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Re-circulation of Schmallenberg virus, Germany, 2019

Kerstin Wernike | Martin Beer

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany

Correspondence

Kerstin Wernike, Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald - Insel Riems, Germany.
Email: kerstin.wernike@fli.de

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Abstract

Schmallenberg virus (SBV), an insect-transmitted orthobunyavirus that induces severe foetal malformation in calves and lambs, was detected for the first time in late summer 2011 in Central Europe. Thereafter, the virus spread rapidly across the continent causing a large epidemic in the ruminant population. In 2019, detection of virus was again reported more frequently in Germany. From March to November, infections of viremic adult animals were noticed. In September, SBV genome was also detected in newborn lambs. Altogether, affected species included cattle, sheep, a goat and a fallow deer. M-segment sequences were generated from viruses detected in viremic cattle and compared to viral sequences from previous years. The genome of viruses detected in the blood of acutely infected adult cattle and sheep, which represent the circulating SBV strains, seems very stable over the course of nine years and in various European countries. The nucleotide similarities of these viruses are as high as 99.4%–100%. The renewed SBV circulation in 2019 in the country, in which the virus was first detected in 2011 and where it circulated again in 2014 and 2016, suggests the establishment of an enzootic status in Central Europe with regular larger waves in a cycle of around 3 years. Therefore, it has to be anticipated that SBV will re-emerge at similar intervals in future, and hence, it represents a constant threat for the continent's ruminant population.

KEYWORDS

arbovirus, epidemiology, phylogenetic analysis, re-emergence, Schmallenberg virus

1 | INTRODUCTION

The insect-transmitted Schmallenberg virus (SBV), an orthobunyavirus of the Simbu serogroup, emerged in late 2011 near the German/Dutch border (Hoffmann et al., 2012), and subsequently caused a large epidemic in the European ruminant population (EFSA, 2012). In cattle, sheep and goats of all age groups, the virus induces a short-lived viremia of only 2–6 days, associated with either no or mild unspecific clinical signs such as fever, diarrhoea or reduced milk yield (Hoffmann et al., 2012; Laloy et al., 2015; Wernike et al., 2013).

However, when immunologically naïve ruminants are infected during a critical period of gestation, SBV can cause abortion, stillbirth or severe foetal malformations that become noticeable several months after the acute infection of the dam. The most common foetal lesions affect the central nervous system, the skeletal muscle and/or the axial skeleton and are summarized as arthrogryposis–hydranencephaly syndrome (Beer & Wernike, 2019). Schmallenberg virus is transmitted between its mammalian hosts by blood-sucking insect vectors, specifically biting midges of the genus *Culicoides* (reviewed in Sick, Beer, Kampen, and Wernike (2019)).

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The negative-stranded RNA genome of SBV is composed of three segments that are named according to their size as large (L), medium (M) and small (S). The L-segment encodes the viral RNA-dependent RNA polymerase, while the S-segment encodes the nucleocapsid protein N and the non-structural protein NSs in alternative overlapping reading frames. The M-segment, which contains the most variable genomic region of SBV (Coupeau, Claine, Wiggers, Kirschvink, & Muylkens, 2013; Fischer et al., 2013; Wernike & Beer, 2019), encodes the surface glycoproteins Gn and Gc, as well as the non-structural protein NSm (Elliott, 2014).

Besides Germany and the Netherlands, SBV reached Belgium, France, Luxemburg, Italy, Spain and Southern England (EFSA, 2012) within the first season of the insect vectors responsible for SBV transmission, that is during late summer and autumn of 2011. In ruminants kept in the core regions of the epidemic, SBV caused a very high seroprevalence of approximately 70% to nearly 100% (Elbers et al., 2012; Wernike et al., 2014). Nevertheless, SBV re-appeared in the following year and spread across the British Isles, reached Scandinavia and Eastern European countries, crossed the Alps and spread to Southern Europe as far as Greece (Bradshaw et al., 2012; Chaintoutis et al., 2014; Fernandez-Aguilar et al., 2014; Lazutka et al., 2014; Mason et al., 2013; Steinrigl et al., 2014; Wisloff, Nordvik, Sviland, & Tonnessen, 2014). Despite the high seroprevalences induced in European livestock, the virus still circulated in the 2013 *Culicoides* vector season, but presumably at low levels as it was only sporadically detected (Friedrich-Loeffler-Institut, 2015). However, in summer and autumn 2014, SBV reappeared once more to a greater extent in continental Europe and, as a consequence, the incidence of offspring displaying SBV-induced malformations increased in the following winter (Wernike, Hoffmann, Conraths, & Beer, 2015). In 2015 and the subsequent winter, SBV cases were reported only sporadically, but in the 2016 vector season, the virus circulated once again on a larger scale in the European *Culicoides* midges and ruminant population (Kesik-Maliszewska, Larska, Collins, & Rola, 2019; ProMED-mail, 2016; Wernike & Beer, 2017). To what extent SBV circulated also in 2017 or 2018 remained mostly unknown (Collins, Doherty, Barrett, & Mee, 2019), however, in 2019, SBV cases were again frequently reported to the German authorities, being only the 'tip of the iceberg' due to the very short viremia (Hoffmann et al., 2012; Wernike et al., 2013) and the therefore very low probability of detecting infected ruminants during the viremic phase.

