

Spread of Schmallenberg virus infections in the ruminants in Poland between 2012 and 2013

Magdalena Larska, Julia Kęsik-Maliszewska, Aleksandra Kuta

Department of Virology, National Veterinary Research Institute, 24-100 Pulawy, Poland
m.larska@piwet.pulawy.pl

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Abstract

The aim of the study was to evaluate the prevalence of Schmallenberg virus (SBV) specific antibodies in ruminants from 13 Polish provinces, sampled between January 2010 and August 2013. A total number of 1813 serum samples from cattle, sheep, goats, mouflon, wild and farmed cervids, and European bison were tested by ELISA for viral nucleoprotein antibodies. First SBV seropositive animals were identified in August 2012 (1.6%), and the percentage increased gradually, reaching 57.1% in December of this year. The proportion of seropositive animals in 2013 at the level of 34.2% increased tenfold in comparison to 2012 (3.4%), which was particularly significant at the level of individual provinces. In 2013, the highest percentages of SBV seropositive animals were found in Dolnoslaskie (92.3%), Podlaskie (82.3%), and Zachodniopomorskie (80.9%) provinces. Significant associations between the seroprevalence and province of origin, month, ruminant species, and insect vector activity were found, while no dependence of animal age on seropositivity was observed. The differences between SBV seropositive large and small ruminants suggested the involvement of some vector exposure factors, which needs to be further investigated.

Key words: ruminants, Schmallenberg virus, serology, ELISA, Poland.

Introduction

In August 2011, Schmallenberg virus (SBV) was first identified in Germany and the Netherlands at the outbreaks of unknown disease of cattle. The animals showed non-specific clinical signs such as drop of milk yield, watery diarrhoea, and increased body temperature (11). The causative agent was identified as a new virus, which belonged to *Orthobunyaviridae* genus, not yet reported in Europe. The origin of SBV remains unclear; however, a recent retrospective study revealed the presence of SBV antibodies in cattle in Turkey already in 2006, five years before the appearance of the virus in Western Europe (1). The viruses genetically related to SBV are common in Asia, Africa, and Australia, and they all belong to arboviruses, pathogens transmitted by insect vectors. The SBV vectors are *Culicoides* spp. midges also involved in the transmission of bluetongue virus (BTV). Ruminants are the most susceptible hosts of SBV infection; however, specific antibodies have been reported in camelids, swine, wild boars, and dogs. The greatest losses due to SBV infection are associated with the malformations, stillbirths, and mortality of the

newborns during the first 24 h. The increased fertility problems in cows such as sterility, foetal absorptions, late abortions, which may prolong the intercalving interval, together with a transient decrease in milk yield and moderate diarrhoea may suggest SBV infection. While in cattle the symptoms often remain unnoticed, losses in sheep and goats may be more severe, affecting up to 50% of progeny, which has been also observed in Polish sheep flocks (16). In spite of many analogies to BTV, SBV spreads faster across Europe (5, 22), which may be related to the biology of *Culicoides* spp. or the differences in the virus replication in the vector (14). The presence of SBV has been reported by EFSA in 25 European countries in May 2013, which included over 8000 herds of cattle, sheep, goats, buffaloes, wild cervids, European bison (13), and New and Old World camelids (5). The seroprevalences in cattle and small ruminants reached up to 100%. Similarly to BTV, SBV overwintered in many geographic areas (6, 21, 25), including Poland, and reappeared again in 2012 and 2013 spreading across the whole continent (5). While BTV ability to survive the winter has not been explained, the transovarial transmission of SBV in the midges has been suggested (14).

SBV infection in Polish cattle was first reported in August 2012 (15). The outbreak coincided with the introduction of two French bulls into the herds in Goleniow (Zachodniopomorskie province) and Czeszchowa districts (Ślaskie province), which were SBV positive during the quarantine. The virus spread within the herds quite rapidly. Forty percent of cattle were infected two weeks after the introduction of the infected bull in the Goleniow herd, while over 90% of the animals in the herd were seropositive few months later. The infections in both herds were asymptomatic. The presence of virus RNA was also confirmed in *Culicoides obsoletus*, which were caught in August 2012 in Goleniow district, 5 km away from the examined herd (15).

Additionally to the direct losses in the cattle reproduction, increasing risk of SBV infections resulted in the introduction of new restrictions concerning the trade of live ruminants, their embryos, and semen from the countries where the presence of SBV has been confirmed to the countries free of the disease, including the countries with unknown epizootic situation. Because reporting of suspected cases is not obligatory, the farmers prefer not to cope with any possible veterinary restrictions of the herd if the case is reported, which makes it difficult to assess the current epizootic situation of SBV in Poland. Therefore, the aim of this study was to determine the seroprevalence of SBV infections in wild and domestic ruminants in our country between 2010 and 2013.

