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Investigation of *Chlamydia abortus* from aborted ewes in Hakkâri province and its districts by enzyme-linked immunosorbent assay (ELISA)

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ABSTRACT

In this study, it was aimed to determine the cases of *Chlamydia abortus* in 180 ewes that aborted during lambing period in different flocks in Hakkari province and it's district in Türkiye. Blood samples were collected from ewes that had aborted, using the jugular vein in accordance with the technique. The serum obtained from the blood samples collected in biochemical serum tubes was tested for antibodies against *Chlamydia abortus* using an ELISA test kit (Sunlong sheep *Chlamydia abortus* ELISA test kit). According to the ELISA test results, 8 samples (4.4%) out of 180 samples collected from Hakkari province and its districts tested positive for *C. abortus*. The distribution of samples that tested positive for *C. abortus* according to the ELISA test results was as follows: 4 sheep in the central district of Hakkari, 3 sheep in the district of Yüksekova, and 1 sheep in the district of Şemdinli tested positive for *C. abortus*. As a result, for the first time, data on the prevalence of *C. abortus* in sheep farms in Hakkari province center, Yüksekova and Şemdinli districts were obtained. The data obtained were shared with the animal health branch directorates in the agricultural directorates in Hakkari provinces and districts. Especially in the last decade, it will contribute to the development of activities for the development of preventive measures against *C. abortus*, which is one of the most important factors causing abortion in sheep farms.

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Introduction

Abortions are among the main causes of productivity losses in small ruminants. Nutritional deficiencies, traumatic causes, bacteria and viral agents play a role as causes of abortion in sheeps. (Gülaydın et al., 2023). Particularly bacterial agents are mainly cause of abortions in different countries around the World (Abnaroodheleh et al., 2021). Enzootic abortions in sheep and goats are widespread throughout the world and *Chlamydia abortus*, a bacterium of significant importance in the livestock industry, has been the subject of comparatively less research attention than other bacterial agents causing abortion (Abnaroodeh et al., 2021; Esmaeili et al., 2015). However, recent studies have indicated that it may be as important in the sheep industry as other bacterial agents. *Chlamydia abortus* is recognized as a genital tract pathogen and an important cause of reproductive failure, especially in dense sheep flocks (Huang et al., 2013). It is emphasized that *C. abortus* is an important cause of abortions with endemic course in many regions of the world, including countries neighboring Turkiye (Malal, 2020). Furthermore, *C. abortus* is recognized as one of the identified zoonotic chlamydia species (Giannitti et al., 2016). Similarly, in human medicine, it has been reported that *C. abortus* causes abortions in humans and this poses a danger to public health (Malal, 2020).

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The diagnosis of *C. abortus* in aborted sheep is based on laboratory analysis using direct or indirect methods. There are difficulties in identifying the causative agent due to the need for cell culture and experienced personnel, and because the procedures are challenging and time-consuming. Therefore, serological tests are widely used in the diagnosis of *C. abortus* (Çaya et al., 2006). Determining the pathogens that cause abortions in sheep, knowing the environmental variables and detailed information related to factors such as care and nutrition are important in the prevention of abortions. One of the major causes of lamb losses and abortions worldwide is infection with *C abortus*, described as enzootic abortion of ewes (OEA) (Longbottom et al., 2013). Infected sheep shed large numbers of *C. abortus* agents into the external environment via feces, urine, nasal and eye discharge (Jonker and Michel 2023), placenta, uterine discharge and abortion material (Sargison et al., 2015; Fayez et al., 2021). It is reported to be transmitted by ingestion of contaminated grasses or inhalation of air in the environment contaminated by the discharge and rarely by mating (Marzok et al. 2023; Jonker and Michel 2023). For the diagnosis of the disease, pathogen isolation in vaginal, conjunctival and rectal specimens from aborted cases and fetal tissue (lung, liver, brain, spleen, kidney and abomasum) specimens from waste lambs, indirect serological tests (complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA)) or immunofluorescence directly in cell culture, immunohistochemistry and DNA -based methods (polymerase chain reaction and DNA microarray) (Aldama et al., 2022; Kalender et al., 2013). For the diagnosis of the disease, pathogen isolation is made from samples taken from vaginal, conjunctival and rectal regions in abortion cases and from fetal tissue (lung, liver, brain, spleen, kidney and abomasum) samples from aborted lambs, indirect serological tests; complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA) or direct cell culture (immunofluorescence, immunohistochemistry and DNA -based methods (polymerase chain reaction and DNA microarray) (Aldama et al., 2022; Kalender et al., 2013). Enzyme-linked immunosorbent assay (ELISA) commercial ELISA tests are available that detect antibodies against specific antibodies of *C. abortus* in the serum of affected sheep. However, the disadvantages of these commercial tests include interspecies cross-reactions (*C. abortus* and *C. pecorum*) that make specific diagnosis difficult (Aldama et al., 2022).

