

A review on Schmallerberg virus infection: a newly emerging disease of cattle, sheep and goats

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ABSTRACT: Schmallerberg virus (SBV) infection is an emerging infectious disease of ruminants first described in Germany in November, 2011. Since then it has spread very rapidly to several European countries. The disease is characterised by fever, reduced milk production and diarrhoea in cattle and abortions, stillbirths and foetal abnormalities in sheep and goats. SBV is an enveloped, negative-sense, segmented, single-stranded RNA virus, classified in the genus *Orthobunyavirus* of the Bunyaviridae family, and is closely related to Akabane, Aino and Shamonda viruses. As of now there is no vaccine available for SBV, which poses a serious threat to naive ruminant population. Owing to its recent discovery, our understanding of Schmallerberg viral disease and its pathology and pathogenesis is limited. This article reviews the data reported so far on this emerging disease with regard to aetiology, epidemiology, pathogenesis, pathology, diagnosis and control and discusses the future scenario and implications of the disease.

Keywords: abortion; congenital malformation; emerging infection; pathology; pathogenesis; ruminants; Schmallerberg virus; stillbirths

Contents

- | | |
|---|---|
| 1. Introduction | 5. Pathogenesis |
| 1.1 History and geographic distribution | 6. Pathology |
| 2. Aetiology | 7. Diagnosis |
| 3. Epidemiology | 7.1. Viral isolation and identification |
| 3.1. Host range | 7.2. Differential diagnosis |
| 3.2. Transmission | 8. Prevention and control |
| 4. Clinical manifestations | 9. Conclusion |
| | 10. References |

1. Introduction

The term Emerging Infectious Diseases describes new or unrecognised diseases, those that are spreading to new geographic areas and hosts, as well as those that are re-emerging (Marsh 2008). Schmallerberg virus infection is a newly emerging infectious disease of ruminants in Europe which spreads through *Culicoides* midges bites. It is characterized by fever, inappetence, decreased milk production, loss of condition and diarrhoea in cattle and abortion and stillbirths associated with con-

genital malformations in sheep and goats (Gibbens 2012). The Schmallerberg virus (SBV) belongs to the genus *Orthobunyavirus* in the Bunyaviridae family which comprises hundreds of viruses pathogenic to vertebrate and invertebrate hosts. Vaccines for SBV are not available, which poses a serious threat to naive populations of ruminant livestock. Owing to its recent discovery, our understanding of Schmallerberg viral disease and its pathology and pathogenesis is limited. The present review discussed the data reported thus far on this newly emerging disease.

1.1. History and geographic distribution

In the border region of Germany and the Netherlands, between August and October 2011, adult cattle were observed to exhibit unusual clinical manifestations characterised by mild to moderate fever, anorexia, significantly reduced milk yield, loss of condition and diarrhoea, with affected animals recovering in two to three weeks (Gibbens 2012). Then, from December, 2011 onwards, abortions and stillbirths associated with foetal deformities predominantly of limbs and the skull were seen in sheep, goat and cattle in the Netherlands, Germany and Belgium. Diagnostic testing for common diseases was negative. However, in November 2011, after ruling out the presence of other pathogens, scientists at the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (FLI), Germany isolated and sequenced viral genetic material from the affected cattle and identified a new virus. Since then in Germany alone, 2439 animals comprising 1427 cattle, 963 sheep and 49 goats have tested positive for SBV as of 7th May, 2013 (FLI 2013a). Similarly, in United Kingdom, 1753 animals comprising 1257 cattle, 492 sheep and two goats, one red deer and one alpaca have tested positive for SBV as of 24th April, 2013 (AHVLA, 2013). In a retrospective study in Turkey, employing indirect ELISA to screen 1362 serum samples collected from slaughterhouse animals between 2006 and 2013, the overall seroprevalence for SBV infection was found to be 24.5%, comprising 39.8% cattle, 1.6% sheep, 2.8% goats and 1.5% Anatolian water buffaloes, which indicated that the exposure of domestic ruminants to SBV in Turkey may have occurred up to five years prior to the first recorded outbreak of the disease in 2011 (Azkur et al. 2013). In Belgium, a serological survey of 1082 sheep and 142 goats to detect SBV-specific antibodies by ELISA revealed a 98.03% overall between-herd seroprevalence in sheep and a 40.68% within-herd seroprevalence in goats (Meroc et al. 2013b). So far, infections with SBV have been detected in Germany, the Netherlands, Belgium, the United Kingdom, France, Italy, Luxembourg, Spain, Denmark, Estonia, Switzerland, Ireland, Northern Ireland, Norway, Sweden, Finland, Poland, Austria, Switzerland and Turkey (Elbers et al. 2012; Azkur et al. 2013; FLI 2013a; Larska et al. 2013; Meroc et al. 2013a,b; Sailleau et al. 2013). According to unconfirmed reports there could be infection in further European countries. Biting midges infected

