UNIVERSITY OF LIVERPOOL

The Epidemiology and Surveillance of *Culicoides*-borne Diseases of Ruminants in the UK

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By

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Abstract

Between 2006 and 2011 two *Culicoides*-borne diseases of ruminants emerged in Europe: bluetongue virus serotype-8 (BTV-8) and Schmallenberg virus (SBV). This thesis sought to answer questions arising from this developing disease landscape, to better inform policymakers, stakeholder groups and disease modellers.

SBV spread rapidly through Europe, reaching the UK in January 2012. However, in 2014 no cases were reported. It was unknown if this was a lack of circulation, or a lack of reporting. A freedom from disease study was designed. 1444 sheep, born between October 2014 and April 2015, were sampled from 131 farms from Cornwall to Kent. Samples were tested by ELISA for antibodies against SBV, 5 positive samples were confirmed negative by VNT. Circulation of SBV in 2015 in the south of England was concluded to have been unlikely.

Like SBV, BTV-8 had circulated throughout Europe, only to be controlled by movement restrictions and vaccination. Subsequently, Europe was declared BTV-8 free in 2010 and vaccination production halted. In 2015 BTV-8 re-emerged in Europe. An online questionnaire determined that respondents from smaller farms, those that had previously vaccinated against BTV-8 and those who were deemed to be 'risk adverse' were all more likely to want to vaccinate, and more willing to pay more to vaccinate. Voluntary vaccination only achieved an 80% uptake if vaccination was free and after BTV-8 cases were reported in the UK despite 90% of farmer respondents stating they believed it important to keep BTV-8 out of the UK. Not all farmers vaccinated all of their flock/herd previously. This survey highlights the complex issues surrounding voluntary vaccination at the farm perceived risk versus cost level.

The mechanisms for how either virus successfully overwintered are still poorly understood. A cross-sectional study demonstrated that *Culicoides* vectors are active during peak lambing periods inside lambing sheds. A longitudinal study the following lambing season demonstrated that *Culicoides* were more abundant indoors than outdoors, and demonstrated activity of gravid and parous *Culicoides* over the winter. This demonstrates a possible mechanism for overwintering of BTV-8 and SBV in the south of England.

SBV re-emerged in 2016. A questionnaire was designed to determine the impact of SBV on the 2016/2017 lambing period. The impact was found to be highly comparable to a previous study of the 2012/2013 outbreak. Additionally SBV confirmed and suspected farms were more likely to have mated earlier in the season. If SBV continues to re-emerge cyclically then the impact of disease will continue to be significant unless intervention is taken.

These studies have added to our understanding of, and farmer response to, the SBV and BTV-8 outbreaks, and added to policymakers, stakeholders groups and disease modellers knowledge.

This thesis is based on research carried out in the Department of Epidemiology and Population Health at the University of Liverpool.

The work in this thesis is my own, with the following exceptions:

In Chapter 2 confirmatory VNT testing of ELISA positive samples was undertaken by Dr Anna La Rocca at the APHA.

The questionnaire design in Chapter 5 was agreed by the author, Dr Jennifer Duncan, Dr Rachael Tarlinton (University of Nottingham) and Amanda Carson (APHA).

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List of Abbreviations

Common abbreviations are defined below, specialist abbreviations are defined below and in text.

AHDB	Agriculture and Horticulture Development Board
AHSV	African Horse Sickness Virus
AHVLA	Animal Health and Veterinary Laboratories Agency
APHA	Animal and Plant Health Agency
ANOVA	Analysis of Variance
BTV	Bluetongue virus
BTV-x	BTV Serotype x (e.g. BTV-8)
°C	Degrees Celsius (Centigrade)
CCHF	Crimean-Congo haemorrhagic fever
DB	Dissemination barrier
DEFRA	Department for Environment, Food & Rural Affairs
dsRNA	Double-stranded RNA
EC	European Commission
EFSA	European Food Safety Authority
EHDV	Epizootic haemorrhagic disease virus
EIP	Extrinsic Incubation Period
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FUW	Farmers' Union of Wales
GB	Great Britain
GIS	Geographic Information System
IQR	Interquartile Range
ITR	Indoor Trapping Rates
ITS1	Internal Transcribed Spacer 1
MEB	Mesenteron Escape Barrier
MIB	Mesenteron Infection Barrier
mRNA	Messenger RNA
NFU	National Farmers' Union
NSA	National Sheep Association

NUTS	Nomenclature of Territorial Units for Statistics
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
R_0	Basic reproduction rate
RH	Relative Humidity
RNA	Ribonucleic acid
ROC	Receiver Operating Characteristic
RT-PCR	Reverse-Transcriptase PCR
SBV	Schmallenberg virus
SEM	Scanning Electron Micrograph
SGEB	Salivary Gland Escape Barriers
SGIB	Salivary Gland Infection Barriers
SNT	Serum Neutralisation Test
ssRNA	Single stranded RNA
SVS	Sheep Veterinary Society
TSWV	Tomato Spotted Wilt Virus
UK	United Kingdom
VBD	Vector-borne disease
VNT	Virus Neutralisation Test
WNV	West Nile virus

Common taxonomic abbreviations are defined below:

Obsoletus Complex	*Culicoides obsoletus* and *Culicoides scoticus*
Obsoletus Group	*Culicoides obsoletus*, *Culicoides scoticus*, *Culicoides dewulfi* and *Culicoides chiopterus*

Chapter One

Introduction and Literature Review

1.1 Vector-borne diseases

A vector-borne disease (VBD) can be defined as an infection transmitted by the bite of infected haematophagous arthropod species (European Centre for Disease Prevention and Control, 2016) (discussed in (Wilson *et al.*, 2017)). The most notorious are those known to cause substantial loss of life: mosquitoes transmitting malaria, West Nile virus (WNV) and Zika; ticks transmitting Crimean-Congo haemorrhagic fever (CCHF); midges transmitting bluetongue virus (BTV) and Schmallenberg virus (SBV). It has been estimated that between 1990 and 2000, approximately 30% of emerging infectious diseases were vector-borne (Jones *et al.*, 2008). As arthropods are ectothermic, climatic factors influence survival and reproduction, limiting distribution ranges, abundances and affecting the suitability of the vector to transmit pathogens (Takken and Knols, 2017). Increases in disease range and outbreaks are therefore sensitive to climate changes, with several reviews considering the spread of disease with projected climatic change (Altizer *et al.*, 2013; Githeko *et al.*, 2000; Klasen and Habedank, 2008; Medlock and Leach, 2015; Metcalf *et al.*, 2017; Rogers and Randolph, 2006).

Climate is not the only factor affecting VBD distribution; habitat suitability, land use, pesticide practices, public health policy, host density and accidental transportation are all important factors affecting the spread and establishment of VBDs (Kilpatrick and Randolph, 2012; Klasen and Habedank, 2008). Additionally VBDs, if introduced to a new area through infected host transportation, can establish in novel vector species, resulting in rapid range expansions, as observed for Chikungunya, WNV and the *Culicoides*-borne BTV (Charrel *et al.*, 2007; Wilson and Mellor, 2009).

1.2   *Culicoides*-borne diseases

Culicoides Latreille 1809, biting midges are small biting flies of the family Ceraptopogonidae (order: Diptera) (Kettle, 1977). As 96% of female *Culicoides* are believed to be obligate blood-feeders, the importance of *Culicoides* as vectors of disease are well established (Mellor *et al.*, 2000). They are known to transmit more than 50 viruses of both veterinary and human health importance, of which just under half of these viruses have no other known arthropod vector (Mellor *et al.*, 2000; Wittmann and Baylis, 2000). *Culicoides* have a global distribution (with the exception of Antarctica and New Zealand) and are considered the largest genus of the Ceratopogonidae (21.5% of all species) (Borkent, 2014). The importance of the different *Culicoides* species varies both globally and locally, with variations in geographic ranges, abundance and vector competency.

Species within the *Culicoides* can present a serious biting nuisance to humans (such as *Culicoides impunctatus* Goetghebuer in Scotland), and animals ('sweet itch' in horses), act as a vector for 12 protozoan and 18 filarial nematodes and, most importantly, a vector for viruses (Mellor *et al.*, 2000). Several viruses transmitted by *Culicoides* midges are of public health, animal health and economic importance including African Horse sickness, Akabane virus, bluetongue virus, Oropouche virus and Schmallenberg virus.

1.2.1 Biology of *Culicoides*

The family Ceratopogonidae is within the Nematocerous suborder of the Diptera. This suborder contains many species of disease importance, including: mosquitoes (*Culicidae*), black flies (*Simuliidae*) and sand flies (*Psychodidae*) (Oosterbroek and Courtney, 1995). *Culicoides* are amongst the smallest of these haematophagous flies, typically only 1-3mm in length (Mellor *et al.*, 2000). The lifecycle of *Culicoides* midges consists of eggs, four larval instars, pupa to imago (adult); this is usually completed in 2-6 weeks depending on species and environmental conditions (Mellor *et al.*, 2000).

The adult lifespan is temperature dependent, but typically short. Most survive for less than 10-20 days, however occasionally they may survive far longer (44-90 days) (Mellor *et al.*, 2000). Males do not blood feed, however most females must take a blood meal to provide enough protein for egg development. Some species are autogenous and as such are able to lay the first batch of eggs without a blood meal (as is the case with *C.impunctatus* and *C.circumscriptus* Kieffer) although development of subsequent egg batches still requires a blood meal in these species (Boorman and Goddard, 1970; Carpenter *et al.*, 2006b). Females are able to oviposit 2-4 days after taking a blood meal, with multivoltine species potentially able to complete 3-4 gonotrophic cycles in their lifetimes, although survival for 1-2 cycles is more likely (Mills *et al.*, 2017a; Mullens and Schmidtmann, 1982).

Fecundity varies dramatically between species, with eggs typically oviposited in batches of between 30-450 eggs. They are typically laid white, before turning dark brown to black, they are small (350-500μm), slender (65-80μm) and 'cigar like' (Carpenter *et al.*, 2013; EFSA, 2007b; Mellor *et al.*, 2000). Immature stages usually require a moist environment for development (note 1.2.5 *Culicoides*-borne diseases: Breeding Sites) and as such the larvae are vermiform, swimming with an undulating,

snake-like motion (Mellor *et al.*, 2000). Duration again varies between species, according to nutritional richness of the breeding sites and temperature, from as little as 4 days to several weeks, and much longer in temperate climates where the fourth larval instar enters diapause for the winter months (Downes, 1962; Hill, 1947; Mellor *et al.*, 2000). Pupation is short, typically lasting 2-3 days but can last up to 4 weeks (Mellor *et al.*, 2000).

Adult *Culicoides* are mostly crepuscular, with females taking flight to seek a mate, blood meal or oviposition site (Mellor *et al.*, 2000). Antennae allow *Culicoides* to detect host-derived odours, such as phenol, lactic acid, 1-octen-3-ol and carbon dioxide, and *Culicoides* own derived pheromones which play a role in 'inviting' behaviour for some species and mating (Blackwell *et al.*, 1992b, 1994; Downes, 1968; Logan and Birkett, 2007) (Figure 1.1). *Culicoides* have been found to feed on a wide range of animals namely through biting observation studies and blood meal analysis (Hair and Turner jr, 1968; Lassen *et al.*, 2011, 2012; Martínez-de la Puente *et al.*, 2015; Ninio *et al.*, 2011a; Pettersson *et al.*, 2013; Santiago-Alarcon *et al.*, 2012). Some species are preferential in their host selection and as such are relatively specialised, such as *Culicoides testudinalis* Wirth & Hubert, a specialist freshwater turtle feeder (Grogan *et al.*, 2009). Many species, however, are known to be opportunistic feeders. This opportunistic feeding may facilitate virus transmission, particularly between livestock and wild species (such as deer), or even potentially to humans (Purse *et al.*, 2015). Variations in the type and number of olfactory sensilla on the antennae and palps is thought to reflect host preference and may be responsible for an observable split between mammalian and avian feeders (Braverman *et al.*, 2012; Isberg *et al.*, 2013).

Figure 1.1: Scanning Electron Micrograph (SEM) of the head of a female *Culicoides festivipennis* Kieffer

The flight range is usually only a few hundred metres from the breeding sites, with flight inhibited by wind speeds in excess of 3 meters per second (m/s), although further upwind distances (>3km) have been recorded from mark-release-recapture studies (Kirkeby *et al.*, 2013; Kluiters *et al.*, 2013; Lillie *et al.*, 1981; Sanders *et al.*, 2011b, 2017). However adults in flight can be passively dispersed much further, crossing even large water bodies (Burgin *et al.*, 2013; Eagles *et al.*, 2013).

There are believed to be 1342 extant, and 44 extinct, species of *Culicoides* identified worldwide, with the taxonomy recently reviewed (Borkent, 2014; Harrup *et al.*, 2015). Identification of *Culicoides* from other Ceratopogonidae is possible due to their distinct wing characteristics (Mellor *et al.*, 2000) (Figure 1.2). The further identification of *Culicoides* to species level can be achieved through wing patterns, with multiple keys developed (Bellis *et al.*, 2015; Mathieu *et al.*, 2012; Papp and Darvas, 1997; Rawlings, 1997; Root and Hoffman, 1937). Certain species, such as *Culicoides obsoletus* Meigen, *Culicoides scoticus* Downes & Kettle, *Culicoides dewulfi* Goetghebuer and *Culicoides chiopterus* Meigen, are morphometrically similar (often collectively referred to as the Obsoletus group). Although identification through morphology is possible to an extent by those highly trained, these species are often only identified to group or complex (referring to just *C.obsoletus* and *C.scoticus*) level. Species level identification requires the use of molecular techniques (Harrup *et al.*, 2015; Pagès *et al.*, 2009).
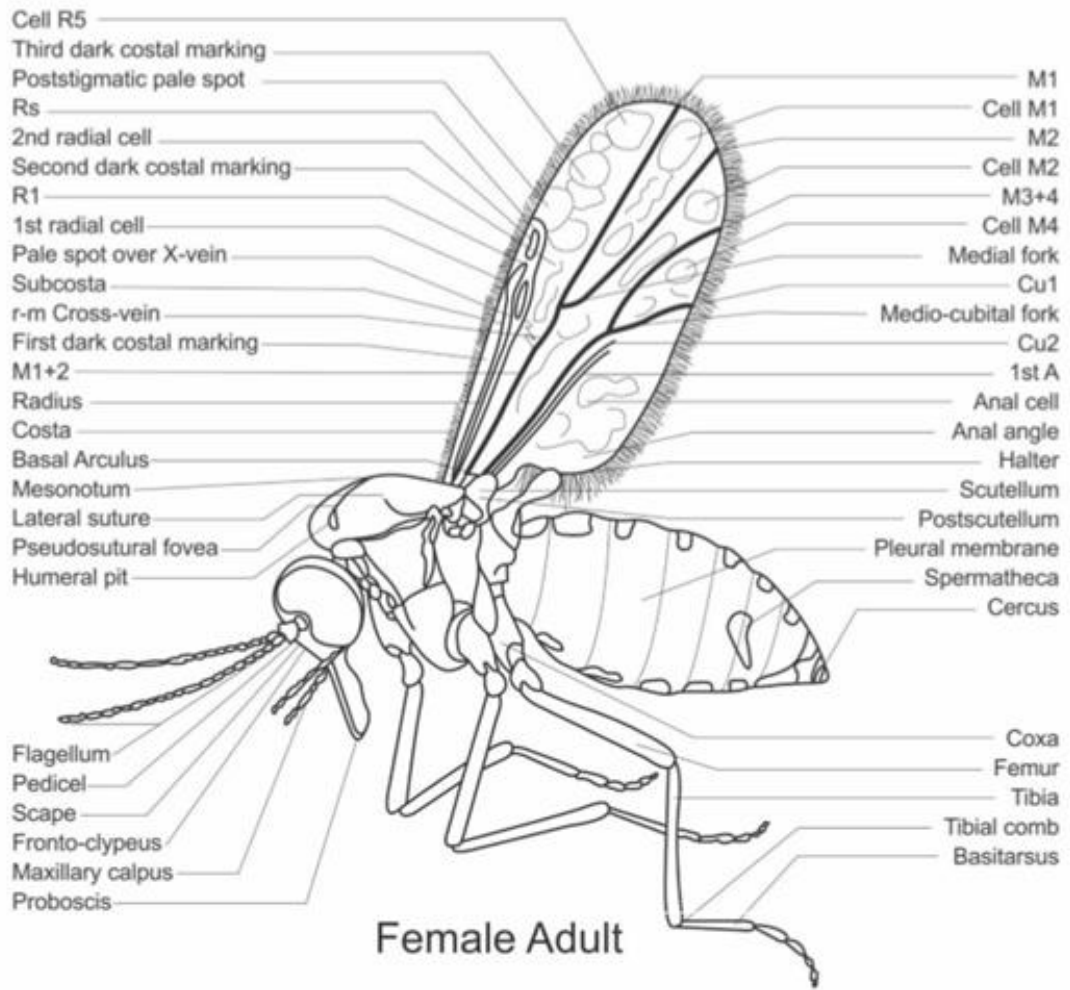
Figure 1.2: *Culicoides* anatomy and wing morphology.

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1.2.2   *Culicoides* as vectors

To be considered a vector for a virus a *Culicoides* needs to not only consume an infected blood meal, but for the virus to infect and replicate within the epithelial cells of the midgut, disseminate into the haemocoel and eventually infect the salivary glands where the virus can then be transmitted again. The time taken between initially ingesting the virus and the virus becoming transmissible is termed the extrinsic incubation period (EIP) (Mills *et al.*, 2017a). As the internal temperatures of *Culicoides* vary with the environment (i.e. they are poikilothermic) the EIP is highly temperature dependent (Carpenter *et al.*, 2015).

The ability for *Culicoides* to act as vectors can be described in terms of vector competency and vector capacity. Vector competency refers to the ability of a vector to be infected by a virus, to support replication and/or development, and to transmit the virus (Carpenter *et al.*, 2015). Typically, vector competency is determined within the laboratory environment to allow for controls and due to the complexities caused by multiple unknown parameters in the field. The results of such studies are then extrapolated to field settings (Mullens *et al.*, 2004). Vector capacity, on the other hand, considers the vectors ability to transmit the virus at a population level. Survival rates, biting rates, species density and EIP of the *Culicoides* are all incorporated into vector capacity, as is vector competence. By incorporating the behavioural and environmental factors, as well as biochemical and cellular factors, the importance of a vector can be considered. For example *Culicoides brevitarsis* Kieffer is an inefficient vector (it has a low vector competency), however, as it is highly abundant with a high biting rate, it has a high vector capacity and is considered to be of major importance to BTV transmission in Australia (Kelso and Milne, 2014). Due to the complexities associated with vector competency, including numerous factors within the vector, virus and host, it is unsurprising that vector competency differs both between species of *Culicoides*, as well as between

individuals of the species (Carpenter *et al.*, 2015). Selection experiments in colony species have allowed the creation of refractory lines, demonstrating an element of genetic heritability to vector competence, although the complete exact underlying factors are still currently unknown (Fu *et al.*, 1999; Mills *et al.*, 2017a; Tabachnick, 1991).

There are multiple infection barriers for the virus to pass prior to transmission (Figure 1.3). The first of these is the mesenteron infection barrier (MIB): the infection of the midgut epithelial cells. Having successfully entered and replicated, infectious virus particles then must escape the mesenteron (MEB: mesenteron escape barrier). The importance of the mesenteron as a barrier to virus transmission was first observed in the 1930s, where investigations of the non-vector species *Circadulina mbila* Naudé (a species of leaf-hopper) was found to be able to transmit maize-streak virus after the mesenteron was punctured (Storey, 1933). Subsequently the same was demonstrated for Eastern equine encephalomyelitis, where *Aedes aegypti* Linnaeus mosquitoes were only able to act as vectors after the mesenteron was punctured in blood engorged females (Merrill and Tenbroeck, 1935). This bypassing of mesenteric infection barriers by mechanical rupturing means that co-infection of *Culicoides* with filarial worms can result in vector competency. This has been observed with laboratory colonies of *Culicoides nubeculosus* Meigen, a species unable to transmit BTV under usual experimental conditions, but vector competent if co-infected with *Onchocerca cervicalis* (Mellor and Boorman, 1980). Equally the MEB can be bypassed by a phenomenon coined the 'leaky gut' phenomenon, observed when rearing larval *Culicoides sonorensis* Wirth & Jones at higher temperatures, resulting in higher infection rates (Mellor *et al.*, 1998). Having successfully escaped the mesenteron, the virus must evade the hosts defences (the dissemination barrier) to reach and enter the salivary glands, where the virus must enter the salivary glands and replicate prior to virus

transmission. The virus may be unable to enter the salivary glands, if salivary gland infection barriers (SGIB) exist, or may be unable to exit the salivary glands into the host if salivary gland escape barriers (SGEB) exist.

Comparisons of bluetongue virus serotype-1 'transmission competent' and 'transmission refractory' colonies of *Culicoides variipennis* Coquillett demonstrated the existence of dissemination barriers, but a lack of evidence for SGIB or SGEB, as intrathoracic injections always resulted in disseminated infection and detectable virus in the saliva (Fu *et al.*, 1999). The assumption that SGIB or SGEB do not exist in other *Culicoides* species, or in response to other viruses, is unproven, although the lack of these barriers has been repeatedly observed in *C.sonorensis* and reported for both *Culicoides imicola* Kieffer and other northern European species (Bowne and Jones, 1966; Carpenter *et al.*, 2015; Jennings and Mellor, 1989; Mills *et al.*, 2017b; Veronesi *et al.*, 2013a, 2013b). Interestingly there is increasing research emerging of the important role of *Culicoides* saliva in the modification of the structure and infectivity of Orbiviruses and modulating host immune response (Darpel *et al.*, 2011; Drolet and Lehiy, 2014; Pages *et al.*, 2014).

Transovarial transmission, the transmission of virus to the ovaries, has not yet been demonstrated for BTV infections of laboratory colonies (Ballinger *et al.*, 1987; Jones and Foster, 1971). Despite this transovarial transmission should not be automatically ruled out. BTV RNA has previously been detected in *C.sonoresis* larvae and pupae, and SBV RNA has been reported in nulliparous wild *Culicoides* (*C.punctatus* Meigen and *C.obsoletus/scoticus* complex) albeit at high Ct values corresponding to sub-transmissible infection (Larska *et al.*, 2013b; White *et al.*, 2005).
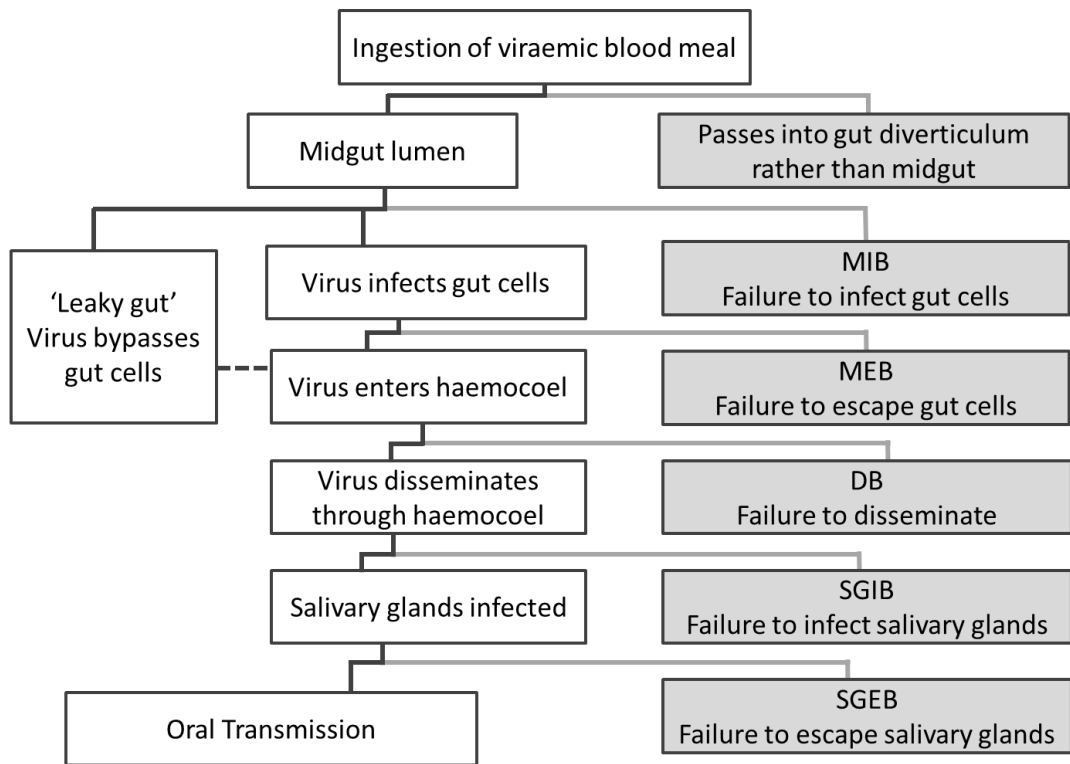
Figure 1.3: Barriers to the infection of arthropods to viruses. Within *Culicoides* any MIB, MEB and DB that may be present can be bypassed through intrathoracic inoculation. This observation subsequently infers these to be the likely factors affecting vector competency of *Culicoides* in the field. Such studies also demonstrate the apparently low importance of SGIB and SGEB to vector competency in *Culicoides*. Adapted from Mellor *et al.,* (2009)

1.2.3 Climatic variables

Temperature affects many stages of vector development and is the key driver of arbovirus transmission. The precise effects of temperature are complicated, and vary between *Culicoides* species, virus species/strain, and a combination of the two (Mellor *et al.*, 2009c). On the one hand, increased temperature promotes a higher frequency of blood feeding (due to increased rate of egg development and quicker lifecycle duration) and an increased rate of virus replication and dissemination within the vector, therefore increasing the likelihood of virus transmission. On the other hand, increased temperatures reduce the adult survivorship, reducing the likelihood of transmission (Purse *et al.*, 2015). At lower temperatures virus replication and dissemination slows considerably and may even stop within the vector, however the lifespan of the adult may be significantly extended (to a point). These interactions complicate the understanding of temperature on VBD transmission and overwintering potential (Mellor *et al.*, 2009c). Specifically considering the colonised, and therefore most researched, BTV vector *C.sonorensis*, the following can be said for the effect of temperature on BTV transmission and vector survival:

- Adult survivorship declines rapidly above 28°C (10% survival at 10 days). At 10-20°C adult survivorship was higher (80-90% alive after 18-24 days) (Wellby *et al.*, 1996). This is in agreement with other work (Hunt *et al.*, 1989; Lysyk and Danyk, 2009).

- Increased adult activity has been observed at warmer temperatures, whereas activity appears to reduce below 10°C (Barnard and Jones, 1980; Linhares and Anderson, 1990; Mullens, 1985; Nelson and Bellamy, 1971).

- A shorter gonotrophic cycle (time required for eggs to develop) has been shown at higher temperatures, therefore increasing the feeding rate: observed mean egg development of 2 days at 30°C, and 10 days at 13°C (Mullens and Holbrook, 1991). This correlates to the estimated gonotrophic

cycle in southern California of 3-4 days in hot summer months and up to 14 days in the cooler winter months (Gerry and Mullens, 2000).

- Eggs have been shown to be the most cold tolerant life stage (survival after 1 hour exposure to -20°C) whereas larvae suffer complete mortality <4°C and pupae <10°C (McDermott *et al.*, 2017).

- Rapid virus development at high temperatures (BTV-11 virogenesis reported in just 1 day in some individuals at 32°C) (Mullens *et al.*, 1995).

- Temperatures below 15°C have been shown to inhibit the development of BTV-11 (at least within the 22 days of the study) (Mullens *et al.*, 1995).

The smaller Palearctic species have been understudied in respect to temperature limits, likely due to the inability to colonise UK vector species. A comparison study of the development times of colonised *C.sonorensis* and *C.nubeculosus* observed both a quicker development of all life stages and higher survivorship of adult *C.nubeculosus* at colder temperatures to *C.sonorensis* (Wittmann, 2000). Taken together, these factors all affect the ability for a vector to transmit a virus. It is necessary, therefore, to determine at which temperature vectors are able to take a blood meal, oviposit and feed again and, at the same time, the temperature for the virus to complete its EIP within the vector. Temperature may also affect the ability for species to become vector competent: immature stages of *C.nubeculosus* reared at 33°C became competent vectors for BTV (13.4% demonstrated oral infection, despite 0% at 25-30°C) (Wittmann, 2000). This phenomenon was also observed for African Horse Sickness virus (AHSV), perhaps suggesting that the gut wall was compromised at higher temperatures: the 'leaky gut phenomenon' (note 1.2.2 *Culicoides*-borne diseases: *Culicoides* as vectors and Figure 1.2) (Mellor *et al.*, 1998; Wittmann, 2000).

Immature life stages are particularly susceptible to desiccation, however due to their small size humidity also affects the survival of adult *Culicoides.* Therefore activity of

Culicoides has also been linked to humidity, demonstrated to increase at higher relative humidity (Blackwell, 1997; Mellor *et al.*, 2000; Walker, 1977). Indeed this may explain the crepuscular activity of many species, exploiting the lower desiccation risk presented by being active at these times, particularly in arid environments (Mellor *et al.*, 2000).

Wind speed also affects *Culicoides* due to their size. *Culicoides* have been shown to be able to fly upwind in speeds up to 3m/s, although decreased flight activity has been demonstrated with higher wind speeds (2.2m/s for *C.imicola* in Kenya) (Blackwell, 1997; Carpenter *et al.*, 2008b; Walker, 1977). Passive dispersal of *Culicoides* is thought to be the route of long distance migrations and disease introductions (Alba *et al.*, 2004; Burgin *et al.*, 2013; Gloster *et al.*, 2007, 2008; Kluiters *et al.*, 2015; Sanders *et al.*, 2011b; Sedda and Rogers, 2013). Radar observations consistently demonstrate mass take-offs of insects at dusk into fast moving wind streams (Reynolds *et al.*, 2008) and *Culicoides* have been recovered at height (>200m) (Chapman *et al.*, 2004; Sanders *et al.*, 2011a). Passive is perhaps not the correct description of this dispersal, as insects undergo active flight to reach the wind streams, within the wind streams, and to return to ground level (Reynolds *et al.*, 2008; Sanders *et al.*, 2011a).

1.2.4 British species of *Culicoides*

Over 40 species of *Culicoides* in Britain have been described previously in the literature (Campbell and Pelham-Clinton, 1960; Edwards, 1926; Gould *et al.*, 2006; Scottish Natural Heritage, 2017) with 51 species identified so far in Britain by the *Culicoides* Reference Laboratory at the Pirbright Institute (Marion England, personal communication, January 29, 2018). Undoubtedly new species will continue to be described over time, with the discovery of species so far undescribed (Guichard *et al.*, 2014).

1.2.4.1   British Culicoides species that are vectors

In total, 38 species of *Culicoides* have been implicated in virus transmission, of which 24 species are considered to act as putative vectors for BTV worldwide (Meiswinkel *et al.*, 1994; Wittmann and Baylis, 2000).

Prior to 1998 *C.imicola* was thought to be the only important *Culicoides* vector species in Europe (Mellor *et al.*, 2009c). The species is Afro-Asiatic, with the European range of the species limited to the Mediterranean (Conte *et al.*, 2009). However the importance of Obsoletus group *Culicoides,* a widespread palaearctic midge species, became clear as BTV outbreaks, particularly BTV-8 in 2006 (discussed in 1.4 Bluetongue virus) spread well outside of the known European range *of C.imicola*. The increasing use of molecular markers allowed greater determination of field-caught species implemented as potential vectors during this period (Carpenter *et al.*, 2015). However the need to pool *Culicoides* for testing prevented the calculation of the infection rate for each species, and in many cases the studies were unable to state exactly which species may have been infected (Carpenter *et al.*, 2015). It was not until the more recent 2011/2012 SBV outbreak (discussed in 1.3 Schmallenberg virus) that screening for competent individuals vastly improved. A study in the Netherlands screened small pools of decapitated heads for evidence of virus transmission, with bodies stored allowing for later repeat testing of the individuals within the positive pools. This allowed the exact number of positive individuals to be identified within the positive pools, with positive individuals' speciated using the 18S internal transcribed spacer 1 (ITS1). This further confirmed *C.obsoletus, C.scoticus* and *C.chiopterus* as putative vectors for SBV and allowed the estimation of field infection rates (Elbers *et al.*, 2013b). Table 1.1 summarises the different species that have been implicated in disease transmission in Britain and Europe.

Table 1.1: *Culicoides* species implicated in disease transmission in Europe

Species	Virus	Implicated by	References
Obsoletus group*	Schmallenberg virus	PCR	(Balenghien *et al.*, 2014; Elbers *et al.*, 2013b; Goffredo *et al.*, 2013; Larska *et al.*, 2013b, 2013c; Rasmussen *et al.*, 2012)
	Bluetongue virus	Virus Isolation	(De Liberato *et al.*, 2005; Savini *et al.*, 2005)
	African Horse Sickness	Virus Isolation	(Mellor *et al.*, 2009a)
Culicoides obsoletus	Schmallenberg virus	PCR	(De Regge *et al.*, 2012; Elbers *et al.*, 2013b)
	Bluetongue Virus	PCR	(Foxi *et al.*, 2016)
Culicoides scoticus	Schmallenberg virus	PCR	(Elbers *et al.*, 2013b)
	Bluetongue Virus	PCR	(Foxi *et al.*, 2016)
Culicoides dewulfi	Schmallenberg virus	PCR	(De Regge *et al.*, 2012)
	Bluetongue virus	PCR	(Meiswinkel *et al.*, 2007)
Culicoides chiopterus	Schmallenberg virus	PCR	(Balenghien *et al.*, 2014; De Regge *et al.*, 2012; Elbers *et al.*, 2013b)
Culicoides pulicaris Linnaeus	Schmallenberg virus	PCR	(Balenghien *et al.*, 2014; De Regge *et al.*, 2012)
	Bluetongue virus	Virus Isolation	(Caracappa *et al.*, 2003)
	African Horse Sickness	Virus Isolation	(Mellor *et al.*, 2009a)
Culicoides punctatus	Schmallenberg virus	PCR	(Larska *et al.*, 2013b, 2013c)
Culicoides imicola	Schmallenberg virus	PCR	(Balenghien *et al.*, 2014)
	African Horse Sickness	Virus Isolation	(Mellor *et al.*, 2009a)
	Bluetongue virus	Virus Isolation, PCR	(De Liberato *et al.*, 2005; Foxi *et al.*, 2016)
Culicoides nubeculosus	Schmallenberg virus	PCR	(Balenghien *et al.*, 2014; Veronesi *et al.*, 2013b)
Culicoides newsteadi Austen	Schmallenberg virus	PCR	(Balenghien *et al.*, 2014)
	Bluetongue virus	PCR	(Foxi *et al.*, 2016)

* where Obsoletus group *Culicoides* were tested, rather than species level

British species are typically crepuscular, with peak adult activity therefore occurring around dusk and/or dawn, although some diurnal activity may occur particularly on warm overcast days (Blackwell, 1997; Sanders *et al.*, 2012). Hourly trapping determined dusk (with a peak in the mean numbers caught at 10pm) and dawn (with a peak between 5am-7am) to be the most active times for adult *C.impunctatus* when collecting using multiple light traps (Blackwell, 1997). This is similar to the peak activity recorded for *C.obsoletus* complex in the Netherlands through hourly sweep net catches (between 10pm-2am and 3am-5am), but differs to peak catches of *C.chiopterus* (peak catches between 6am-9am and 8pm-10pm) and *C.dewulfi* (peak catches between 5am-6am, 7am-8am and 8pm-10pm) (Meiswinkel and Elbers, 2016). This observed difference highlights the known bias of light trap catches: they are not necessarily representative of the adult *Culicoides* population (Carpenter *et al.*, 2008b). In particular *C.chiopterus* and male *Culicoides* are known to be underrepresented by light traps (Carpenter *et al.*, 2008b; Venter *et al.*, 2009). Despite the bias introduced by using light traps, this trap type represents the lowest intensity in labour for the researcher for collecting adult *Culicoides*, as once set the trap can be left in position, only visiting the trap to collect samples. Additionally light traps typically provide the largest catch size and the greatest diversity of species but are not thought to accurately represent *Culicoides* biting rates (Carpenter *et al.*, 2008b; Viennet *et al.*, 2011). Sweep netting, drop traps and direct aspiration of adult *Culicoides* off host animals are significantly more labour intensive and can be poorly tolerated by animals nearby, however these trapping techniques are typically more

representative of the species actively feeding on the animals (Viennet *et al.*, 2011).

Again these techniques underestimate male *Culicoides*, those not currently seeking

a blood meal and those with certain host preferences (for example, preferential

avian feeders if using mammal hosts as attractants) (Nevill *et al.*, 1988; Viennet *et*

al., 2011). Adult *Culicoides* (predominately male *Culicoides* and female *Culicoides*

when not seeking a blood meal) may also be active to seek nectar sources

(Downes, 1958). Hourly catches of *C.impunctatus* analysed for the presence of

carbohydrates suggested peak nectar feeding activity to occur between 3am-7am

and 10pm-12am, corresponding roughly to the reported peak hourly activity

reported in the same study (Blackwell, 1997).

Emergence trapping of *Culicoides* from breeding sites has typically demonstrated a

greater representation of male *Culicoides* compared to other trapping techniques

(Steinke *et al.*, 2014; Thompson *et al.*, 2013). Although likely more representative of

the female:male sex ratio, this trapping technique again underrepresents certain

species, particularly those where the breeding sites are poorly described, and gives

no detail on the age structure of the adult *Culicoides* population (Birley and

Boorman, 1982; Harrup *et al.*, 2013). Emergence trapping has highlighted the

synchronicity of the spring emergence in overwintering *Culicoides* in the UK and

northern Europe (González *et al.*, 2013; Thompson *et al.*, 2013). Light traps also

demonstrate this 'spring flush' with large catches of predominantly nulliparous

Culicoides observed early in the season; typically in April and May in the UK

(Blackwell *et al.*, 1992b; González *et al.*, 2013; Holmes and Boorman, 1987;

Meiswinkel *et al.*, 2014; Sanders *et al.*, 2011b; Searle *et al.*, 2014). The

synchronicity of this mass spring emergence of *Culicoides* from overwintering sites

is likely due to temperature and/or photoperiod cues, however so far the exact

prompts are unknown (Lühken *et al.*, 2015; Searle *et al.*, 2014; Vinogradova, 2007;

White *et al.*, 2017). Lühken *et al.,* (2015) observed no emergence of overwintering

Culicoides bought into the laboratory when held at 10°C for 27 days, in contrast to

relatively quick emergence (mean peak emergence = day 13) when samples were

held between 20-25°C. The study also reported observing no effect of photoperiod

on overwintering *Culicoides* emergence at either temperature extreme (Lühken *et*

al., 2015). However, it should be noted that the study only addressed two extremes

in temperature and photoperiod within the laboratory environment. This has still left

much to be explored and certainly has not completely ruled out photoperiod as one

of the potential drivers of *Culicoides* synchronous spring emergence in the field.

Future studies should look to further address this question of emergence cues,

possibly through manipulating photoperiod in the field, or through emergence

experiments in laboratory incubators using field collected overwintering larval

Culicoides.

The seasonal activity of adult *Culicoides* in the UK is typically observed from spring

emergence (usually in April/May) through to autumn (November). However, the

exact seasonal dynamics vary between species, location and trapping techniques.

For example a previous light trap study run between 1979-81 in the south east of

England observed Obsoletus group *Culicoides* to be active between April and

November, with shorter seasons observed for *C.pulicaris* and *C.punctatus* (between April and October) and *C.impunctatus* (mid-May to mid-September) (Holmes and Boorman, 1987). A larger study of 12 sites across England in 2008 observed a similar season for Obsoletus group *Culicoides* but a longer season for *C.pulicaris* and *C.punctatus* with season length varying between sites with species abundance (Sanders *et al.*, 2011b). Peaks in abundance throughout the season infer generations, with UK species having been observed to peak once (suggesting these species to be univoltine), twice (bivoltine) or three times (trivoltine) (Blackwell *et al.*, 1992a; Hill, 1947; Holmes and Boorman, 1987; Sanders *et al.*, 2011b; Thompson *et al.*, 2013; White *et al.*, 2017). Understanding the seasonality and activity of adult *Culicoides* throughout the year is paramount to understanding the timing and spread of *Culicoides* borne diseases in the absence of viraemic livestock movement (Baylis *et al.*, 1997; Sanders *et al.*, 2011b). After all, this overwintering period represents a time of low, or no, vector activity (termed the 'vector free period' and described further in relation to bluetongue outbreaks in section 1.4.7 Bluetongue: Control measures and vaccination), providing a theoretically safe time for animal movement during disease outbreaks. Currently there is a lack of detailed information on the factors associated with overwintering and emergence in UK vector species. A greater understanding of the seasonal temperature limits of *Culicoides* at all life stages is necessary, particularly outlining the cold tolerance, diapause mechanisms and emergence cues in UK species (Purse *et al.*, 2015).

1.2.5   Breeding sites

A wide range of breeding sites have been described for *Culicoides*. These can be divided into three broad categories: water-saturate soil, fresh dung and moist decaying matter (including manure) (Kettle and Lawson, 1952). Typically immature stages are found within 8cm of the surface (0-5cm) where, depending on species, they are able to either prey on macroscopic invertebrates (i.e. nematodes and immature insects), or consume detritus and microbiota (i.e. organic matter, bacteria, fungi, algae) (Uslu and Dik, 2006).

