Chlamydia abortus infection in goats in the southwest of Iran

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SUMMARY

Chlamydia abortus that causes abortion and fetal death in mammals, including goats and sheep, has major economic and general hygiene effects worldwide. This study intended to investigate the prevalence of Chlamydia abortus infection and its associated risk factors in goat herds in Khuzestan province, in the southwest of Iran. To that end, 368 goat serum samples were randomly collected from different cities of Khuzestan province (Susangerd 49, Izeh 60, Hendijan 80, Dezful 58, Shoushtar 60 and Ahvaz 61) and were evaluated by ELISA assay. The seroprevalence rate of Chlamydia abortus was found to be 5.71% (95CI; 3.34–8.08%). Logistic regression showed that the odds of infection between age groups based on year and infection was 0.88 (95CI; 0.64–1.2) (P>0.05). The findings also revealed that the odds of infection in the female goats increased to 1.29 (95CI; 0.17–10.07) (P<0.05) compared to that in males. In comparison to goats without any history of abortion, the odds of infection in goats with a history of abortion was 1.74 (95CI; 0.37–8.04). The relative frequency of infection in the cities of Shoushtar, Izeh, Dezful, Hendijan, Ahvaz and Susangerd were found to be 6.67, 3.33, 5.17, 5, 13.11 and zero percent, respectively (P>0.05). This study confirmed the presence of Chlamydia abortus in the goat herds of Khuzestan province. Accordingly, preventive and controlling measures need to be considered by the health authorities and goat owners so that to reduce the infection's harmful effects.

Keywords: Chlamydia abortus, Epidemiology, Goat, Serology

RÉSUMÉ

Infection par Chlamydia abortus chez des chèvres dans le sud-ouest de l'Iran

Chlamydia abortus provoque la perte et la mort du foetus chez les mammifères. Cette maladie s'observe chez les chèvres et les moutons, ses conséquences économiques et sanitaires sont très importantes. Cette étude vise à analyser la prévalence de Chlamydia abortus et les risques des facteurs d'infection qui y sont liés sur les troupeaux de chèvres dans la province du Khuzestan dans le sud-ouest de l'Iran. À cette fin, 368 échantillons de sérum de chèvres ont été prélevés au hasard dans les différentes villes de la province du Khuzestan dans les départements: Susangerd 49, Izeh 60, Hendijan 80, Dezful 58, Shoushtar 60 et Ahvaz 61. Les échantillons ont été évalués par le test ELISA. Le taux de séroprévalence de la Chlamydia abortus est de 5.71% (95CI; 3,34 – 8,08%). La régression logistique a montré que les risques d'infection entre les groupes d'âges en fonction de l'année et de l'infection était 0.88 (95CI; 0.64 – 1.2) (P>0.05). Les études chez les chèvres ont également montré que les risques d'infection chez les femelles sont 1.29 (95CI; 0.17 – 10.07) (P>0.05) fois supérieurs aux mâles. Les chèvres ayant déjà connu une perte de leur foetus présentent un risque d'infection à la Chlamydia abortus qui est 1.74 (95CI; 0.37 – 8.04) fois plus élevé. La fréquence relative de l'infection dans les villes de Shoushtar, Izeh, Dezful, Hendijan, Ahvaz et Susangerd est respectivement de l'ordre de 6,67, 3,33, 5,17, 5, 13,11 et de zéro pourcent (P>0.05). Cette étude confirme la présence de la Chlamydia abortus dans les troupeaux de chèvres de la province du Khuzestan. Des mesures de prévention et de contrôle sanitaire doivent être prises par les autorités compétentes du pays et les propriétaires de chèvres afin de réduire les effets néfastes de cette infection.

