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The Frequency of Abortion Caused by *Chlamydia abortus* in Aborted Fetuses of Sheep and Goats in Iran

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ABSTRACT

Background: *Chlamydia abortus*, is one of the most important causes of abortion in small ruminants. Evaluating the frequency of chlamydial infection in abortion of animals is beneficial in epidemiological surveys. The purpose of the present study is to determine the frequency of abortion caused by *Chlamydia abortus* using PCR method.

Methods: A total of 200 fetuses were collected from 150 ewes and 50 does. The samples were collected from abomasal contents and lungs of the fetuses and using PMP gene of *Chlamydia abortus*, PCR was conducted. The infection occurrence with regard to the species of animals, age, gender and type of pregnancy along with age, numbers of pregnancy and numbers of abortion in the aborted animals were statistically analyzed by Chi-squared test, t-test and Spearman correlation coefficient which all were calculated using SPSS version 25.

Results: The bacterium DNA was detected in 47 fetuses. The infection occurrence didn't have significant statistical relationships with the species, age and gender of the fetuses. Chlamydial infection in the twin fetuses was significantly more than the single ones. The infection had statistical relationship with ages and parturition numbers of the aborted animals but not with the numbers of abortion.

Conclusion: Regarding the high frequency of abortion caused by *Chlamydia abortus* in this study (23.5%), it is necessary to boost the information about the prevalence of *Chlamydia abortus* in different regions of our country.

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Introduction

Ovine enzootic abortion (OEA) caused by *Chlamydia abortus* is one of the most important abortive diseases in small ruminants worldwide (1). This gram-negative bacterium causes certain economic losses in Europe, North America and Africa and 45% of ovine abortions in the UK are related to this agent (2). Affected animals usually don't show clinical signs except for abortion and the animals lose the fetuses in their last months of pregnancy (3). The first time that the infection occurs in a naive flock, it may cause abortion in 30-60% of ewes and does. This rate of abortion remained for 2-3 years in the affected flock and during these years, *Chlamydia abortus* infects most of the female animals. After that, the rate of abortion declines to 10-15% (3). Aborted sheep and goats excrete *Chlamydia* through the discharges or the bacterium may be transmitted by aborted fetuses. As *Chlamydia abortus* is a zoonotic agent, pregnant women may abort when exposing to excretion of infected animals (4).

OEA has been reported as the predominant abortifacient bacterium throughout the world (5). In an eight-year investigation which was performed to detect the causative agents of abortion in goats, *Chlamydia abortus* had taken the most rate of abortion in 23% of cases (6). In the report of veterinary investigation diagnosis analysis (VIDA) in 2013, *Chlamydia abortus* was the most important causes of abortion (45%) compared to the other agents (3).In another study in 2014, 41.7% of aborted small ruminants in Namibia were infected with *Chlamydia abortus* (7).

Pursuant to a few studies have been done in Iran, *Chlamydia abortus* has a significant role in small ruminant's abortion (8-10). Ghorbanpour et al. in 2007 reported *Chlamydia abortus* infection in 8.9% of aborted sheep in Ahvaz province (11). In a study in 2011 which investigated the presence of *Chlamydia abortus* in flocks, Esmaeili et al. found 25.6% seropositive small ruminants in seven provinces of Iran (10).

According to the role of *Chlamydia abortus* in small ruminant's abortion, knowledge about the frequency of the infection in different provinces is needed for making control programs. This requires reliable diagnostic methods as what makes the base of strategies against infectious abortion in sheep and goats, is the detection of the main causes (9). There are various diagnostic tests with different sensitivity and specificities for the detection of *Chlamydia abortus*, though the bacterium is neglected in many laboratories because *Chlamydia* spp. don't grow in routine bacteriological media and isolation of them is difficult (5). Chlamydiosis is routinely diagnosed by direct smears stained with methods such as modified Ziehl-Neelsen's (MZN) but, staining is neither sensitive nor specific, and because of the similarity of *Chlamydia* appearance with *Brucella* and *Coxiella burnetii*, there is a possibility of mis-diagnosis (12).

In the other hand, molecular procedures such as PCR are easy, fast and sensitive methods for the detection of *Chlamydia*, so these properties make them preferred and reliable methods for designing national strategic decisions in combating OEA (5). In the present study, we demonstrated the frequency of chlamydial infection in the fetuses belonged to aborted ewes and does. In this research, PCR was conducted in order to evaluate the infection rate of Iranian flocks to OEA.

