella burnetii*. This study aimed to detect the relationship of *C. burnetii* seropositivity between farm animal owners and their animals. Blood serums of 20 farm animal owners, 32 cow and 88 sheep were investigated with indirect Immuno Fluorescent Assay (IFA) using *C. burnetii* phase I and II antigens. Milk samples of the same animals were tested for *C. burnetii* by PCR. The serological test results of animals and their owners were compared by statistically methods to reveal the interdependence and correlations. The seropositivities of IgG antibodies against *C. burnetii* were 90.0% for farm animal owners, 53.1% for cows and 63.6% for sheep. All of the animal owners were consuming dairy products made from their own animals raw milk. However, as shown by PCR results, none of the tested cows and sheep were responsible for shedding of *C. burnetii* through their milks. Although, there was no correlation between the shedding pattern and serological results of animals, there was a significant correlation between the serological results of animals and their owners for both phase I and II antigens against *C. burnetii*. There are statistically important relationships between farm animals and their owners about phase I and phase II IgG titration levels against *C. burnetii*. Moreover, there was close dependency between the presence of chronic *C. burnetii* infections in animals and their owners. On the other hand, serological results of milk samples are not in significant correlation with the serologically dependency of animals and their owners.

Key words: *Coxiella burnetii*, cow, sheep, farm animal owners, IFA, PCR, Turkey

INTRODUCTION

Coxiella burnetii is an obligate intracellular microorganism that causes Q fever in humans and animals (Maurin and Raoult, 1999). Ruminants (sheep, goats and cattle) are recognized as the main sources of human infections (Berri *et al*., 2004). Humans are infected mainly by inhalation of contaminated aerosols or by consumption of raw milk or fresh dairy products (Maurin and Raoult, 1999). Drinking contaminated milk has induced sero-conversion in human volunteers without clinical signs (Arricau-Bouvery and Rodolakis, 2005). The antibodies in milk could not always be detected due to the local synthesis against the antigenic stimulation of mammary glands by *C. burnetii* shedded in milk. But this condition is usually as a result of immunoglobulin transfer from blood to milk. Therefore, the milk-antibody titers cannot be used for the detection of *C. burnetii* in milk-shedder females (Rodolakis *et al*., 2007). The IFA (Immunofluorescent assay) remains the reference technique for Q fever diagnosis (Maurin and Raoult, 1999). The microimmunofluorescence test has the advantage of requiring only a small amounts of antigen, allowing the determination of both phase I and II antibodies. In acute cases of Q fever, the antibody level to phase II is usually higher than that to phase I, however in chronic Q fever, vica versa (Fournier *et al*., 1998). Although, Q fever is a disease closely related with occupations such as handling livestock (Casolin, 1999), most of these previous studies concerned with general populations. In the literature, there are few data about the relationship of *C. burnetii* seropositivity both in farm animals and their owners. It aimed to investigate *C. burnetii* seroprevalences in farm animals and their owners and the presence of *C. burnetii* in milk samples. Moreover, in the present study, it aimed to detect possible dependency and correlations among the seropositivity of animals, their milks and owners.

MATERIALS AND METHODS

The present study was planned to address the lack of data on *C. burnetii* from Central Anatolia Region (Kirikkale).

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As *C. burnetii* is a very infectious pathogen, only biosafety level 3 laboratories and experienced personel should be allowed to manipulate contaminated specimens and cultivate this microorganism from samples. Therefore in the present study an indirect IFA for serum samples and PCR for milk samples were preferred.

Samples: Q fever primarily remains as an occupational hazard in personnels who are in contact with domestic animals such as cattles, sheep and less frequently goats (Maurin and Raoult, 1999). Therefore in this study, 20 animal owners who were intensive cow and sheep breeders in rural parts of Kirikkale and have direct contact with their animals were preferred as human sampling group while cows and sheep of this group were taken as the animal sampling group.

A standardized questionnaire was used to obtain information relevant to each person's age, education, ownership of animals (cows and/or sheep) and consuming habits of raw milk and products made from their animals milk. Additional, questions were included to the standardized questionnaire addressing information from the owners regarding the prior health status (tick infestation, abortion and sterility) of the animals.

