SEROPREVALENCE OF CHLAMYDOPHILA ABORTUS IN ABORTING EWES AND DAIRY CATTLE IN THE NORTH-EAST PART OF TURKEY

HALIL IBRAHIM GOKCE, CIHAN KACAR¹, OKTAY GENC², AND MAHMUT SOZMEN³

Department of Internal Medicine, ¹Department of Obstetrics and Gynaecology, ²Department of Microbiology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, 55139, Samsun, Turkey ³Department of Pathology, Faculty of Veterinary Medicine, Kafkas University, 36100, Kars, Turkey
devrek@hotmail.com

Received for publication December 14, 2006.

Abstract

The aim of the study was to determine the seroprevalence of Chlamydia abortus in aborting ewes and cattle in the Kars province in the north-eastern part of Turkey. Thirty sheep flocks and 26 herds of dairy cattle out of 4 districts in the province were examined by ELISA. For this purpose, abortion cases of 236 sheep and 192 cows of different breeds and age were investigated serologically during lambing and calving season. Subsequently, the rate of abortion in ewes and cows were 10.25% (236/2302) and 28.23% (192/680), respectively. Antibodies against C. abortus were found in 46.66% (14/30) of sheep flocks and 26.92% (7/26) cattle herds examined. In the study, 13.98% (33/236) of aborted sheep and 8.33% (16/192) of cattle were positive for antibodies specific to C. abortus. Seroprevalence of C. abortus in sheep and cattle ranged from 5.40 to 18.29% and 4.76 to 12.67%, respectively. In conclusion, C. abortus causes abortion in both sheep and dairy cattle in the north-eastern part of Turkey. It is recommended that seropositive animals should be eliminated from flocks and herds. An appropriate vaccine against C. abortus should also be applied for ewes and cows to reduce the incidences of abortion.

Key words: cattle, sheep, abortion, Chlamydia abortus, seroprevalence.

Abortion has been a serious economic problem in domesticated ruminants worldwide. Several bacterial, viral, parasitic, and nutritional factors cause abortion in animals (6, 12, 16, 22). Chlamydia (C) abortus is one of these abortifacient pathogens in sheep and cattle (6, 14, 15, 20, 21, 27). It causes ovine enzootic abortion in sheep and epizootic bovine abortion in cattle (6, 22). Chlamydia abortus is a Gram-negative, intracellular bacterium that was formerly known as Chlamydia psittaci serotype 1 (10). It is considered as one of the most economically important animal pathogens of domesticated animals, which causes abortion, weak neonates and foetal loss, infertility and mastitis in sheep, goats, and cattle in many countries around the world (6, 7, 22). The bacterium is also a zoonotic agent that causes abortion and other clinical symptoms in humans (24, 28, 29).

Clinical diagnosis is often difficult because the clinical signs and pathological lesions are not specific for C. abortus infection, and can also be observed in abortions caused by other agents. Diagnosis of C. abortus infection can be achieved by various antigen detection techniques, including PCR, histochemical and immunological staining of smears of placental tissue or chlamydial inclusions following the isolation of the organism in cell culture (6, 20, 22, 25). However, all of these tests are dependent on the acquisition of high quality, well-preserved diagnostic material or the specialist culturing competence and experience. On the other hand, a number of serological techniques such as immunofluorescence tests (IFATs), ELISAs, and the complement fixation test (CFT), have also been widely used in veterinary laboratories for detecting the infected animals. However, antigenic cross-reactivity between C. abortus and C. pecorum, as well as with some Gram-negative bacteria like Acinetobacter can cause false positive results in CFT and IFAT (6, 18, 21, 26). The false positive results can be avoided by using more sensitive and specific methods like competitive and indirect ELISA based on a recombinant protein fragment of the C. abortus polymorphic outer membrane protein (POMP). These tests have been suggested to be highly sensitive and specific, showing no cross-reactivity with animals infected with C. pecorum. They were recommended to be suitable for the use in the diagnosis of C. abortus infection in animals and humans (17, 18).

