

Seroprevalence of *Chlamydia abortus* in sheep in Bosnia and Herzegovina

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ABSTRACT

An epidemiological study was carried out to determine the seroprevalence of *Chlamydia abortus* (*C. abortus*) in sheep in Bosnia and Herzegovina. This was the first systematic study of this kind carried out in Bosnia and Herzegovina. Samples were collected during 2012 and 19 sheep flocks, located in the different parts of the country and with a recorded history of reproductive failures (abortion, stillbirths and infertility) were involved. A representative sample from each flock was taken by a simple random sampling allowing the detection of seropositive animals within a flock with 95 % confidence, with expected prevalence rates of 20 %, using the recommendations for determining the required necessary sample size to detect the presence of disease. In total 178 sheep blood sera were tested for the specific antibodies against *C. abortus*, with the use of enzyme-linked immunosorbent assay (CHEKIT* *Chlamydophila abortus* Antibody Test Kit). The results showed that 77 (43.3 %) out of 178 ovine sera were seropositive for *C. abortus* infection, as indicated by the manufacturer's interpretation of the results. The flock was considered to be positive if at least one animal was seropositive. The flock prevalence of *C. abortus* in the examined flocks was 84.2 % (16/19 flocks). The results of the present study indicate that *C. abortus* infection occurs frequently in sheep in Bosnia and Herzegovina.

Key words: Bosnia and Herzegovina, *Chlamydia abortus*, seroprevalence, sheep

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Introduction

Pathogens from the family *Chlamydiaceae* can cause disease in humans and a wide range of animals, including birds, sheep, goats, cattle, pigs, cats, koalas, amphibians and reptiles. They may result in adverse pregnancy outcomes in both humans and animals (POSPISCHIL et al., 2002; BAUD et al., 2008). The family *Chlamydiaceae* contains a single genus *Chlamydia*, comprising all currently known 11 member species (SACHSE et al., 2015).

Ovine chlamydiosis, also known as enzootic abortion of ewes (EAE) or Ovine enzootic abortion (OEA), is caused by an obligate intracellular gram-negative bacteria *Chlamydia abortus* (*C. abortus*), formerly known as *Chlamydia psittaci* serotype 1. *C. abortus*, as etiologic agent of OEA was for the first time reported in 1950 (STAMP et al., 1950).

Ovine enzootic abortion is distributed worldwide and *C. abortus* is a common agent of abortions in small ruminants in numerous countries of Europe (MASALA et al., 2005; RUNGE et al., 2005). According to data from the Veterinary Investigation Diagnosis Analysis (VIDA) database from 2003, in Great Britain, *C. abortus* remained the most commonly diagnosed cause of ovine abortion with 40 % and 44 % of diagnoses in 2003 and 2002 respectively. In Switzerland 39 % of abortions in sheep were caused by this agent (CHANTON-GREUTMANN et al., 2002). There are also reports from countries on the continents of America, Asia and Australia (DUMAN and DURAK 1998).

Ovine enzootic abortion is the most important cause of reproductive failure in sheep and goats manifested as abortion, premature births or weak lambs, which die within a few days after birth (AL-QUDAH et al., 2004; AITKEN and LONGBOTTOM, 2007). Abortions usually occur in the last 2 to 3 weeks of gestation (NIETFELD, 2001).

When the infection enters the flock, the following course of clinical events can be expected: sporadic abortions in the first year, followed by a mass occurrence of abortions in the second year, when more than 30 % of ewes may abort. The disease becomes enzootic from the third year onwards, with abortion registered in 5-10 % of ewes, primarily in those pregnant for the first time, as well as in newly introduced animals (BOREL et al., 2004; GERBER et al., 2007; OIE, 2012).

The main sources of infection in the lambing period are the placentas and foetal fluid of the infected animals, although the infectious agent may be also found in faeces and urine, as well as in the goat's milk (ČISLÁKOVÁ et al., 2007). Ingestion is considered to be the main route of infection, although inhalation may also be a route of transmission (AL-QUDAH et al., 2004; JONES and ANDERSON, 1988; ALJUMAAH and HUSSEIN, 2012). Venereal transmission is suggested to be a less common route (APPLEYARD et al., 1985)

Development of the clinical signs due to *C. abortus* infection depends on the time of infection. Sheep and goats infected 5-6 weeks before parturition may develop the clinical disease during their current pregnancy. Animals infected during the last 4 weeks of gestation and animals infected in the non-pregnant state may develop a latent infection and may develop the clinical signs in a subsequent pregnancy (AITKEN and LONGBOTTOM, 2007). Infected and latently infected sheep may shed *C. abortus* in their reproductive tract several years after infection. In these animals the pathogen was detected in the endometrium, vagina and oviduct (PAPP et al., 1994; PAPP and SHEWEN, 1996). After abortion, most ewes gain immunity and re-breed successfully, but become a reservoir of infection and release bacteria, especially during the subsequent oestrus and lambing periods (RODOLAKIS et al., 1998; LIVINGSTONE et al., 2009).