Material and Methods

A total of 1813 serum samples from sheep (*Ovis aries*) (n = 1066), cattle (*Bos taurus*) (n = 355), free-living (n = 86) and farm (n = 115) red deer (*Cervus elaphus*), goats (*Capra hircus*) (n = 64), European bison (*Bison bonasus*) (n = 61), farm fallow deer (*Dama dama*) (n = 56), and farm mouflon (*Ovis aries*) (n = 10), collected between January 2010 and August 2013 from the 13 provinces, were tested. The domestic ruminants were between six months and 17 years of age. Most samples were derived from BTV monitoring programme in domestic ruminants (n = 1574, 86.8%), while other samples were subjected to the Department of Virology for diagnostic or certification purposes (e.g. involved in BVDV and BoHV-1 eradication programmes). The number of samples collected in the BTV monitoring programme supervised by the veterinary inspection services was estimated for the detection of SBV seroconversion with 95% probability, assuming 20% seroprevalence. The samples were obtained due to the courtesy of the regional veterinary laboratories. All animals included in the study had no history of SBV infection, any SBV suspected cases (such as malformations of the newborns in the seropositive herds with no SBV identification) investigated at the National Veterinary Research

Institute were excluded. The sera from free-living cervids were obtained during the research conducted in the project No. NR12-0127-10/2010 funded by the National Centre of Research and Development. These animals were culled by certificated hunters in accordance with the Act of 13 October 1995 on the Hunting law (Law Gazette 2002.42.372). The European bison were eliminated or chemically immobilised in accordance with the decision of General Director of the Environment.

The commercial serological indirect ID Screen Schmallenberg Virus Indirect Multi-Species ELISA (IDvet, France) for the detection of antibodies against SBV nucleoprotein was used. The assay is based on the blocking of the specific antibodies in a tested serum by the recombinant SBV nucleoprotein (N) antigen coating the microplates from binding to the conjugate – anti-multi-species IgG antibodies produced *in vitro* in a hybrid cell line coupled with horseradish peroxidase. The results were interpreted on the S/P values calculated from the optical density (O.D.) value measured at 450 nm using the following equation: $\text{O.D. sample tested} - \text{O.D. positive control} - \text{O.D. negative control} \times 100$. The S/P values negative control below and equal to 50% were considered negative, S/P value above 60% - positive, and the S/P value between 50% and 60% - doubtful. The sensitivity and specificity of the test for bovine, ovine, and caprine sera against virus neutralisation test declared by the manufacturer were 100% and 99.25%, respectively.

The positive results were confirmed by ID Screen Schmallenberg Virus Competition Multi-Species (IDvet, France), which uses the same viral N antigen as the above mentioned test and the specific monoclonal antibodies, which bind competitively to the antigen. For each sample, the S/N percentage was calculated as follows: $\text{O.D. sample} / \text{O.D. negative control} \times 100$. The samples presenting a S/N value less than or equal to 40% were considered positive, greater than 40% and less than or equal to 50% were considered doubtful, and greater than 50% were negative. Sensitivity and specificity of the assay tested for bovine, ovine, and caprine serum samples, declared by the manufacturer was 97.6% and 100% respectively. Both tests were performed according to the manufacturer's instruction. STATA version 11 software (StataCorp., USA) was used in the statistical analysis and graphs design. The results were expressed as the mean values with 95% confidence interval (CI) calculated for binomial distribution using Wald estimation. The Kruskal-Wallis test was used to define the correlations between the percentage of seropositive animals and parameters, such as animal species, population type (farm or free-living), province of origin, monthly vector activity. The relationship between age and seropositive results was studied using Mann-Whitney test. The significant differences ($P < 0.05$) were described in the value of χ^2 , in number of degrees of freedom (df), and the probability value (P).

Results

Out of 1813 samples, 203 (11.1%; 95% CI 9.7 – 12.6) reacted positively in both ELISAs used for the detection of SBV specific antibodies.

Association between SBV seroprevalence and month. No antibodies against SBV were found in the serum samples collected between January 2010 and August 2012 (n = 186) (Table 1).

Four seropositive sheep (two from Lubuskie and two from Lubelskie provinces), detected in August 2012, which represented 1.6% (95% CI 0.9–2.6) of individuals tested in this month, were the first seropositive animals. Then, the percentage of seropositivity increased quite rapidly. In December 2012, already 57.1% (95% CI 36.5–75.5) of the ruminants tested, including some animals from the eastern provinces, had specific antibodies. During the winter months, the seroprevalence remained at the similar level, and then it began to increase again in the spring of 2013 (Fig. 1.).

Association between SBV seroprevalence and province of origin. The overall percentage of seropositive animals increased tenfold from 3.4% (95% CI 24.1–43.9) in 2012 to 34.2% (95% CI 29.9–38.7) in 2013, which was significantly expressed at the province level (Table 2). The highest percentage of positive seroreagents was recorded in 2013 in Dolnoslaskie (92.3%, 95% CI 64.0–99.8), Zachodniopomorskie (80.9%, 95% CI 73.4–88.4), and Podlaskie provinces

(82.3%, 95% CI 62.1–102.5). The percentage of seropositive animals was related to the province of origin in 2012 ($\chi^2 = 113.4$; 11df; $P = 0.0001$) and in 2013 ($\chi^2 = 210.5$; 7df; $P = 0.0001$). The SBV seroprevalence at the province level is illustrated in Fig. 2.

