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Seroepidemiology of coxiellosis in small ruminant populations of East Azarbaijan province, northwest of Iran

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Abstract

Coxiella burnetii is the cause of a common bacterial disease between humans and animals. The resulting disease from an infection with this agent is referred to as Q fever in humans and as coxillosis in animals. The present research was performed to pinpoint the seroprevalence of coxiellosis in small ruminant (goats and sheep) populations in the East Azarbaijan province, northwest of Iran. Blood samples taken from 184 small ruminants (164 sheep and 20 goats) in various rural parts of the East Azarbaijan province were analyzed using an indirect ELISA test. Sex, age, species, history of abortion, and geographical location were also recorded as risk factors. Out of a total of 184 serum samples analyzed for the presence of antibodies against *C. burnetii*, 39.7% (n = 73) appeared positive, 7% (n = 13) were doubtful, and 53.3% (n = 98) were negative. There were no statistically significant differences among the risk factors, except for sex and geographical area. The study revealed that a relatively high proportion of the tested small ruminants are seropositive to *C. burnetii*. Steps should be implemented to halt the transmission of the disease and reduce the zoonotic risk of *C. burnetii* in the region, taking into account the economic and public health significance of *C. burnetii* for both animals and humans.

Introduction

Coxiella burnetii (*C. burnetii*) is a gram-negative bacterium which goes under the Rickettsiaceae family leading to coxiellosis in animals and Q fever in humans. The initial case of Q fever in Iran was reported in 1952. Since then, human cases and reports of the serum prevalence of the illness in the human population have been reported from various parts of Iran (1). The Q fever has been largely neglected in Iran since 1976, and no reports of human cases and its outbreaks have been published. In 2009, antibodies against Q fever were reported in a patient in southeastern Iran (2). Later studies revealed that Q fever is categorized as an endemic illness in various parts of the country (3). *C. burnetii* results in spore-like structures in the environment and resists severe environmental conditions as well as chemical and physical tensions. The disease has

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been reported in all countries except New Zealand (4). The Center for Disease Control and Prevention (CDC) has categorized *C. burnetii* under group B pathogen, since it can be employed as a biological weapon due to its capability to spread over long distances in a short time. Furthermore, it has been considered as a zoonosis, therefore there can be various hosts for this bacterium. The presence *C. burnetti* has been reported in a vast group of animals including livestock, birds, and pets (5). Low rainfall conditions, as present in most parts of Iran, exacerbate the transfer of this agent. In addition, sandstorms blowing from Iran's western neighbors such as Kuwait and Iraq can also contribute to the transmission of *C. burnetii*. Respiratory transmission has also been reported in Kuwait and Iraq (6). Severe and chronic instances of the illness have recently been reported in Iran (1-3). Small ruminants can be considered as transmitters and reservoirs of the infection to humans, therefore determining the illness in ruminants is vital (7, 8). *C. burnetii* can lead to multiplication in lysosomal vacuoles in phagocytic cells. Moreover, this bacterium alters lipopolysaccharide (LPS) antigens during infection stages I and II (9). It can be observed in two morphological forms, the small cell variant (SCV) is the metabolically active form observed in the host cell (10). Usually, no clinical signs are observed in animals infected with *C. burnetii*. In small ruminants, *C. burnetii* appears to be one of the agents contributing to most abortion cases (11, 12). This pathogen usually results in reproductive problems such as abortion in late pregnancy, stillbirth, weak offspring birth, and premature birth in small ruminants and is also connected with infertility in cattle (13, 14). The aim of this study was to investigate the seroprevalence of coxiellosis in small ruminant populations of the East Azarbaijan province in the northwest of Iran.

Materials and Methods

Sample collection

The cross-sectional study was done from March to November 2023 in various rural areas of the East Azarbaijan province (including Maragheh, Bonab, Malekan, and Ajabshir cities). These cities are located in the northwest of Iran. Samples were taken from the jugular vein of 164 healthy sheep and 20 goats and after blood clotting, the sera were stored in the freezer at -40 °C. The risk factors, including sex, age, species, abortion record, and geographic location were also recorded for each sample.