with SBV have so far been detected in Belgium, Denmark, Germany, Italy, and Norway.

2. Aetiology

Schmallenberg virus (SBV) is an enveloped, negative-sense, segmented, single-stranded RNA virus. As mentioned above it was first detected at the FLI, Germany using metagenomic analysis and named after the German town 'Schmallenberg' from which the first positive samples came. Scientists from the FLI led by Dr. Harald Granzow of the Institute of Infectology visualised SBV using high-resolution electron microscopic analyses of infected cells. The shape of the virus was similar to that of other bunyaviruses; the virus was visible as a membrane-enveloped particle with a diameter of about 100 nm. The membrane enveloped the three segments of the genetic information (FLI 2012).

Preliminary phylogenetic analyses classified SBV as a member of the genus *Orthobunyavirus* in the family Bunyaviridae which is closely related to Akabane, Ainoa and Shamonda viruses; these viruses are all in the Simbu-Serogroup, a subgroup not previously detected in Europe (Hoffmann et al. 2012). Similar to Akabane virus, another Simbu serogroup virus, SBV can cause fatal congenital defects by infection of foetuses during a susceptible stage in pregnancy (Garigliany et al. 2012a).

The genus *Orthobunyavirus* is the largest of five genera in the Bunyaviridae family which comprises over 170 named viruses, many of them pathogenic to humans and animals (Elliott and Blakqori 2011). The members of this genus are arthropod-borne viruses (arboviruses) that are mostly transmitted by mosquitoes and Culicoides biting midges. At least 25 viruses are included in the Simbu serogroup (Calisher 1996), and currently divided into seven species (Akabane virus, Manzanilla virus, Oropouche virus, Sathuperi virus, Shamonda virus, Shuni virus and Simbu virus) that are defined by cross-neutralisation tests and cross-haemagglutination-inhibition tests (Plyusnin et al. 2012). Several viruses in the Simbu serogroup are known to be teratogenic in ruminants. The genome of the genus *Orthobunyavirus* consists of three single-stranded, negative-sense RNA segments named large (L), medium (M), and small (S) segments according to their size. The L RNA segment encodes the RNA-dependent RNA polymerase; the M RNA segment encodes the two surface glycoproteins (Gn

and Gc) and the other non-structural protein (NSm). The Gn and Gc proteins act as antigenic determinants and are recognised by neutralizing antibodies. The S RNA segment encodes the nucleocapsid protein (N) and non-structural protein (NSs) which plays a role in complement fixation (Elliott and Blakqori 2011; Goller et al. 2012; Yanase et al. 2012) and also in modulating the innate immune response of host cells (Elliott et al. 2013; Varela et al. 2013). Of the three segments, the M RNA segment is the most variable among orthobunyaviruses. Genetic reassortment occurs naturally among these viruses, which results in the emergence of new virus strains with potential alterations in their antigenicity, virulence and host range (Bowen et al. 2001; Elliott and Blakqori 2011).