Materials and Methods

Sample collection

A total of 200 fetuses were collected from 150 ewes and 50 does which belonged to flocks with the abortion rate of more than 10%. The information about fetal age, gender and being single or twin, was recorded. The fetuses were examined for the presence of macroscopic legions. The samples were collected aseptically from abomasal content and lung of the fetuses.

Conventional Polymerase Chain Reaction

DNA was extracted from the abomasal contents and the homogenized lungs using CinnaGen DNA extraction kit. Conventional PCR was conducted according to Laroucau et al. in 2001 (13). The target fragment in this study was polymorphic membrane protein (PMP) associated gene of *Chlamydia abortus*.

Forward and reverse primers included ATG AAACATCCAGTCTACTGG and TTGTGTAG TAATATTATCAAA respectively. PCR reactions were performed using 2 µM of each primer, 200 µM of each dNTPs, 3 mM MgCl2 and 0.5 U Taq DNA polymerase. The final volume of reaction mixture amounted to 25 µl including 20µl master mix and 5µl template DNA. Amplification was carried out in the automated DNA thermal cycle using the following cycling parameters: Denaturation at 94°C for 3 min, subsequently 35 cycles of 94°C for 1 min, annealing at 55°C for 1 min and primer extension at 72°C for 1 min. The final extension performed at 72 for 10 min. *Chlamydia abortus* DNA amplified to give a fragment of 300 bp.

Statistical analysis

The infection occurrence with regard to species, age, and gender of the fetuses, age, parturition number, and abortion number of the aborted animals and the type of pregnancy (single or twin) was statistically analyzed using Chi-squared test, t-test and Spearman correlation coefficient which all were calculated using SPSS version 25. $P<0.05$ was considered significant.

Result

The studied animals had at least 1 abortion in their history (Table 1) and they didn't show any clinical signs before abortion. The age of 117 fetuses was more than 3 months and the others (83 fetuses) were younger. The fetuses included 100 males and 100 females. One hundred and sixty-four aborted fetuses were single and the others (36 fetuses) were twins. PCR detected *Chlamydia abortus* in 47 fetuses (23.5%). There wasn't any significant relationship between the PCR positive results and species (P-value=0.773), age (P-value=0.127) and gender (P-value=0.868) of the fetuses. While there was a significant difference in getting the infection between the single and twin fetuses (P-value= 0.001, OR= 3.432, 95% CI: 1.6-7.37) in a way that the property of being healthy in the singles was 3.43 times more than the twins (Table 2.). There wasn't any meaningful correlation between the fetal infection and the numbers of abortion in the aborted ewes and does (spearman rho= 0.077, P-value= 0.278). While the relationship between fetal infection and the parturition numbers and the age of the aborted animals was significant (Table 3). Average age of the non-infected sheep and goats was more than the infected ones. The infection rate decreased by increasing the parturition numbers.

Discussion

Sheep and goat in a way that the rate of infection (23.5%) was higher than most of the previous studies had been done in Iran (9, 14, 15). Heidari et al. using PCR reported *Chlamydia abortus* as the prominent cause of abortion in the south of Iran compared to other infectious causes, but the infection rate was lower than our study (11%) (14). In another research in 2019 in the southwest of Iran, the seroprevalence of *Chlamydia abortus* among goats was 5.71% (15). In a recent study in 2017, *Chlamydia* was isolated from 25% of swab samples belonged to sheep and goat flocks in Iran (9).

Table 1. Number and frequency of abortion in the studied animals.

The number of abortion in the animals	Frequency	Percent	Cumulative Percent
0	11	5.5	5.5
1	158	79.0	84.5
2	25	12.5	97.0
3	5	2.5	99.5
4	1	0.5	100.0
Total	200	100.0	

Table 2. *Chlamydia abortus* infection of the fetuses detected by PCR based on variables including species, age, sex, and being single or twin. The significance of differences was analyzed using Chi-squared test, t-test and Spearman correlation coefficient.

Variable		Number	Percent
Species	Sheep	36	76.6%b
	Goat	11	23.4%
Age of aborted fetus	<3Month	15	31.9%
	>3Month	32	68.1%b
Fetus sex	Male	23	48.9%
	Female	24	51.1%b
Litter size	Single	31	19.0%
	Twin	16	44.0%a

a: Significant, b: Non-significant

Table 3. Analysis of the correlation between the PCR results and the numbers of parturitions, abortions and, ages of the aborted ewes and performed by t-test. There is just a significant relationship between chlamydial infection, age and the number of parturition, but not the number of abortion.