Association between ELISA value and year.

A significant increase in the proportion of seroconverted animals between 2012 and 2013 is also well illustrated by the graphs of the S/P values (Fig. 3). In 2012, only a few samples reached optical density values above the cut off in the ELISA, and were considered positive, whereas in the next year higher numbers of animals obtained much higher S/P values (the second peak appeared at the graph) (Fig. 3).

Association between SBV seroprevalence and vector activity. The relation between the percentage of SBV seropositive animals and the activity of *Culicoides* spp. was statistically significant ($\chi^2 = 104.2$; 8df; $P = 0.0001$) (Fig. 4). A sudden increase in SBV seroprevalence from August until December 2012 was positively associated with the midges activity in 2012, despite the shift between the two charts. In the winter, when *Culicoides* spp. activity decreased, the percentage of seropositive remained stable ranging from 32.5% in November 2012 to 24.4% in March 2013. The second increase in seroprevalence, which began in April 2013, and reached the highest values of 92.31% in July 2013, overlapped the vector activity peak of 2013.

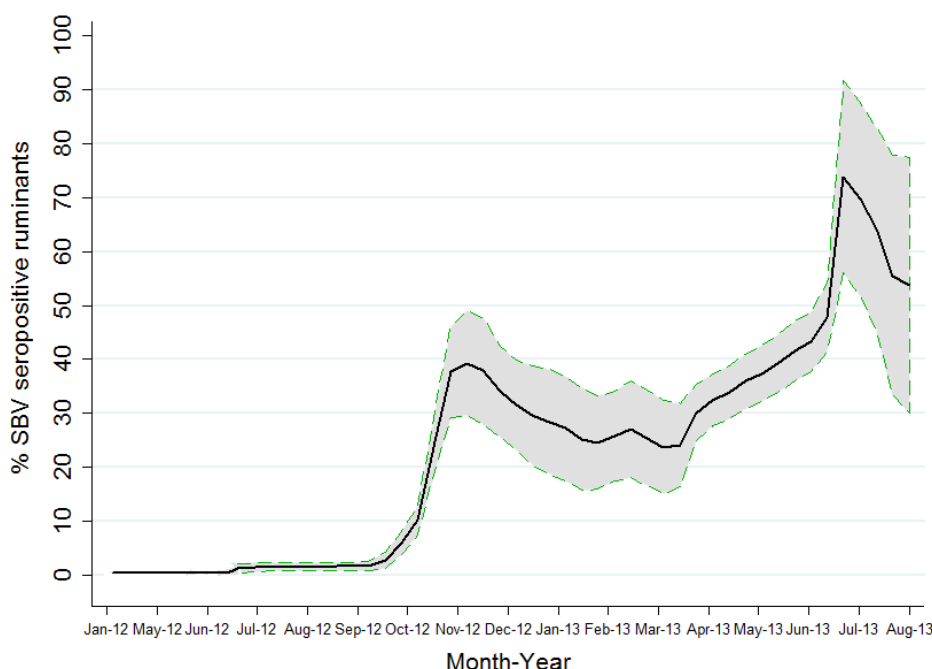


Fig. 1. Diagram of the percentage of seropositive animals for Schmallenberg virus in respect to the month of sampling. The black smoothed curve expresses the estimated mean percentage of monthly seroprevalence function, while the gray area limited by the green dashed lines stands for Wald 95% confidence interval (CI) with $P < 0.05$

Table 1. Descriptive statistics of SBV seropositive ruminants by month and year of sampling

Year	Month	N	n seropositive	%	SD*	95% CI**
2010	January	16	0	0	0	0
	February	10	0	0	0	0
2011	March	7	0	0	0	0
	April	6	0	0	0	0
	December	18	0	0	0	0
	January	62	0	0	0	0
2012	May	12	0	0	0	0
	June	13	0	0	0	0
	July	42	0	0	0	0
	August	888	14	1.6	0.4	0.8–2.4
	September	210	3	1.4	0.8	0–3.0
	October	2	1	50	35.3	0–100
	November	43	14	32.55	7.1	18.6–46.6
	December	21	12	57.1	10.8	36.0–78.3
2013	January	62	12	19.3	5	9.5–29.2
	February	6	4	66.7	19.2	28.9–100
	March	41	10	24.4	6.7	11.2–37.5
	April	65	13	20	49.6	10.2–29.7
	May	260	99	38.1	3	32.2–44.0
	June	13	12	92.3	7.4	77.8–100
	July	13	8	61.5	13.5	35.1–88.0
	August	3	1	33.3	27.2	0–86.7
Total		1.813	203	11.1	0.7	9.7–12.6

*standard deviation; **95% Wald confidence interval

Table 2. Descriptive statistics of SBV seropositive ruminants by provinces and year of sampling

