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Misinterpretation of Schmallenberg virus sequence variations: the sample material makes the difference

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Abstract

In recent reports about the molecular epidemiology of Schmallenberg virus (SBV), an orthobunyavirus affecting ruminants, it was proposed that the observed sequence variability within the viral M-segment might be higher in sheep than in cattle. However, these analyses are highly biased by the sample material from which the publicly available sequences were generated. While from cattle predominantly blood samples from acutely infected animals were studied, the vast majority of ovine samples originate from malformed fetuses or newborn lambs. Therefore, the observed sequence variability is misinterpreted since the samples from malformed fetuses and lambs do not reflect circulating SBV.

Keywords Phylogenetic analysis · Mutation · Host · Malformation

Dear Editor,

The insect-transmitted Schmallenberg virus (SBV), an orthobunyavirus (order *Bunyavirales*) of the Simbu serogroup, emerged in late 2011 in the German/Dutch border region [1] and subsequently caused a large epidemic in the European ruminant population [2]. Besides the major target species cattle, sheep, and goats, the host range comprises various captive and wild ruminants and some further ungulates and zoo animals [3]. Depending on the time of infection, the virus induces two distinct clinical presentations. In domestic ruminants of all age classes, SBV causes a short-lived viremia, sometimes associated with mild, transient disease characterized by fever, diarrhea, or decreased milk production. In contrast, persistent infections of fetuses during a vulnerable phase of their development can result in abortion, premature birth, or severe congenital malformation [4].

Since its unexpected emergence, SBV has been investigated comprehensively. Besides studies aiming at the biology, epidemiology, and pathogenesis of SBV infections, several phylogenetic studies have been published. Like in typical orthobunyaviruses, Schmallenberg virions consist of six proteins, which are encoded by a tripartite, negative-stranded RNA genome. The L-segment encodes the RNA-dependent RNA polymerase, while the S-segment encodes the nucleocapsid (N) protein and in an alternative overlapping reading frame the non-structural protein NSs. The M-segment encodes the viral glycoproteins Gn and Gc as well as the non-structural protein NSm [5]. Within the M-segment, a region of high-sequence variability has been identified, which at the time of its identification seemed to be geographically and host species independent [6, 7].

During recent studies aiming at the molecular epidemiology of SBV [8–10] it was proposed that the sequence variability of SBV might be higher in sheep than in cattle. However, a major drawback of these studies is the strong bias caused by the sample material from which the selected sequences were generated. The vast majority of sequences generated from blood samples of acutely infected animals originate from cattle. From acutely infected, viremic sheep only one original sample and the corresponding cell-culture isolate were sequenced (GenBank acc. numbers KC108883 and KP731871). In contrast, nearly every SBV sequence publicly available at NCBI GenBank that was generated from malformed fetuses or newborns originates from sheep, while virus variants from only three malformed calves (acc.

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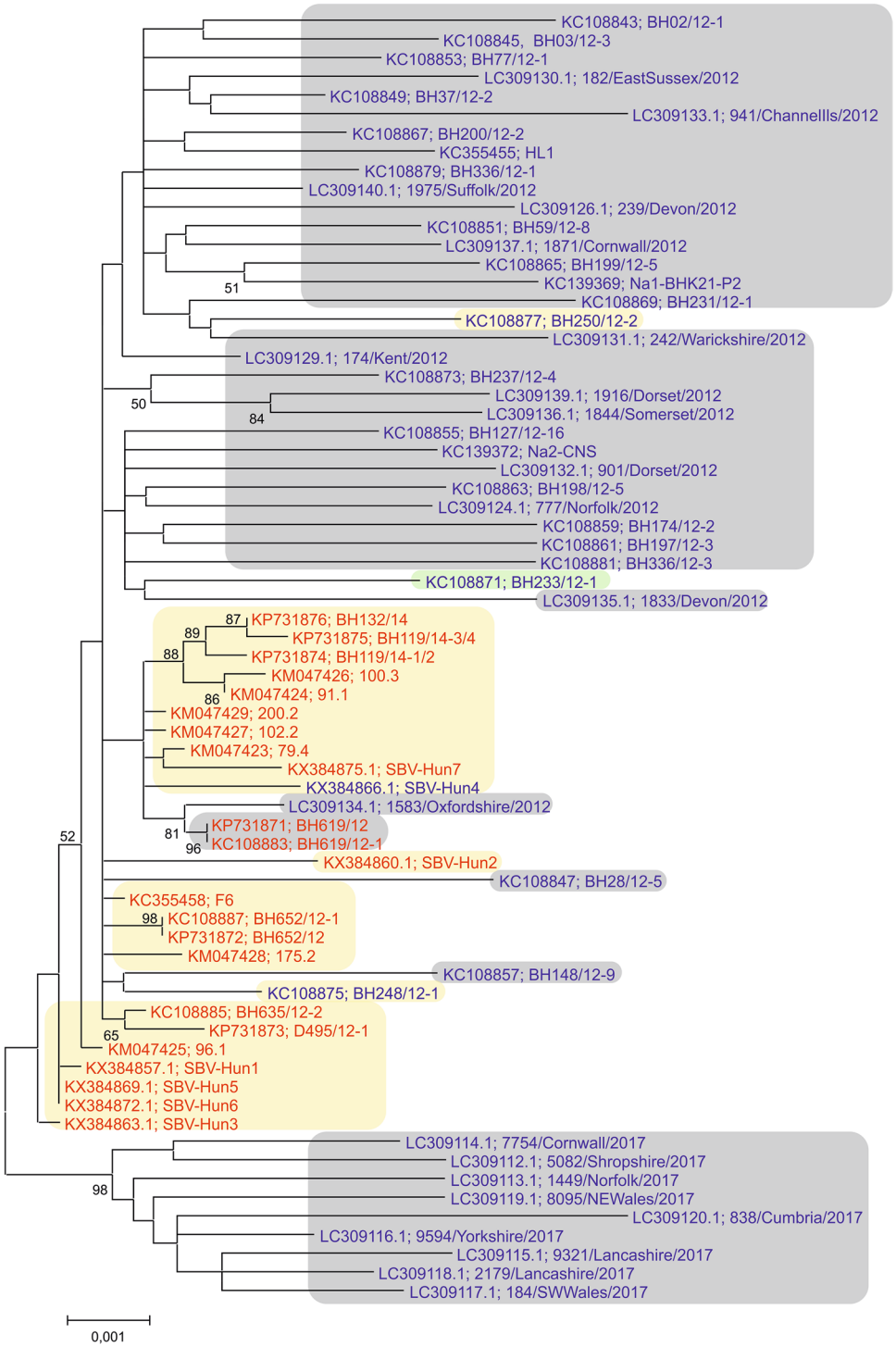