Hakkâri province is located in the Eastern Anatolia Region of Turkiye and is characterized by the geographical and seasonal characteristics of this region (Bingöl and Aygün 2014). Hakkâri province is located on a land with a high altitude and when we look at the flora pattern it has, it has very favorable pastures for animal husbandry (Hakkâri Provincial TOM, 2024), Looking at the number of sheep in Hakkâri province, it is seen that there are 476,462 sheep according to the data of 2022 (GAP, 2023) and in the light of these data, it is seen that sheep breeding activity is important in Hakkâri province.

In this study, it was aimed to investigate *C. abortus* by Enzyme-Linked Immunosorbent Assay (ELISA) in aborted sheep in Hakkâri Province and its districts and to provide a guide to veterinarians and sheep breeders in prevention and control strategies by determining their prevalence.

Materıal and Method

Animal materials: The animal material of this study consisted of 180 ewes between the ages of 2-6 years and in the lambing period, which were reared in Hakkâri city center and its districts, and which aborted except for traumatic reasons. In order to provide the necessary blood samples for the study, cooperation was established with veterinarians in the center and other districts of Hakkâri province. In line with the information of veterinarians working in public and private clinics in the center and districts of Hakkâri province, the existing sheep farms in the region were identified. The owners were first informed about the causes of abortion cases in sheep and the owners were informed. By ensuring cooperation between the owners and veterinarians, the flocks with aborting sheep were identified in line with the reporting of the presence of aborting sheep in their holdings by veterinarians and sheep breeding business owners. After taking the necessary biosecurity measures for sampling (mask, apron, gloves, boots, etc.), the ear tag number of the identified sheep was first recorded. Afterwards, the patient registration form was filled in for the sheep with abortion.

Animals and ethical approval: Approval for this study was obtained from the Animal Experiments Local Ethics Committee of the Van Yüzüncü Yıl University (Date: 27.07.2023, Number: 2023/09-01).

Collection of blood samples: The sheep were properly restrained and blood samples were collected from the Vena jugularis with 10 ml sterile syringes according to the technique. The blood samples were filled into 5 ml serum biochemical tubes. The serum biochemistry tube in which the blood sample was filled was numbered and the blood sampling process was terminated from the aborted sheep. Blood samples were taken from 180 aborted sheep in Hakkâri province and its districts, excluding traumatic abortion cases. Distribution of blood samples taken from sheep flocks according to districts in Figure 1.

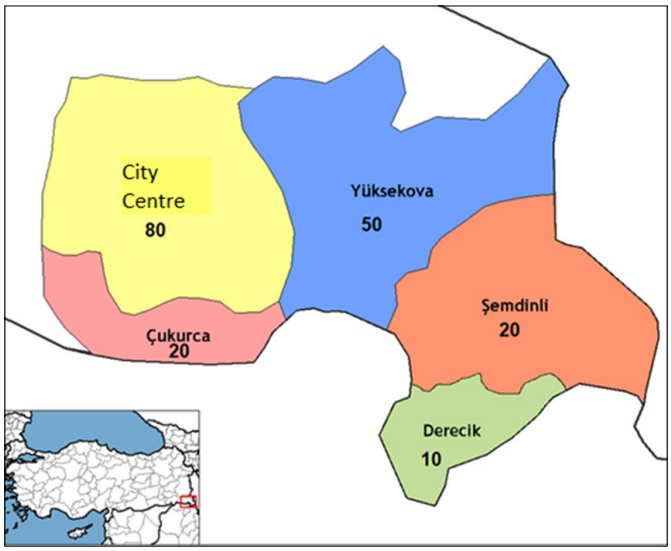

Figure 1. Distribution of blood samples taken from sheep flocks according to districts.

