An investigation on the potential role of Q fever and chlamydiosis of ovine abortion

Aliye Gülmez Sağlam[1]*, Elif Çelik[1], Gökhan Koçak[2], Seda Gökdemir[1], Muazzez Yeşilyurt[3] Semra Kaya[4]

[1]Kafkas University, Faculty of Veterinary Medicine, Department of Microbiology, Kars, Türkiye
[2]Iğdır University, Faculty of Applied Sciences, Iğdır, Türkiye
[3]Siirt University, Faculty of Veterinary Medicine, Department of Microbiology, Siirt, Türkiye
[4]Kafkas University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Kars, Türkiye

*Corresponding: aliye.saglam@kafkas.edu.tr

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Abstract
Chlamydia abortus and *Coxiella burnetii* are among the significant pathogens that result in economic losses in small ruminants, particularly sheep, on a global scale. Both agents have been linked with disorders of the reproductive system in animals and are among the primary causes of abortion cases. This study aimed to evaluate the prevalence of Q fever and ovine enzootic abortion (OEA) in aborted sheep during lambing seasons. Samples of blood and vaginal swabs were obtained from sheep flocks in the Iğdır province. In total, both blood samples and vaginal samples from 100 aborted sheep were analyzed for *C. abortus* and *C. burnetii*. Both agents were investigated by ELISA in serum and by direct PCR in vaginal swabs. The results of the study indicate that 44% of the sheep sera exhibited positive antibody reactivity to *C. burnetii.* Five out of 100 sera samples from sheep tested were positive for ovine enzootic abortion. In addition, three samples were serologically positive for both agents. Molecular analyses of vaginal swabs were negative for both agents. The results of this study confirm the existence of exposure of sheep flocks in the Iğdır province to both agents. The detection of Q fever and OEA in abortive sheep indicates that these pathogens carry a risk of infection in humans due to their zoonotic properties.

Keywords: *Aborted sheep, chlamydiosis, Q fever, seroprevalence*

INTRODUCTION

Sheep breeding, which is an important part of the animal husbandry sector, is quite common in Eastern Anatolia, Türkiye (Ertaş et al., 2022). In our country, sheep are raised especially to meet the red meat deficit (Öztürkler, 2015), and many products such as milk, wool, and leather are also obtained (Ertaş et al., 2022). The issue of abortion represents a significant challenge for the livestock industry, particularly in the case of small ruminants. The loss of fetuses and subsequent reduction in milk production can result in considerable economic losses for the livestock industry. It is established that the occurrence of abortion in animals has an impact on human health and animal welfare (Karagül et al., 2019; Sebestiani et al., 2018). Several different factors, including both infectious and non-infectious agents may cause abortions. Infections are the main factor in abortions and can sometimes co-infection in the same cases (Santos et al., 2022; Sebastiani et al., 2018). Bacterial agents that are responsible for abortion in domestic mammals include *Brucella*, *Salmonella, Coxiella burnetii, Campylobacter,* and *Chlamydia* species (Abnaroodheleh et al., 2021; Sebastiani et al., 2018). In studies conducted in Türkiye, although abortion cases are generally caused by brucellosis, it has been demonstrated that *Chlamydia abortus* and *C. burnetii* are bacteria capable of causing abortions, with significant detection rates in ovine and caprine populations (Gülmez Sağlam and Şahin, 2016; Karaca et al., 2009; Karagül et al., 2019). The importance of identifying these diseases is related to their prevalence as causative agents and their potential for transmission between animals and humans.

Coxiella burnetii is a Gram-negative bacterium that can infect many animals, including livestock, domestic and wild mammals, and other bird and cold-blooded animal species. Q fever is a potential cause of infection in the reproductive system of ruminants (Agerholm, 2013; Alkahachi et al., 2020). It is postulated that the majority of human cases are contracted via domestic animals (Ramo et al., 2022; Wolf et al., 2020). In a study conducted by Kaplan et al. (2024) in Erzurum province, *C. burnetii* was detected in 14% of the milk samples offered for sale and revealed that animal products may be important in terms of public health.

Ovine enzootic abortion (OEA) is a disease of considerable economic importance to sheep and goat farming worldwide. It is caused by *C. abortus* and affects sheep and several other ruminants (Borel et al., 2018). Small ruminants infected with *C. abortus* may abort three weeks before parturition. However, some animals do not show any preliminary clinical signs of impending abortion. Nevertheless, in certain animals, the presence of vaginal discharge or changes in behavior may be observed 48 hours before the onset of abortion (Villagra et al., 2015). The contamination of the environment continues by vaginal discharge for up to two weeks (Rodolakis et al., 2015).

