ORIGINAL ARTICLE

Investigation of zoonotic infections in risk groups in Ordu University Hospital, Turkey

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Abstract

Aims: Zoonotic diseases, which are a major public health problem in our city, have a negative impact on public health and also cause economic losses due to yield losses of animals and deaths. This study was carried out to determine the seroprevalence of tularemia, bartonellosis, brucellosis, Q fever, and cystic echinococcosis in the risk groups for zoonotic infection.

Subjects and Methods: Ninety serum samples were taken from people in the risk groups in covering veterinarian, butchers, farmers and examined with the following tests: Microagglutination test for tularemia, indirect fluorescent antibody test (IFAT) for bartonellosis, standard tube agglutination test for brucellosis, IFAT IgG for Q fever, and enzyme-linked immunosorbent assay IgG test for cystic hydatid.

Statistical Analysis Used: The Chi-square analysis was used to assess, and the logistic regression analysis was used to identify the risk factors.

Results: The analyzed all serum samples were found to be seronegative for tularemia, bartonellosis, and hydatid cyst antibodies. When analyzed for *Coxiella burnetii* with IgG antibody titers, it was determined that 23 samples (25.6%) were seropositivity. When brucellosis was analyzed with serological tests for *Brucella*, it was positive in seven samples (7.8%).

Conclusions: In this study, examined in the risk groups in which it is located along black sea coast of Turkey for tularemia, bartonellosis, and hydatid cysts, seropositivity was not found. When *Brucella* was tested, 7.8% was found to be positive, and when analyzed in terms of Q fever, 25.6% of people were determined to be seropositive. In conclusion, in our region, Q fever seropositivity was found to be higher in the risk groups. Therefore, most of the zoonotic disease look like not so common in the region, out of tularemia.

Key words: Bartonellosis, brucellosis, cystic echinococcosis, Q fever, seroprevalence, tularemia

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Introduction

Zoonotic diseases are infectious diseases that are transmitted between species from animals to humans. Q fever, tularemia, bartonellosis, brucellosis, and cystic echinococcosis are among the important zoonotic infections affecting both humans and animals. Q fever, an acute or chronic zoonotic illness caused by the bacterium *Coxiella burnetii*, has received international attention in recent years, primarily due to a large-scale outbreak in the Netherlands from 2007 to 2010 involving more than 4000 human cases and the euthanasia

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of 50,000 goats, one of the primary reservoirs for the bacterium.[1] The number of human Q fever cases reported annually to the Centers for Disease Control and Prevention has increased from 17 cases with the onset in 2000 to 167 cases with onset in 2008.[2] Approximately, 3% of the US adult general population is seropositive for *C. burnetii*. Goats, sheep, and cattle are the principle sources of human infection. The primary mode of transmission is inhalation of pathogen-contaminated aerosols from excreta, especially birth products. Parturient cats, kittens, dogs, and other animals have also been associated with human cases. *C. burnetii* is easily dispersed into the air and airborne transmission of the disease to people living over a mile from the animal sources has been reported. Humans are highly susceptible to *C. burnetii* infection, with an infectious dose of one to ten organisms. Person-to-person transmission occurs rarely. These factors make it difficult to define what constitutes an exposure to Q fever and to identify potential human cases during an investigation.[3]

Tularemia is a zoonotic infectious disease which shows a worldwide distribution and manifests with different clinical symptoms. The clinical picture in humans varies from fulminant to life-threatening pneumonia or septicemia.[4] The disease is transmitted by direct contact with tissues or body fluids of infected animals, arthropod bites, ingestion of contaminated food or water, or inhalation of aerosols.[5]

Bartonellosis is a zoonotic disease that is caused by *Bartonella* species, mostly *Bartonella henselae*, often seen in people with impaired immunity, characterized by angiomatous skin lesions, and may also be accompanied by systemic involvement.[6,7] It may cause cat scratch disease, bacillary angiomatosis, fever, endocarditis, neurological syndromes, carrion's disease, and trench fever in humans. Although cats are the natural reservoir of *B. henselae*, it has also been isolated from many animal species.[8] It should be considered in terms of risk factors, especially for those feeding pets (cat, dog, rabbit, etc.,) at home, working in the garden, farming, or involved with agriculture, and hunter.

