Seroprevalence of Schmallenberg virus in the United Kingdom and the Republic of Ireland: 2011–2013

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Abstract

Since its identification in late 2011, *Schmallenberg virus* (SBV) spread rapidly across Europe. Using archived samples from domestic ruminants collected between October 2011 and June 2013, the seroprevalence in the United Kingdom (UK) and Republic of Ireland (IE) was estimated using a serum neutralisation test. There was no significant difference ($P > 0.05$) in seroprevalence between sheep and cows suggesting that neither species is significantly more at risk of SBV infection in the UK. A single 2011 sample tested positive; the sample was taken in November from a cow in Wiltshire. There was a steady increase in overall seroprevalence during the first three quarters of 2012, which then more than doubled in quarter 4 (October–December), which may reflect a peak of vector activity. By the end of June 2013, overall seroprevalence was around 72%. However, although seroprevalence was over 50% in Wales and southern and central counties of England, it was below 50% in all other areas of the UK and IE. This suggests that there were still substantial numbers of animals at risk of infection in the latter half of 2013.

Keywords

Schmallenberg virus; SBV; seroprevalence; arbovirus.

Introduction

Schmallenberg virus (SBV) is an arbovirus of the Orthobunyavirus genus that is transmitted by biting midges (*Culicoides* spp.). Since its identification at the end of 2011 (Hoffmann et al., 2012), SBV has spread rapidly throughout mainland Europe. SBV infection of adult ruminants appears to be sub-clinical or mild; causing watery diarrhoea, fever and reduced milk production (Muskens et al., 2012). However, infection of animals during pregnancy causes arthrogryposis–hydranencephaly syndrome (AHS), which results in congenital malformations, abortions and stillbirths (Tarlinton et al., 2012).

Although the original identification of SBV infection was made following observation of acute signs in adult dairy cattle from late summer 2011 (Hoffmann et al., 2012), the majority of SBV infections are reported due to the appearance of AHS in calves and lambs. The first cases of AHS were reported in the Netherlands in November and December 2011 and in Belgium in 2012 (Garigliany et al., 2012; van den Brom et al., 2012). By comparison with the related Akabane virus (Kirkland et al., 1988), it is suspected that SBV causes AHS only if infection occurs in the mid-stages of pregnancy (Tarlinton et al., 2012). Therefore, it is assumed that when AHS is observed, SBV must have been circulating several months previously. This is supported by the initial detection of SBV in France in January of 2012 on the basis of malformed lambs (Dominguez et al., 2012) with subsequent retrospective analysis identifying seropositive animals sampled in October 2011 (Zanella et al., 2013). SBV infection in the United Kingdom (UK) was first identified in malformed lambs from farms in south-eastern coastal regions (Kent, East Sussex, Norfolk and Suffolk) in January 2012 (APHA, 2012; Roberts, 2012). Studies of Belgian ruminants found that almost all animals were seropositive for SBV at the end of 2011 (Meroc et al., 2013a; Meroc et al., 2013b). Although the duration of acquired immunity for SBV remains unknown, it was speculated that herd immunity would prevent a second epidemic in 2012. In a follow-up

study, anti-SBV antibody titres remained high in animals one year later and very few clinical cases were reported in 2012 (Meroc et al., 2013c).

The aim of this study was to determine the rate and extent of geographical spread of SBV from its first emergence in the UK up to the introduction of an inactivated SBV vaccine by testing archived serum samples from ruminants for SBV-specific antibodies.

Materials and Methods

Archived samples were obtained from the nutritional monitoring analytical services (NUVetNA) located at the School of Veterinary Medicine and Science (University of Nottingham). The study was approved by the University of Nottingham's School of Veterinary Medicine and Science Ethics Committee. Sample details (species of origin, location and date of sampling) were obtained from the NUVetNA database. Serum was used for the majority of the testing but where serum was not available, plasma was used.

Virus neutralisation tests (VNT) were carried out as described in Loeffen et al. (2012) using virus strain BH80/11-4 (species *Schmallenberg virus*, genus *Orthobunyavirus*, family *Bunyaviridae*) (kindly provided by M. Beer, Friedrich-Loeffler Institute) with the minor modification that cells were fixed by the addition of 100% ethanol and stained using 0.1% v/v methylene blue in water. Positive and negative controls (samples previously tested with the SBV IDscreen indirect ELISA [IDvet, France] by [BioBest Laboratories, UK]) were tested in parallel with every batch of VNTs.

Seroprevalence maps were generated as choropleth maps in ArcGIS Explorer (Esri, USA). Statistical analysis was performed using a two-tailed Fisher's exact test in GraphPad Prism v6 with the threshold of P set at 0.05.

Results

Serum samples from 1108 ruminants were retrieved from 34 counties (England: 24; Wales: 3; Northern Ireland: 3; Scotland: 2; Republic of Ireland [IE]: 2). The sampling dates covered the period from October 2011, prior to the first recorded cases of SBV in the UK (APHA, 2012; Sedda and Rogers, 2013), until the end of June 2013. Of the 851 cattle and 251 sheep tested, 396 (46.5%) and 161 (64.1%), respectively, were seropositive.

