



Investigation of *Coxiella burnetii* and *Chlamydia abortus* Antibodies in Sheep in Düzce Region

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ABSTRACT

Aim: The aim of this study is to investigate the *Coxiella burnetii* and *Chlamydia abortus* antibodies in sheep in Düzce province. These two pathogens are causative agents of Query fever and Enzootic abortion of ewes, respectively which are related to abortion in small ruminants. Economic loss due to the abortions which are caused by microbiological agents is considerably high in many countries.

Material and Methods: Blood samples of sheep were collected from Düzce province of Turkey for serological tests. Commercial enzyme-linked immunosorbent assay (ELISA) was used for the detection of specific antibodies to *C. burnetii* and *C. abortus*. A total of 72 samples obtained from 14 herds located in 12 villages/districts of 5 towns in Düzce province were utilized in this study. Samples were collected from 2-5-year-old female animals without an abortion history.

Results: Total seroprevalence of Enzootic abortion of ewes and Query fever was found to be 20.83% and 26.38%, respectively. However, the proportion of seropositive herds was found to be 42.8% for Enzootic abortion of ewes and 50% for Q fever, which is higher than total seroprevalence. 6 flocks out of 14 were found to be seropositive for both of the diseases simultaneously.

Conclusion: This study confirms the exposure of sheep herds in Düzce province to *C. burnetii* and *C. abortus* and provides original seroprevalence data about Düzce. The data gathered are beneficial to evaluate the possible connection of two investigated abortogenic pathogens in sheep abortion cases in Düzce province.

Keywords: Abortion; *Chlamydia*; *Coxiella burnetii*; seroprevalence; Q fever.

Düzce İlinde Koyunlarda *Coxiella burnetii* ve *Chlamydia abortus* Etkenlerine karşı Antikorların Araştırılması

ÖZ

Amaç: Bu çalışmanın amacı Düzce ilindeki koyunlarda, *Coxiella burnetii* ve *Chlamydia abortus* etkenine karşı spesifik antikorları araştırmaktır. Q humması ve koyunların enzootik abortusu hastalıklarının ayrı ayrı nedeni olan söz konusu 2 patojen, küçük ruminantlarda yavru atmalar ile ilişkilidir. Mikrobiyal etkenlerin neden olduğu yavru atmalara bağlı ekonomik kayıplar dünyanın birçok ülkesinde kayda değer seviyededir.

Gereç ve Yöntemler: Çalışma kapsamında serolojik testler için koyun kan örnekleri Düzce ilinden toplandı. Ticari ELISA kitleri, *C. burnetii* ve *C. abortus* etkenlerine karşı spesifik antikorların tespiti için kullanıldı. Düzce ilinin 5 ilçesindeki 12 köy veya mahallede yer alan 14 sürüden alınmış 72 numune çalışmada kullanıldı. Numuneler, 2-5 yaş aralığında yavru atma geçmişi olmayan dişi hayvanlardan alındı.

Bulgular: Sırasıyla koyunların enzootik abortusu ve Q humması hastalıkları için total seroprevalans %20,83 ve %26,38 olarak bulundu. Fakat seropozitif sürü oranı, total seroprevalansdan daha yüksek oranda sırasıyla koyunların enzootik abortusu ve Q humması hastalıkları için %42,8 ve %50 olarak bulundu. 14 sürü içinde 7 sürüde her iki hastalık için seropozitif hayvanlar tespit edildi.

Sonuç: Çalışma sonuçları Düzce ilindeki araştırılan koyun sürülerinin *C. burnetii* ve *C. abortus* etkenlerine maruz kaldığını doğrulamaktadır.

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Elde edilen verilere göre, Düzce ilinde koyun yavru atma vakalarında çalışmada araştırılan iki abort etkenin muhtemel bağlantısının değerlendirilmesi faydalı olacaktır.

Anahtar Kelimeler: Abort; *Chlamydia*; *Coxiella burnetii*; seroprevalans; Q humması.

