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Exposure to Schmallenberg virus in Irish sheep in 2013

D. J. Barrett, S. J. More, R. G. O' Neill, D. M. Collins, C. O'Keefe, V. Regazzoli, D. Sammin

MALFORMED fetuses attributable to Schmallenberg virus (SBV) were found in 49 cattle herds and 30 sheep flocks exclusively in the southern and eastern parts of Ireland (Barrett, D., More, S. J., O'Neill, R., Bradshaw, B., Casey, M., Keane, M., McGrath, G. & Sammin, D. (Submitted) Prevalence and distribution of exposure to Schmallenberg virus in Irish cattle during November 2012 to November 2013). National bovine serological studies late in 2012 and 2013 confirmed exposure to SBV was effectively confined to the south-east (Barrett and others, submitted). It was unclear whether the distribution of seroconversion in cattle reflected the situation in sheep. Several studies have shown that *Culicoides* species preferentially feed on cattle rather than sheep (Ninio and others 2011, Ayllon and others 2014, Eibers & Meiswinkel 2014), leading to lower levels of seroconversion in sheep flocks than in neighbouring cattle herds (Gache and others 2013). It was anticipated that SBV would continue to spread across the country over the second (2013) vector season, similar to the experience in mainland Europe (Garigliany and others 2012, Veldhuis and others 2013, Balmer and others 2014). The objectives of this study were to determine the geographical distribution of SBV exposure in Irish sheep before and during the 2013 vector season, and to determine if SBV was active in 2013 in flocks where SBV infection was previously confirmed.

Two studies were conducted, each in different sets of sheep flocks. In the first study, serological samples were collected in 32 sentinel flocks sampled on two or three occasions at six-week intervals between May 2013 and September 2013. The flocks were distributed across the country (15, 8 and 9 flocks from the south-east, midlands and north-west, respectively), and had

been volunteered through Sheep Ireland (www.sheep.ie) (n=24) and veterinary practitioners (n=8). For welfare and legislative reasons, individual adult sheep were blood sampled only once. In the second study, serological samples were collected in 14 sheep flocks where SBV infection had been confirmed in malformed fetal lambs the previous spring. In these flocks, samples were collected from 15 lambs aged between 8 months and 10 months on a single occasion, in November 2013. For both studies, it was estimated that a minimum number of 13 samples would be required per round of sampling, to ascertain freedom from infection assuming a flock prevalence of 40%, a test sensitivity of 80% and a test specificity of 99%. In practice, 15 animals were sought at each round of sampling.

For both studies, sera were tested using commercially available test kits, an indirect ELISA (Idexx Laboratories) and a competition ELISA (ID.vet), each according to the manufacturer's instructions. In the second study, sera were additionally tested by serum neutralisation test (SNT), with a threshold titre of 1:16 used to determine SBV seroconversion (Loeffen and others 2012, Bouwstra and others 2013).

In the first study, there were 17 (53%) seropositive flocks, including all 15 in the east and south-east (Fig 1), with no subsequent change in SBV status. A single seropositive animal was found in two flocks: in counties Sligo and Meath, in the north-west and north-east, respectively. These two seropositive animals had been purchased from flocks in the south-east region in 2012 and it was assumed these animals had seroconverted before movement.

In the second study, two (14.3%) flocks had multiple seropositive lambs. Each flock had used vaccination and the lambs were seropositive by both ELISA methods but not by SNT. Two other flocks each had a single seropositive lamb; in both cases, the lamb was seropositive to SNT and one or both ELISA methods (Table 1).

In Irish sheep flocks, seroconversion to SBV virus was confined to the south and south-east of the country with no further circulation in 2013, similar to earlier results from Irish cattle (Barrett and others, submitted). In considering the probability of transmission of SBV from the south and east coast to locations further inland, it is noteworthy that the prevailing wind direction in Ireland is from the south-west and as such this would not have facilitated long-distance windborne spread of biting midges in a north-westerly direction. Further spread would be largely reliant on local transmission dynamics. Regional differences in availability of suitable vectors is also unlikely to explain the limited geographical spread of SBV as a previous study demonstrated several potentially bluetongue-competent *Culicoides* species were abundant and widely available throughout Ireland, especially in the northern half of the country (McCarthy and others 2010). However the timing of the initial incursion of SBV, which is considered to have occurred during the latter half of the vector active season (O'Neill and others 2014), may provide an explanation. Scottish modelling has shown that SBV introduction late in the vector season under climatic conditions similar to Ireland markedly reduced the spread of infection compared with an introduction earlier in the vector season (Bessell and others 2013). That study also showed that mean Scottish summer temperatures facilitate only limited spread, as vector life cycles are very temperature dependent. Temperatures in the south-east of Ireland in the summer and autumn of 2012 were approximately 1°C less than the 30-year average (Anon 2015). Therefore the incursion of SBV relatively late in the vector season in a year with below average temperatures was likely to have curtailed the spread of Irish SBV in 2012.

