



SEROLOGICAL EVIDENCE OF *COXIELLA BURNETII* INFECTION AMONG COMPANION DOGS IN FARS PROVINCE, SOUTH IRAN

A. ESMAILNEJAD¹ & M. ABBASZADEH HASIRI²

¹Department of Pathobiology, ²Department of Clinical Studies; School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Summary

Esmailnejad, A. & M. Abbaszadeh Hasiri, 2017. Serological evidence of *Coxiella burnetii* infection among companion dogs in Fars Province, South Iran. *Bulg. J. Vet. Med.*, 20, No 4, 377–384.

Coxiella burnetii is an important zoonosis at a global scale. The epidemiological role of dogs in transmission of Q fever has recently been demonstrated, but there is still a dearth of information on the subject. The aim of present study was to evaluate the occurrence and seroprevalence of *Coxiella burnetii* infection among companion dogs population in Fars province, South Iran. Blood samples were collected from 181 asymptomatic dogs, mostly referred to Veterinary Hospital of Shiraz University for regular vaccination. The IgG antibody detection against *C. burnetii* was made by indirect Enzyme-linked Immunosorbent Assay (ELISA), employing *C. burnetii* phase I and II antigens. A logistic regression model was developed to analyse multiple risk factors associated with seropositivity. Specific antibodies against *C. burnetii* were detected in 14 (7.7%) cases, 12 with S/P% of 20–50% and 2 with S/P% greater than 80%. Prevalence was significantly higher in adult dogs above 5 years (18.18 %; 2 out of 11) compared with dogs between 1 and 5 years (7.86 %; 7 out of 89) and less than 1 year (6.17%; 5 out of 81) ($P=0.043$). Prevalence was also higher in male dogs (11.21 %; 12 out of 107) than in female (2.7 %; 2 out of 74) ($P=0.035$). Breed, type of housing, type of food and exposure to other farm animals showed no significant differences between positive and negative cases ($P>0.05$). The results of this study showed the presence of *C. burnetii* infection among the companion dogs population in Iran, which could be a public health concern for humans. In areas like Iran, where human cases of Q fever are not common or remain unreported, the public health implications of Q fever seroprevalence in dogs are quite significant.

Key words: *Coxiella burnetii*, ELISA, dog, Iran, Q fever

INTRODUCTION

Coxiella burnetii is a Gram-negative, obligatory intracellular bacterium that causes Q fever, a significant worldwide zoonosis (Angelakis & Raoult, 2010; Andoh *et al.*,

2013). *C. burnetii* infects a wide range of animals including mammals, birds, arthropods (mainly ticks) as well as humans (Tissot-Dupont & Raoult, 2008). Domes-

ticated ruminants including cattle, sheep and goats are the commonest animal reservoirs of *C. burnetii* for human infection. Infected dogs and cats have also been implicated as possible additional reservoirs for Q fever transmission (Buhariwalla *et al.*, 1996; Angelakis & Raoult, 2010; Sykes, 2013). The organism is shed in high numbers in the urine, faeces, milk, and especially in amniotic fluids and placenta materials during parturition. Due to the resistance of the bacteria to heat, drying, and many common disinfectants, they can survive for long periods in the environment and transmit to the new host (Angelakis & Raoult, 2010; Porter *et al.*, 2011).

The main route of *C. burnetii* transmission to humans is inhalation of contaminated aerosols, but it may occur through the consumption of raw infected milk and dairy products (Khalili & Sakhaee, 2009; Andoh *et al.*, 2013; Sykes, 2013). Dogs and cats might be infected by consumption of placenta or milk from infected ruminants, or by the aerosol route. Contact with infected wildlife reservoir such as rodents, rabbits and tick bites is considered to have a low potential for *C. burnetii* transmission (Angelakis & Raoult, 2010; Andoh *et al.*, 2013; Sykes, 2013; Meredith *et al.*, 2015). Q fever infection in animals is mainly subclinical, though some reproductive tract diseases including late abortions, stillbirth, infertility and metritis have been reported (Buhariwalla *et al.*, 1996; Porter *et al.*, 2011; Sykes, 2013). Therefore, the exact prevalence of the infection has remained undetermined in most parts of the world, including Iran.

