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Prevalence and risk factor of Q fever among veterinary students in Iran

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Abstract. Q fever, caused by *Coxiella burnetii*, is a zoonosis with a worldwide distribution. This study is aimed to determine seroprevalence of Q fever and to identify the correlation between 8 risk factors for Q fever among students at Faculty of Veterinary Medicine in the study in Iran. In the present study, 121 blood samples (serum) were taken from students and tested using indirect diagnostic ELISA kit. The data were analyzed using descriptive statistics and 95% confidence interval, Chi-square statistical test, and logistic regression. Results showed that 34.7% were positive from all the serum samples. Results of the regression test showed that correlation only between age (P-Value = 0.038) and sex (in women; P-Value = 0.05, OR = 2.22 95% CI = [1.00 - 4.90]) with positive serum titer of acute Q fever. According to the results, high seroprevalence of Q fever was observed among the veterinary students. This problem can be solved by taking more careful preventive measures against this disease in the training centers and veterinary students.

INTRODUCTION

Q fever is a bacterial disease which is common between humans and animals; so, it is necessary to pay more attention to this disease in the society. This disease is caused by Gram negative, rod-shaped, obligate intracellular bacterium called *C. burnetii* (Maurin & Radult, 1999). Humans are typically infected with this disease through the inhalation of infected aerosols caused by childbirth, feces, and urine of infected animals and, to a lesser extent, via dermal scratches, mucous membranes, and consumption of raw milk or infected dairy (Belti *et al*., 2000; Shannon & Heinzen, 2009). Q fever usually has no special clinical symptoms in humans; however, in its acute form, it appears as a self-limiting flu-like illness with the incubation period of 1 to 3 weeks accompanied by high fever, fatigue, contusion, muscular pains, chills, sweating, headache, anorexia, pneumonia, hepatitis and encephalopathy. Chronic form of Q fever is followed by symptoms including chronic fatigue and endocarditis and infection in pregnant women can cause miscarriage and premature birth (Massung *et al.*, 2011). Q fever in humans is an occupational disease, which mostly occurs in ranchers, abattoir workers, veterinarians, and generally people who are in contact with the livestock and animal products (Maurin & Radult, 1999). Q fever is caused by various sources including livestock, wild mammals, birds, fish, and arthropods. Among all the sources, sheep, goat, dairy cow, and pets are considered as the main sources of this disease for humans (Angelakis & Raoult, 2009; Kim *et al*., 2005). In nature, two infection cycles have been identified for *C. burnetii*: the first one occurs in arthropods (especially, ticks), wild

mammals, and birds and the second occurs in the livestock. Ticks are the primary source of this bacterium, which spreads the infection among wild animals and transfers it to domestic animals. Since this bacterium has considerable durability and stability in extracellular environment, the infection in animals, especially domestic ones, through the inhalation of infected aerosols is as effective as the spreading tick. Infection is rarely transferred from a human to another human; typically, humans are not directly infected with ticks (Maurin & Radult, 1999). Q fever is diagnosed by cultivation, molecular methods, and serology. Among the serological methods, Complement fixation test (CFT), Indirect immunofluorescences antibody test (IFAT) and Enzyme linked immune sorbent assay (ELISA) are the most commonly utilized assays. Due to its higher sensitivity, acceptable characteristics, and economical nature, ELISA method is more common than the other mentioned tests; this method can be used for the diagnosis of anti-phase I and anti-phase II antibodies in *C. burnetii* (Angelakis *et al*., 2014). The available evidence obtained from various studies in the southeast of Iran (Naderinejad *et al*., 2014; Aflatoonian *et al*., 2014; Khalili *et al*., 2010; Khalili *et al*., 2014; Khalili & Sakhaee, 2009). Implies that Q fever is endemic in animals and humans and is considered an occupational disease in humans. Also, despite the high contact of veterinary students with animals, no studies have been performed in this area on Iranian veterinary students; therefore, the present work is aimed to investigate the sero-prevalence of Q fever (phase II) and its correlation with 8 risk factors using a domestic ELISA kit developed at Faculty of Veterinary Medicine among veterinary students at Shahid Bahonar University of Kerman.

MATERIALS AND METHODS

Samples

In this study, first, the blood samples were taken from 121 veterinary students in six different grades of veterinary studies at Shahid Bahonar University of Kerman along with completed questionnaires (including first and last names, age, sex, education, knowledge about Q fever, place of residence, records of being in contact with animals and their secretions, keeping animals at home, and consuming unpasteurized dairy) in order to conduct indirect ELISA assay for phase II *C. burnetii*. Then, the blood tubes were transferred to the microbiology laboratory at Faculty of Veterinary Medicine. In order to separate the serum content, the blood samples were centrifuged at the speed of 3500 g for 10 min. Then, the separated serum samples were transferred to the micro-tubes and numbered to be kept in a freezer at -20°C until the day when ELISA test was due.

