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Serological survey of Schmallenberg virus in wild boar (*Sus scrofa*) from Southern Poland

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Summary

The aim of the study was to assess the prevalence of Schmallenberg virus in free-ranging wild boar in the southern provinces of Poland. The most recent paper on this subject used samples collected more than a decade ago. In our study tissue fluid samples were obtained post-mortem from 129 wild boar culled during the 2023/2024 hunting season in the following Polish voivodeships: swietokrzyskie (n = 38), malopolskie (n = 35), lubelskie (n = 32), opolskie (n = 16), and dolnoslaskie (n = 8). The samples were then subjected to serological testing using a commercially available ID Screen Schmallenberg virus Competition Multi-species cELISA kit. Positive and doubtful results were retested using an indirect ELISA kit. A positive test result was obtained for 2 out of 129 (1.6%) tissue fluids. A further three samples were positive in the screening test but were not confirmed. These results are consistent with similar studies conducted in other European countries, as well as with previous Polish works on this subject, and suggest a low-level presence of SBV among Polish wild boar, and thus their relatively limited role in the transmission and circulation of Schmallenberg virus.

Keywords: Schmallenberg virus, wild boar, serology, Poland

Schmallenberg virus (SBV) is a vector-borne pathogen belonging to the *Orthobunyavirus* genus within the *Peribunyaviridae* family. It is mainly transmitted by *Culicoides* spp. biting midges (10), which are also known for their significant role as vectors of widely discussed bluetongue and epizootic hemorrhagic disease viruses (22). SBV predominantly affects domestic ruminants, including cattle (*Bos taurus*), sheep (*Ovis aries*), and goats (*Capra aegagrus hircus*) and has been associated with severe clinical symptoms, such as fever, diarrhea, reduced milk production, and congenital malformations (4, 13). Thus far, Schmallenberg disease is considered to be non-zoonotic (26). It was first identified in 2011 in German cattle and has since rapidly spread across Europe (3, 13), leading to considerable concerns regarding its potential impact on both animal health and agricultural productivity. After the initial epidemic of 2011 and 2012, the virus has repeatedly re-emerged in Europe (7, 14, 29), expanding its range into further regions and has been described to be a cyclical problem (20). Throughout the first and subsequent outbreaks, SBV has been confirmed in Poland, primarily in farm ruminants (15), but its presence has also been reported in non-domesticated species, particularly wild ungulates (17, 21). Simultaneously, similar studies conducted in other European countries revealed numerous comparable cases of SBV-specific antibodies presence in wildlife, primarily in cervids, but also including wild boar (*Sus scrofa*) (2, 9, 28). It is known that free-ranging animals can serve as potential reservoirs or indicators of SBV circulation in the environment (24). In 2017, the first study on SBV-specific antibodies presence in wild boar in Poland was published, revealing very low seroprevalence results (0.99%) based on samples gathered between 2012 and 2014 (16). Since then, no further research on SBV in Polish wild boar has been done. Due to their lower seroprevalence rates in comparison to domestic ruminants and other free-ranging animals, wild boar are not the first choice for SBV testing; however, because they are widely distributed in Europe and are known to interact with both wild and domestic animal populations, their inclusion in surveillance programs remains to be needed. To date, wild boar seem to be more susceptible to SBV infection than domestic pigs (*Sus domesticus*) (9, 24). Assessing the danger of transmission to domestic livestock and putting in place efficient control measures may be made easier with an understanding

of the virus seroprevalence in wildlife. While most of the studies have mainly focused on domesticated species, with limited data available on the prevalence of SBV in wild-ranging animals, especially in recent years, this study aims to fill this gap by investigating the presence of SBV-specific antibodies in Polish wild boar, based on material collected in 2023 and 2024.