Brucellosis is a zoonosis endemic in Turkey and is usually transmitted from raw milk and milk products.[9] This disease is common as an occupational disease in veterinarians, farmers, butchers, shepherds, and abattoir workers who are in direct contact with animals.[10] Brucellosis is an important public health problem that causes human deaths and serious economic losses all over the world as well as in Ordu, Turkey.[11] Human brucellosis causes difficulty for clinical diagnosis due to the variety of its clinical symptoms and being asymptomatic. In this case, diagnostic dilemma as patients could be asymptomatic or symptomatic with symptoms ranging. *Brucella* bacteria enter the human body through the skin, mucous membranes, conjunctiva, respiratory, and gastrointestinal tract. It is frequently seen in people who have direct contact with animals including professionals and laboratory workers.[9]

Cystic echinococcosis is a zoonotic disease that causes a major public health problem and leads to serious economic losses because of its prevalence in animals in our country.[12] The two most important species causing the disease in humans are *Echinococcus granulosus* and *Echinococcus multilocularis*. It is often transmitted to humans from infected dogs by the fecal-oral route when the eggs are ingested in contaminated food and water. The parasite eggs, settles in other organs, especially the liver and lungs causing cystic echinococcosis.[13]

Therefore, it is important to determine the incidence and prevalence of zoonotic infections both in humans and in animals. This study was carried out to determine the seroprevalence of the major/some zoonotic infections among at-risk groups in Ordu, which is located at the north area part of Turkey.

Subjects and Methods

In this research, purposeful sampling (risk groups; veterinarian, butchers, farmers, etc.,) and full-count technique were used to recruit participants in the selected study area. Ninety serum samples were taken from people, including 25 veterinarians, from the risk group patients from family medicine policlinics between September and December 2012. *Francisella tularensis* microagglutination test (MAT) for tularemia, *B. henselae* indirect fluorescent antibody test (IFAT) for bartonellosis, standard tube agglutination (STA) test for brucellosis, *C. burnetii* IFAT IgG for Q fever and enzyme-linked immunosorbent assay (ELISA) IgG test for cystic hydatid were completed.

For MAT two-fold serial dilutions (1/10–1/5120) of the serum, samples were prepared in the U-based microplate. Serum-free final pits were used for antigen control. An equal amount of coated antigen was added to it, and thus, 1/10 and 1/20480 dilutions of serum were obtained. The microplate was covered and incubated for 24 h at 37°C in a humid environment. Agglutination reaction was evaluated with the naked eye and reading mirror. The subsidence of antigen-antibody complex in the pits as "lace or umbrella" style and the supernatant being completely clear were considered positive; the presence of sediment as a smooth-edged button gathered in the center of the pit surrounded by diluent was considered negative.[14]

Brucella antibodies in serum samples were detected by the STA test.[15] The diagnosis of brucellosis was based on clinical findings (fever, sweating, muscle weakness, arthralgia, appetite loss, and weight loss) and positivity of a standard serum agglutination test titer of ≥1/160 as an indicator of *Brucella*.

IFA kit (C. *burnetii* IFA IgG, Vircell, Spain) was used to determine the IgG antibodies against C. *burnetii* Phase II. For IgG antibodies ≥1:16 titers was considered positive as an indicator of contact or infection.[16] Phase II IgG ≥1:64 titers was evaluated as positive in terms of acute Q fever. Hydatidosis ELISA IgG (Vircell SL, Spain) kit was used for the determination of antibodies against *E. granulosus*.[17]

Chi-square analysis (Fisher's exact test) was applied to analyze whether or the Q fever seropositivity statistically depends on gender, age, occupational group, time of work, and symptoms. Besides, the multiple logistic regression analysis was used to identify the risk factors that affect Q fever seropositivity. Descriptive statistics and qualitative variables are shown as the number of cases and (%). A $P < 0.05$ was considered statistically significant.

Table 1: *Coxiella burnetii* IgG antibody titers

	Total number	Prevalence
Negative	67	74.4
1/2048	1	1.1
1/1024	3	3.3
1/512	1	1.1
1/256	2	2.2
1/128	8	8.9
1/64	8	8.9
Total	90	100.0

The authors assert that all procedures contributing to this work comply and with necessary permissions from the provincial health directorates have been obtained.

Results

The analyzed 90 serum samples (76 male and 14 female) were found to be seronegative for tularemia, bartonellosis, and hydatid cyst antibodies. When analyzed for C. *burnetii* with IgG antibody titers, it was determined that 67 samples (74.4%) were seronegative, with 1 (1.1%) person: 1/2048, 3 people (3.3%) 1/1024, 1 person (1.1%) 1/512, 2 people (2.2%) 1/256, 8 people (8.9%) 1/128, and 8 people (8.9%) 1/64 titer seropositivity [Table 1]. When analyzed for brucellosis by using the serological tests for *Brucella*, seven samples (7.8%) were found to be positive.