Initial characterisation of SBV showed that the M- and L-segment sequences most closely resembled those of Aino virus and Akabane virus sequences, whereas the N gene was most closely related to Shamonda virus (Hoffmann et al. 2012). Further studies revealed that SBV is a reassortant, with the M RNA segment from Sathuperi virus and the S and L RNA segments from Shamonda virus (Yanase et al. 2012). On the other hand, Saeed et al. (2001) described Shamonda virus as a reassortant virus comprising the S and L segments from Sathuperi virus and the M segment from the unclassified Yaba-7 virus. A very recent study by Goller et al. (2012) showed that SBV is most likely not a reassortant, rather one of the ancestors of Shamonda virus, which itself is a reassortant with the S and L genomic segments from SBV and M segment from an unclassified virus, thus fully supporting the conclusions of Saeed et al. (2001). The authors even suggested the reclassification of Shamonda virus into the species Sathuperi virus and its renaming as Peaton virus or Sango virus. However, a study published in May, 2013, based on the crystal structure of the bacterially expressed SBV nucleoprotein to a 3.06-Å resolution, reported that Schmallerberg virus (SBV) has a novel mechanism for viral RNA encapsidation and transcription (Dong et al. 2013). These detailed insights into the phylogeny of SBV could be the basis for the development of efficient, cross-protective vaccines.

The infectivity of SBV is lost or significantly reduced after exposure to temperatures of 50–60 °C for 30 min. The virus is also susceptible to common disinfectants such as 1% sodium hypochlorite, 2% glutaraldehyde, 70% ethanol and formaldehyde and does not survive outside the host or vector for long periods.

3. Epidemiology

According to epidemiological investigations, reinforced by what is already known about the genetically related Simbu serogroup viruses, SBV affects ruminants (OIE 2013). Initial epidemiological analyses in Germany showed that the spatial density of outbreaks in sheep holdings was statistically significantly associated with the population density of sheep, with transplacental infections taking place since mid-September 2012 (Conraths et al. 2012). The seasonal peak of the transplacental SBV infections coincided with the peaks of the BTV-8 infections observed in 2006 and 2007 as well as with the maximum levels of BTV-8-infected biting midges detected in 2007. Current knowledge of the epidemiology of the phylogenetically closest relatives of the SBV (Shamonda, Sathuperi, Aino and Akabane viruses) is not exhaustive enough to predict whether the current outbreak of Schmallerberg virus is the prelude to endemicity or to a two year-long outbreak before the infection “burns out” when serologically naïve animals are no longer available (Garigliany et al. 2012b). It is possible that cyclic epizootic reemergences may occur in future, either synchronised with a global decrease of herd immunity or due to antigenic variants escaping the immunity acquired against their predecessors.

3.1. Host range

SBV has been isolated or confirmed by PCR in cattle, sheep, goat, bison roe deer and red deer, whereas the serological presence of SBV antibodies has been detected in roe deer, red deer, alpaca (new-world camelids), mouflons and water buffalo (Azkur et al. 2013; Conraths et al. 2013). According to the current data, infection with SBV is more efficient in sheep than in cattle (FLI 2013a). So far, no evidence has been found for the zoonotic potential of this virus, although some members of the Simbu serogroup such as Oropouche virus are zoonotic.

3.2. Transmission

SBV is transmitted through haematophagous insect vectors, especially through *Culicoides* midges bites. SBV has been detected in pools of *Culicoides* biting midges, and many *Culicoides* species including the *C. obsoletus* complex, *C. dewulfi* and *C. chiop-*

terus, *C. dewulfi*, *C. pulicaris* have been found positive for SBV (Carpenter 2012; De Regge et al. 2012; Rasmussen et al. 2012; van den Bergh, 2012; Veronesi et al. 2013). All of these are found, often together, throughout Northern Europe, including the UK. It may be noted that *Culicoides obsoletus* is the primary vector of Bluetongue virus, especially serotype 8 (BTV-8), in northern Europe. Naive animals infected with SBV virus have been detected to have viral RNA in their blood for several days (Wernike et al. 2013), indicating that biting insects may acquire the virus and can then transmit to other susceptible animals during blood feeding. SBV also transmits vertically across the placenta and vertical transmission from females to their offspring is of particular importance as SBV has been shown to be involved in congenital malformations in lambs, goat kids and calves (De Regge et al. 2013).