A blood sera and a milk samples for each 32 cows and 88 sheep were collected. Before milk sampling, the teats were cleaned and the first two jets of milk were discarded. Milk samples from quarters were collected aseptically in sterile tubes. All of the blood serums and milk samples used in this study were stored a -20°C until the assays. There was no indication of mastitis in the cows and sheep examined and milk samples showed physiological consistency. Serologically positive or negative cows can shed *C. burnetii* through their milk (Lorenz *et al*., 1998; Muramatsu *et al*., 1997). Therefore, both seropositive and seronegative milk samples of cows and sheep were investigated by PCR in the present study.

Serology of blood samples: All the blood serum samples of humans and animals were evaluated by a commercial IFA kit (Fuller Laboratories, Q fever IFA IgG Antibody Kit, USA) for the *C. burnetii* phase I and II antibodies recommended as the manufacturer's instructions. Results were invigilated with a fluorescence microscope (Olympus BX50 with a fluorescence attachment/Japan). The presence of bright apple-green fluorescent rickettsiae at the serum dilution 1:16 or higher was considered as a positive result of the test. Cutoff points were used for phase I antigen titer as ≥1:128 for acute Q fever and for phase II antigen titer as ≥1:128 and <1:800 for chronic Q fever, respectively (Anonymous, 2008).

Reference *C. burnetii* DNA: *C. burnetii* 9 milk isolates which were in 0.9% saline with a concentration of 1×10^{10} particles mL^{-1} and treated with proteinase K overnight and subsequently heated for 15 min at 100°C to inactivate the proteinase K was kindly provided by Dr. Gulay Altay (Ankara Nuclear Research Center in Agriculture and Animal Science, Turkish Atomic Energy Authority, Ankara, Turkey).

DNA extraction from milk samples: Milk samples of 1 mL were centrifuged at 13,000 g for 60 min then cream and the milk layers were removed (Kennerman *et al*., 2008). The pellet was washed twice with sterile water and DNA extraction was performed with the QIAmp DNA mini kit® (Qiagen S.A., Courta-boeuf, France) according to the instructions of the manufacturer. Briefly, the pellet was subsequently treated with proteinase K at 56°C overnight in the suitable lysis buffer and the enzyme was inactivated by heating to 70°C for 10 min. Ethanol (0.525 vol. 96%) was added and the mixture was applied onto a QIAmp spin column. After two rounds of washing with provided washing buffers, the DNA was eluted with 200 μL of the supplied elution buffer. About 5 μL of the DNA solutions were used as template DNAs in the PCR assay.

Oligonucleotides: Primers Trans1 (5'-TAT GTA TCC ACC GTA GCC AGT C-3') and Trans2 (5'-CCC AAC AAC ACC TCC TTA TTC-3') derived from a transposone-like repetetive region of the *C. burnetii* genome were purchased from Integrated DNA Technologies (Coralville, USA). The expected amplification product size of the target sequence was 687-bp (Kim *et al*., 2005).

PCR assay: PCR was performed as described previously in a total volume of 50 μL containing the following: 5 μL of 10×PCR buffer (Bioron GmbH, Ludwigshafen, Germany), 5 μL of 25 mM $MgCl_2$ (Bioron), 1.25 μL of 10 mM dNTP mixture (Bioron), 0.5 μL of each primer which contains 100 pmol μL^{-1} and 0.4 μL of 5 U Taq DNA polymerase (Bioron) (Rodolakis *et al*., 2007). The volume of the master mix was adjusted to 45 μL with sterile ultra pure water and 5 μL of template DNA was added. The touchdown PCR assay for DNA amplification was carried out in a thermocycler (Eppendorf mastercycler gradient, Hamburg, Germany) with the parameters described previously (Kennerman *et al*., 2008). Reference DNA extract and sterile ultra pure water was included to all assays as positive and negative controls, respectively.