Although habitats are typically poorly defined, partially due to the complexities in collecting and identifying immature stages, the UK fauna has been described to a larger extent than elsewhere (Harrup *et al.*, 2013; Hribar, 1989; Kettle and Lawson, 1952). Early studies identified the immature stages of *C.chiopterus* and *C.dewulfi* as developing in cattle dung; no other habitats have yet been described for these two species (Kettle and Lawson, 1952). In contrast, *C.obsoletus* appears less specialised, typically found in high carbon:nitrogen ratio soils (reflecting decomposition of the organic matter and mineralization) with presence in substrates favouring increased moisture levels and pH (Harrup *et al.*, 2013; Zimmer *et al.*, 2010). These breeding sites include marshes, swamps and acid grassland, through to rotting vegetable matter, manure, silage residues, leaf litter and damp debris inside tree holes (including banana stumps) to name but a few (González *et al.*, 2013; Kettle and Lawson, 1952; Thompson *et al.*, 2013; Zimmer *et al.*, 2008, 2013a, 2013b, 2014). Historically less was known about the breeding sites of *C.scoticus*, partially due to the inability to reliably differentiate adults from *C.obsoletus* using wing morphology (Harrup *et al.*, 2015). Breeding sites had been reported for several fungal species, mud ruts, silage residues and marshy areas (Buxton, 1960; Campbell and Pelham-Clinton, 1960; Zimmer *et al.*, 2010). With the increasing use of PCR speciation woodland leaf litter, areas surrounding open water and

organically enriched substrates have also been identified as immature development sites for *C.scoticus*, overlapping many of the known *C.obsoletus* habitats (Harrup *et al.*, 2013).

The breeding sites of *C.pulicaris* appear to include marshes, swamps, water body embankments, waterlogged areas/runoff zones, open pasture, manure and in manure enriched soils (Campbell and Pelham-Clinton, 1960; González *et al.*, 2013; Harrup *et al.*, 2013; Kirkeby *et al.*, 2009). The habitats of *C.punctatus* overlap to an extent; including marshland and muddy swamps, organically enriched bare mud, manure enriched soils, and waterlogged areas and meadows (González *et al.*, 2013; Harrup *et al.*, 2013; Kettle and Lawson, 1952; Kirkeby *et al.*, 2009).

In northern Europe species are generally considered to overwinter as fourth instar larvae within breeding sites (Mellor *et al.*, 2000; Vinogradova, 2007). As all vector species have been recorded to develop in dung and manure, with several species' breeding sites encompassing silage and manure enriched soils, it is perhaps unsurprising that emergence of vector species has been recorded inside animal housing (Ninio *et al.*, 2011b). Currently it is uncertain as to whether this overwintering of the larvae represents true diapause (controlled endogenously by biological mechanisms combined with environmental signals) or rather a transient state of quiescence (controlled exogenously by environmental factors) (Lühken *et al.*, 2015; Vinogradova, 2007; White *et al.*, 2017).

1.3   Schmallenberg virus

In summer and autumn 2011, cattle in Germany and the Netherlands presenting with pyrexia, diarrhoea and a reduced milk yield, were tested for all endemic and emerging viruses. No known pathogen was isolated from the samples. Metagenomic analysis identified a new *Orthobunyavirus*, of the Simbu serogroup, subsequently named Schmallenberg virus after the geographic origin of the samples tested (Hoffmann *et al.*, 2012).

1.3.1   Genetic analysis and evolution of SBV

Orthobunyaviruses are one of five genera within the family *Bunyaviridae* (Figure 1.4). The Bunyaviridae are segmented, negative- sense single-stranded RNA viruses, which encompass viruses of plant, veterinary and public health importance (Saeed *et al.*, 2001; Walter *et al.*, 2011). With the exception of Hantaviruses, Bunyaviruses are transmitted by arthropod vectors, in particular *Culicoides* biting midges, mosquitoes, *Phlebotomus* sandflies, ticks and, in the case of *Tospovirus*, thrips (Elliott, 1997; Walter *et al.*, 2011).
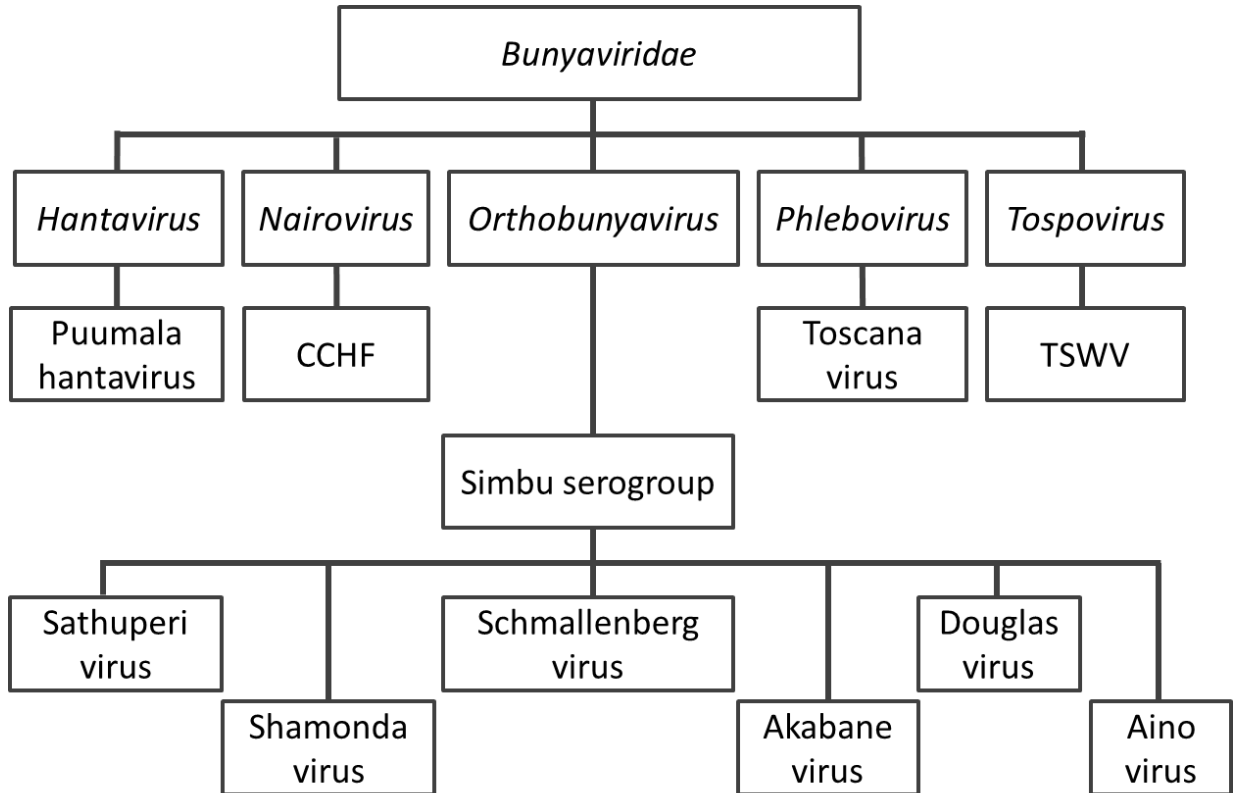
Figure 1.4: The *Bunyaviridae* taxonomy with selected examples of plant, veterinary and public health importance. CCHF: Crimean-Congo Haemorrhagic Fever, TSWV: Tomato spotted wilt virus.

1.3.1.1   Structure

The genome of viruses within the Bunyaviridae consist of three segments, named to reflect their relative nucleotide length: large (L), medium (M) and small (S) (Walter *et al.*, 2011). Within each genus the overall segment length is similar and the encoding of protein products is commonly expressed (Walter *et al.*, 2011). The three segments of Orthobunyaviruses encode four structural proteins and an additional two non-structural proteins, which are only encoded in *Orthobunyavirus, Tospovirus* and *Phlebovirus*:

- The L segment encodes the RNA-dependent RNA polymerase L protein which results in RNA replication and mRNA transcription products. The L protein also has an endonuclease activity which cleaves cellular messenger RNAs to produce capped primers for initiating viral messenger RNAs transcription ('cap snatching').

- The M segment encodes a polyprotein precursor that is cleaved into two glycoproteins embedded in a lipid bilayer (Gn and Gc) which are responsible for viral attachment, hemagglutination, cell fusion and the induction of neutralizing antibodies. The M segment additionally encodes the non-structural protein, NSm, of unknown function.

- The S segment encodes the nucleocapsid protein and additional non-structural protein (NSs) in an overlapping open reading frame. The primary role of the N protein is to encapsidate the products of replication to form the ribonucleoprotein complex, whilst the role of NSs is to modulate the host-cell antiviral response, interfering with innate immunity. It has been demonstrated that although the NSs is not essential for SBV replication, a virus lacking the NSs is strongly attenuated in experimental mice models. (Briese *et al.*, 2013; Doceul *et al.*, 2013; Goller *et al.*, 2012; Varela *et al.*, 2013; Walter *et al.*, 2011).

1.3.1.2 Phylogeny

There are more than 170 viruses of 48 defined species within the Orthobunyavirus genus, divided into 18 serogroups (*9th Report International Committee on Taxonomy of Viruses*, 2011; Doceul *et al.*, 2013). SBV resides within the Simbu serogroup, alongside other viruses known to cause congenital malformations, stillbirths and abortions in ruminants (note Table 1.2) (Goller *et al.*, 2012; Varela *et al.*, 2013). Initial analysis indicated that SBV had a 69% identity with the L segment of Akabane virus, 71% with Aino virus M segment and a 97% identity with the S segment of Shamonda virus (Hoffmann *et al.*, 2012). Later studies have demonstrated different potential relatives depending on the segment sequence used for comparison. One study suggested a high identity of SBV M segment with Sathuperi and Douglas viruses, whereas another showed SBV S and L segments displayed a greater identity to that of Shamonda virus, with phylogenetic analysis placing SBV in the Sathuperi virus species (Goller *et al.*, 2012; Yanase *et al.*, 2012). It has been postulated that SBV is a possible ancestor of Shamonda virus, which inversely contains the S and L segments of SBV, with the M segment of an unknown virus, suggesting the circulation of SBV far prior to the 2011 European outbreak (Goller *et al.*, 2012).

Full genome analysis, comparing the genome of blood samples collected from adult cattle in 2011 to samples collected in 2014, was recently undertaken in Germany. This study found high stability in the S and L segments, with very few nucleotide substitutions observed (Wernike *et al.*, 2015). Furthermore, the most variable genome segment, the M segment, was found to also have a high stability in the samples tested, despite the identification of a highly variable region within the Gc coding sequence in previous studies of malformed newborns (Coupeau *et al.*, 2013; Fischer *et al.*, 2013; Wernike *et al.*, 2015) .

It is important to note, that until 2011, no Simbu serogroup virus had been detected within Europe (Yanase *et al.*, 2012). Despite studies further defining the relationship of SBV in relation to other viruses within the Simbu serogroup, this analysis does not currently help identify the origin of the disease.

Table 1.2: Selected *Bunyaviridae* viruses of human animal and plant importance.

Genus	Virus	Range	Vector(s)	Host(s)
Hantavirus	Puumala	Europe	**	Humans
Nairovirus	CCHF	Europe, Africa, Asia	Ticks (*Hyalomma*)	Humans
	Nairobi sheep	Africa, Asia	Mosquito (*Culex*), Ticks (*Dermacentor, Haemaphysalis, Ixodes* and *Rhipicephalus*)	Sheep, goats
Orthobunyavirus	Aino*	Asia, Australia	*Culicoides*, Mosquitoes (*Culex*)	Cattle, sheep, goats
	Akabane*	Africa, Asia, Australia	*Culicoides*, Mosquitoes (*Aedes, Culex*)	Cattle, sheep, goats
	Cache Valley	N.America	Mosquitoes (*Aedes, Culex, Culiseta*)	Sheep
	Douglas*	Asia, Australia	*Culicoides*	Cattle
	La Crosse	N.America	Mosquitoes (*Aedes*)	Humans
	Oropouche	S.America	*Culicoides*	Humans
	Peaton	Asia, Australia	*Culicoides*	Cattle, sheep
	Sathuperi*	Asia	*Culicoides*, Mosquitoes (*Culex*)	Cattle
	Schmallenberg*	**Europe**	***Culicoides***	**Cattle, sheep, goats**
	Shamonda*	Africa, Asia	*Culicoides*	Cattle
	Tahyna	Europe	Mosquitoes (*Aedes, Culiseta*)	Humans
Phlebovirus	Rift Valley	Africa	Mosquitoes (*Aedes, Anopheles, Culex* and *Mansonia*), *Culicoides*	Humans, cattle, sheep, goats
	Toscana	Europe	Sandflies (*Lutzomya Phlebotomus* and *Sergentomyia*)	Humans
Tospovirus	TSWV	Europe	Thrips (*Thripidae*)	Plants

*Simbu serogroup. **Rodent reservoir host. CCHF: Crimean-Congo Haemorrhagic Fever, TSWV: Tomato spotted wilt virus. Adapted and expanded from Horne and Vanlandingham 2014, Yanase *et al.,* 2003.

1.3.2 Origin

The exact origin of SBV remains unknown. However, the emergence of SBV, and previously BTV-8 (covered later in 1.4 Bluetongue virus) from the same region of northwest Europe, suggests a similar route of introduction. Several authors have suggested accidental importation as the most likely route, as the region is synonymous with international trade (Carpenter *et al.*, 2013; Saegerman *et al.*, 2010). This includes the accidental transport of *Culicoides* with animal, human or plant transport. Studies investigating *Culicoides* importation are currently limited, most likely due to the small size and fragile nature of adult *Culicoides*. A 2005 study in China demonstrated the potential for accidental *Culicoides* importation through international sea trade, finding *Culicoides* were active in 9/70 ships in Qinhuangdao port, China (Nie *et al.*, 2005).

Cut flowers have been postulated as a possible transfer mechanism, with harvesting, preparation and transport to global destinations all within 48-72 hours (Mintiens *et al.*, 2008; Oura and El Harrak, 2011). These flowers, particularly roses, are rarely treated with pesticides and are typically shipped in cooled environments (0-1°C) to ensure freshness (Hulst, 2004). Preliminary investigations by the author and Kluiters (unpublished work) determined that *Culicoides* species were active throughout the flower development process prior to shipping. This seems a likely possible route, with Kenyan flowers alone making up approximately 50% of all flowers sold at the Dutch auctions. This potential route needs further investigation as the Kenyan flower industry predicts a continued 5% annual growth in exported flowers and has increased direct exportation to UK supermarkets, receiving 25% of the 133,658 tons of flowers exported in 2016 (Kenya Flower Council, 2017).

1.3.3 Susceptible species

Susceptible species are those that can support replication of an infectious agent, where natural cases of infection have been observed, or experimental infection has been demonstrated via a natural pathway (EFSA, 2014). For SBV, the EFSA summarises the identification of susceptible species into the following categories (EFSA, 2014):

- Species where SBV and clinical signs have been observed (natural or experimentally)

- Species where SBV RNA has been directly detected

- Species where antibodies against SBV have been detected (indirect detection)

As such a wide range of species have been described as susceptible to SBV infection. These species include domestic ruminants, wild and exotic ruminants and some non-ruminant species including members of the *Camelidae, Equidae, Hippopotimidae, Rhinocerotidae, Suidae, Tapiridae* and *Carnivora* (note Appendix I: Species susceptible to SBV infection). Clinical signs have been demonstrated both naturally and experimentally in domestic ruminants (Ganter *et al.*, 2013; Helmer *et al.*, 2013b; Laloy *et al.*, 2015; Martinelle *et al.*, 2015; Wernike *et al.*, 2013a, 2013b, 2014a). The pathology of such species, namely cattle, sheep and goats, is covered in the following section (1.3.4 Schmallenberg virus: Pathogenesis).

Despite a lack of clinical signs, domestic camelids have been found to be seropositive for antibodies against Schmallenberg virus, with high within-farm seroprevalences reported (Jack *et al.*, 2012; Schulz *et al.*, 2015). Experimental infection of 3 llamas (*Lama glama* (Linnaeus, 1758)) and 3 alpacas (*Vicugna pacos* (Linnaeus, 1758)) reported short viraemias (1-4 days) and no clinical signs (Schulz *et al.*, 2015).

Experimental infections of both pigs and poultry found no evidence for virus replication (EC, 2014; Poskin *et al.*, 2014b). However serological surveillance of wild boars in Europe have since detected antibodies against SBV, suggesting that wild boars are susceptible to infection (Kęsik-Maliszewska *et al.*, 2017; Mouchantat *et al.*, 2015).

The susceptibility of wild cervids to Schmallenberg virus has been confirmed by several serological surveillance studies in the UK, France, Germany, Sweden, Poland and the Netherlands, and are summarised in Appendix I (Barlow *et al.*, 2013; Chiari *et al.*, 2014; EC, 2014; Laloy *et al.*, 2014; Larska *et al.*, 2014; Malmsten *et al.*, 2017). A young elk (*Alces alces* (Linnaeus, 1758)) presenting with pneumonia and dermal oedema was found to be positive for SBV RNA by real-time RT-PCR. However, as this was a singular and unusual presentation of disease, the authors were unable to determine if the clinical signs observed were associated with the SBV infection or if other pathogens were responsible (Larska *et al.*, 2013a). To the author's knowledge, no evidence of abortions or malformations have been reported in wild cervids; however, studies on the impact of SBV on wild and domesticated species of cervids are lacking (Laloy *et al.*, 2014; Malmsten *et al.*, 2017).

The susceptibility of exotic species, particularly rare species present in zoos, was of concern. Many animals held across Europe represent valuable conservation collections where reductions in reproductive success, or death of an adult, would represent serious losses to the survival prospects of the species. Serological surveillance in the UK, France and the Netherlands was completed, highlighting several species' susceptibility to SBV (summarised in Appendix I) (EC, 2014; Laloy *et al.*, 2016; Molenaar *et al.*, 2015). This testing confirmed susceptibility in endangered ungulate species, most notably in Asian elephants (*Elephas maximus* (Linnaeus, 1758)). Again, no evidence has been reported of abortions or foetal malformations in zoo collection species. However, as many collections throughout

Europe are housed outside, these species are at risk from ongoing future VBD transmission (Molenaar *et al.*, 2015).

Unusually, domesticated dogs (*Canis familiaris* (Linnaeus, 1758)) have been found to be susceptible to SBV infection. A Swedish study tested 86 female dogs for antibodies against SBV, of which one dog (2 samples) returned positive for both competitive ELISA and SNT. No clinical signs had been reported in this dog (Wensman *et al.*, 2013). However, one of five puppies presented with neurological symptoms (ataxia, exotropia as well as severe torticollis on necropsy) was tested for SBV, with positive real-time RT-PCR results in the necropsied cerebellum and positive SBV antibody response described in the mother (ELISA and VNT). Follow up of the kennel found 1 other adult dog positive for antibodies by both ELISA and VNT, but puppies from the second dog had not presented with any clinical signs and were not tested (Sailleau *et al.*, 2013). Despite a third study finding no serological evidence for SBV in dogs (in an area that had reported a high SBV seroprevalence in ruminants (Garigliany *et al.*, 2013)), it would appear that dogs are susceptible to SBV.

Infection in horses appears unlikely, with no serological evidence collected from 92 horses in an area with high ruminant seroprevalence in the UK (EC, 2014). Equally, there is no evidence currently for natural SBV susceptibility in wild carnivores or small mammals (rodents and shrews) (Mouchantat *et al.*, 2015). Studies addressing the zoonotic potential of SBV found no evidence to suggest human susceptibility to SBV infection (Ducomble *et al.*, 2012; Reusken *et al.*, 2012). Type I interferon receptor knock-out mice can be experimentally infected with SBV, allowing their use as a small animal model for *in vivo* studies (Wernike *et al.*, 2012a).

1.3.4 Pathogenesis

1.3.4.1 Clinical signs in adult domestic ruminants

Clinical signs in adult ruminants are typically mild. Adult sheep and goats can be asymptomatic. However diarrhoea and nasal discharge have been reported in clinically infected sheep and a reduced milk yield has been anecdotally reported for milking sheep in the Netherlands (Lievaart-Peterson *et al.*, 2015; Wernike *et al.*, 2013b). No fever peaks have been reported for any experimentally infected sheep or goats (Laloy *et al.*, 2015; Wernike *et al.*, 2013b).

Adult cattle may present with acute fever (>40°C), diarrhoea, loss of appetite, pyrexia and reduced milk yield (up to a 50% reduction in production) (Doceul *et al.*, 2013; EFSA, 2012; Veldhuis *et al.*, 2014b; Wuthrich *et al.*, 2016). Clinical signs are typically short in duration, lasting a few days to a week (EFSA, 2014; Wernike *et al.*, 2013b, 2014a).

1.3.4.2 Viraemic period

The viraemic period is believed to be typically short-lived. Experimental studies have demonstrated a viraemic period lasting between 3 to 5 days in sheep and 3 to 4 days in goats (Laloy *et al.*, 2015; Wernike *et al.*, 2013b). No difference in the duration and level of viraemia was observed under different SBV dilution dosages in experimental infections of sheep. However, dosage did affect the number of animals that became infected (Poskin *et al.*, 2014a). Unlike the viraemic period of infectious serum, viraemia in lymph nodes, particularly the mesenteric lymph nodes and spleen, appears persistent, with SBV RNA detected in lymph nodes 44 days post-inoculation (Poskin *et al.*, 2014a; Wernike *et al.*, 2013b). No SBV genomic RNA was discovered in the lymph nodes of experimentally infected goats and bucks.

However, the mesenteric lymph nodes had not been collected as part of the study (Laloy *et al.*, 2015). SBV RNA has also been isolated from the ovary of a single sheep and goat, although the implications of these findings are currently unknown (Laloy *et al.*, 2015; Wernike *et al.*, 2013b).

The viraemic period for cattle appears to last between 2 to 6 days in experimental studies (Hoffmann *et al.*, 2012; Wernike *et al.*, 2013a). Like sheep, persistent viraemia of the lymph nodes has been reported for cattle (at least 5 weeks post-inoculation persistence) (Wernike *et al.*, 2012b, 2013a).

Natural infection studies have observed longer viraemia in some animals, with bimonthly sampling demonstrating that 20% of lambs remained SBV RNA positive across the 2 week sampling period (Claine *et al.*, 2013). Nevertheless, to date this appears to be the only study repeatedly testing animals for SBV RNA over time in naturally infected flocks/herds.

Antibodies against SBV typically develop 1-2 weeks after initial infection in sheep, 9 days to 2 weeks in goats and 10 days to 3 weeks in cattle (Laloy *et al.*, 2015; Poskin *et al.*, 2014a; Wernike *et al.*, 2013a, 2013b). Early longitudinal studies on anti-SBV antibody persistence presented evidence of long term protection (18-24 months for adult cattle) (Elbers *et al.*, 2014; Wernike *et al.*, 2013a). However, it has since become apparent that duration of immunity is complex, with loss of anti-SBV antibodies reported for 10% of cattle in a German dairy herd within 3 years (Wernike *et al.*, 2015c).

1.3.4.3 Clinical signs in offspring

Although infection of adult ruminants rarely results in clinical signs, infection of a naïve pregnant ruminant with SBV can result in abortions, still births and foetal malformations. Typical foetal or neonate physical malformations present as joint deformity (arthogryposis), joint immobility/ bone fusion (ankylosis), twisting of the neck (severe torticollis), curved spine (kyphosis, lordosis scoliosis), a shortened jaw (brachygnathia inferior) and/or underdevelopment of the central nervous system (hypoplasia). Central nervous system hypoplasia may be mild to severe, with microencephaly, hydranencephaly and spinal cord and cerebellar hypoplasia all described in the literature (Doceul *et al.*, 2013; Garigliany *et al.*, 2012; Hahn *et al.*, 2013; Herder *et al.*, 2012; van den Brom *et al.*, 2012). Observed neurological disorders include blindness (amaurosis), incoordination (ataxia) and behavioural abnormalities. Commonly these disorders result in intra-uterine death or death immediately after birth. However, not all offspring in multiple births may be affected (Doceul *et al.*, 2013). For example, in the case of twins, one may present with physical malformations, whereas the other may remain viable; equally one may present with physical malformations and the other may present neurologically (Doceul *et al.*, 2013).

The observed teratogenic effects are similar to those observed for the related Akabane and Aino viruses (Hashiguchi *et al.*, 1979; Kirkland *et al.*, 1988; Konno *et al.*, 1982; Kurogi *et al.*, 1975; Tsuda *et al.*, 2004). Akabane is particularly well studied, and infection of the foetus is known to occur between days 28-36 in sheep, 30-50 in goats and 76-174 in cattle (Kirkland *et al.*, 1988). The severity of the foetal malformations depends at which point during gestation the infection occurs; with the greatest clinical signs observed if infection coincides with the differentiation of neuronal tissues (Konno *et al.*, 1982; Parsonson *et al.*, 1977).

An experimental infection study demonstrated that SBV infection of ewes at days 45 or 60 of gestation resulted in high placental colonisation, but no clinical signs were subsequently observed in the lambs (Martinelle *et al.*, 2015). Experimental infection of pregnant ewes 107 days after mating also did not result in any clinical signs or the detection of SBV RNA, in the lambs (Rodríguez-Prieto *et al.*, 2016). Observations of natural infections of SBV in cattle found no malformation in calves born to animals that were infected between days 75-175 of gestation (Wernike *et al.*, 2014b). Nevertheless, it has been proposed that malformed and dead calves born after SBV infection at between 60-144 days were due to SBV infection despite the inability to extract SBV RNA (Wernike *et al.*, 2014a). It is therefore believed that only a small proportion of susceptible animals infected during the vulnerable period of gestation will go on to present with clinical signs in the lambs/calves. At a population level the rate of stillbirths and malformations caused by foetal SBV infection has been reported to be as low as 0.5% in Dutch dairy herds (Veldhuis *et al.*, 2014a).

1.3.5 Transmission routes

1.3.5.1 Vector transmission

The known *Culicoides* vectors of SBV have been outlined in Table 1.1. Colony line vector competency study of *C.nubeculosus* estimated about a 3% competency, similar to the rate reported in the same line for BTV (Veronesi *et al.*, 2013a, 2013b). This was consistent with field collections of *C.nubeculosus*, estimating a minimum infection rate of 4% (Balenghien *et al.*, 2014). It should be noted that the minimum infection rate is calculated as the ratio of the number of positive pools to the total number of *Culicoides* in the sample: assuming only one infected individual is present in a positive pool. The minimum infection rate is thought to be valid when the infection is relatively rare within the *Culicoides* population, but is a poor estimate

when pool sizes are particularly large and/or infection rates are high as it will underestimate the true *Culicoides* infection (Bustamante and Lord, 2010; Gu *et al.*, 2003).

SBV minimum infection rates for other field caught species are thought to range from 0.1-4.0% for the Obsoletus complex, 0.14-1.0*%* for *C.chiopterus*, 1.1% for *C.dewulfi*, 0.29% for *C.punctatus* and 0.37-1.12% for *C.pulicaris* (Balenghien *et al.*, 2014; De Regge *et al.*, 2012; Elbers *et al.*, 2013b; Goffredo *et al.*, 2013; Larska *et al.*, 2013b; Rasmussen *et al.*, 2012). Although it is important to consider the likelihood of differing vector competency amongst different geographic vector populations, studies of BTV-9 competency in UK Obsoletus complex found competency varied regionally (0.4-7.4%) (Carpenter *et al.*, 2006a). The SBV minimum infection rate for *C.imicola* is particularly low, around 0.04%, highlighting the importance of other species in SBV transmission (Balenghien *et al.*, 2014).

Mosquitoes have not been demonstrated to transmit SBV (Balenghien *et al.*, 2014; Scholte *et al.*, 2013). Experimental infection studies of *Culex pipiens* Linnaeus 1758 and *Aedes albopictus* (Skuse 1895) have demonstrated SBV transmission with intrathoracic inoculation, but not oral inoculation, suggesting a MIB and/or MEB in these species (Balenghien *et al.*, 2014; Manley *et al.*, 2015).

1.3.5.2 Semen

SBV RNA has been isolated from bovine semen (Hoffmann *et al.*, 2013; Kęsik-Maliszewska and Larska, 2016; Ponsart *et al.*, 2014; Schulz *et al.*, 2014; Van Der Poel *et al.*, 2014). The detection of SBV RNA in the semen appears to be independent from SBV viraemia, with a high proportion of positive bulls found to excrete SBV RNA in the semen over a prolonged period in one study (>2 months in 50% of SBV RNA semen positive bulls) (Hoffmann *et al.*, 2013). A wide variability has been reported in naturally infected bulls, with some evidence of variability

between breeds (Ponsart *et al.*, 2014). Although SBV RNA positive semen has been demonstrated to be infectious when injected subcutaneously, the implications of insemination as a transmission route remain unknown (Ponsart *et al.*, 2014; Schulz *et al.*, 2014). Any transmission at insemination would occur outside of the vulnerable period of gestation; therefore it is unlikely direct offspring would be infected. The greatest risk presented by positive SBV RNA semen samples is the potential risk of initiating future SBV outbreaks if accidentally stored and used outside of periods of SBV circulation.

So far no studies have demonstrated SBV RNA in semen of bucks or rams (Laloy *et al.*, 2015).

1.3.5.3 Vertical transmission in ruminants

SBV RNA has been detected in the central nervous system of clinically presenting calves born alive, several days after birth (Garigliany *et al.*, 2012; Peperkamp *et al.*, 2012). However no SBV RNA has been detected in the blood or skin of these animals, a necessary prerequisite to vector transmission. Additionally SBV RNA has not been isolated from healthy lambs, kids or calves, suggesting that live SBV within the central nervous system of these clinically affected animals is likely to be an epidemiological dead-end (EFSA, 2014).

External placenta and umbilical cords have been found to be positive for SBV RNA (Balseiro *et al.*, 2015; Bilk *et al.*, 2012; Poskin *et al.*, 2017). Although this has been deemed an unlikely route of pseudo-vertical transmission as experimental studies have demonstrated no transmission of SBV by the oral route (Wernike *et al.*, 2013a). Currently there is no evidence of SBV RNA in milk, however again as transmission via the oral route appears unlikely, this would be an unlikely route for transmission (EFSA, 2014).

1.3.6 Distribution

After its initial description SBV was rapidly reported across Europe in 2011 and 2012 (Figure 1.5). In the UK, the first reports of SBV cases occurred in the south east of England in January 2012, spreading in a north westerly direction, with 358 foetal cases confirmed in 2012 and 43 foetal cases confirmed the following year (Figure 1.5) (Table 1.3) (AHVLA, 2013; Harris *et al.*, 2014). By 2013 a total of 8,730 herds and flocks from 29 countries had reported SBV in Europe, covering a climatic range from the Mediterranean basin to north of 65° latitude (Balseiro *et al.*, 2015; Chenais *et al.*, 2013; EFSA, 2013; Monaco *et al.*, 2013; Wisløff *et al.*, 2014). The seroprevalence and surveillance of SBV has been further discussed within Chapter 2.
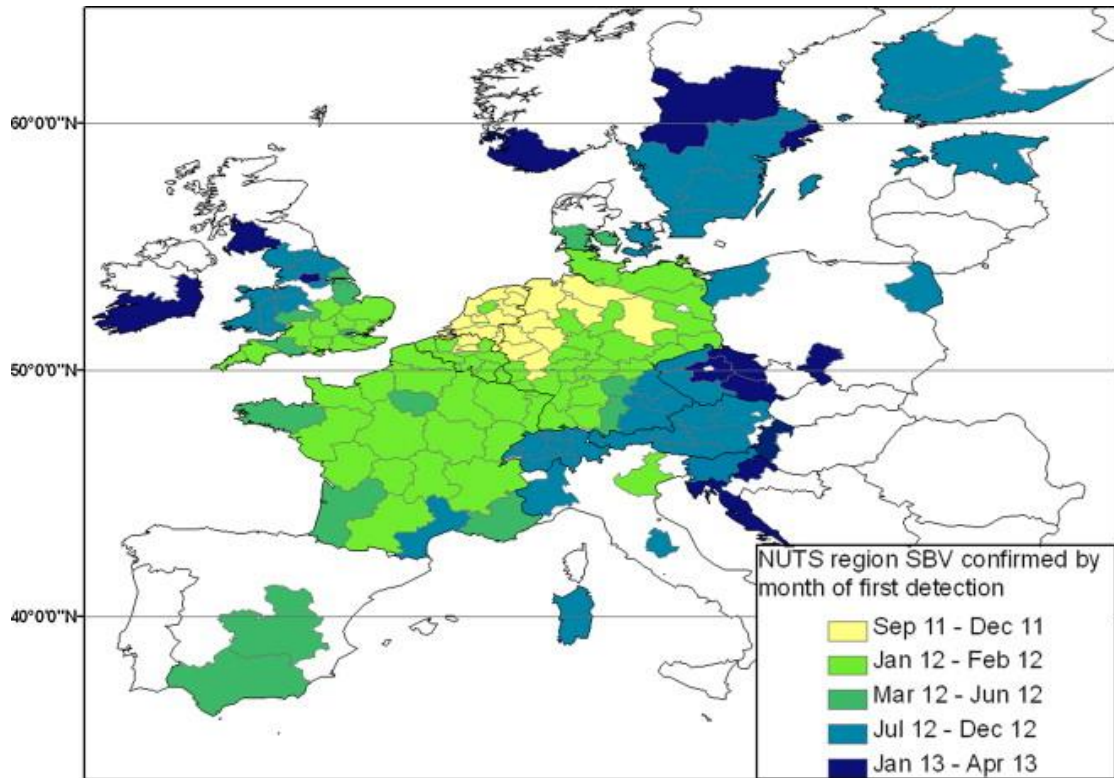
Figure 1.5: Reported NUTS2 regions with at least one SBV herd/flock confirmed by direct detection, by period of first report. From Afonso *et al.,* 2014.

Table 1.3: The number of foetal cases confirmed by the APHA (previously Animal Health and Veterinary Laboratories Agency AHVLA) in sheep and cattle in England, Wales and Scotland from (AHVLA, 2013).

Year	Confirmed cases in GB
2012	358
2013	43
2014	0*
2015	Chapter 2
2016	Chapter 5

* APHA personal communication.

Further investigations have since provided possible evidence for SBV circulation, or a related Simbu serogroup virus, in Mozambique, Tanzania and Jordan, suggesting a far wider distribution than just Europe (Abutarbush *et al.*, 2017; Blomström *et al.*, 2014; Levin, 2015). However, where ELISA tests have been used it is important to note the potential for cross reactivity with other Simbu serogroup viruses, preventing complete confidence in SBV being the agent detected.

1.3.7 Vaccination

Vaccinations came to the market in 2013, with products supplied by MSD Animal Health, Merial and Zoetis. These vaccinations were based on inactivated virus. Studies have since demonstrated that this type of vaccination appears to be effective in preventing SBV viral replication if challenged 3 weeks after a single dose (Hechinger *et al.*, 2014; Wernike *et al.*, 2013d). The exact duration of immunity these vaccines confer is unknown, with manufacturers stating immunity for 12 months in cattle and 6 months in sheep (Zulvac) (European Medicines Agency, 2017b). As with all vaccines, production heavily relies on demand for the product.

1.4 Bluetongue virus

Bluetongue virus (BTV) is an Orbivirus encompassing 27 confirmed serotypes worldwide (Jenckel *et al.*, 2015; Schulz *et al.*, 2016). Most of these serotypes are vector-borne. An estimation by Bath at the 2nd International Congress for Sheep Veterinarians in 1989 put the worldwide cost of BTV at around $3 billion annually (as cited in More *et al.*, 2017).

1.4.1 Genetic analysis and evolution of BTV

Orbiviruses are one of 15 genera within the Reoviridae (Figure 1.6). The Reoviridae are double-stranded RNA (dsRNA) viruses and are considered one of the largest families of viruses (King *et al.*, 2012; Roy, 2017). These viruses have icosahedral symmetry where the linear dsRNA segments are surrounded by concentric layers of capsid proteins (King *et al.*, 2012). Unsurprisingly, as the family is so large, viruses of the Reoviridae are transmitted between hosts in a variety of ways; some replicate in both arthropod vectors and animal or plant hosts (orbiviruses, coltiviruses and fijiviruses, to name but a few), others infect insects through the fecal-oral route (cypoviruses), whilst others are transmitted between hosts through the respiratory or fecal-oral routes (orthoreoviruses and rotaviruses) (King *et al.*, 2012). Even within the orbivirus genus the arthropod vector and host species varies, with midges, ticks and mosquitoes all implicated in transmission depending on the virus (Roy, 2017).
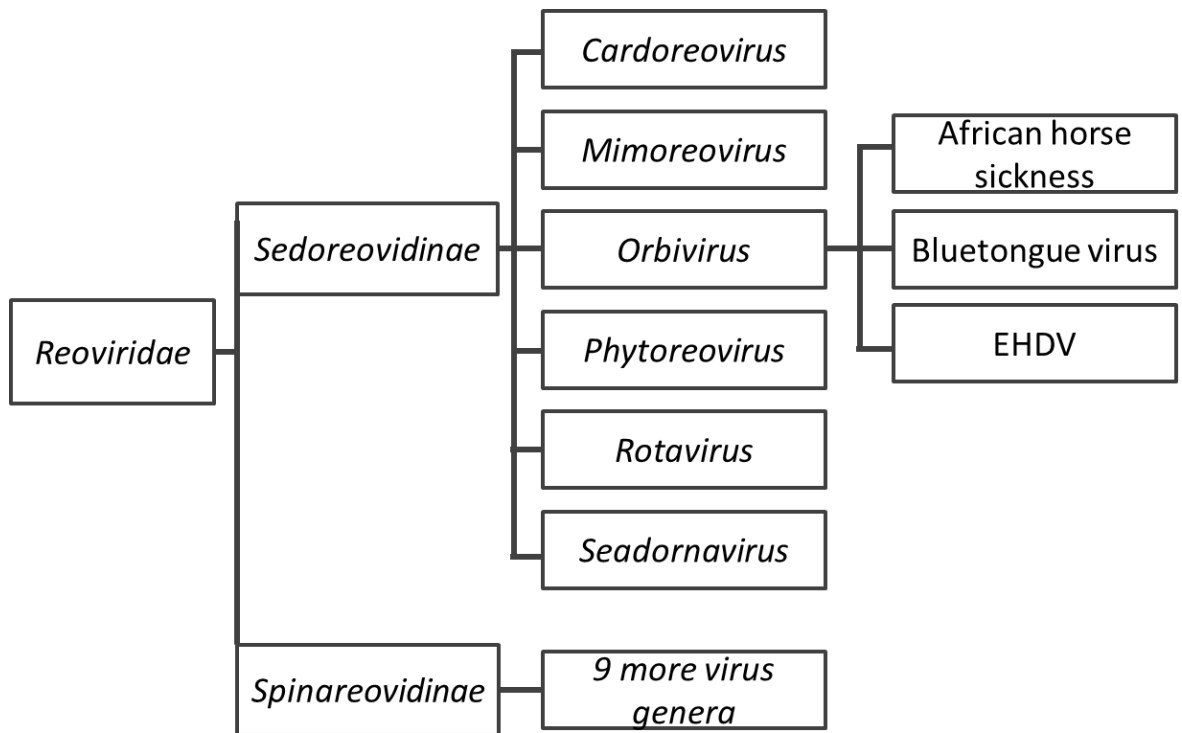
Figure 1.6: The *Reoviridae* taxonomy and selected examples of viruses. EHDV: Epizootic haemorrhagic disease virus.

1.4.1.1 Structure and serotypes

Viruses within the Orbivirus genus contain a genome of 10 segments of dsRNA (King *et al.*, 2012). BTV is a nonenveloped spherical virus with an icosahedral capsid consisting of 7 structural proteins. The virion is made up of the subcore (comprised of 60 VP3 dimers) which acts as a scaffold protein for the core surface layer (260 VP7 trimers). VP7 is the group specific antigen for the orbiviruses. The outer capsid layer consists of 60 VP2 trimers and 120 VP5 trimers. It is the triskelion-like spikes of the VP2 that allows virus attachment and VP5 penetrates the membrane allowing entry into the host cell (Mertens *et al.*, 2004; Nason *et al.*, 2004; Zhang *et al.*, 2010) (Figure 1.7). The serotype BTV specificity is based on recognition of the VP2 by neutralising antibodies (Hassan and Roy, 1999; Mertens *et al.*, 2004) .VP5 is also required to exit the late endosome into the cytosol (Hassan *et al.*, 2001). The core particle does not disassemble.

VP2 trimer

VP5 trimer

VP7 (T13) trimer

P3 (T2) decamer

Transcriptase complex
VP6 hexamer?
VP1(Pol) monomer
VP4(Cap) dimer

10 segments of dsRNA, as 4 stacked spirals of five-fold axes
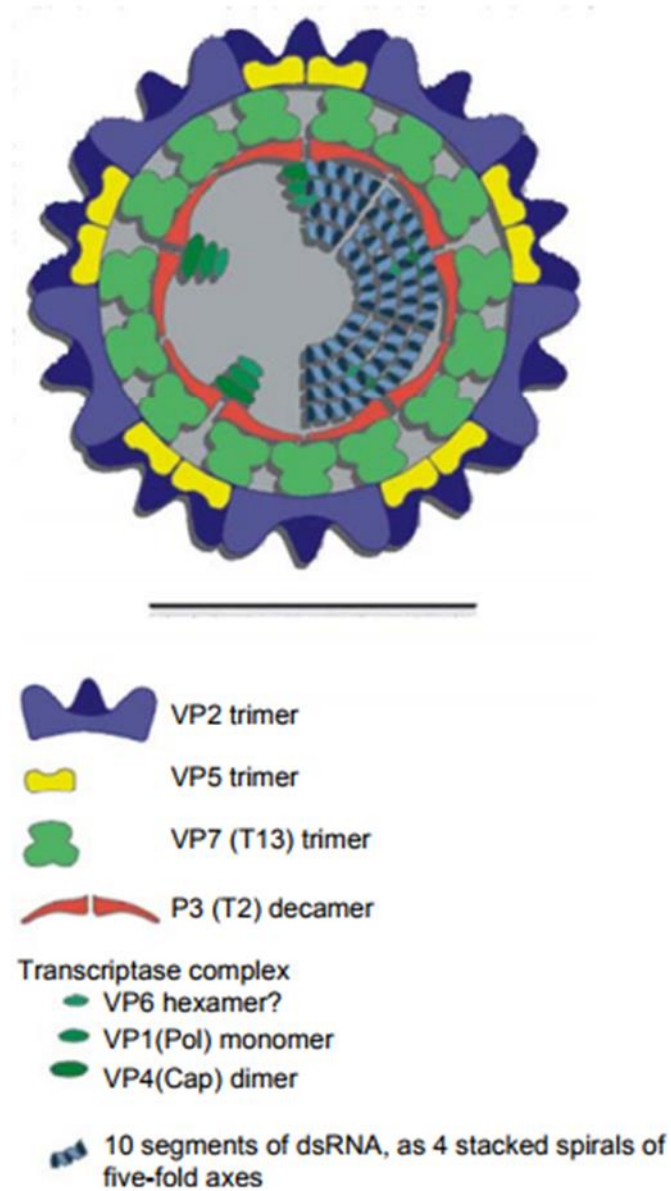
Figure 1.7: A schematic diagram of the structure of BTV-8. From Mertens *et al.,* 2004.