Material and methods

Material collection took place in five Polish Voivodeships: swietokrzyskie (n = 38), malopolskie (n = 35), lubelskie (n = 32), opolskie (n = 16) and dolnoslaskie (n = 8) during the 2023/2024 hunting season (Fig. 1). A total of 129 wild boar were sampled. The animals were both males (n = 60) and females (n = 69) from 0.5 to 4 years of age (mean = 1.5; median = 1) and were all legally culled. Tissue fluid from skeletal muscles was obtained post mortem, transported to the laboratory, and then stored at –80°C pending analysis. All samples were serologically tested using an Enzyme-Linked Immunosorbent Assay (ELISA) method. The presence of SBV-specific antibodies was determined with a commercially available ID Screen Schmallenberg virus Competition Multi-species cELISA kit (Innovative Diagnostics, Grabels, France), where the 96-well microplates are coated with the recombinant SBV nucleoprotein antigen. Each sample was distributed to a separate well and then sealed and incubated for 45 minutes at 37°C. Afterwards, all microplates were washed multiple times, and the horseradish peroxidase-labeled conjugate was added for another 30 minutes of incubation at room temperature. After that, another washing procedure was performed. Next, the substrate (3,3',5,5'-Tetramethylbenzidine) was added to each well, and the microplates were left in the dark for 15 minutes. Once the time was up, the colorimetric reaction was terminated by the stop solution. Results were measured at a wavelength of 450 nm using an EPOCH spectrophotometer (BioTek Instruments Inc., US). The detected optical densities (OD) were converted into S/N values using the given equation: S/N% = OD sample/OD negative control × 100, and then interpreted as follows: S/N ≤ 40% positive, S/N > 50% negative, 40% < S/N ≤ 50% doubtful. To verify the results, positive and doubtful samples were retested using an indirect ELISA kit (ID Screen Schmallenberg virus Indirect Multi-Species, Innovative Diagnostics) in accordance with the manufacturer's guidelines. Since that was a confirmatory test, each sample was analyzed in duplicate using even and odd columns. OD values of each sample were read at 450 nm, the same as before, and then calculated as follows: S/P% = netOD sample/netOD positive control × 100, with net being the difference between even and odd columns. If the S/P% value was above 60%, the result was considered positive. In addition, 95% confidence intervals (95% CI) were calculated for seroprevalence using Sterne's exact method.

Results and discussion

The initial screening showed 5/129 (3.9%; 95% CI: 1.54-8.79) positive tested wild boars. Three of them had been culled in swietokrzyskie (male, 1 year old, Bukowa; female, 0.5 year old, Rytwiny Klasztorna; female, 2 years old, Beszyce) and the other two in lubelskie (female, 1 year old, Lublin; male 2 years old, Kutki) (Fig. 1). To verify the results, those five individuals were retested with a subsequent confirmatory test using a different ELISA assay, which identified two seropositive samples (2/129; 1.6%; 95% CI: 0.28-5.64), both from swietokrzyskie (male, 1 year old, Bukowa; female, 2 years old, Beszyce) (Fig. 1). Those two sites are approximately 7 kilometers apart. Our study was conducted using tissue fluid. While not the main method, it has been used in a number of other studies, has been shown to have high sensitivity, to generate similar results to serum, and is particularly useful in free-ranging animals, where the collection of blood samples may prove difficult (1, 5, 34). Information on the prevalence of Schmallenberg virus in wild boar is limited. Among livestock, infections are only found in ruminants, and experimental infections in pigs have not led to replication of the virus (25). Consequently, although antibodies to SBV can be found in wild boar, this species is of secondary interest to researchers. If at all, studies on wild boar are conducted as part of a broader review of non-domestic animals, such as red deer, roe deer, or mouflon, in an area (6, 12, 14, 23, 27, 30). A recent meta-analysis by Dagnaw et al. (8) included only three papers on wild boar (with one of the papers cited actually not involving wild boar). Our study covers an under-researched topic. We found the presence of SBV antibodies in 2 out of

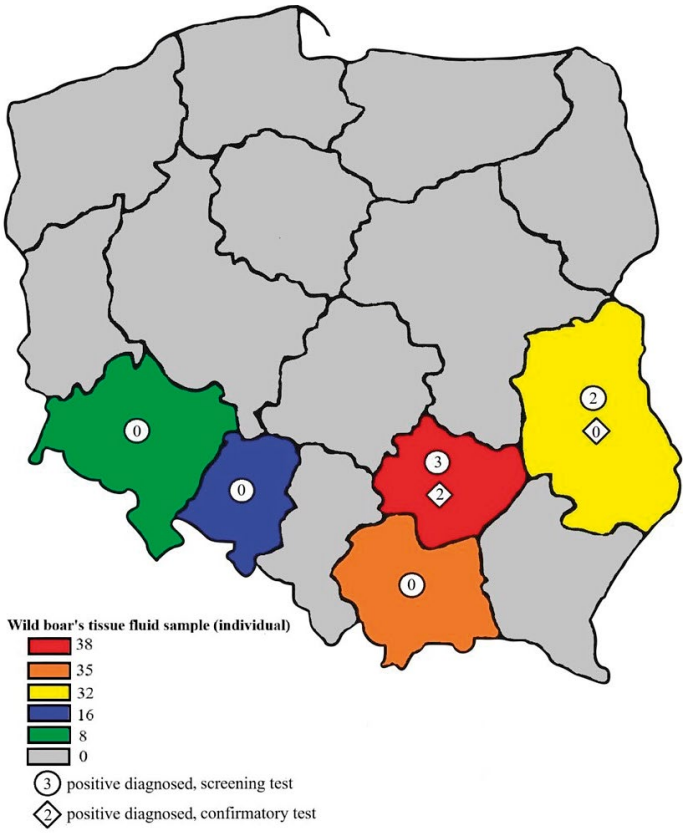

Fig. 1. Map presenting the number of wild boar's tissue fluid used in research and the number of positive diagnosed samples