Collection of blood serum: Blood serum samples were taken from aborted ewes during lambing period and filled into biochemical serum tubes and then serum was removed by centrifugation at 3000 RPM (Revolutions per minute)/10 minutes. After centrifugation, the sera were collected in eppendorfs. The serum samples were then stored at -20 ^{o}C until analysis.

Examination of blood serum: The collected serum samples were analyzed by ELISA test kit (Sunlong sheep *Chlamydia abortus* ELISA test kit, Zhejiang, China) and analyzed by ELISA reader (SEAC SIRJO S, Burladingen, Germany).

Statistical analysis: For descriptive statistical analysis of the data obtained, the rates and frequencies of the agents were determined. SPSS (version-21) statistical package program was used in the calculations.

Results

According to the statistical analyses based on the ELISA results, the rate of *C. abortus* was found to be 5% in Hakkari city center, 6% in Yüksekova district and 5% in Şemdinli district. In addition, the average distribution rate of *C. abortus* seropositive in Hakkari and its districts was 4.4% (Table 1).

Based on the flocks of aborted sheep included in the research, it was reported by the breeders that 2 flocks in Hakkari provincial center and 2 flocks in Yüksekova district were vaccinated with brucella vaccine, while other flocks were not vaccinated with any protective vaccine. Moreover, it was determined that the study was the first pregnancy period of the aborted ewes. According to the information provided by the sheep farm owners during the research, it was determined that there was an increase in abortion cases in the flocks compared to the previous year. Proportional distribution of samples taken and analyzed with *C. abortus* ELISA test kit according to the districts is shown Table 2.

Table 1. Samples taken by settlement and number of herds sampled

Settlement area	Number of samples taken	Number of herds that taken samples
Center	80	16
Yüksekova	50	12
Şemdinli	20	7
Çukurca	20	5
Derecik	10	3
Total	180	43

Table 2. Proportional distribution of samples taken and analyzed with *C. abortus* ELISA test kit according to the districts.

Settlement area	Number of samples taken	Number of positive samples	Number of negative samples	Positive %
Center	80	4	76	5
Yüksekova	50	3	47	6
Şemdinli	20	1	19	5
Çukurca	20	0	20	0
Derecik	10	0	10	0
Total	180	8	172	4.4

Discussion

Members of the Chlamydiaceae family are a major public health concern with their worldwide prevalence and can infect many animals, especially sheep (Huang et al., 2013). Although *C. trachomatis* and *C. psittaci* were the first chlamydiae identified, many other chlamydia species were identified in the following period. *C. abortus*, first known as C. *psittaci* serotype 1, has been reported to be one of the most important etiologic agents in abortion cases worldwide (Giannitti et al., 2016). Chlamydial infection causes multiple diseases in livestock in numerous studies conducted worldwide (Kaltenboeck et al., 2005). Chlamydia members fall into the category of difficult to culture. Historically, cell culture or isolation in chicken embryos has been considered the 'gold standard' (Jonker and Michel, 2023). However, the diagnosis and screening of *C. abortus* infection in aborting sheep is largely based on antibody detection in serum samples (Wilson et al., 2009). In recent years, studies aiming to identify immunoreactive proteins for ELISA tests and the development of vaccine prototypes against diseases caused by Chlamydia species affecting small ruminants have gained considerable importance due to their public health, animal welfare and economic importance (Aldama et al., 2022). Many studies have been conducted worldwide to detect *C. abortus* in aborting sheep and the presence of the agent has been detected