A major characteristic of *C. burnetii* is its antigenic variation (called phase variation) which is due to the partial loss of lipopolysaccharide antigens. Phase I anti-

gens are very infectious and a single bacterium may infect a human, while Phase II forms are less infectious (Hotta *et al.*, 2002; Angelakis & Raoult, 2010). This antigenic variation is valuable for serological differentiation between chronic and acute Q fever. It is considered that the presence of anti-phase II antibodies reveals only the acute phase of Q fever, whereas high levels of both phase I and phase II antibodies are characteristic of chronic disease (Boni *et al.*, 1998).

The epidemiological role of dogs in Q fever has recently been demonstrated, but there is still a dearth of information on the subject. The aim of the study described here was to evaluate the occurrence and seroprevalence of *Coxiella burnetii* infection among companion dogs population in Fars province, South of Iran. For this purpose, the IgG antibody titre against *C. burnetii* infection was determined using purified phase I and phase II antigens, which resemble the chronic disease pattern. Since dogs may serve as a possible reservoir of infection for human beings, the public health implications of these results are quite significant.

MATERIALS AND METHODS

Area of study and sample population

The present study was conducted on 181 blood samples collected from companion dogs in Shiraz, South of Iran. Shiraz is located at an altitude of 1484 m above sea level and its climate is semi-arid, with mild winters and warm summers (Saboochi *et al.*, 2012). The studied dogs were presented to Veterinary Hospital of Shiraz University, between December 2014 and September 2015, mostly for regular vaccination. All applicable national and institutional guidelines for the care and use of animals were followed. Amongst the

specimens, 107 were male dogs and 74 were female. The studied dogs were classified based on the age into 3 groups (<1 year; 1–5 years; and >5 years) and based on the breed into 2 groups (pure and mix-breed). At the time of blood collection, 21 dogs showed respiratory or gastrointestinal signs, but all were asymptomatic and revealed no clinical signs of Q fever, including fever, lethargy and reproductive disorders (Table 1). Each serum sample was accompanied by a questionnaire to be completed by the owner and included information related to the place where dogs were kept, type of food and exposure to other dogs and farm animals. None of the animals included in this study had been vaccinated against the Q fever and no *Coxiella* vaccine is available in Iran.

Laboratory methods

Blood samples were collected from the cephalic veins and sera were separated and stored at -20°C until serological assays. Immunoglobulin G (IgG) class antibodies against *C. burnetii* were detected using indirect enzyme-linked immunosorbent assay (ELISA) method, employing Phase I + II purified antigens (IDVet, Innovative Diagnostic, France). Sera were prepared at 1:10 dilution and specific antibodies were measured using a peroxidase-labelled anti-multi-species IgG antibody conjugate. The diagnostic specificity and sensitivity of the ELISA test were 100%, while the correlation of ELISA with PCR and CFT tests was 92.86% and 94.44%, respectively.

Interpretation of the test

Sera were tested using a commercial indirect ELISA kit modified by the manufacturer for using in dog population. The test results were considered valid if the mean optical density (OD) of the two positive

controls (OD_{PC}) was greater than 0.350, and the ratio of the mean OD value of the positive and negative controls (OD_{PC} and OD_{NC}) was greater than 3 ($\text{OD}_{\text{PC}}/\text{OD}_{\text{NC}} > 3$). The sample/positive control (S/P) percentage was calculated by the formula: $[\text{OD}_{\text{sample}} - \text{OD}_{\text{NC}}] / [\text{OD}_{\text{PC}} - \text{OD}_{\text{NC}}] \times 100$.

