Transboundary and Emerging Diseases

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SHORT COMMUNICATION

Follow-up of the Schmallenberg Virus Seroprevalence in Belgian Cattle

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Summary

Schmallenberg virus (SBV), which emerged in Northwestern Europe in 2011, is an arthropod-borne virus affecting primarily ruminants. Based on the results of two cross-sectional studies conducted in the Belgian ruminant population during winter 2011–2012, we concluded that at the end of 2011, almost the whole population had already been infected by SBV. A second cross-sectional serological study was conducted in the Belgian cattle population during winter 2012–2013 to examine the situation after the 2012 transmission period and to analyse the change in immunity after 1 year. A total of 7130 blood samples collected between 1st January and 28 February 2013 in 188 herds were tested for the presence of SBV-specific antibodies. All sampled herds tested positive and within-herd seroprevalence was estimated at 65.66% (95% CI: 62.28–69.04). A statistically significant decrease was observed between the beginning and the end of 2012. On the other hand, age-cohort-specific seroprevalence stayed stable from 1 year to the other. During winter 2012–2013, calves between 6 and 12 months had a seroprevalence of 20.59% (95% CI: 15.34–25.83), which seems to be an indication that SBV was still circulating at least in some parts of Belgium during summer–early autumn 2012. Results showed that the level of immunity against SBV of the animals infected has not decreased and remained high after 1 year and that the spread of the virus has slowed down considerably during 2012. This study also indicated that in the coming years, there are likely to be age cohorts of unprotected animals.

Introduction

Schmallenberg virus (SBV) is an arthropod-borne virus which emerged recently in North-Western Europe and is closely related to viruses from the Simbu serogroup, part of the family *Bunyaviridae*, genus *Orthobunyavirus*. (Hoffmann et al., 2012). SBV was shown to be responsible for abortions, stillbirths and congenital malformations which started occurring during autumn 2011 in domestic ruminants all across Europe (Garigliany et al., 2012a; Herder et al., 2012; Van den Brom et al., 2012; Doceul et al., 2013). The detection of SBV in biting midges (*Culicoides* spp.) collected during summer and autumn of 2011 suggests the central role of the vectors in transmitting the virus (De Regge et al., 2012; Rasmussen et al., 2012; Elbers et al., 2013; Larska et al., 2013).

Based on the results of two cross-sectional studies conducted in the Belgian ruminant population (cattle, sheep and goat) during winter 2011–2012 (WS 2012), we concluded that at the end of 2011, almost the whole population

had already been infected by SBV (Méroc et al., 2013a,b). In consequence, we had extrapolated from the known epidemiology of similar viruses of the Simbu serogroup as well as from the results of an experimental study carried out recently (Wernike et al., 2013) that the majority of the host population should have developed post-infection protective immunity against SBV. Even if the duration of acquired immunity against SBV is unknown, because of this, it was expected that Belgium was not going to experience a new epidemic during the lambing and calving season of 2012–2013. Indeed, since that time, very few clinical cases have been confirmed by RT-qPCR (H. Van Loo and L. Delooz, personal communication). A second cross-sectional serological study was conducted in the cattle population during winter 2012–2013 (WS 2013) to examine the situation after the 2012 transmission period (summer and autumn 2012) and to analyse the change in immunity since the previous screening.

Materials and Methods

Sampling design and diagnostic methods

Sample size was calculated to reach a desired level of confidence of 95%, a desired precision of the estimate of 5% and a design (between-herd) prevalence of 90%. The latter was chosen based on the results of the serological screening performed in the Belgian cattle population during winter 2011–2012 (99.76%; 95% CI: 98.34–99.97) (Méroc et al., 2013b). To account for any herd dropout, a total of 200 cattle herds (dairy, beef or mixed herds) (not allowing for dropout, $n = 139$) were randomly selected from the cattle census sampling frame extracted from the central identification and registration system of the Belgian Federal Agency for the Safety of the Food Chain (SANITEL). The sample selection was stratified by province proportionally to the number of herds present in each province**.** In each of the selected herd, maximum 40 animals were sampled proportional to the average age distribution, according to the following scheme: 10 animals of 6–12 months of age; 10 animals of 12–24 months of age; and 20 animals >24 months of age.