Although the Q fever seropositivity was not statistically significant ($2 = 1.107$, $P = 0.243$) depending on gender, the odds ratio (OR) of Q fever in men was found to be 291 times more than for women. In spite of Q fever, seropositivity not being statistically significant ($\chi^2 = 3.365$, $P = 0.339$) depending on age. However, according to the results of the logistic regression analysis, compared with the people 30 years and below, the risk of Q fever seropositivity was found to be 4.308 times more for people between 31 and 40 years; the risk of Q fever was found to be 2.545 times more for people of 41–50 years; and the risk of Q fever

Table 2: Potential risk factors associated with Q fever seropositivity in the logistic regression equation

Variable	Number	Total number	Prevalence	Exp(B)	95% CI for Exp(B)	*P*
Sex						
Male	21	76	27.6	2.291	0.472-11.112	0.304
Female	2	14	14.3	-	-	
Age (years)						
≤30	2	16	12.5	-	-	-
31-40	8	21	38.1	4.308	0.769-24.143	0.097
41-50	8	30	26.7	2.545	0.471-13.770	0.278
≥51	5	23	21.7	1.944	0.327-11.558	0.465
Occupation						
Veterinary	7	27	25.9	-	-	-
Buthcer	4	20	20.0	0.714	0.177-2.877	0.636
Farmer	10	34	29.4	1.190	0.383-3.699	0.763
Hunter	2	9	22.2	0.816	0.136-4.898	0.824
Working years in the profession						
≤5	1	11	9.1	-	-	-
6-10	3	12	25.0	3.333	0.292-38.082	0.333
11-15	4	16	25.0	3.333	0.319-34.830	0.315
16-20	3	13	23.1	3.000	0.265-33.974	0.375
≥21	12	38	31.6	4.615	0.529-40.279	0.166
Symptoms						
No	12	51	23.5	-	-	-
Yes	11	39	28.2	1.277	0.493-3.306	0.615

The final model fit was tested using Hosmer-Lemeshow test. The Hosmer-Lemeshow statistic has a significance of 0.467 which means that it is not statistically significant, and, therefore, our model is quite a good fit. CI=Confidence interval

seropositivity was found to be 1.944 times more for people above the age of 51. In addition, the Q fever seropositivity was not statistically significant ($\chi^2 = 0.645$, $P = 0.886$) depending on profession. However, the results of the logistic regression analysis showed the risk of Q fever in butchers and hunters was lower than for veterinarians and farmers [Table 2]. Moreover, the Q fever seropositivity was not statistically significant ($\chi^2 = 2.339$, $P = 0.674$) depending on the time of work. However, according to the results of the logistic regression analysis, the OR of Q fever seropositivity was identified in people who have more than 5 years professional experience as approximately three-fold or more than the people who are new in their profession (≤5 years) [Table 2].

Discussion

Considering that the zoonotic infections constitute more than half of community-acquired infections, today animal-borne diseases constitute a serious threat to human health.[3]

Seroepidemiological studies conducted in different regions of Turkey have shown that although seroprevalence of brucellosis varies according to risk groups, it ranges from 2.9% to 33%.[18,19] In our study, seropositivity was detected at 7.8% in patients in the risk groups. Moreover, in studies in different regions, it has been reported at rates ranging from 20% to 33% in veterinarians, 6.2–25% in farmers, 2–5.7% in slaughterhouse and dairy employees, and 2.9–21% in butchers.[20] In the study by Kilic *et al.*,[20] while finding the seropositivity rate as 19% in veterinary and 4.6% in slaughterhouse workers, in all risk groups, the rate was identified as 7.2%. Seropositivity was detected at 7.8% in patients in the risk groups, and this data is consistent with seroprevalence in our country. The reason for this situation may be due to the conditions of animal breeding depending on the regional characteristics and prevalence rates in animals.

Tularemia, which spreads worldwide, can cause very different clinical manifestations in humans from asymptomatic cases to life-threatening septicemia. In recent years, tularemia has become a re-emerging infection in Turkey with epidemics and also sporadic cases.[21] In our country, cases have been reported from almost all regions especially in the Black Sea and Marmara Regions. Generally, it is seen in rural areas where the of animal husbandry is greater and hygienic conditions are not feasible; the disease is rarely seen in people who live in cities.[22] There have been some difficulties in determining the incidence of tularemia because it is not listed as a notifiable disease list in many countries. The disease is poorly recognized and therefore often missed, a portion of cases are not reported, especially in children and adults can be observed to have it in the form of unidentified moderate infections missed by clinicians or the disease is asymptomatic.[23] For all these reasons, the incidence of tularemia in the world is not fully known. A total of 431 tularemia cases were recorded in Turkey in 2005, but a significant reduction was observed in the number of the cases in the next 3 years; the number of patients decreased to 71 in 2008. The number of cases increased again in 2009 and this continued in subsequent years. The number of cases reached 428, 1531, 2151, and 607 in 2009, 2010, 2011, and 2012, respectively.[24] Despite the increasing number of tularemia cases, there have been no cases reported from Ordu in recent years. Therefore, in our study, seropositivity was not detected in any of the patients in the risk groups.

