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REVIEW

Schmallenberg virus: continuing a trend?

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Abstract: Since its first isolation in Germany in 2011 the *Schmallenberg* strain of the *Sathuperi virus* has been detected across northern Europe. The virus is associated with neurological disease, abortion, and congenital deformities in ruminants, particularly among sheep and cattle. The rapid dissemination of the pathogen is reminiscent of the transmission of another midge-borne arbovirus, *Bluetongue virus*, which also spread to Europe on several separate occasions between 1998 and 2007. In this review we detail the characteristic features of the *Schmallenberg virus* and consider whether the virus highlights a European vulnerability for other midge-transmitted pathogens.

Keywords: Schmallenberg, Sathuperi, Culicoides, bunyavirus, midge, bluetongue

Schmallenberg virus: virus isolation and related viruses

Schmallenberg virus was first identified by next generation sequencing of complementary deoxyribonucleic acid (cDNA) prepared from the plasma of cattle with fever and reduced milk yield near Schmallenberg in Germany in November 2011.[1] Cattle with similar clinical signs were also reported at the same time in the Netherlands. Reverse transcription-polymerase chain reaction (RT-PCR)-based tests were developed, and testing of archive samples revealed no evidence for the presence of the virus before 2011.[2] From December 2011, *Schmallenberg virus* was associated with an epizootic of deformed calves and lambs across Europe with cases in France, Germany, Belgium, The Netherlands, the United Kingdom, Switzerland, Luxembourg, Italy, Denmark, and Spain.[2] Initial sequence analysis placed *Schmallenberg virus* as a novel virus in the *Simbu* serogroup of the genus *Orthobunyavirus* in the family *Bunyaviridae*.[1,3] More recent sequencing analysis places the *Schmallenberg virus* within the species of the *Sathuperi virus*.[4]

The *Simbu* serogroup contains 25 viral species and is one of the largest serogroups within the *Orthobunyavirus* genus.[5–8] Other viruses in the serogroup include the human pathogen *Oropouche virus*, which is mosquito-transmitted and causes a nonfatal dengue fever-like illness in the Caribbean and in South America, and *Akabane virus*, which causes epizootic congenital malformations in ruminants (Table 1). Phylogenetically, *Sathuperi virus* is the most closely related to *Shamonda*, *Akabane*, and *Aino* viruses, which are all transmitted by midges of the *Culicoides* genus and also cause disease in ruminants.[4,8] Although insect transmission studies with *Schmallenberg virus* have not yet been reported, the virus has been detected by RT-PCR from pools of *Culicoides* midges within affected areas.[9–11] *Sathuperi virus* has also been reported

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Table 1 Simbu serogroup viruses infecting humans and ruminants

Virus species	Virus	Vertebrate host	Arthropod vector	Vertebrate clinical signs	Refs
Akabane	*Akabane, Tinaroo, Sabo, Yaba-7*	Cattle, Sheep, goats, buffalo, pigs (horses, camels, Zebra)	*Culicoides*	Reduced milk yield (cattle), abortions, stillbirths, congenital abnormalities of limbs, back and head, arthrogryposis and hydranencephaly	8,12,96–107
	Iriki	Described in cattle	*Culicoides*	As type strain but also causes epizootic encephalitis in calves	108–112
Shuni	*Aino, Kaikalur*	Cattle, sheep	*Culicoides, Mosquitoes (Culex)*	Abortion, congenital abnormalities (scoliosis, necrotizing encephalopathy)	111–116
Iquitos		Humans	*Culicoides*	Fever	95
Oropouche		Humans	*Culicoides*	Fever	94,117,118
Sathuperi	*Sathuperi*	Cattle	*Culicoides, Mosquitoes*	None reported	12–15
	Douglas	Cattle, (buffalo, sheep, goats, deer)	*Culicoides*	None reported	33,119,120
	Schmallenberg	Cattle, Sheep, Goats (Alpacas, Red Deer, Roe Deer)	*Culicoides*	Reduced milk yield (cattle), abortions, stillbirths, congenital abnormalities of limbs, back and head, arthrogryposis and hydranencephaly	1,2,20,23,24
Shamonda	*Shamonda, Peaton, Sango*	Cattle (Sheep, Horses, Buffalo, goats, pigs)	*Culicoides*	None reported	12,121,122