at different rates in different countries (Malal, 2020; Orjuela et al., 2022; Hireche et al., 2022). Similarly, in various studies conducted in different parts of Türkiye, high rates of *C. abortus* were detected in various regions. The incidence of *C. abortus* varies across countries and can reach 30% in susceptible herds (Büyük et al., 2020). The rate of *C. abortus*-induced abortions in Turkiye's border neighbors was reported to be 35.8% in Bulgaria, 14.9% in Greece, and 11.0% to 38.0% in Iran (Malal, 2020). Turkiye and it was reported that the seroprevalence of the agent was detected at high rates according to the regions and caused significant financial losses (Öztürk et al., 2006). When the studies conducted in different regions of Turkiye were examined, these rates were determined as 20.8% in Düzce province and 32.0% in Burdur province (Öztürk et al., 2016), 19.05% in Kars province, 23% in Mersin province, 16% in Gaziantep province, 24% in Kahramanmaraş province, 3% in Şanlıurfa province, 14% in Kilis province, 3% in Hatay province, 1% in Adana province, 2% in Adıyaman province and 1% in Osmaniye province (Çaya et al., 2006). In a study conducted in the Black Sea region covering 8 provinces, the fetuses of 393 aborted ewes were examined and 65 ewe fetuses (16.5%) were found to be seropositive for *C. abortus* (Akpınar et al., 2024). Looking at the studies conducted worldwide, *C. abortus* seroprevalence was reported to be 21.5% in Brazil, 31.1% in Mexico and 20.9% in Tibet (Esamaeili et al., 2015). In a study conducted with 440 sheep in Germany, 7% (31 sheep), in a study conducted with 304 sheep in Britain, 30.9%, in Taiwan, 23.8%, in Tunisia, 8.7%, in a study conducted with 199 sheep in Algeria, 6.5% (13 sheep) were seropositive for *C. abortus* infection (Esamaeili et al., 2021). It is thought that differences in *C. abortus* infection rates in all of these studies may be due to differences in animal breeds, sampling methods and times, differences in detection methods, environmental conditions of each country and differences in infection rates (Esmaeili et al., 2021).

It is stated that Hakkari province constitutes 2.46% of Turkiye's meadow and pasture area with 369,610 hectares of meadow and pasture area and this situation is of great importance in shaping the tendency towards sheep-goat breeding in the region (Demir and Seçkin, 2022). In this study, it was observed that no study was conducted to detect *C. abortus* in Hakkâri province and its districts. In Hakkâri province, where sheep farming is an important field of endeavor, it was seen that it is important to carry out a study for the detection of *C. abortus* bacteria, which causes enzootic sheep abortions that cause great economic losses. In this study, it was aimed to determine the presence of *C. abortus* by ELISA test kit by extracting serum from blood samples taken from sheep that aborted during lambing period in Hakkâri province and its districts.

Conclusion

According to the statistical data obtained in this study, as a result of the analysis of serum samples taken from 180 ewes that were not vaccinated against *C. abortus* and aborted during the lambing season in Hakkâri province, *C. abortus* ELISA test positivity was detected in approximately 8 samples (4.4%) throughout the province. Out of 80 samples taken from Hakkari provincial center, 4 samples (5%) out of 80 samples, 3 samples (6%) out of 50 samples taken from Yüksekova, 1 sample (5%) out of 20 samples taken from Şemdinli were positive for *C. abortus* ELISA test. C. abortus ELISA test was negative in Derecik and Çukurca districts. According to these results, it was observed that the prevalence of *C. abortus* infection was higher in Yüksekova district of Hakkari province compared to the provincial center and Şemdinli district, and this prevalence was equally distributed in the provincial center and Şemdinli district. In conclusion; although the presence of *C. abortus* infection in Hakkari province and its districts was low, seropositivity was detected in some areas. The presence of *C. abortus* infection in the region indicates that importance should be given to taking protective measures against the agent. In this context, necessary protection and control programs were recommended to both the public and producers.

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