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Case Report

First genomic detection of Peaton virus in a calf with hydranencephaly in Israel

Adi Behar*, Binyamin (Boris) Leibovich*, Nir Edery†, Tohru Yanase‡ and Jacob Brenner§

*Division of Parasitology, Kimron Veterinary Institute, Beit Dagan, Israel, †Division of Pathology, Kimron Veterinary Institute, Beit Dagan, Israel, ‡National Institute of Animal Health, National Agriculture and Food Research Organization, Kagoshima, Japan and §Division of Virology, Kimron Veterinary Institute, Beit Dagan, Israel

Abstract

Simbu serogroup are arbo- viruses which are mainly transmitted by *Culicoides*. Two members of the Simbu serogroup, Akabane and Shuni viruses, have been isolated from congenitally malformed ruminants in Israel. A recent serosurvey revealed that Israeli ruminants have been exposed to several additional Simbu viruses, including *Shamonda* and *Sathuperi* that seems to be circulating in Israel. In April 2017, an apparently healthy one-month-old male calf was transferred to the Kimron Veterinary Institute. A few days later, the calf was reported to be slow to respond to its surroundings and was not able to feed on its own. Blindness was observed upon clinical examination. RNA of the small, medium and large segments of Simbu serogroup viruses were amplified and sequenced from the testis tissues and from the Cerebrospinal fluid (CSF). During post-mortem examination, hydranencephaly was defined. Phylogenetic analysis of all three segments of Simbu serogroup viruses showed that the sequences detected in the Israeli calf were virtually identical to Peaton virus (PEAV). PEAV was also detected in two pools of *Culicoides imicola* trapped at two different locations in Israel. This is the first genomic detection of PEAV outside Australia and Japan. These results are of epidemiological significance, as they demonstrate that PEAV is circulating in Israel and affects cattle. Consequently, these results are also of relevance to a potential spread of Simbu serogroup viruses into Europe.

Keywords: Simbu serogroup viruses, arbovirus, Peaton virus, congenital malformations, Weak calf syndrome, hydranencephaly.

Correspondence: Adi Behar, Department of Parasitology, Kimron Veterinary Institute, Beit Dagan, Israel. E-mails: adib@moag.gov.il; adibehar@gmail.com

The Simbu serogroup viruses, one of the largest serogroups within the genus *Orthobunyavirus* of the family *Peribunyaviridae*, comprises at least 24 antigenically different, but serologically related viruses that are transmitted mainly by *Culicoides* biting midges (De Regge 2017; Hirashima *et al.* 2017). Several Simbu serogroup viruses have been shown to cross the placenta of ruminants to the developing fetus and to cause outbreaks of abortion, stillbirth and malformations (St George & Standfast 1989; Tsuda *et al.* 2004; Yanase *et al.* 2010; Hoffmann *et al.* 2012; Golender *et al.* 2015; Brenner *et al.* 2016). The congenital malformations seen at birth are known as arthrogryposis hydranencephaly syndrome and correlate with the stage of pregnancy at which the mother is infected (St George & Standfast 1989; De Regge 2017). Brain damage can range from microscopic to mild or severe malformations and includes hydranencephaly, microencephaly and polio-encephalomyelitis (St George & Standfast 1989; Brenner *et al.* 2016). In cattle, severe brain malformations may occur if the dam is infected between 76 and 106 days of pregnancy. Surprisingly, some calves with little more than an intact brain stem are born alive. They are often blind, poorly responsive to external stimuli and have a weak suckling reflex. Nevertheless, they may be capable of standing and getting around, and are able to develop physically if