Province	2012					2013				
	N	n seropositive	%	SD*	95% CI**	N	n seropositive	%	SD*	95% CI**
Dolnoslaskie	-	-	-	-	-	13	12	92.3	7.4	77.8–100
Lodzkie	8	1	12.5	11.7	0–35.4	-	-	-	-	-
Lubuskie	218	13	6.0	1.6	2.8–9.1	-	-	-	-	-
Lubelskie	337	10	3.0	0.9	0.1–4.8	6	0	0.0	0	0
Malopolskie	14	0	0.0	0	0	-	-	-	-	-
Mazowieckie	66	1	1.5	1.5	0–4.5	-	-	-	-	-
Podlaskie***	95	7	7.4	2.6	2.1–12.6	17	14	82.4	9.2	64.2–100
Pomorskie	22	8	36.4	10.2	16.3–56.5	46	4	8.7	4.1	0.5–16.9
Slaskie***	35	0	0.0	0	0	60	14	23.3	5.5	12.6–34.0
Swietokrzyskie	44	0	0.0	0	0	-	-	-	-	-
Warminsko-mazurskie***	16	3	18.8	9.7	0–37.9	2	1	50.0	35.3	0–100
Wielkopolskie	404	0	0.0	0	0	208	24	11.5	2.2	7.1–15.9
Zachodniopomorskie***	34	1	2.9	2.9	0–8.6	110	89	80.9	3.7	73.6–88.2
Total	1 293	44	3.4	0.5	2.4–4.4	462	158	34.2	2.2	30.0–38.7

*standard deviation; **95% Wald confidence interval; ***significant differences in percentage of SBV seropositive ruminants between 2012 and 2013 ($P < 0.05$)

Association between SBV seroprevalence and ruminant species. Considering the total number of samples tested, the highest percentage of seropositivity was observed in cattle (37.7%; 95% CI 29.5–42.8), then in European bison (36.1%; 95% CI 28.7–50.2), while the lowest - in goats (3.1%; 95% CI 0.1–7.2) and in sheep (2%; 95% CI 0–4.2) (Table 3). Differences in the proportion of seropositive ruminants according to species were statistically significant in 2012 ($\chi^2 = 136.9$; 6df, $P = 0.0001$) and in 2013 ($\chi^2 = 42.1$; 4df, $P = 0.0001$). The association between seroprevalence and

type of the population (free-living or farm) was significant in 2012 ($\chi^2 = 229.6$; 1df, $P > 0.001$), but insignificant in 2013 ($\chi^2 = 1.4$, 1df, $P = 0.23$). In 2013, the percentages of SBV seropositive free-ranging (26.9%; 95% CI 15.8–39.1) and farm (35.6%; 95% CI 27.3–45.6) animals were very similar; however, when all ruminants sampled after August 2012 (after the first detection of antibodies) were considered, the seroprevalence of free-living European bison and deer was significantly higher than in domestic animals.

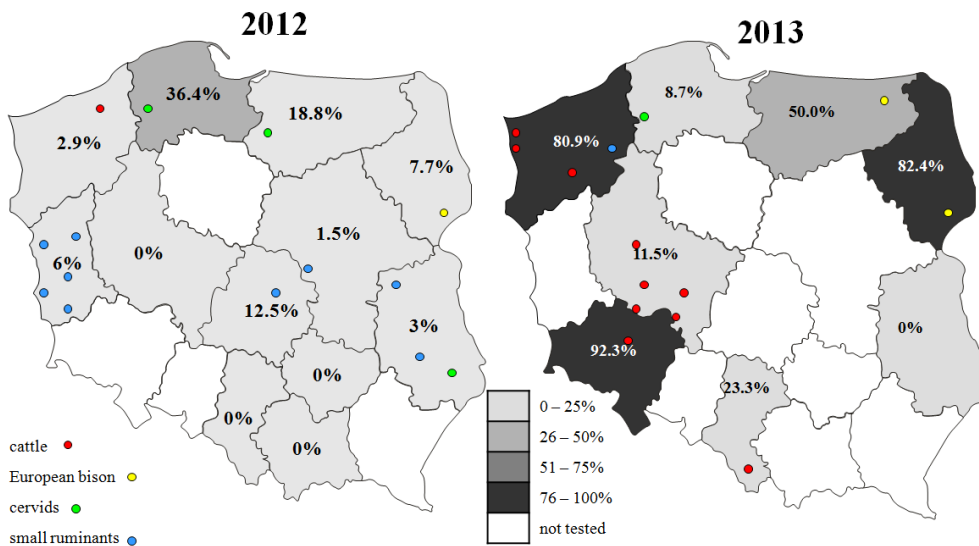


Fig. 2. Maps of SBV seroprevalence at the province level including the infected species (color dots) in 2012 and 2013