The 10 segments of dsRNA can be repeatedly transcribed by 3 core-associated enzymes: VP1, VP6 and VP4. VP1 is a RNA polymerase, VP6 acts as a viral helicase, an ATPase and has a role recruiting the 10ssRNA genome segment to the sub-core prior to the synthesis of dsRNA by VP1 (Matsuo and Roy, 2011; Mertens, 2004). VP4 was one of the first proteins shown to exhibit all of the enzymatic activities to form a 'cap' structure (Sutton *et al.*, 2007).

The final three dsRNA segments encode non-structural proteins: NS1, NS2, NS3 and NS4 (Roy, 2017). NS1 enhances viral protein synthesis by preferentially promoting the translation of BTV RNA within the host cytoplasm, whilst NS2 recruits the protein components and BTV RNA for packaging, replication and core assembly (Roy, 2017). This process is further facilitated by NS3. NS3 has also been demonstrated to mediate virus release within insect cells, most likely in the *Culicoides* midgut and salivary glands (therefore allowing the virus to bypass any MEB or SEB) (Mertens *et al.*, 2004). NS4 was identified relatively recently; it is believed that the likely function of this non-structural protein is to modulate host interferon response and as an interferon antagonist, a likely key determinant of viral virulence (Ratinier *et al.*, 2016).

1.4.2 History

Bluetongue virus has been described since the late 18th century in South Africa and was regarded as an African disease until 1943 when an outbreak was recorded in Cyprus (Erasmus and Potgieter, 2009). Retrospectively, it was suspected that outbreaks had been occurring on the island since at least 1924. Approximately 2500 sheep died during the 1943 outbreak, with some flocks experiencing 70% mortality (Gambles, 1949; Polydorou, 1978). This focussed international attention on the disease, and outbreaks were subsequently described in Palestine, Turkey and Israel over the following 6 years (Gambles, 1949; Shimshony, 2004). This international attention also led to the realisation that the American disease 'soremuzzle' was actually bluetongue virus serotype 10, previously identified in South Africa (Hardy and Price, 1952; McKercher *et al.*, 1953). More strains were identified in North America, and at the same time an outbreak of BTV-10 occurred in Portugal, rapidly spreading to Spain, continuing in the Iberian Peninsula clinically affecting both sheep and cattle until 1960 (de Diego *et al.*, 2014). This outbreak was again severe with disease reported in approximately 180,000 animals. Stringent

movement restrictions, vaccinations and culling resulted in a 20 year break from disease, until BTV-4 was recorded in Greece in 1979 (Bréard *et al.*, 2007; de Diego *et al.*, 2014).

In 1958 BTV-16 was reported in Pakistan and outbreaks of different serotypes have since occurred across both Pakistan and India (Prasad *et al.*, 2009). BTV-20 was identified in *Culicoides* caught in the Northern Territory of Australia in 1977, and subsequently another 7 serotypes were identified. Australia, however, has mostly remained free from disease as sheep are not typically reared in the north and east of the country where the serotypes have been confirmed (within the range of the known vector: *C.brevitarsis*), although trade restrictions have had a significant impact (Kirkland, 2004). In 1994 a review questioned if BTV should still be considered an emerging disease and if it was justified to continue its inclusion on List A of the OIE International Zoosanitary Code (now the OIE list of notifiable terrestrial and aquatic animal diseases) (Gibbs and Greiner, 1994).

Unfortunately between 1998 and 2005, outbreaks of BTV once again occurred in Europe (Mellor *et al.*, 2008 further reviewed in Purse *et al.,* 2005) (Figure 1.8). Two major systems appeared to predominate throughout the outbreaks, one in the eastern basin, where BTV serotypes 1, 4, 9 and 16 were identified and one in the western basin, where outbreaks of BTV-1, 2, 4 and 16 were recorded. In the eastern basin it is important to note that BTV-2, 4 and 16 had previously been reported in Syria, Jordan and/or Israel, with the westward transmission through Turkey well documented (Shimshony, 2004; Taylor, 1985; Taylor and Mellor, 1994; Yonguç *et al.*, 1982). These fringe areas are linked to both north Africa and Europe through traditional livestock trade routes. One example, 'ruminant street', represents a corridor between south Asia and Europe formed from the connected ruminant populations of Pakistan, Afghanistan, Iran and Turkey (Purse *et al.*, 2005). High and low pressure weather systems, of the lower atmosphere, also drive winds across

the region, demonstrating the longstanding potential for BTV to enter Europe, via

infected livestock movement or wind-dispersal of infected *Culicoides* (Purse *et al.*,

2005). The western basin outbreaks, although sudden, all remained within the range

of the known vector, *C.imicola*, with transmission most likely due to wind dispersal

of infected *Culicoides* from north Africa (Mellor *et al.*, 2008).
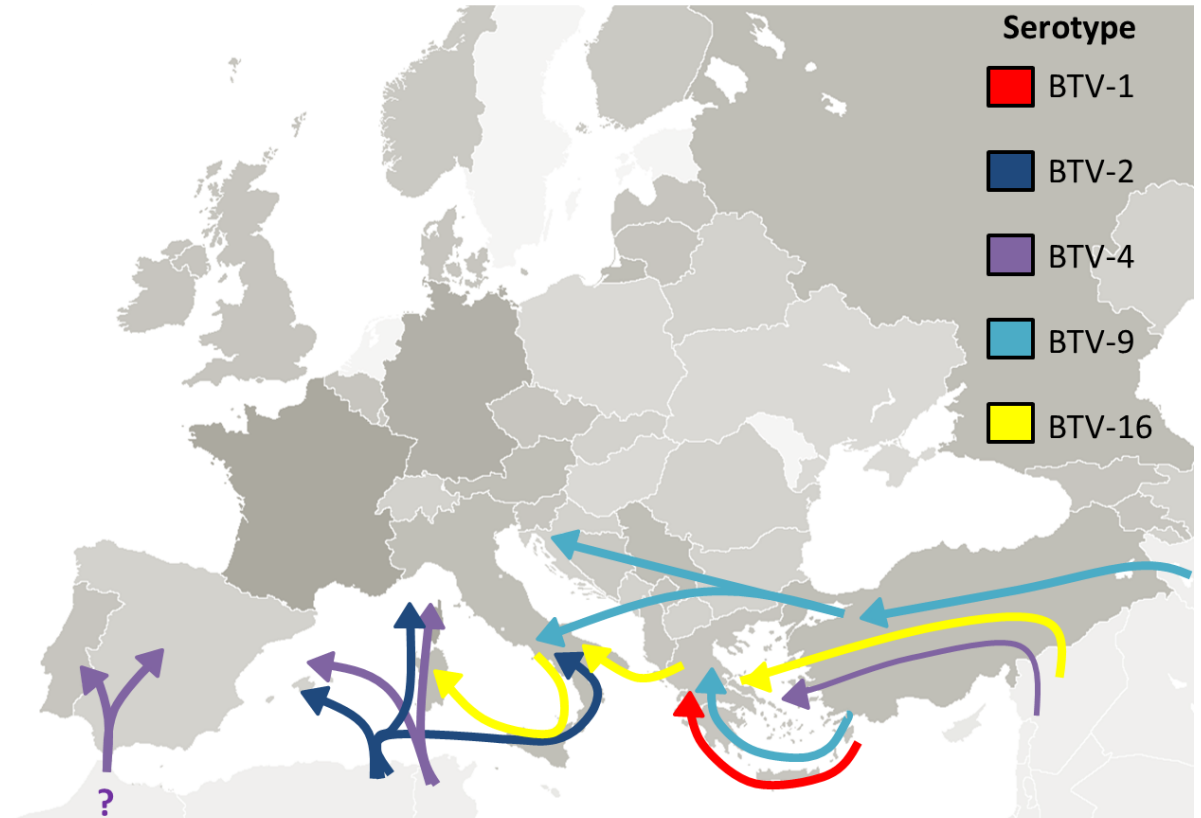
Figure 1.8: Outbreaks of BTV in Europe 1998-2005.

1.4.3 BTV-8 2006 outbreak

On the 14th August 2006 an outbreak of BTV-8 was detected in the Maastricht area of the Netherlands (EFSA, 2007a; OIE, 2006). The disease spread quickly along an east–west axis to include Belgium, Luxembourg, much of north west Germany and northern border of France by the end of 2006 (EFSA, 2007c; Mellor *et al.*, 2009b). The disease successfully overwintered, continuing to spread the following year. By August 2007, BTV-8 reached the UK, with roughly 100 holdings infected by the end of 2007 (Defra, 2007a). The observed losses from BTV-8 were higher in 2007, with 1/6th of Belgium's national flock succumbing to the disease (Wilson and Mellor, 2008). By early 2009 BTV-8 had been introduced into 18 countries across Europe (Wilson and Mellor, 2009). In 2010 the OIE declared France free of BTV-8, signalling the end of the 2006 BTV-8 outbreak (Sailleau *et al.*, 2015).

This outbreak represented the most northerly reports of BTV worldwide, and extended far beyond the northern limits of *C.imicola* (EFSA, 2007a). Although other serotypes had been previously active in the Mediterranean basin, BTV-8 had not been described in the region, with phylogenetic analysis indicating sub-Saharan African origin (Maan *et al.*, 2008). The exact route of origin again remains unknown, but the potential importation of infected *Culicoides* was deemed possible (EFSA, 2007a).

1.4.4   Susceptible species

Clinical signs have been demonstrated both naturally and experimentally in domestic ruminants (Backx *et al.*, 2007; Dal Pozzo *et al.*, 2009; Darpel *et al.*, 2007; Elbers *et al.*, 2008a, 2008d). The pathology of these species, namely cattle, sheep and goats are covered in the following section (1.4.5 Bluetongue virus: Pathogenesis).

Domestic camelids have been experimentally infected with BTV-8, with mild clinical signs observed in both llamas and alpacas (Schulz *et al.*, 2012a). A cross sectional study in Germany has also demonstrated antibodies against BTV-8 in camelids (Schulz *et al.*, 2012b). The viraemia in these species is thought to be short, with mild, if any, clinical signs reported, however BTV-8 related fatalities have been described for both llamas and alpacas (Henrich *et al.*, 2007; Schulz *et al.*, 2012b).

Antibodies against BTV-8 have been reported from a range of wild cervids (Casaubon *et al.*, 2013; Chatzopoulos *et al.*, 2015; Falconi *et al.*, 2011; García-Bocanegra *et al.*, 2011; Linden *et al.*, 2008, 2010; Lopez-Olvera *et al.*, 2010). Samples collected from red deer (*Cervus elaphus* Linnaeus 1758) in southern Belgium in 2007 demonstrated an antibody seroprevalence of 40.4% within the wild population (Linden *et al.*, 2008). Seroprevalence of antibodies against BTV-8 appear typically lower amongst wild roe deer (*Capreolus capreolus* Linnaeus 1758), Ibex *(Capra ibex* Linnaeus 1758*)* and southern chamois (*Rupicapra pyrenaica* Bonaparte 1845) than red deer (Casaubon *et al.*, 2013; Falconi *et al.*, 2011; Linden *et al.*, 2010). Crucially, experimental studies have demonstrated that BTV-8 RNA can be detected in red deer blood for extended periods whilst displaying only mild, if any, clinical signs. This highlights the potential importance of wild cervids to act as reservoirs for disease and in maintaining the sylvatic cycle (Lopez-Olvera *et al.*, 2010).

Natural BTV infection of various serotypes has been reported for several carnivore species worldwide (EFSA, 2007a; Holekamp *et al.*, 1994). However the BTV-8 outbreak resulted in the infection and death of 2 Eurasian Lynx in a Belgian zoo. Both animals had been fed ruminant foetuses and stillborns from BTV-8 confirmed farms (Jauniaux *et al.*, 2008). This suggests carnivores are potentially susceptible to BTV-8 transmission via the oral route. Although studies exist for carnivore susceptibility to other BTV serotypes, this is, to the author's knowledge, the only known report for BTV-8 in Europe.

1.4.5 Pathogenesis

1.4.5.1 Clinical signs in adult domestic ruminants

BTV is primarily considered a disease of sheep (MacLachlan, 2011). The clinical severity of BTV infection varies with BTV serotype and breed of sheep, with indigenous breeds from endemic regions rarely displaying clinical signs of disease (EFSA, 2007a). European wool and mutton breeds have been reported to be particularly susceptible to BTV infection (EFSA, 2007a). The case fatality for the BTV-8 outbreak in Europe reached 30-50% in sheep, although less than 10% of infected animals were thought to present with clinical signs (Darpel *et al.*, 2007).

Experimental infection of poll Dorset sheep with BTV-8 demonstrated varying severity in clinical signs between animals (Darpel *et al.*, 2007). This, combined with the known variation between breeds complicates diagnosis based on clinical signs alone. Broadly clinical signs from day 5 after infection included pyrexia (>40°C), hyperaemia of the buccal, labial and nasal mucosa, facial oedema, early signs of conjunctivitis and hyperaemia of the coronary band. From 1 week, post-infection facial oedema, hyperaemia and lameness became more severe and respiratory distress became apparent, although the degree to which each animal was affected varied (Darpel *et al.*, 2007). The description of lethargy, nasal/oral discharge,

dysphagia, dyspnoea, oedema of the head and haemorrhages of the oronasal mucosa have since been replicated in other experimental studies (including Texel and Swiss sheep breeds) and in the field (Backx *et al.*, 2007; Elbers *et al.*, 2008c; Worwa *et al.*, 2010).

Experimental infections of goats with BTV-8 described milder clinical signs than for sheep, but included lethargy, dysphagia, diarrhoea and lameness to varying degrees (Backx *et al.*, 2007). Initially no clinical signs in goats were reported in the field, despite previously high morbidity and mortality during outbreaks of BTV-2 (Elbers *et al.*, 2008d). However, an outbreak was confirmed in a dairy goat herd in the Netherlands, presenting with a drop in milk yield and fever, with individual cases of oedema, nasal discharge and erythema of the udder skin (Dercksen *et al.*, 2007).

Typically, cattle are considered to be sub-clinical for BTV infections, with clinical cases normally only associated with novel serotype outbreaks in naïve populations. During the European BTV-8 outbreak, cattle also presented clinically, indicative of the naïve status of this population. Case fatality was estimated to be up to 10% in cattle, although again less than 10% of infected animals were thought to present with clinical symptoms (Darpel *et al.*, 2007). Reported clinical signs for BTV-8 infected cattle included crusts/lesions of the nasal mucosa, erosions of the oral mucosa, salivation, fever, conjunctivitis, coronitis, muscle necrosis and limb stiffness (Elbers *et al.*, 2008b). Experimental infections resulted in similar clinical manifestations (Dal Pozzo *et al.*, 2009). Median recovery times for both infected sheep and cattle from clinical disease has been reported to be 2 weeks (Elbers *et al.*, 2008d)

1.4.5.2 Viraemic period

The viraemia of BTV is highly cell associated as viral replication first occurs in dendritic cells, mononuclear phagocytes and endothelial cells (Barratt-Boyes and Maclachlan, 1994; Hemati *et al.*, 2009). It is only during the later stages of viraemia that BTV is exclusively associated with the red blood cells (Singer *et al.*, 2001). Viraemia in sheep is typically first detected 3 days after initial infection. It is possible to continue to detect BTV RNA by PCR for more than 100 days after initial infection (140-160 days in cattle). However, the maximal duration of viraemia determined infectious to *C.sonorensis* was 21 days after initial infection for both cattle and sheep (Bonneau *et al.*, 2002; Katz *et al.*, 1994).

1.4.6   Transmission routes

1.4.6.1 Vector transmission

Vector transmission of BTV is the main transmission route. The putative *Culicoides* vectors of BTV have been outlined in Table 1.1. Studies quantifying vector species competence are generally lacking from this period, with methodologies revisited and improved upon during the 2011 SBV outbreak (note section 1.3.5.1 Schmallenberg virus: Vector transmission) (Carpenter *et al.*, 2015). Field caught *C.scoticus* were shown to be capable of replicating BTV-8 to high viral loads when fed sheep blood spiked with virus of a reasonable titre ($10^{6.5}$ Tissue Culture Infectious Dose 50/ml) (Carpenter *et al.*, 2008a).

1.4.6.2 Semen

Semen is considered a viable transmission route for BTV, and as such semen production and trade is carefully handled under several EU standard directives and the OIE Terrestrial Animal Health Code (EFSA, 2007a; Gard *et al.*, 1989). Naturally infected bulls have been shown to excrete BTV-8 in collected semen samples (Vanbinst *et al.*, 2010). Infection has been demonstrated to reduce semen quality transiently, with recovery to normal levels several months after the onset of clinical signs (Kirschvink *et al.*, 2009; Leemans *et al.*, 2012; Müller *et al.*, 2010).

1.4.6.3 Vertical transmission in ruminants

Vertical transmission of BTV-8 from cow to calf has been observed in the Netherlands and Northern Ireland, where BTV-8 RNA positive calves were born to PCR negative but seropositive cattle (Menzies *et al.*, 2008; Santman-Berends *et al.*, 2010; van Wuijckhuise *et al.*, 2008). Experimental studies have demonstrated the transplacental transmission of BTV-8, with a calf born displaying clinical signs of disease and successful isolation of BTV-8 from the blood, prior to colostrum intake (Backx *et al.*, 2009). The increased reports of hydraencephaly in cattle foetuses further demonstrated the ability for the virus to successfully cross the placenta (De Clercq *et al.*, 2008; Vercauteren *et al.*, 2008). Experimental infections also demonstrated the ability of BTV-8 to cross the placenta in ewes and goats, with high transmission rates noted if infected mid-gestation (Belbis *et al.*, 2013; van der Sluijs *et al.*, 2011). Not all BTV-8 positive offspring displayed clinical signs, with studies describing healthy viraemic offspring, which would present a risk for the ongoing transmission of BTV-8 (Santman-Berends *et al.*, 2010; van Wuijckhuise *et al.*, 2008).

Pseudo-vertical transmission has been demonstrated experimentally, with infection of BTV-8 negative calves after intake of colostrum spiked with BTV-8 blood (Backx

et al., 2009). This phenomenon has also been observed for natural BTV-11 transmission in a Californian sentinel dairy (Mayo *et al.*, 2010). Circumstantial evidence for transmission of BTV-8 through consumption of placental tissue has been described in the literature (Menzies *et al.*, 2008). This is certainly a possible pathway, as the oral cavity and oesophagus of type I interferon receptor- deficient mice have been demonstrated to be susceptible to BTV-8 infection, suggesting a potential entry route for oral infection (Calvo-Pinilla *et al.*, 2010).

1.4.7 Control measures and vaccination

Control and eradication of BTV in Europe is under Council Directive 2000/75/EC with the rule for implementation adopted under Commission Regulation No 1266/2007 (EC, 2017). Under an outbreak scenario, three zones are applied, namely: a radius zone, a protection zone and a surveillance zone (EC, 2017).

All holdings within the 20km radius zone are regularly visited, with animals clinically examined and pathology and laboratory testing to confirm disease. All susceptible animals are held at the holding, with no export or import of animals.

Within the 100km protection zone a surveillance programme must be implemented, with serological screening of sentinel ruminants and entomological monitoring. Vaccination may be applied depending on the strategy applied. Animal movement is restricted to the zone unless it has been demonstrated that the virus is not circulating.

The 50km surveillance zone is similar to the protection zone. Vaccination with live attenuated vaccines is not permitted.

To allow movement of animals during an ongoing outbreak a 'vector free period' can be established, under which movement is allowed to resume. A 'vector free period' is handled under Annex V of the EC 1266/2007. It can be declared through:

- Providing evidence of no BTV circulation within the area, through surveillance or other evidence suggesting a halt in BTV

- A lack of vector activity, demonstrated through entomological surveillance

- In the absence of evidence determining a maximum threshold, the absence of *C.imicola* and the collection of less than 5 parous *Culicoides* per trap must be used.

- Additionally, temperature thresholds, defined in relation to the ecological behaviour of *Culicoides* vectors, can be applied

(European Commission, 2007).

The Department for Environment Food and Rural Affairs (DEFRA) has provided a Great Britain (GB) BTV disease control strategy (Defra, 2014). This outlines the application of the above restriction zones if BTV is confirmed, and the banning (without licence) to movement of semen, ovum or embryos outside of the restriction zones (Defra, 2014). Movement of animals is allowed within and between the surveillance and protection zones if animals show no signs of disease on the day of transport. Determination of the 'vector free period' is again as described above. Voluntary vaccination of sheep and cattle against BTV-1, BTV-2, BTV-4 and BTV-8 using inactivated vaccines is allowed outside of restriction zones and whilst GB is free of disease (Defra, 2014).

Vaccination using an inactivated vaccine against BTV-8 has previously proved beneficial for GB (Szmaragd *et al.*, 2010). No cases of BTV-8 were reported in 2008 in GB, having encouraged a voluntary vaccination programme, unlike in other European countries that year. Modelling has suggested that the vaccination programme led to reduced incidence, extent of spread and outbreak size, with a high level (>80%) uptake of vaccination deemed the most important factor for controlling BTV-8 spread (Szmaragd *et al.*, 2010). Currently (August 2017) two

companies, Merial and Zoetis, have vaccines against BTV-8 on the market in the UK. The onset of immunity is stated by the manufacturers to be 21-25 days after the full dose. The efficacy of these vaccines was found to be good in a challenge experiment, even providing protection after only a single vaccination in sheep (as per manufacturers recommendations) (Defra, 2014). The duration of immunity from vaccination is stated to be 12 months in both sheep and cattle (European Medicines Agency, 2016, 2017a, 2017c, 2017d, 2017d)

1.5   Differential epidemiology and diagnosis of SBV and BTV-8

Key characteristics of both Schmallenberg virus and bluetongue virus have been
briefly summarised in table 1.4 adapted from Carpenter *et al.,* and described in
detail in earlier sections of this chapter (Carpenter *et al.*, 2013).

Table 1.4: A summary of the key characteristics of Schmallenberg virus and
bluetongue virus, adapted from Carpenter *et al.,* 2013. More detail can be located in
the sections noted in italics

Schmallenberg virus	Bluetongue virus
Orthobunyavirus	Orbivirus
Identified using metagenomic sequencing during 2011 in Germany *1.3.1*	Identified as a filterable agent during early 20th century in South Africa *1.4.2*
Infects many ruminant species and antibodies have been identified in many more ungulate species *1.3.3*	Infects all ruminant species investigated to date and surveys have identified additional host species that may also be able to sustain transmissible virus *1.4.4*
Clinical disease in sheep and cattle characterised by congenital deformities in young born to adults infected in their first trimester. Economic impact limited to individuals *1.3.4*	Clinical disease severe in sheep and deer with milder signs in cattle. Economic impact can be huge: total cost of the BTV-8 incursion in Europe is likely to exceed 1000 million Euros *1.4.5*
Detected in Palearctic region. Possible evidence of circulation outside of Europe *1.3.6*	Virtually worldwide distribution between latitudes 35°S to 45°N including temperate regions with seasonal absences of *Culicoides* adults *1.4.2*
Culicoides are the only vector identified to date *1.3.5.1*	*Culicoides* act as primary biological vector and involvement of other vectors is thought to be epidemiologically negligible *1.4.6.1*
No evidence of animal-to-human transmission	No evidence of animal-to-human transmission
Not notifiable. Preventative vaccinations produced *1.3.7*	Notifiable disease. EC disease control strategy implementation and vaccination programmes *1.4.7*

A stark difference between the European outbreaks of SBV and BTV-8 was the respective rate of spread (Rossi *et al.,* 2015). SBV spread rapidly both within and between farms, likely facilitated by the short incubation period of SBV despite the shorter associated viraemia (1.3.4.2 Schmallenberg virus: Viraemic period) compared to BTV-8 (1.4.5.2 Bluetongue virus: Viraemic period). Modelling of SBV transmission further determined a high probability of host to vector transmission than BTV-8 (14% compared to estimates of roughly 1% in field-caught *Culicoides)* (Carpenter *et al.,* 2006, 2008; Gubbins *et al.,* 2014). SBV also appears to replicate quicker (0.03 per day-degree) and at lower replication temperature threshold (12.3C) than reported for other BTV serotypes (Carpenter *et al.,* 2011; Gubbins *et al.,* 2014). The spread of BTV-8 was further limited by mandatory notification, vaccination programmes and strict movement restrictions, implemented at great financial cost (1.4.7 Bluetongue virus: Control measures and vaccination and further discussed in Chapter 3). SBV, in contrast, is not notifiable, with preventative vaccination optional and subsequent costs incurred by individuals rather than at a governmental level (1.3.7 Schmallenberg virus: Vaccination and further discussed in Chapter 5).

The clinical signs of both diseases can be vague in adult animals, especially when only mild clinical signs present. Indeed, the unspecific signs of either disease could indeed be mistaken for each other: reduction in milk production, lethargy, nasal discharge, fever, still births and abortions.

BTV-8 could be misdiagnosed as the related epizootic haemorrhagic disease, or foot and mouth, with all causing lesions/erosions around the mouth and lameness (Arzt *et al.*, 2011; Stevens *et al.*, 2015). The abortions, still births and foetal malformations associated with SBV could be misdiagnosed as toxoplasmosis, bovine virus diarrhoea virus/Border disease, herpesviruses, or even other Simbu

serogroup viruses: Aino, Akabane or Shamonda (Agerholm *et al.*, 2015; Esteves *et al.*, 2016).

Other factors, such as genetic factors (spider lamb syndrome), nutritional conditions (Vitamin A deficiency), mineral deficiencies (copper, calcium, manganese) or toxins and chemicals (pregnancy toxaemia, lead poisoning, *Veratrum californicum* Durand toxicity, wild *Lupinus spp.* Linnaeus, *Conium maculatum* Linnaeus*, Nicotiana spp.* Linnaeus) could also lead to similar clinical signs (Dittmer and Thompson, 2015). As alternative diagnosis exists, particularly in the case of SBV, conformational testing of suspected cases is paramount.

1.6   Surveillance techniques

The monitoring and surveillance of diseases is paramount to the effective planning and implementation of evidence-based strategies and policies for disease prevention and control (Amato-Gauci and Ammon, 2008). Both disease monitoring and disease surveillance require the collection, validation, analysis and interpretation of health and disease data, with the latter typically inferring a direct link between collection and intention to take actions (usually associated with a pre-defined action plan for stakeholders) (Amato-Gauci and Ammon, 2008). The collection of such data may be active and/or passive. Active monitoring and surveillance puts an emphasis on the active role of the investigator in data collection, with typically a more targeted approach to recruitment. Such studies can prove expensive in terms of both investigator time and monetary expense. Examples of active monitoring/surveillance includes both serological studies and monitoring/surveillance of vector populations. Passive monitoring and surveillance on the other hand involves the reporting of suspect cases, typically by the animal owner (which may be voluntary or mandatory in nature) rather than by the investigator that requires the information, and subsequently is sometimes referred to as 'reactive'. This type of monitoring/surveillance requires the disease of interest to produce clinical signs, as subclinical disease will not be recognised by those reporting. Equally if the disease is stigmatised, not considered a serious problem by the owners (possibly due to a lack of disease awareness) or there is no perceived benefit to the owner (due to a lack of adequate compensation or a lack of engagement within the community (i.e. does not feel a common responsibility)) then a reliance on a passive approach is unlikely to result in success (Doherr and Audigé, 2001).

Questionnaires and surveys are usually implemented as part of passive monitoring/surveillance due to the comparatively low cost of the technique. There are multiple approaches to administering questionnaires and surveys, all with different advantages and disadvantages depending on the population of interest. The different modes of questionnaire administration have been reviewed and discussed in depth by Bowling (2005). Briefly these modes of administration can be broadly characterised into face-to-face interviews, telephone interviews, postal surveys and online surveys, with the cost to the investigator reducing as the list progresses (in terms of both time and financial cost of implementation in a UK model). Face-to-face interviews are considered the least burdensome for the participant, as only basic verbal and listening skills are necessary to participate. However, the person delivering the questionnaire needs to be mobile, will require training to deliver the questionnaire without biasing responses and to interpret and analyse the given responses which can be extremely time consuming in both delivery and analysis. Telephone interviews require a greater auditory demand and access to a telephone, but still only require basic verbal and listening skills. Investigators again may require training to deliver, interpret and analyse the responses and again there is a time burden for both delivering the interview and interpreting results. Postal surveys are even more burdensome for participants, excluding those with visual impairments, those lacking dexterity and require reading and writing skills. These surveys can be financially costly compared to the other administration techniques due to the combined outward postal costs and need to provide return postage. Investigators expend time entering responses for analysis and comprehending handwritten text. Online surveys are potentially less burdensome for participants thanks to advances in technology (allowing for both narration and dictation) however they do require access to a computer and to be computer literate (Bowling, 2005). A previous study conducted in 2001 demonstrated a more complete response rate compared to paper versions

(Johnson *et al.*, 2001). Online surveys are also the most convenient and the cheapest method for investigators to administer, with multiple sites offering free question hosting and most academic institutes able to provide a questionnaire facility and/or support in online questionnaire design if specific requirements are needed. Time costs are minimal, with responses directly inputted by the respondent and typically in a downloadable format ready for analysis.

Bias is a common issue of questionnaires and surveys, with a total of 48 types of bias identified, categorised and discussed in a literature review by Choi and Pak (Choi and Pak, 2005). Immediately each administration technique has introduced bias to the study through coverage and response rates. To administer face-to-face, telephone and postal surveys the target population must be known, with up-to-date contact information accessible for the target group. For farming communities in the UK previous studies have utilised databases held by a commercial telephone database, veterinary institutions, levy boards, farming unions, farming supply companies and farm assurance schemes (Angell *et al.*, 2014; Cross *et al.*, 2009; Garforth *et al.*, 2013; Hall and Wapenaar, 2012; Richens *et al.*, 2015). None of these databases represents complete coverage and all have the potential to bias responses (i.e. those signed up to farm assurance schemes need to meet certain standards on farm and as such biosecurity and welfare may well be higher, more information on farm assurance programmes can be found on GOV.UK (Food Standards Agency, 2012)). As already noted online surveys necessitate access to a computer and proficiency in use. Defra concluded 90% of farms in England had access to a computer in 2012, of which 70% reported proficiency in computer use (Defra, 2013). The proportion of farms with access to computers is likely to have increased since 2012, in line with the rest of Great Britain (from 80% in 2012 to 90% 2017) suggesting a good potential coverage for online surveys (Office for National Statistics, 2017).

Response rates vary by study topic, questionnaire length and administration design. Previous responses by UK farmers to postal questionnaires are typically low, with 18-29% reported (Angell *et al.*, 2014; Cresswell *et al.*, 2014; Hall and Wapenaar, 2012; Harris *et al.*, 2014). Online questionnaires are hampered by the inability to accurately establish response rates and as such are typically not included in study results. This is a major drawback to online studies however they have the potential to reach a greater proportion of the target population if advertised carefully. It should be noted that advertisement of studies again introduces the potential for bias in respondent selection. Face to face interviews, and to a lesser extent telephone interviews, typically rely on high response rates and in-depth responses from relatively few respondents (Bennett and Balcombe 2011). This type of survey is frequently applied within the social sciences and is increasingly popular for investigating attitudes, insights, motivators and opinions within an epidemiological context (Richens *et al.*, 2015; Tongue *et al.*, 2017). Farmer attitudes and perceptions are also addressed in qualitative questions within postal and online questionnaires, typically through the analysis of open ended questions (Cresswell *et al.*, 2014; Cross *et al.*, 2009; Hall and Wapenaar, 2012; Harris *et al.*, 2014; Richens *et al.*, 2015; Tongue *et al.*, 2017).

1.7   Summary and objectives

In five years, between 2006 and 2011 two *Culicoides* borne diseases of ruminants emerged for the first time in Europe. Both diseases spread rapidly throughout Europe, causing significant economic, animal health and animal welfare concerns. Whilst considerable progress has been made towards understanding the epidemiology of both diseases, questions still occur in response to a changing disease landscape.

This thesis; 'The epidemiology and surveillance of *Culicoides* borne diseases of ruminants in the UK' aimed to address some of the major questions that arose from this changing disease situation, to better inform policy makers, stakeholder groups and disease models. This thesis aimed to address the following objectives:

- To investigate the current situation of Schmallenberg virus in the south of England. (Chapter 2)
- To determine the likely uptake of voluntary vaccination under different price bands and changing disease scenarios. (Chapter 3)
- To investigate the activity of *Culicoides* vectors, both indoors and outdoors, over the winter. (Chapter 4)
- To determine the impact of Schmallenberg virus re-emergence on the national flock and to compare this impact to the reported impact of Schmallenberg virus in 2012. (Chapter 5)

Chapter Two

A freedom from disease study: Schmallenberg virus in the south of England in 2015

This chapter 2 has been published in the *Veterinary Record* (see Appendix II):

Stokes, J. E., Baylis, M., and Duncan, J.S. (2016) A freedom from disease study: Schmallenberg virus in the south of England in 2015. *Veterinary Record* 179 (17), pp. 435–435. DOI:10.1136/vr.103903.

2.1 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 3 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, the author 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; 5 samples from 4 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.

2.2 Introduction

In November 2011 a novel Orthobunyavirus, of the Simbu serogroup, was identified by metagenomic analysis of cattle presenting with diarrhoea, pyrexia and reduced milk yield in Germany (Hoffmann *et al.*, 2012). The virus was subsequently named Schmallenberg virus (SBV), after the geographic origin of the samples tested. SBV spread rapidly, reaching England within 3 months of initial outbreak, with the southern-most counties of England all reporting outbreaks of Schmallenberg virus between 2012 and 2013 (EFSA, 2012). Like several viruses of the Simbu serogroup, and the unrelated bluetongue virus serotype 8 (BTV-8), SBV is transmitted by *Culicoides* biting midges. It is thought that the initial incursion into the UK was via wind dispersal of SBV infected *Culicoides* from France 113 days before the first report of a malformed lamb (Elbers *et al.*, 2013a; Sedda and Rogers, 2013).

Since its initial discovery, SBV has been detected throughout Europe (EFSA, 2014) in domestic cattle, sheep, goats and numerous species of wild ruminants, including camelids. Recently a high frequency of samples from hunted wild boar in Germany were found to have SBV specific antibodies (collected 2011/2012) (Mouchantat *et al.,* 2015). Additionally there is a single report of SBV specific antibodies in a dog, but other studies have failed to find evidence of infection in carnivores (Mouchantat *et al.*, 2015; Wensman *et al.*, 2013). 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 (Elbers *et al.*, 2012; Gache *et al.*, 2013; Nanjiani *et al.*, 2013). Herd level prevalence of UK sheep in 2012/2013 was found to range between 40-90% (Nanjiani *et al.*, 2013).

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 (Beer *et al.*, 2013; Sailleau *et al.*, 2013). 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 (EFSA, 2012; Martinelle *et al.*, 2015). Foetal or neonate malformations typically present as arthrogryposis, scoliosis, kyphosis, severe torticollis, brachygnathia and hypoplasia of the central nervous system (Doceul *et al.*, 2013). The hypoplasia may be mild to severe, resulting in microencephaly, hydranencephaly and spinal cord and cerebellar hypoplasia (Doceul *et al.*, 2013; van den Brom *et al.*, 2012). Behavioural and/or neurological disorders are also frequently noted, with lung hypoplasia sometimes observed (Lievaart-Peterson *et al.*, 2012). In the case of twins it is possible for only one to present with malformations, whilst the other remains viable, or for one twin to present with arthrogryposis, whereas the other presents with neurological abnormalities (Doceul *et al.*, 2013).

A recent study on the duration of immunity in experimentally infected adult sheep has demonstrated SBV specific IgG antibodies detectable for over one year after a single challenge with SBV (Poskin *et al.*, 2015). Additional evidence exists of acquired immunity against reinfection in naturally infected sheep, as well as evidence of maternally derived antibodies in suckling lambs (Rodríguez-Prieto *et al.*, 2016). Whilst experimentally infected cattle have been demonstrated to remain immune to reinfection for at least 56 days (Wernike *et al.*, 2013a).

Four cases of SBV were confirmed on the 16th of January 2012 in England (Harris *et al.*, 2014). Voluntary reporting recorded 81 and 87 serologically confirmed cases in UK sheep in 2012 and 2013 respectively (AHVLA, 2013), 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 (Veldhuis *et al.*, 2015). A German study reported a recurrence of SBV in cattle in 2014, despite an apparent decrease in cases the previous year (Wernike et al. 2015).

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.

2.3 Materials and methods

All animal work was reviewed and approved by the University of Liverpool Veterinary Research Ethics Committee (VREC310) and carried out under a Home Office Project Licence (PPL 70/8529). All farmers gave informed written consent and were reminded of their right to withdraw from the study at any point.

To calculate the number of farms needed to substantiate a prevalence of 2.5% or below the software package FFD was implemented in *R* (Kopacka, 2011). As sheep occur within flocks, a two-stage cluster analysis was used to estimate both the number of flocks and the number of sheep within each flock to be sampled; individual sampling was selected to allow the test sensitivity to remain the same across flocks. The α-error threshold was set to 0.05 (5%). An intra-herd prevalence of 20% was set, this is lower than the prevalence recorded in several large scale continental studies, but closer to the lower range reported in a 2013 UK study (Elbers *et al.*, 2012; Méroc *et al.*, 2013a; Nanjiani *et al.*, 2013; Veldhuis *et al.*, 2013), herd sensitivity of Se_{herd}=90% was set (EFSA, 2014), with a known test sensitivity of Se_2= 97.2% (Bréard *et al.*, 2013). The total number of sheep holdings in the southern-most counties of England was extracted from the Department for Environment, Food & Rural Affairs (Defra) 2010 census: a total of 6,495 sheep holdings were registered. This determined a necessary sample size of 11 sheep per holding collected from 131 holdings to detect prevalence below 2.5% with 95% confidence. Holdings were recruited for the study through the National Sheep Association (NSA) South West show, NSA magazine and large animal veterinary practices (Figure 2.1). The number of holdings sampled per county was stratified based on Defra 2010 census data: Cornwall (*n*=18), Devon (*n*=67), Dorset (*n*=12), Hampshire (*n*=5), Sussex (*n*=20) and Kent (*n*=9).

Figure 2.1: Map of the south of England showing the distribution of sampled farms. Exact farm location has been jittered and enlarged to prevent individual participant identification. Ten farmers asked that their farm location was not mapped.

Blood samples were collected between 15th September 2015 and 11th December 2015 from the jugular vein of 12 sheep per holding (11, plus 1 to account for failures). Sampling began after the spring and summer peaks in midge activity, with the majority of samples collected after the final autumn peak in midge activity (Sanders *et al.*, 2011b). All sampled sheep were born after October 2014 and were more than six months old at time of sampling to exclude animals with immunity following infection in 2012, 2013 or 2014 and to avoid maternal antibodies. This assumption is based on the maternal antibodies of calves lasting less than six months for both SBV and Akabane virus (Elbers *et al.*, 2014; Tsutsui *et al.*, 2009). Serum was extracted from the blood samples and analysed by a commercially available SBV antibody ELISA (ID Screen® Schmallenberg virus indirect, IDvet,

France) as per the manufacturer's instructions. Negative and positive controls, supplied by the manufacturer, were included on each plate to allow individual plate validation and calculation of S/P% (sample to positive control percentage). Serum samples were considered negative if the S/P% was up to 50% calculated as per manufacturer's instructions. Samples returning an S/P% greater than 50% were sent to the APHA to be confirmed by a Virus Neutralisation Test (VNT). An equal number of samples returning an S/P% less than 50% were also sent as blind controls; they were selected randomly using the RANDBETWEEN function in Microsoft Excel to determine farm number and then sample number from farm. Two positive controls were used in the VNT, with VNT titres of 1/40 and 1/80 respectively. Samples were determined to be negative if the VNT titre was greater than 1/5 based on the minimum dilution undertaken at the APHA (La Rocca, personal communication).

2.4 Results

A total of 1,572 sheep from 131 holdings were sampled between September 15th and December 11th 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, 8 hill, 8 upland, 10 had flocks across lowland, hill and/or upland pastures and 2 holdings declined to answer or were unsure.

Half (50.0%) of farmers (57/124, seven farmers declined to answer) reported that they had previously suspected cases of SBV infection in their flocks in the form of birth of lambs showing typical congenital abnormalities. Of these 57 farmers, 12 had cases which were diagnosed by a vet but not laboratory confirmed, while 13 had cases that were diagnosed by a vet and laboratory confirmed as SBV. In the remaining 32 suspect case farms none had disease diagnosed, either by a vet or laboratory.

Only 13.7% (17/124) of farmers stated they had vaccinated their sheep against SBV; 15 farmers stated they vaccinated in 2013, whilst 2 farmers vaccinated in both 2013 and 2014. 1 farmer vaccinated only their cattle against SBV but not their sheep.By contrast, only 1.6% (2/124) of the farmers stated that they had had cases of bluetongue virus (BTV) on farm, with 78.2% (97/124) stating they had vaccinated against BTV for at least 1 year.

A total of 11 sheep from each holding were tested by ELISA for antibodies against SBV (1,444 samples in total). Overall 9 samples, from 8 holdings, returned doubtful or positive (S/P% >50%) results for antibodies to SBV when tested by ELISA. These samples were retested by ELISA, with 5 samples, from 4 holdings returning positive for antibodies against SBV. No antibodies were detected in these 5 samples when tested by VNT at the APHA (Table 2.1).