C. abortus is zoonotic and extremely hazardous for pregnant women in whom it can cause life threatening illness and abortions (JORGENSEN, 1997; LONGBOTTOM and COULTER, 2003). There are reports of this disease in children and adults with flu-like symptoms. Populations at risk are also veterinarians, ranchers and others who come in contact with the aborted fetuses and infected material (AITKEN, 1986; HADLEY et al., 1992).

Considering the importance of chlamydiosis for both animal and public health, and taking into the account the lack of data about the presence of the infection in sheep in our country, the aim of this study was to perform a seroepidemiological investigation associated with infection by *C. abortus* of sheep flocks in Bosnia and Herzegovina.

Materials and methods

Sampling. A total of 178 blood samples were collected from 19 sheep flocks with a history of reproductive failure during the lambing season. Sample collection began in March 2012 and continued until December 2012. The sheep population was from the five regions of Bosnia and Herzegovina (east, west, north, south and central Bosnia and Herzegovina). The samples were stratified and weighed according to the animal populations distribution in the five regions. Sample size (the required number of flocks and ewes) was estimated at a 95 % confidence level with predicted prevalence of 20 %, having in mind the estimated number of sheep flocks in Bosnia and Herzegovina, using the recommendations for determining the required necessary sample size to detect the presence of disease (PFEIFFER, 2000). The flocks involved in this study consisted of five to thirty animals kept in extensive conditions.

Five millilitres of blood samples were collected by jugular venepuncture from each animal into vacutainer tubes. After clotting the samples at room temperature, the sera were separated and stored at -20 °C until testing for the presence of antibodies against *C. abortus*.

Serology procedure. The enzyme-linked immunosorbent assay (CHEKIT* *Chlamydia abortus* Antibody Test Kit) was obtained from IDEXX Switzerland AG, Switzerland. This is one of the tests certified for detection of enzootic abortion in ewes in Germany, and it exhibits specificity of 100 % and sensitivity of 95 % (GOETZ et al., 2005; LENZKO, 2012). The assay was performed according to the manufacturer's instructions.

The analysis took place at the Veterinary Faculty of the University of Sarajevo, following the procedure described below. Appropriately diluted (1:400) sera samples and positive and negative control sera were put into the wells of an ELISA microtiter plate, coated with inactivated *C. abortus* antigen, and incubated at 37 °C for 60 min. The plate was washed with the CHEKIT wash solution from the kit three times, and after that CHEKIT-CHLAMYDIA-Anti-Ruminant-IgG, monoclonal and labelled with horseradish peroxidase, added to each well. After incubation at 37 °C for 60 min in a humid chamber, the plate was again washed three times. The CHEKIT-TMB-Substrate was added to each well and incubated at room temperature (18-25 °C) for 15 min. The reaction was stopped by adding CHEKIT-Stop Solution per well. The plates were read within two hours after the addition of the stop solution. The results were read using an ELISA reader (SUNRISE; Version: V 4.51) at 450 nm wavelength filter, as recommended. The optical density (OD) of the positive control, as well as the OD samples, was corrected by subtracting the OD of the negative control.

The samples were then analysed in relation to the negative and the positive controls, as recommended by the manufacturer. Samples giving OD values ≥ 40 % were considered positive, OD values between ≥ 30 % and ≤ 40 % were considered suspect.

Results

The results of the serological examination confirmed the occurrence of antibodies against this pathogen. The flock was considered to be positive if at least one animal was seropositive. From the total of 19 examined flocks, 16 (84.2 %) were positive and 3 (15.8 %) were suspect (Table 1).

Table 1. Seroprevalence of *C. abortus* in sheep according to flock size in Bosnia and Herzegovina

Size of flocks	Number of flocks	Samples tested	Seropositive flocks		Suspect flocks	
			No	%	No	%
1-10	7	58	6	85.7	1	14.3
11-20	10	100	8	80.0	2	20.0
21-30	2	20	2	100.0	-	-
Total	19	178	16	84.2	3	15.8

From the total of 178 examined sheep blood sera, 77 (43.3 %) were positive and 28 (15.7 %) were suspect. The prevalence of chlamydial antibodies in the examined sheep flocks from different regions of Bosnia and Herzegovina was very high and ranged from 25 % to 90 % (Table 2).

Table 2. Total number of examined samples according to regions of Bosnia and Herzegovina

Region of B&H	Number of samples (blood sera)	Seropositive samples ¹		Suspect samples ²		Negative samples ³	
		No	%	No	%	No	%
Nord B&H	47	17	36.2	9	19.1	21	44.7
West B&H	62	28	45.2	9	14.5	25	40.3
East B&H	28	7	25.0	5	17.8	16	57.1
Central B&H	31	16	51.6	5	16.1	10	32.2
South B&H	10	9	90.0	0	0	1	10.0
Total	178	77	43.3	28	15.7	73	41.0

¹Samples giving %OD of ≥ 40 %; ²Samples giving %OD ≥ 30 % to < 40 %; ³Samples giving %OD < 30 %

Discussion

Ovine enzootic abortion is distributed worldwide and, according to the relevant literature, chlamydial infection is the leading cause of reproductive failure in sheep and goats in numerous countries, ranging from 20 % to over 70 % (AITKEN, 2000; LENZKO et al., 2011; OIE, 2012).