The blood samples were collected by farm veterinarians and were then sent to the regional laboratories 'Association Régionale de Santé et d'Identification Animales' (ARSIA) and 'Dierengezondheidszorg Vlaanderen' (DGZ) where serum was tested for the presence of SBV-specific antibodies using an ELISA assay (ID Screen® Schmallenberg virus Indirect ELISA kit; Montpellier, France; Bréard et al., 2013). Results were expressed as S/P percentage (S/P%) using the optical densities (OD) from the ELISA reader ($S/P\% = OD_{sample}/OD_{positive\ control} \times 100$). A cut-off prescribed by the manufacturer was used to assign the samples into a category (positive, negative and doubtful). Samples which presented an S/P% lower or equal to 50%, between 50% and 60%, and >60% were, respectively, considered as negative, not interpretable and positive. In this study, the doubtful results were considered as positive in the data analysis.

Statistical methods

A marginal model, the generalized estimating equations (GEE) (Liang and Zeger, 1986), which takes into account the resulting correlation among animals, was used to estimate within-herd seroprevalence with 95 per cent confidence intervals (95% CI). An exchangeable working correlation matrix was assumed. Design effect was taken into account by weighting each observation by the inverse of the sampling probability. The age category variable (6–12, 12–24 and >24 months) was introduced as an independent variable in the GEE model to study the effect of the factor on the SBV status. Models were fitted in STATA® software version 10.0 (StataCorp, College Station, TX, USA).

Farm X and Y coordinates were extracted from SANITEL. A purely spatial normal model was used to scan for clusters of sampled flocks with either high or low levels of within-herd seroprevalence (SaTScan 8.2.1.; Kulldorff, 1997), and only clusters with *P*-value below 0.05 were to be considered as statistically significant.

Results and Discussion

A total of 7130 blood samples collected between 1st January and 28 February 2013 in 188 herds were tested for antibodies against SBV. Four thousand four hundred and seventy samples turned out to be positive and 372 samples had a value of S/P% which was not interpretable. Considering a herd to be positive if at least one sample in the herd was positive, all herds in the current study were seropositive.

The mean within-herd seroprevalence was estimated at 65.66% (95%CI: 62.28–69.04). One year earlier, during WS 2012, the SBV seroprevalence in the Belgian cattle population was 86.3% (95% CI: 84.75–87.71) (Méroc et al., 2013b); therefore, a statistically significant decrease was observed between the beginning and the end of 2012 ($P < 0.05$). As presented in Fig. 1, the predicted values of within-herd seroprevalence in positive herds ranged from 35% to 92%, but were skewed to the left. The median and mode values were 72% and 76%, respectively. After 1 year, the distribution has globally shifted towards lower values of within-herd seroprevalence.

As described in Table 1, the distribution of seroprevalence according to age category indicates a statistically significant ($P < 0.001$) association between the age

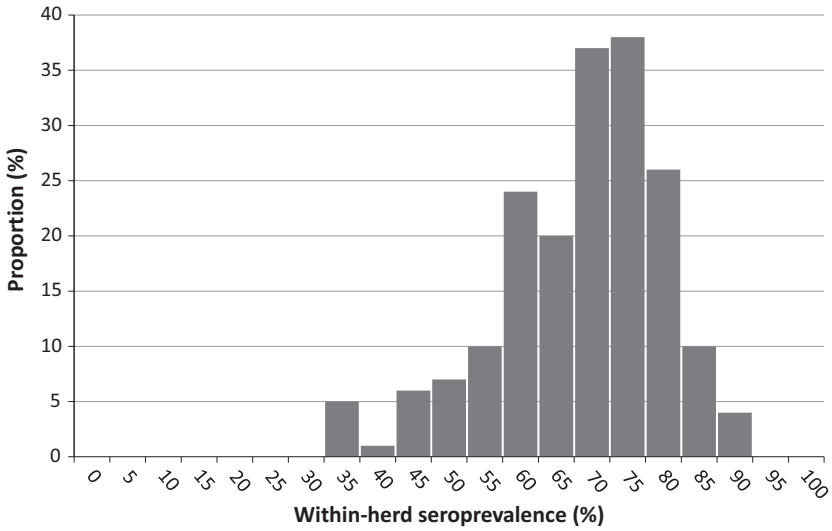

Fig. 1. Density-scale histogram of within-herd seroprevalence estimates (%) in Belgian cattle herds seropositive for Schmallenberg virus-specific antibodies.

