Veterinary Microbiology 176 (2015) 97–108

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Genetic stability of Schmallenberg virus *in vivo* during an epidemic, and *in vitro*, when passaged in the highly susceptible porcine SK-6 cell line

Martin A. Hofmann *, Markus Mader, Franziska Flückiger, Sandra Renzullo

Institute of Virology and Immunology IVI, Development, Sensemattstrasse 293, CH-3147 Mittelhäusern, Switzerland

ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form 23 December 2014
Accepted 8 January 2015

Keywords:
Schmallenberg virus
Bunyaviridae
Nucleotide sequencing
Genetic stability
SK-6 cells
Virus isolation

ABSTRACT

Schmallenberg virus (SBV), an arthropod-borne orthobunyavirus was first detected in 2011 in cattle suffering from diarrhea and fever. The most severe impact of an SBV infection is the induction of malformations in newborns and abortions. Between 2011 and 2013 SBV spread throughout Europe in an unprecedented epidemic wave. SBV contains a tripartite genome consisting of the three negative-sense RNA segments L, M, and S. The virus is usually isolated from clinical samples by inoculation of KC (insect) or BHK-21 (mammalian) cells. Several virus passages are required to allow adaptation of SBV to cells *in vitro*. In the present study, the porcine SK-6 cell line was used for isolation and passaging of SBV. SK-6 cells proved to be more sensitive to SBV infection and allowed to produce higher titers more rapidly as in BHK-21 cells after just one passage. No adaptation was required. In order to determine the *in vivo* genetic stability of SBV during an epidemic spread of the virus the nucleotide sequence of the genome from seven SBV field isolates collected in summer 2012 in Switzerland was determined and compared to other SBV sequences available in GenBank. A total of 101 mutations, mostly transitions randomly dispersed along the L and M segment were found when the Swiss isolates were compared to the first SBV isolated late 2011 in Germany. However, when these mutations were studied in detail, a previously described hypervariable region in the M segment was identified. The S segment was completely conserved among all sequenced SBV isolates. To assess the *in vitro* genetic stability of SBV, three isolates were passage 10 times in SK-6 cells and sequenced before and after passaging. Between two and five nt exchanges per genome were found. This low *in vitro* mutation rate further demonstrates the suitability of SK-6 cells for SBV propagation.

1. Introduction

Schmallenberg virus (SBV) was named after the community in Germany where it was first isolated in late 2011 from a cow showing diarrhea, fever, and a drop in milk production (Hoffmann et al., 2012). Since then SBV has swept across Europe in an unprecedentedly fast epidemic. By summer 2013 the ruminant population of almost all European countries was infected by SBV, and the virus continued to spread eastwards, reaching as far as the Baltic's and Greece (ProMED-mail-reported SBV case on Sept. 23rd, 2013). Despite the high seroprevalence in ruminants in Europe, new SBV cases have been detected in Germany and The Netherlands in 2014 (ProMEDmail no. 20141121.2978286 from November 13th). The origin of the SBV causing the first outbreak in Germany is still not known. In a very recent report (Blomström et al., 2014) the

* Corresponding author. Tel.: +41 0 31 848 92 11; fax: +41 0 31 848 92 22.
E-mail address: martin.hofmann@ivi.admin.ch (M.A. Hofmann).

http://dx.doi.org/10.1016/j.vetmic.2015.01.010
0378-1135/

presence of SBV antibodies was described in cattle, sheep and goats in Mozambique, Africa, with a seroprevalence between 47 and 100%. Although these findings have to be confirmed, they represent the first indication from where SBV could have been introduced into Europe.

Cattle infected with SBV show various degrees of clinical symptoms such as diarrhea, fever, drop in milk production. These symptoms usually last only a few days before the animals recover completely (Beer et al., 2013; Doceul et al., 2013). Adult sheep and goats usually exhibit few clinical signs. However, the most serious impact of SBV is the infection of the fetus in pregnant animals, resulting in abortion or death of newborn calves, lambs and kids (Garigliany et al., 2012). This is often accompanied by extensive malformations of the limbs and in the brain (Hahn et al., 2012). Although domestic and wild ruminants represent the main mammalian host species, the virus can also cause clinical disease in dogs (Sailleau et al., 2013). Although SBV-specific antibodies in wild boars have been reported (Desmecht et al., 2013), a recent study showed that pigs are inefficient hosts for SBV infection and transmission (Poskin et al., 2014).