2 | MATERIALS AND METHODS

For this study, SBV reports of the year 2019 were extracted from the German Animal Disease Reporting System TNS (= 'Tierseuchen-Nachrichtensystem', <https://www.fli.de/en/services/information-systems-and-databases/tsn/>). For reporting to TNS, an SBV case is defined by the isolation of the virus in cell culture or the detection of viral RNA by RT-PCR or the detection of antibodies in newborn animals before colostrum intake. Suitable antibody detection systems

include neutralization tests and FLI-licensed commercially available ELISAs. Detection of SBV genome by (real-time) RT-PCR in January and February 2019 ($n = 3$) presumably represented the outcomes of foetal infections after acute infections of the dams in the previous insect vector season, that is summer and spring of the year 2018. Therefore, only reports from March onwards were considered as SBV infections of the 2019 insect vector season.

In addition, eight bovine blood or serum samples originally tested in the context of pre-export examinations were submitted to the German National Reference Laboratory (NRL) at the Friedrich-Loeffler-Institut to confirm an SBV infection and to further characterize the re-emerging viruses (sample identifiers 2019BVD04502 to 2019BVD04504 and 2019BVD04507 to 2019BVD04511). From these samples, viral RNA was extracted using the QIAamp Viral RNA Mini kit (QIAGEN) according to the manufacturer's recommendations and tested by an S-segment-based real-time RT-PCR (Bilk et al., 2012).

To evaluate the sequence variations among SBV variants circulating in Europe between 2011 and 2019, M-segment sequences were generated from four of the samples from 2019 and compared to SBV sequences generated between 2011 and 2018 (source: NCBI GenBank). For that, the open reading frame of the M-segment of samples 2019BVD04502, 2019BVD04508, 2019BVD04509 and 2019BVD04510 was sequenced as described previously (Fischer et al., 2013; Wernike et al., 2015) (primer sequences are available on request) directly from the clinical specimens. The newly generated sequences and SBV M-segment sequences deposited in NCBI GenBank were aligned using Geneious Prime, version 2019.2.3 (Biomatters Ltd), and a maximum-likelihood tree (Hasegawa-Kishino-Yano model (Hasegawa, Kishino, & Yano, 1985); 500 bootstrap replicates) was generated using MEGA X version 17.0.5 (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). In order to visualize the sample material/sequence correlation, sample materials from which the viral sequences have been generated were indicated in the phylogenetic tree by different colours. The sequences generated in the present study have been submitted to the GenBank databases under accession numbers MN954714 to MN954717.

3 | RESULTS AND DISCUSSION

3.1 | Re-circulation of Schmallenberg virus in 2019

At 26 March 2019, an SBV-positive RT-PCR result in a dairy cow older than 2 years was reported to TNS, representing most likely the first reported SBV case of the 2019 insect vector season. Thereafter, 13 further reports were made comprising 22 animals of all age groups (Table 1). From March to November, infections of adult animals were reported, while in September, SBV genome was also detected in newborn lambs. Altogether, affected species included cattle, sheep, a goat and also a fallow deer (Table 1).

