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# Prevalence of *Chlamydia abortus* Infection in Aborted Sheep and Goats in Kerman Province, Southeast of Iran

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## ABSTRACT

In recent years, *C. abortus*, the etiological agent of ovine enzootic abortion, has been associated with many cases of lamb loss in sheep and goat farms in Iran. However, there is a lack of epidemiological data regarding Chlamydia-related abortion in this region. Accordingly, we aimed to investigate the prevalence of *C. abortus* and the associated risk factors in the small ruminants of Kerman Province, southeast Iran. For this purpose, we collected 134 vaginal swab samples from 70 sheep and 64 goats that had experienced abortion. Following DNA extraction from samples, we amplified the POMP90-3 gene of *C. abortus* using PCR to confirm *C. abortus* presence, and then one positive sample was selected for sequencing. The results indicated an overall C. abortus prevalence rate of 21.6%, with 20.3% prevalence in goats and 22.8% in sheep. We observed a higher incidence rate in animals with a higher number of parturition; however, no significant corelation was observed between the prevalence rate of *C. abortus* and species. In addition, sampling location was considered a risk factor associated with *C. abortus* infection. This study highlighted *C. abortus* as a threat to small ruminants' reproduction in Kerman Province, which deserves constant monitoring and multi-faceted preventive strategies.

## Keywords

*Chlamydia abortus, Kerman province, Ovine enzootic abortion, PCR*

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## Abbreviations

C. abortus: Chlamydia abortus
MOMP: Major outer membrane proteins
POMP: Polymorphic outer membrane proteins
PBS: Phosphate-buffered saline
PCR: Polymerase chain reaction
OEA: Ovine enzootic abortion



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## Introduction

C. *abortus*, a Gram-negative bacterium belonging to the family Chlamydiaceae, is an obligate intracellular pathogen responsible for OEA or EAE. The disease burdens considerable economic loss in small ruminant farms if it affects enormous cases called abortion storms [1-4]. *C. abortus* transmits through any environmental exposure to the bacteria released by infected animals, abortion materials, or post-partum secretions, which poses health concerns for pregnant women and wild animals [5, 6]. Spillover of *C. abortus* through domestic and wild animal reservoirs has made controlling the disease difficult [5].

In the initial stage of *C. abortus* infection, bacteria colonize in the lymphatic tissues and then disseminate to other organs, resulting in several implications, such as pregnancy loss (abortion) and birth to stillborn if the infection occurred in the late stage of pregnancy (5-6 months) [3, 7-9]. Otherwise, bacteria enter the latency phase and may cause abortion in the second year of pregnancy [10]. Various approaches are available for confirming *C. abortus* in diagnostic laboratories. Methods for the direct identification of the agent, such as *C. abortus* isolation from clinical samples, staining the smears of fecal samples or vaginal swabs, and immunological staining of the organism, are either outdated or non-convenient [11-15]. Serological tests, including CFT and ELISA, are used for the indirect diagnosis of C. abortus [16]. These techniques identify the presence of chlamydial antibodies in the sera of infected animals. However, they have been replaced with molecular methods to improve the detection of C. abortus. Molecular identification methods, such as PCR, real-time PCR, and DNA microarray are highly sensitive approaches due to targeting different biomarker sequences, namely conserved regions, MOMP, POMP genes, or the intergenic space between the 16S and 23S rRNA genes [17-20].

Although *C. abortus* distributes worldwide, the reported distribution of C. abortus is far from the true infection prevalence [5] because of the variability in the sensitivity and specificity of the diagnostic tests and a lack of *C. abortus* epidemiological information, especially in developing countries in Asia and Africa [21]. OEA is endemic in Iran, and several studies previously reported the incidence of the disease in sheep and goats in some areas of this country [22-25]. In the present study, we attempted to investigate the prevalence rate and associated predisposing factors of *C. abortus* infection in aborted sheep and goats of Kerman Province in Iran to provide valuable insight into bacteria spillover in this region.

### Abbreviations-Cont'd

EAE: Enzootic abortion of ewes
CFT: Complement fixation test
ELISA: Enzyme-linked immunosorbent assay

## Result

### *Identification of C. abortus*

Among 134 vaginal swabs collected from sheep and goats, 16 sheep (22.8%) and 13 goats (20.3%) were confirmed to be positive for C. abortus based on the amplification of the POMP 90-3 gene (220 bp) in PCR (Figure 1). The PCR results were validated by sequencing and blasting one PCR product, which showed the highest similarity with the POMP 90-3 gene of C. abortus that was previously registered on NCBI under the accession number ACD10929.1.