129 (1.6%) individuals. This is comparable to a similar study conducted in Poland by Kęsik-Maliszewska et al. (16), who found SBV antibodies in 0.99% of wild boar tested, as well as to studies conducted in Spain by García-Bocanegra et al. (12) and Jiménez-Ruiz et al. (14), who found 2.8 ± 3.1% and 2% positivity in the tested individuals, respectively. Such numbers suggest that wild boars are not an important reservoir of the virus and likely only face incidental exposure to infection. On the other hand, Desmecht et al. (9) and Mouchantat et al. (24) presented a more refined picture of the situation. They found a relatively high seroprevalence in wild boar caught in 2011, 27% in Desmecht and 33% in Mouchantat, respectively, which then dropped to 11% in 2012. The Mouchantat et al. study continued for the following year, when 0 seropositive wild boars were found. However, the low study sample in the last year, n = 36, must be taken into account. Taking into account the low seroprevalences noted in other studies, it is likely that a similar figure of about 2% would have been achieved with a larger sample size. As mentioned earlier, the case is significantly different in free-living ruminants. Recent work by Milićević et al. (23), conducted in Serbia, showed a 31.4% seroprevalence among red deer. A large-scale study of ruminants in 21 French departments described the scale of animal contact with the virus in the years 2011-2014. In the first season of the study, it is possible to observe both areas where the disease is already widespread, such as Côte d'Or (seroprevalence 30 ± 15% among red deer) or Haute Marne (49 ± 13%), as well as pathogen-free, such as Pyrenees Atlantiques, where seroprevalence of 0 ± 11% was recorded among red deer. In the second year, virtually all areas showed high seroprevalences, from 33 ± 9% for ibex in Haute Savoie up to 85 ± 12% for red deer in Haute Corse, and a small sample of 9 roe deer in Côte d'Or, where all of the animals tested positive. In the last survey season, an overall decrease in seroprevalences could be observed compared to the previous period, although a few areas that were at the bottom of the pile in the 2012-2013 season showed increases instead, such as the aforementioned Haute Savoie ibex population which jumped from 33 ± 9% to 72 ± 13% (27). In Poland, a study on European bison carried out by Kęsik-Maliszewska et al. (17) showed the presence of SBV antibodies in 81% of the animals tested. Another group of studies concerns the distribution of the virus in the population of its major vectors, the biting midges from the *Culicoides* genus. SBV infections have been confirmed in *C. obsoletus*, *C. scoticus*, *C. dewulfi*, *C. chiopterus*, *C. pulicaris*, *C. punctatus*, and *C. imicola*, with *C. obsoletus* and *C. scoticus* being identified as the main vectors in Poland (18). Adult female midges become infected by ingesting blood from a viraemic animal. In addition, there is some evidence to suggest vertical transmission of the virus among *Culicoides*. An increased prevalence of SBV-positive midges has been observed in years when increased numbers of cases of the disease have been reported among farmed and free-living animals (18, 31).

Overall, studies carried out across continental Europe find seroprevalences in the range of several to tens of per cent, which fluctuate in waves over 3-4 year periods caused by changes in the circulation of the virus and herd immunity of the animals (15, 30, 33). In light of the enzootic presence of the virus in Europe (11, 19, 32), the cyclical presence of the virus in populations of *Culicoides* (31) and the changes in seroprevalence observed among free-living ruminants, it may be puzzling why, apart from the papers by Desmecht et al. (9) and Mouchantat et al. (24), it has not been possible to observe the occurrence of SBV antibodies in more than a few per cent of the wild boar tested. There is a lack of multi-year studies conducted, beyond the initial 2011-2013 period, on the basis of which it would be possible to determine whether these cycles are equally noticeable in their population, or whether, after the first wave, the ability of the virus to induce an immune response in wild boar has decreased. Our results, as well as those of similar studies, indicate the low importance of wild boar in the transmission and circulation of Schmallenberg virus. However, a multi-year study would allow assessment of their exact role as a reservoir in light of the cyclical changes in the virus's presence.

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