This article has been accepted for publication in The Veterinary Record, 2020, following peer review, and the Version of Record can be accessed online at http://dx.doi.org/10.1136/vr.105869

Cross-sectional study of British wild deer for evidence of Schmallenberg virus infection

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Keywords: Schmallenberg virus, deer, Culicoides, vector-borne disease

Word count: 2418

ABSTRACT

Background: Schmallenberg virus (SBV) is an orthobunyavirus carried by *Culicoides* biting midges that cause reproductive problems in adult ruminants when infected during their gestation period. SBV was first detected in ruminants in the UK in 2011/12 and then again in 2016. The reason behind the 2016 re-emergence of SBV is unknown, but one possibility is that it can be maintained in wildlife, such as deer. SBV has been detected at high seroprevalence in deer in a number of European countries, but only once in the UK in a single region.
Methods: The purpose of this study was to survey wild deer across Great Britain for recent evidence of SBV. Deer hunters were recruited for the purpose of providing post-mortem blood samples to be tested for SBV antibodies.
Results: The seroprevalence of SBV in the British wild deer population was 13.8%; found in red, roe, muntjac and fallow deer species, with more in deer further south.
Conclusion: These results support the growing concern that SBV is now endemic in Great Britain and highlight the need to know the role of wildlife in SBV transmission.

INTRODUCTION

Schmallenberg virus (SBV) is an orthobunyavirus discovered in Germany in 2011 and carried by *Culicoides* biting midges; these insects are also vectors of bluetongue viruses (BTV) (1). From its initial discovery, SBV took just 2 years to reach 27 European countries (2). The outbreak resulted in financial losses to farmers as SBV affects domestic ruminants, namely sheep and cattle (3). In adult animals, infection causes subclinical or mild disease but exposure of pregnant females during key periods of gestation can cause abortion, stillbirth and congenital malformations in offspring (4). Treatment is symptomatic only and so recommended options for prevention include import bans, the use of insecticides, mating livestock to ensure the critical gestation period occurs outside of peak vector season and minimizing exposure to the vector in endemic areas (4).

The south and south-east of England were the most affected parts of the UK in the 2011 outbreak (5). Following what was reported to be a period of absence in 2015 (6), September 2016 saw the return of SBV to England (7). In contrast to the first UK outbreak, SBV reached further north in this second outbreak, with 95 premises in England, 42 in Wales and 2 in Scotland affected before April 2017 (7).

SBV has also been detected in deer and has shown high seroprevalence in a number of European countries (8). The first evidence of SBV in UK deer during the first outbreak was reported in 2012 (9) but only 66 samples from a single region of England were tested. The manifestation of this disease in deer is unknown, as is the possibility of transfer between species. The nature of rearing wild ruminant offspring and the lack of close monitoring mean that disease surveillance in wild deer is generally poor.

Deer may be important reservoirs of SBV between and during outbreaks, contributing to the threat that SBV poses to Great Britain and its large farming industry. The purpose of this study was to investigate evidence of SBV in British wild deer, determining its seroprevalence, geographical distribution and risk factors.

MATERIALS AND METHODS

Study design
Ethical approval was granted by the University of Liverpool Veterinary Ethics Board prior to commencement of the study (VREC707). Sample size was determined by initially estimating the seroprevalence of SBV in wild deer in Europe from published data. Studies on deer SBV seroprevalence in European countries, including Belgium, Netherlands, France, Poland, Italy and Spain, revealed seroprevalences as high as 40—50% in certain species. Many of these estimates were obtained during active outbreaks and may be higher than would be expected during inter-epidemic periods. Geographical similarity meant that the 21% seroprevalence recorded in Great Britain (9) and 9.9% in Ireland (10) were considered to be the most relevant. As the more recent and widespread investigation; the seroprevalence recorded in Ireland was deemed the most reliable basis for this study Therefore, to estimate sample size, we assumed an initial seroprevalence of 10%. Given diagnostic test sensitivity of 97.7% and specificity of 99.7%, we determined that 157 samples were required to estimate seroprevalence with 5% precision and 95% confidence.

Participant recruitment
Deer hunters were recruited to provide post-mortem blood samples to be tested for SBV antibodies. The British Deer Society, Deer Initiative and British Association for Shooting and Conservation advertised to hunters by email, telephone or visit. All licensed deer hunters were considered eligible to participate. Interest was confirmed by email detailing the latitude and number of samples they aimed to supply. Following a high level of recruitment, deer hunters were selected based on their latitude to give wide coverage of Great Britain.