Table 2.1: SBV ELISA and VNT test results in samples that returned a positive ELISA test result, holding ID, county of farm, breed of sheep, birth and sample dates, ELISA titre (S/P%), VNT result, previous self-reporting of suspected cases on farm by farmer and if the farm vaccinated against SBV in 2013. Samples 10-14 are negative controls for the VNT

Sheep ID	Holding ID	County	Breed	Born	Sampled	S/P% ELISA	S/P% ELISA retest	VNT result	Prev. SBV Suspected	SBV Vacc. 2013
1	21	Dorset	Texel	March 2015	September 2015	166.55*	9.82†	Not Tested	No	Yes
2	25	Dorset	Poll Dorset	January 2015	September 2015	64.93*	46.40†	Not Tested	No	No
3	46	Hampshire	Hampshire	December 2014	October 2015	60.77*	44.41†	Not Tested	Yes‡	No
4	48	Cornwall	Roussin	February 2015	October 2015	55.43*	39.54†	Not Tested	Yes	No
5	59	Devon	Highlander	March 2015	October 2015	78.14*	120.62*	Negative	No	No
6	108	Sussex	Dorset	February 2015	November 2015	110.32*	80.97*	Negative	Yes‡	No
7	113	Sussex	Charolais	February 2015	November 2015	90.61*	127.66*	Negative	Yes	No
8	113	Sussex	Charolais	February 2015	November 2015	66.45*	125.91*	Negative		
9	121	Cornwall	Lleyn X Texel	March 2015	November 2015	117.97*	123.79*	Negative	No	No
10	12	Devon	Poll Dorset	December 2014	September 2015	3.19†	Not Tested	Negative	Yes	No
11	50	Cornwall	Zwartble	March 2015	October 2015	3.93†	Not Tested	Negative	No	No
12	99	Kent	Charolais	March 2015	November 2015	8.46†	Not Tested	Negative	Yes	No
13	106	Sussex	Charolais	March 2015	November 2015	2.50†	Not Tested	Negative	Yes‡	No
14	115	Sussex	Southdown X Dorset	April 2015	November 2015	5.18†	Not Tested	Negative	Yes‡	No

* ELISA positive S/P%, †ELISA negative S/P%, ‡laboratory confirmed cases of SBV on farm

2.5 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 (Veldhuis *et al.*, 2015).

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 9 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 (Laloy *et al.,* 2014) tested by both the indirect ELISA used here and the VNT (Bréard *et al.*, 2013; Laloy *et al.*, 2014). 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 (Loeffen *et al.*, 2012) .

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 (EFSA, 2014; Méroc *et al.*, 2013b; Veldhuis *et al.*, 2013; Wernike *et al.*, 2014b). 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 (Gubbins *et al.*, 2014b). Even with low levels of SBV circulation and few susceptible

hosts on farm, previous studies have demonstrated eventual seroconversion of these individuals (Elbers *et al.*, 2013a). 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 (Wernike *et al.*, 2015). 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 (Sailleau *et al.*, 2015). 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 (Ruiz-Fons *et al.*, 2014). 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 (Rossi *et al.*, 2015). 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 (Poskin *et al.*, 2016). 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.

Chapter 3

The reported willingness of farmers to vaccinate against BTV-8

3.1 Abstract

Bluetongue virus serotype 8 (BTV-8) is a *Culicoides* borne disease of ruminants. Clinical signs of disease range from lethargy and weight loss, to oedema of the head, mouth and tongue, to death. The summer of 2006 witnessed the first European outbreak of BTV-8 and by August 2007 the disease had reached the UK. Movement restriction zones and surveillance zones were quickly introduced, and in May 2008 a voluntary vaccination scheme was launched. Unlike the rest of Europe, the UK reported no further cases in 2008. At the end of August 2015, a new clinical case of BTV-8 was reported in France. As vaccination was not currently available to UK farmers at this time, this study aimed to investigate the demand for vaccine within the UK farming community. The factors associated with willingness to pay to vaccinate, and the interest in vaccinating under a changing BTV-8 outbreak was also investigated. Univariable and multivariable logistic regression demonstrated higher willingness to vaccinate if respondents were from smaller sized farms (odds ratio (OR) 0.21, 95% confidence interval (CI) 0.07-0.61, P=0.004), had previously vaccinated against BTV-8 (OR 4.88, 95% CI 1.42-16.73, P=0.012), or were more 'risk adverse' farmers (OR 0.39, 95% CI 0.22-0.69, P=0.001). Ordinal logistic regression modelling additionally determined these respondents to be more willing to pay to vaccinate. Voluntary vaccination only achieved an 80% uptake if vaccination was free and after BTV-8 cases were reported in the UK, despite 90% of farmer respondents stating they believed it important or extremely important to keep BTV-8 out of the UK. 17.5% of farmers stated they only vaccinate some (<50%) of their flock/herd against BTV-8 previously. This survey highlights the complex issues surrounding voluntary vaccination at the farmer perceived risk versus cost level.

3.2 Introduction

Bluetongue virus (BTV) is a non-contagious vector-borne *Orbivirus* (family: *Reoviridae*) which infects ruminants and camelids (Wilson and Mellor, 2009). The World Organisation for Animal Health (OIE) records BTV as a listed disease, due to the potential for rapid spread and severe socioeconomic losses that occur within the sheep and cattle industries. This means any suspected BTV clinical signs must be notified to the relevant authorities; for farmers, livestock owners and veterinarians in the UK this requires the immediate reporting of suspicious clinical signs for investigation by government veterinary inspectors (Defra, 2014). There are 27 BTV serotypes currently known worldwide. The severity of clinical signs vary between BTV serotypes as well as host species and breed, with many infections remaining subclinical. However signs considered typical of BTV infection can include fever, lethargy, salivation, dyspnoea, lameness, nasal discharge, oedema and ulceration of the oral membranes (Cross *et al.*, 2009; Elbers *et al.*, 2008b). These clinical signs tend to be observed more frequently in sheep than cattle, with some European wool and mutton breeds, considered particularly at risk (Darpel *et al.*, 2007; Koumbati *et al.*, 1999; Wilson and Mellor, 2009).

Several common species of *Culicoides* biting midges have been identified as vectors of BTV within Europe (EFSA, 2008; Maan *et al.*, 2012; Schulz *et al.*, 2016; Wilson and Mellor, 2009). Prior to 1998 only sporadic, brief, incursions of BTV into southern Europe had occurred from sub-Saharan Africa, the Middle East and Turkey. However 1998 saw the first spread of BTV serotype 9 (BTV-9) into mainland Europe via Greek islands close to Turkey, followed by outbreaks of BTV-1, BTV-2, BTV-4 and BTV-16 over the following years. In August 2006 BTV-8 was identified in Northern Europe for the first time (van Wuijckhuise *et al.*, 2006). This outbreak spread through parts of The Netherlands, Belgium, Germany, France and

Luxembourg, and returned the following year, reaching the UK in September 2007 (Gloster *et al.*, 2008; Mintiens *et al.*, 2008). Transmission, facilitated by Obsoletus and Pulicaris group *Culicoides*, infected cattle and sheep across Suffolk, Norfolk, Essex, Cambridgeshire, Kent and Surrey by December the same year (Carpenter *et al.*, 2009; Gloster *et al.*, 2008; Mehlhorn *et al.*, 2007; Mellor *et al.*, 2008). Through the implementation of movement restrictions, surveillance zones and a voluntary vaccination scheme, in contrast to elsewhere in Europe, the UK reported no cases of BTV-8 in 2008 (Burgin *et al.*, 2009; Defra, 2007b; Szmaragd *et al.*, 2010). In 2010 the OIE declared France free of BTV-8, signalling the end of the 2006 BTV-8 outbreak (Sailleau *et al.*, 2015).

Five years after the incursion of BTV-8, a new vector-borne disease of ruminants swept through Europe, called Schmallenberg (Hoffmann *et al.*, 2012). Caused by a novel *Orthobunyavirus* (Simbu seorgroup) named Schmallenberg virus (SBV), it spread rapidly across Northern Europe. Like BTV-8, SBV is spread by *Culicoides* biting midges, yet the rate of spread was faster, with the first reports of SBV in the UK in January 2012, within a mere 3 months after initial discovery (Sedda and Rogers, 2013). The expansion of SBV across Europe far exceeded the northern range of the previous BTV-8 outbreak (Afonso *et al.*, 2014). Adult ruminants show only mild clinical signs, however infection of a naïve animal during the vulnerable stages of gestation can result in still birth, abortion and foetal malformation (Beer *et al.*, 2013; Doceul *et al.*, 2013). Unlike BTV-8, SBV was not made a notifiable disease in the UK, with surveillance reliant on voluntary reporting and post-mortem testing by the Animal and Plant Health Agency (APHA). In 2012 the virus overwintered, with more cases reported in 2013, however no cases were confirmed in 2014 or 2015 (APHA, personal communication) with prevalence determined to be between 0-2.5% in sheep in the south of England in 2015 (Chapter 2). SBV has

since re-emerged in Europe, with confirmed cases recorded across the UK (APHA, 2017).

In August 2015 a case of BTV-8 was identified in sheep in Central France (Sailleau *et al.*, 2015). Following surveillance a further 173 cases were identified by February 2016 (Roberts *et al.*, 2016; The International Disease Monitoring Team, 2017). Through the use of models and expert opinion the probability of BTV-8 introduction to the UK in 2016 through infected midges on the wind was deemed to be low in May (5-10%), medium in July (30-60%) and high in September, assuming the virus spread to Northern France (Roberts *et al.*, 2016).

There are no known means of effectively controlling the *Culicoides* population on farm. Therefore, protection from BTV and SBV is reliant upon successful vaccination. The production and subsequent availability of a vaccination is a product of the perceived demand for vaccination. The aim of this study was to investigate the current demand for vaccination within the UK farming community, and factors associated with decision making. Previous reported vaccination history against BTV-8 and SBV is also described and perceptions towards vaccination as a disease prevention method are explored.

These findings will contribute to dialogue between farmers, veterinarians, the pharmaceutical industry and policymakers, and help inform disease models and policy decisions in regards to voluntary vaccination programmes.

3.3 Materials and methods

This study was reviewed and approved by the University of Liverpool Veterinary Research Ethics Committee (VREC422).

3.3.1 Survey Design

An online questionnaire was developed using SurveyMonkey software (www.surveymonkey.com, Portland,Oregon, USA) comprising 5 sections (Appendix III).

The first section comprised a demographics section to determine farm location (county), species kept (sheep, cattle, or both sheep and cattle) and the number of livestock owned (by species, age and sex to convert to Livestock Units (LU)). The second section collected information on previous vaccination history for BTV-8 and SBV.

In the third section respondents were asked if they were currently planning to place an order for BTV-8 vaccine and, if so, the highest cost per dose they would be willing to pay. Respondents were told to assume that dosage rates were 1 per sheep and 2 per bovine, and were given price ranges based on estimates of the actual costs, wholesale costs and subsidised costs of vaccines sold in 2007 (Bluetongue South West, 2006).

The fourth section presented a matrix of price per dose versus number of animals. Respondents were required to select the maximum price they would be willing to pay per dose and how much of their flock/herd (All, Some (<50%), Most valuable (<10%)) they would choose to vaccinate at that price. This was done for different scenarios, based on the previous 2006/2007 BTV-8 outbreak, with varying distances to the outbreak from the respondent's farm (Table 3.1). This question was

designed so that it could be calculated under which conditions 25%, 50% and 80% voluntary vaccine uptake would be needed under each scenario. These cut-offs were selected as they are the pre-emptive vaccination levels currently used in models of the possible spread of BTV-8, which are in turn used to evaluate the potential risk to UK livestock (Roberts *et al.*, 2016).

Table 3.1: Scenarios based on 2006/2007 BTV-8 outbreak with description as provided in questionnaire.

Scenario	Description
Scenario 1	BTV-8 stays in central France, spreading to all the southern provinces of France
Scenario 2	BTV-8 stays in central France, spreading to the northern provinces of France
Scenario 3	All of France is now BTV-8 positive. The disease has also spread to Belgium, the Netherlands and Southern Germany
Scenario 4	A case of BTV-8 is confirmed in Suffolk
Scenario 5	Cases of BTV-8 are confirmed in Suffolk, Norfolk, Kent, Sussex, Hampshire and Dorset
Scenario 6	Cases of BTV-8 are confirmed in your neighbouring county

In the fifth and final section, respondents were asked about their personal perceptions of BTV-8 vaccination, on a scale of 1 (extremely unimportant) to 5 (extremely important). These questions also allowed respondents to answer 'not sure', to separate impartial respondents from those who did not choose to reply. Respondents were asked their perceptions concerning the importance of (i) vaccination to prevent disease within their own flocks/herds, (ii) vaccination having prevented a larger BTV-8 outbreak in 2007/2008; and (iii) the importance of keeping BTV-8 out of the UK. This final section permitted internal validation of the questionnaire, as respondents who voted both a higher price in Section 4 and also 'extremely unimportant' when asked about vaccination were deemed likely to have just selected the first response for all questions or had misunderstood the task. Where this was the case, the responses were removed from analysis.

Prior to roll out, the questionnaire was piloted by four practicing veterinarians, three researchers and five farmers but no major changes were required. The online survey was launched at the end of April 2016.

3.3.2 Survey Sample

The questionnaire was aimed at any cattle or sheep owners farming within the United Kingdom. Respondents were recruited through social media accounts, newsletters circulated through national farming organisations (National Farmers' Union (NFU) and the Farmers' Union of Wales (FUW)) and circulated by the Sheep Veterinary Society (SVS) amongst its members. The questionnaire ran for just over 9 weeks (26th April- 28th June 2016), finishing prior to the BTV-8 vaccine becoming available, as this was deemed likely to skew questionnaire responses.

3.3.3 Data analysis

All results were downloaded from SurveyMonkey into an Excel spreadsheet on the 28th June 2016. The responses were manually checked to remove responses from non-UK residents, those that did not own any sheep or cattle and any responses that were flagged by the internal validation step.

Maps were created in open source Quantum GIS (QGIS version 2.2.0). All statistical analyses were conducted using *R* (version 3.3.2). Probability values of <0.05 were taken as significant.

For modelling, the following additional variables were created from the data:

- The primary outcome variable for both models was ascertained in section 3: intent to vaccinate.
- Farm size was estimated using data supplied by the farmer (number and type of animals) to calculate the livestock units (LU) for each farm. For female adult sheep an average LU of 0.8 was used to take into account the

different grazing habits due to the complexity of calculating this individually for each farm (Defra, 2010). A farm with an LU of less than 100 was designated 'small' and more than 100 'large' for the purposes of analysis.

- Postcode information was used to group responses into zones based on previous experience of BTV-8 restrictions; with Zone 1 referring to 'Protection zone 1' in place in March 2008, Zone 2 'Surveillance zone' and Zone 3 outside of any restrictions (Defra, 2008) (Figure 3.1).

- Risk scores were calculated for each respondent determined by the earliest scenario they stated they would vaccinate their animals. Scenario questions were therefore used as a proxy for the risk taking behaviour of each respondent, with groupings made to account for small response rates; Very Risk Adverse (those that stated they would vaccinate under Scenario 1), Moderately Risk Adverse (those that stated they would vaccinate under Scenario 2 and 3), Moderately Risky (those that stated they would vaccinate under Scenario 4 and 5) and Very Risky (those that stated they would vaccinate under Scenario 6 or not at all) (Scenarios are presented in Table 3.1).

The primary binary outcome variable was whether the respondent would vaccinate their animals against BTV-8 at the time of questioning (yes/no) if a vaccine had been available. Associations between wanting to vaccinate now and the species kept (sheep/cattle/both), the farm zone, farm size, type of farm (pedigree/commercial), previous BTV-8 vaccination history and risk group were investigated through univariable logistic regression. The linear relationship between risk and primary binary outcome variable was explored.

Multivariable modelling was undertaken using a stepwise elimination procedure where a higher P-value threshold of 0.2 was applied for variable exclusion (Kirkwood and Sterne, 2003). A likelihood ratio test for interaction was completed for

the risk variable and the final model fit was assessed using the Pearson χ^2 before creating a ROC curve to display the predictive ability of the model.

To further explore the explanatory variables identified in the univariable logistic regression the expanded primary outcome variable 'willingness to pay for vaccine' was included in an ordinal logistic model. This represents the expansion of the binary 'no/yes' category to five separate price ranks (no, ≤40p, 40p-80p, 80p-£1, at any cost), marked as 1-5.
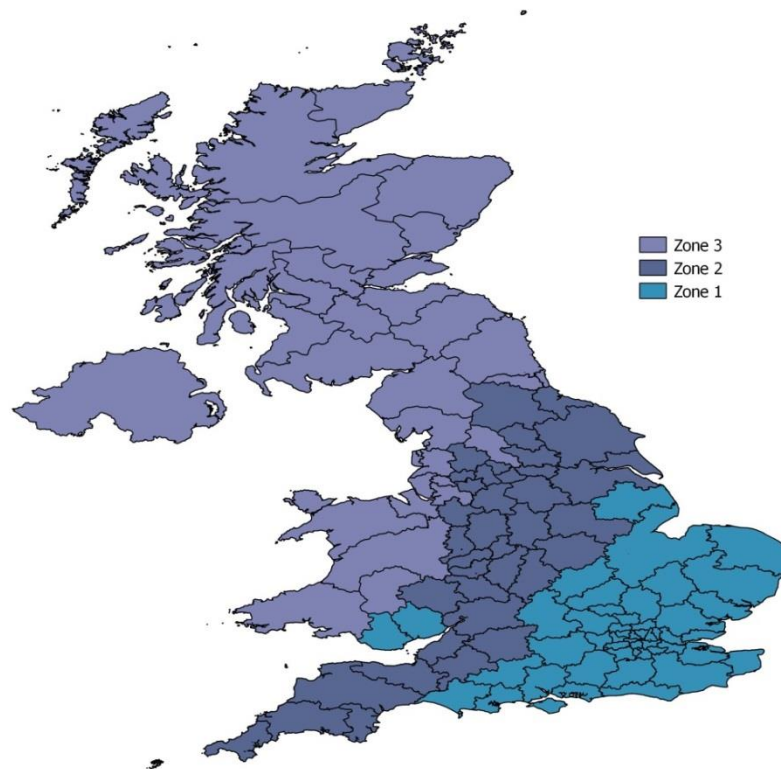
Figure 3.1: Postcode area located in each zone. Zone determined by protection zones and surveillance zones for BTV-8 in place May 2008 (Defra, 2008). *Contains OS data © Crown copyright and database right (2017), contains Royal Mail data © Royal Mail copyright and Database right (2017) and contains National Statistics data © Crown copyright and database (2017).*

3.4 Results

3.4.1 Response rate

A total of 131 participants took part in the questionnaire. Of these, 116 were considered usable and 15 not usable (Table 3.2). Not all respondents answered all questions, and a total of 99 responses were adequately completed for logistic regression analysis.

Table 3.2: Reasons for response removal from final questionnaire analysis and total numbers removed.

Reason for removal	Number of questionnaires
Questionnaire mostly unanswered	7
Not based in the UK	4
Did not own sheep or cattle	3
Questionnaire flagged by internal validation step	1
Total	15

3.4.2 Farm demographics

A greater number of responses were returned from the west of the UK than from the east (Figure 3.2) which matches known density of sheep and cattle holdings (AHDB, 2016b, 2016c). However, a lack of responses from the high density cattle farming areas in the south and east of Scotland, and north of England and high density sheep farming areas in the north east of England may indicate underrepresentation in these areas.
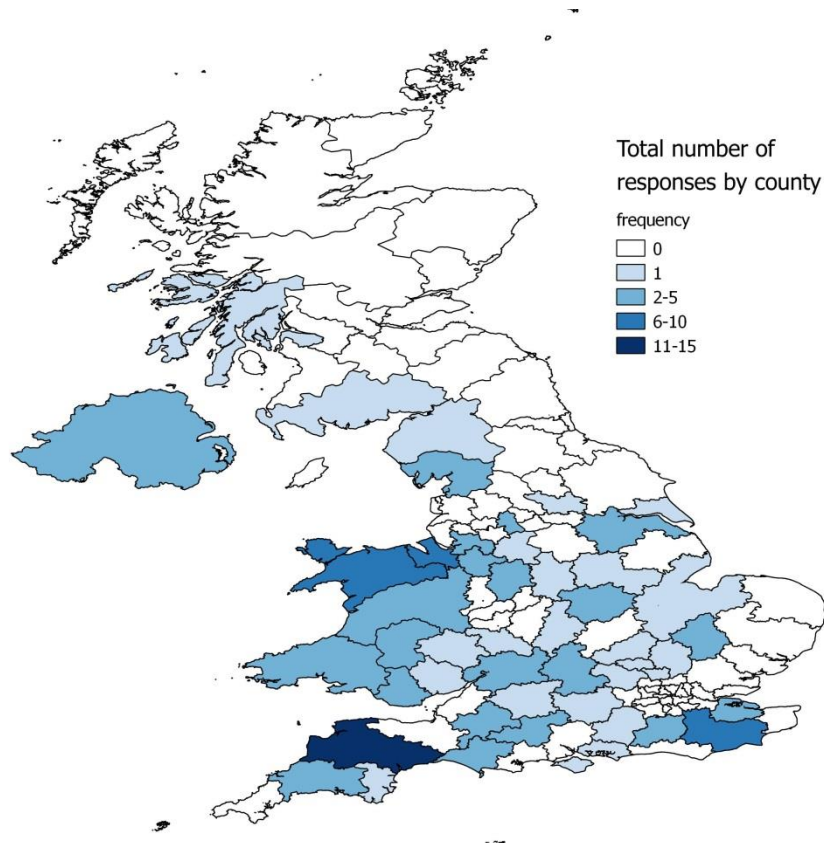
Figure 3.2: Total number of responses per county. *Contains OS data © Crown copyright and database right (2017), contains Royal Mail data © Royal Mail copyright and Database right (2017) and contains National Statistics data © Crown copyright and database (2017).*

Of the respondents, 58 (50%) stated they owned only sheep, 13 (11.2%) owned only cattle and 45 (38.8%) owned both sheep and cattle.

The median number of female sheep (>1 year old) owned by sheep-only farmers was 150 (Inter-Quartile range (IQR) 42-500). The median number of female sheep owned by farmers that owned both sheep and cattle was 500 (IQR 150-750). The mean number of sheep and lambs owned by all farmers was 387 (Figure 3.3).
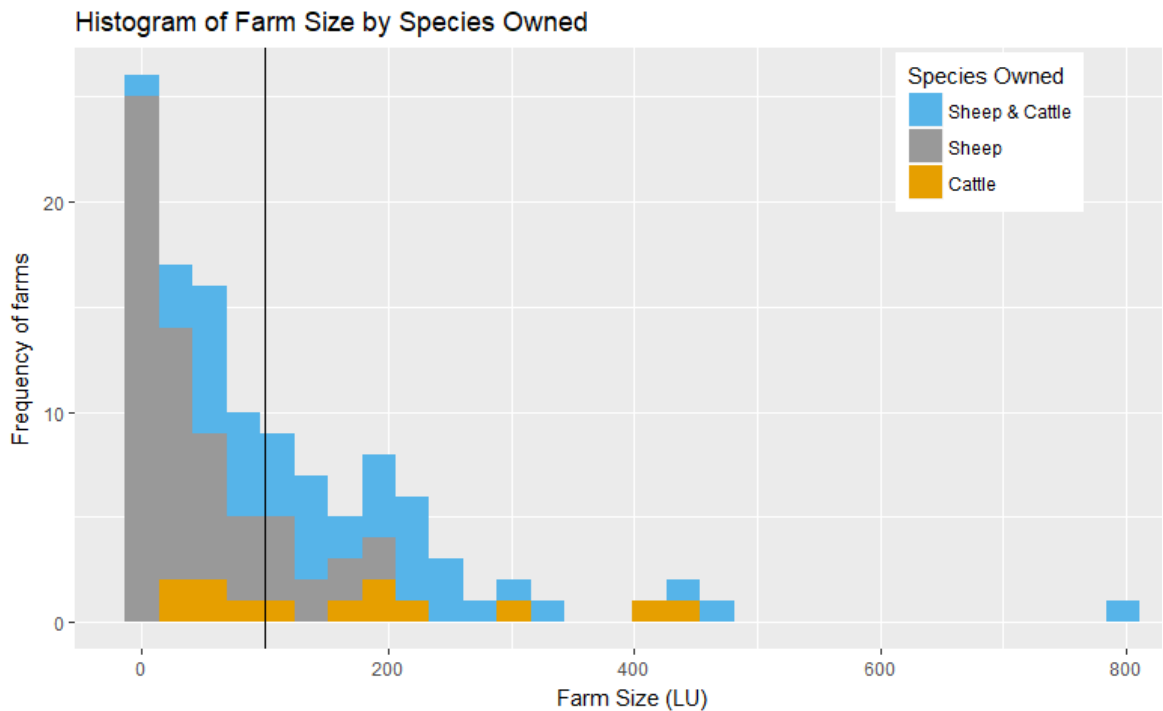
Figure 3.3: A frequency histogram of farm sizes coloured by species owned on farm. LU= Livestock Units. Line separates 'small farms' (n=56) from 'large farms (n=43)

The median number of milking dairy cattle owned by cattle only farmers was 130 (IQR 85-205) whereas the median for those that owned both sheep and cattle was 90 (IQR 68-120). The mean number of dairy cows owned by all farmers was 130 (Figure 3.3).

Only 3 respondents owned both dairy and beef cattle, all of which were from mixed cattle and sheep holdings.

More sheep and beef cattle were owned by farmers that owned both sheep and cattle, whereas a greater number of dairy cattle were owned by those that only owned cattle.

The proportion of owners of pedigree animals responding to the questionnaire was similar across both sheep only and cattle only respondents (Table 3.3). Commercial breeds were more commonly owned than pedigree only, or both pedigree and

commercial stock, for those that owned only sheep or only cattle. Of the owners of

mixed holdings, only one respondent owned pedigree sheep and 6 respondents

owned pedigree cattle, however all owned commercial breeds of the other stock.

Table 3.3: Respondents reporting pedigree/commercial stock by species owned.

Stock owned (n)	Number (%) of participants reporting pedigree/commercial status of stock		
	Pedigree only	Commercial only	Both pedigree & commercial
Sheep only (58)	16 (27.6)	27(46.6)	15 (25.9)
Cattle only (13)	4 (30.8)	5 (38.5)	4 (30.8)
Mixed sheep & cattle (45)	0 (0)	21 (46.7)	24 (53.3)

3.4.3 Previous vaccination history

The proportion of farmers that had previously vaccinated against BTV-8 was higher than those that had previously vaccinated against SBV (Figure 3.4). However, not all respondents vaccinated all of their animals. For those that vaccinated against BTV-8 or SBV, 17.6% and 5.4% of respondents (respectively) stated that they had only vaccinated some of their flock/herd.
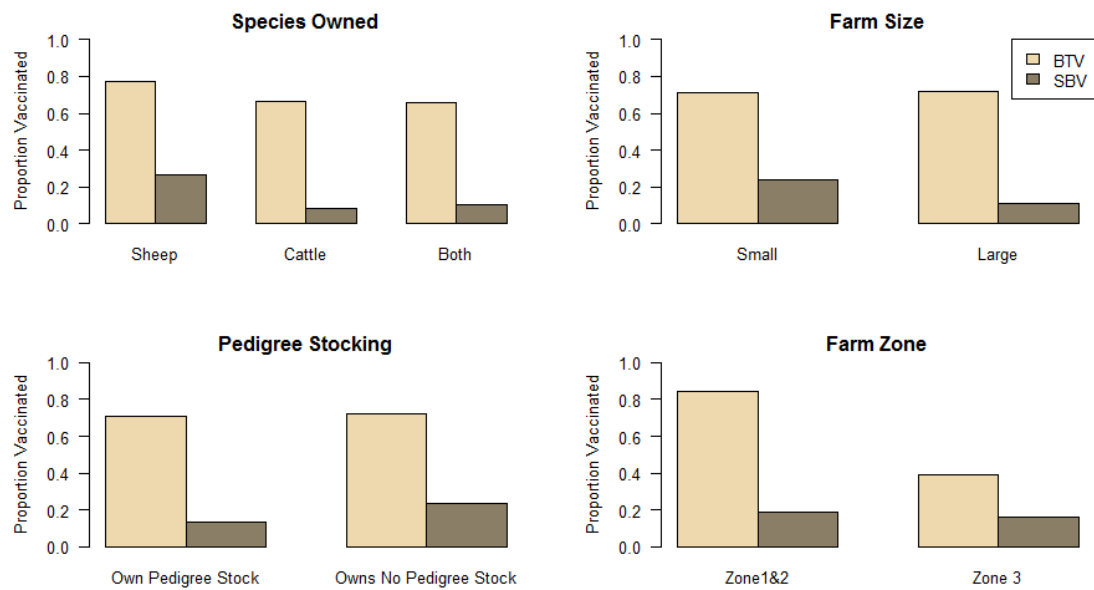
Figure 3.4: Proportion of respondents that reported previously vaccinating against BTV-8 and SBV for the different (a) species owned, (b) size of farm, (c) pedigree stocking, and (d) farm zone. Proportion vaccinated includes those that reported vaccinating only some of their stock.

3.4.4 Willingness to vaccinate

Results of the univariable analysis showed that farmers were significantly (p<0.05) more likely to want to vaccinate if they owned a smaller farm, owned a single species, were located in a previous restriction zone and if they were more risk adverse (Table 3.4). Farmers that had previously vaccinated against BTV-8 were 5.6 times more likely to want to vaccinate again than those that had not previously vaccinated against BTV-8. There was no apparent association between farmers' willingness to vaccinate and owning pedigree-only flocks/herds.

Table 3.4: Univariable analysis of farm demographics and their relationship with respondents' willingness to vaccinate at time of questionnaire.

Variable (number of respondents in group)	Number (%) of farms that would vaccinate now	OR	95% CI	*P* value
Species on Farm				
Sheep (49)*	40 (70.2)			
Cattle (12)	8 (61.5)	0.68	0.19-2.38	0.546
Sheep & Cattle (38)	18 (43.9)	0.33	0.14-0.77	0.01
Farm Type				
Mixed species (38)*	16 (42.1)			
Single species (61)	41 (67.2)	2.82	1.22-6.51	0.014
Location				
Zone 1 & 2 (71)*	48 (67.6)			
Zone 3 (28)	9 (32.1)	0.23	0.09-0.58	0.001
Farm size				
<100LU (56)*	41 (73.2)			
>100LU (43)	16 (37.2)	0.22	0.09-0.51	<0.001
Pedigree status				
No Pedigree (45)*	24 (53.3)			
Own Pedigree (54)	33 (61.1)	1.38	0.62-3.06	0.436
Prior BTV-8 vaccination status				
Didn't Vaccinate (28)*	8 (28.6)			
Vaccinated (71)	49 (69.0)	5.57	2.13-14.57	<0.001
Farmer risk score				
Very Risk Adverse (19)*	15 (78.9)			
Moderately Risk Adverse (36)	28 (77.8)	0.93	0.24-3.62	0.92
Moderately Risky (26)	13 (50.0)	0.27	0.07-1.02	0.054
Very Risky (18)	1 (5.6)	0.02	0.0-0.16	<0.001

. * = baseline, n=99 for each group

Three explanatory variables were retained in the final multivariable model; farm size, previous vaccination history and the risk score of the farmer (Table 3.5). Testing for interaction found no significant interactions between the variables. A Receiver Operating Characteristic (ROC) curve determined a reasonable model fit (ROC area under curve= 0.837) and the Pearson χ^2 goodness of fit test indicted no evidence of a lack of fit (P=0.596).

Table 3.5: Final multivariable model, from 99 full questionnaire responses

Variable	OR	95% CI	P Value
Farm Size	0.21	0.07-0.61	0.004
Prior BTV-8 vaccination status	4.88	1.42-16.73	0.012
Farmer risk score	0.39	0.22-0.69	0.001

3.4.5 Willingness to pay

To examine impact of cost on farmer decision making, the explanatory variables were modelled in an ordinal logistic regression model. Price categories were combined due to low frequency response rates to make the three price categories used as the outcome variable: 1. Would not pay to vaccinate regardless of price, 2. Would vaccinate if the vaccination cost less than 80p, 3. Would vaccinate if the vaccination cost more than 80p. The same three explanatory variables were retained in the final model; farm size, previous vaccination history and the risk score of the farmer (Table 3.8). The assumption of proportional odds for ordinality of the outcome was fulfilled (Likelihood ratio test P= 0.47). The model correctly predicted price outcome approximately 64% of the time.

Owners of smaller farms were approximately four times more likely to vaccinate than owners of larger farms (Table 3.6). Farms that had previously vaccinated were nearly four times more likely to select a higher price category. The odds of a 'Very Risky' farmer stating they would be willing to vaccinate were only 5% that of a 'Very Risk Adverse' farmer.

Table 3.6: Final ordinal model, from 99 full questionnaire responses

Variable	OR	95% CI	P Value
Farm Size	0.19	0.07-0.47	<0.001
Prior BTV-8 vaccination status	3.84	1.26-12.4	0.02
Farmer risk score:			
Very Risk Adverse *ref			
Moderately Risk Adverse	1.47	0.49-4.42	0.49
Moderately Risky	0.41	0.13-1.29	0.13
Very Risky	0.05	0.00-0.36	0.01

3.4.6 Farmer perception of vaccination importance

Just over 85% of respondents stated that they believed vaccination to be important or extremely important to prevent disease within their flocks/herds (Table 3.7). The majority of respondents (80.9%) also stated they believed vaccination was important or extremely important in preventing a larger BTV-8 outbreak in 2007/2008 and 90% of respondents stated they believed it was important or extremely important to keep BTV-8 out of the UK.

A greater proportion of owners of pedigree flock/herds, and owners of small farms, believed vaccination was important or extremely important to preventing disease within their flocks/herds, than owners of non-pedigree flocks/herds and owners of large farms (Appendix IV). Although a smaller proportion of respondents (71.3%) in Zone 3 thought vaccination was extremely important or important in preventing a larger BTV-8 outbreak in 2007/2008, all respondents in Zone 3 believed it was important or extremely important to prevent BTV-8 from entering the UK. Conversely 87.1% of respondents in Zone 1 believed vaccination was important or extremely important in preventing a larger outbreak in 2007/2008, and 85.3% believed it was important or extremely important to prevent BTV-8 from entering the UK.

Table 3.7: Respondents views on the importance of (a) vaccination to prevent disease within their flock/herd, (b) vaccination in preventing a larger BTV-8 outbreak in 2007/2008 and (c) keeping BTV-8 out of the UK.

Question (number of responses)	Proportion (%) of responses				
	Extremely Important	Important	Neither important nor unimportant	Unimportant	Extremely Unimportant
'How important do you believe vaccination is for preventing disease within your flock/herd?' (n=102)	44.1	41.2	4.9	3.9	5.9
'How important do you believe vaccination was in preventing a larger UK BTV-8 outbreak in 2007/2008?' (n=94)	39.4	41.5	6.4	8.5	4.3
'How important do you believe it is to keep BTV-8 out of the UK?' (n=100)	61.0	29.0	5.0	0	5.0

3.4.7 Voluntary uptake of vaccination under different scenarios

The results of the scenario question have been represented graphically as heatmaps (Figures 3.5-3.7). A total of 103 respondents answered for Scenario 1, whilst all other Scenarios provided 102 responses.

Under all 6 scenarios, farmers stated they were more willing to vaccinate all of their stock than just 50% or 10% of them. As the BTV-8 scenarios got closer to the respondents' location (from Scenario 1 to Scenario 6), the percentage of their flock/herd that they would vaccinate increases (Figure 3.5). Full herd/flock vaccination increases from only 11.7% of respondents in Scenario 1, to 22.5% in Scenario 2, 41.2% in Scenario 3, 54.9% in Scenario 4, 66.7% in Scenario 5, and 78.4% in Scenario 6. Despite this, under Scenario 6, where BTV-8 is in the respondents' neighbouring county, 13.7% of respondents would only vaccinate

some of their flock/herd and 2% of respondents would only vaccinate their most valuable animals.
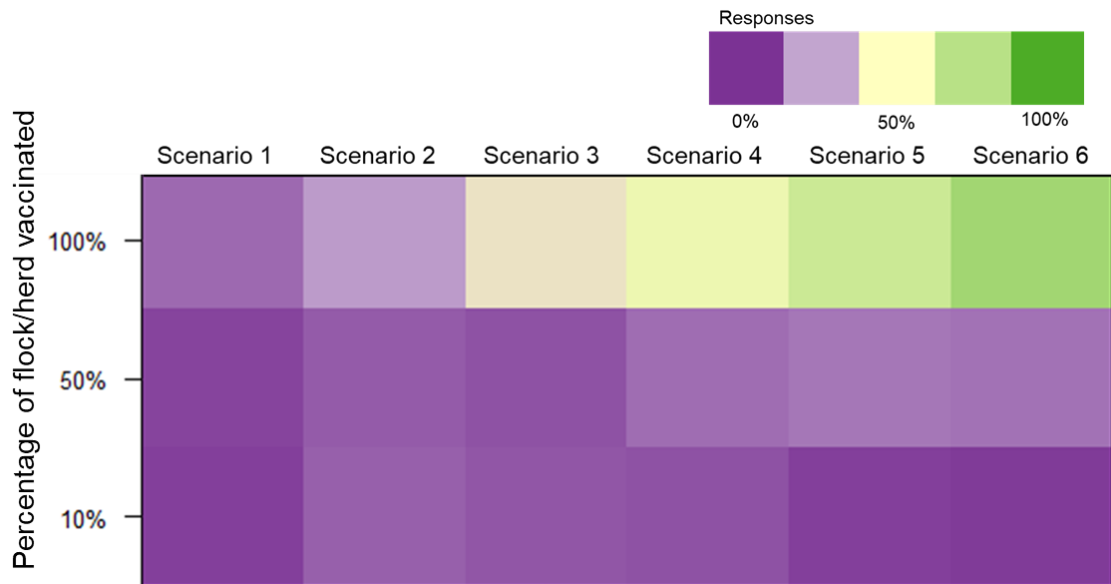
Figure 3.5: Frequency of responses for the percentage of flock/herd that would be vaccinated under each scenario. Percentage of flock vaccinated determined by the farmer selecting the proportion of their flock/herd that they would vaccinate: (All, Some (<50%), Most valuable (<10%)) under Scenarios 1-6 (see Table 1). Number of responses: Scenario 1 (n=103), Scenario 2-6 (n=102).

The price respondents' were willing to pay to vaccinate also increased as the BTV-8 scenarios got closer to the respondents location (Figure 3.6). Only 6.8% of respondents were willing to pay £1 or more per vaccination under Scenario 1. However under Scenario 6 this was nearly 10 times more (65.3%). Interestingly uptake of the middle price (in this case 80p) was low across all scenarios.

If the responses are taken cumulatively, where those that would pay 80p or £1 to vaccinate, would also vaccinate at 40p per dose, then uptake would be just under 20% at Scenario 1 (19.4%), 40.2% at Scenario 2, 55.9% at Scenario 3, 74.5% at Scenario 4 and greater than 80% would be achieved under Scenarios 5 and 6 (84.3, 94.1% respectively).

Pricing at a non-subsidised price (>80p on farm cost per dose in this example) would see uptake drop, with a 20% uptake only reached by Scenario 2 (8.7% Scenario 1, 21.6% Scenario 2), a 50% uptake only reached by Scenario 4 (33.4% Scenario 3, 50% Scenario 4) and an uptake of 80% would not be reached under these prices (64.7% Scenario 5, 76.3% Scenario 6).
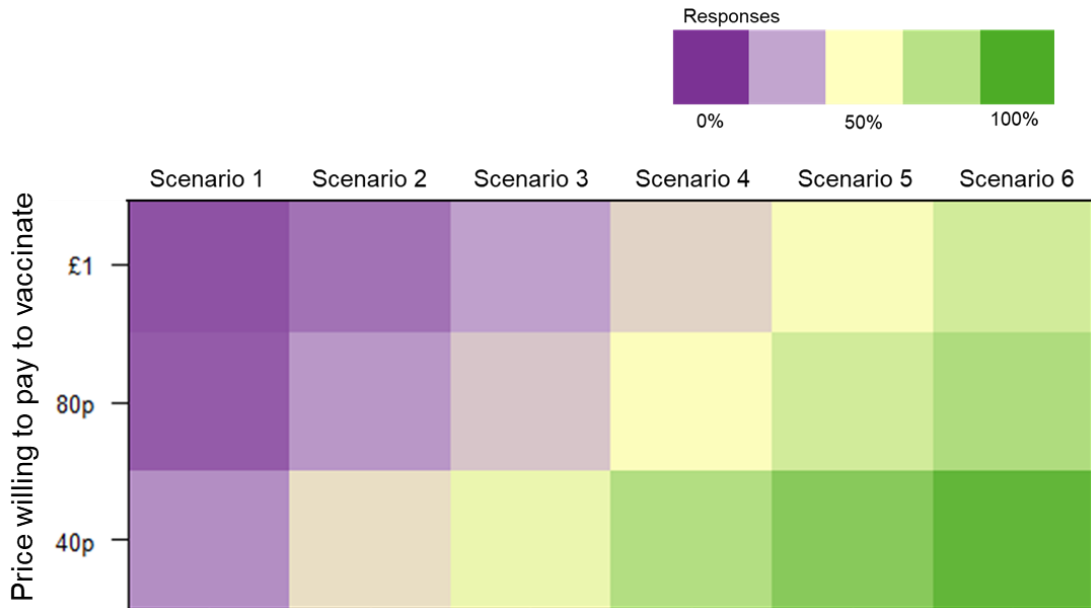
Figure 3.6: Frequency of responses for the price respondents were willing to pay to vaccinate under each scenario. Responses: Scenario 1 (103), Scenario 2-6 (102).

