

VETERINARSKI ARHIV 85 (3), 359-368, 2015

***Chlamydophila abortus* infection in a flock of goats in Bosnia and Herzegovina - a case report**

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KRKALIĆ, L., E. ŠATROVIĆ, T. GOLETIĆ, P. DŽAJA, K. SEVERIN: *Chlamydophila abortus* infection in a flock of goats in Bosnia and Herzegovina. Vet. arhiv 85, 359-368, 2015.

ABSTRACT

The aim of this research was to determine the presence of *Chlamydophila abortus* (*C. abortus*) infection in one flock of goats with a previously recorded history of reproductive failures (abortion, stillbirths, weak born kids) and long-term poor reproductive performances. The affected flock was from the southern region of Bosnia and Herzegovina (B&H) and consisted of 48 goats kept in semi-intensive conditions. Blood samples and vaginal swabs were collected twice during 2012 and 2013 and the sample size was estimated at a 95 % confidence level, with predicted prevalence of 20 %, using the recommendations for determining the required sample size necessary to detect the presence of disease in a flock. A representative sample from this flock was taken by simple random sampling. In the total of 12 blood sera that 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 11 (91.7 %) sera were positive for *C. abortus* antibodies. Vaginal swabs from all animals were analysed by a modified *Chlamydiaceae*-specific rtPCR test, targeting the 23S rRNA gene, to determine the presence of known *Chlamydiaceae*, and three (25 %) samples were positive. These positive samples were subsequently tested with a test targeting the *ompA* gene region (*ompA*-rtPCR) specific for *Chlamydophila abortus*. All three samples were also positive using this test.

Key words: *Chlamydophila abortus*, goats, flock, Bosnia and Herzegovina

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ISSN 0372-5480
Printed in Croatia

Introduction

Chlamydophila abortus (*C. abortus*), the causative agent of a worldwide disease named ovine enzootic abortion (OEA) or enzootic abortion of ewes (EAE), is a Gram-negative obligate intracellular bacterium of the order *Chlamydiales* (BOREL, 2008). Members of the family *Chlamydiaceae* are able to infect a wide range of hosts, including non-human mammals, reptiles and birds. In mammals they cause keratoconjunctivitis, abortion, mastitis, polyarthritis, enteritis and respiratory infections, while in birds the infection is usually respiratory and gastrointestinal in character (VANROMPAY et al., 1995; LONGBOTTOM and COULTER, 2003). Pathogens from the family *Chlamydiaceae* also have zoonotic potential, and infection in humans may result in adverse pregnancy outcomes (BUXTON, 1986).

Chlamydophila abortus (formerly *Chlamydia psittaci* serotype 1) targets the placenta of ewes and goats, causing tissue damage and inflammation, leading to acute placentitis and abortion (AITKEN, 1993). The infection with *C. abortus* is usually asymptomatic in non-pregnant animals, whereas during pregnancy it causes abortion in the last 2 to 3 weeks of pregnancy, while goats may abort at any stage of pregnancy, although miscarriages occur most frequently in the same period (NIETFELD, 2001). Premature births or weak lambs, which die within a few days after birth, are also a common reproductive failure caused by *C. abortus* infection (AL-QUDAH et al., 2004; AITKEN and LONGBOTTOM, 2007). In general, it is known that if *Chlamydiae* are introduced into a naive flock, the losses are much higher in goats (60 %) than in sheep (30 %) (GERBER et al., 2007). In rams and bucks, *C. abortus* can cause orchitis and seminal vesiculitis, resulting in shedding of the organism in the semen, but this mode of transmission is less common (APPLEYARD et al., 1985). Ingestion is considered as the main route of infection, although inhalation can also be a route of transmission. The main sources of infection in the environment are the placentas and foetal fluids of infected animals, and the highest incidence of infections occurs in the lambing period (AL-QUDAH et al., 2004; ALJUMAAH and HUSSEIN, 2012).

Ovine enzootic abortion is a major ruminant infectious disease that may result in unforeseen abortion outbreaks, thereby affecting herd reproduction and production. According to the available literature data, OEA is distributed worldwide and the prevalence rates in different countries range widely from 20 % to over 70 % (AITKEN, 2000; RUNGE et al., 2005; ALJUMAAH and HUSSEIN, 2012; LENZKO, 2012). A study conducted in B&H indicated the presence of this infection in the sheep population (KRKALIĆ et al., 2013), however there are still no data regarding the presence of this infection in goats. Considering the fact that in our country sheep and goats are often kept together in close contact, and taking into account that the pathogen is common for both animal species, there is a need to investigate its presence in the goat population. Therefore, we chose this flock of goats that are kept together with sheep.

