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Short Communication

Isolation of Sango viruses from Israeli symptomatic cattle

Natalia Golender^{1*}, Velizar Bumbarov¹, Avi Eldar¹, Lior Zamir², Boris Even Tov², Gabriel Kenigswald³ and Eitan Tiomkin³

¹Department of Virology, Kimron Veterinary Institute, Beit Dagan, Israel

²Israeli Veterinary Services, Rosh Pina, Israel

³Hachaklait Veterinary Services, Caesarea, Israel

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***Corresponding author:** Natalia Golender, Department of Virology, Kimron Veterinary Institute, Beit Dagan, Israel, Tel: (+972-9681949); Fax: 972-39681788; E-mail: golendern@moag.gov.il

ORCID: <https://orcid.org/0000-0002-9074-7989>

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Abstract

Sango Virus (SANV) is a member of the Simbu serogroup within the order *Bunyavirales*, family *Peribunyaviridae*, genus *Orthobunyavirus*. In the end of October, 2019, two SANV virus were identified and isolated from sera sampled from two symptomatic cows, which manifested fever, milk reduction and diarrhea. Two viruses were isolated in Vero cells. Full genome sequencing of these virus isolates was performed. Genetic and phylogenetic analyses of Israeli SANV strains showed their very close relationship. However, they have significant difference in all three genome segments with a sole known isolated Nigerian SANV Ib An 5077 strain.

Introduction

Sango Virus (SANV) is a member of the Simbu serogroup within the order *Bunyavirales*, family *Peribunyaviridae*, genus *Orthobunyavirus* [1]. The only available SANV Ib An 5077 strain was isolated between 1964–1969 in Ibadan, Nigeria from *Culicoides* spp. [2]. In general, orthobunyaviruses are negative-sense RNA viruses, mostly transmitted by mosquitoes or culicoid flies [3]. The genome of orthobunyaviruses comprises three unique segments of single stranded RNA. The average size of each genome segment is of orthobunyaviruses is about 6.9 kb for the large (L) segment, 4.5 kb for the medium (M) segment and 1.0 kb for the small (S) segment [3]. The S segment encodes the nucleoprotein (N protein), and non-structural protein (NSs), the M segment encodes polyprotein precursor that yields the two external glycoproteins Gn and Gc, and the L segment encodes the RNA-dependent RNA polymerase (RdRp) [3, 4].

Veterinary important representatives of the Simbu serogroup are Akabane (AKAV), Schmallenberg (SBV) and Shuni (SHUV) viruses among others, when two of them – SHUV and AKAV, are endemic in Israel [5]. These viruses are also transmitted

by insect vectors (mostly by *Culicoides* biting midges), are distributed worldwide and are known to cause pregnancy abnormalities and severe fetal malformation summarized under the term “arthrogryposis-hydranencephaly syndrome” [1,6]. Simbu serogroup virus infections can cause two different types of clinical manifestation in ruminants. Acute infections of animals of all age groups are either asymptomatic or mild, associated with unspecific signs like fever, diarrhea or loss in milk yield for a few days, as was seen with Schmallenberg viral infection of domestic ruminants. However, when naïve dams are infected during a critical phase of gestation, severe congenital defects can occur [7,8]. Moreover, AKAV and SHUV can cause neurological diseases and fatalities in affected animals [9,10], when SHUV were detected by molecular methods in several human cases of neurological disease in South African hospital patients [11]. Infected with simbuviruses ruminants usually develop short viremia lasting 2–7 days [12,13].

SANV was probably identified in ruminants only once: in spleen of a springbok, South Africa, which afterwards probably died (the signed sample for investigation was spleen. The phylogenetic tree illustrated relationship with the SANV Ib An 5077 strain by S segment only) [14]. Up today no clinical



disease has been registered in domestic ruminants caused by SANV. The aim of this study is description of clinical signs in affected cattle and genetic and phylogenetic analyses of the novel SANV Israeli strains.

Materials and methods

Field samples

During 2019, a total of 230 plasma, whole blood or serum samples from young and adult sick animals, which were sent to Kimron Veterinary Institute, Beit Dagan, Israel (KVI) for diagnosis of arboviral infections, were tested for simbuviruses (181 sample from cattle, 47- from sheep, and 2 samples from goat).

RNA extraction and qRT-PCR Tests

Viral RNA was extracted using the MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific). All samples were tested for Bovine Ephemeral Fever Virus (BEFV), Epizootic Hemorrhagic Disease Viruses (EHDV), Bluetongue Viruses (BTV) and Simbu serogroup viruses. Thus, for detection of BEFV a method described by Erster was used [15]. EHDV RNA presence was assessed with an Epizootic Hemorrhagic Disease Virus Real-Time PCR Kit (LSI VetMAX, Lissieu, France), according to the instructions of the manufacturer, when BTV RNA presence was assessed with a BTV VetMAX™ BTV NS3 All Genotypes Kit (Applied Biosystems™, Thermo Fisher Scientific Inc., France). qRT-PCR was applied for detection of simbuviruses according to Golender, et al. [5].