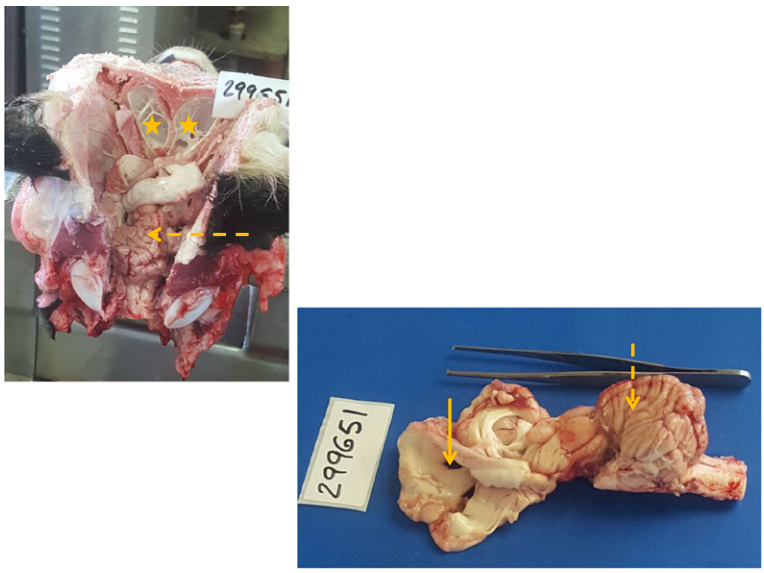

Fig. 1 Gross pathology: Narrow skull cavity with partially developed frontal cerebral lobes (mark with stars). Deformed and reduced cerebral mass (microencephaly), with enlarged internal cisterns (hydranencephaly) (Full arrows). The cerebellum appears normal (dotted arrows).

carefully tended and fed. The colloquial term for them is "dummy calves", and the veterinary term is weak calf syndrome (St George & Standfast 1989; De Regge 2017).

Two members of the Simbu serogroup, Akabane and Shuni viruses, have been isolated from the brains of congenitally malformed ruminants in Israel (Golender *et al.* 2015; Brenner *et al.* 2016). A recent retrospective serosurvey revealed that Israeli ruminants have been exposed to several additional Simbu viruses, including *Shamonda* and *Sathuperi* (Brenner *et al.* 2018). These viruses are considered potential teratogenic agents in ruminants (St George & Standfast 1989; Yanase *et al.* 2012; Hirashima *et al.* 2017), and seem to be circulating in Israel (Brenner *et al.* 2018).

In late April 2017, an apparently healthy 1-month-old male calf was transferred to the Kimron Veterinary Institute (KVI). A few days later, the herdsman reported that the calf was slow to respond to its surroundings and was not able to feed on its own. Blindness was observed upon clinical examination. Epidemiological investigation revealed that the calf was born to a dairy heifer in a large dairy farm located in the central coastal plain of Israel, which is one of 11 farms that are monitored for arboviruses and their vectors by the KVI. The heifer was of the same age as a group of naïve heifers serving as sentinels in 2016. Seroconversion to Simbu serogroup viruses was detected in the sentinels in July 2016 by ELISA (IDEXX Schmallenberg Ab Test Kit, Liebefeld-Bern, Switzerland), indicating that the aforementioned dam may have been exposed to at least one Simbu serogroup virus during the first trimester of her pregnancy. This dam died from dystocia during the calf's birth.

Despite being carefully tended for 5 months, the calf remained weak and in poor body condition. It was humanely sacrificed in the first week of September 2017. Blood samples were taken from the calf upon arrival in April, in the last week of July, and just before euthanasia were negative for Simbu serogroup viruses by real-time PCR (Fischer *et al.* 2013). While opening the calf's skull, it was apparent that the skull cavity was narrower than usual. The cerebellum and brainstem were intact, the cerebrum was absent and the space contained a copious volume of cerebrospinal fluid (CSF) (Fig. 1). This was defined as hydranencephaly. No other gross pathological changes were seen in the internal organs or the skeleton. Total viral nucleic acids were extracted from internal organs (brain, thymus, spleen, liver and