There is no legislation relating to obligatory disease monitoring and vaccination program against OEA in our country.

Materials and methods

Sampling. Blood samples. 12 blood samples were collected from one goat flock with a history of reproductive failure during the lambing season. This flock was from the southern region of Bosnia and Herzegovina and consisted of 48 female goats kept in semi-intensive conditions. The same owner also kept sheep in close contact with the goats. There was a history of brucellosis and Q fever in this flock. Anamnestic information indicated the frequent occurrence of reproductive problems, such as abortions, stillbirths and birth of weak kids. Sample collection took place in December 2012. The sample size was estimated at a 95 % confidence level, with a predicted prevalence of 20 %, using the published recommendations for determining the required necessary sample size to detect the presence of disease (PFEIFFER, 2000). Goats were randomly selected within this flock.

Five millilitres of blood were collected by jugular venipuncture from each animal, into vacutainer tubes. Blood samples were allowed to clot at room temperature before the sera were separated and transported to the Veterinary Faculty in Sarajevo, where they were stored at -20 °C until testing.

Vaginal swabs. Following the completion of the serological examination, the results led us to carry out sampling of vaginal swabs from the same goats. Sampling was conducted in March 2013. The samples were collected using suitable sterile swabs (Dacron swabs with plastic handle). Immediately after collection the swabs were placed in a portable refrigerator at 4 °C for transportation to the laboratory of the Veterinary Faculty in Sarajevo, where they were stored at -80 °C until the DNA extraction procedure could be carried out.

Serology procedure. We used the enzyme-linked immunosorbent assay (CHEKIT* *Chlamydophila abortus* Antibody Test Kit, IDEXX Switzerland AG, Switzerland) to detect the presence of antibodies against *C. abortus* in sera samples. The analysis took place at the Veterinary Faculty of the University of Sarajevo, and the assay was performed according to the manufacturer's instructions, following the procedure described below.

Appropriately diluted (1:400) sera samples and positive and negative control sera were put into the wells of the ELISA microtiter plate, coated with inactivated *C. abortus* antigen, and incubated at 37 °C for 60 min. The plate was washed with CHEKIT wash solution three times and after that the CHEKIT-CHLAMYDIA-Anti-Ruminant-IgG, monoclonal antibody, labelled with horseradish peroxidase, was 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 to

each well and the plates were read within two hours of this addition. 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 positive controls, as recommended by the manufacturer. Samples giving % OD of ≥ 40 % were considered positive. Samples giving %OD ≥ 30 % to < 40 % were considered suspect.

DNA isolation and real-time polymerase chain reaction (rtPCR). DNA isolation was done manually from all vaginal samples, using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following the buccal swab (spin) protocol. DNA was eluted with 150 μ L of PCR grade water and stored at -20 °C before analysis.

The rtPCR tests were performed at Sarajevo Veterinary Faculty, Department of Avian Diseases and Management. First, a modified *Chlamydiaceae*-specific rtPCR test (EHRICHT et al., 2006) targeting the 23S rRNA gene (23S-rtPCR), was used to determine the presence of any known *Chlamydiaceae*, while a second test, targeting the *ompA* gene region (*ompA*-rtPCR), was used because it is specific for *Chlamydia abortus* (PANTCHEV et al., 2009) (Table 1). Both rtPCR tests were conducted on a StepOnePlus (Applied Biosystems) QPCR platform using a QuantiTect Probe RT-PCR Kit (Qiagen), primers and probes listed in Table 1, and the following cycling parameters: 95 °C for 15 min (activation of HotStarTaq® DNA Polymerase), 45 cycles of 95 °C for 15 s and 60 °C for 1 min (annealing and extension). The cycle threshold value (Ct) was calculated automatically.

Results

The results of the serological examination indicated the occurrence of antibodies against *C. abortus*.

Of the 12 goat sera, 11 (91.7 %) were positive and 1 (8.3 %) was negative.

Vaginal swabs from all animals were tested with a modified *Chlamydiaceae*-specific rtPCR test, and the results showed that three animals (25 %) were positive. These positive vaginal swabs were then tested with a second test, targeting the *ompA* gene region (*ompA*-rtPCR), and *C. abortus* was detected in all three swabs (100 %).

