

King, Barnabas and O'Shea Brown, Thomas and Tarlinton, R.E. and Daly, Janet M. (2015) Seroprevalence of Schmallenberg virus in the United Kingdom and Republic of Ireland: 2011-2013. Veterinary Microbiology . ISSN 0378-1135 (In Press)

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- 1 Seroprevalence of Schmallenberg virus in the United Kingdom and the Republic
- 2 of Ireland: 2011–2013
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10 Abstract

11 Since its identification in late 2011, Schmallenberg virus (SBV) spread rapidly across 12 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) 13 14 was estimated using a serum neutralisation test. There was no significant difference (P > 15 (0.05) in seroprevalence between sheep and cows suggesting that neither species is 16 significantly more at risk of SBV infection in the UK. A single 2011 sample tested positive; 17 the sample was taken in November from a cow in Wiltshire. There was a steady increase in 18 overall seroprevalence during the first three quarters of 2012, which then more than doubled 19 in quarter 4 (October–December), which may reflect a peak of vector activity. By the end of 20 June 2013, overall seroprevalence was around 72%. However, although seroprevalence was 21 over 50% in Wales and southern and central counties of England, it was below 50% in all 22 other areas of the UK and IE. This suggests that there were still substantial numbers of 23 animals at risk of infection in the latter half of 2013.

24

25 Keywords

26 Schmallenberg virus; SBV; seroprevalence; arbovirus.

28 Introduction

29 Schmallenberg virus (SBV) is an arbovirus of the Orthobunyavirus genus that is transmitted 30 by biting midges (Culicoides spp.). Since its identification at the end of 2011 (Hoffmann et 31 al., 2012), SBV has spread rapidly throughout mainland Europe. SBV infection of adult 32 ruminants appears to be sub-clinical or mild; causing watery diarrhoea, fever and reduced 33 milk production (Muskens et al., 2012). However, infection of animals during pregnancy 34 causes arthrogryposis-hydranencephaly syndrome (AHS), which results in congenital 35 malformations, abortions and stillbirths (Tarlinton et al., 2012). 36 Although the original identification of SBV infection was made following observation of 37 acute signs in adult dairy cattle from late summer 2011 (Hoffmann et al., 2012), the majority 38 of SBV infections are reported due to the appearance of AHS in calves and lambs. The first 39 cases of AHS were reported in the Netherlands in November and December 2011 and in

40 Belgium in 2012 (Garigliany et al., 2012; van den Brom et al., 2012). By comparison with the 41 related Akabane virus (Kirkland et al., 1988), it is suspected that SBV causes AHS only if 42 infection occurs in the mid-stages of pregnancy (Tarlinton et al., 2012). Therefore, it is 43 assumed that when AHS is observed, SBV must have been circulating several months 44 previously. This is supported by the initial detection of SBV in France in January of 2012 on 45 the basis of malformed lambs (Dominguez et al., 2012) with subsequent retrospective 46 analysis identifying seropositive animals sampled in October 2011 (Zanella et al., 2013). 47 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 48 49 2012 (APHA, 2012; Roberts, 2012). Studies of Belgian ruminants found that almost all 50 animals were seropositive for SBV at the end of 2011 (Meroc et al., 2013a; Meroc et al., 51 2013b). Although the duration of acquired immunity for SBV remains unknown, it was 52 speculated that herd immunity would prevent a second epidemic in 2012. In a follow-up

- 53 study, anti-SBV antibody titres remained high in animals one year later and very few clinical
- 54 cases were reported in 2012 (Meroc et al., 2013c).
- 55 The aim of this study was to determine the rate and extent of geographical spread of SBV
- 56 from its first emergence in the UK up to the introduction of an inactivated SBV vaccine by
- 57 testing archived serum samples from ruminants for SBV-specific antibodies.

58 Materials and Methods

59

(NUVetNA) located at the School of Veterinary Medicine and Science (University of 60 61 Nottingham). The study was approved by the University of Nottingham's School of 62 Veterinary Medicine and Science Ethics Committee. Sample details (species of origin, 63 location and date of sampling) were obtained from the NUVetNA database. Serum was used 64 for the majority of the testing but where serum was not available, plasma was used. 65 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 66 67 Bunyaviridae) (kindly provided by M. Beer, Friedrich-Loeffler Institute) with the minor 68 modification that cells were fixed by the addition of 100% ethanol and stained using 0.1% v/v 69 methylene blue in water. Positive and negative controls (samples previously tested with the 70 SBV IDscreen indirect ELISA [IDvet, France] by [BioBest Laboratories, UK]) were tested in 71 parallel with every batch of VNTs. 72 Seroprevalence maps were generated as choropleth maps in ArcGIS Explorer (Esri, USA).

Archived samples were obtained from the nutritional monitoring analytical services

73 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.

75 Results

76 Serum samples from 1108 ruminants were retrieved from 34 counties (England: 24; Wales: 3;

77 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,

80 396 (46.5%) and 161 (64.1%), respectively, were seropositive.

81 The samples were grouped by year quarter (Q1=winter, Jan–Mar; Q2=spring, Apr–Jun;

82 Q3=summer, Jul–Sep; and Q4=autumn, Oct–Dec) and analysed for seroprevalence by VNT

83 (Fig. 1). Only one 2011 sample, taken from a cow on a farm in South Western England

84 (Wiltshire) during November, tested positive. Antibodies against SBV were found in 14.6%

85 of the animals sampled in the first quarter of 2012 (Q1). Seroprevalence increased steadily in

86 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

98 end of June 2013 there were positive samples from all English counties from which samples99 were obtained.

