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Research article

SEROPREVALENCE OF SCHMALLENBERG VIRUS IN SHEEP IN BELGRADE EPIZOOTIC AREA

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Schmallenberg disease is an infectious disease of cattle, sheep, and goats of viral aetiology caused by the Schmallenberg virus that belongs to the family *Bunyaviridae* and the genus *Ortobunyavirus*. Schmallenberg disease is a vector-borne disease transmitted by midges from the genus *Culicoides* but also by other hematophagous insects. The disease has a seasonal character and most often occurs in the warm months, from late spring to autumn. In this study, we investigated the seroprevalence of Schmallenberg disease in sheep in the epizootic area of Belgrade in the period from 2017 to 2022. A total of 600 sheep serums from the serum bank were tested, 100 serum samples from each year. Sera were tested by commercial ELISA test for the detection of specific antibodies against the Schmallenberg virus. The results showed an average annual seroprevalence of 24.5% in sheep in the Belgrade area in these six years with an increasing trend predicted for the next years.

Keywords: Belgrade area, Schmallenberg disease, seroprevalence, sheep

INTRODUCTION

Schmallenberg virus (SBV) is a novel pathogen known to cause mild clinical signs in adult ruminants, but serious congenital malformation in newborn ones. Susceptible species are cattle, sheep, goats, deer, alpacas and bison. Schmallenberg virus is an enveloped, segmented, negative, single-stranded RNA virus classified in the *Bunyaviridae* family and *Orthobunyavirus* genus [1]. Since its high genetic similarity to the Acabane virus, SBV is classified in the Simbu serogroup [2]. The virus was discovered near the town of Schmallenberg in Germany in 2011 [3]. By the end of the year, the novel Schmallenberg virus was detected on more than 5000 farms in Germany, the Netherlands, Belgium, France, the UK, Italy, and Spain causing acute infections of adult ruminants and malformations in offspring [4]. The disease quickly became enzootic in the western and central parts of Europe. Very soon after, in September

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of 2013, the virus was confirmed in Africa, in the Zambezi Province of Mozambique where the seroprevalence in sheep and goats ranged from 43–97% and a herd seroprevalence of 72–100% [5]. Schmalenberg virus has been circulating in sheep and cattle in Serbia since 2013 [6,7]. Schmallenberg disease (SD) is a vector-borne disease as the virus transmits to insects, primarily *Culex obsoletus* and *Culex scoticus* [8]. However, the role of widely distributed *Aedes albopictus* and *Culex pipiens* in virus replication and transmission was never confirmed [9]. So far, there have been no indications of direct zoonotic transmission from ruminants to humans [10]. The incubation period is 3-5 days. Clinical signs that can usually be observed by the fifth day after the onset include fever, milk drops, inappetence and in some cases diarrhoea, abortions, stillbirths, and congenital malformations, such as arthrogryposis and hydranencephaly [11].

Due to its high impact on animal production and animal welfare, international trade restrictions, particularly in live animals and semen, have been implemented in Europe. However, to more efficiently combat the disease, several inactivated, attenuated and subunit vaccines have been developed. It was shown that only a single immunization with one of the inactivated vaccines completely inhibits viral replication in immunized animals [12]. However, such an approach is not supportive of the DIVA strategy that is nowadays and favourable [13]. The diagnosis of SD is based on laboratory confirmation of the virus or, indirectly, the immune response. For antibody detection, commercial ELISA kits are most often used. It was shown that ID Screen® Schmallenberg virus Indirect Multi-species almost perfectly agrees with the virus neutralization test and should be used as an appropriate substitution for it [14]. However, serology results must be interpreted with caution taking into account the information on the vaccinal status of tested animals. Given the significance of SD and the gap in knowing its prevalence in the Belgrade city area, the main aim of this study was to investigate the seroprevalence of SD in sheep in the Belgrade epizootic area during the last six years, to predict its trend in the future.

MATERIAL AND METHODS

Six hundred serum samples, one hundred per year, from sheep, were tested for the presence of specific antibodies against SBV. Samples from sheep over 12 months of age were collected from 2017-2022 for the national eradication program of Brucellosis and stored in the serum bank of the Institute of Veterinary Medicine of Serbia. Briefly, the sample size was estimated based on a 50000 sheep population, 5% apparent prevalence, 1% precision, and 95% confidence level using Epitools - Epidemiological Calculators online tool [15]. Sheep serum samples were collected from individual smallholder farms on the territory of Belgrade municipalities. There are no large sheep farms in the mentioned area. Blood was sampled from clinically healthy individuals without suspicion of the presence of SBV. It should be noted that

mall backyardholders usually keep both goats and cattle on the same farm and often in the same stalls.