Notes: Host species in brackets indicate species which have been reported to develop an antibody response to virus but development of clinical disease or ability to act as amplifying hosts has not yet been demonstrated for these species. Formal approval of Iquitos virus as a species by ICTV is pending.

to be transmitted by midges in Nigeria and Japan.[12–14] Since *Sathuperi virus* was originally isolated from *Culex* spp. mosquitoes in the Andhra Pradesh and the Tamil Nadu region in India,[15] the role of other arthropods, particularly mosquitoes, in the transmission of *Schmallenberg virus* cannot be ruled out. However, given the repeated isolation of *Schmallenberg* or *Sathuperi* viruses from *Culicoides* spp. midges in Europe,[9–11] Japan,[3,14] and Nigeria,[13] it is likely that they are also the major vector for *Schmallenberg virus* in Europe.

Clinical signs

As with most viruses that can replicate in multiple hosts, disease pathology of *Schmallenberg virus* is dependent on the infected species. Experimental infection of type 1 interferon knockout mice and cattle with *Schmallenberg virus* have both been reported.[1,16,17] In the first cattle study, three 9-month-old calves were inoculated with the virus; one calf developed fever (40.5°C) and one had mucous diarrhea.[1] In this study, viral ribonucleic acid (RNA) was detected by RT-PCR for 2–5 days postinoculation.[1] In the second study, eight 9–18-month-old calves were inoculated with serum from an experimentally infected animal that showed clinical signs (four animals), or 2×10^7 $TCID_{50}$ virus that had been passaged no more than four times in cell culture (four animals). Although no animal developed detectable fever, and only one animal developed mild diarrhea, all virus-inoculated animals developed viraemia detectable for 5–7 days by real time RT-PCR, and all seroconverted by enzyme-linked immunosorbent assay (ELISA) by day 21 postinoculation.[17] Four in-contact control animals that were not inoculated with the virus developed neither detectable nor seroconverted viraemia.

Although *Schmallenberg virus* was originally associated with reduced milk yield and diarrhea in adult cattle, its main clinical impact has been related to teratological effects when pregnant sheep, cattle, and goats have been infected with the virus. Affected fetuses often spontaneously abort and can display arthrogryposis, as well as malformations of the skull, spinal column, and brain including macrocephaly, kyphosis, scoliosis, torticollis, lordosis, cerebellar hypoplasia, hydrocephalus, and porencephaly.[18–21] Fetuses that go to full term can display a range of clinical signs ranging from malformations similar to aborted fetuses to morphologically normal animals with neurological signs such as ataxia, blindness, paralysis, exaggerated movements, and polioencephalomyelitis.[20,22] For wild ruminants, there is evidence that both red deer and roe deer have been exposed

to *Schmallenberg virus* and develop an antibody response.[23] However, in this study, there was no evidence for viral RNA in deer or epizootics of congenital malformations. There is also evidence that alpacas kept in areas where *Schmallenberg virus* is circulating tend to develop an antibody response to the virus,[24] although, to our knowledge, no confirmed cases of clinical disease have been reported in alpacas or other camelids.

The clinical signs of *Schmallenberg virus* infection in sheep, cattle, and goats are consistent with those described for other related Simbu serogroup viruses that infect ruminants, particularly *Akabane virus* and *Aino virus*.[21,25–30] However, some clinical signs reported for Akabane, such as epizootic encephalomyelitis,[31] have not yet been reported for *Schmallenberg virus*. Relatively little description of clinical disease has been reported for *Sathuperi virus*. The virus was first isolated in India in 1955–1957,[15] and it was then detected in Nigeria from the mid-1960s.[12,13] The next report of *Sathuperi virus* isolation was from Japan in 1999.[3] Intriguingly, none of these prior isolations coincided with epizootic outbreaks, nor were clinical signs reported from the source animals. *Douglas virus*, isolated in Australia, is another isolate within the *Sathuperi virus* species.[4,32,33] Although there is a lack of description of clinical disease for *Douglas virus* in ruminants, there is a report comparing the teratogenic potential of *Akabane* and *Douglas* viruses in an embryonated egg model system.[34] In this system, *Akabane* infections led to clinical signs in chicks similar to those seen when the same virus infects ruminants in utero (arthrogryposis in the legs and feet, scoliosis, and retarded development). For *Akabane* disease, clinical signs were noted from doses of 8–300 pfu/egg, depending on the strain. In contrast, the *Douglas virus* only induced similar clinical signs when doses of 1.4×10^5 pfu per egg were used.[34]