Materials
Sample packs were distributed to participants for return to Leahurst Campus, University of Liverpool. A sample pack comprised a padded envelope containing a participant information sheet, study protocol, participant consent form, record sheet, pair of disposable nitrile gloves, disposable pipettes, a blood tube (Sarstedt Ltd, [Leicester, England]), labelled with a unique deer ID number and a disposal bag for used materials. The record sheet was used to obtain additional information for risk factor analysis including the latitude, habitat, age, sex, species and approximate weight of the deer sampled.

Sampling
Blood samples were collected between February 1, 2019, and March 31, 2019. Samples were taken post-mortem from deer shot for routine reasons, ensuring that no deer were killed for the purpose of this study. Each sample received was recorded and checked for a signed consent form before testing. Once the deer ID number was matched to a participant, personal information regarding the deer hunter was kept anonymous and only the deer ID number was used thereafter. Blood samples were refrigerated until they could be centrifuged and the serum frozen. The maximum period for which a sample was left in the fridge before centrifugation was over the weekend when the sample was received late Friday afternoon. Most samples were frozen within 2 hours of receipt at Leahurst.

Testing
Screening for SBV antibodies was done after all the samples were received. In order to avoid poor quality samples yielding false ELISA results, 59 samples estimated to have >50 and <250 mg/dL haemoglobin concentration, according to their colour, were selected for

treatment with HemogloBind (Biotech Support Group, New Jersey, USA). Samples with a higher concentration than this, totalling 78 samples, were discarded immediately, on the assumption that that they were too degraded to be worth treating. Samples with <50mg/dL (28 samples) haemoglobin concentration qualified for use in the ELISA without any further treatment. Eighty-seven samples were therefore tested by ELISA, including 28 that did not require treatment with HemogloBind, were therefore tested by ELISA (ID Screen Schmallenberg virus Indirect Multi-species screening test; IDvet [Grabels, France]) according to the manufacturer's instructions.

Risk factor analysis

The outcome variable, SBV test result, was set as a binary response variable (0= negative; 1 = positive)

Explanatory variables were species, habitat, age, sex, weight, and latitude, defined as follows: species (categorical), red, roe or 'other'; habitat (categorical), deciduous forest, coniferous forest or 'other'; age (categorical), adult (>2 years), yearling (1—2 years) or young (0—1 years); sex (categorical), male or female; weight (continuous, in Kg); latitude (continuous - the latitude of the county town from the county where the sample was obtained - or categorical, divided according to north/south distribution), Northumberland and Cumbria; Yorkshire and Lancashire; or Suffolk, Bedfordshire and Norfolk.

First, univariable analysis of each categorical explanatory variable and the outcome variable was carried out using 'Fisher's Exact test' SISA online tool (https://www.quantitativeskills.com/sisa/statistics/fiveby2.htm). This was chosen instead of chi-squared as there were often expected values of <5. Possible confounding was addressed by stratification.

Second, multivariable logistic regression models were developed using R version 3.5.1 programme. All explanatory variables were included, apart from sex because this variable was unbalanced, with only one positive sample obtained from a male. Weight was log-transformed to reduce right skew. Latitude was continuous. Reference categories of the included categorical variables were 'other' for species, coniferous forest for habitat, and adult for age. There were insufficient sample sizes for us to robustly test for statistical interactions between the different explanatory variables.

RESULTS

A total of 330 sample packs were sent out and 165 (50%) were returned. Due to degradation, only 87 of these samples could be tested, which meant that the desired confidence and precision set out prior to sampling was not achieved.

In the ELISA, the ratio of the optical density for positive controls to negative controls was 4.9. For the ELISA to be valid, it must be greater than 3.

The ELISA resulted in 17 positive and 2 doubtful results. An estimate of 22.1% (confidence interval, 14.5%—32.1%) seroprevalence of SBV in British wild deer was determined if the 2 doubtful results are regarded as positive; and 19.7% (CI 12.6%-29.5%) if the 2 doubtful results are counted as negative.

Virus neutralisation testing (VNT) by the Animal and Plant Health Agency confirmed twelve of these samples as positive for antibodies to SBV, and 7 as negative. Assuming that the VNT is the more accurate test these new results estimate a 13.8% (8.1—22.6% confidence interval) seroprevalence of SBV in British wild deer.

Positive (by VNT and ELISA) and negative results, by risk factor, are summarised in Table 1.

In univariable analysis, two significant associations were found: the probability of being seropositive for SBV was increased if samples were from females or further south (Table 2 and Figure 1). We attempted to address possible confounding between sex and latitude by stratification, testing for an effect of latitude in males and females separately. The effect of latitude was significant in females only (females, $P < 0.02$; males, $P > 0.5$), consistent with confounding, although the sample sizes for males were small, with only a single positive.

