

Seroprevalence of *Chlamydia abortus* infection in sheep and goats in Iran

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Abstract:

BACKGROUND: *Chlamydia abortus* is a zoonotic bacterium that commonly causes abortions in ruminants. This microorganism is one of the most important infectious agents causing abortion and major economic losses in sheep and goats worldwide. **OBJECTIVES:** The objective of this study was investigating the status of *C. abortus* infection among small ruminant flocks of some regions in Iran. **METHODS:** A total of 1440 sera samples from sheep and goats were collected from 113 flocks of 7 provinces and tested with CHEKIT[®]-ELISA for antibodies against *C. abortus*. **RESULTS:** The study detected overall seroprevalence levels of 25.6% for the individual animals, and 81.4% flocks had at least one positive animal. Analysis of different sheep groups based on their age revealed that the highest numbers of infected animals were registered in the 2 years age group ($p < 0.05$). **CONCLUSIONS:** These results indicate that the seroprevalence of *C. abortus* infection in sheep and goats is very high in Iran. Therefore, it is necessary that Iran's veterinary organization set up appropriate surveillance and control programs to reduce economic losses of this disease.

Introduction

Abortion has been a major economic loss for domesticated ruminants worldwide. *Chlamydia abortus* (*C. abortus*) is the obligate intracellular bacterium which affects small ruminant flocks resulting in late-gestation abortion or the birth of weak or dead lambs. The environment would be contaminated by infected placenta and uterine discharges; thereby, the susceptible animal will most probably contract the disease through exposure of these infected materials at this time. In non-pregnant animals, *C. abortus* causes a subclinical infection due to existence in lymphoid tissue where it remains until the beginning of pregnancy (Aitken, 2007).

In addition, the bacterium is known to have zoonotic potential. Pregnant women may have abortions following exposure to animals infected with *C. abortus* (Buxton, 1986).

There are many reports about sheep chlamydiosis status throughout the world. According to Borel's report, 19% of sheep flocks in Switzerland had antibodies against *C. abortus* (Borel et al., 2004). There is one study which revealed that there is around 10 to 20 million pounds economic loss due to ovine enzootic abortion (OEA) in UK annually (Aitken et al., 1990). The Results of Northern Namibia research revealed that the overall seroprevalence level is 25% for the farms and 8% for the individual animals (Samkange et al., 2010).

Despite the economically important losses

due to abortion and foetal loss in sheep and goats, the prevalence of *C. abortus* is not well known in Iran (Ghorbanpoor et al., 2007). The aim of the present study was to estimate the seroprevalence of *C. abortus* infection in small ruminant flocks in Iran.

Materials and Methods

Samples: Serum samples were collected from 1440 sheep and goats in 113 flocks of 7 provinces that were very important in small ruminants breeding. For this purpose, using geographic information system of veterinary organization, >1000 sheep and goat flocks were randomly selected, and all samples were stored at -80°C until testing.

ELISA procedure: The separated sera were tested for IgG against *Chlamydia abortus* by using the Indirect ELISA kit, CHEK-IT®-CHLAMYDIA (IDEXX Laboratories B.V., 1119 NE Schiphol-Rijk, Nederland).

Briefly, test serum and control samples were diluted in CHEKIT wash solution at 1:400 in the wells of microtitre plates that were pre-coated with *C. abortus* antigen.

The plates were then incubated for 60 min at 37°C in a humid chamber and washed. After washing, 100 µL of CHEKIT anti-ruminant immunoglobulin G (IgG) horseradish peroxidase conjugate was added to each well and incubated as stated above. Each well was washed again, and 100 µL of CHEKIT tetramethylbenzidine substrate was added and incubated for 15 min at room temperature. The reaction was stopped with CHEKIT-Stopping Solution and absorbance was measured at 450 nm. The results were calculated using the following formula:

$$PP = \frac{(OD_{\text{sample}} - OD_{\text{neg control}})}{(OD_{\text{pos control}} - OD_{\text{neg control}})} \times 100 \text{ [Eqn 1]}$$

According to guidelines given by the manufacturer, sera with a PP values greater than or equal to 40% was considered positive.