At this stage, it is unclear why *Schmallenberg virus* appears to be much more pathogenic than its closest genetic relatives, *Sathuperi* and *Douglas* viruses.[4] It may simply be a case of a new virus spreading into a naïve population and resulting in rapid amplification and dissemination of the pathogen. Certainly, there is evidence that the transport of pregnant cattle to *Akabane*-endemic areas followed by the returning of these cattle to "naïve" areas can result in significant disease outbreaks.[35] An alternative possibility is that amino acid changes in *Schmallenberg virus* from the parental *Sathuperi virus* have rendered it more pathogenic in ruminants.

Molecular biology

Although *Schmallenberg virus* has only been recently described, the *Orthobunyavirus* genus is one of the best studied genera within the *Bunyaviridae* family in terms of its molecular biology. Orthobunyaviruses have a tripartite negative-sense single stranded RNA genome (Figure 1A). The three genome segments are designated as L, which encodes the viral RNA-dependent RNA polymerase; M, which encodes the viral glycoproteins Gn and Gc and the nonstructural protein NSm; and S, which encodes the nucleocapsid protein, N, and nonstructural protein, NSs.

As a consequence of the segmented nature of the genome, bunyaviruses that share common replication and packaging signals are able to reassort segments when coinfecting the same host. Indeed, based on partial sequence data, *Schmallenberg virus* was proposed to be a reassortant between *Shamonda* and *Sathuperi* viruses.[3] More recent sequencing data suggest that it is *Shamonda virus* that is a reassortant between *Schmallenberg virus* and a third, uncharacterized orthobunyavirus.[4] *Shamonda virus*, has L and S segments that have high levels of sequence similarity to *Schmallenberg virus,* but the M segment of *Shamonda virus* is only distantly related to the M segment of *Schmallenberg* virus.[4] If this is correct, the *Schmallenberg virus* parent of

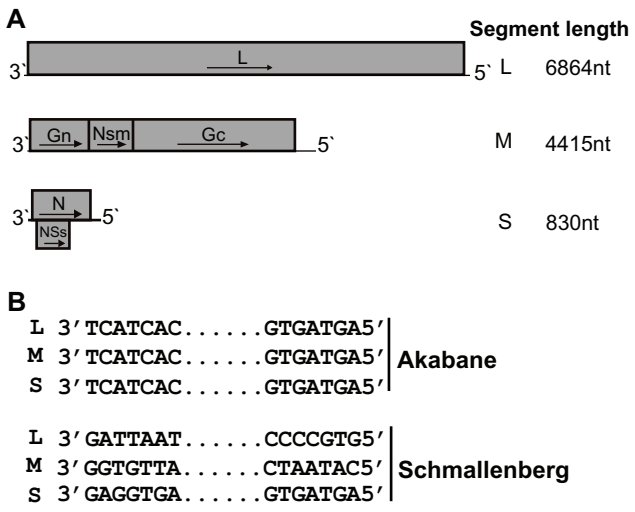

Figure 1 Genome organization of the *Schmallenberg virus*. (**A**) The virus has three strands of negative-sense single stranded RNA (L, M, and S). L encodes the multifunctional cap endonuclease-RNA-dependent-RNA-polymerase enzyme; M encodes two glycoproteins, Gn and Gc, and the nonstructural protein, NSm, encoded as a polyprotein; S encodes the nucleocapsid (N) and, from an alternative frame-shifted start codon, the nonstructural protein, NSs. All three genome segments must be transcribed to positive sense RNA before translation is possible. Arrows indicate the direction of translation. (**B**) The genome terminal nucleotides of the three segments of the *Akabane virus* and *Schmallenberg virus*.