Chlamydia abortus and *C. burnetii* cause clinical findings such as miscarriage, stillbirth, weak offspring, in-

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fertility, and premature birth, which are considered reproductive disorders (Hamedi et al., 2020; Hireche et al., 2016). *C. abortus* and *C. burnetii* can cause co-infection in animals. The risk of transmission of these diseases is higher during birth when high amounts of individual bacteria are released. Infection may occur through inhalation, ingestion, or direct contact with birth fluids or the placenta. Bacteria have also the potential to be shed in milk, feces, and urine. Individuals who have direct contact with infected animals, including farmers and veterinarians, are considered to be at elevated risk of infection (Santos et al., 2022).

Chlamydia abortus and *C. burnetii* the obligate intracellular agents cannot be cultured in standard laboratory media due to their inability to survive and replicate outside of a host cell. Isolation is a prolonged and expensive process that carries inherent risks. A reliable and rapid laboratory diagnosis is essential for the accurate identification of infection within the herd. For laboratory diagnosis of both agents, Identification methods can be divided into two categories: direct and indirect. Direct diagnosis of the causative agent can be made using molecular techniques, isolation, and identification from clinical samples. Serological tests such as ELISA are defined as indirect methods and are used to detect the antibody response to the pathogen in the host (Gülmez and Şahin, 2016; Santos et al., 2022).

In this study, it was aimed to investigate the prevalence of Q fever and ovine enzootic abortion in sheep with an abortion history in the Igdır and to determine the role of these disease agents in abortion cases.

MATERIAL AND METHODS

Field study area

The city of Iğdır is situated within the Erzurum-Kars region of the Eastern Anatolia Region in Turkey. The northern and northeastern border is formed by the Aras River and the Armenian border along the bed of this river. The region is bordered to the east and southeast by Nakhichevan and Iran, to the south by Ağrı province, and the west and northwest by Kars Province.

Sampling

The present study was conducted on sheep with a known background of abortion in the Iğdır province, Türkiye. A total of 100 sheep from 5 different foci, all of which were aged between 2 and 4 years and belonging to the Morkaraman breed, were evaluated in 1 month after abortions. In the study, 5 ml blood samples were taken from sheep's *V. cephalica antebrachii* into one tube one of which contains a silica gel (for serum analysis).

Vaginal swabs were taken from sheep with a history of abortion within the first 30 days postpartum. After the vulva lips were thoroughly cleaned, the lips were separated, and samples were taken from the vaginal wall just anterior to the cervix. To prevent contamination with urine, the swab stick was directed inward from the upper part of the external urethral opening. The swab stick was rotated approximately 4-5 times before being slowly removed. It was then placed in a special medium inside the swab box and sent to the laboratory.

Serological methods

Detection of the Coxiella burnetii

The serum was obtained from the sheep blood samples through the centrifugation of tubes at 3000 rpm for 10 minutes. Thereafter, the ELISA, which is a serological test for the detection of host antibodies against *C. burnetii* and one of the most preferred tests for the detection of this disease, was used. To this end, a Q fever antibody test kit (IDscreen® Q fever indirect multi-species, France) was utilized, following the manufacturer's recommended protocol. The results were analyzed utilizing an ELISA reader at a wavelength of 450 nm. Results were calculated by the formula specified in the kit procedure, as shown below:

An S/P (%) value of more than 80% was considered as strong positive; an S/P value between 50% and 80% was considered positive; an S/P value between 40% and 50% was considered doubtful and an S/P value less than 40% was considered as negative.

Detection of the Chlamydia abortus

The ELISA test kit (CHLMS-MS, ID.vet, France, microwells coated with *C. abortus* specific antigen (Momp)) was used to detect the host response against *C. abortus* according to the instructions of the test kit. An ELISA reader was used to read the results. The results were expressed as a percentage of optical density readings for the test samples. Calculations were performed according to the following criteria: OD% < 50 was defined as negative, OD% > 50 and OD% > 60 were classified as doubtful, while OD% > 60 was considered positive about *C. abortus*.

Molecular Methods

To obtain a direct DNA extract, 100 vaginal swab samples were taken from the aborted animals and extracted using a DNA Mini Kit (PureLink™, K182002, USA). This was done by the instructions provided by the manufacturer.