SBV has been found in bovine semen. The FLI detected SBV-genome in the semen of 11 bulls with a known SBV-antibody status (FLI 2013a). All samples were investigated with an optimised RNA extraction method and a real-time quantitative RT-PCR (RT-qPCR) system developed at the FLI. Whether SBV can be transmitted by SBV-positive semen is still under investigation. However, direct transmission of SBV from animal to animal is very unlikely. It also appears that the virus does not spread through the oral route. In an experimental study in naive cattle infected with SBV orally as well as subcutaneously, viral RNA was detected in serum and blood samples for several days in the subcutaneously infected animals whereas, orally inoculated animals and uninfected controls remained negative throughout the study (Wernike et al. 2013).

Due to the close relationship of SBV with the Sathuperi, Shamonda, Aino, and Akabane viruses, a risk for humans is not to be expected, as zoonotic viruses are rare within this group with the exception of Oropouche virus. Investigations of the Robert Koch-Institute on persons with close contact to infected animals revealed no signs of infection (FLI 2013b). However, further investigations have to be conducted.

4. Clinical manifestations

Two clinical presentations have been observed due to SBV infection. In adult cows, acute infec-

tion results in fever ($> 40\text{ }^{\circ}\text{C}$). The viraemic stage is very short (one to six days) and is followed by anorexia, impaired general condition, a significant reduction in milk yield (up to 50%) and diarrhoea, with full recovery within 2–3 weeks (Gibbens 2012; Hoffmann et al. 2012; DEFRA 2013; FLI 2013b). These symptoms have mainly been observed during the vector-active season (April to November). Usually, disease outbreaks in the affected herds last for two to three weeks; however, the possibility of a different epidemiological presentation cannot be ruled out. There may be no obvious clinical symptoms in adult sheep and goats at the time of infection, although cases of sheep with diarrhoea in the United Kingdom and a reduction in milk in milking sheep in the Netherlands have been reported (DEFRA 2013; FLI 2013b).

Another manifestation of SBV infection is associated with abnormalities in animals born alive or dead at term, stillbirths or aborted following infection of the dam, affecting mainly sheep but also cattle and goats. Congenital malformations in foetuses and new-borns are the major clinical signs and are similar to those seen in Akabane virus infection. These congenital anomalies are classified as arthrogryposis hydranencephaly syndrome (AHS), which includes stillbirth, premature birth, mummified foetuses, arthrogryposis, hydranencephaly, ataxia, paralysis, muscle atrophy, joint malformations, torticollis, kyphosis, scoliosis, behavioural abnormalities and blindness (USDA-APHIS 2012). Transplacental infection with SBV leads to severe congenital malformations such as arthrogryposis, malformation of the vertebral column (kyphosis, lordosis, scoliosis, torticollis) and of the skull (macrocephaly, brachygnathia inferior) as well as variable malformations of the brain (hydranencephaly, porencephaly, cerebellar hypoplasia, hypoplasia of the brain stem) and of the spinal cord in lambs, goat kids and calves. Some animals are born with a normal outer appearance but have nervous signs such as blindness, ataxia, recumbency, inability to suck and convulsions. Foetal deformities vary depending on when infection occurred during pregnancy (Conraths et al. 2013). Field evidence from Europe shows that many animals are infected with SBV without any clinical signs (DEFRA 2013). Typically, the impact in most affected herds or flocks has been low, although a small number of farms have reported more significant losses (DEFRA 2013; FLI 2013a; Azkur et al. 2013; Larska et al. 2013; Meroc et al. 2013a,b).

5. Pathogenesis

There are very limited studies on the pathogenesis and pathology of SBV infection. Experimental infection in cattle and sheep showed an incubation period of between one and four days with viraemia lasting for one to five days (Hoffmann et al. 2012; OIE 2013). A study employing a real time quantitative reverse transcription PCR (RT-qPCR) test on the organ distribution of SBV in spleen, cerebrum, meconium, spinal cord, rib cartilage, umbilical cord, placental fluid from the stomach as well as external placental fluid scraped from the coat of 15 foetal lambs and two calves showing typical malformations, revealed that the external placental fluid, all except for one cerebrum, and the umbilical and the spinal cord to be SBV-positive, suggesting that both the external placental fluid and the umbilical cord could be suitable sample materials for the confirmation of infection with Schmallerberg virus in malformed new-borns (Bilk et al. 2012). Among the different organs tested using rRT-PCR in malformed lambs ($n = 90$) and calves ($n = 81$), brain stem material was found to be the most appropriate tissue for SBV detection although it could also be detected in all other tissues but to a more variable degree (De Regge et al. 2013).