Notes: Like other bunyaviruses, the terminal sequences of *Akabane* are conserved between segments and are complementary. Current sequences for the *Schmallenberg virus* do not match this pattern.

Abbreviations: RNA, ribonucleic acid; L, large; M, medium; S, small.

Shamonda virus must have been circulating in the field since before 1965, when *Shamonda virus* was isolated from cattle in Nigeria.[8,12]

Consistent with its original identification by next generation sequencing, the full genome sequence of all three segments of *Schmallenberg virus* have been published.[1] However, one of the features of bunyavirus genomes is that segments share conserved, complimentary terminal nucleotides at the 5′ and 3′ ends of the RNA segments.[5,36–39] Each of the published terminal sequences for the Schmallenberg viral segments is different (Figure 1B), so it is likely that these sequences are either missing or extended nucleotides. Indeed, in order to recover the replicating *Schmallenberg virus* from cloned cDNA, Elliott et al[40] found it necessary to modify the published sequences to include terminal nucleotides predicted from a consensus of other Simbu serogroup viruses. The modified *Schmallenberg virus* had similar growth in cell culture to the original, unmodified isolate, which was recovered in baby hamster kidney (BHK21) cells after prior incubation (10 days) of sonicated blood with a *Culicoides* (KC) cell line.[1,40]

Bunyaviruses replicate in the cytoplasm of infected cells and assemble in the lumen of Golgi stacks.[41–45] The viral glycoproteins, Gn and Gc, and the nonstructural protein, NSm, are encoded by the mRNA segment. Gn and Gc form a heterodimer that is retained in the Golgi to allow virus assembly.[46–50]

There is evidence for functional interactions between the 5′ and 3′ end of the negative sense viral genomic RNA segments and for the direct interaction between these and the viral nucleocapsid, N, and the RNA-dependent-RNA polymerase, L.[5,45] Transcription occurs in the cytoplasm and involves priming of viral transcripts using short, capped oligonucleotides derived from cellular mRNAs.[51–55] To enable this cap-snatching mechanism, which is analogous to that employed in the nucleus by *Influenza A virus*, the bunyavirus L proteins have cap-dependent endonuclease activity.[53,56] Although there is no direct evidence for this endonuclease activity for the *Schmallenberg virus* L, the protein does contain the conserved motifs associated with this activity in other bunyaviruses (Figure 2). Although bunyavirus transcripts are not polyadenylated, there is evidence from several members of the *Bunyaviridae* family that the untranslated regions of several bunyaviruses contain cis-acting sequences that promote efficient translation of the viral messenger-sense RNA in the absence of a poly-A tail.[57–60]

In mammalian cells, but not in vector insect cells, replication of some bunyaviruses, including *Schmallenberg virus*, results in the shut off of host cell transcription.[40,61–66] This effect is mediated by the viral NSs protein and was initially characterized as a way in which bunyaviruses avoid interferon and other antiviral responses that require transcription. Although not all bunyaviruses encode a NSs ortholog,[45,67] *Schmallenberg virus* does. Furthermore, the recovery of a *Schmallenberg virus* variant in which NSs is deleted resulted in a virus with reduced ability to shut down host cell transcription and increased induction of interferon synthesis in infected mammalian cells.[40]

Spread

With relatively little data on the basic biology of *Schmallenberg virus*, beyond what can be inferred from the replication of other orthobunyaviruses, there have been several recent reviews that have focused on the identification and spread of the virus.[2,19,20,68] Rather than repeat the detailed epidemiological situation, which has been well covered by others, we will limit our discussion to the key features of the European *Schmallenberg virus* outbreak. Firstly, the detection of the virus in 2011 appears to genuinely coincide with the introduction of *Schmallenberg virus* into Europe. Testing of retrospective samples has consistently failed to identify any cases that are positive by PCR or ELISA prior to 2011.[1,2] Despite the intriguing hints from the similarity between *Schmallenberg virus* and two of the three segments of *Shamonda virus* noted