Detection of the Coxiella burnetii

Coxiella burnetii was identified using Trans-PCR that targets IS1111A transposase gene. The Trans I and Trans II primers were obtained and used to target this region of the gene. The expected product is 687 bp (Berri et al., 2000). PCR was performed using 4 µL of each extracted DNA, with a total volume of 25 µL. The final mixture comprised 2 µM of each primer, 200 µM of each dNTP, 3 mM $MgCl_2$, and 0.5 U of Taq DNA polymerase.

DNA amplification was performed using a thermal cycler (Bio-Rad, MJ Mini Gradient Thermal Cycler, PTC-1148). The trans-PCR thermal program was modified by lowering the annealing temperature and 'touchdown' PCR was performed as suggested by Berri et al., (2000). Following the amplification, the products were analyzed with 1.5% agarose gel electrophoresis. Bands with 687 bp in length were considered as *C. burnetii*. *C. burnetii Nine-Mile I* strain obtained from Ankara University, Veterinary Faculty was used as a positive control. ddH_2O was used as a negative control.

Detection of the Chlamydia abortus

PCR analysis targeted the polymorphic membrane protein (pmp) gene of *C. abortus* (Greco et al., 2005). For this purpose, a total volume of 25 µL was used for the PCR reaction, consisting of 2.5 µL 10X PCR Buffer, 0.5 µL dNTP mix, 2 µL $MgCl_2$, 12.75 µL H_2O, 1 µL primers (20 pmol/µL), 0.25 µL Taq polymerase and 5 µL DNA. The reaction's thermal cycling was conducted by optimizing the methodology outlined by Greco. et al., (2005). The amplified products were examined using 1.5% gel electrophoresis, focusing on those with a size of 300 base pairs. *C. abortus* DNA previously obtained from sheep abortion and confirmed by Real-time PCR was used as a positive control (Büyük et al., 2020). ddH_2O was used as a negative control.

RESULTS

This study investigated of *C. burnetii* and *C. abortus* in the sera and vaginal swabs of sheep. Out of 100 sheep with aborted history for *C. burnetii*, 41 were negative, 15 were doubtful, 24 were positive, and 20 were strongly positive (Figure 1). 44 of 100 sheep's sera were determined to be positive for *C. burnetii* (Figure 2). *C. abortus* was investigated by indirect ELISA assay among the sheep's sera. Five out of 100 sera samples from sheep tested were positive for OEA. Three samples positive for *C. abortus* were also positive for Q fever (Figure 3). The molecular analyses of the vaginal swabs were found to be negative for both agents.