A recent study on SBV pathogenesis revealed that the virus is neurotropic in naturally *in utero*-infected lambs and calves (Varela et al. 2013). Analysing tissue sections of brain and spinal cord from a total of eight naturally infected lambs and calves presenting congenital malformations viz. arthrogryposis, brachygnathia inferior, torticollis and curvature of the spine accompanied by muscle hypoplasia and demyelination using immunohistochemistry, the investigators detected the abundant expression of SBV antigen in the cell body and processes of neurons of the grey matter in the brain and also in the grey matter of the spinal cord, thus strongly indicating that SBV replicates in the neurons of the brain and the spinal cord of animals naturally infected with SBV.

Additional SBV pathogenesis studies in an experimental mouse model showed that SBV to replicate abundantly in neurons where it caused cerebral malacia and vacuolation of the cerebral cortex, thus reconfirming its strong neurotropism (Varela et al. 2013). The SBV-induced acute lesions in experimental mice progressed from per-acute haemorrhages at 48 h post-infection to malacia at

72 h that extended to more widespread vacuolation of the white matter at 96–120 h post-inoculation. Strong immunoreactivity for SBV within the cerebral neurons of mice was seen as early as 48 h post-inoculation. The progression of mice brain lesions from bilateral symmetrical vacuolation of the cerebral cortex and mesencephalon to porencephaly and extensive tissue destruction can facilitate understanding of the pathogenesis in ruminants where similar lesions are often observed in aborted lambs and calves in naturally occurring Schmallerberg cases.

The neurons in the brain are the major target for Schmallerberg viral replication in the developing foetus. Sheep foetuses are susceptible to SBV infection during days 28–50 of gestation (European Food Safety Authority 2012), the time frame which coincides with the development of the blood brain barrier (BBB). In sheep the BBB starts to develop between days 50 and 60 of gestation and reaches full development by day 123 (Evans et al. 1974). Thus, it is easy for the virus to access the foetal brain from the 28th day of gestation when the placentomes (functional units of exchange between mother and foetus in the ruminant placenta) develop until day 50 when the BBB starts to develop. This is probably why the disease is mild in adult animals (with intact BBB) with no apparent lesions in the CNS. The malformations and deformities observed in SBV-infected lambs and calves are accompanied by muscle hypoplasia and demyelination. The muscular hypoplasia and muscular defects are mostly secondary to damage of the CNS (Varela et al. 2013).

Developing and using reverse genetic systems to recover the Schmallerberg virus from cloned cDNA, two independent studies have revealed that SBV modulates the host innate immune response by inhibiting IFN production by the host cell at the level of transcription, especially through its NSs protein. NSs acts as a virulence factor which antagonises interferon probably by globally inhibiting host cell metabolism. In this way the host innate immune response is overcome and efficient viral replication is permitted (Elliott et al. 2013; Varela et al. 2013). Similar roles have been identified for the NSs proteins of Rift Valley fever virus (Bouloy et al. 2001) and La Crosse virus (Blakqori et al. 2007).

Serial passage in sheep choroid plexus cells (CPT-Tert) led unexpectedly to, instead of the usual attenuation of viral virulence, the development of mutant SBV (SBVp32) with increased virulence and pathogenicity in suckling mice. This mutant, having accumulated a variety of mutations in all three viral RNA segments, appeared to spread more rapidly than wild

SBV in the brain of infected animals (Varela et al. 2013). Interestingly, most of the mutations in the M segment were localised to the Gc protein which acts as an antigenic determinant in the outer viral surface (principal target of neutralising antibodies). Such mutations generated in Gc in an immunologically unconstrained environment could be associated with an increased cell receptor affinity.