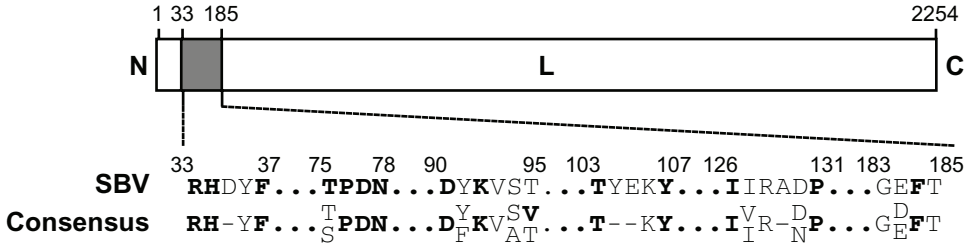

Figure 2 Position and sequence of the putative cap-snatching endonuclease in *Schmallenberg virus* L.

Notes: The position of the putative cap-snatching endonuclease and sequence motifs in Schmallenberg L are indicated. Amino acids in bold in this motif have been characterized as important for nuclease function in the *La Crosse virus*.[56] The consensus sequence shows the variation in these sequences across the L protein of 35 other *orthobunyavirus* isolates.

above, neither the original source of the *Schmallenberg virus* outbreak, nor the field circulation of the *Schmallenberg virus* strain outside Europe have been reported.

Secondly, spread of *Schallenberg virus* has been extremely rapid. This is consistent with the spread of the *Bluetongue virus* in northern Europe in 2006–2009.[69–71] If anything, the spread of *Schmallenberg virus* has been even more rapid, and involves rapid spread even against the direction of the prevailing winds in this region. It is probably worth noting that in controlled infection studies in paleoarctic species of *Culicoides* in which *Bluetongue virus* was fed to midges, only 0.4%–7.4% of midges were susceptible to infection.[72] Therefore, the increased spread of *Schmallenberg virus* may simply be a result of more efficient uptake into the vector. Interestingly, the time of year with peaks of animals testing positive for *Schmallenberg virus* are consistent with those testing positive for *Bluetongue virus* in the recent *Bluetongue virus* type 8 outbreak, which further supports the hypothesis that Schmallenberg disease is primarily midge transmitted.[2] However, at this stage, it is not possible to rule out the possibility that other blood feeding arthropods may play a role in transmission of the virus.

Finally, the Schmallenberg outbreak is ongoing, with continued seroconversion of animals in the past year.[23,73–83] Surveys of immune responses among the camelids and ruminants at the Royal Veterinary College, London to the *Schmallenberg virus* have suggested that although only 3% of ruminants were seropositive in July 2012, whilst 62% had been seroconverted by November 2012 (Brownlie et al, personal communication).[24] The clinical implications and reproductive consequences of this are being studied further.

Future control measures

The degree of control necessary for *Schmallenberg virus* is currently unclear. The lack of clinical disease in other parts of the world – despite evidence from molecular reassortment studies that the virus has been present for some time,[4,12] and the evidence that prior infection provides protection from reinfection[2] – suggests that herd immunity may be one of the biggest factors in controlling the impact of this disease. It may be possible to manipulate this by vaccination, although currently no licensed vaccines are available. Inactivated and attenuated virus vaccines have been shown to be effective for other Simbu serogroup viruses.[84–86] It is also likely that the *Schmallenberg virus* NSs knockout mutant viruses could be the basis for an effective vaccine.[40,87] Clone 13, a naturally occurring NSs deletion mutant of the Rift Valley fever virus has been demonstrated to be an effective vaccine candidate in sheep and cattle.[88,89] However, clone 13 contains a large deletion in NSs, which is possible because the nucleocapsid (N) and NSs genes are coded separately and in opposite directions on the S segment of the Rift Valley fever virus RNA. In *Schmallenberg virus* and other orthobunyaviruses, N and NSs overlap and are encoded by different initiation sites (start codons) in the same viral strand (Figure 1A). Therefore, although it is possible to knock out NSs expression through the insertion of stop codons or via mutation of ATG codons, it is not possible to delete this gene without also deleting the coding sequence for the essential protein, N. *Schmallenberg virus* NSs knockout mutants may, therefore, have more potential to revert than NSs deletion mutants for other bunyaviruses. This may be a serious concern for developing these mutants as potential vaccine candidates. As with all viruses with segmented genomes, there is a further complication that attenuated live virus vaccines may reassort with circulating field strains of the virus.

