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Seroepidemiological feature of *Chlamydia abortus* in sheep and goat population located in northeastern Iran

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Abstract

Chlamydia abortus is a Gram-negative intracellular bacteria responsible for major economic losses due mainly to infection and subsequent induction of abortion in several animal species and poses considerable public health problems in humans. This study was conducted to determine the prevalence of antibody against *C. abortus* in sheep and goat population of Khorasan Razavi province located in northeastern Iran. Four hundred fifty-two (271 sheep and 181 goats) sera samples from 40 sheep/goat epidemiologic units located in 11 counties were selected. Sera were assayed for antibodies against *C. abortus* using ELISA assay. Out of 452 sheep and goat sera, 44 [9.70% (95.00%CI: 7.10%-12.40%)] were positive for *C. abortus* antibodies. 28 out of 40 epidemiologic units (70.00%) and 10 out of 11 counties (91.00%), at least one seropositive sample was found. There was no significant difference between the seropositivity of sheep and goats. Age, sex, and location did not show significant relationship with the test results. The results showed that *C. abortus* was circulating in wide parts of Khorasan Razavi province. Considering the economic and public health importance of *C. abortus*, measures should be taken to help prevent its spread and to reduce the zoonotic risk of *C. abortus* in the studied region.

Introduction

Chlamydia abortus, a Gram-negative intracellular agent from the family of Chlamydiaceae, has been recognized since 1950 as the species responsible for enzootic abortion in ruminants. The bacterium has worldwide distribution. It induces enzootic abortions during the late stages of pregnancy or premature birth of weak animals in ruminants.[1,2] It has been introduced as the most common cause of abortion in sheep and goats in many parts of the world.[3-5] Infection is transmitted orally following exposure of susceptible animals to high levels of infected uterine discharge, aborted material, or contaminated neonates. Venereal transmission of *C. abortus* is uncommon, however, may occur in certain situations.[6,7]

Following C. *abortus*-induced abortion, ewes develop cell-mediated and humoral immunity that prevents abortions in subsequent pregnancies. However, immunity does not eliminate infectious agents. The disease can become chronic and infected animals may excrete the bacteria intermittently for up to three years, increasing the spread of disease.[1,8]

Chlamydia abortus is also recognized as a zoonotic pathogen. Human infection may be acquired from infected products of abortion or parturition or carelessly handled laboratory cultures of the organism. It can cause subclinical infection to acute influenza-like illnesses in humans. Furthermore, documented cases of human placentitis and abortion caused by *C. abortus* of ovine origin, indicate that pregnant women were at serious risk of *C. abortus* infection because the organism was able to colonize in human placenta, causing abortion, stillbirth and maternal illness.[9-12]

The only study which investigated the seroprevalence of *C. abortus* in Iran showed a serologic feature of infection in the west of Iran.[12] Seroprevalence of the bacterium is unknown in the other parts of the country. Considering the economic and public health importance of *C. abortus,* the present study was conducted to determine the seroprevalence of *C. abortus* in sheep and goat population in the northeast of Iran.

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Materials and Methods

Study area. A cross-sectional study was undertaken in Khorasan Razavi province located in north-eastern Iran. Sheep and goat rearing is common in the rural area of the province and it is one of the important sources of income. More than four million sheep and goats have been enumerated in this province based on the agriculture census of 2013. Sheep and goats are routinely kept together. Each rural area with sheep and goat population was registered as a "sheep/goat epidemiologic unit" in the veterinary administration of Khorasan Razavi province.

Sampling procedure. Sample size calculated for the expected *C. abortus* seroprevalence of 25.00%[12] at the absolute precision of 5.00% for a 95.00% confidence level, which was 289. We selected 452 (271 sheep and 181 goats) samples using a cluster random sampling method which was much more than the calculated sample size.

In the first step, out of 28 counties of Khorasan Razavi province, 11 counties were selected randomly. Then from the list of sheep/goat epidemiologic units of the selected counties registered in the province veterinary head office, 40 random units were selected (3-5 units for each county). Finally, 6-18 sheep and goats sera samples were collected from selected units. The proportion of samples collected per counties was in line with the proportion of the sheep/goat population in the respective counties. Sampling was performed from February to April 2017. About 10.00 mL blood samples were collected from the animals' jugular vein. For each animal, location, sex, and age category (under 2, 2-3, and more than 3 years old) were recorded. The age of sheep and goats was recorded as the owners claimed. Samples were transported on ice to the laboratory of Khorasan Razavi head office. They were centrifuged at 1,800 *g* at 4.00 ˚C for 10 minutes to obtain the serum. Sera were stored in the labeled vials at –20.00 ˚C until testing.