Fig. 1 Phylogenetic analysis based on the nucleotide sequences of the M-segment of SBV. The sequences were obtained from NCBI GenBank and are labeled by accession number and isolate name. Sequences labeled in blue were obtained from organ samples of malformed fetuses or newborns and sequences labeled in red originate from the blood of acutely infected animals. Viral sequences generated from bovine samples are highlighted in yellow, while sequences from ovine samples are shaded in gray, and from caprine samples in green. Statistical support for nodes was obtained by bootstrapping (1000 replicates); only values ≥ 50% are shown. The scale bar indicates nucleotide substitutions per site

numbers KC108875, KC108877, and KX384866) and one goatling (acc. number KC108871) were sequenced.

When these sequences are grouped according to the sample materials instead of the animal species, a very high-sequence stability becomes apparent in the genome of viruses present in viremic adult animals [11, 12]. In contrast, a high-sequence variability can be seen in the amino-terminal part of the glycoprotein Gc-encoding region of viruses present in the brain of malformed newborns.

For visualization of the sample material/sequence and host species/sequence correlations, SBV M-segment sequences deposited in NCBI GenBank were aligned and a maximum-likelihood tree (Hasegawa–Kishino–Yano model; 1,000 bootstrap replicates) was generated using MEGA

version 7.0.14 [13]. Subsequently, sample materials and host species from which the viral sequences were generated were indicated in the phylogenetic tree. The genome of viruses detected in the blood of acutely infected adult cattle and sheep over the course of several years and in various European countries seems very stable and represents the circulating SBV strains. In contrast, sequences generated from organ samples of malformed fetuses or newborns exhibit remarkable sequence variations (Fig. 1) and represent non-circulating SBV strains which persisted in these fetuses for weeks or months. This sequence variability seems to be independent of the geographical region or host species from which the samples originated, since even in individual animal flocks highly divergent sequences were observed [8], and the sequence KC108877 from a malformed calf clustered with the virus sequences generated from malformed lambs (Fig. 1).

The observed differences in the frequency of mutations are therefore most likely not related to the animal species, but to the transmission mode and biology of the virus. Insect-transmitted viruses such as SBV have to adapt to two different hosts, i.e., the arthropod vector and the mammalian host. Hence, the high sequence stability of circulating SBV strains, which are detectable in the insect vectors or in the blood of acutely infected animals, might be necessary for transmission from the mammalian host to the insect vector. In contrast, viruses present in malformed fetuses are not transmitted to the insect vectors. Moreover, these viruses replicate in the presence of sometimes very high antibody titers [14], when a fetus is infected once it is immunocompetent or becomes able to develop specific antibodies during an ongoing infection. Therefore, the mutation hot spot was supposed to be involved in immune evasion mechanisms in infected fetuses [6, 15], which is supported by the genome region in which the mutations occur. The envelope glycoproteins Gn and Gc are generally major immunogens of orthobunyaviruses and the genomic region in which the mutation hot spot is located represents a key immunogenic domain of SBV [16, 17].

In summary, the sequence variability observed in SBV's M-segment heavily depends on the sample material from which the viruses are isolated. Consequently, only sequences of circulating strains from acute infections of adult animals or infected insect vectors should be used for the classification of viruses and molecular–epidemiological studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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