Statistical analysis: The chi-squared (χ^2)

test (SPSS for Windows: Release 16.0 standard version, SPSS Inc., Chicago, IL) was used to compare the seroprevalence of *C. abortus* in relation to the age, species, province, and sex of the animals tested. The differences were considered statistically significant when $p < 0.05$.

Results

Of 1440 animals, 368 (prevalence 25.6% 95% CI= 31, 16) were positive and 92 (81.4%) flocks had least one positive animal (Table 1).

The prevalence of *C. abortus* antibodies among females (26.8%) was significantly higher than males (21.2%) and seropositivity in 2 years old animals was higher than 4 years old ($p < 0.05$). (Table 2). There was no significant difference between the prevalence of *C. abortus* antibodies in sheep and goats, but among goats, infection in does was significantly higher than bucks ($p < 0.01$).

There was history of abortion in 81.4% seropositive females and 13.2% of aborted females had at least two pregnancy losses. Seventy one percentages of female animals had abortion history which 26.1% of them were seropositive. The seroprevalence in ewes and rams were 26.5% and 27.4%, respectively, and statistical analysis showed that there was no significant difference ($p > 0.05$)

Discussion

This study demonstrates the first large-scale

Table 1. Seroprevalence of *C. abortus* infection in sheep and goats in Iran.

		Number/ overall	Prevalence (%)	Total
Species	sheep	218/816	26.7	368/1440 (25.6)
	goat	150/624	24	
Gender	Ram	55/201	27.4	368/1440 (25.6)
	Ewe	163/615	26.5	
	Buck	14/124	11.3	
	Doe	136/500	27.2	

Table 2. Seroprevalence of *C.abortus* infection in small ruminant flocks according to age and province in Iran.

PROVINCE	Tehran	Lorestan	Qom	Fars	Bushehr	West Azerbaijan	Khuzestan	TOTAL
Number/overall	29/77	96/292	47/155	84/277	37/189	44/251	31/199	368/1440
(Prevalence %)	(37.7)	(32.9)	(30.3)	(30.3)	(19.6)	(17.5)	(15.6)	(25.6)
AGE(year)	<1	1-2*	2-3	3-4*	4-5	5-6	6-7	
Number/overall	38/233	72/229	66/250	115/405	39/177	23/91	15/55	368/1440
(Prevalence %)	(16.3)	(31.4)	(26.4)	(28.4)	(22)	(25.3)	(27.3)	(25.6)

seroepidemiological investigation on *C.abortus* in Iran's small ruminant flocks. The previous studies performed in abortion agents of Iranian livestock had focused on aborted fetuses that were submitted to the laboratories for further examination. Since Chlamydia isolation is difficult, laboratories ignore it in their investigations, and there is not much information about the role of this organism in sheep and goat abortions in Iran.

Previous work in Iran by Ghorbanpoor et al. indicated that *C.abortus* infections were prevalent in sheep flocks of Khuzestan province, and they reported 9% of the sheep had antibodies against *C.abortus* (Ghorbanpoor et al., 2007). The high rate (25.6%) of seropositivity in this study is in agreement with other studies which demonstrate prevalence of *C.abortus* in different countries. The survey results of Jiménez-Estrada et al. showed that the seropositive rate was 31.1% for healthy sheep in Mexico (Jiménez-Estrada et al., 2008). Pinheiro Junior et al. revealed that 21.5% of sheep in the Brazilian state of Alagoas had antibodies of *C.abortus*. They reported that 77.7% of the flocks had at least one seropositive animal (Pinheiro Junior et al., 2010). Another study found that the prevalence of chlamydial antibodies in Tibetan sheep was 20.9% (Huang et al., 2013).

The seroprevalence in sheep and goats were 26.7% and 24%, respectively, and the statistical analysis showed that there was no significant difference ($p>0.05$), suggesting that *C.abortus* infection had no relationship with species.