Serology. The presence of a specific IgG antibody against *C. abortus* was assayed by an indirect ELISA kit (ID-Vet, Grabels, France). According to the manufacturer's declaration, the sensitivity and specificity of the ELISA kit were 95.00 and 100%, respectively. Positive and negative control sera were provided by the manufacturer. After performing all stages of the test as described by the manufacturer, the optical density (OD) of all wells was read by an ELISA plate reader at 450 nm.

Interpretation of the test result for each sample was performed according to the following formula:

$$OD\ index = \frac{OD\ sample}{OD\ positive\ control} \times 100$$

Sera samples with values greater than 60.00% were considered as positive. An epidemiological unit was considered positive where at least one of the selected animals from the unit was positive.

Statistical analysis. Seroprevalence of antibody against *C. abortus* concerning sex, location, and age categories was reported. Univariate Chi-square test and multivariate logistic regression tests were used to assess the relation of age, sex, and location with seropositivity. All statistical procedures were performed using SPSS (version 21.0; IBM Corp., Armonk, USA)

Results

Forty-four out of 452 sheep and goats samples were positive. Animal-level seroprevalence was 9.70% (95%CI: 7.10-12.40). In 28 out of 40 epidemiologic units (70.00%) and 10 out of 11 counties (91.00%), at least one seropositive sample was found. The proportion of seropositivity at an animal level in the studied epidemiologic units was ranged from 0 to 40.00%. The seroprevalence at an animal level in counties that were investigated in the present study varied from 0 to 13.30% (Fig. 1). The proportion of seropositivity concerning independent variables including sex, county of origin, and age categories are presented in Table 1. The test results did not show any significant relationship with sex ($p > 0.05$), counties of origin ($p > 0.05$) and age categories ($p > 0.05$) in the univariate analysis.

All independent variables entered into the logistic regression model. None of the independent variables showed a significant relationship with seropositivity in the multivariate model.

Seroprevalence in sheep (10.30%; 95.00%CI: 6.70-13.90) and goats (8.80%; 95.00%CI: 4.70-12.90) were not statistically different ($p > 0.05$).

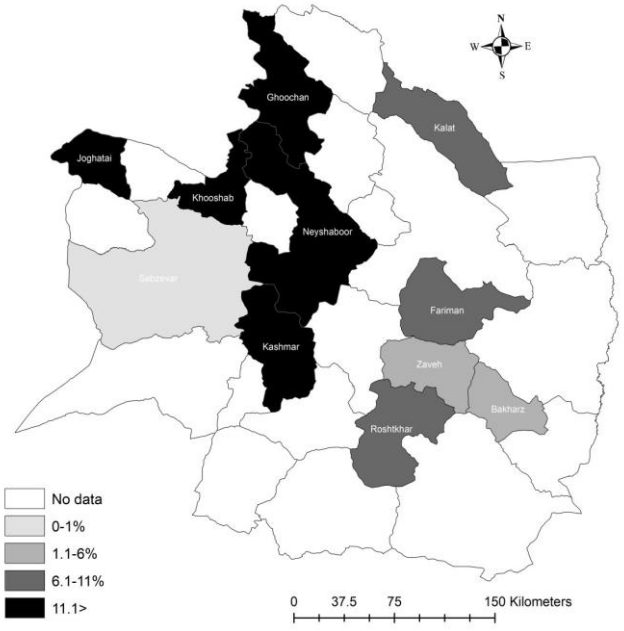

Fig. 1. Chropletic map showing animal level seroprevalence of *C. abortus* in studied counties of Khorasan Razavi province.

Table 1. Animal level seroprevalence of antibody to *Chlamydophila abortus* concerning sex, age, and district for sheep and goat population of Khorasan Razavi provinces, northeast Iran.