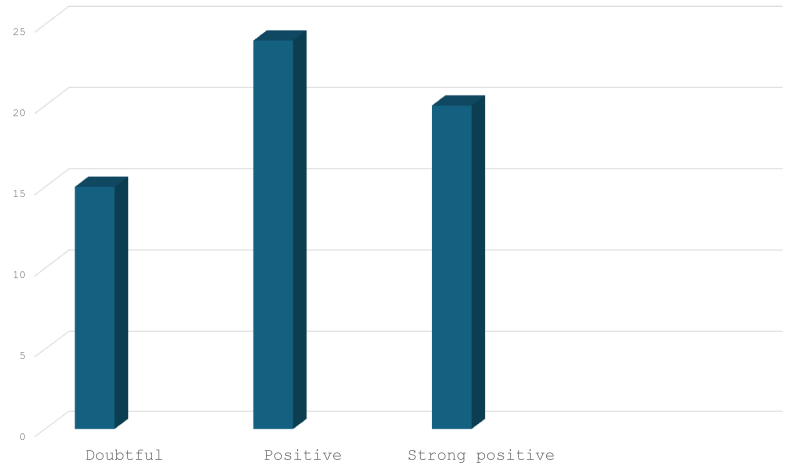

Figure 1. *Results of indirect ELISA for Q fever according to their ODs*

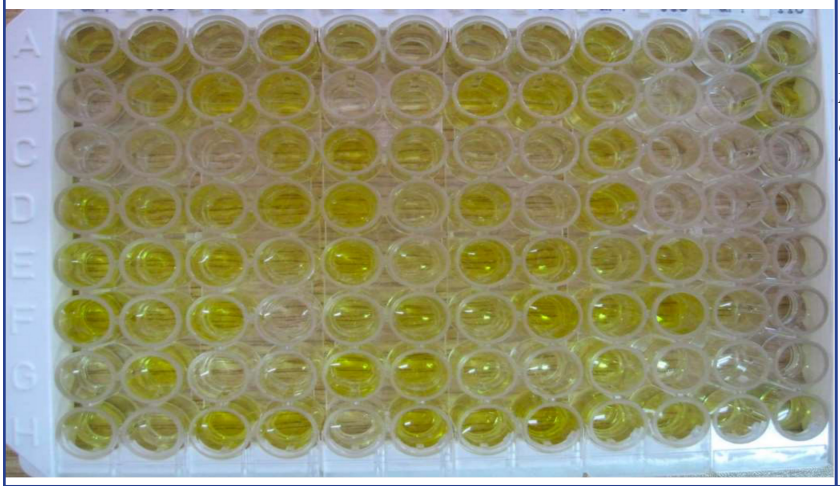

Figure 2. *View of the ELISA on the microplate. A-1: Positive control, B-1: Negative control, other wells show samples with positive and negative reactions*

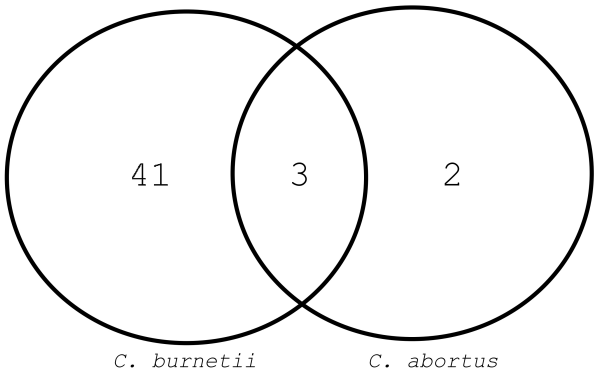

Figure 3. *Total prevalence of both pathogens in Iğdır province*

DISCUSSION

The etiology of abortion in ovine and caprine species is multifactorial, resulting from infection by a range of bacterial, viral, parasitic, and mycotic agents (Ali et al., 2022; Gülaydın et al., 2023). A major concern for breeders is the economic impact of abortion on sheep and goats. This study investigated the occurrence of *C. abortus* and *C. burnetii* infections, which are important abortigenic agents, in aborted sheep in the Iğdır province using serological and molecular methods. This was tested using an indirect ELISA. The observed antibody response indicates a natural immune response to microbial exposure, given that there is no vaccination program against both factors in Türkiye.

Coxiella burnetii is a zoonotic agent that has the potential to infect humans and many domestic animals (McQuiston and Childs, 2002), as well as healthy animals and the environment through aborted fetuses and fetal fluids, and secretions of infected animals (Kılbaş et al., 2023). As a result of reviewing worldwide studies on the prevalence of Q fever in livestock, it has been reported that the seroprevalence of Q fever levels is 15-27% in many countries, regardless of species. According to meta-analyses by Guatteo et al., (2011) and Nokhodian et al., (2017), the seroprevalence rates of *C. burnetii* in sheep and goats are closely compatible with findings from various global meta-analyses and systematic reviews. In Türkiye, Kılbaş et al., (2023) reported an overall prevalence of 13.49% in animals and an average prevalence of 19.1% in sheep for *C. burnetii*. In a study conducted on sheep by Kılıç and Kalander, (2016), the percentage of *C. burnetii* in the Eastern Anatolian region was determined by ELISA to be 16% in aborted sheep flocks and 7.60% in healthy sheep flocks. Karagül et al., (2019) stated that in their study conducted on sheep in Düzce province, the overall rate of seropositivity for Q fever was determined to be 26.38%. However, they determined the seropositive herd rate to be 50%, higher than the total seroprevalence. There are also studies aimed at investigating the status of *C. burnetii* in animals in the Northeast Anatolian region. In a study conducted on small ruminants in this region, *C. burnetii* was detected in 24.4% of sheep (Serifoğlu Bagatir et al., 2021). A study carried out in the province of Kars showed that 43.2% of the sheep were positive for *C. burnetii* using the ELISA (Gülmez Saglam and Şahin, 2016). In the current study, out of 100 sheep with aborted history for *C. burnetii*, 15 samples were found doubtful, 24 were positive, 20 were strong positive and, 41 were negative. 44 of the 100 sheep's sera were determined to be positive in terms of antibodies against phase I and phase II *C. burnetii*. Compared to other national and international studies, the overall positivity rate of *C. burnetii* in this research is higher than that reported previously. Following the study, the seroprevalence of *C. burnetii* was 44%, pointing to a considerable health risk for both animals and humans in these research areas. The importance of detecting *C. burnetii* increases, especially since small ruminants are considered reservoirs for humans.

