Demonstrating freedom from Schmallenberg virus in the South West of England, 2015

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Abstract

In 2011-2012 northern European livestock faced a threat from a newly emerged virus, Schmallenberg virus (SBV), only a few years after a major outbreak of bluetongue serotype 8 (BTV-8). Like BTV-8, SBV is transmitted by *Culicoides* biting midges to ruminants and spread throughout Europe. SBV, however, spread faster, reaching the UK within three months of initial discovery. Adult ruminants show only mild, if any, clinical signs, however infection of naïve ruminants by SBV during the vulnerable period of gestation leads to abortions, still births and foetal malformations. Although some data exists for the prevalence of SBV on UK sheep farms early in the outbreak, we have no information on its current status. Is SBV still circulating in the UK? To answer this we designed a freedom from disease study across the southernmost counties of the UK. During autumn 2015, 1444 sheep, from 131 farms, were tested for antibodies against SBV by ELISA; five samples from four farms were twice found positive by ELISA but were later confirmed negative by VNT. As the sheep were born between October 2014 and April 2015, we conclude it is unlikely that SBV is still circulating in the south of England.

Keywords: *Schmallenberg virus, sheep, freedom, surveillance*

Introduction

In November 2011 a novel Orthobunyavirus, of the Simbu serogroup, was identified by metagenomic analysis of cattle presenting with diarrhoea, hyperthermia and reduced milk yield in Germany (1). The virus was subsequently named Schmallenberg virus (SBV), after the geographic origin of the samples tested. SBV spread rapidly, reaching England within three months of initial outbreak, with the southern-most counties of England all reporting outbreaks of Schmallenberg virus between 2012 and 2013. Like several viruses of the Simbu serogroup, and the unrelated bluetongue virus serotype 8 (BTV-8), SBV is transmitted by *Culicoides* biting midges (2).

Since its initial discovery, SBV has been detected throughout Europe in domestic and wild ruminants (2). European studies, conducted in 2011, 2012 and 2013, found animal level prevalence to range between 8-100% and 8.5-93.3% in cattle and sheep respectively (3-5). Herd level prevalence of UK sheep in 2012/2013 was found to range between 40-90% (3). SBV infections of adult ruminants are generally asymptomatic; however, if infection of a naïve pregnant animal coincides with the vulnerable period of gestation, transmission across the placenta can result in abortions, stillbirths and foetal malformations (6) Studies on the related Akabane virus estimate the vulnerable period to be between days 28 to 56 of pregnancy, however a recent study demonstrated high placental colonisation of SBV when infected at days 45 or 60 of gestation, but a lack of subsequent abortions and malformations observed in the lambs (2). Evidence exists of acquired immunity against reinfection in naturally infected sheep, as well as evidence of maternally derived antibodies in suckling labs (7).

Four cases of SBV were confirmed on 16 January 2012. Voluntary reporting recorded 81 and 87 serologically confirmed cases in UK sheep in 2012 and 2013 respectively, however no cases of SBV were confirmed by PCR in lambs or calves presenting with arthrogryposis by the Animal and Plant Health Agency (APHA) in 2014 or 2015 (APHA, personal communication). A recent study of naïve cattle from the Netherlands detected a low level of SBV (<1%) in 2013 (8). A German study reported a recurrence of SBV in cattle in 2014, despite an apparent decrease in cases the previous year (8,9).

The high circulation of SBV in the UK in 2012 and 2013 followed by a subsequent decline in cases in 2014 and 2015 leads to the question; is this apparent decline in cases in the UK a true decrease in circulation or a lack of reporting? This study aimed to determine if SBV was still circulating in southern-most counties of England in 2015 by examining the serological status of sheep born after the 2014 vector period.

Materials and methods

A two-stage cluster analysis was used to estimate the number of flocks, and the number of sheep per flock to be sampled. This was determined to substantiate, with 95% confidence, a SBV prevalence of below 2.5% for the southern-most counties of England. Using the software package FFD in *R* it was determined 11 sheep from 131 farms would need to be sampled.

Blood samples were collected between mid-September and mid-December 2015 from sheep born the previous October 2014-April 2015. All sheep sampled were older than six months to avoid detection of maternal antibodies, and younger than 14 months to exclude those that may have been exposed to SBV in previous years. A short questionnaire was delivered on farm to determine prior SBV case and vaccination history.