In a study aimed at establishing an *in situ* hybridization method (ISH) to detect SBV mRNA, to evaluate the usefulness of ISH as a complementary diagnostic tool and to further analyse SBV pathogenesis, Hahn et al. (2013) investigated SBV mRNA distribution in the CNS of 82 naturally infected ruminants (46 lambs, two goat kids, and 34 calves), all of which were positive for SBV by qRT-PCR. They employed ISH on various tissues from four lambs, one goat kid, and five calves, including placenta, muscle, eye, heart, aorta, lung, trachea, liver, kidney, spleen, small and large intestine, mesenteric and pulmonary lymph nodes, thymus, adrenal gland, testis, and uterus. SBV mRNA was found in varying amounts, predominantly in neurons of the cerebrum, cerebellum, brain stem, medulla oblongata, and spinal cord of seven lambs, one goat kid, and two calves. Randomly distributed clusters of SBV-positive neurons were frequently found in small ruminants, whereas only single positive cells were found in both calves. SBV mRNA was not found in any peripheral organ. They concluded that neurons are the predominant target in SBV-infected neonates and that the *in situ* detection of SBV mRNA represents a suitable way to study SBV pathogenesis, especially in the active phase of infection, and might enable identification of SBV as the causative agent in cases of CNS inflammation of unknown etiology.

6. Pathology

A study by Herder et al. (2012) described pathologic lesions in 40 sheep, two goats, and 16 cattle naturally infected with SBV as determined by real-time quantitative reverse transcription polymerase chain reaction. The most common gross lesions were arthrogryposis, vertebral malformations, brachygnathia inferior, and malformations of the central nervous system, including hydranencephaly, porencephaly, hydrocephalus, cerebellar hypoplasia, and micromyelia. Histologic lesions included lymphohistiocytic meningoencephalomyelitis in some cases, glial nodules mainly in the mesenceph-

alon and hippocampus of lambs and goats, and neuronal degeneration and necrosis mainly in the brain stem of calves. Micromyelia was characterised by a loss of grey and white matter, with few neurons remaining in the ventral horn in calves. The skeletal muscles had myofibrillar hypoplasia in lambs and calves. The lesions of SBV-associated abortion and perinatal death are similar to those attributed to Akabane virus and other viruses in the Simbu group of bunyaviruses (Herder et al. 2012). In foetal sheep, SBV infection can result in cavitation of the white matter of the cerebrum, cerebellar hypoplasia, mild lymphohistiocytic perivascular encephalitis, and small glial nodules scattered throughout the brain (Peperkamp et al. 2012). On histologic examination of 82 naturally infected ruminants (46 lambs, two goat kids, and 34 calves), Hahn et al. (2013) identified encephalitis, characterised by lymphohistiocytic, perivascular cuffs, in 11 (nine lambs, one goat kid, and one calf) of the 82 animals. All small ruminants showed inflammation, whereas both calves lacked inflammatory changes.

Varela et al. (2013), while investigating whether mice could be used as an experimental model of SBV infection and pathogenesis, inoculated 8–14 two-day old new-born NIH Swiss mice intracerebrally with 400 PFU of wild type SBV, sSBV (synthetic SBV) or cell culture media as a control. All mice inoculated with SBV and sSBV died within eight days post-inoculation while all control mice survived until the end of the experiment. Histopathology of brains collected at 72 h post-infection revealed bilateral symmetrical vacuolation and loosening of the neuropil of the superficial cerebral cortex and the mesencephalon; in particular, small areas of haemorrhage within large areas of malacia (necrosis of brain tissue) in the cerebral cortex arrow. In brains collected at 120 h post-infection there was random multifocal vacuolation of the white matter of the cerebrum with small amount of nuclear debris. There was a minimal, multifocal perivascular infiltrate of lymphocytes in the adjacent grey matter. The presence of SBV was confirmed by immunohistochemistry using a polyclonal antibody against the N protein.