Table 1. Schmallenberg virus within-herd seroprevalence in cattle during screening in winter 2011–2012 (WS 2012) and in winter 2012–2013 (WS 2013), stratified by age category

Age category	WS 2012	WS 2013
6–12 months	64.9% (95% CI: 61.34–68.3)	20.59% (95% CI: 15.34–25.83)
12–24 months	86.79% (95% CI: 84.43–88.85)	61.63% (95% CI: 54.94–68.33)
>24 months	94.4% (95% CI: 93.14–95.44)	84.96% (95% CI: 82.01–87.91)

category of the animal and its serological status; older cattle tend to be more seropositive to SBV than younger cattle. In addition, a significant decrease in seroprevalence was observed in the three different age categories between the two screenings ($P > 0.05$). On the other hand, if we focus on age-cohort-specific results by comparing seroprevalence in 'age category 6–12 months at WS 2012' versus 'age category 12–24 months at WS 2013' and in 'age categories 12–24 months and >24 months at WS 2012' versus 'age category >24 months at WS 2013', the differences are not statistically significant ($P < 0.05$).

Even though the sampled populations differed from one cross-sectional survey to the other, the sampling designs of WS 2012 and 2013 were similar, therefore allowing comparison of the two samplings. Both the overall decrease in seroprevalence and the fact that cohort-specific seroprevalence did not differ from 1 year to the other suggest that (i) the progress of the epidemic has slowed down after the 2011 transmission period and very few cases of new infections have occurred during the 2012 transmission period (summer and autumn 2012) and (ii) the level of immunity against SBV of the animals infected has not decreased and remained high after 1 year.

During winter 2012–2013, calves between 6 and 12 months had a seroprevalence of 20.59% (95% CI: 15.34–25.83). Animals in this age category were born between January and September 2012, hence, after the first vector active season in 2011. In consequence, the level of infection in this cohort provides a snapshot of the 2012 transmission season. Nevertheless, it is possible that some of the calves were infected during gestation (which took place during the 2011 transmission period) and developed an immunological response against the virus. In this case, infection probably occurred after the development of foetal immunocompetence, around the fifth month of gestation (Schultz et al., 1973; Garigliany et al., 2012b). The animals also might have acquired the antibodies after they were fed colostrum. The duration of maternal-derived antibodies against SBV is not yet known, but Tsutsui et al. (2009) studied the duration of maternal immunity against Akabane virus in calves and found that 4–5 months is the estimated age when the maternal antibodies decay with a 90% probability (Tsutsui et al., 2009). Therefore, it is possible that some of the calves in the 6–12 months category still had maternal antibodies during WS 2013. However, a proportion of the new-born animals might have been infected after the 2011 vector transmission season. Indeed, there are now several pieces of evidence that SBV was still circulating in the south of Belgium during summer–early autumn 2012, both in sheep (Claine et al.,

2013) and in the vector population (N. De Regge, M. Madder, I. Deblauwe, B. Losson, C. Fassotte, J. Demeulemeester, F. Smeets, M. Tomme, A. B. Cay, unpublished data). No particular spatial cluster of high or low level of within-herd seroprevalence was identified. Seroprevalence is homogenously distributed across the country, and the previously lower infection rate zone identified in the south of Belgium during WS 2012 (Méroc et al., 2013b) has now vanished. Cattle population in the southern province Luxembourg born during 2011 (i.e. classified in 'age category 6–12 months at WS 2012' and in '12–24 months at WS 2013') had a higher seroprevalence during WS 2013 (78.95%; 95% CI: 59.16–98.73) than at WS 2012 (56.81%; 95% CI: 47.29–66.33). Even though this difference is not statistically significant ($P > 0.05$), these observations tend to corroborate the hypothesis that SBV has circulated in this part of Belgium during the 2012 vector active season. In the other provinces, differences of seroprevalence in this age cohort were all non-significant and with absolute values below 10%.