The document of experimental chlamydial

infections in male genital system of ruminants represents the probability of venereal transmission. Teankum et al. found chlamydiae in semen of bulls, and 34.8% of rams in their survey were seropositive using cELISA (Teankum et al., 2007).

Of a total of 325 male samples from small ruminants that were tested, we could detect antibodies in 21.2% of them. It is very important in flock situation in Iran because there is a traditional belief among farmers in some areas that the probability of female lamb birth will increase if they keep old ram (more than 5 years old). This can cause long maintenance of the infected rams and the spread of infection among the flock. A big problem about slaughtering of ram and buck is the emotional attachment of farmers to them. Sometimes in test and slaughter program, the main rams or bucks of flocks should be culled; however, the farmers or their children prevent slaughtering. This is important since farmers keep the infected animals for a long time, and the animals might spread the infection among the flock.

The association between abortion history and presence of anti-*C.abortus* antibodies was significant in this study. It is because of this fact that when non-pregnant sheep are infected, protective immunity does not develop, and thus infection in these animals leads to abortion (Entrican and Wheelhouse, 2006; Rocchi et al., 2009).

The results showed that 81.4% of the flocks had at least one positive animal, which shows that these flocks are infected and chlamydial infection is widespread in the region.

According to our results, there was a high-

er seroprevalence in 2 and 4 year old animals. Where ewes become infected before 110 days of gestation, they are expected to abort in that pregnancy (Aitken, 2007; Longbottom and Coulter, 2007). Since in endemic areas animals of any age are likely to become infected, it is expected that most abortions occur in the first pregnancy (Entrican et al., 2001). Animals that have aborted due to *C. abortus*, develop protective immunity for about 3 years; therefore, in this situation next abortion will happen in the third or fourth pregnancy.

In spite of the hygienic limitations in traditional breeding system of small ruminants in Iran, our data have clearly noticed a need for more farmer education and sharpness about chlamydiosis. Implementation of control procedures for this disease is very important not only because of its zoonotic importance, but also because of its detrimental impact on economic losses in small ruminants breeding.

In conclusion, the seroprevalence of *C. abortus* in Iran's sheep and goat flocks is high; therefore, it is necessary for veterinary organization to establish suitable epidemiological surveillance to control this disease.

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شیوع سرمی عفونت کلامیدیا آبور توس در گوسفند و بزهای ایران

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چکیده

زمینه مطالعه: کلامیدیا آبور توس یکی از عفونت‌های باکتریایی مشترک انسان و دام است که باعث سقط جنین در نشخوارکنندگان کوچک می‌شود. این میکروارگانیسم یکی از عوامل عمده ایجاد سقط جنین و در نتیجه وارد آوردن خسارت اقتصادی در صنعت پرورش گوسفند و بز در دنیا می‌باشد. **هدف:** هدف از این مطالعه بررسی وضعیت آلودگی به کلامیدیا آبور توس در گله‌های گوسفند و بز برخی مناطق ایران بوده است. **روش کار:** تعداد ۱۴۴۰ نمونه سرم از ۱۱۳ گله گوسفند و بز متعلق به ۷ استان ایران جمع‌آوری شد و با استفاده از کیت CHEKIT[®]-ELISA جهت حضور پادتن علیه جرم کلامیدیا آبور توس ارزیابی شدند. **نتایج:** نتایج حاکی از آلودگی ۲۵/۶ درصدی دام‌ها به این جرم بود و ۸۱/۴٪ گله‌ها دارای حداقل یک دام مثبت بودند. تجزیه آماری نتایج نشان داد که دام‌های ۲ ساله دارای بیشترین آلودگی بودند ($p < 0/05$). **نتیجه‌گیری نهایی:** نتایج این مطالعه نشان داد که آلودگی گله‌ها در ایران با کلامیدیا آبور توس بالا بوده و لازم است که سازمان دامپزشکی کشور برنامه مراقبت و کنترل برای جلوگیری از خسارات ناشی از این بیماری در گله‌ها طراحی نماید.

واژه‌های کلیدی: کلامیدیا آبور توس، شیوع سرمی، نشخوارکننده کوچک

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