Control of bunyaviruses through the control of *Culicoides* midges seems unlikely to be effective; the midges are <3 mm long, thus effective insect proof screening on livestock housing is impractical under field conditions. Furthermore, even cattle dung from inside cattle housing can provide sufficient moisture for the midge to go through its larval stages.[90,91] Insecticides and repellants could have some potential, but the elimination of biting in the field, or potential cervid hosts, would not be affected by these controls. Similarly, controls on the movements of affected livestock are impacted by the fact that *Culicoides* midges can be blown by wind for hundreds of kilometers.[92] Indeed, the recent finding that wind dispersal of *Culicoides* appears to be the most significant factor in the 2006–2009 outbreak of *Bluetongue virus* (another *Culicoides*-transmitted viral disease), it may also have relevance in the spread of *Schmallenberg virus*.[93]

In common with the earlier *Bluetongue virus* outbreaks, the cold winters in northern Europe do not appear to have been a barrier to the overwintering of *Schmallenberg virus*. Whether this is because the virus is maintained in developing fetuses over winter – which can then become the foci for new infections in the spring if they survive – or whether it overwinters by some other mechanism, such as transovarial transmission in *Culicoides* or in alternative long-lived arthropods such as ticks, is currently unclear.

The long-term potential for controlling *Schmallenberg virus* will be dependent on the degree to which the virus can persist in wild ruminants and the availability of suitable vaccines. Eradication in Europe is a theoretical possibility if vaccines were available, but this would require a coordinated

vaccination program across all affected countries due to the rapid spread of the disease from affected areas. Whether vaccination is economically justified will depend on the impact the disease has on the production costs of European farmers, which have not yet been quantified.

Other threats

Although at this stage the long-term economic significance of Schmallenberg disease is unclear, the outbreak does highlight the potential of exotic arboviral diseases to spread rapidly into new areas. It is possible that outbreaks of two *Culicoides* midge-transmitted pathogens (*Bluetongue virus* and *Schmallenberg virus*) occurring within 6 years of each other is a coincidence; however, in the context of the separate and repeated incursions of bluetongue into southern Europe in the 8 years prior to that,[70] it seems more likely that conditions in Europe now favor the transmission of previously exotic midge-transmitted viral diseases.

Fortunately, relatively few zoonotic diseases are transmitted by midges and those that are, such as *Oropouche* and *Iquitos*,[94,95] do not generally result in life-threatening illnesses. Of the animal pathogens, *Bluetongue virus*, *African horse sickness virus*, as well as the *Akabane* and *Aino* viruses are among the most significant in other parts of the world. For the *Bluetongue virus* and *African horse sickness virus*, both viruses exist as multiple serotypes, which do not confer cross-protection. Therefore, animals currently vaccinated against *Bluetongue virus*-11, *Bluetongue virus*-4, or *Bluetongue virus*-8 would not be protected from infection with any of the other 23 serotypes of *Bluetongue virus* in a future outbreak. Similarly, antibodies raised to *Schmallenberg virus* do not efficiently cross-neutralize some other Simbu group viruses, such as *Shamonda* or *Sabo* viruses.[4]

Summary

Since its initial identification by next-generation sequencing, *Schmallenberg virus* has spread rapidly through European domestic ruminants, causing an epizootic of congenital abnormalities. Current data suggest that the virus is a member of the species *Sathuperi virus*, although the severity of the clinical signs reported for *Schmallenberg virus* exceeds any previous Sathuperi outbreak. Controls put in place following the bluetongue epizootic in northern Europe in 2006–2009 have been largely ineffective at controlling the spread of *Schmallenberg virus* infections. The most effective future controls, if necessary on welfare or economic grounds, are likely to be dependent on the development and use of effective vaccines, which are a realistic possibility for this virus. It is likely that outbreaks of other midge-transmitted diseases will occur in Europe in the future, and they will be similarly resistant to controls. Therefore, the development of safe, effective vaccines to these pathogens should be prioritized.

Disclosure

The authors report no conflicts of interest in this work.

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