Virus identification

At first stage virus identification was done by partial sequencing of the S-segment using the same pair of primers as used for simbuvirus detection [5]. For precise virus identification the universal primers for M segment 388 bp fragment enable for detection of both SANV and Peaton (PEAV) viruses were developed (PEAV-M-94F '5'- AAA AGG TGG RAA GTG CTT TTA T-3' and PEAV-M-458R '5- CCA TTT TTT ATT GTA GTT CCT TG-3').

Sequencing, BLAST and phylogenetic analyses

For conventional RT-PCRs the One-Step RT-PCR kit (Qiagen, Hilden, Germany) was used. The complete sequences of all three viral segments were obtained by Sanger sequencing using overlapping pairs of primers (available by request). The cDNA fragments were purified with the MEGAquick-spin Total Fragment DNA Purification Kit (iNtRON Biotechnology, Gyeonggi-do, South Korea) and subsequently sequenced by standard Sanger methods in both directions using an ABI 3730xl DNA Analyzer (Hylabs, Rehovot, Israel). The resulting nucleotide sequences were assembled and nucleotide (nt) sequences were aligned and pairwise compared by using Geneious version 9.0.5 (Biomatters, Auckland, New Zealand). Phylogenetic trees were constructed using the Mega X software [16].

Virus isolation

African green monkey kidney cells (Vero) were applied as described previously [17]. In brief, monolayer Vero cells were

inoculated with sera samples and examined daily for evidence of cytopathic effect (CPE). Two passages were performed. The cells, which were infected with field samples (sera), were frozen at -80°C after 5 days of incubation. The full CPE was observed in both cases at 2nd passage on day 3 after infection.

Results

Clinical signs

Whole blood and sera samples from three fattening cows (Moshav Ma'ale Gamla, the Golan Heights, October 27, 2019) and the same kind of samples from five milking cows (Moshav Kanaf, the Golan Heights October 30, 2019) were sent to KVI. Sick cows demonstrated fever, hypersalivation, milk reduction and diarrhea. Two specific adult cows (one- age was unknown, the second- 7 years-old cow), which were found viremic for simbuviruses, manifested fever, milk reduction and diarrhea. The 7 years-old cow was soon slaughtered because of bad improvement in body condition and milk production after the disease. Notably, the only single sample was identified as positive for SHUV samples from all other samples, which were sent during 2019 from symptomatic adult and young domestic ruminants: from an adult cow shortly after abortion from Moshav Ma'ale Gamla, which blood was sampled at July, 2, 2019.

qRT-PCR results

Blood samples collected from three sick cows from Moshav Ma'ale Gamla, showed that one cow was positive for BTV, one- for simbuviruses, and one was negative in all tests. Blood samples collected from five sick cows from Moshav Kanaf, showed that three cows were positive for BTV, one- for simbuviruses, one was negative in all tests. No mixed infections were identified (full data on BTV is not shown). Pan-Simbu RT-qPCR positive samples showed cycle threshold 22.34 and 17.9.

Virus isolation

Two strains ISR-2061/3/19 and ISR-2097/1/19 were isolated. CPE was observed on the second passage of both samples. Virus replication was confirmed basing on Pan-Simbu RT-qPCR and sequencing.

Genetic and phylogenetic analyzes

Partial sequence of S segment used for identification of the virus [16], showed the same similarity to SANV and Peaton (PEAV) viruses. The universal primers for M segment 388 bp fragment, enable for detection of both SANV and PEAV showed 92.25% and 92.19% of nucleotide (nt) identity to SANV IbAn 5077 strain following by Australian PEAV cs322 strain with 86.86–86.75% of nt identity.

The complete sequences of ISR-2061/3/19 and ISR-2097/1/19 strains have been deposited in GenBank under accession numbers MZ285919 – MZ285921 and MZ285922 – MZ285924, respectively.

BLASTn and phylogenetic analysis showed belongings of Israeli strains to SANV by all three segments (Figure 1). Both