Of 11 seropositive animals, three of them (27.3 %) were positive in both ELISA and *ompA*-rtPCR.

Table 1. Primers and TaqMan probes used for real-time PCR detection (adapted from EHRICHT et al., 2006; PANTCHEV et al., 2009)

rtPCR test	Designation	Nucleotide sequence (5' - 3')	Amplicon size (bp)
23S-rtPCR	Ch23S-F	CTG-AAA-CCA-GTA-GCT-TAT-AAG-CGG-T	111
	Ch23S-R	ACC-TCG-CCG-TTT-AAC-TTA-ACT-CC	
	Ch23S-p	FAM ¹ - CTC-ATC-ATG-CAA-AAG-GCA-CGC-CG -TAMRA ²	
OmpA-rtPCR	CpaOMP1-F	GCA-ACT-GAC-ACT-AAG-TCG-GCT-ACA	82
	CpaOMP1-R	ACA-AGC-ATG-TTC-AAT-CGA-TAA-GAG-A	
	CpaOMP1-S	FAM ¹ - TAA-ATA-CCA-CGA-ATG-GCA-AGT-TGG-TTT-AGC-G -TAMRA ²	

¹ - FAM - 6-Carboxyfluorescein; ²-TAMRA 6-carboxy-tetramethylrhodamin

Discussion

Numerous studies have demonstrated the worldwide presence of this disease in small ruminants. In the United States of America, *C. abortus* was found to be the most common cause of infectious abortion in goats, while in the United Kingdom it is estimated that approximately 50 % of all diagnosed abortions have a chlamydial cause (LONGBOTTOM et al., 2002). In South Africa, from a total of 24 examined goat breeding farms, six (25 %) farms had at least one positive animal and were considered positive, while the seroprevalence on these farms ranged from 2.4 % to 54 % (SAMKANGE et al., 2010). *Chlamydophila abortus* is also a common cause of infectious abortions in goats in numerous countries of Europe (MASALA et al., 2005; RUNGE et al., 2005). Swiss studies, performed between 1996 and 1998, revealed that *C. abortus* was the most-commonly involved infectious agent in sheep and goat abortion cases (39 % and 23 %, respectively) (BOREL et al., 2004). In serological surveys, conducted for five consecutive years (1996-2000) in Slovakia by TRÁVNICEK et al. (2001), the seroprevalences for chlamydia in goats were 3.94 %, 10.02 %, 2.96 %, 3.96 % and 6.08 %, for each of the years respectively.

KRKALIĆ et al. (2013) conducted serological research in the sheep population in western B&H, with the use of enzyme-linked immunosorbent assay (CHEKIT[®] *Chlamydophila abortus* Antibody Test Kit) and showed that 45.2 % of ovine sera were positive and the flock prevalence was 83.3 %.

For detection of antibodies in this study, we also used an enzyme-linked immunosorbent assay (CHEKIT[®] *Chlamydophila abortus* Antibody Test Kit) obtained from IDEXX Switzerland AG, Switzerland. This is an antibody test with high specificity and sensitivity, and it is certified for detection of enzootic abortions in ewes in Germany

(GOETZ et al., 2005; LENZKO, 2012). It is a fast and accessible indirect serological test, and antibodies can be detected in serum in the last month of gestation and following an abortion or a suspicious birth, for up to 3 months. In performance tests conducted in France (SCHALCH et al., 1998) and in Germany (GOETZ, 2005) this test demonstrated high specificity (100 %) and sensitivity (89-95 %) in both naturally and experimentally infected animals.

The affected goat flock was kept in close contact with sheep and its medical history data indicated the existence of reproductive failures, such as abortions, stillbirths and births of weak kids. Numerous authors report relatively high seroprevalences in flocks with clinical signs of reproductive disorders, especially abortions (APEL et al., 1989; RUNGE et al., 2005; SAMKANGE et al., 2010).

The results of our research showed a high seroprevalence of 91.7 % in this flock. *Chlamydiaceae* were detected in 25 % (3/12) of goats. All *Chlamydiaceae* positive vaginal swabs were then analysed with the second test, targeting the *ompA* gene region (*ompA*-rtPCR) specific for *C. abortus*, and all of them were positive.