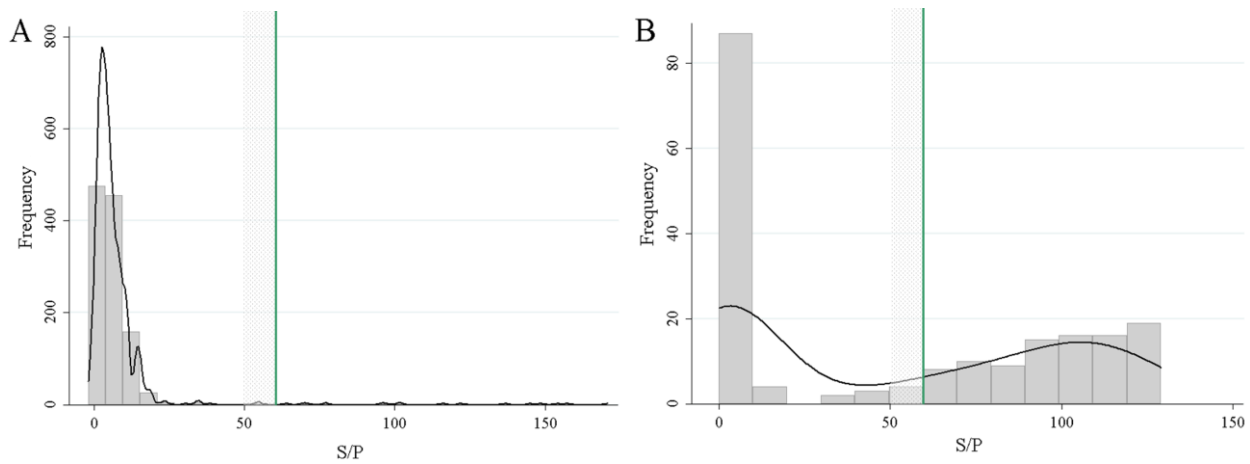


Fig. 3. The histograms of the frequency of S/P values in ID Screen Schmallenberg Virus Indirect Multi-Species ELISA (IDvet, France) for the detection of SBV antibodies in the sera of ruminants tested in 2012 (A) and 2013 (B). The solid black curve represents the Gaussian estimator of kernel density. The results were interpreted as follows: S/P value $\leq 50\%$ was considered seronegative, S/P $> 60\%$ positive (the cut-off value determined by the greed solid line), and the S/P value between 50% and 60% doubtful (marked by dotted area) according to the manufacturer's protocol

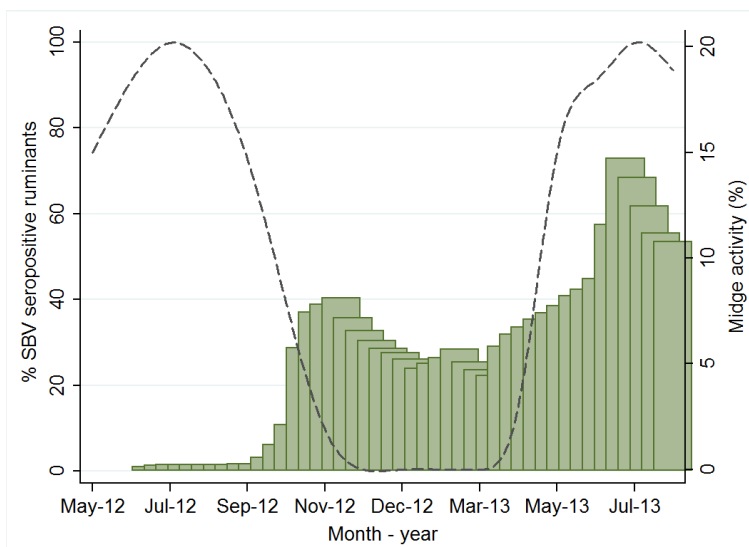


Fig. 4. Graph of the association between the percentage of SBV seropositive animals (columns) and the activity of biting midges of *Culicoides* spp. by month (dotted line). The midge activity was calculated as a monthly percentage of all *Culicoides* midges trapped and counted during 2012 BTV monitoring (18)

Table 3. Descriptive statistics of SBV seropositive ruminants by species and population type tested between January 2010 and August 2013 and between August 2012 and August 2013

Species	2010 - 2013					August 2012 - August 2013				
	N	n seropositive	%	SD*	95% CI**	N	n seropositive	%	SD*	95% CI**
cattle	355	134	37.7	2.5	32.7–42.8	355	134	37.7	2.6	32.7–42.8
European bison	61	22	36.1	6.1	24.0–48.1	25	22	88	6.6	74.3–101.7
fallow deer	56	0	0	0	0	-	-	-	-	-
goat	64	2	3.1	2.2	0–7.4	57	2	3.5	2.4	1.4–8.4
mouflon	10	1	10	9.4	0–28.6	10	1	10	9.4	0–28.6
red deer	201	23	11.4	2.2	7.0–15.8	169	23	13.6	2.6	8.4–18.8
sheep	1066	21	2	0.4	1.1–2.8	1 011	21	2.1	0.4	1.2–3.0
Population type***										
farm	1637	158	9.6	0.7	8.2–11.1	1495	158	10.6	0.8	9.0–12.1
free-ranging	176	45	25.6	3.3	19.1–32.0	132	45	34.1	4.1	25.9–42.3