7. Diagnosis

Clinical diagnosis is made on the basis of clinical manifestations of disease which vary by species. Bovine adults show a mild form of acute disease during the vector season, whereas congenital mal-

formations occur in more species of ruminants including cattle, sheep, goat and bison (OIE 2013). From suspected acute infections in adults, samples of EDTA-preserved blood as well as serum should be collected and transported to the laboratory under cooled conditions. Samples should be collected during the acute stage of clinical infection (e.g., fever, reduced milk yield, diarrhoea). Suspected cases in aborted fetuses or new-born animals are sampled for histopathological, serological and virological examinations as appropriate. From necropsy tissue samples of brain, preferably cerebrum and cerebellum and other parts of brain stem along with spleen and blood are collected, while from live new-born pre-colostral blood and serum and meconium should be collected and transported in cooled or frozen condition (FLI 2013b; OIE 2013). Amniotic fluid (e.g., in swab samples from the skin of malformed animals) and placenta are also suitable as diagnostic material. Detection of SBV-RNA in the meconium is also possible although the detection rate in this material is lower (FLI 2013b). Confirmation of infection is made by viral isolation and detection of SBV sequences using real time PCR on tissues.

7.1. Viral isolation and identification

The Schmallenberg virus can grow in several cell lines derived from various animal species and humans (Varela et al. 2013). SBV grew efficiently in sheep choroid plexus (CPT-Tert) cells, bovine foetal aorta endothelial (BFAE) cells, human 293T, canine MDCK and hamster BHK-21 and BSR cells, reaching titres of 10 PFU/ml at 48 h post-infection and induced cytopathic effect (CPE) in most cell lines except in the BFAE cell (Varela et al. 2013). Of all the cell lines, sheep CPT-Tert cells were the most suitable for SBV culture. In these cells well-defined plaques of approximately 3 mm in diameter were observed 72 h post-infection.

The FLI has developed a real-time quantitative reverse transcription PCR (RT-qPCR) test for rapid detection of Schmallenberg virus that has been made available to institutions in Belgium, France, England, the Netherlands, Italy, and Switzerland. (Bilk et al. 2012; Fischer et al. 2012; FLI 2013a). Meanwhile, a test system for antibody detection is also available. Other supplemental confirmatory tests used on a case-by-case basis include indirect immunofluorescence and virus neutralisation

assays (FLI 2013a). In addition, scientists in the Netherlands and Germany have recently developed an antibody-based virus neutralisation test (VNT) that is suitable for mass testing for SBV in cows, ewes and does from herds known to be infected or suspected (i.e., measuring past exposure in animals via antibody production to this virus). This VNT has a specificity of >99% and it is claimed, a sensitivity of very close to 100% (Loeffen et al. 2012). The assay is highly reproducible.

A new indirect ELISA test for SBV has been developed by scientists at IDEXX Livestock and Poultry Diagnostics to identify antibodies to SBV. The test is claimed to have a high specificity (99.5%) and sensitivity (98.1%) and is suitable for screening animals for SBV infection (IDEXX 2013). The ELISA test, based on the recombinant nucleocapsid protein (N) of SBV, was evaluated and validated for the detection of SBV-specific IgG antibodies in ruminant sera by three European Reference Laboratories (Breard et al. 2013). Validation data sets derived from sheep, goat and bovine sera collected in France and Germany ($n = 1515$) in 2011 and 2012 were categorised according to the results of a virus neutralisation test (VNT) or an indirect immunofluorescence assay (IFA). The specificity was evaluated with 1364 sera from sheep, goat and cattle collected in France and Belgium before 2009. The overall agreement between VNT and ELISA was 98.9%, while between VNT and IFA overall agreement was 98.3%, indicating a very good concordance between the different techniques. Although cross-reactions with other Orthobunyaviruses from the Simbu serogroup viruses might occur, the ELISA test is a highly sensitive, specific, robust and validated technique for the detection of anti-SBV antibodies and can be applied for SBV sero-diagnostics and disease-surveillance studies in ruminant species in Europe.

Recently, Hahn et al. (2013) evaluated the usefulness of ISH to detect SBV mRNA in infected tissues as a complementary diagnostic tool and concluded that *in situ* detection of SBV mRNA represented a suitable way to study SBV pathogenesis and might enable identification of SBV as the causative agent in cases of CNS inflammation of unknown aetiology.