Abortion rate is quite high in both sheep and cattle in the Kars province in the north-eastern part of Turkey. However, there are a few published studies on this subject in the province, and the majority of
causative agents of abortion in sheep and cattle are not well studied. In these studies antibodies against Neospora caninum, Brucella abortus, Leptospora hardjo, and L. grippotyphosa were detected in ewes and cows (1, 11). Therefore, the majority of the causative agents of abortion in ewes and cows have not been well established in the Kars province. It is well known that the bacterium is zoonotic and people, particularly pregnant women, who are in close contact with the infected or carrier animals, are also at risk of infection (6, 22, 24, 28, 29). Therefore, it is essential to conduct serological tests to detect infected and carrier animals to keep the flocks and herds free of the infection. The elimination of infected and carrier animals may also be important to reduce environmental contamination, thus limiting the spread of infection and the possible risk of zoonotic transmission to humans.

The aim of the present study was to determine the prevalence of antibodies to C. abortus in aborting ewes and dairy cattle in Kars province in the north-eastern part of Turkey.

Material and Methods

**Samples.** Thirty sheep flocks and 26 herds of dairy cattle out of 4 districts in the Kars province in the north-eastern part of Turkey were examined by an indirect ELISA commercial kit, for the presence of antibodies against C. abortus (Chlamydia psittaci serotype 1). For this purpose, 236 aborting sheep and 192 cows of different age and breeds were under observation. Sheep and dairy farms in Kars (centre) and its three districts (Susz, Selim, and Kagszman), were regularly visited in the lambing and calving season, and blood samples were collected from each animal within 10 d after the abortion.

**ELISA procedure.** C. abortus-specific antibodies were determined in the sera by an indirect ELISA commercial kit (C. abortus serum verification kit, Pourquier Institute, France) according to the manufacturer’s instructions. Briefly, 200 µl of 1:20 dilutions of test samples, negative and positive control sera were added into each appropriate well. After incubation at 37°C for 60 min and washing, an optimal dilution of protein G peroxidase conjugate (100 µl) was added, followed by further incubation for 60 min and washing. A freshly prepared revelation solution (100 µl of enzyme substrate, TMB) was dispensed to each well and the plates were left in the dark at room temperature, before stopping the reaction, by adding 100 µl of stop solution (1M phosphoric acid). Finally, the optical density (OD) of each well was determined with a micro-ELISA plate reader (Tecan-spectra, Austria) at a test wavelength of 450 nm.

The corrected net OD 450 for each serum was calculated by subtracting the OD 450 value of the uncoated well from the OD 450 of the coated well. The results were considered as reliable if the positive control had a minimum uncorrected mean OD 450 value of 0.350, and the ratio between the corrected mean OD 450 value of the positive control and the corrected OD 450 value of the negative control was greater than or equal to 3.5.

S/P percentage for each sample was calculated for the interpretation of test samples as follows: S/P% = corrected OD 450 of the sample / mean corrected OD 450 of the positive control X 100. The results were defined as negative, doubtful, positive, and strong positive, according to the manufacturer’s validation criteria.

**Statistical analysis.** The results were expressed as the percentage of positive levels for both sheep and cattle. Chi-squared test was used to compare the differences in positive levels of test results between districts.

Results

The abortion rate in sheep and dairy cattle were 10.25% (236/2302) and 28.23% (192/680), respectively (Table 1). Antibodies against C. abortus were found in 46.66% (14/30) of sheep flocks and 26.92% (7/26) cattle herds examined. In the study, 13.98% (33/236) of aborting ewes and 8.33% (16/192) of aborting cows were positive for antibodies specific to C. abortus. Seroprevalence of C. abortus in sheep and cattle ranged from 5.40 to 18.29% and from 4.76 to 12.67%, respectively. Seroprevalence was the highest in Kagszman for sheep (18.29%) and in Kars centre for cattle (12.67%) (Table 2). Furthermore, 4.23% (10/236) of ewes’ and 6.77% (13/192) of cows’ sera were found to be doubtful for C. abortus antibodies according to the validation criteria given by the manufacturer (Table 2). Abortion cases were recorded within the 3rd (40.25%), 4th (36.44%), and 5th (23.30%) month of gestation in sheep and within the 6th (40.1%), 7th (25%), and 8th (34.89%) month of gestation in cattle.