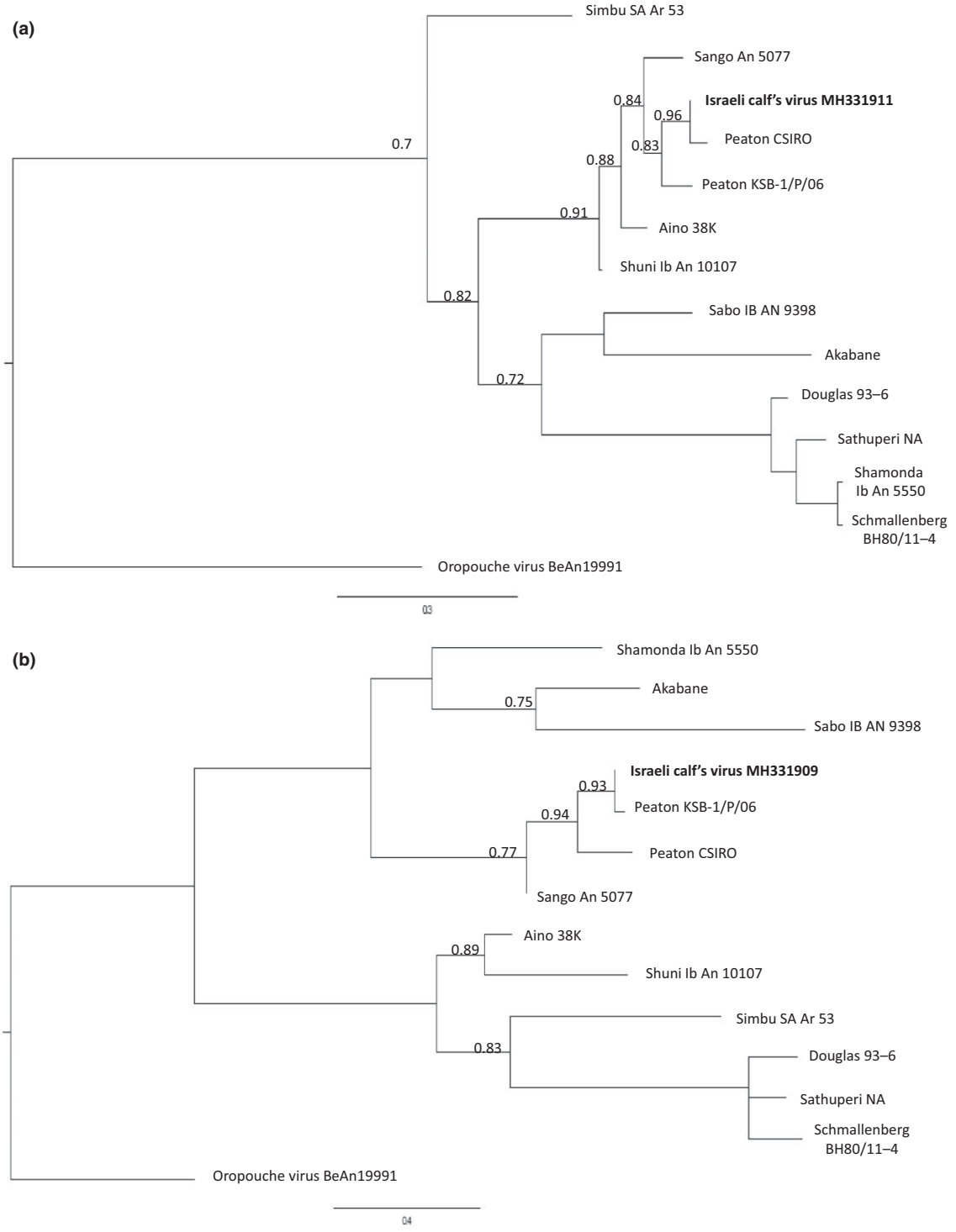

Fig. 2 Rooted maximum-likelihood phylogenetic trees of Simbu serogroup viruses for the S (a), M (b) and L (c) segments, based on a general time-reversible and gamma-distributed rate heterogeneity (GTR_G) model of nucleotide substitution. S, M and L segments of the Israeli calf's virus were compared with the respective sequences from the validated Simbu viruses. Whenever possible, we used Simbu serogroup viruses for which full segment sequences were available. Homologous sequences from Oropouche virus were used as the outgroup. Scale bar indicates estimated nucleotide substitutions. Only bootstrap values greater than 70% are shown.