100 Discussion

101 This serosurvey confirmed the rapid spread of SBV throughout England and Wales and into

102 Scotland, the Republic of Ireland and Northern Ireland during 2012/13. This was probably

103 facilitated by prevailing winds from Europe as modelling of SBV spread across the UK found

104 that the majority of farm-to-farm transmission events were consistent with downwind

105 movement of midges (Sedda and Rogers, 2013).

106 In May 2013 an inactivated whole virus vaccine against SBV was licensed for use in the UK.

107 The vaccine status of the animals from which samples were obtained was unknown and it is

108 not possible to differentiate between animals immunised using this vaccine and naturally

109 infected animals using neutralising antibody responses. Therefore sample testing was

110 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

121 bluetongue virus, which is also transmitted by Culicoides spp. (Hoffmann et al., 2009). SBV 122 RNA has consistently been detected in C. obsoletus, C. scoticus, and C. chiopterus across 123 Europe (Balenghien et al., 2014; De Regge et al., 2012; Elbers et al., 2012); strongly 124 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., 125 126 2013) and C. nubeculosus (Balenghien et al., 2014) but the role of these species in 127 transmission has yet to be confirmed. The seasonal abundance of Culicoides varies dependent 128 on species in the UK (Sanders et al., 2011) and although C. obsoletus complex midges are 129 present in the UK, their prevalence and vector-competence has not been determined. 136 The data presented here suggest that animals are most at risk of SBV-infection during the 137 height of the vector season (August -September). Peak sexual activity for the majority of 138 sheep breeds in the UK is from October through to December, but the breeding season can be 139 advanced to August for January lambing. By inference from studies of Akabane virus 140 infection; only animals infected with SBV during the vulnerable mid-stage of gestation are 141 thought to be at risk of developing AHS (Tarlinton et al., 2012). Therefore, sheep 142 inseminated in August will reach the mid-stage of gestation during the height of the vector 143 season. Thus delaying insemination until late September might be recommended to reduce 144 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).

154 The samples used in this study were submitted for reasons other than suspicion of SBV 155 infection (clinical signs of which are often missed in adult animals); therefore the results are 156 likely to be a true reflection of the level of SBV seroprevalence in the UK. The earliest 157 laboratory-confirmed cases of SBV in the UK were identified from malformed lambs born in 158 December 2011 (Sedda and Rogers, 2013) and January 2012 (APHA, 2012; Roberts, 2012). 159 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 160 161 the ewes giving rise to the first cases of SBV in the UK became infected in October or 162 November 2011. Consistent with this and similar observations in France (Zanella et al., 163 2013), antibodies against SBV were detected in a cow in November 2011 in this study. 164 Seropositive goats, sampled in February 2012, were also identified as part of this study, a year earlier than previously reported (APHA, 2013). 165 166 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 167 168 English and Welsh counties, but that all animals identified by clinical presentation in 169 Scotland had been introduced from other SBV-positive regions of the UK (APHA, 2013). 170 The earliest identified infections of indigenous animals in Scotland were in the South West in 171 December 2012 from bulk tank milk screening (Mason et al., 2013). Furthermore, a 172 retrospective analysis in Ireland reported an SBV seroprevalence of 22.1% in 570 cattle 173 sampled between March and December 2012 from 6 counties which were first to report 174 clinical signs of SBV infection (O'Neill, 2014). Therefore, although it is possible that some or 175 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 isapparent 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.

185 It is not clear to what extent factors such as rearing conditions (indoors or outdoors and 186 stocking density) and the local geography and climate influence the risk of individual farms 187 to infection with arthropod-borne viruses. As the only information available for the samples 188 used in this study were the species of origin, location and date of sampling, it was not 189 possible to assess the impact of potential risk factors such as age, gender or rearing 190 conditions. A study in the Netherlands found no significant age-related different in 191 seroprevalence in cattle over the three regions sampled (Elbers et al., 2012). In the present 192 study when seroprevalence was assessed between cattle and sheep for each year there was no 193 statistically significant difference. Studies in Belgium found that the seroprevalence at the 194 beginning of 2012 was 86.3% in cattle (Meroc et al., 2013b) and over a similar period was 195 84.3% in sheep and 40.7% in goats (Meroc et al., 2013a). However, a study of ruminants in 196 Turkey found that seroprevalence in cattle was over 10-fold higher than in sheep and goats 197 (Azkur et al., 2013). Collectively, these data imply that cattle are highly susceptible to SBV 198 infection and that sheep reared in Western Europe are similarly susceptible. The 199 comparatively low seroprevalence in goats and sheep reared in Eastern Europe could indicate

200 reduced susceptibility of these animals, or that different rearing conditions significantly201 reduce the risk of SBV infection.

202 In conclusion, it is probable that SBV first entered the UK in late 2011 and subsequently 203 spread to Ireland and Scotland with a peak of transmission apparently occurring during 204 autumn 2012. This study suggests that a substantial number of animals remained susceptible 205 to SBV infection in parts of the UK and Ireland in mid-2013. Conversely, it is likely that the 206 majority of animals in some herds or flocks, particularly in the southwest of England, would 207 have antibodies against SBV as a result of previous infection. Furthermore, the findings 208 presented here support the recommendation of putting a ewe to a ram following the peak 209 vector season in cases where the sero-status of the ewe is unknown.

210 Acknowledgements

- 211 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,
- 213 University of Nottingham as an undergraduate research project.

214 Conflict of interest statement

215 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
- 218 of Ireland that tested positive for Schmallenberg virus antibodies in each quarter from
- 219 October 2011 to June 2013.

220

- Fig. 2. Seroprevalence of cattle and sheep for October–December 2011, January–December
- 222 2012 and January–June 2013 (number of animals tested indicated).

- Fig. 3. Schmallenberg virus seroprevalence by county in the United Kingdom and the
- 225 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;
- 227 January–June 2013).

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