*standard deviation; **95% Wald confidence interval; ***farm include domestic ruminants, farm cervids and mouflon, while free-ranging include the red deer and European bison

Discussion

The first detection of SBV seropositive sheep in this study occurred in August 2012 confirming the results of our previous studies on the presumable date of SBV transmission into Poland (13, 15). However, SBV could have probably reached Polish Western borders much earlier. The first clinical case in a bovine newborn diagnosed in Zachodniopomorskie province in November 2012 might have been infected in the spring (12). SBV have spread in Europe faster than BTV-8, despite that the geographic areas of the primary outbreaks overlapped. Within four months after detection of the first seropositive animals in August 2012, the proportion of seropositive animal reached 57.1%. Rapid SBV transmission across the country suggested that the transport of potentially infected animals may have played a greater role than the midges movements only, even accelerated by the wind. Due to the lack of epizootic restrictions on the trade and transport of SBV infected animals inside of the European Union, SBV spread might have been facilitated by import of ruminants from Western European countries into Eastern neighbouring countries. Despite the fact that Poland is often only a transit country, the rest of the areas are located on our territory or close to our borders (Veterinary Officer personal communication), and the imported animals may be the source of infection for our ruminants. Such situation was observed in Białowieża National Park (Podlaskie province) where up to 100% of European bison have remained SBV seropositive since November 2012 (13). Additionally, the involvement of wildlife as the important reservoirs of SBV needs to be considered when analysing the risk of SBV infection for domestic livestock (22).

The highest seroprevalences in Dolnoslaskie (92.3%) and Zachodniopomorskie provinces (80.9%) in

2013 may be explained by the proximity of SBV primary outbreaks in Europe, while high percentage in Podlaskie (82.3%) neighbouring with the Eastern border may be related to the animal transit stops located nearby. In addition, a relatively high percentage of seropositive red deer found in the Lubelskie province (38%) (not shown) may suggest the recent SBV outbreaks somewhere at the border areas of Belarus and Ukraine. However, these countries have not reported cases of SBV yet. High variation in geographical seroprevalence, that was also revealed in other studies (10, 20), suggests that the movement of SBV infected animals might have been the key factor of virus transmission. If the virus spreads only by the insects, a more even distribution of SBV infections and/or their greater concentration around the wetland areas, where the conditions for the habitat and reproduction of the midges are optimal, could be expected.

The first peak of SBV seroprevalence observed in the winter 2012 was shifted with respect to the peak of midges activity, probably due to the later transmission of SBV into the country in late summer, as suggested by this and previous study (13, 15). Using quantitative methods like ELISA, it should be also remembered that the observed seroprevalence does not indicate seroconversion at the time of testing. SBV antibodies appear 10–21 d after infection (11, 26). The half-life of SBV antibodies and their ability to neutralise the virus remain uncertain, except for the seropositive cattle, which is resistant to reinfection for at least eight weeks (24). Similarly to other European countries (6, 21, 25), SBV was able to overwinter in Poland without the active vector. Two possible explanations should be considered including the SBV sylvatic cycle in which the virus is transmitted among the wildlife reservoirs (3, 5, 13, 22), and the survival of the virus in the midge population (25). The latter explained by the possible

transovarial transmission of SBV in *Culicoides* spp. has been proven previously (14). Possibly, some biting midges hibernate inside the farm buildings during the winter in the form of eggs. After the hatching and metamorphosis, the mature females begin to feed in early spring causing the reactivation of the virus and the emergence of further SBV outbreaks at the area (25). Comparable percentage of seropositive animals among farm and free-living ruminants may indicate similar susceptibility to SBV infection. Nevertheless, the seroprevalence in the wild ruminants was higher at the beginning of SBV epidemic in Poland, which again suggests that wildlife are important reservoirs of SBV (3, 13, 22).

The overall SBV seroprevalence in 2012 and 2013 was significantly lower than the prevalence observed in Western European countries, which suggests that Poland is still at the risk of SBV spread, and the occurrence of malformation in the SBV infected neonates might have been expected again in the second semester of 2013 and in 2014. Interestingly, the SBV seroprevalence was higher in the larger ruminants such as cattle and European bison in comparison to the small ruminants. Similar observations were made for other serosurveys. In Belgium, the percentages of seropositive cattle reached 90.8% (for 519 animals tested) (8) and 99.76% (N = 11 635) (19) and in France up to 79% (N = 1715) (7). The seroprevalence reported at the same time in sheep and goats differed significantly. For example, SBV seroprevalence in small ruminants did not exceed 58.7% (N = 1760) and 43.8% (N = 500) in Germany (10). In Greece, the difference between the percentages of seropositive samples from cattle (64.45%, N = 90) and sheep (5.3%, N = 19) also was significant (2). British study has also shown rather low seropositivity in 10 sheep flocks investigated (20). No seropositive ewes were found in some flocks despite confirmation of SBV infection in the lambs originating from the same flocks and grazing close to seropositive cattle farms (20). In the Netherlands, the seroprevalence varied depending on the production type with 98.5%, 63.4%, 89.5%, and 50.8% of seropositive animals among beef cattle (N = 1347), dairy cattle (N = 3030), sheep (N = 2833), and goats (N = 1548), respectively (23). While SBV infection in small ruminants has serious consequences for reproduction, in cattle it can remain subclinical with no or just a few clinical cases in the newborns. However, the observed differences between seroprevalence in cattle and small ruminants are probably not related to the species-specific susceptibility to SBV infection, but rather to the differences in vector exposure factors. Because of the serious implications for sheep and goats breeding (4), more careful and effective prevention methods and control measures implemented might have resulted in a lower seroprevalence in small ruminants (20). Additionally, some studies have shown that *Culicoides* spp. prefer feeding on cattle than on other ruminants.