INTRODUCTION

Abortion in small ruminants is regarded as a significant issue in many countries, which support livestock farming (1-5). Zoonotic organisms leading to abortions in livestock also pose a risk for public health, which should also be taken into consideration (1,3,4,6). Some of the abortogenic microorganisms which cause economic losses for livestock sector and create risks for public health include *Brucella*, *Listeria*, *Coxiella* (*C.*), *Chlamydia* (*C.*), *Leptospira* and *Toxoplasma* (7).

Abortion is generally associated with brucellosis in Turkey; on the other hand, abortions due to *Chlamydia* organisms are considered to be one of the most serious reasons for ruminant abortions (8,9). Query (Q) fever was reported for the first time in 1947 in Turkey and following studies including mainly serological ones stated the risk of the disease for both animal and human health (10,11).

These two pathogens investigated in this study cause similar clinical manifestations such as, abortion, stillbirth, and weak offspring, metritis, infertility, premature birth that are stated as reproductive disorders (12-16). *Chlamydia abortus* not only causes acute flue-like diseases in humans but it also leads to infections such as abortion, even some fatal cases particularly in pregnant women (17,18). Transmission of the organism to people is possible through abortion materials or their working with cultures in the laboratory environment (19).

Q fever like Enzootic abortion of Ewes (EAE) may also affect human health by causing serious cases even death (14). Presence of *Coxiella burnetii* as a causative agent of Q fever can be mainly transmitted to human by aerosol way (13,20) or by consuming fresh dairy products or raw milk (21-23). Sheep and goats which are considered to be main reservoirs are particularly related to human cases (5,21,24). *C. burnetii* has got a wide range of reservoir animals including several domestic and wild animals such as ticks and birds. What is more, infected ruminants can constantly shed the pathogens and they do not show any clinical signs, even simple fever (25,26).

Both of these two pathogens could be acquired by ingestion, sexual transmission and inhalation (14,16). Birth products, vaginal discharges, placenta, feces, semen and urine are the expected sources of shedding the organisms (12,16,20,25). Q fever and EAE cause significant economic losses worldwide except for New Zealand (14,18,21,22,27). It is possible to meet abortions in naive herds in the first year of *C. abortus* exposure and in the second year of transmission, experiencing abortion storms is an expected result (15-17,19). *C. burnetii*, which is considered to be a potential bioterrorism agent and *C. abortus* both pose a zoonotic risk which makes biosafety and biosecurity measures obligatory (19,22,23,27).

Serological diagnosis is beneficial to determine to prevalence for both of the diseases. Because of the

difficulties of clinic diagnosis of Q fever and the risk of working with *C. burnetii* make serological analysis preferential (10,14,19). ELISA is recommended as a valid test for the surveillance studies of EAE by the authority (19). The aim of this study is to determine the antibodies for *C. burnetii* and *C. abortus* simultaneously in sheep in Düzce province and the data gathered are useful for evaluating the abortion cases in livestock in this province.

MATERIAL AND METHODS

The study was carried out in Pendik Veterinary Control Institute (PVCİ), Istanbul, Turkey and the research was approved by the Local Ethics Committee for Animal Experiments, PVCİ, Istanbul, Turkey. 72 sheep blood samples taken from 14 herds which were located in 12 villages/districts of 5 towns in Düzce province were utilized. All the sheep are female, between 2 and 5 years old and they do not have any abortion history. The samples included in this study were selected through random sampling and as this is a preliminary study, the sample size preferred to be relatively small.

Until testing is performed, the sera were kept at -20 °C. Two different ELISA test kits including positive and negative control sera (IDEXX *Chlamydia abortus* Antibody Test Kit, IDEXX Q fever antibody ELISA test kit) were used to determine specific antibodies against *C. abortus* and *C. burnetii* separately. The method was implemented in accordance with the manufacturer's instructions as described in previous studies (13,23,28). The results were interpreted according to the manufacturer's recommendation; namely, samples having a S/P ≤ 30%, between 30-40% and ≥ 40% were labelled as negative, suspect and positive, respectively. The data gathered were illustrated as numbers and percentages

RESULTS

The results of the study revealed that seropositivity in sheep for *C. abortus* and *C. burnetii* was found to be 20.38% (n=15) and 24.67% (n=19), respectively. However, the proportion of seropositive herds for EAE was found to be 42.8% and 50% for Q fever, which is higher than total seroprevalence. 6 flocks out of 14 were found to be seropositive for both of the diseases simultaneously. Table 1 below illustrates the age distribution of the seropositive animals.