The presence of SBV genome could be confirmed in all eight diagnostic submissions. The quantification cycle (C_q) values ranged

TABLE 1 Schmallenberg virus cases reported to the German Animal Disease Reporting System TNS

Species/age group	No. of affected animals	Ascertainment date	Test method
Cattle/dairy cow >2 years	1	26.03.2019	RT-PCR
Sheep/age group not reported	1	30.04.2019	RT-PCR
Cattle/dairy cow >2 years	1	09.07.2019	RT-PCR
Cattle/nurse or mother cow	9	12.07.2019	RT-PCR
Cattle/nurse or mother cow	1	15.07.2019	RT-PCR, antibody ELISA
Fallow deer/age group not reported	1	06.09.2019	RT-PCR, antibody ELISA
Goat/age group not reported	1	12.09.2019	RT-PCR
Sheep/lamb	1	18.09.2019	RT-PCR
Sheep/lamb	1	23.09.2019	RT-PCR
Cattle/dairy cow >2 years	2	11.10.2019	RT-PCR
Cattle/dairy cow >2 years	1	25.10.2019	RT-PCR
Cattle/age group not reported	1	05.11.2019	RT-PCR, antibody ELISA
Cattle/dairy cow >2 years	1	08.11.2019	RT-PCR
Cattle/age group not reported	1	12.12.2019	RT-PCR

between 25.7 and 33.2, which is within the usual range of samples taken from viremic animals (Hoffmann et al., 2012; Wernike et al., 2015). Taking the reports to TSN and the diagnostic submissions to the German NRL for SBV together, 9 of the 16 German federal states were affected (Schleswig-Holstein, Lower Saxony, Brandenburg, North Rhine-Westphalia, Hesse, Rhineland-Palatinate, Saarland, Baden-Wuerttemberg and Bavaria).

When considering the short viremia of only a few days and therefore a very short 'diagnostic window' (Hoffmann et al., 2012), the virus detections most likely represent only a tiny fraction of the actual SBV infections in 2019. Therefore, a renewed SBV circulation to a larger extent has to be assumed in Germany, where the virus was detected in 2011 for the first time and where re-emergence was also reported in 2014 and 2016 (Wernike & Beer, 2017; Wernike et al., 2015). This pattern of re-emergence of SBV, which has been similarly observed in other European countries (Delooz et al., 2017; Kesik-Maliszewska et al., 2019; Larska, 2018), is most likely related to herd seroprevalence (De Regge, 2016; Wernike, Holsteg, Szillat, & Beer, 2018), and it is a well-known phenomenon

from other members of the Simbu serogroup. For instance, there are regular epizootics of Akabane virus, Aino virus or Peaton virus in Japan (Kato, Shirafuji, et al., 2016; Kato, Yanase, et al., 2016) or Akabane virus in Australia (Geoghegan, Walker, Duchemin, Jeanne, & Holmes, 2014). Thus, it has to be anticipated that SBV will re-circulate in Europe to a larger extent at regular intervals of around 3 years in future if no countermeasures are implemented. In consequence, SBV presents a constant threat to the ruminant population of Central Europe.

3.2 | Genetic characterization of re-emerging SBV from 2019

In order to assess the genetic diversity of SBV variants circulating between 2011 and 2019, the diagnostic submissions of 2019 with the highest SBV genome loads (Cq value < 29) were selected for sequencing of the M-segment (sample identifiers 2019BVD04502, 2019BVD04508, 2019BVD04509, and 2019BVD04510) and compared to sequences generated in previous years. In some previous studies, S-segment sequences have been used for comparison of virus variability (Coupeau, Claine, Wiggers, Kirschvink, & Muylkens, 2016; Izzo et al., 2016); however, a very high genetic stability was found (Hofmann, Mader, Fluckiger, & Renzullo, 2015; Wernike et al., 2015). In contrast, the M-segment represents the most variable genomic segment (Coupeau et al., 2013; Fischer et al., 2013; Hofmann et al., 2015; Hulst et al., 2013; Kesik-Maliszewska, Antos, Rola, & Larska, 2018) and therefore was also selected for the present study.

The newly generated sequences of samples collected in 2019 from viremic cattle showed a high similarity of 99.7%–99.9% on the nucleotide level to each other and of 99.6%–99.7% to the first SBV isolate from 2011 (BH80/11-4, GenBank accession number HE649913). Overall, the identities of all compared sequences ranged between 85.4% and 100%, with the lowest percentage of nucleotide similarities caused by large genomic deletions within the N-terminal domain of the Gc protein of viruses detected in malformed foetuses or newborns (Fischer et al., 2013; McGowan et al., 2018). When comparing the viruses detected in the two distinct clinical presentations of SBV, that is viremic adult animals and malformed foetuses, independently from each other, it becomes apparent that the genome of viruses detected in the blood of acutely infected adult ruminants ($n = 31$) remained very stable over the course of 9 years. The nucleotide similarities of these viruses detected Europe-wide varied between 99.4% and 100%. The comparison of German sequences generated from this sample material revealed a gradual genetic drift on a very low level, with some mutations acquired in the year 2014 also present in 2016 and 2019, and three new mutations present in all four samples from 2019 (Figure 1). In contrast to the very stable viral genome found in viremic adult ruminants, M-segment sequences generated from organ samples of malformed foetuses or newborns exhibit remarkable sequence variations, which is reflected in the phylogenetic tree (Figure 2). While the viruses detected in viremic