The cut-off point described in the kit's manual was adjusted for use in ruminants. For interpreting the results obtained in dog population the appropriate cut-off was determined by testing known confirmed seronegative and seropositive samples and defining the background level of antibodies. These samples were not available in our lab, and the IDVet Company could not validate it either. However, regarding the ELISA results obtained in the studied population, background level of antibodies was quite low (most of the sample S/P% were <5), while 2 samples were identified as strongly positive with S/P% >80. So, there was an acceptable separation between the positive and negative samples which could determine the background. In the absence of any validation against a gold standard and in view of these results, samples which were clearly distinct from the background (S/P% >20) were considered as doubtful, in which anti-*Coxiella* antibodies seemed to be present. Accordingly, sera with an S/P% <20% were considered negative; samples with S/P% between 20% and 50% were considered doubtful; samples with S/P% between 50% and 80% – positive and those >80% – strongly positive. Doubtful zone could be attributed either to the seroconversion or to a prior exposure in a dog's history.

Statistical analysis

Relationships between the prevalence of positive cases and age, sex, breed, type of housing, type of food and exposure to

other farm animals were examined using chi-square analysis and Fisher's exact test by SPSS software, version 16 (SPSS, Inc. Chicago, IL, USA). Multiple logistic regression analysis was performed to evaluate the effects of different risk factors on the disease. P value less than 0.05 was considered statistically significant.

RESULTS

Out of the 181 sera samples studied using the ELISA method, 14 (7.7%) had antibodies against *Coxiella burnetii*, 12 with

S/P% of 20–50% and 2 with S/P% greater than 80%. Prevalence was significantly higher in adult dogs above 5 years (18.18%; 2 out of 11) compared with dogs between 1 and 5 years (7.86%; 7 out of 89) and less than 1 year (6.17%; 5 out of 81) (P=0.043). Prevalence was also higher in male dogs (11.21%; 12 out of 107) than in female dogs (2.7%; 2 out of 74) (P=0.035). There were no significant differences between the prevalence of positive cases and breed, type of housing, type of food and exposure to other farm animals (P>0.05) (Table 1).

Table 1. Seroprevalence of *Coxiella burnetii* infection among companion dogs in Shiraz province, Iran

Variable	Category	Number (%) of dogs examined	Number (%) of positive dogs	P value
Age (years)	< 1	81 (44.80)	5 (6.17)	0.043
	1–5	89 (49.20)	7 (7.86)	
	> 5	11 (6.60)	2 (18.18)	
Sex	Male	107 (59.10)	12 (11.21)	0.035
	Female	74 (40.90)	2 (2.70)	
Type of housing	Indoor	19 (10.50)	1 (5.26)	0.21
	Watchdog	146 (80.70)	10 (6.84)	
	Stray	16 (8.80)	3 (18.75)	
Breed	Pure	96 (53.00)	5 (5.20)	0.17
	Mix	85 (47.00)	9 (10.58)	
Exposure to farm animals	Yes	117 (64.60)	7 (5.98)	0.23
	No	64 (35.40)	7 (10.93)	
Type of food	Raw food	76 (42.00)	6 (7.89)	0.95
	Cooked food	95 (52.50)	7 (7.36)	
	Both	10 (5.50)	1 (10.00)	
Clinical signs	Q fever	0	0	–
	Respiratory	9 (4.90)	0	
	Gastrointestinal	12 (6.52)	0	
	No signs	160 (88.39)	14 (8.58)	
Total		181 (100.00)	14 (7.70)	

Table 2. Risk factors in the logistic regression model for *Coxiella burnetii* infection among companion dogs in Shiraz province, Iran

Risk factor	Beta	P value	Odds ratio	95 % CI for odds ratio	
				Lower	Upper
Sex	1.515	0.050	4.547	0.987	20.957
Age	-0.625	0.154	0.535	0.224	1.280
Type of housing	-0.658	0.358	0.518	0.127	2.120
Breed	-0.707	0.224	0.493	0.155	1.568
Exposure to farm animals	-0.790	0.174	0.454	0.142	1.446
Type of food	-0.048	0.926	0.953	0.345	2.634

All positive cases were asymptomatic for Q fever and did not exhibit any respiratory or gastrointestinal signs. To control the potential confounding effects of different risk factors, multiple logistic regression model was built by including significant risk factors associated with Q fever. After including the sex and age variables, it was determined that sex was still a significant factor associated with *C. burnetii* infection in the studied population ($P=0.05$) (Table 2).