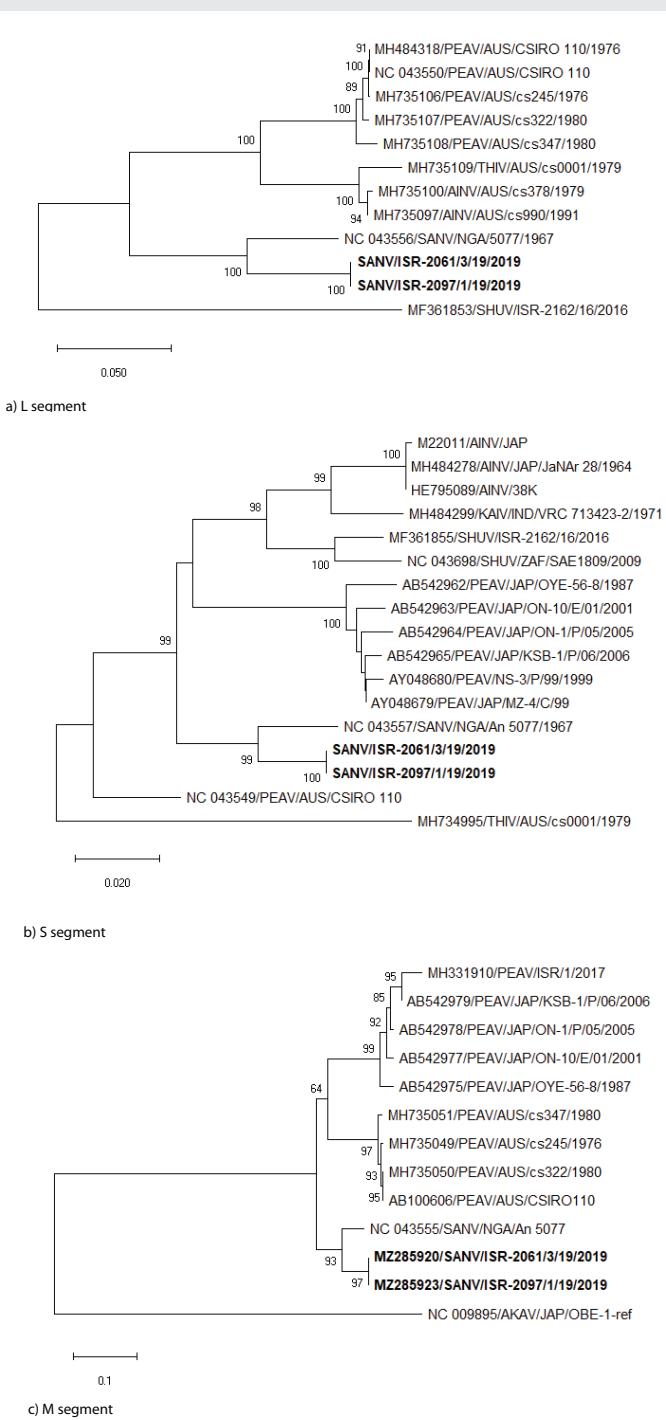


Figure 1: Phylogenetic analysis of Israeli Sango viruses (SANV) based on nucleotide sequences of the L (a), S (b) and M (c) segments and their comparison to other related simbuviruses. The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated (complete deletion option). The sequences obtained from NCBI GenBank are labelled by accession number/virus/location/isolate/year. Sequences generated in the present study are marked by bold fond.

Israeli SANV had identical S segment nt sequences. BLASTn analysis of S segment showed of 96.30% of nt identity with SANV SANV Ib An 5077 followed by PEAV SCIRO 110 strain with

91,86% of nt identity. BLASTp analysis indicated 100% of aa identity by N protein with PEAV SCIRO 110 strain and 95% identity by NSs proteins with PEAV SCIRO 110 strain. BLASTn analysis of M segment of ISR-2061/3/19 and ISR-2097/1/19 showed of 92,54% and 92,58% of nt identity to SANV SANV Ib An 5077 strain following by PEAV KSB-1/P/06 strain with 83,80% and 83,82% of nt identity, respectively. BLASTp analysis of polyproteins of ISR-2061/3/19 and ISR-2097/1/19 strains showed 97,21% and 97,36% identity to SANV, followed by PEAV ON-1/P/05 strain with 92,07% and 92,14% of aa identity, respectively. BLASTn analysis of L segment of ISR-2061/3/19 strains showed of 91,91% of nt identity with SANV SANV Ib An 5077 followed by PEAV SCIRO sc990 strain with 84,61% of nt identity, when analysis ISR-2097/1/19 strain showed of 91,96% of nt identity with SANV SANV Ib An 5077 following by PEAV SCIRO sc990 strain with 84,58% of nt identity. BLASTp analysis of RdRp proteins showed 98,45% of aa identity to SANV Ib AN 5077 strain and 94,23% of aa identity to several different PEAVs of both SANV Israeli strains.

Discussion

Since the first case of SANV isolation in 60-s in Nigeria, SANV was detected one time only in a South African springbok manifesting neurologic signs, which afterwards probably died (the signed sample for investigation was spleen) [14]. Unfortunately, the sequences of this isolate were not published in the GenBank, it is impossible to compare Israeli and South African SANV strains. Identification and isolation of SANV in symptomatic cattle demonstrates ability of additional member of Simbu serogroup to develop mild clinical manifestations of the disease in cattle. As we see in case of SBV infection, it was firstly identified in several European countries as a simbuvirus causing mild manifestation of the disease in cattle [18]. Few months later SBV was identified in aborted and malformed fetuses of domestic ruminants [18]. In spite of the fact that during the next 1,5 year period no cases of SANV were identified neither in aborted fetuses nor in symptomatic animals, further observation after cases of “abortion storms” or neurological cases in cattle have to be done.

Author contributions

Methodology, N.G.; software, N.G.; investigation, N.G. and V.B.; resources, B.E.T., L.Z., E.T. and G.K.; data curation, V.B.; writing—original draft preparation, N.G.; writing—review and editing, V.B.; supervision, A.E.; funding acquisition, A.E. All authors have read and agreed to the published version of the manuscript.

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