Multivariable analysis by logistic regression of all 19 positive and doubtful samples found latitude to be a significant variable: seropositivity was associated with samples from deer at more southern sites. Analysis of the 12 VNT-positive samples also found only a significant association with latitude (see Table 3); as before, samples collected from further south were more likely to be SBV positive.

DISCUSSION

This study provides evidence of continued SBV circulation in deer in Great Britain since 2012 (9), with a number of positive samples from young deer that were likely born in 2018. SBV may be endemic in Great Britain, as it appears to be elsewhere in Europe (11).

A total of 14 ELISA-positive samples out of 66 collected were reported in early Spring 2012 (9); resulting in an estimated 21.2% seroprevalence. Similarly, our ELISA results yielded an estimated 21.8% seroprevalence (19 positive samples out of 87 tested) but confirmatory testing by VNT confirmed only 12 of these to be positive (13.8% seroprevalence). With approximately 2 million UK wild deer a 14—22% seroprevalence suggests that there may be as many as 440,000 deer that have been exposed to SBV. This crude estimate, however, does not take account of different seropositivity rates by latitude; for instance, more deer are present in Scotland, where we expect SBV rates to be lower.

We found that deer in the south are more likely to be SBV positive; this agrees with previous studies reporting a mostly southern distribution of the virus during the 2011 outbreak (12;13). Measures to prevent and control the spread of SBV in Great Britain should therefore focus mostly on ruminants in southern England; although it is important to note that seropositive results were obtained from samples collected as far north as Cumbria and cases have occurred in Scotland.

Sampling from hunted animals is often a poor means of estimating disease prevalence, as there may be a difference between infected and uninfected animals in their availability to hunters. However, we believe this potential source of bias to be minimal for our study of SBV. First, we were detecting antibody responses, which, in most cases, will be from historic

infections. Second, in livestock with active SBV infections the clinical signs are very mild or undetectable, and so unlikely to significantly affect behaviour.

Due to fewer samples being received than expected, and the poor quality of some of the samples, we were unable to adhere to all details of our study protocol. While we had intended that no more than 5 samples should be tested from any single hunter, we tested all samples of sufficient quality.

Antibodies to SBV were detected in both yearling (1-2 years) and young (<1 year) deer, confirming that SBV has been circulating recently in Great Britain. However, persistent maternal antibodies could have given rise to these results in the youngest deer; in Texas, maternal antibodies to bluetongue virus were not shown to disappear until the deer were 23 weeks of age (14). Similarly to the study in Ireland (10), we found higher seroprevalences among older deer (20.5% in adults, 7% in yearlings/young combined), although we did not find a statistically significant effect of deer age on the probability of a sample being seropositive. In an endemic setting, seropositivity rates to a pathogen tend to increase with age, as older individuals have had more opportunity to be exposed to an infection and, therefore, the pattern we have observed is further evidence for endemic transmission. A more highly powered study is recommended to explore in more detail the effect of age on deer seropositivity rates.

Six species of deer live freely in Great Britain; of these, muntjac and Chinese water deer are not found elsewhere in Europe. We received and tested samples from all six; and four were seropositive by VNT. Two of these species (muntjac and fallow) are new reports for SBV in GB. In contrast to all other deer species, muntjac breed all year round. This could help SBV to persist, with susceptible animals entering the population in all seasons; consequently, the presence of muntjac may present an additional disease challenge to Great Britain. In Ireland, positive results were recorded in fallow, sika and red deer (3 of the 4 of the species tested) and in deer of both sexes (10). Neither the Irish nor the present study found evidence that species or sex influence the risk of a deer being SBV seropositive.

Although we received fewer samples than anticipated, advertising for and utilising public participants, specifically those involved in deer management, is an effective way of improving disease awareness, and achieving cost effective surveillance. Additionally, it can ignite interest for involvement in further studies. The fifty percent return rate of samples indicates the need to recruit a substantially larger number of participants than is estimated to power a study.

This study significantly extends the area over which SBV has been reported in British deer. The high seroprevalence of SBV in a large and widespread deer population raises concerns around the welfare of wild deer, has implications for the deer farming industry, and indicates that deer could act as a source of virus for livestock. The apparent endemicity of this *Culicoides*-borne disease in the UK indicates that we should expect continued challenge from this virus, but also heightens concerns about our vulnerability to other viruses spread by the same vectors, including BTV and African horse sickness virus (15). Climate change is likely to further increase the threat posed by these viruses (16).

The re-emergence of SBV in 2016 and its spread to previously untouched areas alert us to the continued threat posed by SBV. Should this pattern of re-emergence continue, then the detrimental impacts caused by the disease will continue (1). The results from this study should motivate further study and surveillance of SBV in deer.

