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Detection of Lumpy Skin Disease Virus in an Asymptomatic Eland (*Taurotragus oryx*) in Namibia

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ABSTRACT: Nasal swabs collected from 40 wild ruminants in Namibia were analyzed by PCR for the presence of lumpy skin disease virus (LSDV) DNA. One sample from an asymptomatic eland (*Taurotragus oryx*) tested positive, providing the first evidence of the presence of LSDV DNA in an eland.

Lumpy skin disease (LSD) is an economically important and emerging viral disease of cattle, transmitted by blood-feeding vectors and caused by the LSD virus (LSDV). Together with sheeppox virus and goatpox virus, LSDV belongs to the genus *Capripoxvirus*, family *Poxviridae* (Tuppurainen et al. 2017). The virus is present in skin nodules, normal-appearing skin, lymph nodes, liver, kidneys, skeletal muscle, mucous membranes of the mouth and nose, saliva, semen, and nasal discharge of infected animals (Tuppurainen et al. 2017). There are some data available on the susceptibility of wild ruminants to LSDV and their role as potential reservoirs of the virus; LSD and antibodies against LSDV have been reported in several wild bovines and antelopes (Davies 1980; Hedger and Hamblin 1983; Greth et al. 1992; Barnard 1997; Le Goff et al. 2009; Lamien et al. 2011; Fagbo et al. 2014). In Namibia, LSD is endemic in cattle, despite vaccination programs, but no information on the presence of LSD in wildlife is available (Molini et al. 2018).

From August to October 2019, nasal swabs were collected from 40 wild ruminants (Table 1) shot during the hunting season on private farms in the Gobabis district, Omaheke Region, eastern Namibia. One of the neighboring properties had reported an LSD outbreak in cattle in 2017 (Molini et al. 2018). None of the wild ruminants had nodules on their skin. The samples were sent, refrigerated, to the Central Veterinary Laboratory of Windhoek for immediate processing. From the nasal swabs, DNA was extracted using a Maxwell®16 buccal swab LEV DNA purification kit (Promega, Madison, Wisconsin, USA) and eluted in 50 µL, following the manufacturer's instructions. All of the DNA samples were tested using a *Capripoxvirus*-specific RPO30 gene-based PCR (Lamien et al. 2011) and real-time PCR (Bowden et al. 2008). Full-length GPCR, RPO30, and EEV glycoprotein (encoded by open reading frame LSDV126) genes were amplified from the extracted *Capripoxvirus*-positive DNA, as previously described (Le Goff et al. 2009; Lamien et al. 2011; Gelaye et al. 2015; Menasherow et al. 2016); primer sequences are listed in the Supplementary Table. Amplicons were visualized on a 1.5% agarose gel, purified using a Wizard SV gel and PCR clean-up system (Promega), and Sanger sequencing was performed by LGC Genomics (Berlin, Germany). The GPCR, RPO30, and EEV glycoprotein gene sequences were submitted under GenBank accession numbers MW115948, MW115949, and MW115950, respectively, and analyzed as described (see Supplementary Material).

Of the 40 swab samples analyzed, only one sample from an eland (*Taurotragus oryx*) tested positive by conventional PCR and real-time PCR for *Capripoxvirus* DNA. From that single positive sample (i.e., LSDV_Nam_201911_01), the complete

TABLE 1. Species and numbers of wild ruminants shot during the hunting season on private farms in the Gobabis district, Omaheke Region, eastern Namibia, August to October 2019, and sampled by nasal swabbing for detection of lumpy skin disease virus using PCR.

List of wild ruminants	No.
Springbok (*Antidorcas marsupialis*)	5
Blue wildebeest (*Connochaetes taurinus*)	6
Black wildebeest (*Connochaetes gnou*)	2
Hartebeest (*Alcelaphus buselaphus*)	4
Oryx (*Oryx gazelle*)	8
Lechwe (*Kobus leche*)	1
Sable antelope (*Hippotragus niger*)	1
Duiker (*Sylvicapra grimmia*)	2
Blesbok (*Damaliscus pygargus phillipsi*)	3
Impala (*Aepyceros melampus*)	4
Greater kudu (*Tragelaphus strepsiceros*)	2
Eland (*Taurotragus oryx*)	2
Total	40

RPO30, GPCR, and EEV glycoprotein gene sequences were amplified and sequenced.

The RPO30 gene sequence of the sample clustered within the LSDV group and had a greater sequence identity to other LSDV field isolates identified in Africa, the Middle East, and Europe than to LSDV Neethling-like viruses, LSDV Haden 1959, LSDV KS1-derived viruses, and LSDV NI2490 (Fig. 1). Similarly, the sequence of the GPCR from LSDV_Nam_201911_01 clustered within the LSDV group (Supplementary Material Fig. S1) clustering within the subgroup comprising LSDV field isolates and LSDV_KS1-derived viruses. Inspection of the multiple sequence alignment of the GPCR identified a 12-nucleotide deletion (see Supplementary Material Fig. S1) characteristic of LSDV field isolates circulating in Africa, Europe, and in the Middle East but absent in the vaccinal strains LSDV_KS1 and LSDV Neethling-like viruses (Le Goff et al. 2009; Gelaye et al. 2015). Likewise, the EEV glycoprotein gene of LSDV_Nam_201911_01 possessed the 27-nucleotide insertion common to recent LSDV field isolates and LSDV_KS1-derived viruses (see Supplementary Material Fig. S2; Menasherow et al. 2016).