ELISA test to detect the C. burnetii antibody

Serum samples were investigated to detect the *C. burnetii* antibody using ID wet ® Q fever indirect multispecies ELISA test kit (ID screen Q fever indirect multi-species ELISA kit, ID vet, France). An ELISA reader was utilized to determine the optic density values. The microplates were read in an ELISA plate reader at 450 nm. Each well's S/P percentage was estimated according to the manufacturer's instructions. Results were finally expressed as a percentage of the optical density of the test sample (%OD) calculated as below:

$S/P\% = (OD_{sample} - OD_{NC}/OD_{PC} - OD_{NC}) \times 100$

NC = Negative control

PC = Positive control

Samples were identified as positive cases if the calculated percentage was >60% (ID vet), and if <50%, the samples were considered negative. Samples calculated to be 50% or ≤ 60% were considered doubtful. The statistical analysis was performed using the IBM SPSS Statistics 25 software (SPSS 25 Chicago Company, USA) and frequency comparisons were executed Chi-square and Fisher's exact test. The difference between the groups was assessed by a Bonferroni supplementary test.

Results

According to the ELISA kit instructions, out of 184 sera analyzed for the presence of antibodies against *C. burnetii*, 39.7% (n = 73) of total cases were positive, 7% (n = 13) were doubtful, and 53.3% (n = 98) turned out to be negative (Table 1). Because of the scarcity of *C. burnetii* infection reports in small ruminants in the East Azarbaijan province, and due to the expression of caution, the doubtful

results were considered as negatives. Risk factors such as age, sex, species, abortion record, and geographic location were also taken into consideration. No statistically significant differences were reported concerning these risk factors, except for geographic area and sex.

Table 1- Risk factors for *C. burnetii* in 184 sheep and goats.

Risk factors	N (%) Positive (n = 73)	Negative (n = 111)	Total Positive (%)	Negative (%)	P-value
Sex					
Female	59 (44.4)[b]	74 (55.6)	32	40.2	0.043
Male	14 (27.5)[a]	37 (72.5)	7.7	20.1	
Species					
Sheep	64 (39)	100 (61)	34.8	55.4	0.606
Goat	9 (45)	11 (55)	4.8	6	
Age					
≤ 2	31 (35.6)	56 (64.6)	16.9	30.4	
2-4	41 (44)	52 (56)	22.3	28.3	0.097
> 4	1 (25)	3 (75)	0.5	1.6	
District					
Maragheh	19 (38)[ab]	31 (62)	10.3	16.8	
Bonab	9 (19.6)[a]	37 (80.4)	4.9	20.1	
Malekan	23 (53.5)[b]	20 (46.5)	12.5	10.9	0.005
Ajab shir	22 (48.9)[a]	23 (51.1)	12	12.5	
History of abortion					
With a history	4 (30.8)	9 (69.2)	-	-	0.385
No history	55 (45.8)	65 (54.2)	-	-	

a-b: In a given column, values with different superscript letters were considered to be significantly different ($p < 0.05$).

Discussion

Coxiella burnetii can result in an abortion rate of 3%-8% in sheep and goats (15). The recent outbreak of *C. burnetii* in the Netherlands also resulted in abortions of up to 60% of pregnant goats in late pregnancy. This pathogen resides in the pregnant dairy animal's placenta and mammary glands. In sheep and goats, the shedding of this bacterium through delivery secretions is considerable (14, 16). Having been approved by the European Food Safety Authority (ESFA), the ELISA method is more sensitive and specific compared to serological methods. This technique is usually preferable over IFA and CFT methods in animals due to its convenience in herd-level screening and its ability to distinguish antibodies of *C. burnetii* (17, 18). The ELISA procedure seems more accurate when employing ruminant antigens compared to tick antigens, and is the recommended test by the ESFA for the ruminants' *C. burnetii* antigen. This method is also able to recognize antibodies against both antigenic *C. burnetii* phases, and the results are either positive, suspected, or seronegative (18, 19). In the present research, the ELISA method was utilized along with the ID vet ® Q fever indirect multi-species ELISA test kit to detect *C. burnetii* antibodies. 39.7% (n = 73) of the total samples were positive. The seroprevalence of *C. burnetii* was estimated at 34.8% for sheep and 4.8% for goat populations. Furthermore, the results revealed that there is a significant difference in the results when associating them with the risk factors of sex and geographic region. This research was the first serology study concerning coxiellosis in small ruminant populations in the East Azarbaijan province. Esmaeili et al. (2019) reported *C. burnetii*

