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Unchanged Schmallenberg virus seroprevalence in the Belgian sheep population after the vector season of 2014 and 2015 despite evidence of virus circulation

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ABSTRACT

Schmallenberg virus (SBV) emerged in North-Western Europe in 2011 and induces congenital defects in ruminants. Many epidemiological studies were undertaken to study the spread of the virus during the first two years after its emergence, but little data is available on the current antibody protection rate against SBV. A cross-sectional seroprevalence study was therefore carried out in the Belgian sheep population and showed that the total seroprevalence against SBV was 26% ($CI_{95\%}$: 21–32) at the end of the vector season of 2015, being significantly lower than the seroprevalence of 84% detected after the outbreak in 2011. Nevertheless, 63% ($CI_{95\%}$: 51–73) of the Belgian sheep flocks still had a certain level of protection against SBV. Despite the fact that PCR detection of SBV in aborted calves in April 2016 evidenced that SBV had circulated in 2015, no change in seroprevalence between 2014 and 2015 was found in the Belgian sheep population.

Schmallenberg virus (SBV) emerged for the first time at the end of 2011 in North-Western Europe and is closely related to viruses of the Simbu serogroup, part of the family Bunyaviridae, genus *Orthobunyavirus* (Hoffmann et al., 2012). *Culicoides* midges have been proposed to be the putative vectors of SBV (De Regge et al., 2012). SBV induces only mild symptoms in adult ruminants but was shown to be responsible for abortions, stillbirths and congenital malformations in cattle, sheep and goats.

After its first identification in Germany in 2011, SBV spread rapidly and widely over a large part of Europe (EFSA, 2014). Belgium was one of the first and most SBV affected countries, whereby virtually all Belgian sheep and cattle herds had been in contact with SBV at the end of the first vector season of 2011 (Méroc et al., 2013; Méroc et al., 2014). Evidence for renewed SBV circulation was found in 2012 and SBV seropositive animals were still detected at each cattle farm at that time but the overall seroprevalence in cattle had dropped from 86% to 65% (Méroc et al., 2015). Since 2013, only limited efforts have been made to follow the SBV situation in Belgium. The absence of SBV detection by qRT-PCR in suspected samples from aborted lambs and calves submitted to the Belgian national reference laboratory CODA-CERVA in 2014 and 2015 indicates that SBV circulation was very limited or absent at that time (Poskin et al., 2016). Interestingly, three SBV suspected aborted calves that were submitted to the Belgian reference laboratory tested positive for SBV by qRT-PCR in April 2016, providing the first evidence of SBV circulation in Belgium since three years (Delooz et al., 2016; N. De Regge, personal communication). All three cases were detected in the southern part of Belgium (Wallonia), with two calves coming from the province of Namur and one from Liège.

A cross sectional seroprevalence study in sheep was performed to determine the seroprevalence against SBV after the vector season of 2015. Sera of 409 sheep coming from 70 farms were collected between October 1st, 2015 and April 1st, 2016. A stratified sampling approach was used proportional with the number of sheep farmers per province in Belgium (Fig. 1). After exclusion of herds with < 4 sheep, a random sampling was applied per province among all Belgian sheep farmers except those participating in the voluntary Maedi-Visna and Caprine Arthritis and Encephalitis program for trade certification (Royal Decree 24-03-1993). All samples originated from sheep > 1 year old and maximum 7 samples per farm were tested for the presence of SBV-