	Infection PCR	N	Mean	Std. Deviation	P-value
Age	Yes	47	3.13	1.244	0.008a
	No	153	3.74	1.394	
Number of parturition	Yes	47	2.30	1.250	0.006a
	No	153	2.90	1.299	
Number of abortion	Yes	47	1.17	0.481	0.62b
	No	153	1.12	0.577	

a: Significant, b: Non-significant

The prevalence of OEA is also high even in countries have used vaccination against the disease (2). The disease was the most common finding in ovine abortion in 2015 (16). The prevalence of *Chlamydia abortus* in Namibia have been recorded 25% and 86% among goat farms (7). In a seroprevalence study in Germany, *Chlamydia abortus* was detected in 49% of sheep in a migratory flock. The researchers related the high frequency of chlamydial abortion to the migratory system of sheep management and their grazing pattern (17). According to another report from VIDA during 2011-2018, the bacterium had the highest percentage among other infectious abortifacient agents (18).

In a study in Mexico in 2014, PCR was conducted on spleen samples of aborted fetuses belonged to seropositive does and 66.6% of the studied flocks were infected with *Chlamydia abortus* (19). We also used PCR which is a rapid method with high sensitivity and specificity and according to the world organization for animal health, it's the method of choice in the detection of OEA (5). The sensitivity of the fragment was targeted in the present study was 10 times higher than the previous methods based on the *ompA* gene (13). Berri et al in 2009, using multiplex conventional PCR detected 3 main causes of abortion including *Chlamydia abortus*, *Coxiella burnetii* and *Chlamydia pecorum* with high sensitivity and specificity (12). In the study of Alem et al. in 2017 in the northwest of Iran, PCR was used for the detection of *Chlamydia abortus* in the tissue pools of the aborted fetuses and 26% of the samples were positive (20).

According to our results, the infection occurrence didn't statistically relate to age, gender and species of the fetuses (Table 2). Bhandi et al. in 2019 reported no relationship in getting Chlamydial infection between male and female goats (21). Moreover, there wasn't any significant relationship between the numbers of abortion in the aborted animals and fetal infection, but the relationship between fetal infection and the age and the parturition numbers of the aborted animals was significant (Table 3). The frequency of chlamydial infection had an inverse relationship with aging and the number of parturition of the studied sheep and goats. In a similar study in Iran, the association of chlamydial abortion with age, gender, history of abortion and geographical region were analyzed. Contrary to our findings, the researchers indicated that there wasn't any significant correlation between chlamydial abortion and the mentioned variables but same as the present results, they found out that as the animals had gotten older, the incidence of the infection had decreased (15). After the first abortion, a protective immunity which prevents abortion develops, but the bacterium may excrete during ovulation and parturition. So these animals often don't show abortion anymore, though they can transmit the disease to healthy sheep and goats (22).

Huang et al. in 2013, reported the infection in 20.9% of sheep in Tibetan and they showed higher infection in female and young sheep than male and adult ones, however, the difference wasn't significant (23). In a seroprevalence study, the seropositivity of animals increased with aging (24), while in Jordan Al-Qudah et al. in 2004 found that age had no significant effect on seropositivity (25).

We also analyzed the impact of the fetuses' age in getting infected with the bacterium and demonstrated *Chlamydia abortus* was present a little more in the fetuses more than 3 months. This may be due to the fact that as pregnancy progresses, the bacterial replication increases in the placenta of affected animal (3).

Salinas et al. in 2008, detected different seroprevalence among species and the region where the samples were taken, though they observed that age and sex didn't have any effects on getting infected with *Chlamydia* (26). Other studies have also shown that chlamydial abortion occurs to a similar extent for goats as sheep (1, 4, 5).

To our knowledge, the present study is the first one which evaluates the statistical relationship between single and twin fetuses. We found out that the property of being healthy in the singles was

3.43 times more than the twins (P-value= 0.001) (Table 2.). Livingstone et al. in 2017 found obviously less pathological lesions in the placenta of single aborted fetuses compared to the twins (27). As pathogenicity of chlamydial abortion is a combination of defects in materno-fetal nutrient, inflammatory response and hormonal regulation disorders (5), twins may be influenced by malnutrition more than single fetuses.

Another variable which was analyzed in our study was the relationship between the infection and the abortions in history of the animals. If *Chlamydia abortus* infects naive sheep and goats which are at pregnancy less than 110 days, the animals will abort but infection after this time results in abortion in the second year of pregnancy. Therefore, it is expected that most animals abort in the second year after the introduction of *Chlamydia* to the flock (2). In contrast, since the infection rate is high in Iran (9, 14, 15), most abortions occur in the first pregnancy of animals (28). In our study chlamydial abortion occurs with no significant difference between the animals which had history of abortion and the animals which experienced their first abortion due to *Chlamydia abortus*.