Serum was extracted and tested for antibodies using a commercially available ELISA (ID Screen® Schmallenberg virus indirect, IDvet, France) per manufacturer's instructions. All samples returning positive were sent to the APHA for VNT testing, along with an equal number of negatives to act as blind controls.

Results

A total of 1,572 sheep from 131 holdings were sampled between 15 September and 11 December 2015. Flock sizes ranged from 20 to 5000, all sheep sampled were born between October 2014 and April 2015. Of the 131 holdings sampled, 103 were lowland flocks, eight hill, eoght upland, 10 had flocks across lowland, hill and/or upland pastures and two holdings declined to answer or were unsure.

One hundred and twenty four farmers answered the short questionnaire. Fifty seven farmers (50%) reported they had previously suspected cases of SBV infection in their flocks. Of these only 13 farmers had cases that were laboratory confirmed. Seventeen (13.7%) farmers reported they had vaccinated their sheep against SBV in 2013, with only two (1.6%) repeating vaccination in 2014.

Of the 1444 samples tested, nine samples, from 8 holdings, initially returned doubtful or positive results for antibodies to SBV when tested by ELISA. These samples were retested by ELISA, with five samples, from four holdings returning positive for antibodies against SBV. No antibodies were detected in these five samples when tested by the 'gold standard' VNT at the APHA.

Discussion

This study found it unlikely that any antibodies against SBV were circulating in the sheep tested. As these sheep were born between October 2014 and May 2015, we can be 95% confident that if SBV was circulating in the south of England in the 2015 vector period, it was present below the 2.5% prevalence threshold designed by this study. Using a similar testing procedure, a study of cattle in the Netherlands determined a maximum possible prevalence of herds to be <1% prevalence in 2013 (8).

The specificity of the commercial ELISA kit used was reported to be 99.8%, giving a likely false-positive rate of ~3 samples of the 1444 tested. Initially nine out of the 1444 samples returned positive by ELISA for SBV-specific antibodies, higher than the calculated test false-positive rate. However other studies have cast doubt on the high specificity of the test if the virus is circulating below the peak outbreak levels, with a false positive rate of 41% reported in wild cervids (10). The use of VNTs as conformational tests for commercial ELISAs is considered advisable due to the high (~99-100%) sensitivity and specificity of the VNT (11).

As observed during the height of the SBV outbreak in Europe, the transmission of SBV is highly efficient, spreading rapidly both within and between flocks (2,12–14). This spread was far faster than that of BTV-8, likely due to the much shorter viraemia, much higher probability of host to vector transmission and SBV's predicted faster replication rate and replication at a lower temperature threshold than BTV-8 (15). Even with low levels of SBV circulation and few susceptible hosts on farm, previous studies have demonstrated eventual seroconversion of these individuals (16). These characteristics of SBV make it also highly unlikely that the five ELISA positive samples were true positives, as that would mean SBV was persisting at a very low prevalence, within a large naïve population. However, this does not mean that it is impossible for SBV to persist at very low levels, particularly if reintroduced late in the *Culicoides* season, as the current knowledge of the epidemiology of SBV is still expanding.

Despite this, surveillance for SBV should continue, with a German study describing a decline of SBV occurrence in cattle in 2013 compared to 2011-2012 seroprevalence, followed by an increase in cases the following year (9). This is a frequent occurrence with midge-borne arboviruses. For example, since the end of the most recent BTV-8 outbreak, the serotype was considered absent from France, with disease free status granted in 2012; only for it to re-emerge in August 2015 (17). It has been postulated that this new outbreak may have re-emerged from wildlife reservoirs, with red deer in Spain previously testing positive for BTV when local livestock remained disease free (18). If this was indeed the case, then greater emphasis should be put on surveillance of wild ruminant populations to determine freedom within this potential reservoir source, particularly as far more wild species have been demonstrated to have SBV-specific antibodies, with far higher prevalence in populations described, than for BTV-8 (19). An alternative to invasive on-farm procedures would be the widespread trapping of *Culicoides* for surveillance, perhaps by bulk testing by county/canton to rapidly test large numbers of the insects. Targeted surveillance could also be utilised, collecting *Culicoides* at sites deemed 'high risk' for possible passive wind transfer from Europe, particularly in the event of recurrence on the continent.

Regardless of the current status of SBV in Europe, this study has highlighted a large, naïve population; susceptible to future potential outbreaks within the south of England. Effective surveillance systems are therefore needed to warn vets and farmers of future disease risks.

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