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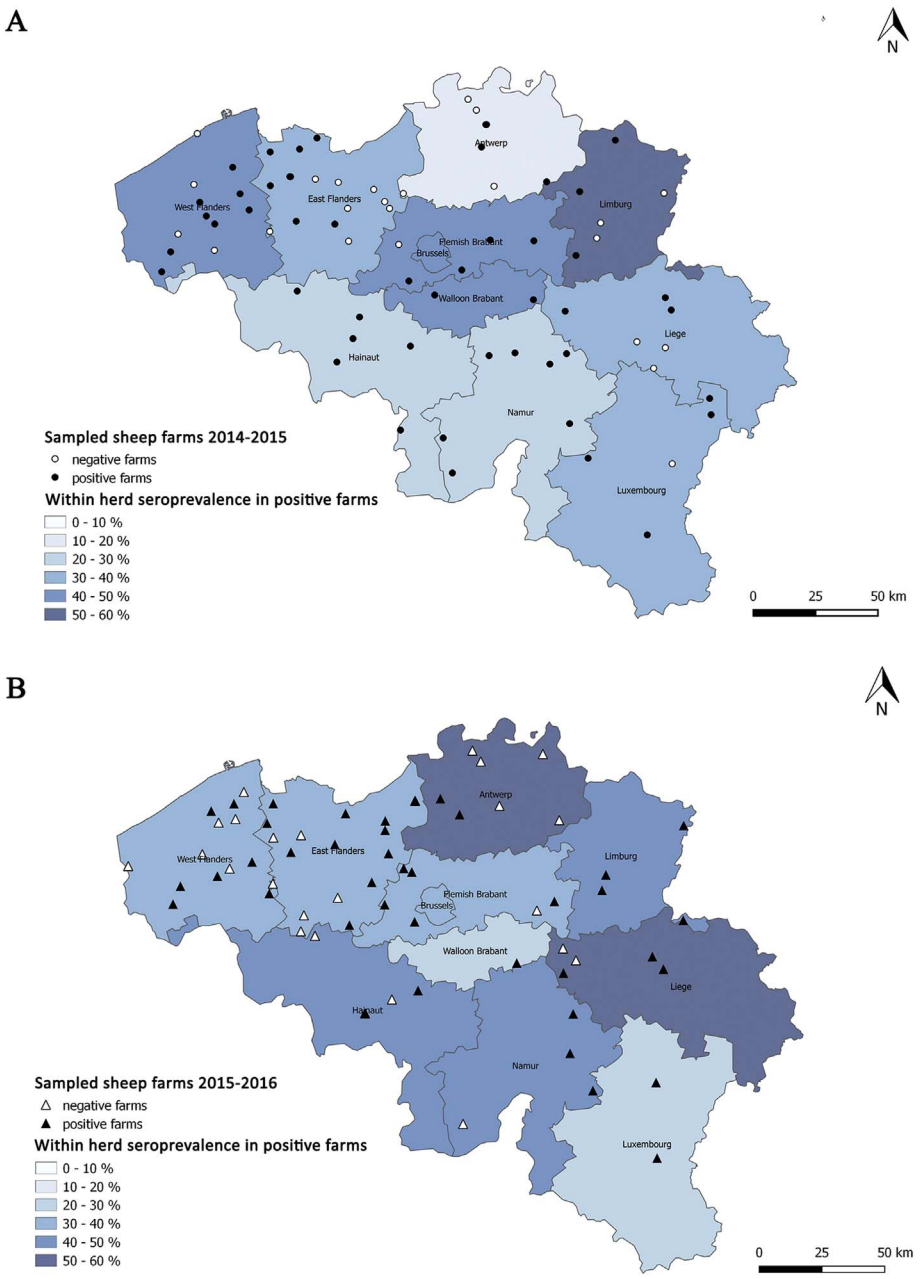

Fig. 1. Geographical distribution of selected sheep farms in 2014 (A) and 2015 (B).

specific antibodies using a commercially available ELISA kit (ID Screen®, Schmallenberg virus, *competition multi-species*, ID-vet, France) following manufacturer's instructions. Based on information provided by the manufacturer, this ELISA has a specificity of 100% ($CI_{95\%}$: 99.6–100) and a test agreement of 97.6% with the ID Screen® indirect ELISA that has a reported sensitivity compared to virus neutralization tests of 97% (Bréard et al., 2013). 20 out of 409 samples scored doubtful in the ELISA and were considered as negative in our analysis.

By analyzing the ELISA data with a generalized estimating equations (GEE) model that takes into account the correlation between animals that belong to the same herd (Méroc et al., 2014), we found an overall SBV seroprevalence of 26% ($CI_{95\%}$: 21–32) after the vector season of 2015 (Table 1). This indicates a clear reduction of SBV seroprevalence in sheep since the SBV emergence in 2011 when an overall seroprevalence of 84% was found in Belgium (Méroc et al., 2013). This seems to be in line with the general assumption that SBV circulation has ceased since the initial outbreak, leading to a decline in the proportion of sheep that have been into contact with the virus. We estimate that this decrease in seroprevalence can merely be explained by replacement of sheep as a part of normal herd management. Yearly replacements rates of 25% are normal in commercial farms (Lievaart-Peterson et al., 2015) and this is in line with a drop in seroprevalence from 84 to 26% in a 4-year time period. The fact that some of the seropositive sheep found in this study lived during the 2011 outbreak makes it also tempting to speculate that antibodies against SBV can persist for a long time. This would be in line with previous observations in sheep under experimental conditions (Poskin et al., 2015) and with findings on long term SBV antibody persistence in cattle (Elbers et al., 2014; Roberts et al., 2014; Wernike et al., 2015). It can however not be completely excluded that the detected antibodies in those sheep come from a (re)infection since the initial 2011 outbreak or from vaccination. The latter is however unlikely since we inquired veterinarians of the regional animal health centers in Flanders (Dierengezondheidszorg Vlaanderen) and Wallonia (Association Régionale de Santé et d'Identification Animales), and none of them was aware of any SBV vaccination in Belgium.

Table 1
Overview of the between-herd, within-herd and total Schmallenberg virus seroprevalence in sheep in different regions and provinces of Belgium.