Table 1. The age distribution of the seropositive animals

Age (years)	Number of animals	Number of seropositive ones (EAE)	Number of seropositive ones (Q fever)
2	27	11	12
3	7	0	0
4	26	3	7
5	12	1	0

As can be seen in Table 1, most of the sheep with seropositive result were in the same age group (2 and 4 years old) for both of the two investigated diseases.

DISCUSSION

Focusing on the predominant infective causes of the abortion is a cost effective approach for producers and this approach provides a safer environment for the public.

It should also be taken into account that the prevalence value of abortogenic agents may vary in different countries and even regions (1).

It is probable to encounter subclinical form of Q fever or persistence of EAE in infected sheep (24). Therefore, conducting regular serological screening at a regional and provincial scale is crucial to determine the most appropriate control measures. Organizing suitable surveillance programs to discover the epidemiological characteristics of these zoonotic agents is the responsibility of veterinary authorities (5,29).

In this study, the total seroprevalence in sheep for *C. abortus* was found to be 20.83%. In the previous studies conducted in Turkey, the total seroprevalence of EAE in sheep varied between 1.81% and 32%. However, the total seroprevalence indicated in more than half of the previous studies is almost 14% or more (8,9,30-34). Thus, the result we gathered in our study (20.83%) is somewhere in between the previously stated values.

Two other studies indicated seropositive herd rate as 46.66%, 80% and 81.4%, which is higher than the total seroprevalence (9,29,32). The proportion of seropositive herd for EAE in our study was observed as 42.8%, which is also higher than the total seroprevalence. The highest number of seropositive animals was at the age of two in this study. In another study, too, a similar result was obtained while investigating the seroprevalence of *C. abortus* in sheep and goats (29).

It is not possible to correlate the positive result with vaccination because a vaccine program against EAE in Turkey has not started yet. The considerably high results due to total seroprevalence and seropositive herds exemplify the risk for the sheep flocks. Establishing a control and eradication programme is a suitable approach to prevent the disease (33). Seroepidemiological studies including other provinces around are needed in order to compare and contrast the results.

The real prevalence of Q fever in herds is expected to be higher because of the carriers which do not show any clinical signs. Therefore, diagnosis of the disease without clinical data in both animals and humans is essential to estimate the real prevalence (14,10). The seroprevalence results of the previous studies carried out in Turkey for livestock vary between 3% and 38.69% (10,13,21-23,35-37). The highest seroprevalence (38.69%) belongs to a study including aborted sheep samples (n=184) (37). The samples of our study were collected from sheep without any abortion history and the results are higher than previous results except for the highest one. There is always a clear difference in terms of seropositivity between animals with and without abortion history (13,35).

The seropositivity rate of the flocks which had at least one positive animal is important to evaluate the incidence of the disease. Two of the previous studies focused on this issue and revealed approximately 4 times higher seropositive rates between herds than the total seroprevalence (13,35). Similar to the findings of these studies, the seropositive herd rate (50%) is nearly twice as much as the total seroprevalence (26.38%) in our study.

Identification of a susceptible host as free from the disease depends on the herd situation and the herd should

not include any serological and clinical evidence of the Q fever (14). For this reason, the animals, which are not tested in the seropositive herds, might continue to pose a risk for the other animals in the herds.

6 flocks out of 14 include seropositive sheep (n=11) for both Q fever and EAE. In another study, 90% of the herds contain positive results due to more than one organism (3). Such kind of sero-survey studies, which investigated the causes of infectious abortions in small ruminants, reported that this situation might be a consequence of mixed infections (3,4,24).

CONCLUSION

Diagnosis of the disease is a significant process to identify the abortion cases in flocks and to build appropriate and effective control measures for prospective outbreaks. The results of this study demonstrate the substantial seroprevalence level for both Q fever and EAV in sheep in Düzce province. These findings indicate the possible risk of the investigated zoonotic agents for both animal and human health. Based on the results gathered, the possible influences of these abortogenic organisms on fertility should be taken into consideration while establishing surveillance and control programs.

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