### *Prevalence of C. abortus infection*

The prevalence rate of chlamydiosis based on different variables, such as animal species, age, number of parturition, and the location was statistically analyzed in the aborted flocks of sheep and goats using the Chi-squared test (Table 1). According to the results, the prevalence of *C. abortus* varied in the different regions ranging from 0% in Bam city to 28.3% in Baft city. Our findings revealed a significant correlation between the geographical area and the level of *C. abortus* in flocks ($p = 0.03$). There was no significant relationship between C. abortus infection and animal species (sheep and goats) ($p = 0.7$), or the age of infected animals ($p = 0.2$). However, the number of abortions in infected animals had a significant correlation with parity ($p = 0.001$).

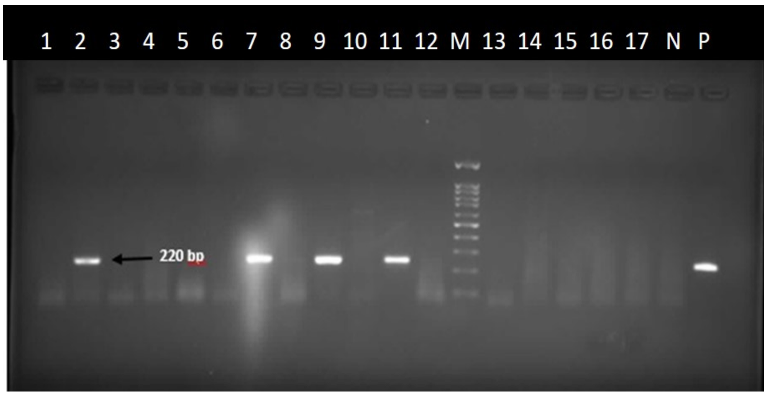


**Figure 1.**
The agarose gel electrophoresis of the POMP 90-3 gene of *C. abortus* isolates.
M: 100 bp ladder; N: negative control (distilled water); P: positive control (*C. abortus*); lanes 1-17: test samples. The observation of a 220 bp band in a sample confirmed *C. abortus* presence.

## Discussion

OEA is an infectious disease with clinical demonstrations in small ruminants, such as sheep and goats [11]. Due to massive economic loss, chlamydial abortion is a global concern in agricultural industries in Europe, North America, Africa, and Iran [21]. There are various laboratory diagnostic techniques for surveying the epidemiology of the disease, such as serological tests and basic detection methods, which provide less sensitivity and specificity for the confirmation of microorganisms. However, molecular methods based on outlining specific genes can reliably identify and differentiate the chlamydial species [17].

In the present study, we identified a high incidence rate of *C. abortus* infection in the Kerman Province of Iran, with ranges of 20.3% and 22.8% in goats and sheep, respectively. The results indicated that various factors, such as geographical location and the number of parturitions, could influence *C. abortus* infection. This observation also highlighted the need for constant genetic and antigenic evaluation of abortion isolates to establish national strategies for preventing the transmission of *C. abortus* in the future.

The prevalence rate of *C. abortus* infection in small ruminants depends on many factors, including the geographical location, size and type of samples taken, animal breed, grazing and management strategies, nutritional deficiency, uncontrolled restriction of a diseased animal movement from infected areas, choice of diagnostic antigen, and studding method [18]. Moreover, aging, species, gender, number of parturition, and geographical region are reported as effective factors in the prevalence of *C. abortus* [25]. Most investigations on the prevalence of OEA in sheep and goats reported an average rate of 20%-37% in Iran [22-25]. In this regard, a survey showed a twice higher prevalence in Chaharmahal and Bakhtiari province. However, some other studies reported a low prevalence of 9% in the south to 11% in the northeast of Iran [26-28]. In neighboring countries, such as Iraq, Arif et al. recorded chlamydiosis in only one of the 30 samples from the aborted ewes (3.3%) in Sulaimani province, which is far from the rate commonly reported in Iran [18]. In the current study, we observed an overall *C. abortus* prevalence of 21.6% among the small ruminants of Kerman province, which was in agreement with most available data in Iran. We also detected diverse incidence rates in different cities, which is consistence with the sero-prevalence of C. abortus in the countries of origin Jordan [29] and China [30]. In contrast with our study, the incidence rate of *C. abortus* infection showed no difference among populations located in different epidemiologic areas of Khorasan Razavi province, northeast of Iran [28]. Another research in the southwest of Iran also showed that the geographical origin of sheep had no significant effect on the incidence of *C. abortus* [31].

Our findings showed that chlamydial infection incidence was higher in ewes with a higher number of parturition. Other studies also reported similar results in Iran and Jordan [25, 29]. The establishment of the latent form of *C. abortus* pathogenesis in non-pregnant infected ewes and the bacteria reactivation and proliferation in the subsequent pregnancy might be the reason for the higher prevalence of infection in ewes with a higher parity [9].

