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SEROPREVALENCE OF *CHLAMYDOPHILA ABORTUS* IN SHEEP IN THE BELGRADE EPIZOOTIOLOGICAL AREA DURING 2019-2021

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Abstract

As one of the most important infective agents of abortion, *Chlamydophila abortus* takes an important place in pathology of ruminant reproductive tract. In sheep, the disease known as enzootic abortion of ewes or ovine enzootic abortion, and is manifested as abortion and accompanied reproductive disorders, thus resulting in significant economic losses worldwide. The characteristics of the pathogen and its zoonotic potential make this disease significant both for animal and public health. Therefore, the presented study aimed to obtain *Chlamydophila abortus* seroprevalence in sheep population in Belgrade epizootiological area. The study was done by testing 552 sheep sera samples from 10 municipalities of Belgrade city, during the 2019-2021 period. Serological examination was performed using ELISA assay (ID Screen® *Chlamydophila abortus* Indirect Multi-species, IDvet, Grabels, France). The obtained results showed *Chlamydophila abortus* seroprevalence of 6% in Belgrade epizootiological area. The largest number of seropositive sheep was found in municipality of Palilula. The detected antibodies against *Chlamydophila abortus* confirmed the circulation of the pathogen in sheep population in Belgrade epizootiological area. The obtained results show the need for further studies and continuous implementation of measures for detection, control, prevention and eradication of the disease.

Key words: *Chlamydophila abortus*, enzootic abortion of ewes, sheep, seroprevalence, Serbia

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SEROPREVALENCIJA *CHLAMYDOPHILA ABORTUS* KOD OVACA NA BEOGRADSKOM EPIZOOTIOLOŠKOM PODRUČJU U PERIODU 2019-2021 GODINE

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Kratak sadržaj

Kao jedan od najznačajnijih infektivnih agensa koji uzrokuju pobačaj *Chlamydophila abortus* zauzima značajno mesto u patologiji reproduktivnog trakta preživara. Kod ovaca, ovo oboljenje je poznato kao enzootski pobačaj ovaca (EAE ili OEA) i manifestuje se pobačajima i pratećim reproduktivnim poremećajima, praveći značajne ekonomske gubitke širom sveta. Karakteristike patogena i njegov zoonozni potencijal čine ovu bolest od velikog značaja ne samo za životinje, već i za javno zdravlje. Stoga je cilj ove studije utvrditi seroprevalenciju *Chlamydophila abortus* u populaciji ovaca na beogradskom epizootiološkom području. Ukupno su testirana 552 uzorka seruma ovaca sa 10 opština grada Beograda, od 2019. do 2021. godine. Serološka dijagnostika je rađena primenom komercijalnog ELISA testa (ID Screen® *Chlamydophila abortus* Indirect Multi-species, IDvet, Grabels, Francuska). Dobijeni rezultati su pokazali seroprevalenciju Chlamydophila abortus od 6% na beogradskom epizootiološkom području. Najviše seropozitivnih ovaca ustanovljeno je na opštini Palilula. Utvrđeno prisustvo antitela protiv *Chlamydophila abortus* potvrdilo je cirkulisanje ovog patogena u populaciji ovaca na teritoriji beogradskog epizootiološkog područja. Dobijeni rezultati ukazuju na potrebu za daljim istraživanjima i kontinuiranom sprovođenju mera za detekciju, kontrolu, prevenciju i eradikaciju ove bolesti.

Ključne reči: *Chlamydophila abortus*, zarazni pobačaj ovaca, ovce, seroprevalencija, Srbija

INTRODUCTION

Chlamydophila abortus is an obligate, intracellular, gram-negative bacteria from the *Chlamydiaceae* family, recognized as the species responsible for enzootic abortion since 1950 (Stamp et al., 1950). The pathogen was previously identified as *Chlamydia psittaci*, serotype 1 (Sachse et al., 2015). *Chla-*

mydophila abortus is one of the most common causes of infectious abortion in ruminants present worldwide, except in Australia and New Zealand. Only in the UK, the disease in sheep is accounted for 44% of diagnosed abortions due to infectious agents, and more than 56% of small ruminant abortions in Spain (García-Seco et al., 2016). In the UK, resulting economic losses are estimated at nearly £20 million each year (Longbottom et al., 2002).