According to Ninio *et al.* (21), the alimentary tract of biting midges contained 54% of bovine blood and only 4% of sheep blood. This was also confirmed by Lassen *et al.* (17), who studied midges in four different areas where various ruminant species were reared. Blood-fed *Culicoides* abdomens contained 77% of cattle, 4% of goat, 0.8%–2.5% of deer, and 0.4% of sheep blood. The concentrations of midge-attracting gases secreted by ruminants, such as carbon dioxide and octenol, density of the hair coat, maintenance and type of bedding should be considered as important selective factors for SBV transmission (9, 10). Meroc *et al.* (19) reported higher seroprevalence in the cattle over 2-year-old and suggested the connection with the longest exposure of this group for the insects bites. The differences in the seropositivity in various age groups can be also associated with the variation of the excretion of *e.g.* carbon dioxide, one of the strongest midge attractants (27). In this study, however, there was no correlation between age of the animal and the presence of antibodies against the SBV, which is in agreement with other studies (6). However, if SBV circulates for a longer period in Poland, the risk of exposure to infection is expected to be higher in older animals.

In summary, the proportion of SBV infected domestic and wild ruminants in Poland since August 2012 was associated with the date of testing and the geographical origin of the animals, as the main SBV infection risk factors were the number of viraemic animals in a given area and the presence of active SBV vector. The trade and animal transport of farm ruminants potentially infected with SBV might have increased the risk of the virus transmission. Especially the rapid Easter bound SBV spread in the autumn/winter 2012 suggests the carriage of virus infected animals by means of transport, rather than natural midge migration speed, even if intensified by wind or other favourable environmental conditions. The research on SBV distribution will be continued in Poland as it seems that SBV is going to circulate continuously, similarly to Akabane virus in Australia, remaining a threat to the health and production of farm and free-living ruminants in Europe.

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References

1. Azkur A.K., Albayrak H., Risvanli A., Pestil Z., Ozan E., Yilmaz O., Tonbak S., Cavunt A., Kadi H., Macun H.C., Acar D., Ozenc E., Alparslan S., Bulut H.: Antibodies to Schmallenberg virus in domestic livestock in Turkey. *Trop Anim Health Prod* 2013, 45, 1825–1828.