DISCUSSION

Results of the present study provide evidence of exposure to *Coxiella burnetii* infection in the companion dogs population in South Iran. Based on our knowledge, no other studies have been conducted on Q fever in dogs in Iran. The present study revealed that 14 out of 181 (7.7%) dogs had serologic evidence of *C. burnetii* exposure. Since no vaccination programme against *C. burnetii* existed in the studied population, positive cases were considered as naturally infected.

The prevalence of *C. burnetii* among dog populations in different areas of the world varies within a wide range. The present seroprevalence was higher than that reported in new states of Federal Re-

public of Germany (3%, $n=1620$) (Kramer, 1991), North Italy (0.87%, $n=802$) (Baldelli *et al.*, 1992), New Zealand (0%, $n=12556$) (Hilbink *et al.*, 1993) and Canada (0%, $n=447$) (Marrie *et al.*, 1985), but lower than reported seropositivity in Australia (11.4%, $n=201$) (Cooper *et al.*, 2011), Southern Croatia (12%, $n=51$) (Punda-Polić *et al.*, 1994), Japan (15%, $n=632$) (Htwe *et al.*, 1992), Nigeria (29%, $n=786$) (Addo & Bale, 1981), Switzerland (31%, $n=388$) (Metzler *et al.*, 1983) and California (66%, $n=316$) (Willeberg *et al.*, 1980). Although there is no information about Q fever in dogs population in Iran, Asadi *et al.* (2012) reported 19.5% and 27.2% seroprevalence of *C. burnetii* in sheep and goats respectively (Asadi *et al.*, 2012). Esmaili *et al.* (2013) also reported seroconversion rate of 23.7% among sheep population in northern Iran (Esmaili *et al.*, 2013).

Traditionally, the acquisition of Q fever has been linked to close contact with farm animal especially to ruminants. However, present research and some other previous reports showed that there was no correlation between seropositive cases and direct contact with farm animals (Gale *et al.*, 2007; Tozer *et al.*, 2014). It was hypothesised that other risk factors (except

farm animals contact) like wildlife contact, tick exposure or pet-owners who worked closely with other farm animals would have a positive association with seropositivity to *C. burnetii* in the pets (Cooper *et al.*, 2011). In present study, breed, type of housing, type of food and presence of clinical signs showed no significant differences between positive and negative cases. Based on the literature, high prevalence of *C. burnetii* has been reported in the urine of dogs and human infection has been directly linked to the pet dogs (Komiya *et al.*, 2003; Tozer *et al.*, 2014). Therefore, no clinical signs of positive cases might have a decisive role in human infections. In a study conducted on the sheep and goat flocks of Iran, native breed of sheep and goats showed significantly higher prevalence of Q fever among studied breeds (Asadi *et al.*, 2014). In present research, similar association between pure and mix breeds of dogs was not proven.

Among risk factors which were investigated in this study, only age and sex showed significant association with the *C. burnetii* infection. The higher prevalence in adult dogs above 5 years might be attributed to more exposure and increase the chance of contact with ticks or other infectious material with time. Seroprevalence of *C. burnetii* was also significantly higher in male dogs, while in most studies conducted on sheep and goats, no significant relationship was seen between gender and infection rate (Asadi *et al.*, 2014). It might be because more male dogs (than females) are kept for working or guarding purposes.

Q fever has been confirmed in different animal species including cattle, sheep, goats, camels, horses, wild rodents and birds in Iran. Therefore, Iran should be considered as an endemic area for Q fe-

ver, where livestock animals play an important role in the transmission of the disease. However, there are few epidemiological studies on human Q fever in Iran (Mostafavi *et al.*, 2012). In the newest study, Khalili *et al.* (2010) detected 24% and 36% phase I and phase II *C. burnetii* specific IgG among febrile patients with suspected brucellosis in southeast Iran (Khalili *et al.*, 2010). The high prevalence of *C. burnetii* in different animal species compared to relatively few reports of human clinical case of Q fever leads to the speculation that most of the cases are sub-clinical and remain misdiagnosed.