Studies estimating the seroprevalence of *C. abortus* in small ruminants have found different but high rates: from 2 % to 43 % in different cantons of Switzerland (BOREL et al., 2004), 36 % in Slovakia (TRÁVNÍČEK et al., 2003), and a range from 21 % to 46 % in different parts of Italy (MASALA et al., 2005). Studies performed in Turkey also indicate different prevalences across the country from 17.95 % in the Kars Region (BAZ, 2000) to 47 % in north eastern Turkey (GOKCE et al., 2007).

It has been mandatory to notify detection of *Chlamydia* species in sheep in Germany since 2005. In a regional study conducted in Lower Saxony, antibodies against *Chlamydia* were detected in 54 % of the examined sheep flocks (RUNGE et al., 2005). Despite vaccination and other measures implemented, OEA is still present in that country. Using an enzyme-linked immunosorbent assay (CHEKIT* *Chlamydophila abortus* Antibody Test Kit) in Thuringian sheep flocks, LENZKO (2012) determined a seroprevalence of 94 %, and using a PCR and DNA microarray test found the presence of *Chlamydiae* in 78 % of the examined sheep flocks, whereas the most frequently found species was *C. abortus* (50 %).

Considering that Bosnia and Herzegovina does not legislate the regular monitoring and surveillance of OEA, and no systematic research into the presence of the disease in Bosnia and Herzegovina's sheep population has been carried out, the aim of this study was to determine the seroprevalence of *C. abortus* in sheep across Bosnia and Herzegovina for the first time.

We used an enzyme-linked immunosorbent assay (CHEKIT* *Chlamydophila abortus* Antibody Test Kit) obtained from IDEXX Switzerland AG, Switzerland. This test was used because it is recommended as a valid test for OEA detection in several European Union countries, exhibiting specificity of 100 % and sensitivity of 95 % (GOETZ et al., 2005; LENZKO, 2012).

This was the first systematic study carried out in Bosnia and Herzegovina. In this research we found the presence of this type of chlamydial infection (*C. abortus*) in sheep with high prevalence rate of 43.3 %, signifying that *C. abortus* infection is highly endemic in sheep flocks in Bosnia and Herzegovina. From a total of 19 analysed flocks, 16 (84.2 %) were positive. The prevalence ranged from 25 % to 90 % in different parts of our country. From the southern region of Bosnia and Herzegovina, only 10 samples of blood sera were analysed although more samples were collected, but due to inadequate transport conditions and consequential haemolysis, they were rejected. In this region, we obtained the highest prevalence value of 90 %.

The results of this study represent a cause for concern and highlight the need for further research into this type of chlamydial infection in sheep and goat populations, primarily because it is a dangerous disease causing major economic losses reflected in reproductive and productive losses in sheep breeding, as well as due to its zoonotic potential. This suggests that a control program should be adopted as soon as possible by the Veterinary State Office, as well as the need to adopt the appropriate legislation for compulsory monitoring and surveillance of this disease, with measures for detection, control, prevention and eradication.

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SAŽETAK

U radu je opisano epidemiološko istraživanje s ciljem utvrđivanja seroprevalencije protutijela za vrstu *Chlamydia abortus* ovaca u Bosni i Hercegovini (BiH). Ovo je prvo sustavno istraživanje ove vrste provedeno u BiH. Uzorci seruma bili su uzeti tijekom 2012. godine, a bilo je obuhvaćeno 19 stada ovaca s registriranim reprodukcijskim poremećajima (pobačaji, mrtvorodenja, neplodnost) diljem države. Reprezentativan uzorak iz svakog stada osigurali smo jednostavnim slučajnim odabirom kojim je omogućeno otkrivanje seropozitivnosti unutar stada na 95%-tnoj razini povjerljivosti, s očekivanom prevalencijom od 20 %, služeći se preporukama za određivanje potrebne veličine uzorka za otkrivanje prisutnosti bolesti. Pretraženo je ukupno 178 uzoraka krvnog seruma ovaca na prisutnost specifičnih protutijela protiv *C. abortus* pomoću imunoenzimnog testa (CHEKIT* *Chlamydia abortus* Antibody Test Kit). Sukladno preporukama proizvođača za tumačenje rezultata, od ukupno 178 pretraženih uzoraka 77 (43,3 %) je bilo pozitivno na *C. abortus*. Stado se smatralo pozitivnim ako je sadržavalo makar jednu seropozitivnu životinju. Prevalencija obuhvaćenih stada na *C. abortus* iznosila je 84,2 % (16/19 stada). Rezultati ovog istraživanja ukazuju na činjenicu da je infekcija vrstom *C. abortus* kod ovaca u Bosni i Hercegovini vrlo proširena.

Cljučne riječi: Bosna i Hercegovina, *Chlamydia abortus*, seroprevalencija, ovce