Detection of the amplification products: A 20 μL aliquot of each resultant PCR product was further analyzed by agarose gel (1.5% Agarose-Basica LE, Prona, Spain) electrophoresis (CSL MSMixi-Duo, Corston, UK), stained

with 0.1 μg mL^{-1} ethidium bromide (BioChemica GmbH, Darmstadt, Germany) at 100 V for 1 h and visualized by a gel documentation and analysis system (Sygene Ingenius, Cambridge, UK).

Statistical analysis: All statistical analysis was performed using SPSS version 15 (SPSS, Chicago, USA). The data were expressed as median value (minimum-maximum). For correlation analysis Spearman correlation analysis was used. Additionally for evaluation of the dependency of the groups Fischer's exact test was used. $p<0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

According to the standardized questionnaire, 20 animal owners aged between 37-55 with education levels of primary school, they were breeders for approximately 20 years. The average animal numbers they owned were 12-15 cows and sheep. All were consuming raw milk and products of their animals. Questions concerning animals revealed that none of the animals were sterile and only sheep had abortion. All cows and sheep were exposed to a general tick infestation.

The seropositivities of phase I and II IgG antibodies against *C. burnetii* were shown in Table 1. According to the results of Spearman correlation analysis there were correlations between phase I of cows and owners ($r = 0.858$, $p = 0.013$), phase II of cows and owners ($r = 0.416$, $p = 0.353$), phase I of sheep and owners ($r = 0.816$, $p = 0.0031$) and phase II of sheep and owners ($r = 0.639$, $p = 0.019$). The detailed serological results of *C. burnetii* in sampling groups were shown in Table 2. Seropositivities of cows, sheep and owners were 53.1, 63.6 and 90%, respectively.

Statistically, a strong association was found between seropositive animals and owners who were in contact with them by Fischer's exact test. There were relations between acute cases of cows and owners ($p = 0.371$) and sheep and owners ($p = 0.371$). Moreover, chronic cases of cows and owners ($p = 0.114$) and sheep and owners ($p = 0.005$) were related to each other.

On the other hand, no relationship was found between seropositivity of cows and sheep for *C. burnetii* and shedding through milk. *C. burnetii* was not found in any of the milk samples by PCR.

Q fever is a zoonotic disease with a worldwide distribution caused by *Coxiella burnetii* (Maurin and Raoult, 1999). Likewise, there are many reports about the presence and prevalance of coxiellosis in Turkey (Gozalan *et al*., 2004).

Q fever infections have previously been described in occupational risk groups such as veterinarians and agricultural workers in Turkey (Cetinkaya *et al*., 2000). The seroprevalence of *C. burnetii* infection found among farm workers in the present study was higher compared to those in Australia (Casolin, 1999) and Turkey (Cetinkaya *et al*., 2000; Sertpolat and Karakartal, 2005). These differences were may be due to the random sampling of the other studies, size of the sampling populations and full time or part time workers.

Table 1: The presence of IgG antibodies against *C. burnetii* (phase I and II) in blood serums of animals and their owners

No. of samples (n)	Phase I antibody titer median (min-max)	No. of phase I positive samples (%)	Phase II antibody titer median (min-max)	No. of phase II positive samples (%)	No. of phase I and II positive samples (%)
Cow (32)	1:64 (0-1:512)	16 (50.0)	1:32 (0-1:512)	16 (50.0)	14 (43.8)
Cow owner (7)	1:64 (1:32-1:512)	7 (100.0)	1:64 (1:32-1:512)	7 (100.0)	7 (100.0)
Sheep (88)	1:128 (0-1:512)	56 (63.6)	1:64 (0-1:512)	48 (54.5)	48 (54.5)
Sheep owner (13)	1:64 (0-1:512)	11 (84.6)	1:64 (0-1:128)	11 (84.6)	11 (84.6)

Table 2: Serological results of *C. burnetii* in sampling groups

	A	AA	CA	SPA	SNA	O	AC	CO	SPO	SNO
Groups	(n)									
C 1	10	3	2	5	5	2	-	-	2	-
C 2	11	1	6	7	4	1	-	-	1	-
C 3	11	2	3	5	6	4	1	3	4	-
Total	32	6	11	17	15	7	1	3	7	-
S 1	12	1	2	3	9	1	-	-	1	-
S 2	12	4	5	9	3	1	-	-	-	1
S 3	12	6	1	9	3	1	1	-	1	-
S 4	12	5	3	10	2	3	-	-	3	-
S 5	12	5	3	11	1	2	-	1	2	-
S 6	15	3	4	7	8	3	-	2	2	1
S 7	13	5	2	7	6	2	1	1	2	-
Total	88	29	20	56	32	13	2	4	11	2