Because *Bartonella* usually causes silent infections which can be easily missed; therefore, both in our country and in the world there is not enough information about the seroprevalence of bartonellosis. Yilmaz *et al.*[7] in a study conducted in Denizli, the seroprevalence of *B. henselae* was found to be 6% in blood donors who were admitted to Pamukkale University Blood Center. Sporadic cases of *B. henselae* are present in our country, and there has been no further data found about seroprevalence of *B. henselae* and the situation in humans. In studies conducted in different countries, the seropositivity of *Bartonella* spp. in healthy individuals and in risk groups has been reported to be between 0.2% and 38.9%.[7,25-27] These different rates are thought to be due to the socioeconomic levels of society, living areas, the presence of animal reservoirs in the environment, climate, and hence there is an impact of climate on vectors and the differences in diagnostic methods. The insufficient data about seroprevalence of *B. henselae* in humans in our country is due to the fact that this bacterium may produce similar clinical findings to many diseases, can be transferred by vectors and our country having suitable climatic conditions for the vectors. The data related to *B. henselae* seroprevalence must be supported by studies from different regions to identify the risk factors for our country. However, in our study, seropositivity was not detected in any of the patients from the risk groups.

Cystic echinococcosis is seen in almost every region of our country and is reported to be seen widely in Marmara, in the West of Central Anatolia and especially in the Eastern Anatolia region.[28] Because the epidemiology of this disease is affected by the socioeconomic level of the population, the region's climate, animal care, and conditions of feeding, there are differences between regions.[29,30] In a study by Yazar *et al.*[30] conducted in Kayseri, they found the positivity was 2.7% with Western blot confirmation test positivity rate determined as 0.9%. Ozkol *et al.*[29] has reported that positivity detected in primary school students in Manisa was 4.3%. Kilic *et al.*[31] in their study in veterinarians determined seropositivity as 2.15% and they confirmed this by using Western blot. Çelebi *et al.*[32] in their study in Ankara determined seropositivity as 1.1% in veterinarians. In this study, we could not find any seropositivity. Although

cystic echinococcosis is a widespread zoonosis in Turkey, the low livestock population in our region could probably explain the zero seroprevalence of cystic echinococcosis.

Although Q fever is common all through the world, the variety of clinical pictures and limited laboratory diagnostic capacity hinders the determination of the true prevalence. In our country, in studies to determine the prevalence of Q fever in people in risk groups; Kılıc *et al.*[20] reported this rate as 28.6% in veterinarians working in Hatay province; Ozgur *et al.*[33] found seroprevalence of 26% in veterinarians in Istanbul, 80% in veterinary health technicians and 33.3% in veterinary students 33.3%. Çelebi *et al.*[32] in a study conducted in Ankara found that the rate of Q fever seropositivity was 30.6% in veterinarians working in pet clinics. A small number of studies have been completed in order to determine the prevalence of Q fever in risk groups in the world. In different studies conducted in the world on risk groups, antibodies developing against *C. burnetii* in veterinarians was found to be 20% in the UK, 25.7% in Switzerland, and 22.7% in Japan.[34,35] While the seropositivity rate of 25.6% that we found in our study was higher than the seroprevalence rates obtained from veterinarians in Aydın, Tokat, and Elazig provinces, it is observed that it is compatible with the studies conducted in Hatay, Istanbul, and Ankara and international studies. The reason for this difference between the studies can depend on variations of the carriage rate in animals, habitations of the animals, breeding conditions depending on the geography, the diagnostic methods, and the titers, which are used for the diagnosis of disease also used for the risk groups.

Conclusion

In our study, when people in the risk groups in our province are analyzed in terms of tularemia, bartonellosis, and cyst hydatid, seropositivity was not detected. In this study, it was determined that there was a high seroprevalence for Q fever and brucellosis in the risk groups. Brucellosis seropositivity was detected in 7.8% of patients in the risk groups. When analyzed in terms of Q fever, due to the presence of seropositivity in 25.6% of people, awareness needs to be raised for workers in the risk groups about zoonotic infections and further research needs to be done to discover the regional epidemiological data. Because Q fever seropositivity was determined at a higher rate in risk groups in our region, we think that it should be considered and investigated in some cases such as pregnancy, immune deficiency, and heart valve lesions, which prepare the ground for Q fever. This study suggests that people, especially those who are in close contact with animals, should be warned and informed about zoonotic infections. In addition, further studies should be performed to fully elucidate the epidemiology of the mentioned zoonotic infections in this region. Developing knowledge and approaches to zoonotic diseases will contribute to the creation of social awareness about these diseases.

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Conflicts of interest

There are no conflicts of interest.

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