Mots-clés: Chlamydia abortus, épidémiologie, chèvre, sérologie

Introduction

The family Chlamydiaceae contains obligate intracellular gram-negative bacteria, with nine species related to Chlamydia genus including: C. trachomatis, C. suis, C. muridarum, C. abortus, C. caviae, C. felis, C. pecorum, C. pneumoniae and C. psittaci [13, 23, 30]. Abortion and birth of weak lambs in affected animals by Chlamydia abortus is one of the traditional hygiene problems in many sheep-rearing countries of the world. Chlamydia abortus strains are widespread among ruminants and have been found to be related to abortion in horses, rabbits, guinea pigs, mice, pigs and humans [8]. Clinical signs of the infection may develop in infected animals before abortion in the last 2–3 weeks of pregnancy. Generally, the percentage of abortion in the first year is low, however; subsequently reaches to 30 and 10% in the second and third years, respectively [27]. Shedding of etiological agents for 3 years has been also described [26]. Although C. pecorum is frequently isolated from the digestive tract of ruminants with no clinical symptoms, it is considered as a causative agent of fertility disorder, conjunctivitis, arthritis, mastitis, and pulmonary inflammation in sheep, goats and cattle [21]. While C. psittaci can cause severe flu-like infections in humans, birds suffer from largely non-specific, sometimes fatal intestinal and respiratory problems [2]. These infections also influence goats and, to a lesser degree, cattle, horses, pigs and deer. However, due to the lack of epidemiological pieces of evidence, little is known about the rate of such infections in goat herds [15]. Nearly 20 years ago, C. pecorum association with small ruminants’ abortion incidents was investigated in the south of France [22]. Chlamydia spp. have a wide range of hosts, but not nearly all carriers develop symptoms of the disease. There is a significant relationship between Chlamydia abortus infection with sex, age and geographical location that the last is more
important in the distribution of infection. It may be due to present susceptible species such as wild animals, differences in climate, health and hygiene program, breeding practices, ease of access to the veterinarian, and knowledge of livestock breeders [3, 20, 24]. The recognition of carrier animals in each area is one of the most important steps to identifying chlamydial infections epidemiology, and subsequently employing the best way to control and prevent such infections. In addition to DNA-based techniques (e.g., polymerase chain reaction and DNA microarray) and RFLP, various diagnostic techniques such as direct microscopic inspection, culture in embryonated chicken eggs or in cell cultures, serological exams for protein detection like complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), immunohistochemistry and direct immunofluorescence could be utilized to recognize chlamydial agents in biological samples [14]. Several studies regarding *C. abortus* in sheep by serology [9], and *Chlamydia psittaci* in pigeons [10] by PCR have been already conducted in Khuzestan province. As serological methods are able to check a large number of samples at a time, they are preferred to another identification techniques for the control and monitoring of the flock. However, one of the disadvantages of this approach is its inability to differentiate active or inactive infections in the first epidemiological surveys. Due to small animal migration from the neighboring provinces of Khuzestan and the lack of quarantine provision on the border of the province, and given the fact that the presence of such agents has been already proven in the province, the aim of this research was to study the seroprevalence of *C. abortus* infection and its associated risk factors in the goat population in Khuzestan province.

**Materials and methods**

**STUDY AREA**

Khuzestan in the southwest of Iran is a tropical province with 64057 Kmh² which is located between latitude 29˚58´ to 32˚58´ N and longitude 47˚42´ to 50˚39´ E.

**SAMPLE SIZE**

The sample size was determined by using the following formula [28]. The minimum sample size for 95% confidence level, 16% expected prevalence [7] and 4% precision were calculated 323 goats, but in this survey 364 goats from 16 flocks were tested.

\[
\text{n} = \frac{(Z_{\alpha/2})^2 \times P(1-P)}{(d)^2}
\]

The number of goats to be sampled was determined individually for each flock using the following formula [28]:

\[
\text{n} = 1 - (1-P)^{1/d} \times \left[ \frac{N - \text{d}}{2} \right] + 1
\]

Where:

- \( n \) = sample size for the flock.
- \( p \) = probability of finding at least one seropositive goat in the flock.
- \( N \) = flock size.
- \( d \) = minimum number of seropositive goats expected in the flock.

The probability of detection of at least one seropositivity in goats in each flock was determined at 95% (\( P=0.95 \)), and the number of seropositivity in goats in each flock (\( d \)) was calculated for the expected prevalence 16% [7].