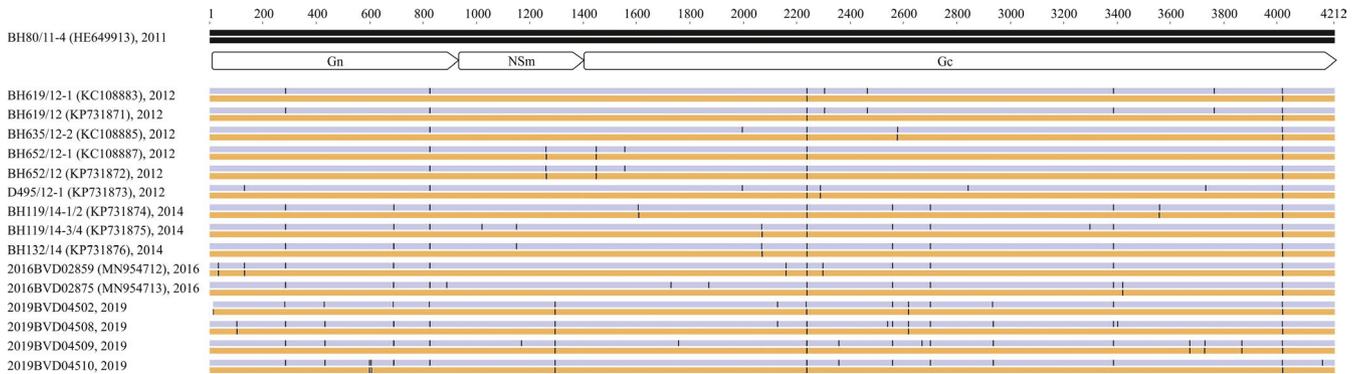


FIGURE 1 Comparison of the M-segment sequences of SBVs detected in acutely infected adult ruminants in Germany between 2011 and 2019. The sequences are named by strain name, NCBI GenBank accession number, year of sample collection. The first isolate from 2011 is used as reference strain. Nucleotide (blue bar alongside the name of each isolate) and amino acid (orange bar) substitutions are highlighted as vertical black lines [Colour figure can be viewed at wileyonlinelibrary.com]