The main agricultural activity in the Gobabis district is extensive beef ranching. Problems related to traditional livestock farming from extended periods of drought, the growth of the wildlife meat industry, and the tourism sector in Namibia have led to the proliferation of private farmlands for game animals. Hence, there are increasing contacts between wild ruminants and cattle that share grazing land and water points, resulting in higher risks of cross-species disease transmission. This should be taken into consideration when developing strategies and guidelines for the management of wildlife-livestock interactions with the aim of limiting disease transmission. Indeed, this will become particularly important for the management of LSD if a wildlife reservoir is ever definitively identified.

An earlier study showed the presence of antibodies to LSDV in eland, but neither the presence of LSDV DNA nor clinical signs of LSD were described (Barnard 1997). Whether eland serve as reservoirs remains unclear. The fact that substantial outbreaks of LSD are not observed in eland or other wildlife in LSD endemic countries suggests that these animals may be more resistant to the clinical disease than domestic cattle. Nevertheless, they might still serve as sources of reinfection of domestic cattle. Sampling wild animals is complicated; extra resources are required, and often diseased animals succumb to either the specific disease or predation before they can be sampled. In the case of LSD, no data on morbidity or mortality in wild ruminants are available. Morbidity in livestock is usually 10% to 20%, but recovery can be slow because of emaciation and secondary infections. Mortality is lower, 1% to 5%. Although only a single sample has been characterized in our study, the findings should encourage further studies through both the active and passive collection of nasal swabs and, where possible, skin nodules and scabs from wild ruminants, to elucidate the susceptibility of these species to LSDV and their role in spreading the disease.

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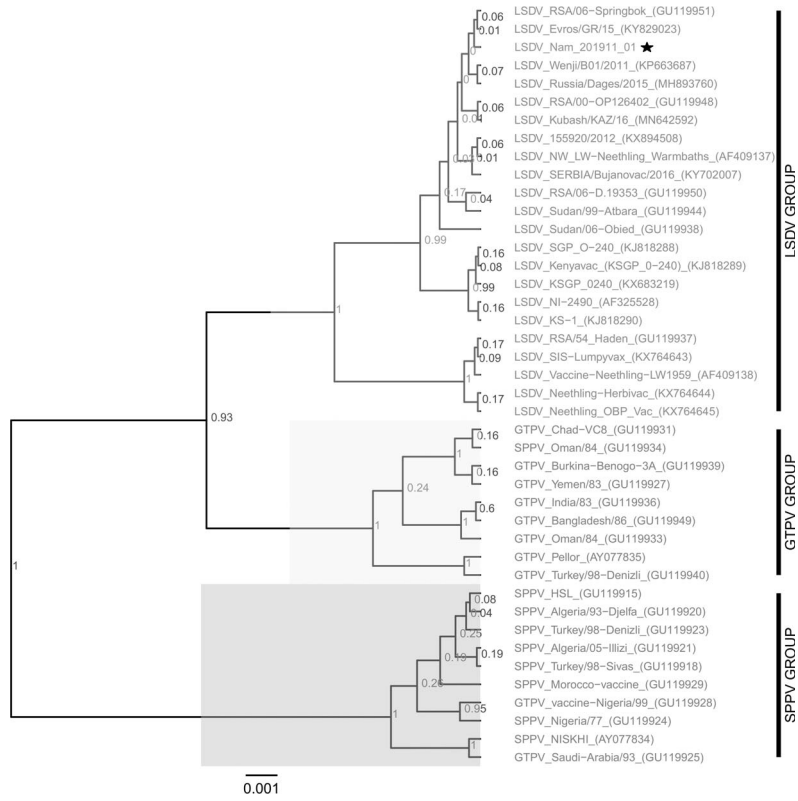

FIGURE 1. Maximum clade-credibility tree based on the complete RPO30 gene sequences of capripoxviruses. The posterior probabilities are plotted as respective node labels. The Namibian LSDV sequence from an eland (*Taurotragus oryx*), one of 40 wild ruminants shot during the hunting season on private farms in the Gobabis district, Omaheke Region, eastern Namibia, during the period August to October 2019, and sampled by nasal swabbing, is highlighted with a star symbol, and reference sequences are represented with their accession numbers. LSDV group=lumpy skin disease virus group; SPPV group=sheep pox virus group; GTPV group=goat pox virus group.

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SUPPLEMENTARY MATERIAL

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