antibody presence in both sheep and goat populations in the Qom province (central Iran) at 35.71% (20). Nokhodian et al (2017) found that the seroprevalence of *C. burnetii* in sheep and goat populations in the Chaharmahal -va- Bakhtiyari province was 2.54% and 2.6%, respectively (21). Lorestani et al (2016) also showed that the seroprevalence of *C. burnetii* in sheep populations in the Lorestan province was 15% (22). Keyvani Rad et al (2014) reported the seroprevalence of *C. burnetii* at 29.8% for goat and 36.5% for sheep populations in the Khorasan Razavi province (northeast of Iran). The age of the animals and geographic location of sampling revealed a statistically significant difference in both species regarding the seroprevalence of *C. burnetii* ($p < 0.05$) (23). Ezatkhah et al (2015) showed that of the animals tested, 33.9% of sheep and 22.4% of goats had antibodies to *C. burnetii* in the southeast of Iran (24). Also, two serology studies performed in Turkey (western neighbor of Iran) in the years 2000 and 2010 revealed that 10.5% and 20% of the sheep were positive, while 44.7% and 81% of the flocks showed at least one positive case, respectively (25, 26). 52% of the goats were reported infected in Oman located in the south of Iran (27). Even though no information, exists regarding the serological outbreaks of the antibody against *C. burnetii* in domestic animals in Afghanistan (eastern neighbor of Iran), infection with *C. burnetii* among US soldiers deployed to Afghanistan proved the existence of *C. burnetii* in this region (28-30). The studied area shares a border with Afghanistan, and both countries sheep and goat flocks cross the border to foraging. Moreover, meat prices are more expensive in Iran compared with Afghanistan which encourages people to import animals illegally. The most crucial contributing factors to the various declarations of *C. burnetii* outbreaks in dairy products worldwide are differences in the weather and environmental conditions of the regions under study, the study method and type, sample types, and the season when sampling occurs. The higher occurrences of the *C. burnetii* antibodies can be due to the difference in weather conditions and types of small ruminant flocks' husbandry. The majority of the East Azarbaijan province is semi-arid, and this may facilitate the dispersion of aerosols. Furthermore, small ruminant flocks accommodated in an enclosed space during the night, can initiate and enhance the transmission of pathogenic agents. The results obtained from this research also revealed that coxiellosis seems endemic in the studied region and has spread throughout this province. Identification of the ratio of seropositive animals, the determination of risk factors of seropositivity, and the dissemination of this information can aid authorities take crucial steps. This may imply that the presence of coxiellosis disease can circulate among farm animals, and can be one of the most important zoonotic bacterial diseases for human populations in this province.

Conclusion

Based on the previously obtained results, coxiellosis is currently considered an endemic disease in various areas of Iran. Because of limited and insufficient studies, this disease is not considered or mistaken for other febrile diseases such as influenza and brucellosis. In the absence of proper management of the disease (including livestock vaccination, control of livestock entry and exit, enhanced monitoring of the production and distribution of dairy products, and the training of livestock farmers for disease control) in the country's livestock population, extensive damage to the country's livestock and human population is expected due to this pathogenic agent.

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Conflict of interest

There is no conflict of interest.

Ethical approval

Not applicable.

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