In sheep, the disease is known as enzootic abortion of ewes (EAE) or ovine enzootic abortion (OEA), causing various reproductive failures, such as abortion, premature or stillbirths, birth of dead or weak lambs with low body weight (Aitken and Longbottom, 2007). Mummification and maceration of the foetuses were also observed. The abortions occur mostly in the last 2-3 weeks of pregnancy, while ewes that have aborted once do not normally abort again as a result of this infection. In non-gravid animals the disease passes as persistent subclinical form. Serohemorrhagic vaginal discharge can be the only clinical symptom, present just occasionally, so the infection often passes unnoticed allowing continuous spread of the pathogen. *Chlamydophila abortus* can cause orchitis and seminal vesiculitis in rams, hence males participate in shedding the pathogen through semen, too (Longbottom and Coulter, 2003). Newborns get infected during the passage through the birth canal of the infected mother (in second gestation), or congenitally in-utero. The transmission of the pathogen is through direct contact with aborted foetuses or genital tract secretions of infected animals, as infected placenta and uterine discharge contain the highest amounts of the pathogen. Moreover, after abortion the pathogen can be shed for several weeks (Rodolakis et al., 1998). Indirectly, the pathogen can be disseminated through contaminated feed and water.

The infection is most commonly diagnosed either by the detection of the specific antibodies using commercially available ELISA kits (or even CFT), or directly by detecting the pathogen, using molecular techniques (conventional and real-time PCR).

Considering its zoonotic potential, all occupationally related staff that are in contact with infected animals or infected material, are at risk of the infection that can be expressed through various manifestations ranging from subclinical infection to influenza-like illness. The disease is particularly dangerous for pregnant women, who are at the highest risk regarding the ability of *Chlamydophila abortus* to colonize the human placenta (Longbottom and Coulter, 2003).

To this day, the data regarding *Chlamydophila abortus* infection on the territory of Serbia are scarce. Therefore, aim of the presented study was to obtain the seroprevalence of *Chlamydophila abortus* in sheep population on Belgrade epizootiological area during the 2019-2021 period and provide novel, updated information about this significant disease.

MATERIAL AND METHODS

In total, 552 sera samples collected from sheep residing in Belgrade epizootiological area, including 10 municipalities, were taken from sera bank between 2019 and 2021 and consequently tested in order to obtain the seroprevalence of *Chlamydophila abortus*. For the purpose of detection of antibodies against *Chlamydophila abortus,* ELISA assay (ID Screen® *Chlamydophila abortus* Indirect Multi-species, IDvet, Grabels, France) was performed, according to the manufacturers' instructions. The optical density was measured with ELISA reader (Tecan), and the samples were considered as positive when S/P ratio was above the cut-off value of 60%. The specificity and sensitivity of the used ELISA kit for small ruminants are 99.5% and 80%, respectively.

RESULTS AND DISCUSSION

The results of the study revealed the presence of the pathogen in Belgrade epizootiological area. The obtained overall *Chlamydophila abortus* seroprevalence in sheep population in Belgrade epizootiological area is 6%. The largest number of *Chlamydophila abortus* seropositive animals (14) was confirmed in municipality of Palilula, while no *Chlamydophila abortus* seropositive animals were found in Obrenovac municipality. The *Chlamydophila abortus* seroprevalence results are presented in Table 1 and 2.

Table 1. The seroprevalence of *Chlamydophila abortus* in sheep population in the Belgrade epizootiological area from 2019 to 2021

Municipality	No. of tested sera	Positive reactions	
		No.	%
Barajevo	54	3	5.5
Grocka	55	4	7.3
Lazarevac	58	2	3.4
Mladenovac	54	2	3.7
Obrenovac	60	0	0
Palilula	60	14	23.3
Sopot	57	1	1.7
Surčin	57	1	1.7
Voždovac	50	2	4
Zemun	47	4	8.5
Total	**552**	**33**	**6.0**

Table 2. The seroprevalence of *Chlamydophila abortus* in sheep population in the Belgrade epizootiological area per year