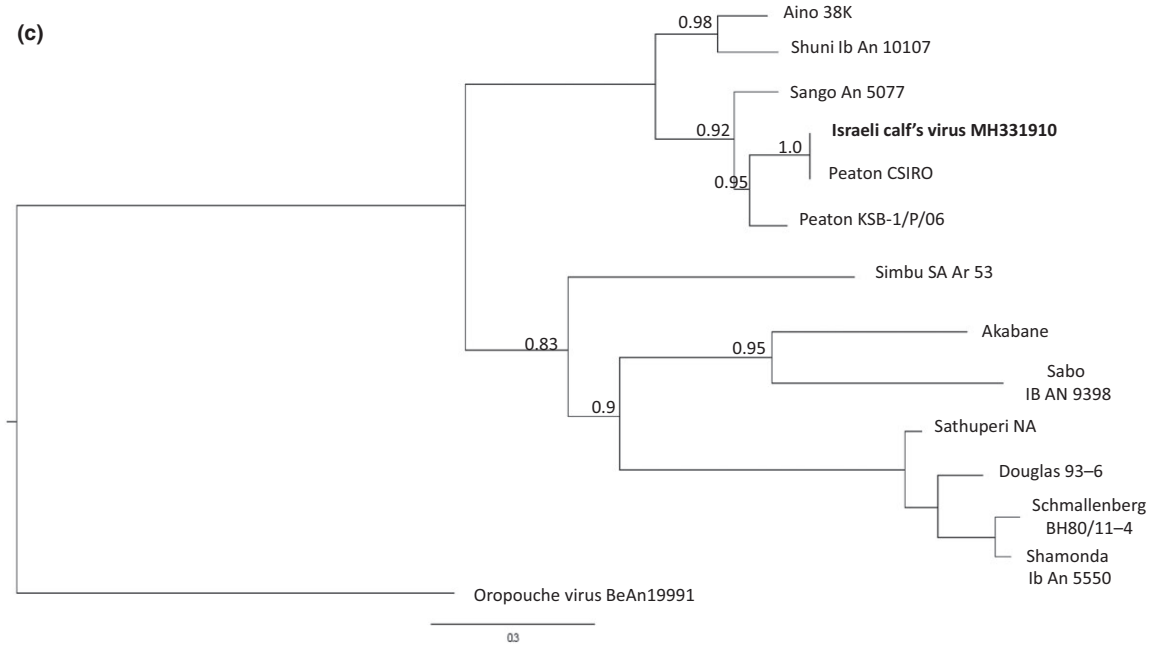

Fig. 2 Continued.

testis) and CSF using the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega). Nested PCR targeting the large segments of Simbu serogroup viruses was performed as follows: Pan Simbu reverse transcription PCR targeting the large RNA segment was conducted according to Fischer *et al.* 2013;. The PCR products served as the template for the nested PCR with primer pairs SimbunestF; 5′-T GATTAGTGAACCAGGGGACTC-3′; and SimbunestR; 5′- GCACTCCATTTTGACATATCAGC-3′ (expected product size ca. 200 bp). Reverse transcription PCR targeting the small and medium RNA segments was conducted according to Hirashima *et al.* 2017 (expected product size ca. 443 bp) and Otani *et al.* 2013 (expected product size ca. 488 bp), respectively. Partial nucleotide sequences of the three segments were amplified only from two samples: CSF and testes. sequence data obtained in this study have been deposited in GenBank with accession no.: MH331909- MH331911. Phylogenetic analysis performed for each segment using maximum likelihood implemented in PhyML (Dereeper *et al.* 2008) showed that the virus sequences detected in the Israeli calf were virtually identical to those of Peaton virus (PEAV) (Fig. 2a–c). The presence of all three segments of this virus also confirms that this is indeed PEAV and not a reassortant containing one PEAV segment. Our attempts to isolate the virus from the CSF were unsuccessful.

This is the first genomic detection of PEAV in a ruminant in Israel. A recent retrospective serosurvey suggested circulation of this virus in Israel between 2008 and 2014 (Brenner *et al.* 2018). Nevertheless, based on serological cross-reactivity, neutralization tests can only differentiate between Simbu serogroup viruses up to a certain level. To specifically identify an individual virus, detection of its genome is necessary. Previous studies on Simbu serogroup viruses have shown that virus isolation from offspring with congenital malformations is difficult and viral RNA is often no longer detectable by real-time PCR in malformed progeny (St George & Standfast 1989; De Regge 2017). Therefore, the effective genomic detection of PEAV from the tissues of a calf with hydranencephaly exhibiting weak calf syndrome has an epidemiological importance, as it shows that

PEAV is present in Israel and affects cattle. Further support for the presence and circulation of PEAV in Israel was provided during the writing of this manuscript: PEAV genomic materials were detected for the first time in two pools of *Culicoides imicola* trapped during summer and autumn of 2017 at two different locations along the Israeli central coastal plain (data not shown). The presence of PEAV presented here might also indicate that other Simbu serogroup viruses such as *Shamonda* and *Sathuperi* may be circulating in Israel as was previously proposed (Zentis *et al.*2012; Brenner *et al.* 2018), and it is only a matter of time until they will be gnomically detected in animals and/or vectors.

Finally, a recent sero-survey has indicated the presence of PEAV in Africa (Mathew *et al.* 2015), but this is the first detection of the virus genetic material outside Australia and Japan (Yanase *et al.* 2010). Thus, our results are of relevance to a potential spread of Simbu serogroup viruses into Europe.

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Conflict of interest

The authors declare they have no conflict of interest.

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 and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

Contributions

Dr. Leibovich and Dr. Brenner preformed all clinical examination and ensure the calf's health. Dr. Edry preformed the post mortem examination and pathology. Dr. Yanase advised on the molecular analysis and assists in writing the manuscript. Dr. Behar is head of KVI'S monitoring system for arboviruses and their vectors, was responsible for the epidemiological investigation and conducted all molecular analysis and insect collections. Dr Behar and Dr. Brenner wrote the manuscript together.

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