**SERA SAMPLES**

The blood samples were randomly collected from 368 non-vaccinated goats (at least 6 month years old) from 16 flocks in the cities of Shoushtar, Izeh, Dezful, Susangerd, Hendijan and Ahvaz in Khuzestan province during October to December 2015. The blood samples were centrifuged at 3000 rpm for 10 minutes to separate sera. Sera samples were then stored at -20°C until used. The variables of sex (male and female), history of abortion (yes or no), age (year) and location (Shoushtar, Izeh, Dezful, Susangerd, Hendijan or Ahvaz), were collected from all goats according to the observations and interviews conducted.

In the total number of 368 samples, there were 346 (94.02%) female and 22 (5.98%) male goats. The mean score and standard deviation of age were 3.12 and 1.42 year, respectively. Accordingly, the animals were divided into three age groups: young (< 2 year old), sub-adult (2 to 3 years old) and adult (≥ 4 years old) according to dental formula [6]. Among the female goats, 6.59% of them had a history of abortion.

**SEROLOGICAL ANALYSIS**

Serum samples were tested for the presence of *C. abortus* antibodies by using ELISA kit manufactured by ID vet (ID vet, France). The optical density (OD) of the samples was measured at 450 nm. Then, the S/P percentage of *C. abortus* antibody was calculated for each sample by multiplication of 100 in output of optical density of the sample and positive control split up. According to the instruction manual, the samples were considered negative if the S/P% were less than 50%, while the samples with the S/P% more than or equal to 50% and less than 60% were considered doubtful and the ones with S/P% more than or equal to 60% were considered positive.

**STATISTICAL ANALYSIS**

The statistical analysis of data was performed using SPSS (Version 16.0; SPSS Inc., Chicago, USA). The association between age, gender, history of abortion and geographic location were analysed by Chi-square test and bivariate and multivariate logistic regression. Differences were considered statistically significant at \( P \leq 0.05 \).
Results

The seroprevalence rate of *C. abortus* was 5.71% (95CI; 3.34-8.08%). The absolute frequency of S/P values for *C. abortus* is presented in Figure 1.

![Figure 1: Absolute frequency of S/P values for Chlamydia abortus in goats in the Southwest of Iran](image)

<table>
<thead>
<tr>
<th>Category</th>
<th>Groups</th>
<th>Prevalence</th>
<th>Odds Ratio</th>
<th>95% CI for OR</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Young</td>
<td>6.9%(4/58)</td>
<td>0.88</td>
<td>0.64-1.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Sub-adult</td>
<td>6.67%(11/165)</td>
<td>0.88</td>
<td>0.64-1.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>4.12%(6/145)</td>
<td>0.88</td>
<td>0.64-1.2</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

The different lowercase letters in each variable represent a significant difference

**Table I:** Prevalence of *Chlamydia abortus* antibodies in goats in the Southwest of Iran based on age

<table>
<thead>
<tr>
<th>Category</th>
<th>Groups</th>
<th>Prevalence</th>
<th>Odds Ratio</th>
<th>95% CI for OR</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>4.54%(1/22)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>5.78%(20/346)</td>
<td>1.29</td>
<td>0.17-10.08</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

The different lowercase letters in each variable represent a significant difference

**Table II:** Prevalence of *Chlamydia abortus* antibodies in goats in the Southwest of Iran based on sex

<table>
<thead>
<tr>
<th>Category</th>
<th>Groups</th>
<th>Prevalence</th>
<th>Odds Ratio</th>
<th>95% CI for OR</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td>Delivered normally</td>
<td>5.45%(17/312)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>History of recently aborted</td>
<td>9.1%(2/22)</td>
<td>1.74</td>
<td>0.37-8.04</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

The different lowercase letters in each variable represent a significant difference

**Table III:** Prevalence of *Chlamydia abortus* antibodies in goats in the Southwest of Iran based on history of abortion

The statistical analysis showed that infection was not correlated with age orders, however decreased with aging (P>0.05). Univariate logistic regression showed that the odds of infection between the age based on year and disease was 0.88 (95CI; 0.64-1.2) and with increased every year, odds of infection decreased 12%. Moreover, 0.5% of fluctuation in the infection was justified by the age factor (Table I).