The samples were grouped by year quarter (Q1=winter, Jan–Mar; Q2=spring, Apr–Jun; Q3=summer, Jul–Sep; and Q4=autumn, Oct–Dec) and analysed for seroprevalence by VNT (Fig. 1). Only one 2011 sample, taken from a cow on a farm in South Western England (Wiltshire) during November, tested positive. Antibodies against SBV were found in 14.6% of the animals sampled in the first quarter of 2012 (Q1). Seroprevalence increased steadily in Q2 and Q3 of 2012, but in Q4, a sharp increase (to 74.4%) was recorded. Seroprevalence remained at around this level in Q1 and Q2 of 2013.

To investigate whether sheep or cattle were more at risk of SBV infection, samples were analysed by year and species (Fig. 2). Seroprevalence for both cattle and sheep increased between 2012 and 2013, but there was no significant difference ($P > 0.05$) in seroprevalence between species by year. In addition to cattle and sheep samples, sera from 6 goats from a farm in Hampshire (sampled in February 2012) were tested, of which 3 were positive for SBV neutralising antibodies.

Annual seroprevalence by county is shown in Figure 3. SBV infection was confirmed in all but 3 counties from which samples were obtained in 2012 and all but 2 counties in 2013. Seroprevalence was higher in the southern counties of England and in Wales than the rest of the UK and IE. These data indicate that SBV spread both northerly and westerly and by the

end of June 2013 there were positive samples from all English counties from which samples were obtained.

Discussion

This serosurvey confirmed the rapid spread of SBV throughout England and Wales and into Scotland, the Republic of Ireland and Northern Ireland during 2012/13. This was probably facilitated by prevailing winds from Europe as modelling of SBV spread across the UK found that the majority of farm-to-farm transmission events were consistent with downwind movement of midges (Sedda and Rogers, 2013).

In May 2013 an inactivated whole virus vaccine against SBV was licensed for use in the UK. The vaccine status of the animals from which samples were obtained was unknown and it is not possible to differentiate between animals immunised using this vaccine and naturally infected animals using neutralising antibody responses. Therefore sample testing was discontinued at the end of the second quarter of 2013.

In this study, there was a steady increase in SBV seroprevalence each quarter from the last quarter of 2011 to the third quarter of 2012. This suggests the possibility of continued viral transmission during the winter months, evidence for which has been reported for the winter of 2012/13 on a German sheep farm and a sheep farm in southern England (Davies and Daly, 2013; Wernike et al., 2013).

There was a sharp increase in seroprevalence between Q3 and Q4 (late autumn/early winter) in 2012 with seroprevalence then remaining stable for the next two quarters (spring and early summer). This suggests a peak in viral transmission during late autumn and early winter. This may reflect the greater abundance of vectors during the autumn. A similar peak of infection in autumn was observed in northern Europe during the 2006–2008 outbreak of

bluetongue virus, which is also transmitted by *Culicoides* spp. (Hoffmann et al., 2009). SBV RNA has consistently been detected in *C. obsoletus*, *C. scoticus*, and *C. chiopterus* across Europe (Balenghien et al., 2014; De Regge et al., 2012; Elbers et al., 2012); strongly implicating them in the transmission of SBV. Other midge species have tested positive for SBV RNA; *C. dewulfi*, *C. pulicaris* (De Regge et al., 2012), *C. punctatus* (Larska et al., 2013) and *C. nubeculosus* (Balenghien et al., 2014) but the role of these species in transmission has yet to be confirmed. The seasonal abundance of *Culicoides* varies dependent on species in the UK (Sanders et al., 2011) and although *C. obsoletus* complex midges are present in the UK, their prevalence and vector-competence has not been determined.

The data presented here suggest that animals are most at risk of SBV-infection during the height of the vector season (August –September). Peak sexual activity for the majority of sheep breeds in the UK is from October through to December, but the breeding season can be advanced to August for January lambing. By inference from studies of Akabane virus infection; only animals infected with SBV during the vulnerable mid-stage of gestation are thought to be at risk of developing AHS (Tarlinton et al., 2012). Therefore, sheep inseminated in August will reach the mid-stage of gestation during the height of the vector season. Thus delaying insemination until late September might be recommended to reduce the risk of AHS.

The seroprevalence for Q4 of 2012 was 74.4%, indicating that the extent of spread of SBV in the UK was similar to that reported in other European countries. SBV seropositive animals were detected in the North East and North West of France for the first time in October 2011 and it took just 3 months for the seroprevalence to reach 80% in both regions (Zanella et al., 2013). A random bulk milk tank survey conducted in Sweden in early 2012 identified a single seropositive farm on the south coast; a subsequent random survey 6 months later found that 75% of 723 herds were seropositive (Chenais et al., 2013). A similar rate of spread and

seroprevalence has also been reported for Belgium (Meroc et al., 2013a; Meroc et al., 2013b) and the Netherlands for the winter of 2011/12 (Elbers et al., 2012).