*C: Cows, S: Sheep, A: Animals, AA: Acute Animals, CA: Chronik Animals, SPA: Seropositive Animals, SNA: Seronegativeanimals, O: Owners, AO: Acute Owners, CO: Chronic Owners, SPO: Seropositive Owners, SNO: Seronegative Owners

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). Moreover animal owners and veterinarians are at special risk groups for this infection (Maurin and Raoult, 1999).

Thomas *et al.* (1995) stated that full time farmers were at least three times more likely to have acquired antibodies than do part time farmers. Psaroulaki *et al.* (2006) reported that a strong association was found between seropositivity for *C. burnetii* in humans that were in contact with sheep and/or cows.

In the present study the animal owners from sampling group were full time in contact with their animals for a period of approximately 20 years. Thus, nearly all animal owners was seropositive for *C. burnetii.* Similarly, according to the results there was a correlation between seropositivities of animal owners and their animals. This correlation might be attributed to wide dissemination characteristics of *C. burnetii* to the environment (Maurin and Raoult, 1999). The results of the questionnaire to animal owners did supported the correlation that the owners were both handlers and consumers of raw milk and raw milk products.

C. burnetii occurs in two antigenic phases called phase I and II and this antigenic difference is important in diagnosis. In acute cases of Q fever, the antibody level to phase II is usually higher than to phase I but in chronic Q fever, the reverse is the case. Additionally, antibodies to both phase I and II antigens have been known to persist for months or years following the initial infection (Fournier *et al.*, 1998). According to the study results, the high antibody response of animal owners to the *C. burnetii* phase I antigen was indicative of chronic infection or convalescence. Also a close relationship between the titration and ratio of phase I and II IgG antibodies of animals and their owners was detected.

In the present study, seropositivities of IgG antibodies against *C. burnetii* were 53.1% for cows and 63.6% for sheep. These results were in agreement with Masala *et al.* (2004) who found the seroprevalence of Q fever in sheep of Italy as 47%. On the other hand several studies have shown that there are significant differences about Q fever seroprevalance which could be resulted from their sampling region in Turkey (Cetinkaya *et al.*, 2000; Kennerman *et al.*, 2008; Kirkan *et al.*, 2008). Moreover, high titers of blood serum seropositivity could be more likely to be associated with an active Q fever but there is no warranty securing seronegative animals is free of the infection. Muramatsu *et al.* (1997) supported this theory with their results. Kennerman *et al.* (2008) reported that absent or low antibody levels could be linked either to long term *C. burnetii* carriage, primo-infection in progress, host immunological status and/or factors related to the strain. Also, Rodolakis *et al.* (2007) stated that the shedding in milk is sometimes intermittent and latently infected cows milk might be PCR negative. The inconsistency between the serological serum and milk PCR results in the study might be attributed this knowledge.

Moreover, the period of coxiella shedding might be vary according to the differences in the transmission route and *C. burnetii* infection might be persistent in dairy herds with little temporal or regional variations Kim *et al.* (2005) and Raoult *et al.* (2005) reported that asymptomatic cows shedded *C. burnetii* exclusively in their milks but ewes with abortions due to Q fever shedded the bacteria mostly by feces and vaginal mucus. This could explain why the acute and chronic Q fever results of sheep owners were higher than cow owners in the present study. Also, the high seropositivity of cow owners in the present study may be caused from raw milk consumption habits of the owners from their cows. According to the significant correlation between the serological results of animals and their owners for both phase I and II antigens against *C. burnetii.*

CONCLUSION

This study concluted that there was a significant relation between animals and their owners. However, PCR results of milk samples were not in correlation with the serological results of animals and their owners. The high seropositivity of cow owners might be related to raw cow milk consumption habits of the owners.