Year	No. of tested sera	Positive reactions	
		No.	%
2019	184	17	9.2
2020	184	7	3.8
2021	184	9	4.9

As one of the most important infectious agents causing abortions, *Chlamydophila abortus* has severe consequences for both animal health and economy. The available data regarding *Chlamydophila abortus* infection on the territory of Serbia are relatively poor. The study covering the three-year-period reported the *Chlamydophila abortus* seroprevalence of 18.6% in 367 tested sera samples from sheep that have aborted (Vidić et al., 2007). The mentioned study was carried out on the territory of AP Vojvodina, in the north of the country. Studies covering other parts of the country have not recently been conducted.

Reported seroprevalence data on *Chlamydophila abortus* in sheep population throughout Europe are various. In Slovak Republic the reported seroprevalence was 11.7% (Cisláková et al., 2007), while in Italy it ranged from 21% to 46.7% (Masala et al., 2005). In neighbouring Bosnia and Herzegovina, the reported *Chlamydophila abortus* seroprevalence in sheep was 43.3% (Krkalić et al., 2016), while in Croatia, the seroprevalence detected in sheep was 19.6%, i.e. 20.5% depending of the kit used (Špičić et al., 2015). The results of our study have shown the *Chlamydophila abortus* seroprevalence in sheep at a lower level of 6%. The results between different municipalities varied from 0 to 23.3%. The most seropositive animals were found in municipality Palilula (14), while no *Chlamydophila abortus* seropositive animals were detected in Obrenovac.

A group of authors, using the same ELISA test kit in Belgium have established seroprevalence of *Chlamydophila abortus* in sheep in the range from 0 to 4.05% in nine different Belgian regions. The established overall seroprevalence rate was of 0.68%, while it was of 6.15% on the herd level (Yin et al., 2014). It was interesting that only herds with fewer than 50 sheep were seropositive, indicating a higher seroprevalence rate in smaller herds (Yin et al., 2014). Contrary to this, in the UK the number of reported cases in sheep was higher on bigger farms with more than 150 animals, compared to those in smaller herds (Longbottom et al., 2012).

Urban environment of Belgrade city and its municipalities, where most of the herds are of smallholder type, could potentially play an important role in explaining lower seroprevalence. Relatively small sample size should also be taken into account. Still, correlation between the herd size and established seropositivity should be investigated further.

Although low *Chlamydophila abortus* seroprevalence was established, the necessity for further continuous implementation of measures for detection, control, prevention and eradication of the disease on the territory of Serbia is advised. Furthermore, according to the current legislation of Veterinary Directorate, Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, every abortion in sheep must be notified and examined for *Chlamydophila abortus* (Official Gazette of the Republic of Serbia, No. 36/2021). Animal owners are required to report every abortion, whereby the further, prescribed testing will be consequently performed. Although not implemented in our country, vaccines against EAE have shown various efficiency. However, outbreaks have been reported even in vaccinated flocks (Jones et al., 1995). Nevertheless, the incidence of abortions and the period of shedding the pathogen were reduced (Montbrau et al., 2020).

Along with its endemic persistence, the disease is difficult to control regarding its periodic recurrence and maintenance of the pathogen in the flock and host animals. Further studies regarding the infection in ruminants on the territory of Serbia are essential in order to ensure more detailed information about *Chlamydophila abortus* infection. Beside serology, molecular diagnostic should provide additional important information.

CONCLUSION

The presented results confirmed the presence of *Chlamydophila abortus* in sheep population in Belgrade epizootiological area. The obtained *Chlamydophila abortus* seroprevalence is of 6%. The results of our study provided novel and updated information about this significant infection regarding the territory of our country.

Further continuous implementation of measures for detection, control, prevention and eradication of *Chlamydophila abortus* on the territory of Serbia are significant for both animal and public health.

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Author's Contribution:

ZZS and MN drafted the manuscript and performed the laboratory tests. JŽ designed the study and together with DV and BK coordinated the work. JŽ and BK revised the manuscript critically and prepared the final draft of the manuscript.

Competing interest

The authors declare that they have no competing interests.

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