Preparing the kits and examining the serum samples

In this study, an indirect ELISA kit developed at Faculty of Veterinary Medicine was used (Naderinejad *et al*., 2014). In summary, 1- 50µl antigen of phase II *C. burnetii* (fully purified Dolfinin, Slovakia) diluted with the ratio of 1.39 was added to all the wells in the ELISA kit micro-plate which was stored inside a refrigerator for one night so that the antigen was attached to the bottom of the wells. 2- In the next step, all the wells in the ELISA kit micro-plate were evacuated and every well was rinsed three times with 400 µl PBS. 3- 150 µl from 2.5% casein solution was added to all the micro-plate wells as the blocker agent and the solution was stored inside the incubator at 37°C for 2 h. 4- Contents of the wells were emptied and every well was rinsed three times. 5- 50 µl from the serum samples diluted with the ratio of 1:500 was added to the micro-plate wells as suspicious, negative control, and positive control serum samples and the kits were stored in the incubator at 37°C for 1 h. 6- Contents of the wells were evacuated and every well was rinsed three times. 7- 50 µl from the 1/2000 diluted antibody conjugate (Serotech, England) was added to all the wells and the micro-plate was stored in the incubator at 37°C for 1 h. 8- Contents of the wells were evacuated and every well was rinsed three times. 9- After the rinsing solution was completely removed from the wells, 50 µl from the substrate TMB

solution was added to all the wells and the micro-plate was stored in the incubator at 37°C for 20 min. 10- Finally, the micro-plates were removed from the incubator and 50 µl from 1M sulfuric acid was added to all the wells to stop the reaction and to prepare the micro-plate for the reading. Then, optical density (OD) of the samples was read by ELISA reader (Anthos 2020, Austria) with the wavelength of 450 nm against the reference filter 620 (Naderinejad *et al.*, 2014). To interpret the results and identify the positive, suspicious, and negative samples, first, doubled values of standard deviation were summed with the mean value of OD for the negative controls, the cut-off value for the designed kit was calculated, and value of OD was obtained as 0.100. 10% higher and lower than the cut-off value determined for the designed kit was considered as, Border Line respectively 0.09 - 0.110 (Belti *et al.*, 2000; Naderinejad *et al.*, 2014). Finally, the overall percentage of the positive results and percentage of positive results in women and men were calculated with the 95% confidence interval (95% CI). Then, correlation of positive serum titer for Q fever (phase II) with 8 risk factors of age, sex, education, knowledge about Q fever, place of residence, keeping animals at home, consuming unpasteurized dairy, and contact with animals and their secretions was analyzed using Chi-square and logistic regression statistical tests (Naderinejad *et al.*, 2014). The analyses were performed in SPSS 19.0 statistical software.

RESULTS

Among the 121 subjects in this study, 42 had Q fever phase II antibodies, according to which the seroprevalence of these antibodies was calculated as 34.70% (95% CI: 26.22% - 43.18%). Also, 22 and 20 out of 51 and 70 female and male participants in this study had Q fever phase II antibodies, respectively, according to which the seroprevalence was calculated as 43.14% (95% CI: 29.54% - 56.74%) and 28.57% (95% CI: 17.99% - 39.15%), respectively (Chart 1). Also, based on the statistical analysis, no correlation was observed between positive serum titer for Q fever (phase II) and the risk factors, except for age and sex. According to the results, risk of infection with this disease was reduced with age; also, risk of infection was greater in women than men (Table 1).

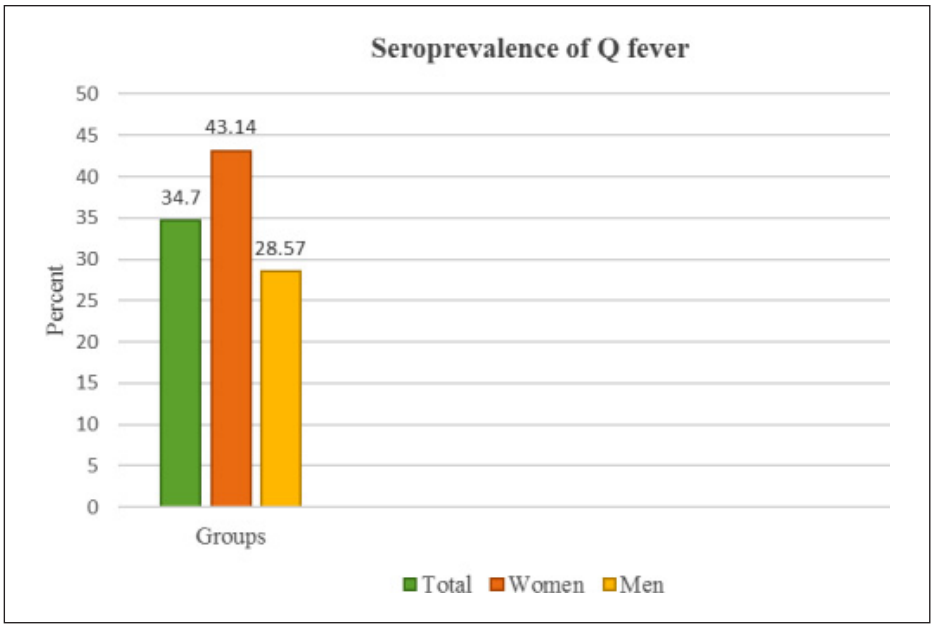

Chart 1. Seroprevalence of Q fever in veterinary students of Shahid Bahonar University of Kerman (2013).