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REFERENCES

Anonymous, 2008. Q Fever (*Coxiella burnetii* infection). Wisconsin Division of Public Health Disease Surveillance Manual. EpiNet, http://hanplus.wisc.edu/EPINET/active_htms/qfever.htm.

Arricau-Bouvery, N. and A. Rodolakis, 2005. Is Q fever an emerging or re-emergeing zoonosis? Vet. Res., 36: 327-349.

Berri, M., E. Rousset, J.L. Champion, N. Arricau-Bouvery, P. Russo, M. Pepin and A. Rodolakis, 2004. Ovine manure osed a garden fertiliser as a suspected source of human Q fever. Vet. Rec., 153: 269-270.

Casolin, A., 1999. Q fever in New South Wales Department of Agriculture workers. J. Occup. Environ. Med., 41: 273-278.

Cetinkaya, B., H.B. Ertas, A. Muz, H. Ongor, H. Kalender, N. Arslan and M. Gurcay, 2000. Seroprevalence of coxiellosis in cattle, sheep and people in the east of Turkey. Vet. Rec., 146: 131-136.

Fournier, P.E., T.J. Marrie and D. Raoult, 1998. Diagnosis of Q fever. J. Clin. Microbiol., 36: 1823-1834.

Gozalan, A., L. Akin, J.M. Rolain, F.S. Tapar and O. Oncul *et al*., 2004. Epidemiological evaluation of a possible outbreak in and nearby Tokat province. Mikrobiyol Bul, 38: 33-44.

Kennerman, E., E. Rousset, E. Golcu and P. Dufour, 2008. Seroprevalence of Q fever (coxiellosis) in sheep from the Southern Marmara Region, Turkey. Comp. Immunol. Microbiol. Infect. Dis., 33: 37-45.

Kim, S.G., E.H. Kim, C.J. Lafferty and E. Dubovi, 2005. *Coxiella burnetii* in bulk tank milk samples, United Satates. Emerging Infect. Dis., 11: 619-621.

Kirkan, S., O. Kaya, S. Tekbiyik and U. Parin, 2008. Detection of *Coxiella burnetii* in cattle by PCR. Turk. J. Vet. Anim. Sci., 32: 215-220.

Lorenz, H., C. Jager, H. Willems and G. Baljer, 1998. PCR detection of *Coxiella burnetii* from different clinical specimens, especially bovine milk, on the basis of DNA preparation with slica matrix. Applied Environ. Microbiol., 64: 4234-4237.

Masala, G., 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: 301-305.

Maurin, M. and D. Raoult, 1999. Q Fever. Clin. Microbiol. Rev., 12: 518-553.

Muramatsu, Y., T. Yanase, T. Okabayashi, H. Ueno and C. Morita, 1997. Detection of *Coxiella burnetii* in cows milk by PCR-Enzyme-linked ýmmunosorbent assay combined with a novel sample preparation method. Applied Environ. Microbiol., 63: 2142-2146.

Psaroulaki, A., C. Hadjichristodoulou, F. Loukaides, E. Soteriades and A. Konstantinidis *et al*., 2006. Epidemiological study of Q fever in humans, ruminant animals and ticks in Cyprus using a geographical information system. Eur. J. Clin. Microbiol. Infect. Dis., 25: 576-586.

Raoult, D., T. Marrie and J. Mege, 2005. Natural history and pathophysiology of Q fever. Lancet Infect. Dis., 5: 219-226.

Rodolakis, A., M. Berri, C. Hechard, C. Caudron and A. Souriau *et al*., 2007. Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine and ovine herds. J. Dairy Sci., 90: 5352-5360.

Sertpolat, M. and G. Karakartal, 2005. The investigation of *Coxiella burnetii* seroprevalence by indirect immunofluorescent antibody test in the healthy blood donors living in the Ýzmir region. Turk. J. Infect., 19: 419-423.

Thomas, D.R., L. Treweek, R.L. Salmon, S.M. Kench and T.J. Coleman *et al*., 1995. The risk of acquiring Q fever on farms: A seroepidemiological study. Occupat. Environ. Med., 52: 644-645.