CONCLUSION

In conclusion, the presence of *Coxiella burnetii* antibodies in dog sera is a public health concern, due to the close contact between dogs and humans, which provides a link between an environmental reservoir and humans. Q fever should be considered a public health problem for humans and animals in Iran. Veterinarians should also pay more attention to this disease and include it within their differential diagnosis. Further studies including molecular detection of *Coxiella burnetii* from companion dogs and cats and isolated ticks in suspected areas are highly recommended. In areas like Iran, where human cases of Q fever are not common or remain unreported, the public health implications of Q fever seroprevalence in dogs are quite significant.

REFERENCES

- Addo, P. & J. Bale, 1981. A serological survey for Q fever in dogs around Zaria. *Nigerian Veterinary Journal*, **10**, 25–26.
- Andoh, M., R. Andoh, K. Teramoto, T. Komiya, T. Kaneshima, A. Takano, H. Haya-

- shidani & S. Ando, 2013. Survey of *Coxiella burnetii* in ticks collected from dogs in Japan. *Journal of Veterinary Medical Science*, **75**, 1115–1117.
- Angelakis, E. & D. Raoult, 2010. Q fever. *Veterinary Microbiology*, **140**, 297–309.
- Asadi, J., M. Kafi & M. Khalili, 2012. Seroprevalence of Q fever in sheep and goat flocks with a history of abortion in Iran between 2011 and 2012. *Veterinaria Italiana*, **49**, 163–168.
- Asadi, J., M. Khalili, M. Kafi, M. Ansari-Lari & S. M. Hosseini, 2014. Risk factors of Q fever in sheep and goat flocks with history of abortion. *Comparative Clinical Pathology*, **23**, 625–630.
- Baldelli, R., C. Cimmino & M. Pasquinelli, 1992. Dog-transmitted zoonoses: A serological survey in the province of Bologna. *Annali dell'Istituto Superiore di Sanita*, **28**, 493–496.
- Boni, M., B. Davoust, H. Tissot-Dupont & D. Raoult, 1998. Survey of seroprevalence of Q fever in dogs in the southeast of France, French Guyana, Martinique, Senegal and the Ivory Coast. *Veterinary Microbiology*, **64**, 1–5.
- Buhariwalla, F., B. Cann & T. Marrie, 1996. A dog-related outbreak of Q fever. *Clinical Infectious Diseases*, **23**, 753–755.
- Cooper, A., R. Hedlefs, N. Ketheesan & B. Govan, 2011. Serological evidence of *Coxiella burnetii* infection in dogs in a regional centre. *The Australian Veterinary Journal*, **89**, 385–387.
- Esmaili, S., E. Mostafavi, M. Shahdordizadeh & H. Mahmoudi, 2013. A seroepidemiological survey of Q fever among sheep in Mazandaran province, northern Iran. *Annals of Agricultural and Environmental Medicine*, **20**, 708–710.
- Gale, M., N. Ketheesan, B. Govan, R. Kennedy & R. Norton, 2007. Q fever cases at a North Queensland centre during 1994–2006. *Internal Medicine Journal*, **37**, 644–646.
- Hilbink, F., M. Penrose, E. Kovacova & J. Kazar, 1993. Q fever is absent from New Zealand. *International Journal of Epidemiology*, **22**, 945–949.
- Hotta, A., M. Kawamura, H. To, M. Andoh, T. Yamaguchi, H. Fukushi & K. Hirai, 2002. Phase variation analysis of *Coxiella burnetii* during serial passage in cell culture by use of monoclonal antibodies. *Infection and Immunity*, **70**, 4747–4749.
- Htwe, K., K. Amano, Y. Sugiyama, K. Yagami, N. Minamoto, A. Hashimoto, T. Yamaguchi, H. Fukushi & K. Hirai, 1992. Seroepidemiology of *Coxiella burnetii* in domestic and companion animals in Japan. *The Veterinary Record*, **131**, 490.
- Khalili, M. & E. Sakhaee, 2009. An update on a serologic survey of Q fever in domestic animals in Iran. *American Journal of Tropical Medicine and Hygiene*, **80**, 1031–1032.
- Khalili, M., N. Shahabi-Nejad & M. Golchin, 2010. Q fever serology in febrile patients in southeast Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **104**, 623–624.
- Komiya, T., K. Sadamasu, M. I. Kang, S. Tsuboshima, H. Fukushi & K. Hirai, 2003. Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. *Journal of Veterinary Medical Science*, **65**, 1047–1048.
- Kramer, M., 1991. Zum Vorkommen, zur Verbreitung und zur Epidemiologie des Q-Fiebers in den neuen Bundesländern der Bundesrepublik Deutschland. *Tierärztliche Umschau*, **46**, 411–416.
- Marrie, T. J., J. Van Buren, J. Fraser, E. V. Haldane, R. S. Faulkner, J. C. Williams & C. Kwan, 1985. Seroepidemiology of Q fever among domestic animals in Nova Scotia. *American Journal of Public Health*, **75**, 763–766.
- Meredith, A., S. Cleaveland, M. Denwood, J. Brown & D. Shaw, 2015. *Coxiella burnetii* (Q-Fever) seroprevalence in prey and predators in the United Kingdom: Evaluation of infection in wild rodents, foxes and