A commercial ELISA kit for detecting specific anti-nucleoprotein antibodies ID Screen SBV Competition Multi-species, Idvet, France, was used to test selected serum samples following the manufacturer's instructions. The disease trend for six years and predicted seroprevalence of SD for 2023 were calculated using the TREND function of MSExcel, which is based on a formula that includes the independent variable (Xt) representing the years of testing and the dependent variable (Yt) representing the number of or settlements or positive cases confirmed in mentioned year.

RESULTS

Out of 600 serum samples, anti-SBV antibodies were detected in 147 samples (24.5%). The average annual seroprevalence ranged from 12% in 2019 to 37% in 2022 (Table 1). With regard to the municipality, the average five-year seroprevalence ranged from 6.7% in Zemun to 44.4% in Sopot. Sheep from three municipalities (Čukarica, Rakovica, Zvezdara) were negative for anti-SBV antibodies during the observed period (Table 1).

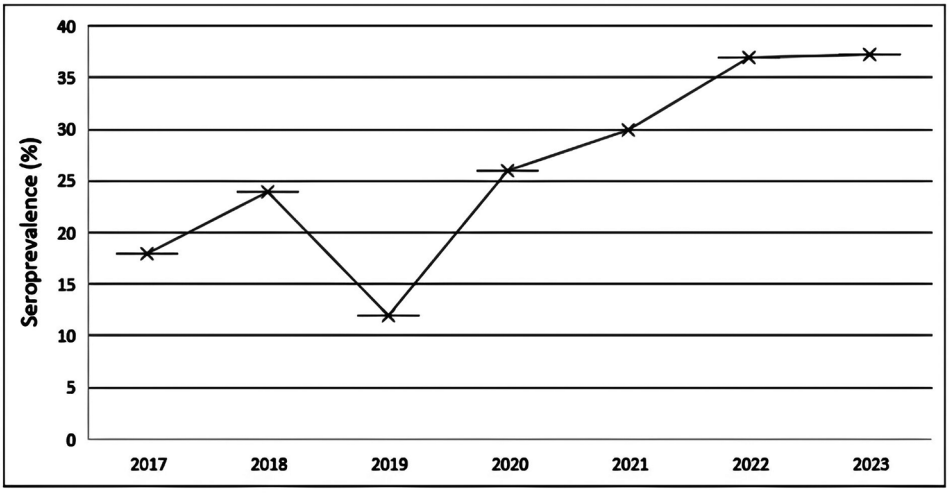

Chart 1. SBV seroprevalence in the Belgrade city area from 2017-2022. with prediction for 2023.

The predicted value of SD seroprevalence for 2023 was calculated using the linear trend function in MS Excel.

Table 1. Summarized results on SD seroprevalence stratified by year and by municipality

Municipality	2017			2018			2019			2020			2021			2022		
	Tested (n)	Pos (n)	Prev (%)	Tested (n)	Pos (n)	Prev (%)	Tested (n)	Pos (n)	Prev (%)	Tested (n)	Pos (n)	Prev (%)	Tested (n)	Pos (n)	Prev (%)	Tested (n)	Pos (n)	Prev (%)
Barajevo	5	2	40.00	5	0	0.00	5	1	20.00	5	2	40.00	5	0	0.00	5	5	100.00
Grocka	2	0	0.00	2	2	100.00	2	0	0.00	2	1	50.00	2	0	0.00	2	0	0.00
Lazarevac	17	5	29.41	17	5	29.41	17	3	17.65	17	4	23.53	17	5	29.41	17	3	17.65
Mladenovac	27	0	0.00	27	6	22.22	27	0	0.00	27	6	22.22	27	11	40.74	27	10	37.04
Obrenovac	27	8	29.63	27	6	22.22	27	3	11.11	27	7	25.93	27	6	22.22	28	12	42.86
Sopot	6	0	0.00	6	3	50.00	6	2	33.33	6	3	50.00	6	4	66.67	6	4	66.67
Surčin	4	2	50.00	4	0	0.00	4	1	25.00	4	2	50.00	4	2	50.00	4	2	50.00
Čukarica	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00
Palilula	2	0	0.00	2	0	0.00	2	2	100.00	2	0	0.00	2	1	50.00	2	0	0.00
Rakovica	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	0	0	0.00
Zemun	5	1	20.00	5	0	0.00	5	0	0.00	5	1	20.00	5	0	0.00	5	0	0.00
Voždovac	2	0	0.00	2	2	100.00	2	0	0.00	2	0	0.00	2	1	50.00	2	1	50.00
Zvezdara	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00	1	0	0.00
SUM	100	18	18.00	100	24	24.00	100	12	12.00	100	26	26.00	100	30	30.00	100	37	37.00