As mentioned above, after the first abortion the affected animals benefit a protective immunity, so the previous abortions may be for other infectious and non-infectious factors. Santos et al., found more seroprevalence in goats with the history of abortion (29). In contrast, Bhandi et al. in 2019 indicated no association between seropositivity and abortion history or other reproductive problems (21).

There might be several abortifacient factors except for *Brucella* which may be the cause of abortion in small ruminants in our country, though the most attention is focused on brucellosis. A study in 2019 in Zimbabwe showed 22% of goats were infected with *Chlamydia abortus*, while all the animals were negative for *Brucella melitensis* (21). Paolo et al. in 2019, using Real-time PCR and nested-PCR detected *Chlamydia abortus* in placenta of aborted goats which all were negative for *Brucella* and *Coxiella* (30).

Findings from our study represented chlamydial infection in 23.5% of aborted fetuses in both ewes and does, indicates the bacterium involvement in abortion of animals in the Iranian flocks. It should be noted that this percentage is obtained from the animals that had aborted their fetuses while based on the OEA infection pattern in the second year of its introduction to a flock and according to the history of abortion in our studied flocks, there may be more infected animals which are shedding the bacterium but are persistent to abortion.

However, there is almost no control plan for OEA in small ruminant's abortion cases in the country. The strategy for prevention of OEA is based on vaccination and proper health management. As Iran doesn't use vaccine against the disease, the implementation of suitable health statues is helpful in this circumstance. Majed et al. in 2018 showed that in the flocks which have good health management the disease is significantly lower than the others (31). Si-Qin S-Y et al. in 2014 explained the important role of the raising system and geographical region on the infection rate (24).

Regarding the high frequency of abortion caused by *Chlamydia abortus* in the samples belonged to different provinces of Iran, there should be appropriate programs particularly in the *Brucella* free flocks in our country to prevent destructive effects of OEA.

Conclusion

Regarding the high frequency of abortion caused by *Chlamydia abortus* in the samples belonged to different provinces of Iran, there should be appropriate programs particularly in the *Brucella* free flocks in our country to prevent destructive effects of OEA.

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Ethics approval and consent to participate

This project was conducted under the ethic approval code 28786/6/7.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Borel N, Polkinghorne A, Pospischil A. A review on chlamydial diseases in animals: still a challenge for pathologists?.*Vet Pathol* 2018; **55**(3): 374-90.
2. Longbottom D, Coulter L. Animl chlamydiosis and zoonotic implications. *J Comp Pathol* 2003; **128**(4): 217-44.
3. Essig A, Longbottom D. *Chlamydia abortus*: new aspects of infectious abortion in sheep and potential risk for pregnant women. *Curr Clin Microbiol Rep* 2015; **2**(1): 22-34.
4. Selim A. *Chlamydophila abortus* Infection in Small Ruminants: A Review. *Asian J Anim Vet Adv* 2016; **11**(10): 587-93.
5. World health organization for animal health, 2018. Enzootic abortion of ewes. Oie, France, Chapter: 3.7.5. pp. 1456-65.
6. Moeller Jr RB. Causes of caprine abortion: diagnostic assessment of 211 cases (1991–1998). *J Vet Diagn Invest* 2001; **13**(3): 265-70.
7. Samkange A, Katsande TC, Tjipura-Zaire G, et al. Seroprevalence survey of *Chlamydophila abortus* infection in breeding goats on commercial farms in the Otavi Veterinary District, northern Namibia. *Onderstepoort J Vet Res* 2010; **77**(1): 1-5.
8. Barati S, Moori-Bakhtiari N, Najafabadi MG, et al. The role of zoonotic chlamydial agents in ruminants abortion. *Iran J Microbiol* 2017; **9**(5): 288.
9. Esmaeili H, Hamedi, M., Madani SA. Isolation of *Chlamydia* spp. from Ewes and Does in Iran.*ArchRazi Inst* 2017; **72**(4): 249-53.
10. Esmaeili H, Bolourchi M, Mokhber-Dezfouli MR. Seroprevalence of *Chlamydia abortus* infection in sheep and goats in Iran. *Iran J Vet Med* 2015; **9**(2): 73-7.
11. Ghorbanpoor M, Seed G, Razieh H. Serological Study on Enzootic Abortion of Ewes in Ahvaz, Iran. *J Anim Vet Adv* 2007; **6**(10): 1194-96.
12. Berri M, Rekiki A, Boumedine KS, et al. Simultaneous differential detection of *Chlamydophila abortus*, *Chlamydophila pecorum* and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. *BMC Microbiol* 2009; **9**(1): 130.
13. Laroucau K, Souriau A, Rodolakis A. Improved sensitivity of PCR for *Chlamydophila* using pmp genes. *Vet Microbiol* 2001;**82**(2): 155-64.
14. Heidari S, Derakhshandeh A, Firouzi R, et al. Molecular detection of *Chlamydophila abortus*, *Coxiella burnetii*, and *Mycoplasma agalactiae* in small ruminants' aborted fetuses in southern Iran. *Trop anim health pro* 2018; **50**(4): 779-85.
15. Borujeni MP, Bakhtiari NM, Hajikolaei MH, et al. *Chlamydia abortus* infection in goats in the southwest of Iran. *RevMed Vet-Toulouse* 2019; **170**(1-3): 9-14.
16. O'Neill LM, O'Driscoll Á, Markey B. Comparison of three commercial serological tests for the detection of *Chlamydia abortus* infection in ewes. *Irish Vet J* 2018; **71**(13): 1-9.