The samples used in this study were submitted for reasons other than suspicion of SBV infection (clinical signs of which are often missed in adult animals); therefore the results are likely to be a true reflection of the level of SBV seroprevalence in the UK. The earliest laboratory-confirmed cases of SBV in the UK were identified from malformed lambs born in December 2011 (Sedda and Rogers, 2013) and January 2012 (APHA, 2012; Roberts, 2012). By comparison with related viruses, it has been proposed that sheep presenting with fetal abnormalities would have been infected 2–3 months previously. Therefore, it is thought that the ewes giving rise to the first cases of SBV in the UK became infected in October or November 2011. Consistent with this and similar observations in France (Zanella et al., 2013), antibodies against SBV were detected in a cow in November 2011 in this study. Seropositive goats, sampled in February 2012, were also identified as part of this study, a year earlier than previously reported (APHA, 2013).

Surveillance statistics published by the Animal and Plant Health Agency (formerly the AHVLA) in February 2013 highlighted that seropositive animals had been identified in all English and Welsh counties, but that all animals identified by clinical presentation in Scotland had been introduced from other SBV-positive regions of the UK (APHA, 2013). The earliest identified infections of indigenous animals in Scotland were in the South West in December 2012 from bulk tank milk screening (Mason et al., 2013). Furthermore, a retrospective analysis in Ireland reported an SBV seroprevalence of 22.1% in 570 cattle sampled between March and December 2012 from 6 counties which were first to report clinical signs of SBV infection (O'Neill, 2014). Therefore, although it is possible that some or all of the seropositive animals from Scotland and Ireland identified in the present study were

previously-infected animals introduced from England, Wales or Continental Europe, it is apparent that the virus spread to the Republic of Ireland and all countries of the UK.

The apparent reduction in seroprevalence between 2012 and 2013 observed in Powys (Wales) and Dumfries and Galloway (Scotland) are unlikely to represent a reversion of animals to a seronegative status as the samples were obtained from different animals in each year. Furthermore, only single samples, both of which were seropositive, were available for Gloucestershire and Cornwall in 2013 giving an apparent 100% seroprevalence for these counties. Therefore these data should be used as a broad indicator of regional seroprevalence and national spread of SBV, not as evidence for farm-level seroprevalence.

It is not clear to what extent factors such as rearing conditions (indoors or outdoors and stocking density) and the local geography and climate influence the risk of individual farms to infection with arthropod-borne viruses. As the only information available for the samples used in this study were the species of origin, location and date of sampling, it was not possible to assess the impact of potential risk factors such as age, gender or rearing conditions. A study in the Netherlands found no significant age-related different in seroprevalence in cattle over the three regions sampled (Elbers et al., 2012). In the present study when seroprevalence was assessed between cattle and sheep for each year there was no statistically significant difference. Studies in Belgium found that the seroprevalence at the beginning of 2012 was 86.3% in cattle (Meroc et al., 2013b) and over a similar period was 84.3% in sheep and 40.7% in goats (Meroc et al., 2013a). However, a study of ruminants in Turkey found that seroprevalence in cattle was over 10-fold higher than in sheep and goats (Azkur et al., 2013). Collectively, these data imply that cattle are highly susceptible to SBV infection and that sheep reared in Western Europe are similarly susceptible. The comparatively low seroprevalence in goats and sheep reared in Eastern Europe could indicate

reduced susceptibility of these animals, or that different rearing conditions significantly reduce the risk of SBV infection.

In conclusion, it is probable that SBV first entered the UK in late 2011 and subsequently spread to Ireland and Scotland with a peak of transmission apparently occurring during autumn 2012. This study suggests that a substantial number of animals remained susceptible to SBV infection in parts of the UK and Ireland in mid-2013. Conversely, it is likely that the majority of animals in some herds or flocks, particularly in the southwest of England, would have antibodies against SBV as a result of previous infection. Furthermore, the findings presented here support the recommendation of putting a ewe to a ram following the peak vector season in cases where the sero-status of the ewe is unknown.

Acknowledgements

The authors would like to thank Dr Nigel Kendall (NUVetNA) for providing the samples used in this study. The study was funded by the School of Veterinary Medicine and Science, University of Nottingham as an undergraduate research project.

Conflict of interest statement

The authors have no conflicts of interest to declare in relation to this manuscript.

Figure legends

Fig. 1. The percentage of serum samples from animals in the United Kingdom and Republic of Ireland that tested positive for Schmallenberg virus antibodies in each quarter from October 2011 to June 2013.

Fig. 2. Seroprevalence of cattle and sheep for October–December 2011, January–December 2012 and January–June 2013 (number of animals tested indicated).

Fig. 3. Schmallenberg virus seroprevalence by county in the United Kingdom and the Republic of Ireland; counties are coloured according to the overall SBV seroprevalence from all samples collected in a given year (October–December 2011; January–December 2012; January–June 2013).

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