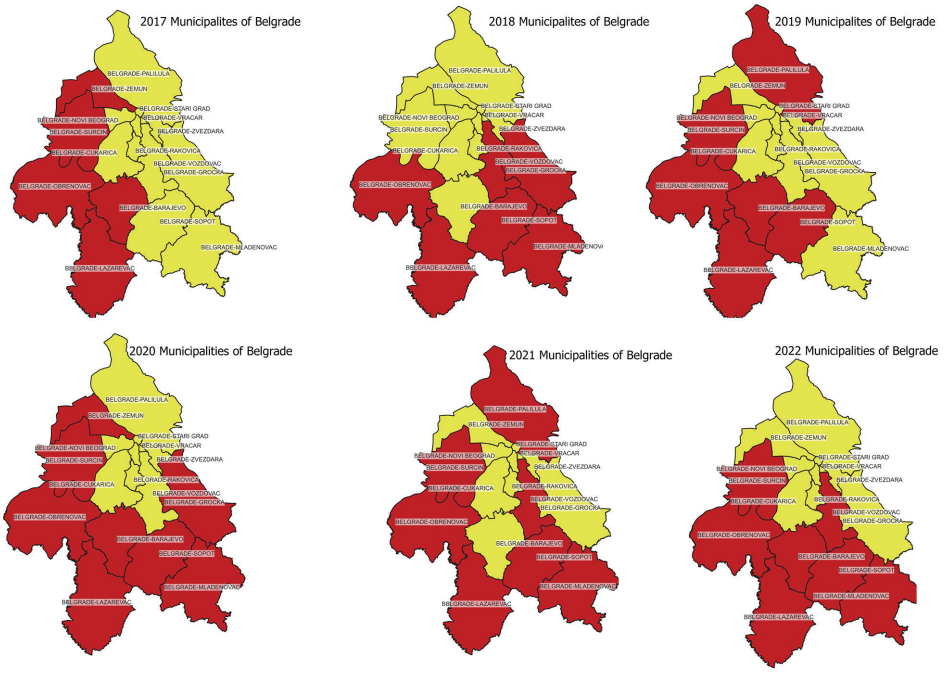

Figure 1. Maps of municipalities with SD seropositive sheep in the Belgrade area 2017 – 2022

DISCUSSION

Schmallenberg virus in Serbia has been circulating at the latest from 2013 when for the first time infection was indirectly confirmed [6]. Vaccination of sheep against the Schmallenberg virus has never been carried out in Serbia. Based on this fact, a positive finding of antibodies against SBV is a consequence of infection. However, this is the first study conducted in the Belgrade city area aiming to determine the SD spread and the prevalence. The metropolitan area of Belgrade is divided into 17 municipalities. Keeping small ruminants is completely prohibited in 3 central municipalities and partially prohibited in the rest of the 14 municipalities thus the samples were stratified in addition to the year of sampling by spatial distribution. The average seroprevalence of 24.5% in Belgrade for the period 2017-2022 is higher than estimated by Bojkovski et al. (2015) [16] who reported the seroprevalence of 13.45% for the Republic of Serbia in previous years but in agreement with the results provided for Vojvodina where the seroprevalence of SD in sheep was 22% in the period 2013-2018 [6]. A similar seroprevalence was reported in other countries such as Poland [17], Switzerland [18], Lebanon [19], and Spain [20]. The results are consistent with the results of SB seroprevalence in Kosovo in sheep (19.2%) in 2014 and 2016. The results of SD seroprevalence in sheep in Albania (0%) are not in agreement with the seroprevalence in Serbia, but they are in agreement with the seroprevalence of 20% in goats in Albania [21].