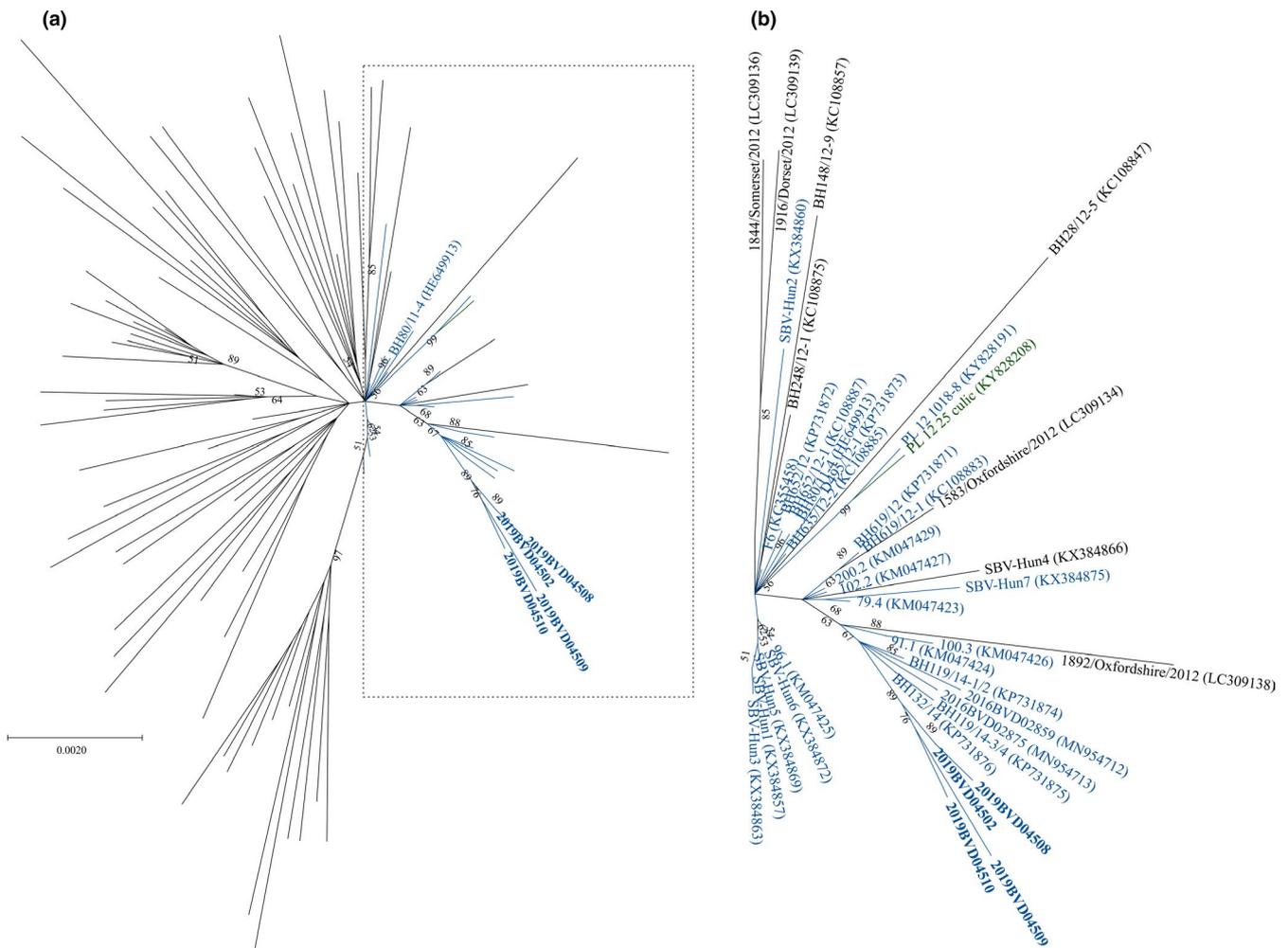


FIGURE 2 Phylogenetic analysis based on the nucleotide sequences of the M-segment of SBV. To compare the sequences generated in the present study (labelled in bold), further SBV M-segment sequences were obtained from NCBI GenBank. Sequences labelled in black were obtained from organ samples of malformed fetuses or newborns and sequences labelled in blue originate from the blood of acutely infected animals sampled between 2011 and 2019 in various European countries. The sequence labelled in green was generated from an infected insect vector. Statistical support for nodes was obtained by bootstrapping (500 replicates); only values $\geq 50\%$ are shown. (a) The sample name and accession number of the first SBV isolate (year 2011) and the sample identifiers of the newly generated sequences (year 2019) are given. The scale bar indicates nucleotide substitutions per site. (b) Subset of figure panel A showing the sample names and accession numbers of all M-segment sequences generated from acutely infected animals since 2011 (blue) [Colour figure can be viewed at wileyonlinelibrary.com]

animal hosts or insect vectors cluster closely together, SBV found in cases of prenatal infections is highly divergent (Figure 2). This sample material/sequence variability correlation has been described before (Hellert et al., 2019; Wernike & Beer, 2019), and it seems to be independent of the host species or geographical region from which the samples originated, as even in individual animal flocks highly divergent viral sequences were observed (Kesik-Maliszewska et al., 2018). Interestingly, the sequence adaptations that accumulate in infected fetuses primarily affect the major antigenic domain of SBV (Hellert et al., 2019). However, these variant viruses were never found as circulating SBV strains, that is in viremic ruminant hosts or insect vectors. Thus, they are most likely artefacts that are not fit for the usual transmission cycle and, as a consequence, cannot be spread.

In conclusion, genetically highly stable SBV again circulated in 2019. Thus, the virus reached an enzootic status in Europe and established a pattern of cyclic re-emergence every 2–3 years. Therefore, it has to be anticipated that SBV will re-appear to a larger extent also in the future at regular intervals if no countermeasures are implemented.

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ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The blood samples were taken by the responsible farm veterinarian in the context of the health-monitoring programme of the farm or in the context of pre-export examinations, and no permissions were necessary to collect the specimens used in the study.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Kerstin Wernike  <https://orcid.org/0000-0001-8071-0827>

Martin Beer  <https://orcid.org/0000-0002-0598-5254>

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