Table 1. Correlation between age and sex with the seroprevalence of Q fever in students of veterinary Faculty of Shahid Bahonar University of Kerman (2013)

Risk factors	Odds Ratio	95% Confidence Interval	P-Value
Age	0.82	0.69 – 0.99	0.038
Sex			
Women	2.22	1.00 – 4.90	0.05
Men	–	1	–

DISCUSSION

Results obtained in this study, conducted for the first time in Iran on veterinary students, showed that the seroprevalence of Q fever was significant among students of Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman with more than one-third of the students having phase II antibodies against infection. Also, this infection was more significant in women and at younger age.

According to the study by Metanat *et al.* (2013), during which the serum samples taken from 105 patients with fever (52 men and 53 women) visiting Bou-Ali Hospital in city of Zahedan, Iran were tested by indirect immunofluorescences method, 35.2% of the subjects had positive serum titer against acute Q fever (Metanat *et al*., 2014). In 2013, Esmaeili *et al*. took 250 serum samples from hunters and their families, butchers, healthcare personnel, and laboratory personnel in Kurdistan Province, Iran and tested them in terms of Ig G (phases I, II) antibody against *C. burnetii* using indirect ELISA method. According to the results, 27.83% of the subjects had positive serum titer against *C. burnetii* with the highest level of seroprevalence of Q fever among butchers with 38% (Esmaeili *et al*., 2014). In another study by Gunal *et al*. (2011-2012) which was conducted in Tokat Province, Turkey, the serum samples of 53 patients with fever (37 men and 16 women from 18 to 65 years old) were tested in terms of Ig G and Ig M antibodies against *C. burnetii* using indirect ELISA method. According to the results, 19 patients (36%) had positive serum titer, among which two patients (4%) had acute Q fever. Also, based on the statistical analysis on the patients with positive and negative serum, no statistical difference was found in relation to age, contact with animals, occupation, place of residence, and rural life (Gunal *et al*., 2013). According to the study by Psaroulaki *et al*. (2006) in Cyprus, the seroprevalence of Q fever (antibody against phase II *C. burnetii*) was investigated in the serum samples of 583 subjects using indirect immunofluorescences (IFA) assay which showed that 52.7% of human samples were positive (Psaroulaki *et al*., 2006). In another study by Derooij *et al.* in the Netherlands, the serum samples from 674 veterinary students were tested for Ig G and Ig M (phases I, II) antibodies against *C. burnetii* using indirect ELISA method. According to the results, 126 students (18.7%) had lg G (phases I, II) antibody against *C. burnetii*, while the highest level of seroprevalence among the veterinary students in Spain, Brazil, California, and Ohio was reported to be within 10% - 40% (MT Derooij *et al*., 2014). Q fever has become a public health problem in the Netherlands since 2006, such that in 2009, Q fever was reported in 2357 human subjects and infection with this disease was continued to be observed until 2010 (Guatteo *et al*., 2011). In the study by Schimmer *et al.* (2009-2010), the serum samples of 268 workers selected from 111 dairy goat farms in the Netherlands were tested in terms of existence of Ig G and Ig M antibodies against Q fever agent using indirect immuno-fluorescences method. According to the results, seroprevalence among the farm workers, couples, and 12-17 years old children was reported as 73.5, 66.7, and 57.1%, respectively (Schimrner *et al*., 2014). In the study by Nielsen *et al.* (1996 - 2002) in Denmark, the seroprevalence among 856 women was reported as 19.7% using indirect immunofluorescences method, from among which 147 subjects were in contact with animals as a part of their profession (Nielsen *et al*., 2013). According to the study by Delcarmen *et al.* (1994-1995) in Spain, the serum samples of the students at Faculty of

Veterinary, University of Zaragoza, were tested by complement fixation method during two stages (in 1994–1995 academic year), which reported seroprevalence as 10.02 and 11.02%, respectively (Delcarmen *et al*., 2000).

CONCLUSION

Strains with different levels of *C. burnetii* acuity have been diagnosed in different parts of the world and epidemic face of the disease is strongly associated with the genotype of *C.burnetii* (Schimrner *et al.*, 2014). Genetic diversity of strains may indicate the fact that *C. burnetii* strains available in Iran have a lower level of acuity and therefore the high serological prevalence in this study and other relevant studies without any record of clinical samples is associated with acuity of common strains in Iran. Considering the results obtained in the present work, which indicated the high seroprevalnce of Q fever (antibody against phase II *C. burnetii*) among the students of Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman and also according to previous studies and serological evidence that Q fever is endemic in humans and animals in the region, it is necessary to take immediate actions against this epidemic disease and preventive measures such as training the ones at risk, vaccination, pest control and spraying in livestock, preventing transportation of suspected animals which have records of genital disease and miscarriage, and using mask in training centers and regions where this disease is widespread in order to prevent the transmission of infection to humans and other animals, especially through aerosols as a major way of bacterial transmission or at least to reduce the occurrence of this disease.

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Conflict of interest:
There is no conflict between the authors.

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