The sampled sheep are of different age categories and therefore we cannot determine when exactly the infection occurred. Sampling was carried out during the first part of the year so that vectors in the current year had an impact on infections only on those sheep sampled at the beginning of summer. Previously sampled seropositive sheep were infected in earlier vector seasons.

Thus SD in the village Progar, municipality Surčin, which is located by the Sava River and two lakes can be considered enzootic since present each year. On the contrary, high seroprevalence was discovered in Sopot in 2021 and 2022 which is not located near extensive water courses, stagnant water, or swampy terrain which could have led to increased vector activity and a potentially higher percentage of SBV infections. The SB presence in Obrenovac, Surčin and Lazarevac was expected because these are suburban municipalities lying on the Sava and Kolubara rivers, and where there are stagnant waters in the plain areas of Surčin municipality as well. The presence of rivers, ponds, stagnant waters, and underwater terrains is an excellent prerequisite for the survival of mosquitoes, which are the main vectors of SBV.

The highest seroprevalence was detected in Sopot, Barajevo, and Voždovac where the largest number of sheep is kept. Though the infection with SBV was not confirmed in three municipalities, it cannot be excluded given the small number of tested animals. However, in these municipalities, the sheep population is rather small, limited to smallholder farms. On the contrary, though 100% seroprevalence was estimated in the municipalities Grocka and Voždovac in 2018, Palilula in 2019, and Barajevo in 2022, these results should also be interpreted with caution due to the relatively small sample size. Taking into account the linear trend in the last 6 years, the seroprevalence prediction for the next year has an increasing trend of SD in sheep in the Belgrade area and we can expect a seroprevalence of 37,2 % in 2023. (Chart 1.)

Based on the timeline and the seroprevalence, it can be concluded that SB appeared in the eastern part of Belgrade which spread to the southern municipalities (Picture 1). The only exception is SB occurrence in the northern municipality of Palilula in 2019 which abounds in stagnant water and abuts the Danube River's largest watercourse.

Though seroprevalence in some municipalities was not consistent during the last 6 years possibly due to the migrations, selling of animals, slaughtering, and sample size, there is a significant increasing trend of SD in sheep in Belgrade. Therefore, SD deserves more attention as well as the need for its more comprehensive monitoring that should include SBV exclusion for each abortion in ruminants in order to estimate its real impact, in particular on reproductive disorders. A larger number of serum samples should be screened for SBV in order to obtain a more accurate picture of seroprevalence and to make the results more relevant. It would be useful to have the results of SBV seroprevalence in sheep in the whole country because the Belgrade District is not a geographically isolated area. Based on the results of the seroprevalence, one should think about the use of the vaccine in areas that are constantly more likely to have a high seroprevalence rate for SVB.

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Authors' contributions

LjV has contributed substantially to the conception and design, analysis and interpretation of data. JMZ and JM have stratified serum samples from the serum bank and carried out the immunoassays ELISA test. DG participated in the design of the study and performed the statistical analysis. JN conceived of the study, participated in its design and coordination, and helped draft the manuscript. VM has been revising it critically for important intellectual content, has made substantial contributions to conception and design, and has given final approval of the version to be published.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SEROPREVALENCIJA ŠMALENBERG BOLESTI KOD OVACA NA EPIZOOTIOLOŠKOM PODRUČJU GRADA BEOGRADA

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Šmalenberg bolest predstavlja zarazno oboljenje goveda, ovaca i koza čiji je uzročnik Šmalenberg virus koji spada u familiju *Peribunyaviridae* i rod *Ortobunyavirus*. Oboljenje je

sezonskog karaktera. Virus prenose vektori - insekti iz roda *Culicoides* ali i drugi hematofagni insekti zbog čega se najčešće javlja u toplim mesecima od kasnog proleća do jeseni. U našem radu smo istraživali seroprevalenciju Šmalenberg bolesti kod ovaca na epizootiološkom području grada Beograda u periodu od 2017. do 2022. godine. Testirano je ukupno 600 uzoraka krvnih seruma ovaca iz banke seruma, po 100 uzoraka iz svake godine. Ispitivanje je izvršeno komercijalnim ELISA testom za dokazivanje prisustva specifičnih antitela protiv Šmalenberg virusa. Rezultati ukazuju na prosečnu godišnju seroprevalencu Šmalenberg bolesti kod ovaca od 24,5% na teritoriji Beogradskog područja sa predviđenim trendom rasta u narednim godinama.