Chlamydia abortus is an important bacterial pathogen because it causes abortions in sheep and can also cause infection in humans. Although it mostly causes infections in small ruminants, it can also infect other animal species (Sillis and Longbottom, 2011). In a study conducted by Hamedi et al., (2020) in Iraq, *C. abortus* was detected in 23.5% of aborted fetuses. In a study conducted in Germany by Runge, (2012), *C. abortus* was detected in 49% of sheep. In Iran, the seroprevalence value of *C. abortus* was reported to be 26.5% in small ruminants. (Esmaeili et al., 2015). Iraninezhad et al., (2020) found 44 (9.70%) of 452 sera positive for *C. abortus* in sheep and goats from Khorasan Razavi province in north-eastern Iran.

In Turkey, most cases of OEA-related abortion in domestic animals have been investigated using serological and molecular methods, and *Chlamydia* spp. have been detected at rates ranging from 1.56% to 32% (Gökçe et al., 2007; Karagül et al., 2019; Kaya and Öztürk, 2020; Küçükayan et al., 2007; Otlu et al., 2007, Öztürk et al., 2016; Türütoğlu et al., 2000). The seroprevalence of enzootic abortion in sheep was found to be 20.83% in a study conducted in Türkiye (Karagül et al., 2019). Çaya et al., (2006), in their study conducted in 9 provinces in the south-eastern and Mediterranean regions, detected *C. abortus* at rates varying between 2.5-30%, and *C. abortus* was detected at significant levels in most provinces. In Kars, the neighboring province of Iğdır, where the current study was conducted, Gökçe et al., (2007) found a seropositivity rate of 13.9% in aborted sheep and 8.33% in cattle. Otlu et al., (2007) found the seropositivity rate to be 5.4% in their study of aborted sheep in Kars. In a study conducted by Öztürk et al., in Burdur province in 2016, the disease prevalence in sheep was determined to be 32.0% by ELISA.

In the current study, serum samples from aborted sheep were tested by ELISA. *C. abortus* was detected in 5 of 100 aborted sheep. The average rate of enzootic abortion in the Iğdır region is low compared to other studies conducted in Türkiye. When results for both *C. burnetii* and *C. abortus* are compared with other studies, geographical location, test type and efficacy, race, sample size and type, grazing strategy and population density may play a role in differences in results (Abushahba et al., 2017; Radostits et al., 2007).

Given that *C. burnetii* and *C. abortus* are obligate intracellular pathogens, laboratory investigation was not feasible. Consequently, this study examined 100 vaginal swabs collected from aborted ewes via molecular analysis for these agents. Both agents were not identified using the molecular method. Among the reasons why molecular methods were negative is that after miscarriage, the shedding of pathogens is intense but decreases over time and is intermittent. Therefore, vaginal swabs do not have a sufficient bacterial load. Serologic tests used in epidemiologic studies show that the organism was previously exposed to the disease but do not show bacteremia or that it is still shedding the agent. Serologic tests and molecular methods may give different results.

CONCLUSION

As a result, it was observed that the seroprevalence of Q fever in small ruminants was high in the Iğdır province. It can be said that *C. burnetii* is one of the main causes of abortion, given the low abortion rate evaluated in the study. The diagnosis of both diseases is an important step in the identification of cases of abortion in flocks and the establishment of effective control measures. The results

of this study show a high incidence of Q fever in sheep in Iğdır province, followed by a lower seroprevalence for OEA. These results underlined the potential risk of the pathogens studied for animal and public health. In the Iğdır province, where the study was conducted, sheep farming is done as family-type enterprises. The animals are cared for, fed and milked using traditional methods. This situation poses a risk of transmission to humans of agents that may be excreted in milk. Based on these findings, prevention and control measures should be based on their potential impact on animals and humans. Additionally, it is recommended that further studies be conducted to improve comprehension of the transmission mechanisms of this pathogen and to develop strategies for the mitigation of the associated risks. Exploring potential reservoirs and intermediate hosts, improving surveillance systems, and enhancing biosecurity measures are crucial steps in controlling the spread of the pathogen.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical statement

The ethical approval of the study was obtained from the Local Ethics Committee for Animal Experiments of Kafkas University (Türkiye) (KAU-HADYEK/2023/168).

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization was carried out by AGS, SK, and GK.; methodology, validation, and data curation, AGS, EÇ, SD, and MY; writing—original draft preparation, AGS, EÇ and SD; writing—review and editing, AGS, SK, and GK. All authors have read and agreed to the published version of the manuscript.

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