2. Chaintoutis S.C., Kiossis E., Giadinis N.D., Brozos C.N., Sailleau C., Viarouge C., Bréard E., Papanastassopoulou M., Zientara S., Papadopoulos O., Dovas C.I.: Evidence of Schmallenberg virus circulation in ruminants in Greece. *Trop Anim Health Prod* 2013 (in press).
3. Chiari M., Sozzi E., Zanoni M., Alborali L.G., Lavazza A., Cordioli P.: Serosurvey for Schmallenberg virus in Alpine wild ungulates. *Transbound Emerg Dis* 2014, 61, 1–3.
4. Claïne F., Coupeau D., Wiggers L., Muykens B., Kirschvink N.: Schmallenberg virus among female lambs, Belgium, 2012. *Emerg Infect Dis* 2013, 19, 1115–1117.
5. EFSA. 2013: <http://www.efsa.europa.eu/en/supporting/doc/429e.pdf>.
6. Elbers A.R., Loeffen W.L., Quak S., de Boer–Luijtz E., van der Spek A.N., Bouwstra R., Maas R., Spierenburg M.A., de Kluijver E.P., van Schaik G., van der Poel W.H.: Seroprevalence of Schmallenberg virus antibodies among dairy cattle. the Netherlands, winter 2011–2012. *Emerg Infect Dis* 2012, 18, 1065–1071.
7. Gache K., Dominguez M., Pelletier C., Petit E., Calavas D., Hendrikx P., Touratier A.: Schmallenberg virus: a seroprevalence survey in cattle and sheep. France, winter 2011–2012. *Vet Rec* 2013, 173, 141.
8. Garigliany M.M., Bayrou C., Kleijnen D., Cassart D., Desmecht D.: Schmallenberg virus in domestic cattle. Belgium. 2012. *Emerg Infect Dis* 2012, 18, 1512–1514.
9. González M., López S., Mullens B.A., Baldet T., Golderazena A.: A survey of Culicoides developmental sites on a farm in northern Spain with a brief review of immature habitats of European species. *Vet Parasitol* 2013, 191, 81–93.
10. Helmer C., Eibach R., Teqtmeyer P.C., Humann–Ziehanke E., Runge M., Ganter M.: Serosurvey of Schmallenberg virus infections in sheep and goat flocks in Lower Saxony, Germany. *Transbound Emerg Dis* 2013, doi: 10.1111/tbed.12161.
11. Hoffmann B., Scheuch M., Höper D., Jungblut R., Holsteg M., Schirrmeier H., Eschbaumer M., Goller K.V., Wernike K., Fischer M., Breithaupt A., Mettenleiter T.C., Beer M.: Novel orthobunyavirus in cattle. Europe, 2011. *Emerg Infect Dis* 2012, 18, 469–472.
12. Larska M., Jasik A., Żmudziński J.F.: Clinical cases of Schmallenberg virus infection in Poland. *Med Weter* 2013, 69, 428–431.
13. Larska M., Krzysiak M., Smreczak M., Polak M.P., Żmudziński J.F.: First detection of Schmallenberg virus in elk (*Alces alces*) indicating infection of wildlife in Białowieża National Park in Poland. *Vet J* 2013, 198, 279–281.
14. Larska M., Lechowski L., Grochowska M., Żmudziński J.F.: Detection of the Schmallenberg virus in nulliparous Culicoides obsoletus/scoticus complex and C. punctatus – the possibility of transovarial virus transmission in the midge population and of a new vector. *Vet Microbiol* 2013, 166, 467–473.
15. Larska M., Polak M.P., Grochowska M., Lechowski L., Związek J.S., Żmudziński J.F.: First report of Schmallenberg virus infection in cattle and midges in Poland. *Transbound Emerg Dis* 2013, 6, 97–101.
16. Larska M., Tarkowska K., Kuta A., Fidler–Kwiatkiewicz E., Ciastek M., Żmudziński J.F.: Clinical picture of Schmallenberg virus infections. *Życie Wet* 2013, 88, 488–492.
17. Lassen S.B., Nielsen S.A., Kristensen M.: Identity and diversity of blood meal hosts of biting midges (Diptera: Ceratopogonidae: Culicoides Latreille) in Denmark. *Parasit Vectors* 2012, 5, 1–9.
18. Lechowski L., Grochowska M.: The report of the monitoring of Culicoides spp. Midges, potential vector of bluetongue virus in Poland in 2012. available at the Chief Veterinary Office, Warsaw, Poland.
19. Méroc E., Poskin A., Van Loo H., Quinet C., Van Driessche E., Delooz L., Behaeghel I., Riocreux F., Hooyberghs J., De Regge N., Caij A.B., van den Berg T., van den Stede Y.: Large-scale cross-sectional serological survey of Schmallenberg virus in Belgian cattle at the end of the first vector season. *Transbound Emerg Dis* 2013, 60, 4–8.
20. Nanjiani I.A., Aitken P., Williams P.: Prevalence of seropositive sheep within flocks where Schmallenberg virus infection was suspected or confirmed. *Vet Rec* 2013, 173, 371.
21. Ninio C., Augot D., Delecalle J.C., Dufour B., Depaquit J.: Contribution to the knowledge of Culicoides (Diptera: Ceratopogonidae) host preferences in France. *Parasitol Res* 2011, 108, 657–663.
22. Tarlinton R., Daly J., Dunham S., Kydd J.H.: Schmallenberg virus: could wildlife reservoirs threaten domestic livestock? *Vet J* 2013, 198, 309–310.
23. Veldhuis A.M., Schaik G., Van Vellema P., Elbers A.R., Bouwstra R., Heijden H.M., van der Mars M.H.: Schmallenberg virus epidemic in the Netherlands: spatiotemporal introduction in 2011 and seroprevalence in ruminants. *Prev Vet Med* 2013, 112, 35–47.
24. Wernike K., Eschbaumer M., Schirrmeier H., Blohm U., Breithaupt A., Hoffmann B., Beer M.: Oral exposure, reinfection and cellular immunity to Schmallenberg virus in cattle. *Vet Microbiol* 2013, 165, 155–159.
25. Wernike K., Kohn M., Conraths F.J., Werner D., Kameke D., Hechinger S., Kampen H., Beer M.: Transmission of Schmallenberg virus during winter, Germany. *Emerg Infect Dis* 2013, 19, 1701–1703.
26. Wernike K., Hoffmann B., Bréard E., Bötner A., Ponsart C., Zientara S., Lohse L., Pozzi N., Viarouge C., Sarradin P., Leroux-Barc C., Riou M., Laloy E., Breithaupt A., Beer M.: Schmallenberg virus experimental infection of sheep. *Vet Microbiol* 2013, 166, 461–466.
27. Wieser–Schimpf L., Foil L.D., Holbrook F.R.: Effect of carbon dioxide on the collection of adult Culicoides spp. (Diptera: Ceratopogonidae) by a new modification of black light New Jersey light traps. *J Am Mosq Control Assoc* 1991, 7, 462–466.