ACKNOWLEDGEMENTS

This study was funded by the University of Liverpool Institute of Veterinary Science as part of a BSc in Veterinary Conservation Medicine, as well as an award from the British Deer Society (BDS). We thank the individuals who supplied blood samples from deer, Alastair Boston of the Deer Initiative for help with participant recruitment, and Giacomo D'Ammando for help with the results analysis. For Figure 1, we acknowledge Office for National Statistics (2011). 2011 Census: boundary data (England and Wales) [data collection]. UK Data Service. SN:5819 UKBORDERS: Digitised Boundary Data, 1840- and Postcode Directories, 1980. http://discover.ukdataservice.ac.uk/catalogue/?sn=5819&type=Data%20catalogue, Retrieved from http://census.ukdataservice.ac.uk/get-data/boundary-data.aspx. Contains public sector information licensed under the Open Government Licence v3

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TABLES

Table 1: Detection of antibody to Schmallenberg virus in deer blood samples by enzyme-linked Immunosorbent assay (ELISA) and virus neutralisation test (VNT). NA – not available.

Risk factor		Not tested	VNT +ve, -ve	ELISA +ve, -ve	VNT % positive	ELISA % positive
County	Cumbria	47	3, 39	8, 34	7.1	19.0
	Yorkshire	37	2, 15	3, 14	11.8	17.6
	Suffolk	4	2, 2	2, 2	50	50
	Northumberland	10	0, 6	0, 6	0	0
	Bedfordshire	12	1, 6	1, 6	14.3	14.3
	Lancashire	5	1, 1	1, 1	50	50
	Norfolk	15	3, 4	4, 3	42.3	57.1
	East Sussex	1	0, 0	0, 0	0	0
	Dumfries & Galloway	2	0, 0	0, 0	0	0
	Wiltshire	1	0, 0	0, 0	0	0
	Worcestershire	1	0, 0	0, 0	0	0
	Dorset	3	0, 0	0, 0	0	0
	Ayrshire	4	0, 0	0, 0	0	0
	Herefordshire	2	0, 0	0, 0	0	0
	NA	2	0, 2	0, 2	0	0
Species	Red	24	1, 13	3, 11	7.1	21.4
	Roe	85	6, 47	11, 42	11.3	20.8
	Muntjac	20	1, 7	1, 7	12.5	12.5
	Fallow	11	2, 3	2, 3	40	40
	Chinese water deer	2	0, 2	0, 2	0	0
	Sika	2	0, 1	0, 1	0	0
	NA	2	2, 2	2, 2	50	50
Sex	Female	110	12, 53	18, 47	18.5	27.7
	Male	34	0, 20	1, 19	0	5
	NA	2	0, 2	0, 2	0	0
Habitat	Coniferous Forest	95	8, 48	13, 43	14.3	23.2
	Deciduous Forest	29	3, 21	5, 19	12.5	20.8
	Grassland	10	1, 3	1, 3	25	25
	Other	10	0, 1	0, 1	0	0
	NA	2	0, 2	0, 2	0	0
Age	Adult	82	8, 31	12, 27	20.5	30.8
	Yearling	31	2, 21	3, 20	8.7	13.0
	Young	30	1, 21	3, 19	4.5	13.6
	NA	3	1, 2	1, 2	33.3	33.3
Total		142	12, 75	19, 68	13.8	21.8

Table 2: Univariate analysis using the Fisher Exact test. For latitude, counties were divided according to north/south distribution; Northumberland and Cumbria; Yorkshire and Lancashire; or Suffolk, Bedfordshire and Norfolk. Significant mid-p values (<0.05) are in bold.

Risk factor	Mid-p value
Latitude	**0.012**
Sex	**0.045**
Habitat	0.714
Species	0.580
Age	0.158

Table 3: Multivariable analysis by logistic regression. Significant P values (<0.05) are in bold. The final model was obtained by removing explanatory variables in order of least significance and resulted in only latitude as a significant predictor; estimates for the other variables were obtained by adding them separately to the final model.

Risk factor	Coefficient	S.E.	P
Latitude	-0.871	0.360	**0.016**
Age(Yearling)	-0.432	0.932	0.643
Age(Young)	-1.338	1.124	0.234
Species(Red Deer)	1.823	1.786	0.307
Species(Roe Deer)	1.404	1.087	0.197
Log(Weight)	0.266	0.388	0.494
Habitat(Deciduous Forest)	0.010	0.760	0.980
Habitat(Other)	0.225	1.252	0.857

Figure 1: Map of counties in England showing the number of deer blood samples positive and negative for SBV by virus neutralisation test (VNT); only counties from which samples were tested are shown. Contains National Statistics data © Crown copyright and database right [2011]. Contains OS data © Crown copyright [and database right] (2011