7.2. Differential diagnosis

The clinical symptoms of acute SBV infection in adults are not specific. All possible causes of

high fever, diarrhoea, milk reduction and abortion should be taken into account including bluetongue, epizootic haemorrhagic disease (EHD), foot and mouth disease (FMD), bovine viral diarrhoea (BVD), border disease and other pestiviruses, bovine herpesvirus 1 and other herpesviruses, Rift Valley fever, bovine ephemeral fever, toxic substances, etc. However, for the malformations of calves, lambs and kids, the diagnosis should take into account other orthobunyavirus infections, bluetongue, pestiviruses, genetic factors and toxic substances.

8. Prevention and control

There is no treatment or vaccine currently available for SBV. As it is a new disease further work is needed to determine what control measures may be appropriate. Since SBV is not a notifiable disease there are no movement restrictions. Current knowledge suggests that acutely infected animals carry the virus during this period of viraemia. The culling of infected livestock has not been attempted, and would in any case be futile. One possible option is to control the *Culicoides* vectors by employing methods such as the application of insecticides and pathogens to habitats where larvae develop; environmental interventions to remove larval breeding sites; controlling adult midges by treating either resting sites such as animal housing or host animals with insecticides; housing livestock in screened buildings; and using repellents or host kairomones to lure and kill adult midges (Carpenter et al. 2008; FLI 2013b). The treatment of livestock and animal housing with pyrethroid insecticide combined with the use of midge-proofed housing for viraemic or high-value animals and reduction of local breeding sites are the best options currently available (Carpenter et al. 2008). However, application of pour-on insecticides has been unsuccessful with no noticeable reduction in the density of biting midges in highly-challenged areas (Bauer et al. 2009). Control of midges is unlikely to be effective given that they are extremely widespread, and appear to be very effective at spreading SBV. Furthermore, the timings of breeding or insemination of female animals can be selected such that the vulnerable stage of pregnancy (analogously to Akabane virus: 4–8 weeks in sheep and approximately 8–14 weeks in cattle) does not lie within the vector-active season.

Fortunately, the prospects for developing a SBV vaccine in the near future do appear to be very good. Different research groups have developed prototypes for inactivated vaccines, none of which has however been granted marketing authorisation yet. Merck Animal Health Company has produced a vaccine based on inactivated wild-type Schmallenberg virus, which should soon be available for use.

9. CONCLUSION

Schmallenberg virus infection is an emerging infectious disease that may cause huge economic losses to livestock ruminants, especially in small ruminant production. In cattle, the disease is usually acute in form with visible clinical manifestations of fever, reduced milk yield and diarrhoea that subsides with the animal recovering in 2–3 weeks. However, in sheep and goats SBV infection is almost asymptomatic with transplacental transmission to foetuses in pregnant animals, causing abortions, stillbirths and a variety of congenital malformations mainly involving the skeletal and nervous systems. Initially, the disease was confined to Northern and Western Europe; however, the detection of SBV antibodies in cattle, sheep, goats and buffaloes (between 2006 and 2013) in a retrospective study in Turkey (Azkur et al. 2013) not only indicates the presence of virus prior to its detection in 2011 in Germany, but also harbingers its spread to Eastern European countries, although the presence of SBV in vector insects in Turkey has not yet been confirmed. As *Culicoides* midges can spread infection across national borders (De Regge et al. 2012; Rasmussen et al. 2012; Veronesi et al. 2013), this may not augur well for landlocked Eastern European and Asian countries which already harbour species of these vectors responsible for dissemination of Akabane and bluetongue viruses in these regions. In the event of SBV spreading to the Far East and South East, particularly in South Asian countries, the virus may severely affect the dense ruminant livestock population in view of the limited resources and containment amenities and favourable geo-climatic conditions for *Culicoides* vectors. As serological and molecular diagnostic tests for SBV infection are easily available, it would be prudent to perform random screening of livestock ruminants as well as vectors in other countries also. This will enable timely containment and control of the disease in cases of its occurrence.

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