In flocks with confirmed SBV infection in the first (2012) vector season, there was no evidence of detectable exposure

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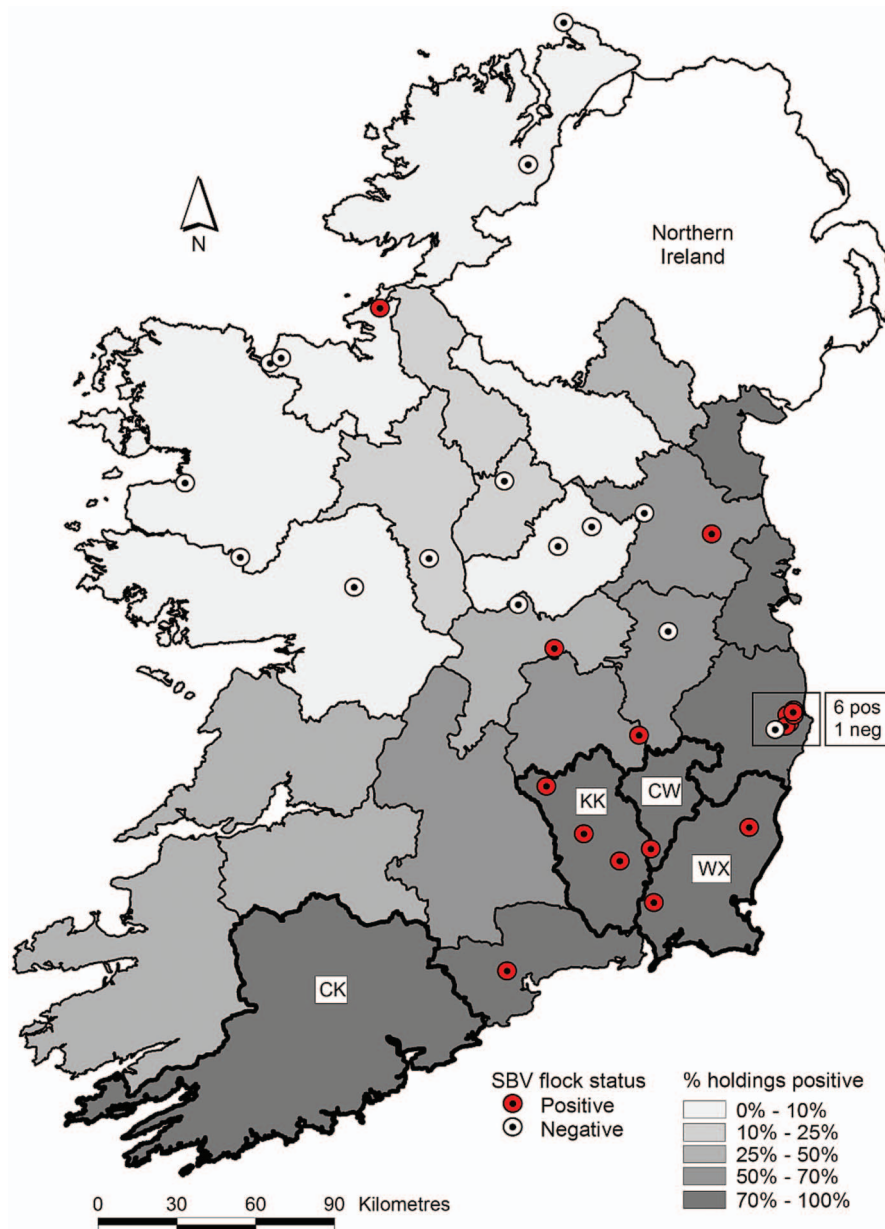


FIG 1: Location of the 32 study flocks in the first study, by Schmallenberg virus (SBV) serological status. County-level evidence of SBV exposure (% holdings positive) was based on serological surveys conducted in cattle during 2012–13 (Barrett, and others, submitted). Counties included in the second study (of 2013-born lambs) are highlighted (Carlow [CW], Cork [CK], Kilkenny [KK] and Wexford [WX])