Figure 2 shows the change in distribution of the semi-quantitative values of the ELISA test (S/P%) between WS 2012 and WS 2013. Globally, the values have shifted to the left. The median values of the seropositive results were 100% and 92.61%, in 2012 and 2013, respectively. Thirteen animals originating from one herd were sampled both during WS 2012 and WS 2013. A decrease in the S/P% value was seen for 11 animals of 13. This observation must be the consequence of a natural decline of active antibodies, linked to the fact that cattle sampled at WS 2012 were probably recently infected at that time. Of the 11 animals, two were seropositive (S/P%>60%) at WS 2012 and then turned out to be seronegative (S/P% >≤50%) at WS 2013. These animals were more than 2 years old at the first WS; therefore, this decrease in antibodies could not be attributed to declining maternal antibodies. Even though this finding emanates from a very limited sample, it might indicate that SBV-specific acquired antibodies may not last for life.

The results of the current study seem to indicate that the spread of SBV in the Belgian cattle population has slowed down considerably during 2012. The findings also demonstrated a long persistence of immunity, as seroconversion against SBV was still detected after 1 year. This is supported by the fact that few new clinical outbreaks have been reported since summer 2012. This study is also an illustration of the fact that even in a host population highly protected by immunity linked to a recent history of infection, principally because of new-borns, part of the population continuously remains susceptible. In other words, in the coming years, there are likely to be age cohorts of unprotected animals. Next to this, the fact that SBV is still present in most neighbouring countries and the possibility that it is being maintained in Belgium for the moment at low levels via other, theoretically less immunized, animal host species such as wild ruminants should be kept in mind. Our study suggests that SBV has been reintroduced in Belgium, but only at very low level 1 year after the first wave of infection and the question of its persistence over the next years remains open. Fur-

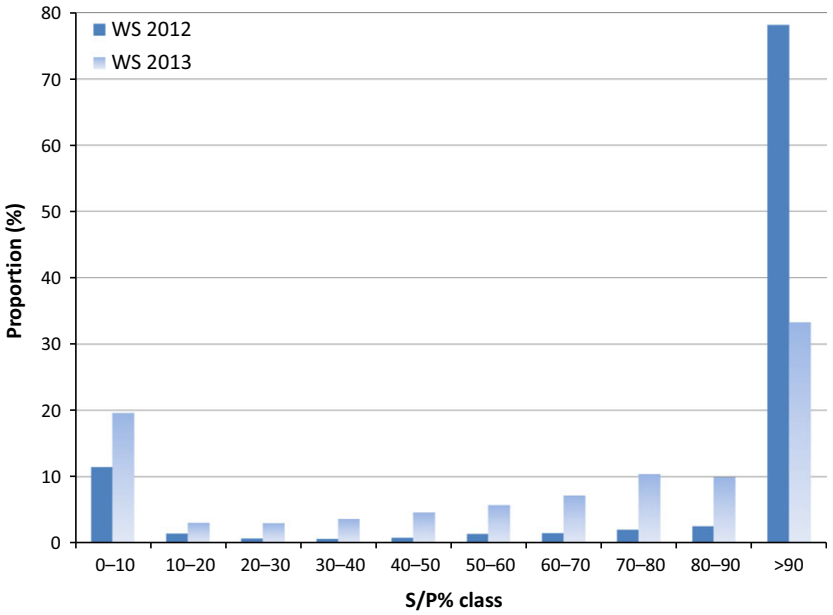

Fig. 2. Distribution of semiquantitative immunological value (Sp ratio(%)) of cattle tested during screening in winter 2011–2012 (WS 2012) and in winter 2012–2013 (WS 2013) for Schmallenberg virus-specific antibodies.

ther studies, including the evaluation of the persistence of virus in the vector population, are necessary to assess the risk of reintroduction of the disease.

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