

## SCHMALLEMBERG VIRUS ANTIBODIES DETECTED IN DAIRY COWS IN THE REPUBLIC OF SERBIA

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### Abstract

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Schmallenberg virus is the emerging pathogen which affects bovine, ovine and caprine species throughout Europe. It was detected for the first time in 2011 in Germany near the town of Schmallenberg (North Rhine-Westphalia). It is classified in family *Bunyaviridae* and *Orthobunyavirus* genus and it is not a zoonotic agent. Due to current epizootiological situation in surrounding countries the serological investigation of the presence of specific antibodies to carried out in the Republic of Serbia. Total of 119 dairy cattle blood sera were collected in May and June 2013 in 5 different locations in the Republic of Serbia. Most of the cows were grazing on pastures, heavily infested by ticks and susceptible for bites of the vectors. The method used in this study was commercial indirect ELISA test for detection of specific Schmallenberg antibodies (ID Screen Schmallenberg virus indirect-IDvet Innovative Diagnostics). From the total of 119 cattle blood sera 16 from two different locations were tested positive and one serum in the third location had doubtful reaction. These are the first results of seropositivity to Schmallenberg virus in the Republic of Serbia. From the results obtained in this study it can be said that the Schmallenberg virus is in circulation in the Republic of Serbia and further investigations are needed in order to obtain virus isolate.

*Key words:* Schmallenberg virus, cattle, antibodies, ELISA, the Republic of Serbia

### Introduction

Schmallenberg virus is an emerging pathogen which affects bovine, ovine and caprine species throughout Europe. It was detected for the first time during 2011 in Germany near the town of Schmallenberg in North Rhine-Westphalia province (Hoffmann et al., 2012). Quickly after, the reports of virus presence came from other European countries like the Neth-

erlands, Belgium, Italy etc. During 2012, most of European countries reported the presence of virus or specific antibodies in cattle, sheep and goats and the virus quickly became endemic in north-western part of Europe (Kaba et al., 2013; Doceul et al., 2013). The isolation of virus in Slovenia at the beginning of 2013 and Croatia brought the possibility that the virus is present also in the Republic of Serbia without registered clinical cases (Toplak et al., 2013; Bedekoviæ et al., 2013).

Schmallenberg virus is an enveloped, negative-sense, segmented single-stranded RNA virus classified in the *Bunyaviridae* family and *Orthobunyavirus* genus. The Schmallenberg virus is closely related to Acabane, Shamonda and Aino virus. Due to sequence data it is classified for now in Simbu serogroup (www.oie.int, 2013). As other viruses of the same family it is arthropode-borne virus and until now it is known that ceratopogonid midges such as *Culicoides spp.* and other haematophagous insects can be vectors of Schmallenberg virus (Mellor et al., 2008; Rasmussen et al., 2012). So far, there have been no indications for direct zoonotic transmission from ruminants to humans (Beer, 2011; Gibbens, 2012). Experimental infection in cattle and sheep showed no clinical signs or mild symptoms at 3 to 5 days post-inoculation with an incubation period of between 1 and 4 days and viraemia lasting for 1 to 5 days (www.oie.int, 2013). Clinical symptoms of Schmallenberg virus infection in cattle include high fever ( $> 40^{\circ}\text{C}$ ), milk drop (to 50%), general depression, loss of appetite and in some cases diarrhea, abortions, stillbirths and congenital malformations, such as arthrogyphosis and hydranencephaly. The virus also affects and wild ruminant are also affected.

The laboratory detection of Schmallenberg virus is based on serology methods such as ELISA, VNT, IFA, isolation of virus on cell culture [*Culicoides variipennis* larvae cells (KC cells) and BHK cells] and molecular biology methods like real-time RT-PCR (www.oie.int., 2013).

Due to the current epizootiological situation in surrounding countries the investigation of seroprevalence to Schmallenberg virus in dairy cattle in the Republic of Serbia was carried out. The goal of this paper is to present the results of serological survey on the presence of specific antibodies to Schmallenberg virus in dairy cattle in the Republic of Serbia in the absence of reported clinical signs of infection.

## Materials and Methods

Total of 119 samples of dairy cows were collected by random choice of location and herds. The sampling was done taking in consideration representative number of investigated cattle in 5 different locations. All animals used to graze on the pastures and they were exposed to the potential vectors of the disease. Most of the animals already had several lactation periods and they were not mixed between herds nor were the newly arrived animals introduced to the herds. There were no reports of any clinical signs of Schmallenberg virus disease in the cattle.