In the final heatmap (Figure 3.7), shading depicts the percentage of flock/ herd vaccinated under the different price and scenario options, combining figures 3.5 and 3.6. The highest proportion of animals that would be vaccinated on the farms was observed under Scenario 6 at a price of £1 or more. The middle price of 80p per dose varied the most in coverage (30-89.3%). The lowest price remained the most stable in terms of coverage, ranging between 72.6% and 88.9%.
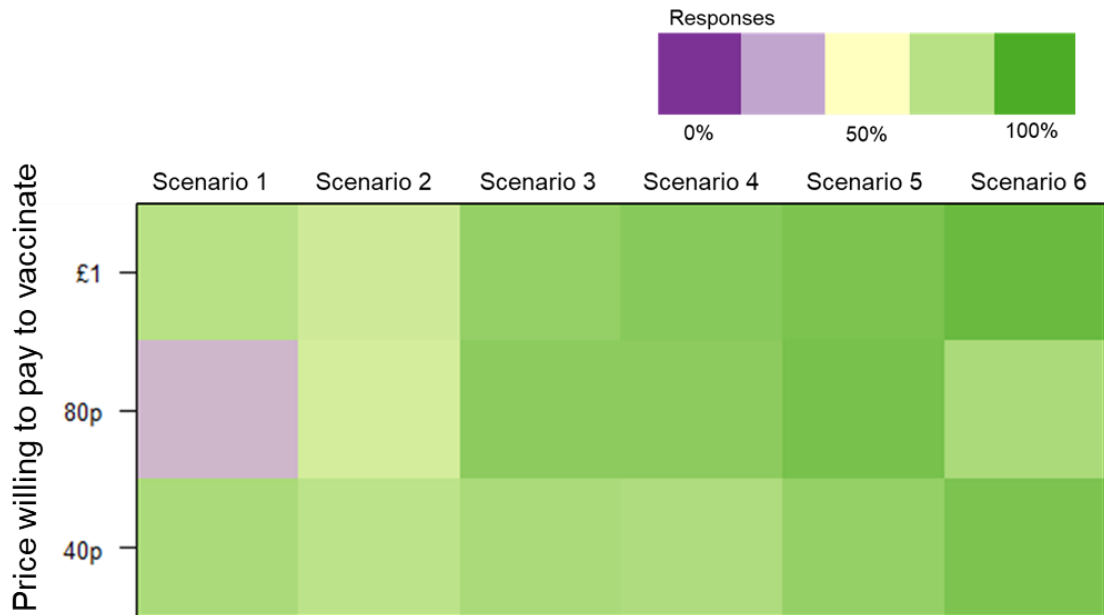
Figure 3.7: The price respondents were willing to pay under the scenarios shaded by the percentage of flock/herd that would be vaccinated (%). Responses: Scenario 1 (103), Scenario 2-6 (102).

In total 5.9% of respondents stated they would not vaccinate at all, at any price, even under Scenario 6: where BTV-8 is confirmed in their neighbouring county. In total 80.6% of respondents stated they would not vaccinate under Scenario 1: BTV-8 stays in central France, spreading to all the southern provinces of France.

3.4.8 Voluntary uptake of free vaccination under different scenarios

Of the 101 respondents that answered this question 10.9% would vaccinate their entire flock/herd under Scenario 1 if the vaccination was free, 31.7% at Scenario 2, 22.8%, at Scenario 3 and 34.6% of respondents would wait until BTV-8 reached the UK (17.8% Scenario 4, 9.9% Scenario 5 and 6.9% Scenario 6).

Taken cumulatively, a 42.6% uptake would be reached by Scenario 2, 65.3% by Scenario 3 and over 80% voluntary uptake would be achieved once BTV-8 reached the UK (83.2% Scenario 4, 93.1% Scenario 5 and 100% Scenario 6).

3.5 Discussion

This study investigated the current demand for vaccine within the UK farming community and identified factors associated with willingness to pay for vaccine. Previous vaccination history against BTV-8 and SBV were described and perceptions towards vaccination explored. The uptake of vaccination under different scenarios was also investigated, determining at which point 25%, 50% and 80% of respondents would vaccinate their flocks/herds.

The relatively low number of responses to the questionnaire limits the study's power, but is highly comparable to other national farmer questionnaires of this length (Cross *et al.*, 2009; Hall and Wapenaar, 2012) (note 1.6 Surveillance techniques). Like other online questionnaires utilising voluntary participation we cannot exclude a motivation response bias. Those that chose to respond may be more interested in vaccinating, or conversely feel negative about previous vaccination schemes (Gethmann *et al.*, 2015). Not all responses could be included in the analysis of all questions due to missing data. This was the result of a trade-off between mandatory responses and drop out, consequently drop out was relatively low, particularly for a questionnaire of this length. In addition, as with many veterinary surveys, the sample of farmers was not randomly selected and therefore the results may not be generalizable to the UK sheep and cattle farming populations. However, for the following reasons the farmers in the sample may be considered typical UK farmers and as such the data presents a useful contribution to our knowledge on farmer's views on BTV and vaccination and raises important issues for the pharmaceutical industry, veterinary profession and government.

Sheep farms were well represented, with lowland, upland, hill, commercial and pedigree flocks all represented in the study. The mean number of sheep owned was

comparable to the UK average (Defra, 2016), whereas smaller cattle farms may have been under-represented by the survey, with a mean of 40 more dairy cows and 11 more beef cattle in this survey than the UK reported average (Defra, 2016). Responses from cattle only farmers were particularly low and consequently the results may be more representative of sheep owning farms. It is equally important to engage cattle farmers and sheep farmers in the context of BTV vaccination. For most BTV strains cattle act as the natural reservoirs, as they are typically asymptomatic and display long viraemias, however during the 2006 BTV-8 outbreak cattle also displayed distinct clinical signs (EFSA, 2007a).

Far more respondents stated they had vaccinated against BTV-8 than for SBV (72.2% compared to 18.8% respectively). This is possibly due to the asymptomatic nature of SBV in adults and the fact that the disease is non-notifiable. Conversely, it could be due to uncertainty regarding the necessity of vaccinating, particularly from respondents that had previously vaccinated against BTV-8.

Of the respondents, more sheep owners stated they vaccinated their animals against both BTV-8 and SBV than cattle only farmers, or those that owned both cattle and sheep. Arguably, sheep are both typically cheaper to vaccinate (normally requiring only one dose of vaccination) and are considered at a greater risk from both diseases, with potentially devastating lambing losses if the first SBV infection coincides with pregnancy, and higher reported BTV-8 morbidity and mortality rates, despite their typically lower financial worth than cattle (EFSA, 2007a; Harris *et al.*, 2014). The number of respondents that stated they had previously vaccinated against BTV-8 is comparable to elsewhere in Europe at the same time (Elbers *et al.*, 2010).

Due to the higher value of pedigree animals and their offspring, it would be expected that pedigree owners would be more likely to vaccinate against BTV-8.

However, this was not observed in this study, where a higher proportion of those owning only commercial stock reported vaccinating against SBV previously than pedigree stock owners. In contrast to surveys of German farmers, respondents from smaller farms in this study were more likely to report that they want to vaccinate against BTV-8 than larger farms (Gethmann *et al.*, 2015).

Companies that manufacture vaccines recommend vaccinating entire flocks/herds to ensure herd immunity, however models should not presume 100% coverage of vaccination against BTV-8 as farmers may not be applying this recommendation on farm. A total of 17.6% of respondents stated they only vaccinated some of their flock/herd when previously vaccinating against BTV-8, and over 15% of respondents stated they would only vaccinate some of their flock/herd (or less) even under the highest risk scenario: 'cases of BTV-8 are confirmed in your neighbouring county'. This has also been described by previous studies, where vaccinating only some animals on farm was determined to be mostly a cost saving exercise (Elbers *et al.*, 2010). This suggests that a voluntary vaccination scheme could be unsuccessful due to low vaccination coverage and has also been noted by European studies (Gethmann *et al.*, 2015). Industry led campaigns should look to raise awareness of this issue, and policymakers and academics should consider this when managing outbreak responses.

It is reassuring to note that respondents were generally willing to pay for vaccination, unsurprisingly, with vaccination uptake increasing for the lower price bracket. However, respondents appeared to evaluate the perceived cost of vaccinating against the perceived risk from disease. Those that had previously vaccinated against BTV-8, and more risk adverse farmers were more willing to pay for vaccination, further highlights this concept. It is important to note that cost is more than just the price of the vaccination; bringing in stock (particularly sheep) can be very time consuming, as can delivering the vaccination (which in itself has

staff/veterinary costs), especially if booster doses are needed later on. Furthermore the farmer may also be taking into account the cost to the animal, such as stress of handling and potential side-effects of vaccination (Garforth *et al.*, 2013), although this may also mean the inverse, protecting their animals from the welfare costs of disease.

The study has shown that the financial cost of vaccination appears to outweigh the risk of not vaccinating for the majority of holdings when the threat is perceived to be low. However, waiting for risk to outweigh cost may result in too little time to deliver vaccine and develop protection, a key concept in the control of BTV-8 that needs to be clearly communicated to farmers and their vets. In this survey less than 40% of respondents would pay top price to vaccinate once BTV-8 reached the UK; lower than the actual uptake reported in the Netherlands in 2009 for a full priced vaccination. However just over 75% would vaccinate at the subsidised prices, similar to the uptake of the subsidised vaccination in The Netherlands in 2010 (Elbers *et al.*, 2010). This is concerning as a risk analysis study in Italy concluded that at least 80% of the susceptible population needs to be immunized to protect the population effectively (Giovannini *et al.*, 2004). The voluntary uptake of a free vaccination by respondents meanwhile would see a reported >80% uptake when BTV-8 reached the UK in this survey. This behaviour is interesting, as when asked how important it is to keep BTV-8 out of the UK 90% of respondents stated important or extremely important.

Although many respondents would wait to vaccinate, potentially putting their farm and their neighbours at risk, the majority of respondents stated they believed vaccination prevented a larger outbreak in 2007/2008. Clearly respondents understand the importance of vaccination in disease control, however perhaps there is a lack of understanding as to how long vaccinations take before livestock become protected, or a lack of knowledge surrounding vector-borne diseases and potential

rate of transmission. Veterinarians play an extremely important role in recommending vaccination to farmers, therefore effective communication between veterinarians and farmers is paramount (Gethmann *et al.*, 2015). However although veterinarians are reported to be seen as credible information sources about vaccination, their advice is not always followed (Garforth *et al.*, 2013). This means that greater farmer engagement should be undertaken at all levels; industry, academia and at policy level, through industry led campaigns (such as the Joint campaign Against Bluetongue (JAB)) and knowledge exchange events.

This survey highlights the complex issues surrounding voluntary vaccination at the farm perceived risk versus cost level. It is apparent that voluntary vaccination would only achieve an 80% uptake if vaccine was free, and only after BTV-8 cases were reported in the UK. This would likely be ultimately too late to protect large numbers of livestock, particularly if conditions were favourable for BTV-8 transmission. Therefore, the key to the success or failure of voluntary vaccination would be the timing of the disease outbreak: a case in the UK outside of the vector period (i.e. an importation case) would likely motivate farmers to vaccinate and a lack of active vectors would result in little disease transmission prior to vaccination protection, however if an outbreak occurred earlier in the season, when vector activity is particularly high, multiple secondary cases would be observed prior to vaccination protection.

Emerging vector-borne diseases are only likely to increase in incidence with increasing global trade and favourable climatic conditions. Inevitably, many of these are likely to come through Europe, placing farmers in the south of England at the forefront of disease prevention. Lethargy of farmers most frequently bearing the cost of preventative vaccination programmes on behalf of the UK livestock industry is likely, and comments to that effect have already been made in this survey. To address this a more inclusive approach is needed: industry could consider

payments to subsidise vaccination, taking a holistic approach to disease prevention and animal welfare as a sector; policymakers should consider the merits of compulsory vaccination programmes, previously subsidised by the European Union, in ensuring national disease prevention, and how this could be made available in the future; greater knowledge exchange should be taking place between vaccine manufacturers, industry stakeholders, policymakers, academics and veterinarians, so that a clear message can be given to farmers as to the risk of emerging and re-emerging vector-borne diseases, when vaccinations should be occurring, and the importance of herd immunity. Knowledge exchange between different groups is particularly important when an outbreak situation is changeable, such as the case with BTV-8.

The appropriateness of voluntary vaccination programmes under changing outbreak situations should be considered; if uptake will not meet the required threshold, or vaccination may occur too late to be preventative, then individual farmers are taking on unnecessary costs. This study suggests that voluntary vaccination in the current case of BTV-8 is unlikely to be efficient. If the threat of BTV-8 transmission from France is determined to be high, then compulsory vaccination, or free vaccination for high risk areas, may be the most effective way of protecting UK livestock.

The current study increases our understanding of farmer motivations to vaccinate. Importantly it highlights factors and trends that are crucial to consider prior to rolling out a voluntary vaccination programme. It is hoped that this study will initiate greater farmer-led discussions prior to disease outbreaks.

Chapter 4

A cross-sectional study of *Culicoides* abundance within lambing sheds over-winter and a longitudinal study inside and outside lambing sheds

4.1 Abstract

Within a five year period two major *Culicoides* borne diseases of ruminants have swept through Europe: bluetongue virus serotype 8 (BTV-8) and Schmallenberg virus (SBV). Both diseases are spread by similar *Culicoides* species, have caused economic and livestock losses, and both managed to overwinter, reappearing the following season. Currently the exact mechanisms for overwintering are unknown, with little evidence for transovarial transmission from adult midges to overwintering larvae. During BTV outbreaks movement restrictions are lifted during vector-free periods. This is briefly defined as less than 5 parous *Culicoides* per trap. This study aimed to investigate the winter activity of *Culicoides* biting midges inside lambing sheds in the south of England. A cross-sectional study was completed between January- April 2016 to establish activity on 21 farms during each farms peak lambing period. The following winter, from November 2016 to April 2017, a longitudinal study was undertaken on 4 farms both inside and outside the lambing sheds. *Culicoides* were found to be active throughout both winters inside lambing sheds. The most abundant species were all putative vector species, with female Obsoletus group *Culicoides* comprising 88.3% of total *Culicoides* caught during the longitudinal study. Parous *Culicoides* were caught every month except January and February in the longitudinal study. Gravid *Culicoides* were caught every month, with the exception of February. This provides strong evidence for ongoing *Culicoides* activity throughout the winter, and therefore demonstrated the potential for ongoing virus transmission throughout the winter.

4.2 Introduction

Within a 10 year period two diseases of ruminants transmitted by *Culicoides* biting midges have emerged, and then re-emerged in northern Europe: Bluetongue virus serotype 8 and Schmallenberg virus (Balenghien *et al.*, 2014; Carpenter *et al.*, 2009). Both diseases have been linked to high economic losses to the sheep and cattle industries, and have negatively impacted animal welfare (Alarcon *et al.*, 2014; Harris *et al.*, 2014; Martinelle *et al.*, 2014; Nusinovici *et al.*, 2013; Pinior *et al.*, 2015; Veldhuis *et al.*, 2014b; Velthuis *et al.*, 2010).

SBV is a novel Orthobunyavirus of the Simbu serogroup (family: *Bunyaviridae*) (Hoffmann *et al.*, 2012). Like other viruses within the Simbu serogroup , SBV is teratogenic if infection of a naïve ruminant coincides with the vulnerable period of gestation (Beer *et al.*, 2013; Lievaart-Peterson *et al.*, 2012). The viraemic period is typically very short (ca.3-5 days) and only very mild clinical signs, if any, are reported for adult ruminants (Laloy *et al.*, 2015; Wernike *et al.*, 2013a, 2013b). SBV spread rapidly across Europe, with particularly high losses reported in early lambing sheep in 2011/2012, and again the following 2012/2013 lambing season (Afonso *et al.*, 2014). Despite a period of extremely low, if any, circulation of SBV since 2014, reports of SBV circulation have once again emerged from Europe, with losses reported again throughout the 2016/2017 lambing season (Collins *et al.*, 2017; Delooz *et al.*, 2016; Sohier *et al.*, 2017).

Five years prior to the 2011 SBV outbreak, bluetongue virus serotype 8 (BTV-8) had suddenly emerged in the same north western region of Europe (Koenraadt *et al.*, 2014; Veldhuis *et al.*, 2016) (sections 1.3.8 Schmallenberg virus: Distribution and 1.4.9 Bluetongue virus: BTV-8 2006 outbreak). The European outbreak of BTV-8 in 2006 was at the time the most northerly outbreak of any BTV serotype (Wilson and

Mellor, 2009). BTV-8 is an *Orbivirus* (family: *Reoviridae*) which is known to cause haemorrhagic disease in sheep, goats and deer (Coetzee *et al.*, 2014). Cattle can act as reservoirs for the disease, but are also associated with clinical disease, although less commonly than sheep. BTV-8 was unusual, however, in there being a marked incidence of clinical disease, including mortality, in cattle (Nusinovici *et al.*, 2013; Thiry *et al.*, 2006; Vercauteren *et al.*, 2008). The viraemic period for BTV-8 can be prolonged (note 1.4.5.2 Bluetongue virus: Viraemic period) but is not necessarily persistent and is considered to have an insufficient duration to overwinter in a single animal (EFSA, 2008). Due to the severe socioeconomic losses associated with BTV-8 and potential for rapid spread, the World Organisation for Animal Health (OIE) records BTV as a listed disease. Outbreaks require the implementation of movement restriction zones, surveillance zones and many countries implement vaccination programmes. These all have high costs, not least the impact of movement restrictions on trade (Tago *et al.*, 2014).

Culicoides are not active the entire season, with the typical seasonality of UK species between April and November (further discussed in section 1.2.4.1 Vector-borne diseases: British *Culicoides* species that are vectors) (Sanders *et al.*, 2011b). To reduce the burden of these restrictions during a multi-year outbreak, a period of movement in the winter is allowed during the defined 'vector free period' (further outlined in 1.4.7 Bluetongue virus: Control measures and vaccination). One of the specific criteria to determine this is as follows:

"Captures of *Culicoides* species proven or suspected to be the vectors of the serotype present in the epidemiologically relevant geographical area below a maximum threshold of vectors collected that shall be defined for the epidemiologically relevant geographical area. In the absence of sound evidence supporting the determination of the maximum threshold, total absence of *Culicoides*

imicola specimens and less than five parous *Culicoides* per trap must be used."
(European Commission, 2007)

In other words, up to 4 parous *Culicoides* can be caught in traps during the so-called 'vector free period' without affecting its status. This threshold is somewhat arbitrary, having been adapted from the original surveillance systems of the Mediterranean basin, designed after incursions of BTV-1, BTV-2, BTV-4 and BTV-16 (1998 onwards) (Carpenter *et al.*, 2009; Wilson and Mellor, 2009). The stipulation of parity is to exclude young *Culicoides* that have yet to take a blood meal (nulliparous), as transovarial virus transmission has yet to be demonstrated and they cannot, therefore, present an infection risk (Meiswinkel *et al.*, 2008a; Mellor, 1990; Wilson *et al.*, 2008). It should be noted that the use of pigmentation to infer parity is becoming increasingly controversial. Newly emerged *C.obsoletus* and *C.imicola* have been observed in separate field studies with pigmented abdomens consistent with the usual definition of 'parous' (Braverman and Mumcuoglu, 2009; Harrup *et al.*, 2013). Despite the use of pigmentation to denote parity likely resulting in the overestimation of parous individuals (particularly near emergence sites) it is currently the best technique for estimating parity and certainly the most feasible to apply to large catches (Harrup *et al.*, 2013).

Culicoides imicola is an afro-tropical species, found between ca.46°N and 35°S, with the European distribution considered limited to the Mediterranean basin (Conte *et al.*, 2009; Versteirt *et al.*, 2017). As such *C.imicola* is not found in the UK. Other known vector species of BTV-8 include the Palaearctic Obsoletus group *Culicoides* (*Culicoides obsoletus, Culicoides scoticus, Culicoides dewulfi* and *Culicoides chiopterus*) and *Culicoides pulicaris,* all of which are common and abundant on UK farms (Carpenter *et al.*, 2006a, 2008a; Dijkstra *et al.*, 2008; Meiswinkel *et al.*, 2007; Vanbinst *et al.*, 2009). These species are also considered vectors of SBV, along with *C.punctatus* and *C.nubeculosus* (Balenghien *et al.*, 2014; De Regge *et al.*,

2012; Elbers *et al.*, 2013a; Goffredo *et al.*, 2013; Larska *et al.*, 2013b, 2013c; Rasmussen *et al.*, 2012).

Since the 2006 BTV-8 outbreak far more research into these Palearctic *Culicoides* species has been undertaken; breeding grounds have been better defined, including the description of breeding grounds inside animal enclosures (Losson *et al.*, 2007; Steinke *et al.*, 2016; Zimmer *et al.*, 2008, 2013a, 2014); climatic variables associated with *Culicoides* feeding rates have been explored (Baylis *et al.*, 2010; Versteirt *et al.*, 2017) and the extrinsic incubation period within colonised species at different temperatures described (Carpenter *et al.*, 2011; Wilson *et al.*, 2008) (discussed in greater depth in sections 1.2.2 *Culicoides*-borne diseases: *Culicoides* as vectors and 1.2.3 *Culicoides*-borne diseases: Climatic variables).

However there is still much to be understood surrounding the overwintering mechanisms of these diseases within the UK. If transovarial transmission of these viruses does not exist within *Culicoides*, and viraemia does not persist within the adult hosts, then survival of the virus is most likely due to on-going low level vector activity during the winter (Losson *et al.,* 2007; Tarlinton *et al., 2012)*. So far other over wintering studies in Europe have demonstrated varying levels of *Culicoides* activity over the winter months, with several describing threshold temperatures prior to the trapping of adult *Culicoides*: mean weekly temperatures of ca.10°C in Germany and Austria (Baldet *et al.*, 2008; Brugger *et al.*, 2016; Clausen *et al.*, 2009; Kameke *et al.*, 2017; Meiswinkel *et al.*, 2008a, 2014). Several authors have even described collections of parous *Culicoides* prior to spring emergence (Baldet *et al.*, 2008; Baylis *et al.*, 2010; Clausen *et al.*, 2009).

Currently the over winter activity of *Culicoides* in the UK remains largely undefined. This study initially aimed to determine if *Culicoides* were active inside lambing

sheds during the winter of 2015/2016 in the south of England. To expand on this study a longitudinal study was undertaken the following year (2016/2017) to:

1. Compare the *Culicoides* activity across years of two farms with 'low *Culicoides* activity' to two farms with 'relatively high *Culicoides* activity' from a geographically comparable area.

2. Allow the comparison of *Culicoides* activity indoors and outdoors once a month for 6 months and to determine if observed *Culicoides* activity was greater indoors than outdoors.

3. To assess the parity rate indoors and outdoors across the winter months.

4.3 Methods

4.3.1 Study period, area and sites: Cross sectional study January-April 2016

A total of 21 sheep farms in the south of England enrolled on the study (Figure 4.1). Insect collections took place between January 2016 and April 2016. Samples were collected over 1 week on each farm during each farms 'peak lambing' period; as peak lambing varied between farms the number of collections each month was not equal.

The degree of barn open-ness (i.e. the number of walls and openings that insects could enter through) was recorded for all farms, as was the number of sheep near to the trap on both set up and take down. The distance from each trap to other species, water, hedges and other favourable *Culicoides* emergence habitat was also recorded for each trap.

All traps were located within 10m of a body of standing water (trough, standing water or waterlogged ground) and within 50m of dung heaps.
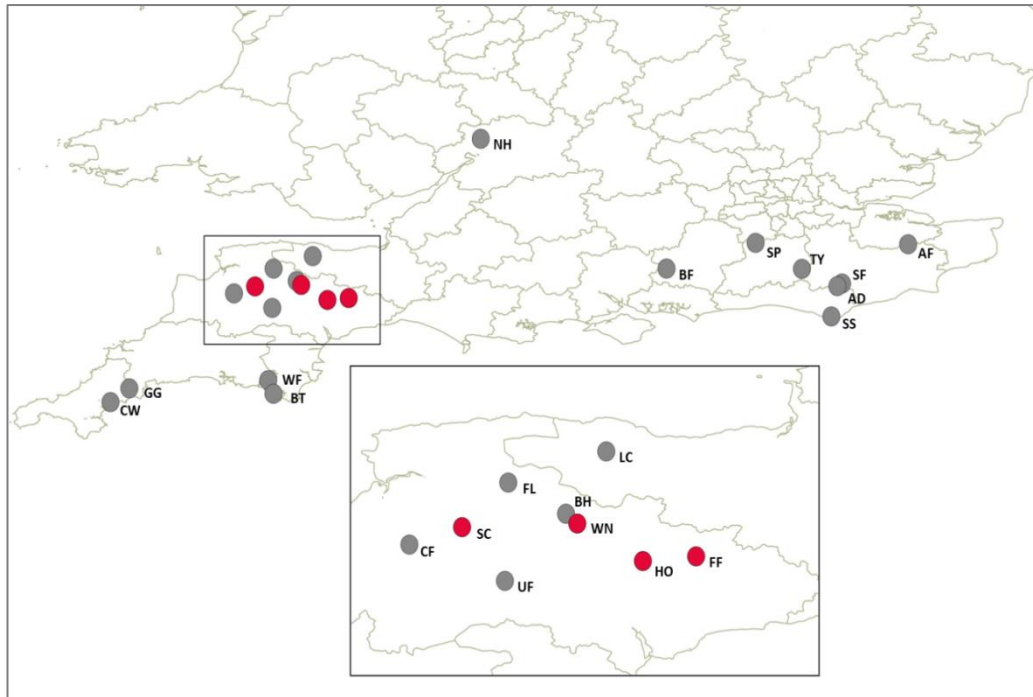
Figure 4.1: Location of farms in the south of England. Red farms are those enrolled both years.

4.3.2 Study period, area and sites: Longitudinal study November 2016- April 2017

A subset of four farms were selected from the initial 2015-2016 cross-sectional study (Figure 4.1). These farms were selected based on proximity to each other and species present (all farms have sheep and cattle on their premises), previous *Culicoides* abundances (Table 4.1: Results section 4.4) and previous Schmallenberg virus history (FF and SC reported no previous suspected SBV cases, HO and WN reported previous suspected SBV on farm). *Culicoides* were collected simultaneously across all farms over 2 nights once a month from November 2016 to April 2017. Again data on livestock abundance, sheep breed, farm characteristics, habitat characteristics and weather and temperature data were collected.

4.3.3 *Culicoides* collection and identification

On all farms *Culicoides* were trapped using mains-only Brandenburg down-draught black light traps, operated to run continuously throughout the study period inside the lambing sheds as previous studies had noted indoor activity of *Culicoides* continued throughout the day (Figure 4.2) (Brugger *et al.*, 2016). For the 2016-2017 longitudinal study indoor traps were placed in the same location as in the previous study. For outside collections additional Brandenburg down-draught black light traps were set up outside of the lambing sheds in fields likely to have animals in for the majority of the study period and within 15m of the lambing shed.

Figure 4.2: Example placement of the Brandenburg down-draught black light trap within a lambing shed.

Samples were collected into beakers containing approximately 150ml of tap water with a drop of detergent to reduce surface tension. Once collected each sample was transferred to 70% ethanol for identification and storage. *Culicoides* were separated using a stereomicroscope from non-*Culicoides* bycatches by morphological features of the wing. *Culicoides* were then separated by wing patterns to species or group level (in the case of the morphometrically cryptic Obsoletus group species: *Culicoides obsoletus, C.scoticus, C.dewulfi* and *C.chiopterus*). Female *Culicoides* were further physiologically characterised by the pigmentation of their abdomen: nulliparous, parous, blood-engorged or gravid (Dyce, 1969).

4.3.4 Weather data

Carbon-51 USB data loggers (Sensormetrix, Reading, UK) were installed within 1m of all indoor traps. The data loggers measured temperature (±0.3°C) and relative humidity (RH) (±3%) every 30 minutes. Outside the barns for the longitudinal study additional wind data loggers (APRS World, LLC, Winona, USA) were installed at 1.5-2m to measure temperature (±0.5°C), relative humidity (±2%), wind speed (±0.1m/s) and wind direction. Wind data loggers recorded at one minute intervals.

4.3.5 Analysis

Farm characteristics, weather data and *Culicoides* species abundances were entered into two Excel documents representing the 2016 cross sectional study and the 2016-17 longitudinal study. Indoor Trapping Rates (ITR) were calculated as total *Culicoides* caught indoors divided by the total *Culicoides* caught both inside and outside, multiplied by 100 (Baldet *et al.*, 2008). All statistical analysis were conducted in R version 3.4.1 (R Core Team, 2017). Spearman's rank correlation coefficient (ρ) was utilised to test the association between numbers of *Culicoides* caught and temperature, humidity and animal numbers as the test is considered robust against outliers and *Culicoides* numbers were not normally distributed

(Shapiro-Wilk test). For categorical data Pearson's Chi-squared (χ^2) tests were completed. A Wilcoxon Signed rank test (*Z*) was used to compare total numbers of *Culicoides* caught inside and outside barns in the 2016/2017 longitudinal study rather than a traditional t-test as the data was found to be not normally distributed (Shapiro-Wilk test). All maps were created in QGIS version 2.2.0 (QGIS Core Development Team, 2017).

4.4 Results

4.4.1 Cross sectional study January-April 2016

Peak lambing was not evenly distributed amongst the farms, resulting in two farms being sampled in January, 4 farms in February, 9 in March and 6 in April (Table 4.1). All traps were within 5m of sheep during the week, with the exception of farm AF which was placed within 20m of the nearest sheep due to restrictions in power source location.

Temperatures inside the barns varied, with lowest average temperatures recorded in February and highest average temperatures recorded in barns in January and April (Table 4.1, Figure 4.3). There was no observed correlation in this study between total female *Culicoides* caught and temperature, humidity or animal abundance (Spearman's rank; P=>0.05).

In total 17,534 *Culicoides* were collected, comprising of the Obsoletus group (19.4%), *C.pulicaris* (79.1%), *C.punctatus* (1.4%), *C.impunctatus* (<0.01%), *C.clastrieri* (<0.01%), and the Achrayi group (<0.01%). A particularly large catch of *C.pulicaris* (n=11,324) occurred on farm AD in April, however *C.pulicaris* abundance was greater than Obsoletus group abundance on 8 of the 21 farms in March and April (Table 4.1).

Culicoides were trapped every month; however no *Culicoides* were recovered from farms NH or LC over the week of trapping. Only Obsoletus group *Culicoides* were recovered from farms HO, WN and SF (Table 4.1). The greatest species diversity was observed on farm FL, which also had the highest sheep breed diversity (over 6 breeds recognised), the greatest number of sheep in close proximity (n=500) and nearby cattle (n=30, within 10m). Female *Culicoides* represented 99.5% of all *Culicoides* caught, of which 10.9% were parous (n=1897), 0.1% gravid (n=19) and 0.2% blood fed (n=26). Parous Obsoletus group *Culicoides* were trapped in January (n=4), March (n=3) and April (n=38). No parous *Culicoides* of any species were caught in February. As traps were collected weekly rather than nightly a mean trap rate per night has had to be calculated (Figure 4.3). This is not ideal as the abundance of *Culicoides* is known to vary drastically between nights. The cut off threshold for *C.pulicaris* was met on farms AD, SP and TY in April. It is possible that more than 5 or more parous *C.pulicaris* were trapped in any one night on farms AF (April), FL and GG (March), however when averaged across the week this was below the threshold. The threshold was not met for any other species during the study period.

Table 4.1: List of farms sampled and the month during which sampling was undertaken to coincide with peak lambing (geographical locations in Figure 4.1). Average, minimum and maximum temperatures were recorded inside each lambing shed, and the total number of species collected is reported. 'Other species' includes Achrayi group species (n=2 FL, n=1 UF), *C.impunctatus* (n=1 TY) and *C.clastrieri* (n=1 FL).

Farm (Month)	Avg Temp (°C)	Min-Max Temp. (°C)	Obsoletus group (% total)	C.pulicaris (% total)	C.punctatus (% total)	Other species (% total)	Total
HO (Jan) †	9.3	2.2-13.9	18 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	18
WF (Jan)	10.3	4.2-14.2	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2
NH (Feb)	4.7	-2.6-15	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
LC (Feb)	4.7	0-10.7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0
WN (Feb) †	5.2	-0.3-10.1	5 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	5
SF (Feb)	4.1	0-10.7	7 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	7
CW (Mar)	9	5.2-10.1	24 (77.4)	7 (22.6)	0 (0.0)	0 (0.0)	31
GG (Mar)	8.7	4.9-16.5	30 (50.8)	26 (44.1)	3 (5.1)	0 (0.0)	59
BT (Mar)	8.9	4.7-12.9	5 (83.3)	1 (1.7)	0 (0.0)	0 (0.0)	6
FF (Mar) †	7.8	4.3-14.2	8 (38.1)	12 (57.1)	1 (4.8)	0 (0.0)	21
SC (Mar) †	7.8	4.1-16.3	1 (25.0)	2 (50.0)	1 (25.0)	0 (0.0)	4
UF (Mar)			0 (0.0)	3 (75.0)	0 (0.0)	1 (25.0)	4
CF (Mar)			2 (4.5)	27 (61.4)	16 (36.4)	0 (0.0)	45
FL (Mar)			6 (21.4)	12 (42.9)	7 (25.0)	3 (10.7)	28
BH (Mar)			4 (80.0)	1 (20.0)	0 (0.0)	0 (0.0)	5
AD (Apr)	9.1	2.4-16.2	1734 (13.1)	11324 (85.8)	135 (1.0)	0 (0.0)	13,193
TY (Apr)	9.3	2.9-15.6	521 (24.8)	1552 (74.0)	23 (1.1)	1 (0.1)	2098
SP (Apr)	8.7	0.1-15.2	986 (51.2)	889 (46.2)	50 (2.6)	0 (0.0)	1925
SS (Apr)	10.7	4.7-20.2	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	3
BF (Apr)	9.1	3.3-19.8	47 (87.0)	7 (13.0)	0 (0.0)	0 (0.0)	54
AF (Apr)	8.3	0.3-17.3	4 (14.8)	7 (25.9)	16 (59.3)	0 (0.0)	27

† indicates farms followed up the following lambing (2016-2017). Temperature data missing for farms UF, CF, FL and BH (data collection failed)
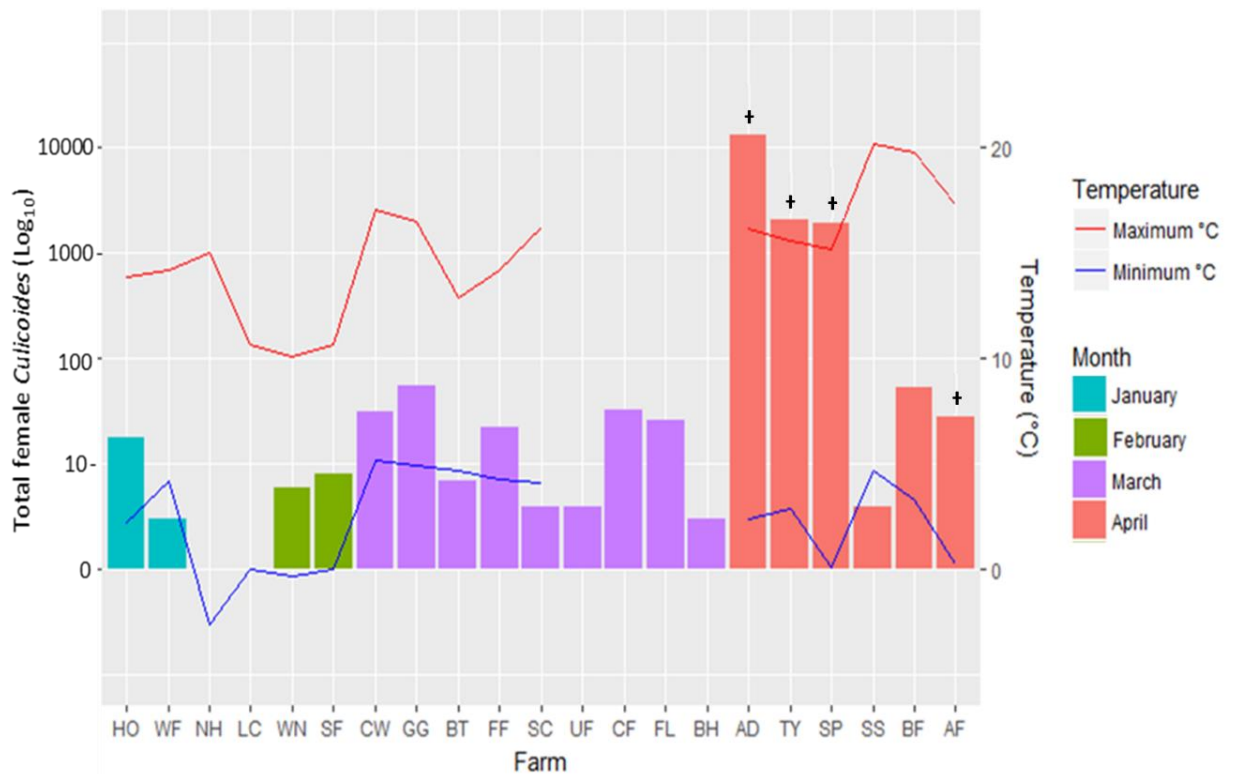
Figure 4.3: The total female *Culicoides* abundance trapped in the lambing sheds for each farm. Minimum and maximum temperatures are reported for the weeks trapping inside each lambing shed. ᵗdenotes farms where the threshold of ≥5 parous *Culicoides* was exceeded. As collections were over 1 week, this represents >28 parous *Culicoides* were collected. Temperature data missing for farms UF, CF, FL and BH. Note: abundance were plotted using a Log_{10} scale

4.4.2 Longitudinal study November 2016- April 2017

All traps (indoor and outdoor) were within 5m of sheep or cattle, with the exception of HO in March (sheep 11-25m away) and SC in December (sheep 25-50m away). There was a diverse range of potential *Culicoides* breeding grounds (leaf litter, dung/manure, leaking water troughs, standing water and/or drainage channels) on all farms, with all traps placed within 10m of any one possible breeding ground source, within 50m of hedgerows and within 100m of woodland.

The minimum temperature recorded outdoors during the study period was -2°C (SC in January), with a maximum of 18.2°C (FF March), whilst the indoor temperature ranged between 0°C and 22.7°C (SC in February and WN in March). As expected,

the average temperatures across all farms were consistently warmer indoors than
outdoors across all sites and months (Table 4.2).

Table 4.2: Combined minimum, maximum and average temperatures for indoors and outdoors over the trapping periods each month.

Month	Indoors			Outdoors		
	Min temp	Max temp	Avg temp	Min temp	Max temp	Avg temp
November	5.4	8.4	6.7	3.3	9.7	6.2
December	7.6	12.4	10.6	6.2	11.4	10.2
January	0.3	8.6	5.7	-2	8.1	3.3
February	0	8.9	5.0	-1.9	11	3.5
March	7.2	15.4	11.6	6	17.9	9.6
April	7.6	12.4	10.6	1.7	15.8	8.6

All temperatures given in degrees Celsius.

A total of 46 collections were made over 6 months (November 2016-April 2017). In
total 540 *Culicoides* were caught belonging to the Obsoletus group (88.3%), or
species *Culicoides pulicaris* (8.5%) and *Culicoides punctatus* (3.1%) (Table 4.3).
Female *Culicoides* represented 91.5% of the total catch, of which the majority were
nulliparous (86.2%). Obsoletus group species were the most common *Culicoides*
found on every farm and comprised more than 80% of all individuals caught both
inside (91.2%) and outside (81.0%) (Table 4.3). A greater proportion of *C.pulicaris*
and *C.punctatus* were trapped outdoors (15.0% and 3.9% respectively) than indoors
(5.9% and 2.8% respectively).

The median number of female Obsoletus group *Culicoides* caught was significantly
higher indoors (6) than outdoors (1) (Wilcoxon signed rank test Z=102.5; P=0.017),
as was the maximum catch (both indoors (n=106) and outdoors (n=39) occurred in
March on farm FF) (Figure 4.4). The ITR for Obsoletus group species (74.0) was
higher than the ITR *for C.pulicaris* (50.0) or *C.punctatus* (64.7). All *C.punctatus*
males trapped in the study were trapped indoors (ITR 100.0) and more Obsoletus
group males were trapped indoors than outdoors (ITR 87.8). The ITR for Obsoletus
group *Culicoides* was not significantly different between the farms: HO (69.2), FF

(70.7), WN (77.3), and SC (79.6) (χ²=1.0, *P*=0.795). Only Obsoletus group

Culicoides were trapped on farm HO throughout the study period, whereas the

highest diversity was observed on farm WN (Table 4.3).

Table 4.3: Total number and species of *Culicoides* trapped both indoors and
outdoors on each farm.