From a total of 11 seropositive goats tested using *Chlamydiaceae*-specific rtPCR and *C. abortus* species-specific rtPCR, only three, or 27.3 % animals were positive for both tests. A negative PCR result with a positive ELISA result can be a consequence of antibodies still present that originated from a previous infection, whereas the pathogen had been eliminated from the host organism or excreted only intermittently (LIVINGSTONE et al., 2009). The intermittent shedding of pathogens occurs in persistently infected animals in vaginal secretions, most often during subsequent oestrus cycles during the periovulation period (LIVINGSTONE et al., 2009). In non-pregnant animals, the antibody level usually drops rapidly off but there are also studies that report a systemic antibody response even when a period of several years has elapsed from the infection and abortion (PAPP et al., 1994). Results of a newer study confirm the presence of elevated levels of *Chlamydia* specific antibodies in sheep in the second year after abortion (LONGBOTTOM et al., 2013).

In the literature it has been postulated that intestinal colonization with *C. abortus* can lead to the formation of circulating antibodies, while excretion in vaginal fluids may not be present (AITKEN and LONGBOTTOM, 2007; GERBER et al., 2007). These facts can complicate the interpretation of positive serological findings. However, in a study conducted by LENZKO (2012), no correlation between the findings of *C. abortus* in faeces and serology was established, which suggests that positive serological findings are not due to enteric *C. abortus* infection.

A converse situation with a negative ELISA and positive PCR result could mean that the animal concerned is a non-pregnant animal in which the antibody level has already fallen. It may also be explained by the fact that only some of the animals form humoral antibodies after the primary infection (GUTTIEREZ et al., 2011; LENZKO, 2012).

Our research confirmed the presence of this disease in a flock of goats. The seroprevalence was extremely high (91.7 %). The determined antigen prevalence was 27.3 % (3/11). This means that in the period of our sampling these three animals excreted *C. abortus* in vaginal secretions. Our study involved only one flock of goats, but the results suggest the need for further systematic research for the presence of this disease in goat populations throughout the country, especially if we take into account the high prevalence in sheep in this country, the zoonotic impact of the causative agent and the risk to public health. Only after systematic research into the presence of this disease in goats in B&H will it be possible to provide recommendations aimed at establishing legislation about measures of surveillance, prevention, control and eradication of this disease.

Acknowledgements

This study was supported by the Ministry of Education and Science of the Federation B&H through the project "Pilot project for research into *Chlamydomphila abortus* (*Cp. abortus*) in sheep and goats in FB&H".

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Received: 24 April 2014

Accepted: 25 November 2014

KRKALIĆ, L., E. ŠATROVIĆ, T. GOLETIĆ, P. DŽAJA, K. SEVERIN: Infekcija vrstom *Chlamydomphila abortus* u stadu koza u Bosni i Hercegovini - prikaz slučaja. *Vet. arhiv* 85, 359-368, 2015.

SAŽETAK

Cilj istraživanja bio je utvrđivanje infekcije vrstom *Chlamydomphila abortus* (*C. abortus*) u stadu koza s reproduktivnim poremećajima (pobačaji, mrtvorodenja, rađanje slabih mladunaca i dugotrajnim lošim reproduktivnim rezultatima). Pretraženo je stado iz južne regije Bosne i Hercegovine (BiH), a čini ga 48 koza držanih u poluintenzivnim uvjetima. Uzorke krvi i vaginalne obriske prikupljali smo u dva navrata tijekom 2012. i 2013. godine, a potreban broj smo odredili koristeći se preporukama za određivanje potrebne veličine uzorka za otkrivanje bolesti u stadu na 95% razini povjerljivosti i očekivanom prevalencijom od 20%. Reprezentativan uzorak osigurali smo jednostavnim slučajnim uzorkovanjem. Od ukupno 12 pretraženih uzoraka krvnog seruma na prisutnost specifičnih protutijela protiv *C. abortus* pomoću imunoenzimnog testa (CHEKIT® *Chlamydomphila abortus* Antibody Test Kit), 11 (91,7%) je bilo pozitivno. Vaginalne obriske svih životinja potom smo analizirali modificiranim *Chlamydiaceae*-specifičnim rtPCR za dokaz 23S rRNA gena. Tri su (25%) bila pozitivna. Potom

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smo pozitivne uzorke pretražili testom za dokaz gena *ompA* (*ompA*-rtPCR) specifičnoga za vrstu *Chlamydophila abortus*. Sva tri uzorka su također bila pozitivna.

Ključne riječi: *Chlamydophila abortus*, koze, stado, Bosna i Hercegovina