Region/Province	Season	Farms (samples)	Between-herd seroprevalence (%)	Within-herd seroprevalence in positive farms (%)	Total seroprevalence (%)
Belgium	2014	80 (531)	70	35	26
	2015	70 (409)	63	41	26
	p-Value		0,39	0,14	0,88
Flanders	2014	53 (351)	62	37	24
	2015	52 (312)	62	39	25
	p-Value		1	0,67	0,72
Wallonia	2014	27 (180)	85	32	30
	2015	18 (97)	66	45	31
	p-Value		0,17	0,04	0,89
West Flanders	2014	24 (97)	71	43	31
	2015	15 (77)	53	40	22
	p-Value		0,45	0,77	0,23
East Flanders	2014	18 (115)	56	31	18
	2015	18 (114)	61	32	21
	p-Value		1	0,84	0,62
Flemish Brabant	2014	6 (41)	83	46	39
	2015	7 (42)	86	37	33
	p-Value		1	0,47	0,62
Antwerp	2014	9 (60)	56	17	10
	2015	7 (46)	29	51	13
	p-Value		0,36	0,13	0,76
Limburg	2014	6 (38)	50	54	26
	2015	5 (33)	100	49	51
	p-Value		0,18	0,8	0,05
Hainaut	2014	6 (41)	100	29	29
	2015	6 (28)	50	44	25
	p-Value		0,09	0,28	0,79
Walloon Brabant	2014	2 (11)	100	41	45
	2015	1 (7)	100	29	29
	p-Value		1	[a]	0,64
Luxembourg	2014	5 (34)	80	36	29
	2015	2 (12)	100	30	25
	p-Value		1	0,73	1
Liège	2014	7 (45)	57	39	24
	2015	5 (32)	60	58	34
	p-Value		1	0,16	0,44
Namur	2014	7 (49)	100	25	33
	2015	4 (18)	75	48	39
	p-Value		0,36	0,09	0,77

[a] Not enough data to calculate this value. A farm was considered seropositive when at least one positive animal was found on that farm. Fisher exact tests were used to assess potential differences in, respectively, between-herd seroprevalence and total seroprevalence between both years. Student *t*-tests were used to compare mean within-herd seroprevalence between both years.

The between-herd seroprevalence was estimated using a generalized linear model. For the purpose of this study, a herd was considered positive if at least one of the sampled animals was positive. We found that 63% ($CI_{95\%}$: 51–73) of the Belgian sheep farms still had at least one SBV positive sheep (Table 1, Fig. 1) after the vector season of 2015. This is clearly lower than the between-herd seroprevalence of 98% after the initial emergence in 2011 (Méroc et al., 2014) but this remaining partial protection in about 60% of the sheep flocks probably helps to explain why no more storms of SBV associated abortions and malformations comparable to the initial outbreak have been observed since 2012.

As described above, the detection of three SBV positive aborted calves in April 2016 indicates that the virus had circulated in 2015. To analyze whether this circulation had let to an increase in seroprevalence compared to the previous year, in analogy to the massive increase in seroprevalence observed during the initial outbreak of 2011, we also determined the seroprevalence after 2014 vector's season. A similar random stratified sampling as described above was therefore applied among farmers participating in the voluntary Maedi-Visna and Caprine Arthritis and Encephalitis Program for trade certification. 531 samples of sheep > 1 year old from 80 farms collected between October 2014 and April 2015 were tested in ELISA. We found an overall seroprevalence of 26% ($CI_{95\%}$: 21–31) and a between herd seroprevalence of 70% ($CI_{95\%}$: 59–79) after the vector season of 2014. Interestingly, no significant differences in between-herd and overall seroprevalence were found at national (Belgium), regional (Flanders, Wallonia) and provincial (West Flanders, East Flanders, Flemish Brabant, Antwerp, Limburg, Hainaut, Walloon Brabant, Luxembourg, Liège and Namur) level between 2014 and 2015 (Table 1). The only significant change found between both years was an increase of the mean within-herd seroprevalence in Wallonia from 32% in 2014 to 45% in 2015. This seems to be in line with the observation that the confirmed SBV cases in 2016 came from Wallonia, and suggests that the virus circulated mostly in the south of Belgium.

Overall, the low number of confirmed SBV abortions by PCR in winter and spring 2015–2016 and the absence of an increase of between-herd and total seroprevalence in sheep between 2014 and 2015 suggest that SBV circulated only to a low level in Belgium in 2015. A first explanation could be that the virus was only reintroduced in Belgium in late autumn of 2015 when the vector population was already decreasing, hindering an extensive virus spread. The fact that no SBV positive aborted lambs were found at the national reference laboratory beginning of 2016 supports the hypothesis that SBV was not circulating during the sheep mating season of late summer and early autumn 2015, the period that newly inseminated ewes are considered to be most vulnerable for SBV infection leading to congenital malformations that are later observed at birth (Martinelle et al., 2015). It can however not be excluded that farmers omitted to submit their malformed lambs to the authorities and that we were therefore not able to detect the positive cases. A second more hypothetical explanation

would be that SBV has established itself since its emergence as an endemic virus that circulates at constant low level in the partly protected ruminant population. This would be in line with observations made for the closely related Akabane virus which has been detected yearly in Japan since its initial detection in 1959, but only sporadically causes outbreaks of congenital malformations. The latter is probably associated with the time needed for the herd immunity to decrease in livestock and/or with the introduction of new virulent mutant strains (Kono et al., 2008).

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