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Short Communication

Identification of *Chlamydophila abortus* in Abomasum Content of Aborted Sheep and Goats by PCR

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Authors' Contribution
ZA conceived and designed the study. ZA, GSG and ZS conducted the lab work. AEH helped in sampling. ZA wrote the article.

Key words
***Chlamydophila abortus*, Aborted sheep fetus, Aborted goat fetus, PCR**

ABSTRACT

Chlamydophila abortus C. abortus cause placentitis, abortion, weak neonates and infertility in sheep and goats worldwide. The aim of this study was to investigate the presence of *C. abortus* in abomasum content of aborted sheep and goat fetuses from provinces of the Central Anatolia of Turkey by PCR. A total of 105 aborted sheep fetuses and 15 aborted goat fetuses were collected from 120 different herds in the lambing seasons 2015-2016. Chlamydial DNA was detected in seven (5.8%) of the 120 abomasum content of fetuses. The 15 aborted goat fetuses were found to be negative for *C. abortus*. The control and vaccination programs for the fight against *C. abortus* infection should be determined and implemented in Turkey.

Enzootic abortion of ewes or ovine enzootic abortion, is caused by *Chlamydophila abortus* (*C. abortus*), characterised with placentitis, abortion, weak neonates and infertility in sheep and goats worldwide (Nietfeld, 2001; Pospischil, 2006). *C. abortus* is member of *Chlamydiaceae* that are obligate intracellular Gram-negative bacteria. It infects macrophages and epithelial cells of humans, mammals and birds and cause broad spectrum of diseases in they (Longbottom and Coulter, 2003). *C. abortus* has the biphasic lifestyle that includes a parasitic intracellular and an infective extracellular phase. This causes the difficulties in control of infections (Pospischil, 2006). Enzootic abortion in goats is similar to infections of ewes and it has also zoonotic potential and economic importance (Pospischil, 2006).

C. abortus is mainly spread by milk, urine, foetal fluids and placenta of animals with abortion (Laroucau *et al*., 2001). Infected animals do not present any clinical signs until abortion or birth. The abortions occur in the last trimester of pregnancy and some of animals deliver very weak lambs or kids. The infection can remain latent for more than 3 years in some animals. The abortion rate is low in effected sheep herds in the first year and this rate is increase in the second and third years (Aitken and Longbottom, 2007).

Clinical diagnosis of the infection in animals is difficult; agent isolation, immunohistochemistry, serological and molecular tests are routinely used for diagnosis. The isolation of bacteria has been accepted as gold standard for diagnosis of chlamydial infection but it has same disadvantages (Aitken and Longbottom, 2007). In the last years, polymerase chain reaction (PCR) methods have been widely used to detection of *C. abortus* in clinical samples (Creelan and McCullough, 2000; Güler *et al*., 2006; Chisu *et al*., 2013; Kalender *et al*., 2013; Abahneh *et al*., 2014). The presence of *C. abortus* infections in Turkey has generally been searched by serological tests. However, there are limited studies on molecular diagnosis of *C. abortus* in abortion materials.

The aim of this study was to investigate the presence of *C. abortus* in abomasum content of aborted sheep and goat fetuses from provinces of the Central Anatolia of Turkey by PCR.

Materials and methods

A total of 105 aborted sheep fetuses and 15 aborted goat fetuses were collected from 120 different herds in the lambing seasons 2015-2016 from Aksaray, Konya,

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Karaman, Isparta, Afyonkarahisar and Niğde provinces. The size of herds was between 40 to 965 animals. *C. abortus* history of the animals was unknown. Abomasum contents of aborted fetuses were taken aseptically as specimen. The samples were transported to the laboratory under cool condition.

Live microorganisms were inactivated by incubating the samples at 95 °C for 15 min in a thermal block. Genomic DNA was obtained from each sample with Vivantis tissue DNA extraction kit, according to the manufacturer's protocol. The quantity and quality of DNA was measured at 260 and 280 nm optical density by a spectrophotometer (Nanodrop, ND1000, USA). The extracted DNA samples were kept at -20 °C until used.