17. Runge M, Binder A, Schotte U, et al. Investigations concerning the prevalence of *Coxiella burnetii* and *Chlamydia abortus* in sheep in correlation with management systems and abortion rate in Lower Saxony in 2004. *Berl Munch Tierarztl Wochenschr* 2012; **125**(3-4): 138-43.
18. Carson A. Abortion in sheep: an update. *Vet Rec* 2018: 528-9.
19. Campos-Hernández E, Vázquez-Chagoyán JC, Salem AZ, et al. Prevalence and molecular identification of *Chlamydia abortus* in commercial dairy goat farms in a hot region in Mexico. *Trop Anim health pro* 2014; **46**(6):919-24.
20. Alem M, Asadpour R, Jafari Joozani R, et al. Molecular Detection of *Chlamydophila abortus* in aborted fetal tissues by using polymerase chain reaction (PCR) in Tabriz, northwest of Iran. *J Cell Mol Res* 2017; **9**(1): 35-8.
21. 21. Bhandi S, Pfukenyi DM, Matope G, et al. Brucellosis and Chlamydiosis seroprevalence in goats at livestock-wildlife interface areas of Zimbabwe. *Onderstepoort J Vet Res* 2019; **86**(1):1-9.
22. Papp JR, Shewen PE, Gartley CJ. Abortion and subsequent excretion of *chlamydiae* from the reproductive tract of sheep during estrus. *Infectimmun*. 1994; **62**(9):3786-92.
23. Huang S, Wu S, Xu M, et al. First record of *Chlamydia abortus* seroprevalence in Tibetan sheep in Tibet, China. *Small Ruminant Res* 2013; **112**(1-3):243-5.
24. Qin S-Y, Yin M-Y, Cong W, et al. Seroprevalence and risk factors of *Chlamydia abortus* infection in Tibetan sheep in Gansu province, northwest China. *Sci World J* 2014; **2014**:1-6.
25. Al-Qudah K, Sharif L, Raouf R, et al. Seroprevalence of antibodies to *Chlamydophila abortus* shown in Awassi sheep and local goats in Jordan. *Vet Med-Czech*2004; **49**(12):460-66.
26. Salinas J, Caro M, Vicente J, et al. High prevalence of antibodies against *Chlamydiaceae*, *Chlamydophila abortus* in wild ungulates using two "in house" blocking-ELISA tests. *Vet Microbiol* 2009; **135**(1-2):46-53.
27. Livingstone M, Wheelhouse N, Ensor H, et al. Pathogenic outcome following experimental infection of sheep with *Chlamydia abortus* variant strains LLG and POS. *PloS one* 2017; **12**(5):1-19.
28. Entrican G, Buxton D, Longbottom D. Chlamydial infection in sheep: immune control versus fetal pathology. *J R Soc Med* 2001; **94**(6):273-7.
29. Santos CS, Piatti RM, Azevedo SS, et al. Seroprevalence and risk factors associated with *Chlamydophila abortus* infection in dairy goats in the Northeast of Brazil, *Pesq Vet Bras* 2012; **32**(11):1082-86.
30. Paolo LAD, Pinedo MFA, Origlia J, et al. First report of caprine abortions due to *Chlamydia abortus* in Argentina. *J Vet Med Sci* 2019; **5**(2):162-67.
31. Majed R, Maab A, Omer A, et al. Preliminary study of seroprevalence of *chlamydophila abortus* amongst cattle in ninavah province. *Adv Anim Vet Sci* 2018; **6**(3):135-8.