SBV is transmitted by insect vectors. It has been shown that several *Culicoides* spp. midges function as main insect vector for SBV transmission (Elbers et al., 2013). Furthermore, it is speculated that also certain mosquito species (*Culex* spp., *Aedes* spp.) which play a role in the transmission of other *Bunyaviridae* such as the Akabane virus that is closely related to SBV could also be capable to transmit SBV (Garigliany et al., 2012). High amounts of SBV RNA found in *Culicoides* indicate that SBV actively replicates during its migration from the midgut to the saliva glands (Rasmussen et al., 2012).

SBV is a new member of the family *Bunyaviridae* that comprises many other arthropod-born viruses. Whereas initial genome sequence data suggested that SBV is a reassortant between Sathuperi and Shamonda virus (Hoffmann et al., 2012), belonging to the Simbu serogroup, phylogenetic analysis using more extensive sequence data now indicates that SBV is most closely related to the Sathuperi virus species (Doceul et al., 2013). For viral RNA detection several RT-qPCR protocols have been published, targeting either the S segment (Bilk et al., 2013) or the L segment (De Regge et al., 2012) of the viral genome.

Several mammalian cell lines such as baby hamster cells (BHK-21), monkey kidney cells (VERO), as well as insect cells (KC) have been described for the isolation and propagation of SBV from viremic serum in cell culture, albeit resulting in low virus titers upon the initial passage (Wernike et al., 2012) and therefore requiring at least a few passages before rapid cytopathic effect (CPE) appears and virus titers rise to $>10^5$ $TCID_{50}$/mL. However, such an adaptation to cell lines usually represents a bottleneck for infection, increasing the chance that the viral genome acquires mutations. Therefore, a cell culture system that allows efficient virus isolation, *i.e.* leading to high virus titers and rapid appearance of CPE in the first passage without the need for serial passaging, is highly desirable.

The porcine kidney cell line SK-6 is widely used for the propagation of many different viruses (Kasza et al., 1972). We have shown that these cells do not produce type I interferon upon stimulation with double-stranded RNA (Ruggli et al., 2003). SK-6 cells have also been shown to maintain the genetic stability of classical swine fever virus for >90 passages (Vanderhallen et al., 1999).

Here we demonstrate that SK-6 cells are highly suitable for SBV isolation and propagation by exhibiting a rapid CPE (within 24–48 h) and yielding high virus titers upon inoculation of the cells with viremic serum. Serial passaging of SBV kept the viral genome virtually unchanged, further supporting the suitability of SK-6 cells.

In addition, nucleotide (nt) sequence comparison of the genome from several SBV isolates collected in Switzerland and other strains mainly from Germany and Belgium demonstrated that SBV evolves rather slowly despite its very rapid spread during the epidemic 2011–2013, and the few identified mutations among the Swiss isolates did not correlate with the geographic or temporal origin of the virus.

2. Materials and methods

2.1. Cells and viruses

The swine kidney cell line SK-6 was kindly provided by M. Pensaert, Faculty of Veterinary Medicine, Gent, Belgium. BHK-21 were obtained from ATCC (no. CCL-10).

2.1.1. High titer viremic sera used for passaging and genetic studies

Seven different SBV isolates with high amounts of viral RNA collected from clinical SBV infection in cattle throughout Switzerland during the epidemic wave of SBV over a time period of four weeks (Table 1) were included in the present study (sample numbers no. 79.4, no. 91.1, no. 96.1, no. 100.3, no. 102.2, no. 175.2, no. 200.2); the latter two samples were collected one week apart from two different animals from the same farm in order to determine the in-herd genetic variability of SBV.

Isolate no. BH80/11-4 from the first SBV outbreak in Germany was kindly provided by Martin Beer, FLI, Germany as cell culture supernatant (one passage in KC cells followed by one passage in BHK-21 cells). The nt sequence of the complete genome of this virus was taken from GenBank (accession numbers HE649912, HE649913, HE649914 for L, M, and S segment, respectively). The German isolate was further passaged in our laboratory once on KC cells, three times on BHK-21 cells and finally once in SK-6 cells. The Swiss SBV isolates were isolated directly in SK-6 cells, using serum from viremic cattle. In order to compare the sequence data from the Swiss isolates with other SBV strains, 32 full-length M (16 from Belgium, 14 from Germany, and 2 from the Netherlands) and 17 full-length L (9 from Germany, 6 from Belgium and 2 from the Netherlands) sequences were retrieved from GenBank (for accession numbers, see Fig. 5)

2.1.2. Low titer viremic sera used for comparison of susceptibility of SK-6 versus BHK-21 cells

Five additional viremic sera with low amounts of viral RNA (no. 91.1, no. 91.2, no. 96.2, no. 96.3, no. 109.1) (Table 3B) were used for the comparison of the susceptibility of SK-6 and BHK-21 cells.