Location A (approximately 70 km distance from Belgrade in direction of Croatia - South Western part of Northern Serbia) was a private extensive breeding type of farm of dairy cattle, grazing and using one pasture and all cows were severely infested by ticks. Location B (approximately 82 km from Belgrade

at northeastern Serbia) included two farms, farm 1 with approximately 20 dairy cows (not infested by ticks and do not graze), and farm 2 around 10 dairy cows (infested by ticks, grazing on the pasture). Location C (central part of the Republic of Serbia) included one private extensive farm where cattle are used for milk and meat production and all animals belong to the domestic cattle breed in type of Simmental. They used to graze on the pasture. There were no evidence of import of animals from abroad, nor were the new animals introduced to the herd. Location D (central northern part of the Republic of Serbia) used to graze on community pasture and they are severely infested by ticks. Location E (eastern north part of Serbia near border with Romania) included 4 herds on private farms where only farms 3 and 4 are in intensive dairy production. All cows grazed on the pasture and they were infested by ticks.

Method used in this study was commercial indirect ELISA test for detection of specific antibodies to Schmallenberg virus - ID Screen Schmallenberg virus indirect (IDvet Innovative Diagnostics).

## Results and Discussion

From the total of 119 cows 16 (13.45%) were reacted positively for the specific antibodies to Schmallenberg virus and 1 result was inclusive. The results and approximate locations are given in Table 1.

From total of 119 tested cattle sera samples from different locations in the Republic of Serbia, we detected antibodies in 16 cows and one was doubtful. The seropositive cows are located at two different locations in the central and central

**Table 1**  
Locations and results of serological survey for presence of specific Schmallenberg virus antibodies in dairy cattle in the Republic of Serbia

Location	Geographical site	No of samples	No of positive samples	Percentage, %
A	South Western part of Northern Serbia	14	0	0%
B	Northeastern Serbia	16	0	0%
C	Central part of Serbia	15	15	100%
D	Central northern part of Serbia	20	1	5%
E	Eastern north part of Serbia	54	0	0%
TOTAL		119	16	13.45%

northern part of the Republic of Serbia. All cows were domestic cattle breed in type of Simmental for combined milk and meat production and had 2-3 lactation periods behind with normal calves delivered. No reproduction problems were registered. There were no signs of appetite lost, cattle freely grazed on pastures and had satisfactory body condition score. Cattle were kept in extensive breeding type with average daily milk production of 15-20 liters. Milk is used for cheese and kajmak production and products are sold in local markets. The usual practice of this breeding type is that cattle are kept on pastures for in barns. Each cow was clinically examined prior to sampling and none of them showed any clinical signs of infection. They were highly infested by tick and were taken for further analysis. Ticks were sampled also from pastures in appropriate way. Within sampling period in the objects where animals stayed neither midges nor mosquitos could not be observed.

People in Serbia do not have economical power to purchase dairy cattle with high milk production rate which also need different herd management and feeding protocols. The herds are usually small and milk production and sell is not an interest of owners.

The goal of our research was to enlarge the knowledge of infectious pathology in the field knowing that haematofageous vectors are highly present. The confirmation of Schmallenberg virus case in Croatia in 2013 (Bedecković et al., 2013) imposed the possibility that the virus was present also in the Republic of Serbia. The data from Serbia indicate that different arboviruses are present in circulation (Lupulović et al., 2011). Our results are in correlation with previous references where we could have expected the positive serology results also for Schmallenberg virus as one of vector-borne viruses.

Central part of the Republic of Serbia, where we the whole herd was seropositive is interestingly known for prevalence of many different vector-borne viruses since 1972 (Bordjoški et al., 1972). Interesting findings are that in north western and north eastern parts of Serbia where cattle also graze on pastures, elevation is lower and average temperature oscillations are larger than in central parts of Serbia, we did not find any animals seropositive are larger than in central parts of Serbia, we found only one seropositive cow from 20 tested samples.

## Conclusions

- Antibodies to Schmallenberg virus were detected in 16 cows from the total of 119 tested samples (13.45%).
- No clinical case of Schmallenberg virus infections has been observed in Serbia yet.
- The high infestation with ticks is established in cows grazing on pastures. Presence of other vectors is also possible but remains to be determined.

- Further investigation is needed in order to have full picture of epizootiological situation in the fields and propose the measures for disease control and eradication in future.

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