- domestic cats using a modified ELISA. *Transboundary and Emerging Diseases*, **62**, 639–649.
- Metzler, A., J. Nicolet, H. Bertschinger, R. Bruppacher & J. Gelzer, 1983. Verbreitung von *Coxiella burnetii*: eine seroepidemiologische Untersuchung bei Haustieren und Tierärzten. *Schweizer Archiv für Tierheilkunde*, **125**, 507–517.
- Mostafavi, E., H. Rastad & M. Khalili, 2012. Q Fever: An emerging public health concern in Iran. *Asian Journal of Epidemiology*, **5**, 66–74.
- Porter, S. R., G. Czaplicki, J. Mainil, Y. Horii, N. Misawa & C. Saegerman, 2011. Q fever in Japan: An update review. *Veterinary Microbiology*, **149**, 298–306.
- Punda-Polić, V., S. Poljak, A. Bubić, N. Bradarić & Z. Klismanić-Nuber, 1994. Antibodies to spotted fever group rickettsiae and *Coxiella burnetii* among domestic animals in southern Croatia. *Acta Microbiologica et Immunologica Hungarica*, **42**, 339–344.
- Saboohi, R., S. Soltani & M. Khodaghali, 2012. Trend analysis of temperature parameters in Iran. *Theoretical and Applied Climatology*, **109**, 529–547.
- Sykes, J. E., 2013. *Canine and Feline Infectious Diseases*. Elsevier Health Sciences, Louis, Missouri.
- Tissot-Dupont, H. & D. Raoult, 2008. Q fever. *Infectious Disease Clinics of North America*, **22**, 505–514.
- Tozer, S., S. Lambert, C. Strong, H. Field, T. Sloots & M. Nissen, 2014. Potential animal and environmental sources of Q fever infection for humans in Queensland. *Zoonoses and Public Health*, **61**, 105–112.
- Willeberg, P., R. Ruppanner, D. Behymer, S. Haghighi, J. Kaneko & C. Franti, 1980. Environmental exposure to *Coxiella burnetii*: A sero-epidemiologic survey among domestic animals. *American Journal of Epidemiology*, **111**, 437–443.

Paper received 10.03.2016; accepted for publication 10.06.2016

Correspondence:

Dr. Mohammad Abbaszadeh Hasiri
Assistant Professor of Small Animal Internal
Medicine
Department of Clinical Studies,
School of Veterinary Medicine,
Shiraz University, P.O. Box 1731,
Shiraz 71345, Iran
fax: +98 71 32286950
tel: +98 71 36138911
e-mail: abbaszadeh@shirazu.ac.ir,
vetmail@gmail.com