Discussion

In the present study, 2302 ewes and 680 cows were examined and 10.25% of the ewes and 28.23% of the cows were found to have aborted during the lambing and calving season. Moreover, antibodies against C. abortus were found in almost 46.66% of sheep flocks and 26.92% of cattle herds examined. In addition to this, C. abortus-specific antibodies were detected in 13.98% of ewes and 8.33% of cows. Seroprevalence of C. abortus in sheep and cattle ranged from 5.40 to 18.29% and 4.76 to 12.67%, respectively. Seroprevalence was highest in Kagszman for sheep (18.29%) and in Kars centre for cattle (12.67%).

The abortion rate is quite high in both sheep and cattle in the Kars province. However, there is very limited published data on the abortion in ewes and cows in the province. In a single study, seroprevalence of brucellosis and leptospirosis in aborting cattle was reported to be 68.1% and 23.9%, respectively (11).
### Table 1
The percentage (number) of abortion in sheep flocks and cattle herds

<table>
<thead>
<tr>
<th></th>
<th>Sheep</th>
<th></th>
<th></th>
<th>Cattle</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>District</td>
<td>Number of flocks</td>
<td>Number of ewes</td>
<td>Abortion rate (%)</td>
<td>District</td>
</tr>
<tr>
<td></td>
<td>Kars (Centre)</td>
<td>6</td>
<td>865</td>
<td>7.97 (69/865)</td>
<td>Kars (Centre)</td>
</tr>
<tr>
<td></td>
<td>Selim</td>
<td>11</td>
<td>560</td>
<td>13.21 (74/560)</td>
<td>Selim</td>
</tr>
<tr>
<td></td>
<td>Kagzman</td>
<td>11</td>
<td>627</td>
<td>13.07 (82/620)</td>
<td>Kagzman</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>2302</td>
<td>10.25 (236/2302)</td>
<td>Total</td>
</tr>
</tbody>
</table>

### Table 2
Percentage (number) of the seropositivity for *C. abortus* antibodies in aborting ewes and cows

<table>
<thead>
<tr>
<th></th>
<th>Sheep</th>
<th></th>
<th></th>
<th>Cattle</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>District</td>
<td>Percentage of positive sheep</td>
<td>Percentage of doubtful ewes</td>
<td>Percentage of negative ewes</td>
<td>District</td>
</tr>
<tr>
<td></td>
<td>Kars (Centre)</td>
<td>17.39 (12/69)</td>
<td>7.24 (5/69)</td>
<td>75.36 (52/69)</td>
<td>Kars (Centre)</td>
</tr>
<tr>
<td></td>
<td>Susuz</td>
<td>18.18 (2/11)</td>
<td>9.09 (1/11)</td>
<td>72.72 (8/11)</td>
<td>Susuz</td>
</tr>
<tr>
<td></td>
<td>Selim</td>
<td>5.40 (4/74)</td>
<td>0 (0/74)</td>
<td>94.59 (70/74)</td>
<td>Selim</td>
</tr>
<tr>
<td></td>
<td>Kagzman</td>
<td>18.29 (15/82)</td>
<td>4.87 (4/82)</td>
<td>76.82 (63/82)</td>
<td>Kagzman</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>13.98 (33/236)</td>
<td>4.23 (10/236)</td>
<td>81.77 (193/236)</td>
<td>Total</td>
</tr>
</tbody>
</table>

Furthermore, 8.2% of Simmental cows imported from Germany and their offspring were found to be positive for *N. caninum* antibodies, while local breeds were seronegative in the Kars province (1). The prevalence of *C. abortus* worldwide has been reported to range from 5% to 39% in sheep (2, 5, 6,) and from 5% to 20% in cattle (4, 6, 9). In Turkey, there are a few published reports on abortion caused by *C. abortus*. In these studies, the prevalence of *C. abortus* has been shown to range between 17% and 20% (3, 8) in aborting ewes. There is a single study on *Chlamydia psittaci* in aborting sheep in the Kars province. In this study, *Chlamydia psittaci*-specific antibodies were detected in 19.05% and 17.95% of aborting sheep by CFT and ELISA, respectively (3). However, chlamydial LPS antigen was used in this study and cross-reactivity with *C. pecorum* and other Gram-negative bacteria was not eliminated. Therefore, seropositivity for *Chlamydia psittaci* could be due to cross-reactivity, and the result may not represent the real seropositivity for *C. abortus* in aborting ewes.