Variables	Levels	No.	Positive No. (%)	*p*-value*
Sex	Male	101	8(7.90)	0.48
	Female	351	36(10.30)	
Age	< 2	139	16(11.50)	0.55
	2 - 3	198	16(8.10)	
	> 3	115	12(10.40)	
County	Ghoochan	32	4(12.50)	0.56
	Kalat	47	5(10.60)	
	Zaveh	35	2(5.70)	
	Kashmar	30	4(13.30)	
	Neyshaboor	92	12(13.00)	
	Fariman	43	4(9.30)	
	Roshtkhar	38	4(10.50)	
	Sabzevar	37	0(0.00)	
	Khooshab	22	3(13.60)	
	Joghatai	44	5(11.40)	
	Bakharz	32	1(3.10)	
Total		452	44(9.70)	

*Univariate analyses (Chi-square test).

Discussion

The results of the present study provided a general insight of the seroprevalence of *C. abortus* in small ruminant population located in northeastern Iran. The presence of antibodies against *C. abortus* showed a widespread distribution of this zoonotic pathogen. We found 91.00% of counties and 70.00% of epidemiologic units of the study area exposed and affected by the *C. abortus*.

The apparent and real seroprevalence at animal level was 9.70 and 10.20%, respectively.[13] Considering the duration of persistence of antibody in exposed animal and average longevity of sheep and goats in the area,3-4 years, annual incidence risk of *C. abortus* infection according to the relationship between prevalence and incidence indices (prevalence ≈ incidence × duration of disease), would be about 3.00%.[13] Substantial role of the bacterium in the induction of sheep/goat abortion has been confirmed in several investigations.[3-5,14] Thus, concerning a large number of sheep and goat population in Khorasan Razavi province, economic losses due to an abortion caused by *C. abortus* is considerable. Furthermore, there are several reports of human infection by *C. abortus* in different parts of the world.[9-11] Therefore, there is a risk of infection for the human population in this region, especially for women in rural area who are routinely at risk of exposure with animal reservoirs.

In comparison with other serological surveys on sheep and goats of western Iran that reported a seroprevalence of antibodies against *C. abortus* as 26.00%, seroprevalence in the north-east is lower (9.70%).[12] In the west of the country, the density of sheep and the goat population is higher and the nomadic system is more common than the studied area. Thus, the chance of exposure to contaminated material or grazing in the contaminated pasture seems to be greater.

Seroprevalence of antibody against *C. abortus* in small ruminants has been reported in other countries, ranging from 4.80% in Italy to 11.70% in the Slovak Republic, 21.50% in Brazil and 21.80% in Jordan.[15-18] Comparison of seroprevalence of the present study with the mentioned studies is not logical because of differences in study design, inclusion criteria, management, rearing system, and detection methods applied.

Although the proportion of the positive sample in female sheep and goats was higher than male animals, sex was not associated significantly with the chance of seropositivity. Also, the seroprevalence of antibodies against *C. abortus* was not statistically different among age categories. Similar results were reported by McCauley *et al*. who studied on seroprevalence of *C. abortus* in Australian sheep and Cubero-Pablo *et al*. who reported seroepidemiology of chlamydial infection of wild ruminants in Spain.[19,20]

Seroprevalence of *C. abortus* in the mountainous semi-arid region of the studied area with a greater density of sheep and goat population located in the north and center of the province was higher than the arid region of south and east, however, differences were not statistically significant.

All these results indicated that *C. abortus* is endemic and circulating in wide parts of Khorasan Razavi province, like other parts of the country. It is therefore important to watch out suspect animals and perform monitoring to control the spread of the disease between animals. The study region has a shared border with Afghanistan and Turkmenistan where farm animals cross the border illegally. There is no documented information on sheep/goat chlamydiosis in these countries, however, accurate inspection of animal transport would help control the spread of infection.

Also, ovine chlamydiosis is an important zoonotic agent, affecting pregnant women, even with indirect contact with infected sheep or goats, principally in rural areas, and especially when simple sanitary rules were not correctly followed. Therefore, awareness of women in rural area who are in close contact with domestic small ruminants is necessary to reduce the chance of transmission from animals to humans. Furthermore, the determination of the cause of abortion in women must be taken into account to denote the importance of the agent in human abortion.

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Conflict of interest

The authors declare that they have no conflict of interest.

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