Farm	Trap location	Obsoletus group (% total)	C.pulicaris (% total)	C.punctatus (% total)	Total *Culicoides* trapped
HO	Outdoors	22 (100)	0 (0.0)	0 (0.0)	22
	Indoors	49 (100)	0 (0.0)	0 (0.0)	49
FF	Outdoors	53 (88.3)	6 (10.0)	1 (1.7)	60
	Indoors	128 (98.5)	1 (0.8)	1 (0.8)	130
WN	Outdoors	30 (62.5)	13 (27.1)	5 (10.4)	48
	Indoors	102 (82.3)	13 (10.5)	9 (7.3)	124
SC	Outdoors	19 (82.6)	4 (17.4)	0 (0.0)	23
	Indoors	74 (88.1)	9 (10.7)	1 (1.2)	84
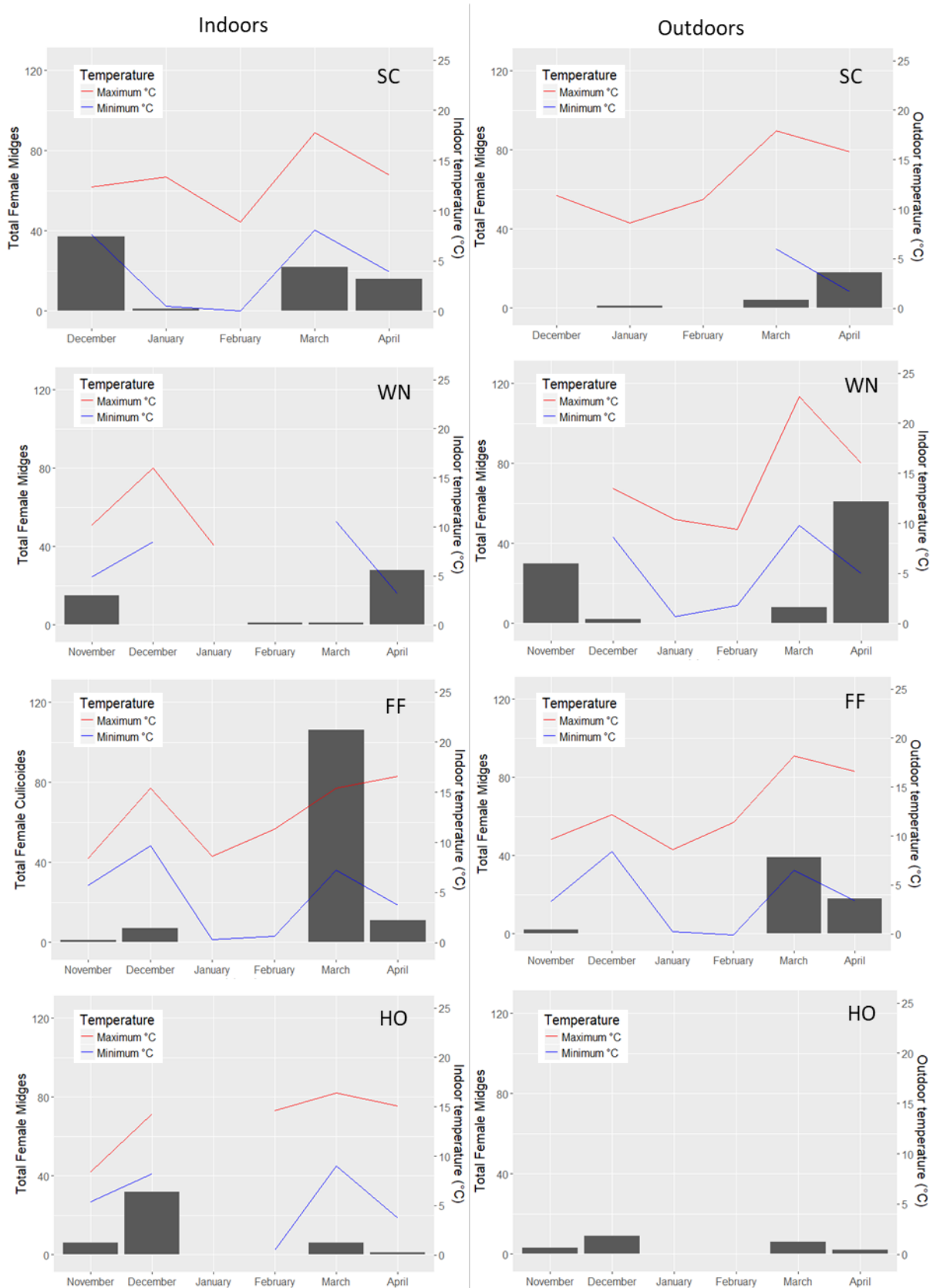
Figure 4.4: Total female *Culicoides* caught indoors and outdoors on each farm by month of trapping. Breaks in weather data represent issues with (2) power, (2) corrupted data.

Culicoides were trapped every month, but the threshold of >5 parous

*Culicoides/*night was only met outside on one farm: WN in November (Figure 4.4).

No parous *Culicoides* were caught on any farm in January or February. These two

months represented very low catches (a total of 2 and 1 *Culicoides* respectively),

interestingly both of the *Culicoides* caught in January were gravid Obsoletus group,

both collected from farm SC (1 indoors, 1 outdoors) (Figure 4.5). Only 1 blood fed

Obsoletus female was trapped (indoors on SC in April); gravid Obsoletus group

Culicoides were found indoors every month except February. Gravid Obsoletus

group *Culicoides* were only caught outdoors on HO in December and April (Figure

4.5). Parous *Culicoides* were caught in November, December and April both indoors

and outdoors. An additional parous Obsoletus group *Culicoides* was caught indoors

in March (Figure 4.5).
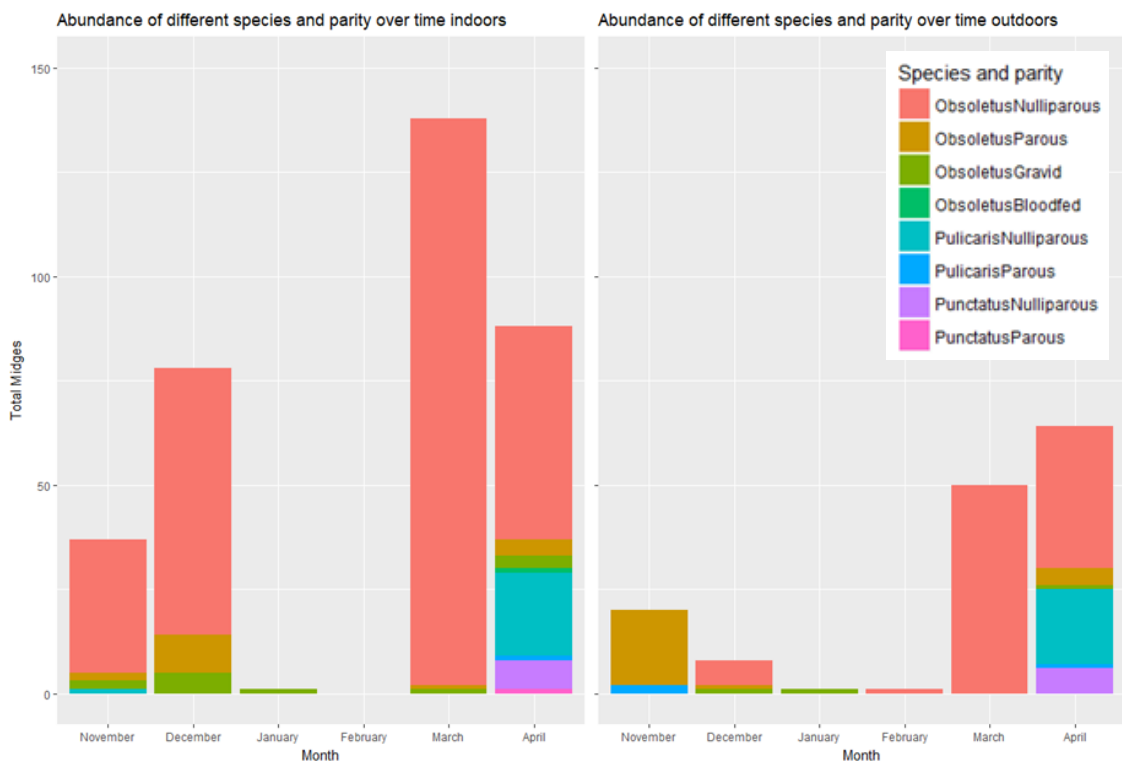
Figure 4.5: Abundance of *Culicoides* species and their parity status by month for both indoors and outdoors.

There was a moderate correlation between total female *Culicoides* caught and minimum and maximum temperature (Spearman's rank $\rho=0.46$; $P<0.01$ and Spearman's rank $\rho=0.59$; $P<0.001$ respectively). The correlation with minimum temperature was more significant for the indoor catches than outdoor (indoor: Spearman's rank $\rho=0.67$; $P<0.01$, outdoor $P>0.05$), whilst the reverse was true for the maximum temperature (indoor $P>0.05$, outdoor Spearman's rank $\rho=0.58$; $P<0.01$). No correlation was found between total female *Culicoides* caught and minimum RH or total sheep present indoors or outdoors. There was a moderate significant negative association between the total female *Culicoides* trapped outdoor and outdoor average RH (Spearman's rank $\rho=-0.53$; $P=0.03$).

Farms had been selected from the previous study as having 'low *Culicoides* activity' (farms WN and SC), or 'relatively high *Culicoides* activity' (farms HO and FF). There was no significant difference in the number of *Culicoides* caught throughout the study between the previous years 'low' or 'relatively high' *Culicoides* activity farms indoors, outdoors or in total (χ^2; $P>0.5$).

4.5 Discussion

This study provides evidence for continued *Culicoides* activity throughout the winter months within lambing sheds in the south of England. However, this study cannot determine the length of the vector free period, or daily *Culicoides* activity during the trapping period, as sampling was completed in blocks over several days and trapping periods represent either snapshots for individual farms, or once monthly repeated sampling. As the most abundant species of *Culicoides* observed in this study are known vectors of both SBV and BTV-8, this study highlights a potential mechanism for overwintering of these viruses within the south of England that needs to be further explored.

Culicoides were found to be active throughout winter in both years. Previous studies have also reported constant activity of *Culicoides* indoors during the winter, adding weight to active *Culicoides* vectors acting as a mechanism for the overwintering of viruses (Clausen *et al.*, 2009; Losson *et al.*, 2007). A weekly average threshold of ca.10°C has been previously described ahead of adult *Culicoides* activity (Kameke *et al.*, 2017; Lühken *et al.*, 2015). The maximum temperatures recorded inside all lambing sheds in 2016 surpassed 10°C, even when the average temperatures dropped considerably in February. Conversely in the 2016-2017 study maximum temperatures indoors ranged between 8.4-15.4°C, with average temperatures only 0.2-2°C warmer indoors than outdoors. December and March were particularly mild in the 2016-2017 study period; both averaged >10°C indoors and December averaged 10.2°C outdoors. Prior studies have also demonstrated activity possible despite below zero temperatures, provided the freezing period is relatively short; with an average of 20 midges per trap per night recorded in November 1992 and March 1993 at Pirbright (Surrey, UK) despite minimum temperatures well into the

minus degrees and the lowest maximum temperature remaining below 10°C the entire winter (Rawlings and Mellor, 1994).

Interestingly there was no difference in *Culicoides* abundance between the farms enrolled on the longitudinal study. The farms had been specifically chosen for direct comparison; all farms had both sheep and cattle present on farm, with multiple potential breeding sites in close proximity to all traps; the farms were geographically close, to reduce the difference in outdoor climatic conditions, and to allow the synchronous deployment of traps (difference between first and last trap approximately 3 hours at midday). This in turn allowed a reliable comparison of the indoor and outdoor catches. This study found total Obsoletus group *Culicoides*, *C.pulicaris* and *C.punctatus* to be more abundant indoors than outdoors throughout the winter, suggesting no strong exophily in these species. Others have suggested that *Culicoides*, particularly Obsoletus group *Culicoides*, are neither purely exophilic or endophilic, instead reacting to environmental factors (Baldet *et al.*, 2008). In the present study the same appears to be true for *C.pulicaris* and *C.punctatus,* where others have also observed a slight endophily in the winter for these species (Kameke *et al.*, 2017), despite others proposing a strong exophilic behaviour of these species (Baldet *et al.*, 2008; Meiswinkel *et al.*, 2008b).

Parous *Culicoides* were trapped in January, March and April in 2016 and November, December, March and April in the 2016/2017 study. This is an observation that has been mirrored elsewhere in Europe (Clausen *et al.*, 2009). The trapping of parous *Culicoides* over the winter, particularly in December, January and March, suggests that either older parous *Culicoides* are able to survive the winter, or that temperatures allow the emergence, feeding, mating, oviposition and survival of *Culicoides* throughout the winter months. The former allows the potential maintenance of virus within the *Culicoides* vector across the season (providing viraemic hosts exist), whilst the latter suggests the possibility of on-going

transmission across the winter. Indeed previous studies have demonstrated the increase in longevity of *Culicoides* species with decreasing temperatures and the ability of Obsoletus group *Culicoides* to easily recover from short (10 day) periods at 4°C in the laboratory (Goffredo *et al.*, 2004; Wittmann *et al.*, 2002). Furthermore a report of sheep positive for SBV RNA in January 2013 does exist (Wernike *et al.*, 2013c). The study by Wernike *et al.,* noted that temperatures had ranged between 5-9°C for several consecutive days during the period the sheep tested positive for SBV RNA and that a singular Obsoletus group *Culicoides* had been collected that month (although the particular individual trapped was negative for SBV). When tested again 4 weeks later SBV antibodies were detected, and other previously negative animals had SBV antibodies by the end of February 2013 (Wernike *et al.*, 2013c). Despite typically short viraemic periods for both SBV and BTV-8, exceptions have been recorded (note 1.3.4.2 Schmallenberg virus: Viraemic period and 1.4.5.2 Bluetongue virus: Viraemic period), with 10% of lambs remaining SBV viraemic across 2 weeks in one study (Claine *et al.*, 2013). Taken collectively this certainly provides strong evidence for the potential for ongoing transmission throughout the winter.

Finding gravid *Culicoides* indoors over the winter further suggests oogenesis may occur within the lambing sheds. This is perhaps not surprising considering the wealth of potential breeding habitats provided within the lambing sheds (Ninio *et al.*, 2011b). However, species identification by PCR would be beneficial to determine the ratio of the different Obsoletus groups species. For example, *C.dewulfi* and *C.chiopterus* are known to breed preferentially in cattle dung, providing a potential control method if particularly active inside sheds in close proximity to, or also housing, cattle over the winter (as the case on farms FF and WN) (Ninio *et al.*, 2011b; Steinke *et al.*, 2016). Male *Culicoides* were trapped in low numbers indoors in November, December, March and April, whilst only a very small number were

collected in November, December and April outdoors, again further adding to the possibility of ongoing breeding of these multivoltine species. In 2016 an abrupt increase in adult *Culicoides* was observed in the light traps in April: a synchronous 'spring flush'. The same dramatic increase was not observed the following year, although this is likely due to a relatively early trap in April and seasonal variation in emergence. Other UK studies have also observed an earlier emergence of *C. punctatus* and *C.pulicaris* compared to Obsoletus group *Culicoides,* with peaks of adult activity typically recorded in in April and May in the UK (with a second peak in abundance usually observed in September/October) (Sanders *et al.*, 2011b; Searle *et al.*, 2014).

Species abundance was low across years, with Obsoletus group and the species *C.pulicaris* the most abundant on farm. These species are also the most abundant on UK farms in summer trapping, and other overwintering studies have reported abundance of these species (Baldet *et al.*, 2008; Kameke *et al.*, 2017; Meiswinkel *et al.*, 2014). It is important to note that black light traps are likely to be biased in attractiveness to different species, and therefore are unlikely to be completely representative of the species present (Carpenter *et al.*, 2008b; Koenraadt *et al.*, 2014; Viennet *et al.*, 2011). They are also known to catch only a fraction of the *Culicoides* active in the area, which means that the *Culicoides* abundance reported here may be far lower than the actual number of *Culicoides* active over the winter period. However, black light traps are currently the only suitable method for surveillance on farm for any long period of time.

Ideally to determine the activity of *Culicoides* over the winter daily catches would be undertaken both indoors and outdoors across multiple farms. This is rarely undertaken due to the amount of time and expenses associated with achieving such a comprehensive study as the speciation of Obsoletus group species by PCR adds to the overall cost of study and the collection of climatic factors adds to the

complexity of analysis (Brugger *et al.*, 2016). Future studies should continue to tease apart the complexities of *Culicoides* activity over winter. This includes further studies to determine if older *Culicoides* are still active indoors in winter and if breeding and oviposition throughout the winter is ongoing, possible through daily collections of adult *Culicoides.* Additionally it should be determined if emergence is ongoing throughout the winter months, possible through weekly emergence trapping on known larval development sites. A greater understanding of these factors is necessary to understand the ongoing successful overwintering and subsequent re-emergence of *Culicoides*-borne diseases of ruminants.

Chapter 5

The impact of Schmallenberg virus on the 2016/2017 lambing season in the UK

5.1 Abstract

Schmallenberg virus (SBV) causes abortions, still births and foetal malformations in naïve ruminant populations. The impact of the initial outbreak on British sheep farms, across lambing 2011-2012 has been previously investigated, with higher farmer perceived impacts, lamb and ewe mortality reported on SBV affected farms. After several years of low, or no, circulation the national sheep flock population once again became vulnerable to SBV infection. Re-emergence of the disease was confirmed in autumn 2016. This study invited sheep farmers to answer a questionnaire designed to determine the impact of SBV during the 2016/2017 lambing period. Higher impacts from neonatal lamb mortality, lambing mortality, dystocia and associated ewe deaths, and higher perceived impacts on sheep welfare, financial performance and emotional wellness were reported on SBV confirmed (n=59) and SBV suspected (n=82), than SBV not suspected (n= 74), farms. Those affected by SBV reported being less likely to farm sheep again next year. The results from the present study are largely comparable to the findings reported for the 2011/2012 outbreak. Additionally, although few farmers (20.4%) reported having ever vaccinated against SBV, the majority (78.3%) stated they would vaccinate at <£1 per dose. Furthermore, the earlier mating period of SBV confirmed and SBV suspected farms provides supportive evidence for the suggested UK SBV time period of disease re-emergence. If SBV transmission continues to be cyclical in nature, the associated animal welfare and economic costs to the UK sheep farming industry will continue to be significant every few years if intervention is not taken.

5.2 Introduction

Schmallenberg virus (SBV) is a single stranded negative-sense RNA virus, belonging to the Simbu serogroup of the *Orthobunyavirus* genus (family: *Bunyaviridae*). This serogroup includes several diseases of animal health importance, including the Aino, Akabane and Shamonda viruses (Lievaart-Peterson *et al.*, 2012; Saeed *et al.*, 2001). Like other viruses within the Simbu serogroup, SBV infects ruminants, is teratogenic, and is transmitted by *Culicoides* biting midges (Hirashima *et al.*, 2017; Yanase *et al.*, 2012, 2005).

The first reports of SBV came from Germany and the Netherlands in autumn 2011 where cattle presenting with diarrhoea, pyrexia and a reduced milk yield tested negative for all known bovine pathogens. A metagenomic approach determined the novel causative agent, subsequently named after the location of the tested samples: Schmallenberg (Hoffmann *et al.*, 2012). Following this initial description, reports of SBV quickly emerged throughout Europe, with transmission facilitated by the dispersal of the *Culicoides* vectors by wind and a completely naïve host population (Gubbins *et al.*, 2014b).

Infections of adult ruminants are typically either asymptomatic or present with only mild clinical signs, as observed in cattle (Hoffmann *et al.*, 2012; Wernike *et al.*, 2013a, 2013b). However if a naïve animal is infected for the first time during the vulnerable period of gestation, infection can result in still births and foetal abnormalities, including arthrogryposis and hydranencephaly (Beer *et al.*, 2013). Infection early in pregnancy has also been linked to lower conception rates, abortions and a reduction in weaning rates (Barrett *et al.*, 2015; Helmer *et al.*, 2013a; Luttikholt *et al.*, 2014; Saegerman *et al.*, 2014; Wernike *et al.*, 2013b). These associated clinical signs of disease are particularly problematic for block breeders,

with high reported losses from the disease in early lambing sheep in 2011/2012 (Afonso *et al.*, 2014; Bessell *et al.*, 2014; Dominguez *et al.*, 2012; Luttikholt *et al.*, 2014; Roberts *et al.*, 2014).

Several studies, including economic modelling studies, have considered the economic impact of SBV to European member states during the initial outbreak (Alarcon *et al.*, 2014; Hasler *et al.*, 2015; Martinelle *et al.*, 2014; Raboisson *et al.*, 2014; Saegerman *et al.*, 2014). Overall the economic cost of SBV was considered relatively low, with the highest costs associated with the disease control measure in which semen trade was restricted (Conraths *et al.*, 2013). However, farm level disease incidence is known to vary significantly, as does the resulting impact. The UK Animal and Plant Health Agency (APHA) found 6% of farmers on SBV confirmed or SBV suspected farms were less likely to farm sheep again the next year, compared to only 1.8% of farmers whose flocks/herds had been unaffected by SBV (Harris *et al.*, 2014). Economic costs may also be higher than originally considered due to the difficulties in quantifying certain types of losses. For example, higher barren rates and reduced fertility are reported in some studies (Barrett *et al.*, 2015; Dominguez *et al.*, 2014; Luttikholt *et al.*, 2014). Furthermore, due to the associated deformities, dystocia is relatively common, potentially resulting in additional losses of ewes whilst birthing malformed lambs (van den Brom *et al.*, 2012). Critically, all studies estimating the impact of SBV have acknowledged the issue of underreporting; SBV is not a notifiable disease, with farmers from many European Member States voluntarily submitting samples and paying for confirmation testing and therefore accurate estimates of the true impact of disease are hard to establish (Afonso and Conraths, 2014; EFSA, 2012).

The unpredictable and intermittent nature of SBV has impacted on farmer uptake of vaccination; the main effective control measure. During the initial 2011-2013 SBV outbreak, high seroprevalences were recorded in Europe, with farm-level

seroprevalences ranging between 94.7-98.2% (Gache *et al.*, 2013; Méroc *et al.*, 2013a; Veldhuis *et al.*, 2013).This high seroprevalence rate was associated with a high predicted basic reproduction ratio (R_0), high prevalence of the *Culicoides* vector and high transmission rate between host and vector (Gubbins *et al.*, 2014b). Having circulated and successfully overwintered between the 2011/2012 and 2012/2013 lambing seasons, SBV reports in the UK in 2014 dropped precipitously. Several studies in Europe described very low circulation between 2014-2015 (Chapter 2; Stokes *et al.*, 2016; Veldhuis *et al.*, 2015; Wernike *et al.*, 2015). These studies highlighted large SBV naïve populations vulnerable to reinfection, particularly as time progressed and vaccinations became no longer available; the sheep population became susceptible to widespread re-infection in the event of SBV emergence. Re-emergence of Simbu serogroup viruses is not uncommon. Akabane virus circulates in Australia, with large outbreaks every 10-15 years when climatic conditions are particularly favourable (allowing range expansion of the vector *C.brevitarsis*), or naïve animals are bought-in to endemic areas (Kirkland, 2002). The unrelated BTV-8 (*Orbivirus*: *Reoviridae*) has re-emerged in Europe after a 5 year hiatus (Sailleau *et al.*, 2015).

Despite vaccines against SBV being rapidly bought to the market during the outbreak, uptake in the UK was found to be relatively low (Stavrou *et al.*, 2017). Previous published work by the author reported only 13.7% farmers in the south of England stated they had vaccinated against SBV in 2013, with this dropping to only 1.6% vaccinating again in 2014. In contrast 78.2% of the same farmers stated they had vaccinated against bluetongue virus serotype 8 (BTV-8) five years previously (n=124) (Chapter 2).

After 3 years of low SBV circulation SBV re-emerged in Europe; by December 2016 deformed lambs were confirmed positive for SBV in the UK (APHA, 2017). With the vaccines withdrawn from the market due to poor uptake, and the duration of natural

immunity unknown, the UK national flock was likely to be highly susceptible to infection.

This study aimed to measure, and compare, the impact of the 2016/2017 re-emergence of SBV on sheep flocks to the impact reported during the initial 2011/2012 outbreak. Expanding on a study following the initial outbreak (Harris *et al.*, 2014) a questionnaire was designed to determine the impact of SBV during the 2016/2017 lambing period on lamb and ewe losses, farmer perceived emotional, financial and welfare costs and views on vaccination (Harris *et al.*, 2014).

5.3 Materials and methods

This study was reviewed and approved by the University of Liverpool Veterinary Research Ethics Committee (VREC537).

5.3.1 Survey design

In order to compare the impact of SBV on the 2016/2017 lambing season to the impact reported during the 2011/2012 lambing season the questionnaire was designed to closely match that of Harris *et al.,* (Harris *et al.*, 2014). Additional questions were designed by the author. The questionnaire was piloted by four sheep farmers and feedback was incorporated into the final questionnaire. Voluntary participation in the questionnaire allowed any sheep farmer within the United Kingdom to participate. The final version was launched online on the 24th of March 2017 using SurveyMonkey (California, USA). The online questionnaire was publicised periodically through Twitter, with support from AHDB Beef and Lamb, Sheep Veterinary Society and the APHA. A link to the online questionnaire was also handed out by veterinary students from both Universities whilst on Easter lambing placements. A further 250 questionnaires were sent out by the APHA to farmers that had submitted samples for SBV testing in England and Wales on the 1st of June 2017.

A total of 32 questions were asked to determine farm demographics, lambing productivity and mortality, ewe mortality, previous vaccination history, the farms' SBV status and the farmers perception towards the impact of SBV on the flock welfare, financial performance and the farmers own emotional wellbeing. The farms SBV status was determined by responses to two questions within the questionnaire and author opinion of additional comments. The categories were:

1. SBV confirmed: Farms where a suspected lamb was confirmed positive for SBV by laboratory testing (implying sample confirmed positive by PCR). Answered 'Yes' to 'Do you believe your flock was infected by Schmallenberg virus this year (2016/2017)?' and answered 'confirmed by laboratory testing of a lamb' when asked 'how was this confirmed'.

2. SBV suspected: SBV was suspected by the farmer or their veterinarian. This includes farms that had positive testing of ewe blood samples (implying sample confirmed positive by ELISA), and those that had lambs sent off for testing (with relevant clinical signs) that were not confirmed positive (by PCR).

3. SBV not suspected: No report of suspected SBV. Answered 'No' to 'Do you believe your flock was infected by Schmallenberg virus this year (2016/2017)?' and had no samples sent for testing. If responded 'No' but had samples sent for testing that returned negative, then changed to Category 2: SBV suspected.

5.3.2 Data analysis

All online results were downloaded from SurveyMonkey into an Excel document on the 19th of June 2017 (Appendix V). All paper versions were manually entered into this Excel document to create a master copy. Responses were checked for consistency and insufficiently completed responses were removed from the working copy.

5.3.3 Mortality definitions & Impact Scores

To allow direct comparison of the impact of SBV on this 2016/2017 lambing season to the impact previously reported for the 2011/2012 lambing season the same calculations, definitions and lamb and ewe mortality scores were used as described previously (Harris *et al.*, 2014). Briefly the following calculation definitions were repeated for analysis here:

Lamb mortality (%) = 100*(Lambs dead from any cause within 1 week/ Total lambs born)

Lamb mortality impact scores (defined and calculated above):

1. 0-<5%

2. 5-<10%

3. 10-<20%

4. 20-<40%

5. ≥40%

Lambing mortality (%) = 100*(Lambs dead from any cause within 1 week / Non-barren ewes)

Ewe mortality (%) = 100*(Number of ewes that died during lambing/ Non-barren ewes)

Ewe mortality impact scores (defined and calculated above):

1. 0-<0.5%

2. 0.5-<1%

3. 1-<5%

4. 5-<10%

5. ≥10%

Combined impact scores= (Lamb mortality impact score) + (Ewe mortality impact score)

The responses for farm demography, lambing productivity, lamb mortality, ewe mortality, impacts of SBV and the farmers' impact perception questions were compared across SBV category. All maps were created in QGIS version 2.2.0 and all statistical analyses were completed in R, version 3.4.1 (QGIS Core Development Team, 2017; R Core Team, 2017). Analysis of variance (ANOVA) and Tukey's HSD post hoc tests were used to compare differences across the SBV categories for continuous data. If the Levene's test for homogeneity of variance was significant the alternative Welch test was used with a Games-Howell post hoc test. For categorical data, including the impact mortality scores, perception questions and previous vaccination history, Pearson's Chi-squared (χ^2) tests were completed. Where the assumptions of the χ^2 were violated a Fisher's Exact test was used.

5.3.4 Malformation definitions

Farmers were asked to describe any malformations seen in any lambs on farm, regardless of whether SBV was suspected or not. These descriptions were then coded separately by the author into the five groups previously determined by Harris *et al.,* (twisted limbs, curved back, jaw deformities, deformed head and nervous signs) and 'Other' (Harris *et al.*, 2014). Themes resulting from the 'Other' group created two more groups: fused joints and eye related deformities. The coding was undertaken blind; the SBV category was masked during coding to reduce the possibility of bias. The coded results were then combined; those that did not match exactly (n=33) were reviewed.

5.4 Results

In total 318 respondents participated in the survey, 232 online and 86 via post (postal response rate 34.4%). All 86 postal responses were included in the survey, however only 129 of the online survey were determined to be useable as 103 respondents did not complete the questionnaire in sufficient detail to be included, leaving 215 useable responses. Not all participants answered every question.

5.4.1 Farm demographics

The majority of respondents were from the west of England and Wales (65,0%, 139/214 responses). In total 27.4% of respondents were from SBV confirmed farms, 38.1% from SBV suspected farms and 34.4% from SBV not suspected farms (n=215) (Figure 5.1). There was no significant difference between the SBV categories and the flock type on farm (P= 0.17, Table 5.1) with a total of 56.5% of respondents defining their flock as crossbreeds/commercials. There was a tendency towards a difference between the SBV categories and farm type (P= 0.07, Table 5.1), specifically there was a greater proportion of upland/hill farms in the SBV not suspected category than the SBV suspected category.

SBV Confirmed Farms

Proportion of
responses (%)

0.0
0.1 - 5.0
5.1 - 10.0
10.1 - 15.0
15.1 - 20.0
20.1 - 25.0

SBV Suspected Farms

SBV Not Suspected Farms
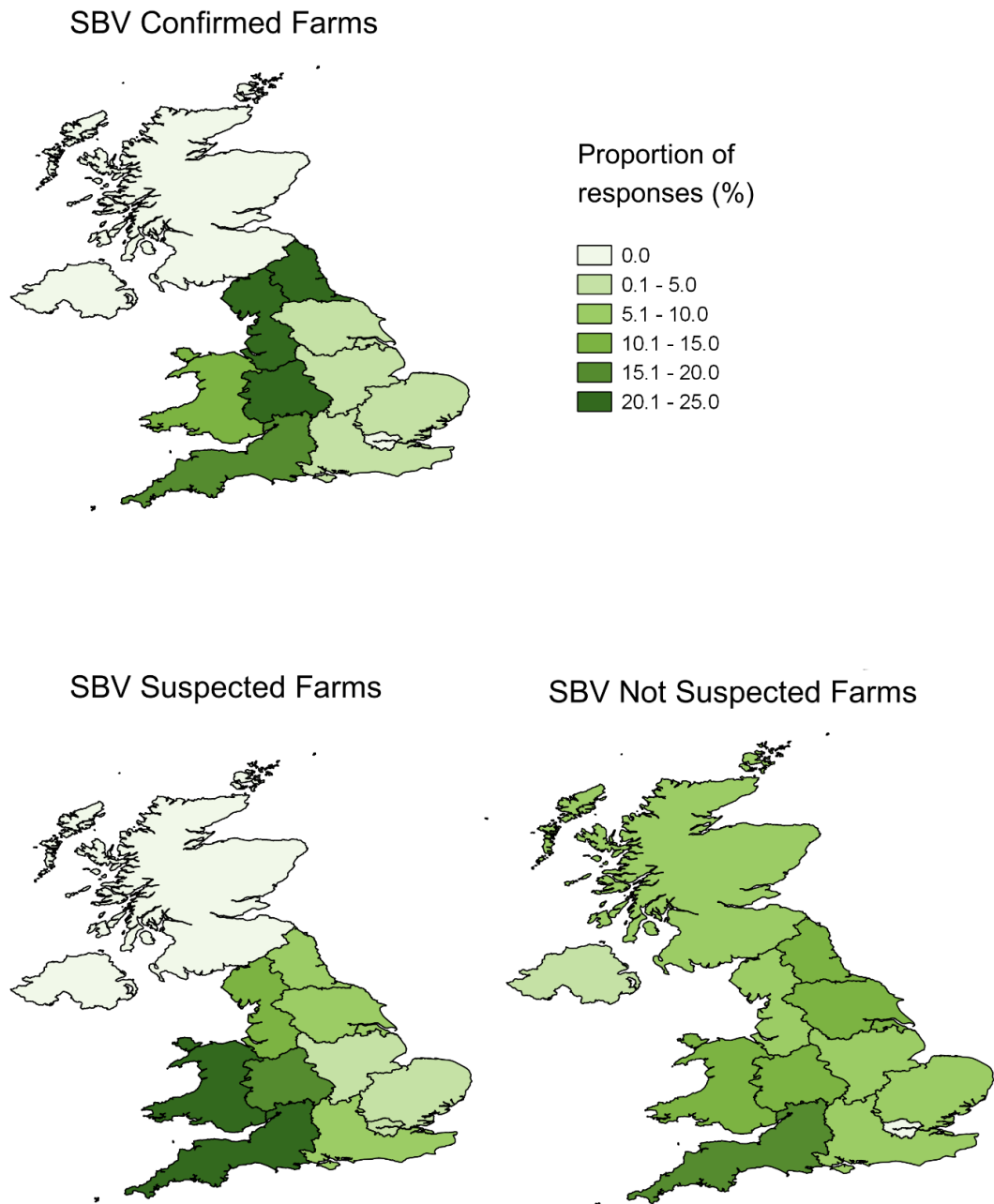
Figure 5.1: Proportion of responses by region for each of the SBV categories. Total responses: SBV confirmed farms (n=59), SBV suspected farms (n=82) and SBV not suspected farms (n=74)

Table 5.1: Farm type and flock type by SBV category

Description (n)	SBV confirmed n=59 (%)	SBV suspected n=82 (%)	SBV not suspected n=74 (%)	P value
Farm type (214)*				**0.07**
Lowland (164)	47 (79.7)	67 (82.7)	50 (67.6)	
Upland/Hill (50)	12 (20.3)	14 (17.3)	24 (32.4)	
Flock type (214)*				**0.17**
Crossbreeds/ Commercials (121)	39 (66.1)	45 (55.6)	37 (50.0)	
Pedigree/Pure Bred (93)	20 (33.9)	36 (44.4)	37 (50.0)	

* Farmers had to select one option to describe their flock. Not all farmers answered every question. Percentages may not equal 100 due to rounding.

5.4.2 Breeding seasons, scanning rates and lambing percentages

The earliest reported date for the ram to be put in with the ewes was the 18th of May 2016; the latest date of ram removal was the 16th of April 2017. The reported duration of the mating season was similar, but slightly shorter for SBV not suspected farms when compared with SBV confirmed and suspected farms (Table 5.2).

The start dates for mating were grouped into 4 categories: 'May/June' (spring/ early summer), 'July/August' (mid-summer), 'September/October' (early autumn) and 'November/December' (late autumn/winter) to allow comparison by SBV category for different seasonal mating strategies. There was a significant difference between the mating start dates on SBV confirmed and SBV suspected farms compared to SBV not suspected farms (P=<0.001; Post-hoc test with Bonferroni's correction P=<0.001) with earlier mating start dates reported on SBV confirmed and SBV suspected farms (Figure 5.2).

The median duration of lambing season was significantly different across the categories, with SBV not suspected farms recording a median lambing duration of 24.5 days less than SBV confirmed farms (P=<0.001, Table 5.2).

There was no significant difference between the SBV categories and barren rates,

scanning percentages or lambing percentages (Table 5.2).

Table 5.2: Farm breeding demographics by SBV category.

Summary description	SBV confirmed (n=59)	SBV suspected (n=82)	SBV not suspected (n=74)	*P* value
Mating season				0.09
Number of responses	58	79	69	
Earliest start date	10/06/2016	18/05/2016	28/07/2016	
Latest end date	02/02/2017	01/04/2017	16/04/2017	
Season duration:				
Median (days)	77	61	56	
Min (days)	15	14	21	
Max (days)	174	264	148	
IQR	50.3-96.5	42.0-88.5	41.0-84.0	
Lambing season				<0.001*
Number of responses	58	79	64	
Earliest start date	30/10/2016	10/10/2016	03/01/2017	
Latest end date	02/06/2017	04/06/2017	30/06/2017	
Season duration:				
Median (days)	64.5	52.0	40.0	
Min (days)	9	6	5	
Max (days)	161	153	115	
IQR	38.3-88.8	40.0-81.0	26.8-57.3	
Tupped ewes that were barren (%)				0.561
Number of responses	48	57	38	
Median	3.7	4.3	3.2	
Min	0	0	0	
Max	27.3	35.2	66.7	
IQR	1.9-6.3	2.7-7.3	1.9-5.1	
Lambing percentage				0.725
Number of responses	59	72	63	
Median	174.3	173.0	166.7	
Min	100.0	110.2	50.0	
Max	212.4	242.9	264.4	
IQR	157.6-185.0	152.3-185.9	146.2-185.6	
Scanning percentage				0.750
Number of responses	50	58	41	
Median	175.0	172.5	176.0	
Min	118.0	100.0	100.0	
Max	223.0	214.0	250.0	
IQR	160.0-188.0	159.3-187.0	160.0-187	

ANOVAs were conducted except where Levene's test determined non-homogeneity of variance (*) where instead the alternative Welch ANOVA was conducted
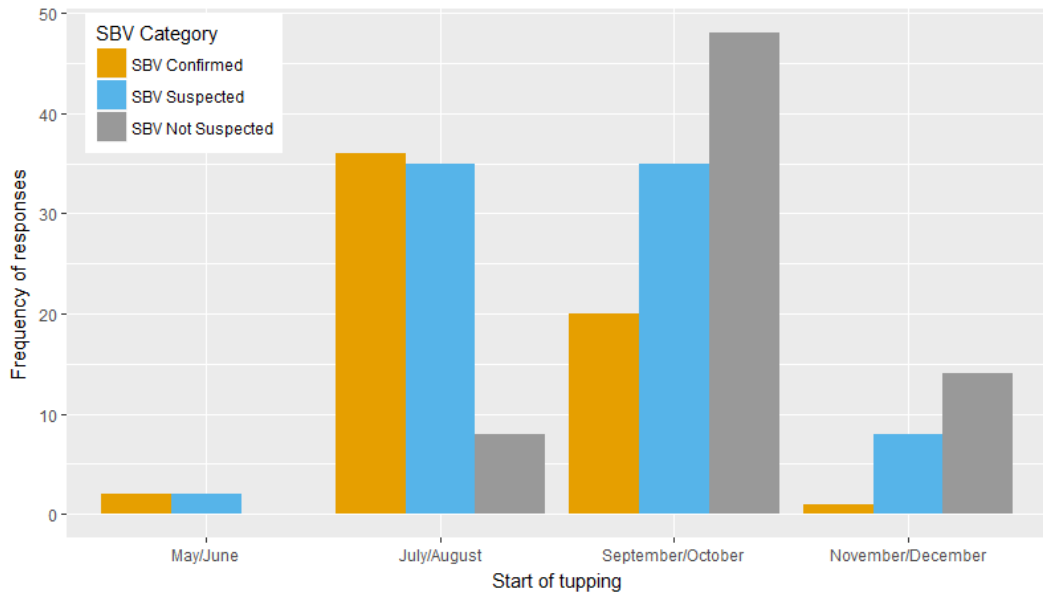
Figure 5.2: A significant difference (Fishers exact P<0.001; Post-hoc test with Bonferroni's correction P=<0.001) was found between the mating start dates on SBV confirmed and SBV suspected farms compared to SBV not suspected farms.

5.4.3 Lamb mortality

Significantly higher lamb mortality was observed on SBV confirmed farms (median of 9.1 lamb deaths per 100 born) and SBV suspected farms (median of 7.6%) than on SBV not suspected farms (median of 5.7%) (P=<0.001, Table 5.3).

Lambing mortality was also significantly higher on SBV confirmed farms (median of 15.2 lamb deaths per 100 pregnant ewes) than SBV suspected (median 12.7%) or SBV not suspected farms (median 8.4%) (P=<0.001, Table 5.3).

Particularly high lambing mortality (more than 40% lambing mortality) was observed more frequently on SBV confirmed farms (13.8%) and SBV suspected farms (8.3%) than on SBV not suspected farms (3.2%). Far more outliers were observed for SBV confirmed farms than SBV not suspected farms (Figure 5.3).

Table 5.3: Lamb mortality and lambing mortality by SBV category

Summary	SBV confirmed (n=59)	SBV suspected (n=82)	SBV not suspected (n=74)	P value
Lamb mortality (per lambs born)				**<0.001**
Number of responses	56	70	50	
Median	9.1	7.6	5.7	
Min	0	0	0	
Max	63.4	47.4	28.6	
IQR	6.8-15.2	4.5-13.1	1.5-9.1	
Lambing Mortality (per pregnant ewes)				**<0.001**
Number of responses	56	70	50	
Median	15.2	12.7	8.4	
Min	0	0	0	
Max	126.8	100.0	53.3	
IQR	10.9-24.8	8.1-20.7	2.3-15.2	

* Tukey HSD were performed for all significant ANOVAs to determine the observable difference
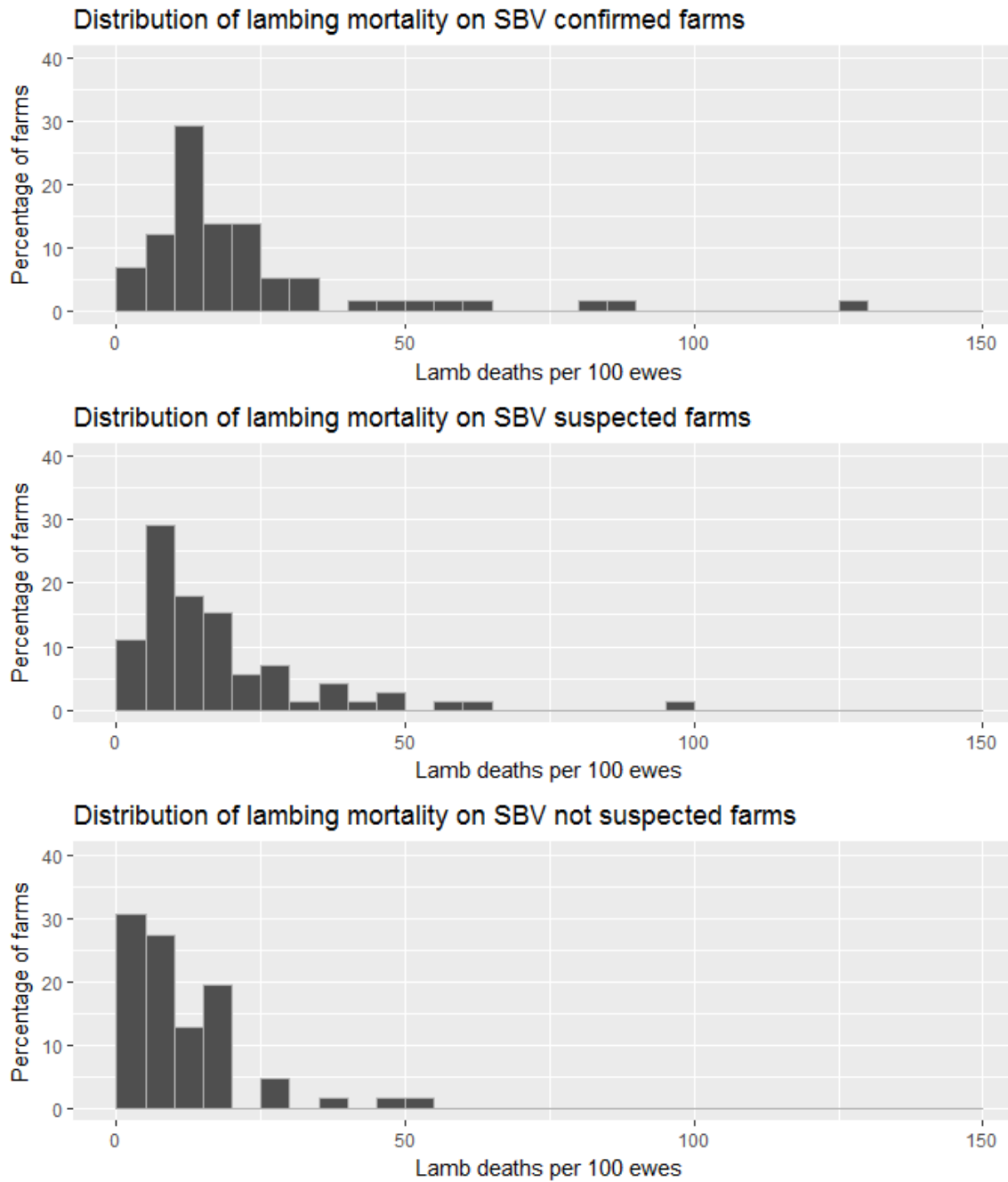
Figure 5.3: Distribution of lambing mortality (%) (lamb deaths per 100 ewes) by SBV category

5.4.4 Abnormalities in lambs

A greater number of malformations were reported by SBV suspected than SBV confirmed or SBV not suspected farms. The most frequently reported malformation was twisted limbs on SBV confirmed and SBV suspected farms, a curved back was the most frequently reported malformation on SBV not suspected farms (Figure 5.4). The most common reported eye deformities on SBV confirmed and suspected farms (n=9) were a lack of eyes (3 and 2 reports respectively) and blindness (1 and 2 reports respectively). One farmer that did not suspect SBV on farm also reported a lack of eyes on one lamb.