According to Table 1, the age of animals is not a predisposing factor for *C. abortus* prevalence. In agreement with

**Table 1.**
Prevalence rate of C. abortus in sheep and goats from different regions of Kerman Province, Iran

| Variables | No. | Number of positive samples | Prevalence (%) | *p-value* |
|---|---|---|---|---|
| **Animal species:** | | | | |
| Sheep | 70 | 16 | 22.8 | |
| Goat | 64 | 13 | 20.3 | 0.7[b] |
| Total | 134 | 29 | 21.6 | |
| **Number of parturition:** | | | | |
| < 2 | 59 | 4 | 6.7 | 0.001[a] |
| 2 – 4 | 40 | 10 | 25 | |
| > 4 | 35 | 15 | 42 | |
| **Age (yr):** | | | | |
| < 2 | 45 | 12 | 26.6 | |
| 2 – 4 | 50 | 7 | 14 | 0.2[b] |
| > 4 | 39 | 10 | 25.6 | |
| **City:** | | | | |
| Baaft | 60 | 17 | 28.3 | |
| Bam | 10 | 0 | 0 | |
| Bardsir | 25 | 4 | 16 | 0.03[a] |
| Kerman | 14 | 2 | 14.2 | |
| Shahr-e Babak | 25 | 6 | 24 | |

a: significant difference ($P < 0.05$)
b: insignificant ($P > 0.05$)


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this result, Iraninezhad et al. [28] and Cubero et al. [32] recorded no significant correlation between age and the epidemiology of chlamydial infection. Contrary to our study, a positive relationship between the age of aborted animals and the chance of positivity for *C. abortus* was mentioned in other reports [25, 30].

According to our results, although the chlamydial infection rate was higher in sheep than in goats, this difference was not significant (Table 1). Similarly, previous studies showed that species was not a risk factor for the occurrence of chlamydial infection [23, 25, 28, 30, 33]. In this regard, a difference was reported by other researchers in the infection incidence between sheep and goats [34-36]. For example, a higher rate of chlamydial infection was observed in sheep compared to goats in Taiwan [37].

### Conclusion

This study was the first report on the prevalence of C. abortus infection among goats and sheep in Kerman province of Iran. According to PCR results, C. abortus was responsible for 22.8% and 20.3% of abortion incidence in sheep and goats, respectively. This finding indicates the circulation of *C. abortus* among small ruminants in Kerman province, which poses serious public health concerns.

## Materials and Methods

### Sample collection

During the lambing season of 2022, 134 vaginal swab samples were collected from 70 sheep and 64 goats with a history of abortion in different cities of Kerman province in the southeast of Iran. The samples were suspended in 500 µl of sterile PBS and then transferred on ice to the Microbiology Laboratory at the Faculty of Veterinary Medicine of the Shahid Bahonar University of Kerman. The samples were stored at -20°C for DNA extraction.

### DNA extraction

The DNA was extracted from vaginal samples using DNA extraction commercial kit (Cinaclon, Iran) according to the manufacturer's instructions. The extracted DNA was quantified by a NanoDrop spectrophotometer (Epoch, BioTek Instruments Inc., USA) at the wavelength of 260 nm, and stored at -20˚C for further analysis.

### PCR verification

To detect C. abortus, PCR was performed on the extracted DNA to amplify the POMP 90-3 gene with specific primers (F:5'–TTTTCAGGATCCTATTGTCCTCCAGGCA–3' and R:5'–GTGAATTCATCAGCATAAATAGCCCCG–3') [14]. The PCR reaction mix was prepared at a final volume of 20 µl, including 10 µl master mix (Amplicon, Denmark), 4 µl template DNA, 0.5 µl of each forward and reverse primer, and 5 µl distilled water. The amplification was initiated with 3 min of denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 30 seconds, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. the PCR amplicons were visualized by agarose gel electrophoresis 1.5% and exposed to a UV light to detect the POMP 90-3 gene (220 bp).

### POMP 90-3 gene sequencing

In the next step, the PCR product of one Chlamydia-positive sample was subjected to sequencing (Macrogen Inc., South Korea) to confirm the amplified POMP 90-3 gene. After receiving DNA fragments of the POMP 90-3 gene, they were trimmed and then assembled using DNAstar software. The final consensus of the received sequence was compared to any relevant sequence in the NCBI database using BLAST.

### Statistical analysis

The sample size was calculated using the online software https://www.calculator.net/sample-size-calculator.html, with a confidence level of 95% and desired absolute precision of 10%. The SPSS for Windows (version 25.0; IBM Corp., Armonk, USA) was applied to perform statistical analysis. The rate of abortion between the investigated groups was explained as percentage of all the sampled animals. The effect of independent risk factors, such as sampling location, number of parturition, animal species, and age on the prevalence of *C. abortus* infection was analyzed by Chi-squared test. The differences in prevalence were considered significant at $p < 0.05$.

## Authors' Contributions

S.A. collected samples, carried out the analysis of samples, data analysis, and wrote the manuscript. M.G. designed the study, supervised the project, revised the data analysis, and critically revised all parts of the manuscript. E.M. supervised the laboratory works. N.E. formal analysis, writing—review and editing. M.A.S. formal analysis, writing—review and editing.

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## Competing Interests

The authors declare that there is no conflict of interest.

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