C. abortus specific primers were used in PCR assay for amplification of *C. abortus* DNA (Crealan and Cullough, 2000). The sequence of forward primer was 5′-TGG TAT TCT TGC CGA TGA-3′ and reverse primer was 5′-GAT CGT AAC TGC TTA ATA AAC CG-3. This primer pairs showed a single amplicon that was 479 bp.

The PCR were performed in a total reaction volume of 25 µL containing 2.5 µL of 10 X PCR buffer, 250 µM each of the dNTPs (Fermentas, Vilnius, Lithuania), 1.5 mM $MgCl_2$, 1.25 U of Taq DNA polymerase (Fermentas, Vilnius, Lithuania), 2.5 µL of template DNA, and 1 µM of each primer (IDT, USA). DNA amplification was performed in a thermalcycler (Ependorf, Mastercycler *gradient,* Germany) with the following steps: 1 X 5 min at 95 °C, 40 X 1 min at 94 °C, 1 min 50 °C, 2 min 72 °C, and a final extension at 72 °C for 7 min. DNA of *C. abortus* S26/3 strain and nuclease free water was used as positive and negative controls, respectively. The amplified PCR products were analyzed by electrophoresis on 1.5% agarose gel, and the gel was then stained with ethidium bromide (0.5 µg/mL) and photographed.

Results and discussion

A total of 120 abomasal contents of the aborted fetuses were collected from the provinces of Central Anatolia of Turkey. Chlamydial DNA was detected in seven (5.8%) of the 120 abomasum content of fetuses by PCR. The 15 aborted goat fetuses were found to be negative for *C. abortus*. A 479 bp PCR product was amplified from seven (6.7%) out of the 105 aborted sheep fetuses. Among the 7 positive samples, 3, 3, and 1 were collected from Aksaray, Konya and Niğde provinces, respectively. The samples belong to Isparta, Karaman and Afyonkarahisar provinces were found to be negative for *C. abortus* (Table I).

In this country, limited studies have been conducted for molecular diagnosis of *C. abortus* in abortion materials. Kalender *et al.* (2013) examined 71 sheep and goat aborted fetuses by PCR method and reported that 9.8% of the samples were positive for *C. abortus*. Güler *et al.* (2006) investigated *C. abortus* DNA in 172 samples of stomach contents from sheep and goat aborted fetuses and 94 vaginal swab samples and detected it in 6 (3.5%) stomach contents and 7 (7.5%) vaginal swab samples. In the present study, *C. abortus* DNA was detected in seven (5.8%) of the 120 abomasum content of sheep and goat fetuses by PCR. This result is in harmony with the previous studies. Ovine enzootic abortion is still endemic in Central Regions of Turkey and causes abortion in sheep herds.

Table I. Distribution of samples among provinces.

Province	Sheep fetuses		Goat fetuses	
	Positive	Negative	Positive	Negative
Aksaray	3	33	0	3
Konya	3	38	0	4
Niğde	1	13	0	3
Isparta	0	4	0	0
Afyonkarahisar	0	5	0	5
Karaman	0	5	0	0
Total	7	98	0	15

In Iran, a total 300 aborted sheep and goat fetuses were investigated by PCR and 11% of samples were found positive for *C. abortus* (Heidari *et al*., 2018). In Jordan, Abahneh *et al.* (2014) examined 66 placental tissues and 15 vaginal swabs collected from aborted ewes by PCR and found that 38 (58 %) placental samples and 13 (87 %) vaginal swabs were positive for chlamydial DNA. Chisu *et al.* (2013) reported that 3 (7.5%) *C. abortus* DNA were detected from ovine aborted placental tissues by PCR in Italy. In United Kingdom, 231 ovine aborted tissues (placenta, fetal lung and fetal abomasum content) were investigated for *C. abortus* by PCR and 73 (31.6%) of samples were found to be positive (Creelan and McCullough, 2000). In our study, the prevalence of ovine enzootic abortion was detected as 5.8%. This rate was lower than result of previous studies that were conducted in Iran, United Kingdom, Jordan, and Italy.

Conclusions

The findings of this study indicated that *C. abortus* plays an important role in sheep abortion cases in the Central Anatolia. The control and vaccination programs for the fight against *C. abortus* infection should be determined and implemented in Turkey.

Acknowledgemens

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Statement of conflict of interest

The authors have declared no conflict of interest.

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