TABLE 1: Serological survey of 2013-born lambs in flocks where Schmallenberg virus (SBV) had been confirmed by PCR in Spring 2013 (study 2)

Flock	County	No tested	Indirect ELISA (prevalence)	Competition ELISA (prevalence)	SNT (prevalence)	Vaccinated
A	Carlow (CW)	15	0	0	0	No
B	Cork (CK)	15	0	0	0	No
C	Cork (CK)	15	0	0	0	No
D*	Cork (CK)	7	0	0	0	No
E	Kilkenny (KK)	15	0	0	0	No
F	Kilkenny (KK)	15	0	0	0	No
G	Kilkenny (KK)	15	0	0	0	No
H	Kilkenny (KK)	15	0	0	0	No
I	Kilkenny (KK)	15	0	0	0	No
J	Kilkenny (KK)	15	1 (7%)	0	1 (7%)	No
K	Wexford (WX)	15	4 (26%)	7 (47%)	0	Yes
L	Wexford (WX)	15	1 (7%)	1 (7%)	1 (7%)	No
M	Wexford (WX)	15	6 (40%)	5 (33%)	0	Yes
N	Wexford (WX)	15	0	0	0	No

*Number of lambs sampled less than the 13 required to substantiate freedom of SNT, serum neutralisation test

among 2013-born lambs. This study was carried when maternal antibodies were presumed to have waned (Elbers and others 2014). While there was evidence of seroconversion in two lambs in separate non-vaccinated flocks, occasional antibody detections among lambs more than six months of age have been attributed to persistent maternal antibodies (L. van Wuyckhuise, personal communication) or false positives (Veldhuis and others 2015). There is a possibility this seroconversion occurred as a result of exposure to SBV in utero. It is interesting to note the variable antibody response among sheep vaccinated for SBV.

The spatial distribution of seroconversion in sheep closely mirrored both the spatial distribution of confirmed cases of clinical SBV in sheep and the distribution of exposure SBV among cattle. This suggests that future serological studies in cattle for SBV, and other vector borne viral pathogens of ruminants, might reasonably be used to predict the exposure in sheep, as bovine samples are more readily available and midges have a known preference for cattle over sheep (Ayllon and others 2014, Elbers and Meiswinkel 2014). The incomplete seroconversion in flocks exposed to SBV during 2012 and the lack of SBV seroconversion among 2013-born lambs in those flocks would suggest that a sizeable proportion of the current sheep population in south-east Ireland is immunologically naive to SBV should re-emergence or reintroduction occur.

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Context

Fetal malformation due to Schmallenberg virus (SBV) was diagnosed in 49 cattle herds and 30 sheep flocks in the south and south east of Ireland in 2013. Serological and pathological studies in cattle, and pathological studies in sheep indicated SBV exposure was confined to the south and south east of Ireland. It was anticipated that SBV exposure would spread north westwards over the course of the 2013 vector season. The objectives of this study were to determine the geographic distribution of SBV exposure in Irish sheep before and during the 2013 vector season, and to determine if SBV was active in flocks where SBV infection had been previously confirmed.

Main conclusion

There was no further increase in the geographic extent of exposure to SBV during the course of 2013, nor was there evidence of SBV transmission during 2013 in flocks where SBV had been previously confirmed.

Approach

In the first study, serological samples were collected in 32 sentinel flocks between May and September 2013. In the second study, serological samples were collected in 14 sheep flocks where SBV infection had been confirmed previously. For both studies, sera were tested using commercially available test kits, an indirect ELISA and a

competition ELISA. In the second study, sera were also tested by a serum neutralisation test.

Results

In the first study, there were 17 seropositive flocks, including 15 in the south east, with no change in the SBV status of the 32 sentinel flocks during the study. There was limited evidence of seroconversion among lambs born in 2013 in flocks where SBV had been confirmed previously.

Interpretation

The spatial distribution of seroconversion to SBV in Irish sheep was effectively confined to the south and south east of the country, with no further geographical expansion of SBV exposure during 2013. Furthermore, there was limited evidence of SBV circulation within flocks where SBV had been confirmed the previous spring. The introduction of SBV to Ireland relatively late in the vector season is considered to have curtailed the spread of SBV in 2012.

Significance of findings

A sizeable proportion of the current sheep population in Ireland is immunologically naive to SBV. The spatial distribution of seroconversion in sheep closely mirrored the spatial distribution of exposure to SBV among cattle, which suggests that future serological studies in cattle for SBV, and other vectorborne viral pathogens of ruminants, might reasonably be used to predict exposure in sheep.



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