The ELISA technique used in the present study is based on a recombinant antigen, a 80-90 kDa protein that is specific for *C. abortus* and that it does not cross react with *C. pecorum* (17, 18). Therefore, the results presented herein seem to be confident and indicate the presence of *C. abortus*-specific antibodies in both aborting ewes and dairy cows in the Kars province. Infected females shed vast numbers of infective *C. abortus* organisms at the time of abortion or parturition, particularly in the placenta and uterine discharges. The organisms are also shed in milk, faeces, and nasal and ocular discharges of aborting animals. *C. abortus* can also be shed in semen and transmitted to cows, possibly leading to embryonic death or infertility (6, 7, 14, 15, 20, 22, 23). Furthermore, wild animals may also serve as reservoir for this organism and play a role in the contamination of the environment and spread of the disease (4, 13). Susceptible animals are infected through ingestion or inhalation of *C. abortus*-infected material, as a result of contamination of lambing and
calving pens or of pasture by foetal membranes and discharges. In non-pregnant animals, the organism can exist in a latent form, possibly in lymphoid tissue, where it remains until at least the onset of pregnancy (6, 9, 22). However, the infection cannot be diagnosed either serologically or by direct detection of the pathogen (e.g., modified Ziehl-Neelsen staining, PCR) until the time of abortion, when infectious organisms are excreted and maternal C. abortus antibody titres rapidly increase. Although ewes develop an immunity and do not repeat further C. abortus-induced abortion, they may continue to excrete infectious organisms during oestrus or subsequent lambing, thereby continuing to contaminate the environment and spread the infection (6, 22).

The winter season is very long in the Kars province, and the flocks and herds are kept indoors during the lambing and calving season. Pregnant and non-pregnant animals, the animals which aborted, and newborns are mostly kept in the same pens. Furthermore, the animals are closely congregated during the parturient period, which results in contamination of pens, equipments, and feeds. Aborted foetuses and placenta are allowed to be consumed by dogs, resulting in contamination of the environment with the organisms. Therefore, keeping all the animals crowded in the same pen during lambing and calving seasons, and contamination of the environment may play a role in the increased incidence of abortion caused by C. abortus in Kars. Furthermore, rams and bulls used for breeding are not examined for the presence of abortifacient agents in the province. These infected or carrier animals used for breeding may also transfer abortifacient pathogens such as C. abortus to ewes and cows. In addition to this, regular serological tests are not applied to flocks and herds to eliminate infected or carrier animals. Absence of a proper control programme to keep flocks and herds free of infection and lack of a vaccination schedule against C. abortus, may also explain the high prevalence of abortion obtained in this study in both ewes and cows in Kars.

In conclusion, the result of the present study indicates that C. abortus causes abortion in both sheep and dairy cows in the Kars province. It is well-known that detection of infected and carrier animals will allow appropriate control measures to be taken to reduce environmental contamination, thus limiting the spread of infection, financial losses, and the possible risk of zoonotic transmission to humans. Therefore, it is recommended that a reliable serological test should be applied regularly to flocks and herds, and seropositive animals should be eliminated. Furthermore, a proper vaccination schedule against C. abortus should also be applied for sheep and cattle, to reduce the incidence of abortion caused by this bacterium.

Acknowledgments: The authors gratefully acknowledge the Prime Ministry State Planning Organisation (DPT) of Turkish Republic for its financial support. The authors would also wish to thank Mrs. Catherine Akca for proofreading the manuscript.

References
17. Livingstone M., Ertrican G., Wattegedera S., Buxton D., McKendrick I.J.: Antibody responses to recombinant