At least one malformation in at least one lamb was described by 84.7% (50/59) of SBV confirmed farms, 87.8% (72/82) of SBV suspected farms and 31.1% (23/74) of SBV not suspected farms.
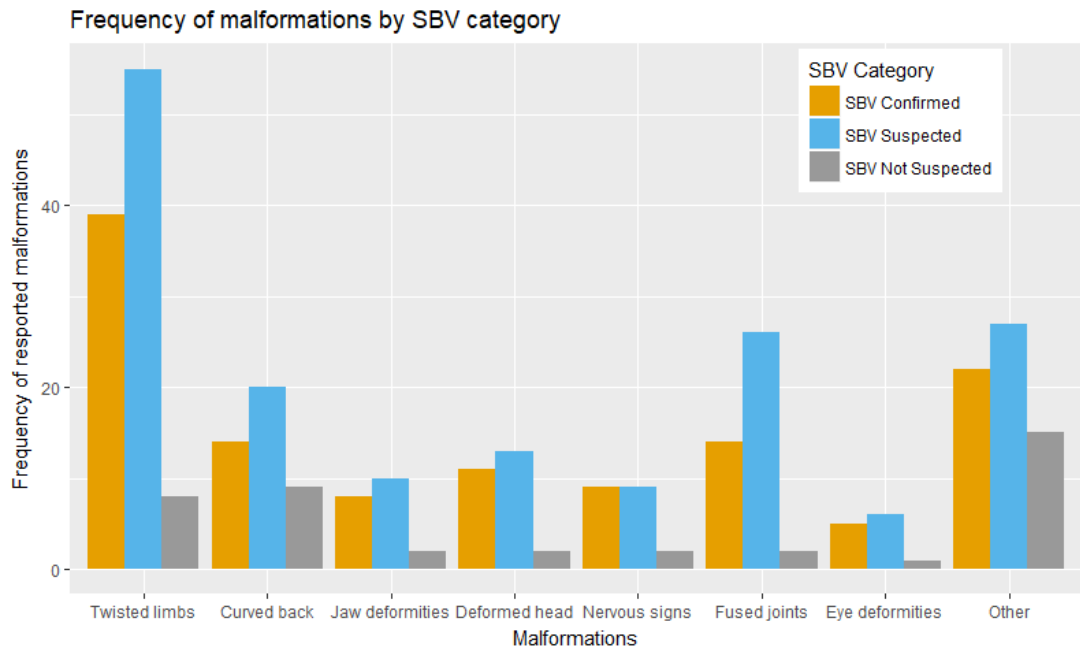
Figure 5.4: The farm level frequency of reported malformations, by SBV category. As farmers may have described a lamb as having multiple malformations (i.e. 'twisted limbs and an undershot jaw') the frequencies do not sum to the total number of farmers describing malformations. Not all farmers answered all questions. Under 'other' the following abnormalities were reported by the farmers: for SBV confirmed: weak (4), small lamb (2), no muscle on back (2), missing ears (2), long legs (1), still born 'rotten' (1), cyst on head (1) and large testicles (1); for SBV suspected: long legs (3), weak (3), no bone structure (3), cyst on head (2), internally deformed (2), two heads (1), protruding spine (1), Short legs (1), small lamb (1), still born 'rotten' (1), missing ears (1); for SBV not suspected: stiff neck (2), long legs (1), small lamb (1), thin legs (1), internal organs external (1), conjoined (1).

5.4.5 Ewe losses

Ewe mortality during the lambing period was not significantly different across the SBV categories (Table 5.4), however the number of ewes that died whilst giving birth to a deformed lamb was significantly different between the groups (P=0.011). In total 30.9% (n=17) respondents from SBV confirmed farms reported one or more ewe deaths due to birthing a malformed lamb, similarly 26.4% (n=19) reported the same for SBV suspected farms, whilst only 5.6% (n=3) of respondents on SBV not suspected farms reported any ewe deaths due to birthing malformed lambs (Table 5.5).

The difference in the number of caesarean sections between categories was significant (P=0.008), with 32.6% (n=15) of respondents on SBV confirmed farms reporting 1 or more caesarean sections due to birthing a deformed lamb, 24.5% (n=12) of respondents reporting the same on SBV suspected farms, compared to no caesareans due to birthing deformed lambs on SBV not suspected farms (Table 5.5).

There was also a significant difference (P <0.001) between the number of respondents reporting farmer assistance of one or more ewes during lambing due to birthing a deformed lamb; 80% (n=32) on SBV confirmed farms, 78.2% (n=43) on SBV suspected farms, and only 33.3% (n=9) on SBV not suspected farms (Table 5.5).

Table 5.4: Total ewe deaths by SBV category

	Farms	Total ewes	Ewes died during lambing		Ewes died during birth due to malformations in lamb	
	n	n	n	%	n	%
All	215	54,938	859	1.56	145	0.26
SBV confirmed	59	16,865	312	1.85	66	0.39
SBV suspected	82	25,156	359	1.43	65	0.26
SBV not suspected	74	12,917	188	1.46	14	0.11

Table 5.5: Ewe mortality and assisted births by SBV category

Summary	SBV confirmed (n=59)	%	SBV suspected (n=82)	%	SBV not suspected (n=74)	%	*P* value
Number of breeding ewes that died during the lambing period							**0.108**
0	16	28.6	25	32.5	25	41.0	
1-5	19	33.9	34	44.2	28	45.9	
6-10	7	12.5	8	10.4	3	4.9	
>10	14	25.0	10	13.0	5	8.2	
Number of ewes that died giving birth to a deformed lamb							**0.011**
0	38	69.0	53	73.6	51	94.4	
1	4	7.3	7	9.7	1	1.9	
>1	13	23.6	12	16.7	2	3.7	
Number of ewes that gave birth to deformed lambs alone							**0.482**
0	25	55.6	25	47.1	17	65.4	
1	7	15.6	13	24.5	5	19.2	
>1	13	28.9	15	28.3	4	15.4	
Number of ewes assisted by farmer because of a deformed lamb							**<0.001**
0	8	20	12	21.8	18	66.7	
1	4	10	15	27.3	8	29.6	
>1	28	70	28	50.9	1	3.7	
Number of ewes assisted by vet because of a deformed lamb							**0.082**
0	28	60.9	34	66.7	24	88.9	
1	10	21.7	10	19.6	3	11.1	
>1	8	17.4	7	13.7	0	0.0	
Caesarean sections because of deformed lamb							**0.008**
0	31	67.4	37	75.5	24	100	
1	11	23.9	5	10.2	0	0	
>1	4	8.7	7	14.3	0	0	

Percentages may not add to 100 due to rounding

5.4.6 Impact Mortality Scores

There was a significant difference between lamb mortality scores across the SBV categories, with a higher proportion of SBV confirmed farms and SBV suspected farms having a higher lamb mortality score than SBV not suspected farms (Table 5.6).

There was no significant difference in mortality impact scores for ewes or combined lamb/ewe scores across the SBV categories (Table 5.6).

Table 5.6: Impact mortality scores for lambs (1-5), ewes (1-5) and combined scores (2-10) by SBV category

Summary	SBV confirmed (n=59)	%	SBV suspected (n=82)	%	SBV not suspected (n=74)	%	*P* value
Lamb Mortality Impact Score	(58)		(72)		(62)		**0.022**
1	9	15.5	23	31.9	29	46.8	
2	24	41.4	26	36.1	21	33.9	
3	15	25.9	13	18.1	7	11.3	
4	7	12.1	7	9.7	5	8.1	
5	3	5.2	3	4.2	0	0.0	
Ewe Mortality Impact Score	(56)		(77)		(61)		**0.635**
1	19	33.9	28	36.4	27	44.3	
2	5	8.9	12	15.6	6	9.8	
3	28	50.0	28	36.4	22	36.1	
4	2	3.6	7	9.1	5	8.2	
5	2	3.6	2	2.6	1	1.6	
Combined Mortality Impact Score	(55)		(68)		(55)		**0.127**
2	4	7.3	11	16.2	17	30.9	
3	10	18.2	10	14.7	10	18.2	
4	9	16.4	16	23.5	7	12.7	
5	15	27.3	18	26.5	12	21.8	
6	11	20.0	7	10.3	5	9.1	
7	4	7.3	2	2.9	2	3.6	
8	0	0.0	2	2.9	2	3.6	
9	1	1.8	0	0.0	0	0.0	
10	1	1.8	2	2.9	0	0.0	

5.4.7 Farmer Perceived Impacts

There was a significant difference between SBV category responses to the farmer perceived impact of SBV on the welfare of the flock, financial performance of the flock and farmers emotional wellbeing ($P<0.001$) (Table 5.7).

In total 10.2% of farmers on SBV confirmed farms and 3.7% of farmers on SBV suspected farms reported that they were less likely to farm sheep again next year because of SBV. No farmer reported being less likely to farm sheep again next year because of SBV from SBV not suspected farms (Table 5.7).

Table 5.7: Perceived impact of SBV on the flocks' welfare, the financial performance of flocks, the farmers' emotional wellbeing and whether the respondent intends to give up sheep farming due to the impact of SBV this year by SBV category

Summary	SBV confirmed (n=59)	%	SBV suspected (n=82)	%	SBV not suspected (n=74)	%	*P* value
Impact of SBV on sheep flocks welfare	(58)		(81)		(67)		**<0.001**
No impact	11	19.0	31	38.3	60	90.0	
Strong positive Impact	0	0.0	1	1.2	0	0.0	
Some positive impact	1	1.7	1	1.2	0	0.0	
Some negative Impact	34	58.6	34	42.0	5	7.5	
Strong negative Impact	12	20.7	14	17.3	2	3.0	
Impact of SBV on sheep flocks financial performance	(58)		(81)		(67)		**<0.001**
No impact	9	15.5	29	35.8	59	88.1	
Strong positive Impact	0	0.0	0	0.0	0	0.0	
Some positive impact	1	1.7	1	1.2	1	1.5	
Some negative Impact	31	53.4	36	44.4	7	10.4	
Strong negative Impact	17	29.3	15	18.5	0	0.0	
Impact of SBV on farmers' emotional wellbeing	(58)		(81)		(66)		**<0.001**
No impact	16	27.6	27	33.3	43	65.2	
Strong positive Impact	0	0.0	0	0.0	0	0.0	
Some positive impact	1	1.7	0	0.0	1	1.5	
Some negative Impact	23	39.7	37	45.7	21	31.8	
Strong negative Impact	18	31.0	17	21.0	1	1.5	
Less likely to sheep farm next year because of SBV	(59)		(82)		(69)		**0.014**
	6	10.2	3	3.7	0	0.0	

5.4.8   Other species on farm

Of the 215 respondents, 113 owned cattle as well as sheep (34/59 SBV confirmed farms, 39/82 SBV suspected farms, 40/74 SBV not suspected farms) of which 40 respondents reported observing abortions, stillbirths or foetal malformations in their cattle (13/34 SBV confirmed farms, 13/39 SBV suspected farms, 12/40 SBV not suspected farms). There was no significant difference between the proportion of abortions, still births or foetal malformations of cattle across the SBV categories (P=0.239).

A total of 11 respondents owned goats as well as sheep (2/59 SBV confirmed farms, 2/82 SBV suspected farms, 7/74 SBV not suspected farms), of which 2 respondents reported observing abortions, stillbirths or foetal malformations (1/2 SBV confirmed farms [1/10 of herd] and 1/2 SBV suspected farms [1/3 of herd]). Again there was no significant difference between the proportion of abortions, still births or foetal malformations of goats across the SBV categories (P=0.109).

Camelids, both Alpacas (4 farms) and Camels (1 farm) were also owned. No abortions, still births or foetal malformations were reported for these species. None of the respondents owned deer, however several reported wild deer on their land, including Sika, Fallow and Roe deer species.

5.4.9 Previous Vaccination History

There was no significant difference between SBV category and previous reported vaccination history (P=0.558). The majority of respondents had never previously vaccinated against SBV (79.6%), with the most reported vaccinations against SBV occurring in 2013 (13.3%) (Figure 5.5).
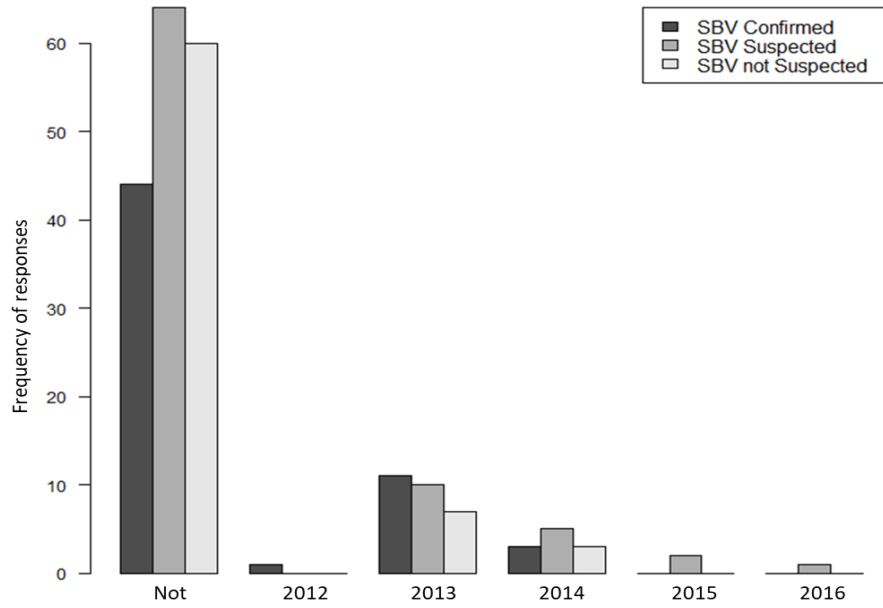
Figure 5.5: Frequency of reported vaccination history by SBV category

5.4.10 Current Demand for Vaccination

There was a small but significant difference between the SBV categories and the price they would be willing to pay to vaccinate now against SBV (P=0.046). A higher proportion of respondents from SBV not suspected farms stated they would not vaccinate than respondents from SBV confirmed or SBV suspected farms (Table 5.8). Roughly a third of respondents from SBV confirmed and SBV suspected farms stated they would consider vaccinating now if the vaccine cost less that £1, whereas just over a quarter of respondents from SBV not suspected farms would do the same.

Table 5.8: Respondents willingness to vaccinate against SBV at different prices for different SBV categories.

Summary	SBV confirmed (n=59)	%	SBV suspected (n=81)	%	SBV not suspected (n=67)	%	*P* value
Would you consider vaccinating your sheep against Schmallenberg virus if it was available now?							**0.046**
No	11	18.6	13	15.9	21	31.3	
Yes, if it cost less than £1	19	32.2	29	35.8	18	26.9	
Yes, if it cost between £1-2	8	13.6	19	23.5	16	23.9	
Yes, if it cost between £2-3	12	20.3	13	16.0	5	7.5	
Yes, if it cost between £3-4	2	3.4	4	4.9	0	0.0	
Yes, if it cost between £4-5	7	11.9	3	3.7	7	10.4	

5.5 Discussion

This study has investigated the impact of SBV re-emergence on the 2016/2017 lambing season in the UK, allowing for comparisons to the initial impact on the 2011/2012 lambing season described previously (Harris *et al.*, 2014). The respondents can be considered to be typical of the UK sheep farming community, as the distribution of farm responses reflects the density of sheep holdings in England and Wales (AHDB, 2017). All major types of sheep farm were represented; hill, lowland, upland, pedigree and commercial farms; and the respondents represented a range of farm sizes (3-3,500 breeding ewes).

In the present study the effects of SBV, reported by farmers, were increased neonatal lamb mortality, lambing mortality, dystocia and associated ewe deaths. In addition, farmers from SBV confirmed and suspected farms perceived that SBV had a significant negative impact on sheep welfare, the farms financial performance and their own emotional wellbeing. Farmers whose flocks were affected by SBV reported that they were less likely to sheep farm again next year. Additionally, SBV confirmed and SBV suspected farms typically described an earlier mating period than SBV not suspected farms, providing supportive evidence for the suggested time period of disease re-emergence in the UK. The findings of the impact of the 2016/2017 SBV outbreak on sheep farms reported in the present study are largely comparable to the findings reported in the 2011/2012 outbreak, with the exception of ewe mortality. A comparative summary of results are presented in Table 5.9 and are discussed below.

Table 5.9: A comparison table to directly compare the results of both studies for the studied factors

Factor	Harris *et al.,* 2011/2012 Study	This 2016/2017 Study
Percentage of tupped ewes that were barren	No difference in median numbers between SBV confirmed (4), suspected (4.3) or not suspected (3.3) farms	No difference in median numbers between SBV confirmed (3.7), suspected (4.3) or not suspected (3.2) farms
Mating season	N/A	Difference between mating start date groups between SBV categories (Figure 2.)
Lambing season	No difference in median days between SBV confirmed (49.5), suspected (48.5) or not suspected (44.5) farms	Difference in median days between SBV confirmed (64.5), suspected (52.0) and not suspected (40.0) farms
Lambing Percentage	No difference in median numbers between SBV confirmed (169.1%), suspected (166.7%) or not suspected (164.2%) farms	No difference in median numbers between SBV confirmed (174.3%), suspected (173.0%) or not suspected (166.7%) farms
Scanning Percentage	N/A	No difference in median numbers between SBV confirmed (175.0%), suspected (172.5%) or not suspected (176.0%) farms
Lamb mortality	Higher mortality SBV confirmed (10.4%), suspected (7.0%) than not suspected (5.3%)	Higher mortality SBV confirmed (9.1%), suspected (7.6%) than not suspected (5.7%)
Lambing mortality	Higher mortality SBV confirmed (18.2%), suspected (11.3%) than not suspected (8.6%)	Higher mortality SBV confirmed (15.2%), suspected (12.7%) than not suspected (8.4%)
Number of breeding ewes that died during the lambing period	More ewes dying on SBV confirmed (66.7%), SBV suspected (67.1%) than not suspected (54.5%) farms	No difference SBV confirmed (71.4%), suspected (67.5%) or not suspected (59%) farms
Number of ewes died giving birth to deformed lambs	More dying on SBV confirmed (36.9%), suspected (16.8%) than not suspected (7.2%) farms	More dying on SBV confirmed (30.9%), suspected (28.4%) than not suspected (5.6%) farms
Number of ewes that gave birth to deformed lambs alone	N/A	No difference between SBV confirmed (44.4%), suspected (52.9) or not suspected (34.6%) farms
Number of ewes assisted by farmer because of a deformed lamb	N/A	More ewes assisted on SBV confirmed farms (80%), suspected (78.2%) than not suspected (33.3%) farms
Number of ewes assisted by vet because of a deformed lamb	More ewes assisted on SBV confirmed farms (35.8%), suspected (19.5%) than not suspected (4.8%) farms	No difference between SBV confirmed (39.1%), suspected (33.3%) or not suspected (11.1%) farms
Number of caesarean sections because of deformed lambs	More caesareans on SBV confirmed (12.3%), suspected (11%) than not suspected (1.6%) farms	More caesareans on SBV confirmed (32.6%), suspected (24.5%) than not suspected (0%) farms
Lamb Impact Mortality Score	Difference in lamb IMS between SBV categories	Difference in lamb IMS between SBV categories
Ewe Impact Mortality Score	Difference in sheep IMS between SBV categories	No difference between SBV categories
Combined Impact Mortality Score	Difference in combined IMS between SBV categories	No difference between SBV categories
Farmer Perceived Impact of SBV on sheep welfare *	Higher impact (4 or 5) on SBV confirmed (36.8%), suspected (17.8%) than not suspected (0.5%) farms	Higher negative impact on SBV confirmed (79.3%), suspected (59.3%) than not suspected (10.5%) farms
Farmer Perceived Impact of SBV on financial performance *	Higher impact (4 or 5) on SBV confirmed (32.8%), suspected (20.1%) than not suspected (2.3%) farms	Higher negative impact on SBV confirmed (82.7%), suspected (62.9%) than not suspected (10.4%) farms
Farmer Perceived Impact of SBV on farmers emotional wellbeing *	Higher impact (4 or 5) on SBV confirmed (49.3%), suspected (25.6%) than not suspected (6.5%) farms	Higher negative impact on SBV confirmed (70.7%), suspected (61.7%) than not suspected (33.3%) farms
Less likely to sheep farm next year because of SBV	No difference between SBV confirmed (5.7%), suspected (5.9%) than not suspected (1.8%) farms	Higher numbers less likely to sheep farm next year on SBV confirmed (10.2%), suspected (3.7%) than not suspected (0%) farms

Colours indicate similar findings (blue) or different findings (orange) across studies. No colour indicates a lack of data for comparison or a difference in methodology (*) between studies preventing direct comparison. Differences were at the P=<0.05 significance. Data summarised for 2011/2012 outbreak (Harris *et al.*, 2014)

Mating start dates and lambing season duration were found to be significantly different between the SBV categories. Importantly, SBV confirmed and SBV suspected farms typically start mating in July/August (61% and 44% respectively) compared to SBV not suspected farms, where the majority (69%) reported mating in September/October. This would put the vulnerable period of gestation (approximately days 28-56 of pregnancy) for these later mated flocks largely outside of the autumn activity peak of the SBV *Culicoides* vector species (EFSA, 2012; Sanders *et al.*, 2011b). As SBV was determined to have circulated in *Culicoides* in August 2016 in Belgium (Sohier *et al.*, 2017), and due to confirmed SBV malformations in lambs in England beginning in December (in the south), and peaking in January and February 2017 (cases across England) (APHA, 2017), it is likely that SBV circulated widely in England in September/October 2016 (APHA, 2017; Sohier *et al.*, 2017). If this was indeed the case those mating in August/September 2016 would be expected to be the worst affected (depending on geographic location and localised *Culicoides* activity) which appears to agree with the observations reported in this study.

Questions have been raised regarding the impact of SBV on early reproductive losses in sheep flocks (Dominguez *et al.*, 2014). However, sound evidence for this is lacking. In the present study there was no difference in the reported barren ewe rate between SBV categories, nor was a difference reported in the previous 2011/2012 study. In fact the reported median barren rates reported here (3.7%) were very similar to those reported for the 2011/2012 outbreak (4%) (Harris *et al.*, 2014) and although these barren ewe rates are higher than industry guidelines (Phythian *et al.*, 2014), they appear to be typical of UK sheep flocks (AHDB, 2016a; Hybu Cig Cymru / Meat Promotion Wales, 2011). Furthermore studies in the Netherlands also failed to find associations between SBV infection and early ewe reproductive performance (Luttikholt *et al.*, 2014).

The lamb mortality and lambing mortality was significantly higher on SBV confirmed and SBV suspected farms, with almost double the median lamb mortality percentage and lambing mortality percentage on SBV confirmed farms compared to SBV not suspected farms (median of 15.2% and 9.1% respectively compared to median of 8.4% and 5.7%). These results were very similar to those reported across the same SBV categories in the UK during 2011/2012 lambing (Harris *et al.*, 2014). European studies assessing the impact of SBV on lamb mortality have found similarly high lamb mortality rates on SBV confirmed flocks compared to those on SBV not suspected flocks (in Belgium (13.2% compared to 9.5%) and the Netherlands (13.9% compared to 8.3%)), with French studies reporting lamb mortality on SBV positive farms at 13-14% (Dominguez *et al.*, 2012, 2014; Luttikholt *et al.*, 2014; Saegerman *et al.*, 2014). Previous studies of lamb mortality in UK flocks, prior to the 2011 SBV outbreak, are similar to those reported on the SBV not suspected farms median neonatal lamb mortality (9%; IQR 5.9-12.3% (Binns *et al.*, 2002)) and similar to reported industry figures (AHDB, 2016a).

It would be expected that the increased lamb mortality on SBV affected farms is largely an effect of the associated congenital malformations on the lamb's ability to adapt to post-natal life. For example, the ability to stand, suckle and keep up with the ewe will affect the risk of starvation, hypothermia and infection. A number of congenital malformations were associated with SBV infection in the present study. Twisted limbs were the most frequently reported malformation of lambs on SBV confirmed and SBV suspected farms, followed by curved backs and deformed heads. This is in agreement with the previous UK experience of SBV. Indeed the descriptions of malformations reported by farmers were the same across both outbreak years (Harris *et al.*, 2014).

Congenital malformations in lambs are not exclusive to SBV infection and can be the result of a wide range of tetragenic, genetic or nutritional factors (Dittmer and

Thompson, 2015) and indeed congenital malformations on SBV not suspected farms were reported here. Therefore, it is important that farmers have samples tested when a case of SBV is suspected on farm. This helps to prevent all malformations from being assumed to be SBV, ensuring that the cause is properly investigated and diagnosed. The data from such testing also provides passive surveillance, which can act as an alert for a reduction in circulation and immunity. Indeed, it was a lack of confirmed samples in 2014, paired with farmer statements, which prompted the author to previously examine SBV circulation within southern sheep flocks, highlighting a large naïve population prior to the re-circulation of SBV (Chapter 2).

Overall there was no difference in ewe mortality across SBV category in the present study. Although unsurprisingly, both ewe mortality associated with birthing malformed lambs and the number of assisted births (both by the farmer and by caesarean) were greater on SBV affected farms due to birthing malformed lambs. More caesarean sections were also reported on SBV confirmed and SBV suspected farms in this study (33% and 25% respectively) than reported during the initial 2011/2012 outbreak (12% and 11% respectively) (Harris *et al.*, 2014). Certainly it appears that the delivery of malformed lambs presents increased risk to ewe health, although we did not find evidence for an impact on overall ewe mortality as has previously been reported (Harris *et al.*, 2014). This could be for a number of reasons; for example, a different study population, or improved farmer/veterinary awareness of the risk to ewe health, and therefore earlier appropriate obstetrical intervention.

The costs associated with dystocia, and the additional costs associated with veterinary assisted births and caesarean sections are significant economic outputs for many farmers. The exact economic costs associated with SBV are difficult to estimate, partially due to the variations in value of stock and variable costings of

veterinary intervention and drug costs, but mostly due to the underreporting of disease both within and between farms. A study of the impact in Belgium considered the secondary associated costs of dystocia, namely the administration of anti-inflammatories and/or antibiotics, estimating the mean percentage of animals per flock treated to be 18.5% in SBV positive flocks and the average cost per animal to be €50.4 (roughly £40 in 2012) (Saegerman *et al.*, 2014). Further studies into the impact of SBV in the UK should consider including questions on secondary costs including treatment of ewes damaged during birthing malformed lambs and direct costs associated with necessary on-farm veterinary intervention.

As would be expected the perceived welfare, economic and emotional impact of SBV to farmers was generally high on SBV confirmed and suspected farms, and low on SBV not suspected farms. The greatest reported negative impact was on farmers' emotional wellbeing. As SBV is no longer novel, the distressing nature of the associated malformations are well known amongst farming communities, it is likely that this awareness, along with potential previous experience of the disease, is likely to have contributed to the high proportion of reports of negative emotional impact of SBV, even on unaffected farms. A total of 4.3% respondents stated they were less likely to sheep farm next year because of SBV. This is comparable to the proportion of respondents stating the same after the previous SBV outbreak (3.7%) (Harris *et al.*, 2014).

Although the data set does not provide a complete description of the 2016/17 UK outbreak, it is interesting to note that the geographic distribution of SBV confirmed farms in this sample did deviated from the previous 2011/2012 outbreak distribution. In 2011/12 the outbreak began in the south east of the UK and rapidly spread in a north westerly direction reaching the majority of England and Wales up to the Scottish Border (AHVLA, 2013). Here, despite the survey being distributed nationally, the majority of SBV positive farms were located in the west of England

and Wales. Although one cannot be certain, the difference in response distribution between the surveys could reflect the distribution of SBV cases in 2016/17 and could indicate a different route or timing of disease introduction (APHA, 2017).

This survey also explored the impact of SBV on abortions, still births or malformations in any other ruminant or camelid species they had on farm. Over half of respondents owned cattle, however even on SBV confirmed farms, the number of abortions, still births or foetal malformations in cattle remained low, with a greater proportion reported on SBV not suspected farms. The reported incidence of arthrogryposis hydraencephaly syndrome in Europe has generally been lower for cattle than sheep (1-4% compared to 3-7% respectively) and as cattle have longer breeding lives and are typically bred throughout the year, this aspect is unlikely to be as obvious in cattle as sheep (Afonso *et al.*, 2014). A total of 11 respondents owned goats, of which 2 reported observing abortions, stillbirths and foetal malformations. Again these were not significantly associated with SBV category. Little is known about the impact of SBV on goat herds in the UK, camelids or other non-commercial ruminants. Future studies should look to assess the impact of SBV on these species and exotic ruminant species. However, if the prevalence of malformations associated with SBV is low, together with the small sample size of mixed species farms here, this study may not have been of sufficient power to detect any difference.

Previous vaccination history against SBV was also explored. Few respondents stated that they had vaccinated against SBV previously (20.2%). However over 78% of respondents stated they would consider paying to vaccinate against SBV if the vaccine was available. Interestingly more respondents from SBV confirmed farms stated they had vaccinated in 2013 than from SBV suspected or SBV not suspected farms. This does not infer a lack of vaccine protection, as the majority of the sheep on farm are unlikely to have been those vaccinated in 2013, but rather restates the

necessity in continuing vaccination programmes, particularly of young stock and replacement ewes.

Under-reporting of SBV cases is recognised as an issue for measuring the impact of the disease on populations (Afonso *et al.*, 2014).The number of suspected cases in this study that were not sent off for testing, in areas where confirmed positives exist, also highlights the potential extent of the under-reporting of disease. Surveys such as this also represent the only way to estimate the potential extent of impact on farm of non-notifiable diseases, as although many farmers will send off a single suspected case for testing, it would be unusual, due to the cost and cost of time during an extremely busy period, to send off all suspected cases on farm for confirmation testing (Afonso *et al.*, 2014). Additionally as infection of adult ruminants results in vague, if any, clinical signs, it is likely that many farms that did not suspect SBV infection this season have in fact been infected, but outside of the vulnerable period of gestation. If this is the case, this may account for why no significant differences were observed between SBV categories and early oestrus factors such as barren rates, lambing percentages and scanning percentages.

The results of this survey clearly demonstrate an impact of SBV on the 2016/2017 lambing season, comparable to that reported for the 2011/2012 lambing season (Harris *et al.*, 2014). If SBV transmission continues to be cyclical in nature, the associated animal welfare and subsequent economic costs to the UK sheep farming industry will continue to be significant every few years if intervention is not taken. Controlling the *Culicoides* vector has so far appeared unfeasible, subsequently the importance of timely vaccination, or changes in the timing of mating periods will continue to be necessary to reduce the impact of future SBV outbreaks. National surveillance programmes, particularly collaborative surveillance programmes with European member states, are increasingly important for the application of timely vaccination programmes; however vaccination production ceases when demand is

low. Future studies should aim to address this cyclical epidemiology, particularly

identifying where the virus persists between outbreaks, and the overwintering

mechanism of the disease.

Chapter 6

Discussion

The aim of this thesis was to address some of the major questions arising from a changing *Culicoides*-borne disease situation, to better inform policy-makers, stakeholder groups and disease modellers.

SBV rapidly spread throughout Europe in 2011/2012, reaching a high prevalence within ruminant populations (EFSA, 2014). As the viraemia was short, and R0 high, this transmission rate was unsustainable, resulting in a decline in reported cases with an increasingly immune ruminant population (Gubbins *et al.*, 2014a; Laloy *et al.*, 2015; Wernike *et al.*, 2013b). Chapter 2 demonstrated this lack of apparent circulation in the south of England in 2015. This, along with complementary studies in mainland Europe, demonstrated a severe decline in circulation after a period of high circulation (Veldhuis *et al.*, 2015; Wernike *et al.*, 2015). More importantly this study highlighted a large naïve population at risk from future re-emergence. The publication of these findings were incorporated into policy documents and reported by stakeholder groups (APHA, 2016a, 2016b; EurekAlert!, 2016; Mount Vets Farm Practice, 2017; MRCVSonline, 2016; PHYS.ORG, 2016; Technology.org, 2016; The Wood Veterinary Group, 2017). As the vaccination had been taken off of the market, due to low demand, there would be limited ways to protect this vulnerable population if re-emergence occurred.

Like SBV, BTV-8 had circulated throughout Europe, only to be controlled through strict movement restrictions and vaccination campaigns by 2010 (Caporale, 2008; Defra, 2014; EC, 2017). This again had left a large population naïve to infection, therefore lacking the immunity of BTV endemic species, resulting again in the potential for morbidity and mortality on re-emergence (Roberts *et al.*, 2016). In August 2015 BTV-8 re-emerged in Europe (Sailleau *et al.*, 2015). As with SBV, the vaccine had been taken off the UK market due to a lack of demand during the 5 year absence from disease. With ongoing virus transmission in France, and an estimated 80% likelihood of disease incursion before the end of 2016, the demand

for vaccine in the UK was unknown (Roberts *et al.*, 2016; The International Disease Monitoring Team, 2017). Chapter 3 sought to better understand the demand for BTV-8 vaccination in the UK, the price farmers would be willing to pay for vaccine and how close the BTV-8 outbreak would have to get to study participants before they would vaccinate their herd/flock.

The study determined that although the majority of respondents (90.0%) felt it was important to keep BTV-8 out of the UK, very few respondents (33.4%) stated they would pay £1 per dose to vaccinate before BTV-8 reached the UK. This reported willingness to vaccinate, however, was higher if the vaccine was subsidised to 40p a dose (55.9%), and much higher if vaccination was free (65.3%). Smaller farms, those that had previously vaccinated against BTV-8 and those that were deemed 'risk adverse' were more likely to both want to vaccinate and to be willing to pay a higher price to vaccinate. The decision to vaccinate appears complex, and a greater, more comprehensive study is needed to further identify, scrutinise and disentangle the intricate factors at play. Such a study should look to utilise sociological approaches and interview techniques, perhaps expanding on examples by Richens *et al.,* (2015) and Bennett and Balcombe (2011) for exploring perception to vaccination strategies and willingness to pay to vaccinate.

A potential factor that previous disease models have not considered has been highlighted by the study: the percentage of the individual flock/herd vaccinated. Although 72.2% of respondents stated that they had previously vaccinated against BTV-8, 17.6% had vaccinated less than half of their flock/herd, likely in an attempt to save on costs. This is in conflict with vaccine manufacturers recommendations to ensure herd immunity. Disease models should therefore not assume complete vaccine coverage on every farm. Future studies should look to determine why this practice is ongoing on farms, and look at factors to encourage vaccine uptake.

Alternatively, studies should look to quantify this practice, allowing this practice to be included in disease model simulations.

A limitation of the study was the relatively small sample size and underrepresentation of cattle-only holdings. This may be due to the low perceived threat of BTV-8 to cattle. However, sheep and mixed holdings were well represented and there was a wide distribution in responses. With more responses the importance of farm location could have been investigated further. Although several comments demonstrated a difference in demand between farms deemed 'at low risk' (i.e. more northerly farms) and those at a comparable 'high risk' (i.e. farmers in the south east of England), no measurable difference was determined in the study. These comments raised important questions that should be further considered: should southern UK farmers pay to vaccinate for effectively the 'greater good'? Is the perceived financial burden of vaccinating against emerging European VBD making southern farmers despondent towards voluntary vaccination campaigns? These are important questions that require stakeholder and policymaker engagement prior to the next VBD outbreak, especially as both BTV-8 and SBV have not only successfully re-emerged, but have also successfully overwintered across outbreak years.

The survival of BTV-8 and SBV mechanism overwinter in northern Europe is still relatively unknown. The known viraemia for SBV is certainly too short for the virus to successfully overwinter in one host and although BTV-8 viraemia has been shown to be prolonged, the persistence is unknown (EFSA, 2008; Laloy *et al.*, 2015; Wernike *et al.*, 2013a, 2013b). SBV again shows no evidence of vertical transmission, and unlike BTV-8, pseudo-vertical transmission has been ruled out (EFSA, 2014). Circumstantial evidence for pseudo-vertical transmission exists for BTV-8 (via the consumption of placental tissue) however it has been deemed unlikely that this is the only pathway for BTV-8 overwintering as SBV also manages

to successfully persist (Backx *et al.*, 2009; EFSA, 2008). Vector free period studies in Europe have certainly demonstrated ongoing low level activity of *Culicoides* vector species throughout the winter; during mild winters, in mild climates and inside animal housing (Baldet *et al.*, 2008; Baylis *et al.*, 2010; Brugger *et al.*, 2016; Clausen *et al.*, 2009; Kameke *et al.*, 2017; Meiswinkel *et al.*, 2008a, 2014). However, the activity of *Culicoides* inside lambing sheds over the winter in the UK has yet to be explored: Chapter 4 sought to investigate this activity during peak lambing, when the greatest number of potential hosts would be indoors, and to compare the activity of *Culicoides* indoors and outdoors over the winter period. This study demonstrated active *Culicoides* vector species throughout the winter months in the south of England. Importantly, parous *Culicoides* were caught throughout the winter with the exception of the months of January and February. Gravid *Culicoides* were also successfully trapped, with collections every month but February. This suggests that either older parous *Culicoides* are able to survive the winter, likely due to increasing longevity with decreased temperatures (Goffredo *et al.*, 2004; Wittmann *et al.*, 2002). Or alternatively, the winter temperatures during the study allowed for the continued emergence, feeding, mating and oviposition of adult *Culicoides* throughout the winter months. Either scenario would present a potential route for virus overwintering, either through maintenance of the virus within older *Culicoides*, or on-going transmission. The case of a SBV RNA positive sheep in January provides evidence for this on-going transmission (Wernike *et al.*, 2013c). This study is unable to conclude which of these mechanisms is most likely, as trapping only occurred once a month during the longitudinal study. Future studies should look to increase the frequency of trapping, ideally alongside complementary emergence trapping from potential breeding and larval overwintering sites. This would help to separate the ongoing activity of surviving older *Culicoides* from activity caused by ongoing emergence throughout the winter.

A further limitation of the study was the use of light traps; light traps have been demonstrated to significantly underrepresent *C.chiopterus* and underestimate abundance of *Culicoides* (Carpenter *et al.*, 2008b). If *Culicoides* are severely underestimated by light traps then catching even one parous *Culicoides* indoors could represent significant *Culicoides* activity within the barn. Quantification of this underestimation for the different *Culicoides* species is essential. The application of multiple trapping methods in future studies, including light catches, emergence traps and direct collection, could help to address this issue. The data reported here is also only a representation of sheep holdings in the south of England, with a particular focus on the south west. Previous studies have described differences in *Culicoides* abundances between different animal holdings (Kameke *et al.*, 2017). As cattle dung represents viable development habitats for *Culicoides* vector species, it is likely that greater numbers of *Culicoides* would be trapped on cattle holdings (Harrup *et al.*, 2013; Kettle and Lawson, 1952). Some cattle are even housed all year round, representing potentially particularly favourable habitats for *Culicoides* species. Farm management practices, such as bedding types, animal waste management and insecticidal use are all likely to further impact *Culicoides* abundance and activity over the winter. Future studies should look to explore these factors in greater detail. Trapping regularly throughout the winter would be beneficial, with studies demonstrating large differences in *Culicoides* abundance and activity between days and at different times throughout the day (Brugger *et al.*, 2016). These factors should be better classified in a UK context; however the continued activity of *Culicoides* demonstrated in this study, along with the observed transmission of SBV and BTV-8 across years, demonstrates the potential for ongoing *Culicoides* activity as the overwintering mechanism at play.