The prevalence of *C. abortus* in female and male goats were found to be 5.78% and 4.54%, respectively. However, Chi square test showed that the difference was not significant (P>0.05). The odds of infection in females was 1.29 (95CI; 0.17–10.07) compared to males'. Furthermore, 0.05% of fluctuation in infection was justified by the gender factor (Table II).

The statistical analysis showed that there was no statistically significant difference between infection and history of abortion (P>0.05). In comparison to goats without any history of abortion, the odds of infection in goats with a history of abortion was 1.74 (95CI; 0.37–8.04). Besides, 0.4% of fluctuation in infection was justified by the history of abortion (Table III). As Table 4 shows, infection rates varied among different cities; that is, Ahvaz with 13.11% and Susangerd with zero percent were the most and the
least infected places, respectively. In effect, the infection and location were not significantly related (P>0.05); however, 8.6% of fluctuation of infection was justified by the geographical locations of the herds (Table IV).

Multivariate logistic regression showed that 8.7% of fluctuation in infection was justified by the factors such as age, gender, history of abortion and geographical location. However, in backward stepwise logistic regression none of them were significantly related to infection (P>0.05).

Discussion

In this survey, seroprevalence rate of Chlamydia abortus was 5.71%. There are several reports from different regions of Iran confirming the presence of Chlamydia abortus infection. In this regard, Ghorbanpoor et al. (2007) reported 8.9% prevalence in 145 aborted sheep in the city of Ahvaz by ELISA [9]. In a similar vein, Esmaeili et al. (2015) evaluated the seroprevalence of Chlamydia abortus infection in 1440 sheep and goats from 7 provinces of Iran were 37.7 %, 30.3%, 32.9%, 30.3%, 19.6%, 17.5% and 15.6%, in Tehran, Qom, Lorestan, Fars, Bushehr, West Azerbaijan and Khuzestan, respectively [7]. The lower frequency of infection in Khuzestan as compared with other provinces could be related to different weather conditions and flock management systems in such provinces, and also to the effects on transmission, distribution and survival of Chlamydia spp. in the environment. The weather of Khuzestan province is warm and warmer than the other provinces of Iran. It may be due to negative effect of this climate situation on horizontal transmission of Chlamydia abortus.

In addition to Iran, the prevalence of Chlamydia abortus infections was considered in different countries by adopting various methods. For instance, in countries such as Egypt (68%), Italy (48-58%), Bosnia and Herzegovina (91.7%) and some areas of Brazil (21.5-50%) and China (66.17%) high frequencies of the infection were reported. [3, 16, 12, 18, 19, 25]. However, in countries like Costa Rica (0.28-4.4%) and Poland (4.2%) low frequencies of the infection were reported. [3, 16, 12, 18, 19, 25]. In effect, the infection was the highest in Ahvaz (13.11%) and the least in Susangered (0%). These cities do not differ in terms of climate, therefore the differences in the rate of C. abortus may be related to livestock management systems, number of animals in the herd, entrance of infected animal to the herd.

The presence of C. abortus infection is documented in Khuzestan province, it is expected that preventive measures should be taken to control and restrict such infection in animal population of the province. Improving the quality of livestock management systems and adopting closed systems in the maintenance and quarantine of animals could have a positive impact on the control of the infection. Additional

<table>
<thead>
<tr>
<th>Category</th>
<th>Groups</th>
<th>Prevalence</th>
<th>Odds Ratio</th>
<th>95% CI for OR</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Susangerd</td>
<td>0%(0/49)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hendijan</td>
<td>5%(4/80)</td>
<td>0.27-8.62</td>
<td>0.89-21.54</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Dezful</td>
<td>5.17%(3/58)</td>
<td>0.26-9.83</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoushtar</td>
<td>6.67%(4/60)</td>
<td>0.37-11.76</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ahvaz</td>
<td>13.11%(8/61)</td>
<td>0.89-21.54</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| The different lowercase letters in each variable represent a significant difference

Table IV: Prevalence of Chlamydia abortus antibodies in goats in the Southwest of Iran based on location
studies are also needed to identify the influence of \textit{C. abortus} on abortion in goats, as well as the risk of its transmission to humans through animal products.

**Acknowledgments**

This study was financially supported by a grant from the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran.

**References**