The re-emergence of BTV-8 in 2015, and subsequent re-emergence of SBV in 2016 demonstrate the circular epidemiology of these *Culicoides*-borne diseases (APHA,

2017; Sailleau *et al.*, 2015). Indeed Akabane, a SBV related Simbu serogroup virus, exists at low levels in Japan, causing large outbreaks at 4-6 year cycles (Kono *et al.*, 2008). If this is to be the case will the impact be comparable each time to the initial outbreak, or will it be reduced? This question was addressed by Chapter 5, which compared the reported impact of SBV on UK sheep flocks this 2016/2017 lambing, to a published record of reported impact during the 2012/2013 outbreak (Harris *et al.*, 2014). Like the previous study, higher lamb mortality, higher lambing mortality, higher ewe mortality due to birthing deformed lambs and higher numbers of caesarean sections were reported on SBV confirmed and SBV suspected farms than SBV not suspected farms. This has also been echoed by European studies (Dominguez *et al.*, 2012, 2014; Luttikholt *et al.*, 2014; Saegerman *et al.*, 2014). Farmers across both outbreaks perceived higher impacts on the welfare of their sheep, their flocks' financial performance and their own emotional wellbeing. This study also demonstrated that those from SBV confirmed and SBV suspected farms started mating earlier than those that did not suspect SBV on farm in 2016/2017. This further supports the common belief that earlier lambing flocks are more affected by SBV than those that lamb later. This is likely due to the observed seasonal activity of adult *Culicoides*, with peaks in activity typically observed in the spring (April/May) and again later in the year (September/October) (Sanders *et al.,* 2011b). In turn this supports the hypothesis that moving lambing to later in the year would potentially reduce the clinical signs of disease in sheep flocks, and therefore reduce the burden of SBV on the sheep farming industry (Sheep Veterinary Society, 2013). Although theoretically feasible, this in practice is unlikely to be integrated into practice, as mating times are typically dictated by breed, ewe body condition, farm land type and supplier demand.

As the study was opportunistic, through the use of an online questionnaire, the prevalence of SBV could not be calculated. Further studies should seek to rectify

this, by either calculating the necessary sample size to determine prevalence (although this study would be considerable in size, cost and time) or by choosing a smaller study area and attempting to contact all farms. The latter would be particularly useful in determining the level of under-reporting for an area. However the feasibility of such a study would be challenging (due to the nature of underreporting and study compliance), and the subsequent results would only be applicable to the study area. A further limitation of the study described in Chapter 5 was the lack of temporal data; knowing when clinical signs were observed would have potentially allowed the likely spread of the SBV outbreak to be mapped. This would have been a useful addition to the study. Despite these limitations, the study demonstrated comparable impact of the 2016/2017 SBV outbreak to the impact previously described for the 2012/2013 outbreak. This finding is particularly important to policymakers and stakeholder groups, as it demonstrates the need for ongoing surveillance for SBV and research into potential control measures.

This thesis set out to react to a changing disease landscape, to inform policymakers and stakeholders as to the current situation. A population at risk from disease re-emergence was described. The complex issues surrounding individual farmer risk versus cost analysis towards voluntary vaccination under a changing disease scenario was highlighted. More collaborative work is needed here to understand this important disease prevention strategy and how to increase uptake. The ongoing activity of *Culicoides* vectors throughout the winter was identified: a potential mechanism for virus overwintering. The importance of indoor habitats should be further investigated, with frequent (ideally daily) collections to determine the exact factors influencing abundance over winter both indoors and outdoors. Further work should seek to identify the exact number of adult *Culicoides* active indoors, utilising a range of different trapping techniques and investigate the ramifications of vector control measures on population abundance at this time of year. Finally, this all

becomes incredibly important when considering that the impact of SBV, as demonstrated here, appears to be the same across emergence years. This represents the ongoing costs of continued disease emergence without improved preventive disease control measures.

Greater understanding of the cycles of disease epidemiology and potential control measures is in everyone's best interest. Undoubtedly, SBV circulation will again reduce to low levels as immunity within the ruminant populations increases, before once again re-emerging when immunity declines. The frequency of this likely cycling is currently unknown. Based on the recent re-emergence this may be as frequent as every 3 years. In between outbreak years more studies are needed to determine the exact level to which the national immunity must drop before re-emergence can occur, continue to explore the likely rate of vaccination uptake amongst the farming community and how to increase voluntary uptake, the exact abundance and activity of *Culicoides* over the winter months and potential ways to reduce the impact of re-emergence and tackle under-reporting.

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Appendix I: Species susceptible to SBV infection
Detection of SBV in different species (Domestic ruminants not included).

Species	Antibodies against SBV	SBV RNA	Clinical signs	Countries	Notes
American Bison	X			UK	Zoo (*Bovidae*)
Arabian oryx	X			UK	Zoo (*Bovidae*)
Banteng	X			UK	Zoo (*Bovidae*)
Blackbuck	X			UK	Zoo (*Bovidae*)
Congo Buffalo	X			UK	Zoo (*Bovidae*)
European Bison	X			UK, Poland	Zoo & wild (*Bovidae*)
Gaur	X			UK	Zoo (*Bovidae*)
Gemsbok	X			UK	Zoo (*Bovidae*)
Greater Kudu	X			UK	Zoo (*Bovidae*)
Impala	X				Zoo (*Bovidae*)
Lesser Kudu	X			UK	Zoo (*Bovidae*)
Nile lechwe	X			UK	Zoo (*Bovidae*)
Nubian goat	X			UK	Zoo (*Bovidae*)
Red forest Duiker	X			UK	Zoo (*Bovidae*)
Roan antelope	X			UK	Zoo (*Bovidae*)
Scimitar-horned oryx	X			UK	Zoo (*Bovidae*)
Sitatunga	X			UK	Zoo (*Bovidae*)
Waterbuck	X			UK	Zoo (*Bovidae*)
Yak	X			UK	Zoo (*Bovidae*)
Axis Deer	X			UK	Zoo (*Cervidae*)
Bongo	X			UK	Zoo (*Cervidae*)
Brow antlered deer	X			UK	Zoo (*Cervidae*)
Chinese water deer	X			UK	Zoo (*Cervidae*)
Elk		X		Poland	Wild (*Cervidae*)
Fallow deer	X			Germany, Poland, Netherlands	Wild (*Cervidae*)
Hog deer	X			UK	Zoo (*Cervidae*)
Moose	X			UK	Zoo (*Cervidae*)
Moufflon	X			Germany	Wild (*Cervidae*)
Pere David's deer	X			UK	Zoo (*Cervidae*)
Philippine spotted deer	X			UK	Zoo (*Cervidae*)

Red deer	X			Belgium, France, Germany, Poland	Wild (*Cervidae*)
Reindeer	X			UK	Zoo (*Cervidae*)
Roe deer	X			Germany Netherlands	Wild (*Cervidae*)
Sika deer	X			Germany	Wild (*Cervidae*)
Swamp deer	X			UK	Zoo (*Cervidae*)
Asian Elephant	X			UK	Zoo (*Elephantidae*)
Donkey	X			UK	Zoo (*Equidae*)
Grevy's Zebra	X			UK	Zoo (*Equidae*)
Onager	X			UK	Zoo (*Equidae*)
Poitou donkey	X			UK	Zoo (*Equidae*)
Przewalski's horse	X			UK	Zoo (*Equidae*)
Shetland pony	X			UK	Zoo (*Equidae*)
Reticulated giraffe	X			UK	Zoo (*Giraffidae*)
Rothschild giraffe	X			UK	Zoo (*Giraffidae*)
Pygmy hippo	X			UK	Zoo (*Hippopotamidae*)
White Rhinoceros	X			UK	Zoo (*Rhinocerotidae*)
Indian Rhinocerus	X			UK	Zoo (*Rhinocerotidae*)
Babirusa	X			UK	Zoo (*Suidae*)
Red river hog	X			UK	Zoo (*Suidae*)
Wild Boar	X			Germany Netherlands	Wild (*Suidae*)
South American tapir	X			UK	Zoo (*Tapiridae*)
Alpaca	X	X		Austria, Germany, UK	Domestic (*Camelidae*)
Bactrian camel	X			UK	Zoo (*Camelidae*)
Llama	X	X		Germany, UK	Domestic (*Camelidae*)
Dog	X	X	X	France	Domestic (*Carnivora*)

Table created from data from (EC, 2014; Jack *et al.*, 2012; Larska *et al.*, 2013a; Molenaar *et al.*, 2015; Schulz *et al.*, 2015; Steinrigl *et al.*, 2014)

Appendix II: Stokes *et al.,* (2016)

Paper
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A freedom from disease study: Schmallenberg virus in the south of England in 2015

Jessica Eleanor Stokes, Matthew Baylis, Jennifer Sarah Duncan

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 naive ruminants by SBV during the vulnerable period of gestation leads to abortions, stillbirths and fetal malformations. Although some data exist 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, the authors 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; 5 samples from 4 farms were twice found positive by ELISA but were later confirmed negative by virus neutralisation test. As the sheep were born between October 2014 and April 2015, the authors conclude that it is unlikely that SBV is still circulating in the south of England.

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 (Hoffmann and others 2012). 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 southernmost counties of England all reporting outbreaks of SBV between 2012 and 2013 (EFSA 2012). Like several viruses of the Simbu serogroup and the unrelated bluetongue virus serotype 8 (BTV-8), SBV is transmitted by *Culicoides* biting midges. It is thought that the initial incursion into the UK was via wind dispersal of SBV-infected *Culicoides* from France 113 days before the first report of a malformed lamb (Elbers and others 2013, Sedda and Rogers 2013).

Since its initial discovery, SBV has been detected throughout Europe (EFSA 2014) in domestic cattle, sheep, goats and numerous species of wild ruminants, including camelids. Recently, a high frequency of samples from hunted wild boar in Germany were found to have SBV-specific antibodies (collected 2011/2012) (Mouchantat and others 2015). Additionally, there is a single report of SBV-specific antibodies in a dog, but other studies have failed to find evidence of infection in carnivores (Wensman and others 2013, Mouchantat and others 2015). European studies, conducted in 2011, 2012 and 2013, found animal-level prevalence to range between 8 to 100 per cent and 8.5 to 93.3 per cent in cattle and sheep, respectively (Elbers and others 2012, Gache and others 2013, Nanjiani and others 2013). Herd-level prevalence of UK sheep in 2012/2013 was found to range between 40 and 90 per cent (Nanjiani and others 2013).

SBV infections of adult ruminants are generally asymptomatic; however, if infection of a naive pregnant animal coincides with the vulnerable period of gestation, transmission across the placenta can result in abortions, stillbirths and fetal malformations (Beer and others 2013, Doceul and others 2013). Studies on the related Akabane virus estimate the vulnerable period to be between days 28 and 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 (EFSA 2012, Martinelle and others 2015). Fetal or neonate malformations typically present as arthrogryposis, scoliosis, kyphosis, severe torticollis, brachygnathia and hypoplasia of the central nervous system (Doceul and others 2013). The hypoplasia may be mild to severe, resulting in microcephaly, hydranencephaly and spinal cord and cerebellar hypoplasia (van den Brom and others 2012, Doceul and others 2013). Behavioural and/or neurological disorders are also frequently noted, with lung hypoplasia sometimes observed (Lievaart-Peterson and others 2012). In the case of twins, it is possible for only one to present with malformations, while the other remains viable, or for one twin to present with arthrogryposis, whereas the other presents neurologically (Doceul and others 2013).

A recent study on the duration of immunity in experimentally infected adult sheep has demonstrated SBV-specific IgG antibodies detectable for over one year after a single challenge with SBV (Poskin and others 2015). Additional evidence exists of acquired immunity against reinfection in naturally infected sheep, as well as evidence of maternally derived antibodies in

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suckling lambs (Rodríguez-Prieto and others 2014). While experimentally infected cattle have been demonstrated to remain immune to reinfection for at least 56 days (Wernike and others 2013).

Four cases of SBV were confirmed on January 16, 2012 (Harris and others 2014). Voluntary reporting recorded 81 and 87 serologically confirmed cases in UK sheep in 2012 and 2013, respectively (AHVLA 2013); 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 naive cattle from the Netherlands detected a low level of SBV (<1 per cent) in 2013 (Veldhuis and others 2015). A German study reported a recurrence of SBV in cattle in 2014, despite an apparent decrease in cases the previous year (Wernike others 2015).

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 following 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 whether SBV was still circulating in the southernmost counties of England in 2015 by examining the serological status of sheep born after the 2014 vector period.

Materials and methods

All animal work was reviewed and approved by the University of Liverpool internal ethics committee (VREC310) and carried out under a Home Office Project Licence (PPL 70/8529). All farmers gave informed written consent and were reminded of their right to withdraw from the study at any point.

To calculate the number of farms needed to substantiate a prevalence of 2.5 per cent or below the software package, FFD was implemented in R (Kopacka 2011). As sheep occur within flocks, a two-stage cluster analysis was used to estimate both the number of flocks and the number of sheep within each flock

to be sampled; individual sampling was selected to allow the test sensitivity to remain the same across flocks. The α-error threshold was set to 0.05 (5 per cent). An intraherd prevalence of 20 per cent was set, which is lower than the prevalence recorded in several large-scale continental studies, but closer to the lower range reported in a 2013 UK study (Elbers and others 2012, Nanjiani and others 2013, Veldhuis and others 2013, Méroc and others 2013b); herd sensitivity of Se_{herd}=90% was set (EFSA 2014), with a known test sensitivity of Se_2=97.2% (Bréard and others 2013). The total number of sheep holdings in the southernmost counties of England was extracted from the Department for Environment, Food & Rural Affairs (DEFRA) 2010 census: a total of 6495 sheep holdings were registered. This determined a necessary sample size of 11 sheep per holding collected from 131 holdings to detect prevalence below 2.5 per cent with 95 per cent confidence. Holdings were recruited for the study through the National Sheep Association (NSA) South West show, NSA magazine and large animal veterinary practices (Fig 1). The number of holdings sampled per county was stratified based on DEFRA 2010 census data: Cornwall (n=18), Devon (n=67), Dorset (n=12), Hampshire (n=5), Sussex (n=20) and Kent (n=9).

Blood samples were collected between September 15, 2015, and December 11, 2015, from the jugular vein of 12 sheep per holding (11, plus 1 to account for failures). Sampling began after the spring and summer peaks in midge activity, with the majority of samples collected after the final autumn peak in midge activity (Sanders and others 2011). All sampled sheep were born after October 2014 and were more than six months old at time of sampling to exclude animals with immunity following infection in 2012, 2013 or 2014 and to avoid maternal antibodies. This assumption is based on the maternal antibodies of calves lasting less than six months for both SBV and Akabane virus (Tsutsui and others 2009, Elbers and others 2014). Serum was

FIG 1: Map of the south of England showing the distribution of sampled farms. Exact farm location has been jittered and enlarged to prevent individual participant identification. Ten farmers asked that their farm location was not mapped

extracted from the blood samples and analysed by a commercially available SBV antibody ELISA (ID Screen SBV indirect, IDvet, France) as per the manufacturer's instructions. Serum samples were considered negative if the S/P% (sample to positive percentage) was up to 50 per cent calculated as per manufacturer's instructions. Samples returning an S/P% greater than 50 per cent were sent to the APHA to be confirmed by a virus neutralisation test (VNT). An equal number of samples returning an S/P% less than 50 per cent were also sent as blind controls; they were selected randomly using the RANDBETWEEN function in Microsoft Excel to determine farm number and then sample number from farm. Two positive controls were used in the VNT, with VNT titres of 1/40 and 1/80, respectively. Samples were determined to be negative if the VNT titre was greater than 1/5 based on the minimum dilution undertaken at the APHA (La Rocca, personal communication).

Results

A total of 1572 sheep from 131 holdings were sampled between September 15 and December 11, 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, 8 hill, 8 upland, 10 had flocks across lowland, hill and/or upland pastures and 2 holdings declined to answer or were unsure.

Half (50.0 per cent) of farmers (57/124, seven farmers declined to answer) reported that they had previously suspected cases of SBV infection in their flocks in the form of birth of lambs showing typical congenital abnormalities. Of these 57 farmers, 12 had cases that were diagnosed by a vet but not laboratory confirmed, while 13 had cases that were diagnosed by a vet and laboratory confirmed as SBV. In the remaining 32 suspect case farms, none had disease diagnosed, either by a vet or laboratory.

Only 13.7 per cent (17/124) of farmers stated that they had vaccinated their sheep against SBV; 15 farmers stated they vaccinated in 2013, while 2 farmers vaccinated in both 2013 and 2014. One farmer vaccinated only their cattle against SBV but not their sheep.

By contrast, only 1.6 per cent (2/124) of the farmers stated that they had had cases of BTV on farm, with 78.2 per cent (97/124) stating that they had vaccinated against BTV for at least one year.

A total of 11 sheep from each holding were tested by ELISA for antibodies against SBV (1444 samples in total). Overall nine samples, from eight holdings, returned doubtful or positive (S/P per cent > 50 per cent) 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 VNT at the APHA (Table 1).

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 per cent confident that if SBV was circulating in the south of England in the 2015 vector period, it was present below the 2.5 per cent 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 per cent prevalence in 2013 (Veldhuis and others 2015).

The specificity of the commercial ELISA kit used was reported to be 99.8 per cent, giving a likely false-positive rate of ~3 samples of the 1444 tested. Initially 9 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 per cent reported in wild cervids (Laloy and others 2014) tested by both the indirect ELISA used here and the VNT (Bréard and others 2013, Laloy and others 2014). The use of VNTs as conformational tests for commercial ELISAs is considered advisable due to the high (~99–100 per cent) sensitivity and specificity of the VNT (Loeffen and others 2012).

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 (Veldhuis and others 2013, Méroc and others 2013a, EFSA 2014, Wernike and others 2014). 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 (Gubbins and others 2014). Even with low levels of SBV circulation and few susceptible hosts on farm, previous studies have demonstrated eventual seroconversion of these individuals (Elbers and others 2013). 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 naive population. However, this

TABLE 1: Schmallenberg virus (SBV) ELISA and virus neutralisation test (VNT) results in samples that returned a positive ELISA test result, holding ID, county of farm, breed of sheep, birth and sample dates, ELISA titre (S/P%), VNT result, previous self-reporting of suspected cases on farm by farmer and if the farm vaccinated against SBV in 2013

Sheep ID	Holding ID	County	Breed	Born	Sampled	S/P% ELISA	S/P% ELISA retest	VNT result	Previous Suspected SBV cases on farm?	Vaccinated flock against SBV in 2013
1	21	Dorset	Texel	March 2015	September 2015	166.55*	9.82†	Not Tested	No	Yes
2	25	Dorset	Poll Dorset	January 2015	September 2015	64.93*	46.40†	Not Tested	No	No
3	46	Hampshire	Hampshire	December 2014	October 2015	60.77*	44.41†	Not Tested	Yes‡	No
4	48	Cornwall	Roussin	February 2015	October 2015	55.43*	39.54†	Not Tested	Yes	No
5	59	Devon	Highlander	March 2015	October 2015	78.14*	120.62*	Negative	No	No
6	108	Sussex	Dorset	February 2015	November 2015	110.32*	80.97*	Negative	Yes‡	No
7	113	Sussex	Charolais	February 2015	November 2015	90.61*	127.66*	Negative	Yes	No
8	113	Sussex	Charolais	February 2015	November 2015	66.45*	125.91*	Negative		
9	121	Cornwall	Lleyn X Texel	March 2015	November 2015	117.97*	123.79*	Negative	No	No
10	12	Devon	Poll Dorset	December 2014	September 2015	3.19†	Not Tested	Negative	Yes	No
11	50	Cornwall	Zwartble	March 2015	October 2015	3.93†	Not Tested	Negative	No	No
12	99	Kent	Charolais	March 2015	November 2015	8.46†	Not Tested	Negative	Yes	No
13	106	Sussex	Charolais	March 2015	November 2015	2.50†	Not Tested	Negative	Yes‡	No
14	115	Sussex	Southdown X Dorset	April 2015	November 2015	5.18†	Not Tested	Negative	Yes‡	No

Samples 10–14 are negative controls for the VNT
*ELISA positive S/P%
†ELISA negative S/P%
‡Laboratory-confirmed cases of SBV on farm

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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 with 2011–2012 seroprevalence, followed by an increase in cases the following year (Wernike and others 2015). 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 (Sailleau and others 2015). 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 (Ruiz-Fons and others 2014). 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 (Rossi and others 2015). 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 (Foskin and others 2016). Targeted surveillance could also be used, 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, naive 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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Appendix III: BTV8 questionnaire

Introduction

Bluetongue virus serotype 8 (BTV-8) has recently re-emerged in France. The virus infects ruminants, including sheep, cattle, deer, goats and camelids. This is the same serotype that caused an outbreak in mainland Europe in 2006, reaching England in 2007. The virus is spread by *Culicoides* biting midges- the same biting flies that spread Schmallenberg virus in 2011/2012.

BTV-8 is a notifiable disease. Cattle carry BTV-8 but clinical signs aren't always common. Sheep however may develop ulcers in the mouth, discharge from the nose and/or swelling of the mouth, head and the skin around the horn of the foot. During the 2006/2007 outbreak in Europe it was estimated that BTV-8 mortality for sheep was about 6%, with the disease estimated to cause mortality in only 2% of cattle infected. More clinical signs and information for cattle and sheep can be found here: https://www.gov.uk/guidance/bluetongue.

Previous control focused on voluntary vaccination and protection zones, restricting animal movement to within zones. Vaccination was encouraged through TV and media campaigns, with DEFRA eventually subsidising the wholesale cost of the vaccine by 50%. The vaccine used in 2007/2008 is not currently being produced: this means that it may be 8 weeks or more between initial demand for a vaccine and the first vaccines becoming available for sale.

This survey aims to find out what demand currently exists among UK farmers for access to the vaccine, and what potential conditions would affect this demand. Your participation in the study is voluntary. By completing the questionnaire you give us consent to analyse and publish the anonymised results. All answers will be treated confidentially and you can end the questionnaire at any time. The results of this study will be published online in a scientific research journal.

1. Name:

2. Name of Farm:

3. What country is your farm based in?
   - [ ] England
   - [ ] Scotland
   - [ ] Wales
   - [ ] Northern Ireland
   - [ ] I am not based in the UK

4. What are the first 2 letters of your postcode?
   (e.g. our postcode is CH64 7TE so we would put CH)

5. Do you own?
   - [ ] Sheep (>>Section: Background: Sheep)
   - [ ] Cattle (>>Section: Background: Cattle)
   - [ ] Sheep & Cattle (>>Section: Background: Sheep & Cattle)
   - [ ] I do not own any sheep or cattle

Background: Sheep

6. Are your sheep:
   - ☐ Pedigree
   - ☐ Commercial
   - ☐ I own both Pedigree and Commercial sheep

7. How many of each of the following do you own?

Adult female sheep (>1 year old)

Adult male sheep (>1 year old)

Lambs (<1 year old)

8. Did you vaccinate your sheep against bluetongue virus in 2008/2009?
   - ☐ Yes
   - ☐ No
   - ☐ Some of my flock, but not all
   - ☐ I did not own sheep in 2008/2009

9. Did you vaccinate your sheep against Schmallenberg virus in 2012/2013?
   - ☐ Yes
   - ☐ No
   - ☐ Some of my flock, but not all
   - ☐ I did not own sheep in 2012/2013

10. Any additional comments you would like to make about this section

Background: Cattle

11. Are your cattle:
    - ☐ Pedigree
    - ☐ Commercial
    - ☐ I own both Pedigree and Commercial cattle

12. Are your cattle:
    - ☐ Beef
    - ☐ Dairy
    - ☐ Both beef and dairy
    - ☐ Other (please specify)

13. How many of each of the following <u>dairy</u> cattle do you own?

Adult young stock (Calf- prebulling)	
Bulling heifers	
In calf heifers	
Milking	
Bulls	

14. How many of each of the following <u>beef</u> cattle do you own?

Breeding females (>2 year old)	
Adult bulls (>2 year old)	
Young stock (<2 year old)	

15. Did you vaccinate your cattle against bluetongue virus in 2008/2009?
    - ☐ Yes
    - ☐ No
    - ☐ Some of my flock, but not all
    - ☐ I did not own sheep in 2008/2009

16. Did you vaccinate your cattle against Schmallenberg virus in 2012/2013?
    - ☐ Yes
    - ☐ No
    - ☐ Some of my flock, but not all
    - ☐ I did not own sheep in 2012/2013

17. Any additional comments you would like to make about this section

Background: Sheep & Cattle

18. Are your sheep:
    - ☐ Pedigree
    - ☐ Commercial
    - ☐ I own both Pedigree and Commercial sheep

19. How many of each of the following do you own?

Adult female sheep (>1 year old)	
Adult male sheep (>1 year old)	
Lambs (<1 year old)	

20. Did you vaccinate your sheep against bluetongue virus in 2008/2009?
    - ☐ Yes
    - ☐ No
    - ☐ Some of my flock, but not all
    - ☐ I did not own sheep in 2008/2009

21. Did you vaccinate your sheep against Schmallenberg virus in 2012/2013?
    - ☐ Yes
    - ☐ No
    - ☐ Some of my flock, but not all
    - ☐ I did not own sheep in 2012/2013

22. Are your cattle:
    - ☐ Pedigree
    - ☐ Commercial
    - ☐ I own both Pedigree and Commercial cattle

23. Are your cattle:
    - ☐ Beef
    - ☐ Dairy
    - ☐ Both beef and dairy
    - ☐ Other (please specify)

24. How many of each of the following <u>dairy</u> cattle do you own?

Adult young stock (Calf- prebulling)

Bulling heifers

In calf heifers

Milking

Bulls

25. How many of each of the following <u>beef</u> cattle do you own?

Breeding females (>2 year old)

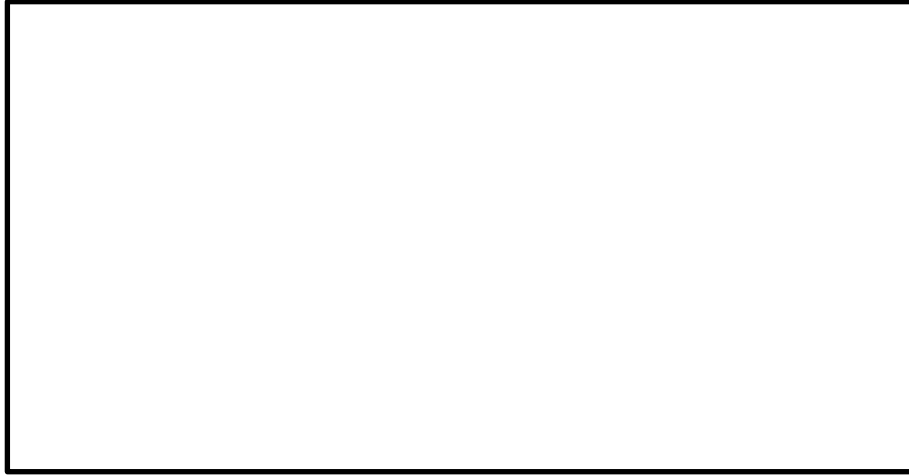Adult bulls (>2 year old)

Young stock (<2 year old)

26. Did you vaccinate your cattle against bluetongue virus in 2008/2009?
- ☐ Yes
- ☐ No
- ☐ Some of my flock, but not all
- ☐ I did not own sheep in 2008/2009

27. Did you vaccinate your cattle against Schmallenberg virus in 2012/2013?
- ☐ Yes
- ☐ No
- ☐ Some of my flock, but not all
- ☐ I did not own sheep in 2012/2013

28. Any additional comments you would like to make about this section

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Current BTV-8 situation: Sheep

BTV is currently in central France, Defra's risk assessment states it is likely an outbreak will occur this summer (2016). Defra also states that a cold spring and summer would reduce this risk. A vaccine is not currently being manufactured, and it would likely take around 2 months to begin producing vaccine if there was sufficient demand.

29. Are you currently intending to place an order to vaccinate your sheep against BTV8? (Assume sheep will have one dose/animal)

    ☐ Yes at any cost* (please see next question)
    ☐ Yes if it costs £1 per dose or less
    ☐ Yes if it costs 80p per dose or less
    ☐ Yes if it costs 40p per dose or less
    ☐ Yes, but only if the vaccination is free
    ☐ No I am not planning to vaccinate.

    Please tell us why you are not currently considering vaccinating:

30. If you answered 'Yes at any cost', what would be the highest price per dose you would be willing to pay?

Current BTV-8 situation: Cattle

BTV is currently in central France, Defra's risk assessment states it is likely an outbreak will occur this summer (2016). Defra also states that a cold spring and summer would reduce this risk. A vaccine is not currently being manufactured, and it would likely take around 2 months to begin producing vaccine if there was sufficient demand.

31. Are you currently intending to place an order to vaccinate your cattle against BTV8? (Assume cattle will have two doses/animal)
    - ☐ Yes at any cost* (please see next question)
    - ☐ Yes if it costs £1 per dose or less
    - ☐ Yes if it costs 80p per dose or less
    - ☐ Yes if it costs 40p per dose or less
    - ☐ Yes, but only if the vaccination is free
    - ☐ No I am not planning to vaccinate.

        Please tell us why you are not currently considering vaccinating:

32. If you answered 'Yes at any cost', what would be the highest price per dose you would be willing to pay?

Current BTV-8 situation: Sheep & Cattle

BTV is currently in central France, Defra's risk assessment states it is likely an outbreak will occur this summer (2016). Defra also states that a cold spring and summer would reduce this risk. A vaccine is not currently being manufactured, and it would likely take around 2 months to begin producing vaccine if there was sufficient demand.

33. Are you currently intending to place an order to vaccinate your sheep against BTV8? (<u>Assume sheep will have one dose/animal</u>)

    ☐ Yes at any cost* (please see next question)

    ☐ Yes if it costs £1 per dose or less

    ☐ Yes if it costs 80p per dose or less

    ☐ Yes if it costs 40p per dose or less

    ☐ Yes, but only if the vaccination is free

    ☐ No I am not planning to vaccinate.

        Please tell us why you are not currently considering vaccinating:

34. If you answered 'Yes at any cost', what would be the highest price per dose you would be willing to pay?

35. Are you currently intending to place an order to vaccinate your cattle against BTV8? (<u>Assume cattle will have two doses/animal</u>)

- ☐ Yes at any cost* (please see next question)
- ☐ Yes if it costs £1 per dose or less
- ☐ Yes if it costs 80p per dose or less
- ☐ Yes if it costs 40p per dose or less
- ☐ Yes, but only if the vaccination is free
- ☐ No I am not planning to vaccinate.

Please tell us why you are not currently considering vaccinating:

36. If you answered 'Yes at any cost', what would be the highest price per dose you would be willing to pay?

Scenarios: (repeated for all)

The following scenarios are to determine what conditions would need t be in place for your to consider vaccination worthwhile

37. For each scenario please tick the conditions that would need to be in place for you to vaccinate.

For example: If BTV-8 spreads to the south of France I would want to vaccinate my most valuable stock, but only if the vaccination costs less than 40p per dose. I should tick: 'vaccination costs 40p per dose' and 'I would only vaccinate my most valuable' for the scenario 'BTV-8 stays in central France, spreading to all southern provinces of France'

Scenarios	Price of vaccination per dose (assume sheep=1 dose/animal, cattle= 2 doses/animal)					
	£1	80p	40p	I would vaccinate some of my flock (<50%)	I would vaccinate my most valuable (<10% of flock)	I wouldn't vaccinate
BTV-8 stays in central France, spreading to all southern provinces of France.						
BTV-8 stays in central France, spreading to the northern provinces of France.						
All of France is now BTV-8 positive. The disease has also spread to Belgium, the Netherlands and Southern Germany.						
A case of BTV-8 is confirmed in Suffolk.						
Cases of BTV-8 are confirmed in Suffolk, Norfolk, Kent, Sussex, Hampshire and Dorset						
Cases of BTV-8 are confirmed in your neighbouring county						

38. Any comments you would like to make about this section:

Final Questions: (repeated for all)

These questions are on your perceptions towards vaccination.

39. How important do you believe vaccination is for preventing disease within your flock/herd?

40. How important do you believe vaccination was in preventing a larger UK BTV-8 outbreak in 2007/2008?

41. How important do you believe it is to keep BTV-8 out of the UK?

Debrief

Thank you for your time. Your answers will help to build a better understanding of likely BTV-8 vaccine use under differing situations. This will inform disease models and, in turn, affect the way we analyse disease risk. Anonymised study results will be published in a scientific peerreviewed journal.

 If you have any further questions or concerns about this study, please contact us using one of the addresses below. If you wish to withdraw at any time please email jstokes@liverpool.ac.uk with the name you entered at the beginning of the study and the county your farm is in, all your responses will then be deleted immediately and you will be sent an email confirming that this action has been taken.

Student Investigator: Jessica Stokes jstokes@liverpool.ac.uk
Principal Investigator: Professor Matthew Baylis baylism@liverpool.ac.uk
University of Liverpool,
Leahurst campus
Chester High Road
Neston
CH64 7TE

42. If you would like a summary of our findings please enter an email address we can send the summary to.
    Your email address will be kept safe and will only be used to send you the

    findings of the study.

Appendix IV: supplementary BTV-8 tables

How important do you believe vaccination is for preventing disease within your flock/herd?

	Proportion (%) of respondents answering (n=102)		
	Extremely important or important	Neither important nor unimportant	Extremely unimportant or unimportant
Total responses	85.3	4.9	9.8
Farm size			
Small	91.3	0	8.7
Medium	94.9	0	5.1
Large	72.5	12.5	15
Farm type			
Sheep	89.1	0	11.0
Cattle	81.8	9.1	9.1
Both sheep & cattle	80.6	11.1	8.4
Zone			
1	77.2	2.9	20
2	89.5	2.6	7.9
3	89.7	10.3	0
Pedigree			
Owns Pedigree	94.3	1.9	3.8
Owns No Pedigree	75.6	8.2	16.4

How important do you believe vaccination was in preventing a larger UK BTV-8 outbreak in 2007/2008?

	Proportion (%) of respondents answering (n=94)		
	Extremely important or important	Neither important nor unimportant	Extremely unimportant or unimportant
Total responses	80.9	6.4	12.8
Farm size			
Small	75.0	15.0	10
Medium	86.8	2.6	10.5
Large	77.8	5.6	16.6
Farm type			
Sheep	84.3	5.9	9.8
Cattle	90.9	0.0	9.1
Both sheep & cattle	71.9	9.4	18.7
Zone			
1	87.1	0.0	12.9
2	83.4	8.3	8.4
3	71.3	11.1	18.5
Pedigree			
Owns Pedigree	48.5	7.8	13.8
Owns No Pedigree	83.8	4.7	11.7

How important do you believe it is to keep BTV-8 out of the UK?

	Proportion (%) of respondents answering (n=100)		
	Extremely important or important	Neither important nor unimportant	Extremely unimportant or unimportant
Total responses	90.0	5.0	5.0
Farm size			
Small	95.5	0.0	4.5
Medium	94.7	2.6	2.6
Large	82.5	10	7.5
Farm type			
Sheep	96.2	0.0	3.8
Cattle	90.9	0.0	9.1
Both sheep & cattle	80.5	13.9	5.6
Zone			
1	85.3	2.9	11.8
2	87.2	10.3	2.6
3	100	0	0
Pedigree			
Owns Pedigree	90.4	3.8	5.8
Owns No Pedigree	89.6	6.3	4.2

Appendix V: SBV impact questionnaire
Questionnaire to measure the impact of the 2016/2017 Schmallenberg virus

outbreak on sheep farms (Paper Version)

You are being invited to complete a questionnaire on the impact of the Schmallenberg virus outbreak on sheep farms this lambing season (2016/17).

The results of the questionnaire can be used to help further our knowledge of the virus and the problems it is causing to farmers and their flocks.

We would ask you to complete the questionnaire regardless of whether you have had any clinical signs of Schmallenberg virus in your flock or not.

The purpose of the questionnaire is to measure;

- How many flocks were, or were not, affected
- Where and when the virus was active in the UK
- What effect the virus had on ewe and lamb losses
- Assessment of impact of SBV infection on lamb and ewe losses
- Estimate the cost of Schmallenberg virus in UK
- Your opinion on potential control measures that could be used (including vaccination)

The survey is anonymous, but we ask for the first two letters of your farms postcode only to be able to map the spread of disease, but your farm will remain anonymous and will not be identifiable. If you would like a summary of the findings of the survey please provide us with a contact email at the end of the survey.

The survey should only take about 15-20 minutes to fill in but it would be helpful if you had an idea of your scanning and lambing figures before starting. You don't have to answer every question but as much information as possible is helpful to us.

By continuing with the survey you consent that the anonymous information provided

- will be anonymised and treated confidentially
- will be used for a research study and written in a report for publication
- may be presented at research conferences or meetings
- that you can request to see a copy/summary of the completed study
- that you can request to see your own information written down/kept during the process of data collection.

Thank you very much for participating in this study. If you have any questions or requests with regards to the study please do not hesitate to contact us:

 Ms Jess Stokes: jstokes@liverpool.ac.uk
Dr Rachael Tarlinton: rachael.tarlinton@nottingham.ac.uk

Please complete the questionnaire for each separate sheep flock that you are responsible for. Alternatively please answer the questionnaire for your "main" flock.

1. What are the first two letters of your postcode?

   [   ]

2. Type of sheep farm:
   - ☐ Lowland
   - ☐ Upland/Hill

3. Is your flock
   - ☐ Pedigree or pure bred
   - ☐ Crossbreed or commercial
   - ☐ Milk sheep

4. How many breeding ewes did you put to ram this (2016/2017) lambing season?

   [   ]

5. **What breed are your ewes?**

   [   ]

6. What date did the rams go in with the breeding ewes?

> [/   /   ]   (DD/MM/YYYY)

7. What date were the rams removed?

> [/   /   ]   (DD/MM/YYYY)

8. Were the ewes scanned during pregnancy?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

9. If the ewes were scanned in pregnancy please fill in your date of scanning (please skip if no/don't know)

> [/   /   ]   (DD/MM/YYYY)

10. If the ewes were scanned in pregnancy what was your scanning percentage this lambing season (2016/2017)?

> [   ]

11. What was your scanning percentage last year (2015/2016 lambing season)? (please skip if you did not scan)

> [   ]

12. How many barren ewes did you have at scanning this season (2016/2017)?

> [   ]

What date did lambing start for your 2016/2017 lambing period?

| / / | (DD/MM/YYYY)

13.   What date did lambing end, or is expected to end for your 2016/2017 lambing period?

| / / | (DD/MM/YYYY)

14.   How many ewes do you have left to lamb? (please put 0 if you have no ewes left to lamb)

15.   Please enter the TOTAL number of lambs for the 2016/2017 lambing season:

Aborted or stillborn

Died within 1 week of birth

Reared for more than 1 week

16. Please enter the total number of lambs for the 2016/2017 lambing season where Schmallenberg virus WAS suspected (including deformities and nervous signs) that:

Aborted or stillborn

Died within 1 week of birth

Reared for more than 1 week

17. How were the numbers of lambs in Q16 and Q17 calculated?
☐ Estimated
☐ From records

18. If you have had malformed lambs in the 2016/2017 season please describe the types of problems they had:

19.   For ewes that produced one or more deformed lambs (2016/2017) please give us the number of lambings that:

Lambed on own

Assisted by yourself/farm hand

Assisted by a vet

Caesarian section

20.   Total number of breeding ewes (2016/2017) that:

Died during the lambing period

Died during lambing because of

difficulties with giving birth to

deformed lambs

21. Do you have other ruminant animals on your property? Please fill in the numbers in each category for 2016/2017

Cattle

Goats

Alpaca

Llama

Deer (give species)

Other

22. Have you had any aborted, stillborn or deformed young in any of these species? Please fill in the number for the most recent birthing

Cattle

Goats

Alpaca

Llama

Deer (give species)

Other

23. Do you believe your flock was infected by Schmallenberg virus this year (2016/17)?
☐ Yes
☐ No

Comments (optional):

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24. If yes, was this tested (please tick):
☐ Confirmed by laboratory testing of a lamb
☐ Confirmed by laboratory testing of a ewe blood samples
☐ Suspected by a vet, but not confirmed by laboratory testing
☐ Suspected by yourself, but not confirmed by laboratory testing
☐ Other

Any details you would like to share (optional):

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25.   Have you ever had testing done for Schmallenberg virus (please tick all that apply):
☐ Yes malformed lambs
☐ Yes malformed calves
☐ Yes in ewes
☐ Yes in individual cows
☐ Yes bulk milk tank
☐ No, I have never had any testing for Schmallenberg virus

If yes, which years have you tested for Schmallenberg virus?:

26.   What impact do you think Schmallenberg virus has had on the welfare of your flock this lambing 2016/2017? (please tick)

☐ Strong positive impact
☐ Some positive impact
☐ No impact
☐ Some negative impact
☐ Strong negative impact

27.   How important do you think Schmallenberg virus will be on the financial performance of the sheep flocks on your farm this year?

☐ Strong positive impact
☐ Some positive impact
☐ No impact
☐ Some negative impact
☐ Strong negative impact

28.   During lambing, did the potential threat of Schmallenberg virus affect you, your lambing staff or your family in terms of emotional wellbeing?

☐ Strong positive impact
☐ Some positive impact
☐ No impact
☐ Some negative impact
☐ Strong negative impact

29.   Has Schmallenberg virus meant that you are less likely to sheep farm next year?

☐ Yes
☐ No

30.   Have you ever vaccinated your sheep for Schmallenberg virus? If yes please tick all years that apply:

☐ No, I have never vaccinated against Schmallenberg virus
☐ Yes I vaccinated in 2016
☐ Yes I vaccinated in 2015
☐ Yes I vaccinated in 2014
☐ Yes I vaccinated in 2013

31.   Would you consider vaccinating your sheep against Schmallenberg virus if it was available now?
☐ No
☐ Yes if it cost less than £1
☐ Yes if it cost between £1-2
☐ Yes if it cost between £2-3
☐ Yes if it cost between £3-4
☐ Yes if it cost between £4-5

32.   Any other comments?

33.   Please leave your email address if you wish to receive a copy of the survey results and an update on Schmallenberg virus impact and research

Thank you for participating in our research.

If you have any questions or concerns, please contact us:

Ms Jess Stokes: **jstokes@liverpool.ac.uk**
                0151 794 6093
                University of Liverpool,
                Leahurst campus,
                Neston,
                CH64 7TE

Dr Rachael Tarlington: rachael.tarlinton@nottingham.ac.uk