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Schmallenberg Virus in Zoo Ruminants, France and the Netherlands

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DOI: http://dx.doi.org/10.3201/eid2212.150983

To the Editor: Schmallenberg virus (SBV), a new orthobunyavirus of the family *Bunyaviridae*, emerged in August 2011 in northwestern Europe (*1*) and spread to most parts of Europe by *Culicoides* vectors (*2*). Most infections are asymptomatic in adult ruminants, yet fever, milk drop, and diarrhea have been reported (*1*). SBV is responsible for congenital malformations in newborn calves, lambs, and goat kids and has also been associated with abortions and early embryonic losses (*3*). The virus affects domestic livestock, but antibodies to SBV have also been found in free-ranging wild ruminants in several European countries (*3–6*) and in wild and exotic ruminants kept in captivity in the United Kingdom and in Austria (*3–5*). We carried out a study to investigate the exposure to SBV of wild and exotic ruminants born in Europe and kept in 1 zoological park in France and 1 in the Netherlands.

We tested 42 serum samples (from 39 animals) collected between 2011 and 2014 in the Safaripark Beekse Bergen (SPBB, Hilvarenbeek, the Netherlands) and 18 serum samples (from 15 animals) collected between 2013 and 2015 in the Ménagerie du Jardin des Plantes, Muséum National d'Histoire Naturelle (MJP, Paris, France). First, we determined the presence of SBV-specific antibodies in the samples by ELISA (ELISA ID Screen SBV Competition; ID Vet, Grabels, France) and by virus neutralization test (VNT) according to a protocol previously described (*7*). The 2 methods gave identical results except for 5 samples found negative by ELISA and positive by VNT. Thirty (55.6%) of 54 animals were found to be seropositive by VNT, which is regarded as the standard for SBV detection (Table). Antibodies to SBV were found in 11 (73.3%) of 15 animals from MJP and 19 (48.7%) of 39 animals from SPBB. Positive results were found in samples collected every year during 2011–2015; the earliest positive result was found in a sample collected in September 2011 (SPBB).

Several seropositive ruminants from MJP were either born in Paris or transferred to Paris from another park in Europe before 2010, which suggests that they were exposed to SBV in Paris. SBV antibodies were found in 3 consecutive samples collected in October 2011, September 2012, and March 2013 from a sable antelope (*Hippotragus niger niger*) in SPBB but also in 3 consecutive samples collected in October 2013, February 2014, and September 2014 in a bharal (*Pseudois nayaur*) from MJP. These data suggest that SBV antibodies can persist for ≥1 year in these 2 species.

We then performed SBV-specific quantitative reverse transcription PCR targeting the small segment (*8*) of the virus on every sample. One sample from an SBV seronegative

Table. Results of virus neutralization testing for Schmallenberg virus among exotic and wild ruminants from 2 zoological parks in France and the Netherlands, 2011–2015*

Common name (species)	No. positive/no. tested	Year(s) of sampling	Animal ages at sampling		Zoological park
			Seropositive	Seronegative	
African buffalo (*Syncerus caffer caffer)*	1/1	2013	3 y		SPBB
Arkal urial sheep (*Ovis aries arkal)*	1/1	2014	5 y		MJP
Axis deer (*Cervus axis)*	0/2	2011–2014		ND, ND	SPBB
Bharal (*Pseudois nayaur)*	2/4	2013, 2014	1 d, 7 y,† 8 y†	10 d, 2 y	MJP
Blackbuck (*Antilope cervicapra*)	0/6	2014		7 mo, 7 y, 15 y, ND, ND, ND	SPBB
Blue wildebeest (*Connochaetes taurinus taurinus)*	3/5	2011	1 y, 6 y, 13 y	1 y, 13 y	SPBB
Common eland (*Taurotragus oryx*)	0/1	2014		1 y	SPBB
Gaur (*Bos gaurus*)	1/1	2015	3 y		MJP
Gemsbok (*Oryx gazella gazella*)	1/1	2011	17 y		SPBB
Markhor *(Capra falconeri*)	2/3	2014	1 y, 10 y	1 y	MJP
Nyala (*Tragelaphus angasii*)	1/2	2012	5 y	ND	SPBB
Père David's deer (*Elaphurus davidianus*)	1/1	2011	15 y		SPBB
Persian fallow deer *(Dama mesopotamica*)	1/1	2013	8 y		SPBB
Pygmy goat (*Capra aegagrus hircus*)	0/1	2014	2 y		MJP
Red forest duiker (*Cephalophus natalensis*)	0/1	2011, 2012		7 y,† 8 y†	SPBB
Rocky mountain goat (*Oreamnus americanus*)	1/1	2014	17 y		MJP
Sable antelope (*Hippotragus niger niger*)	3/3	2011, 2012, 2013	4 y, 5 y,† 6 y,† 7 y,† 10 y		SPBB
Springbok (*Antidorcas marsupialis*)	3/5	2011, 2014	5 y, 14 y, ND	4 y, 5 y	SPBB
Vietnamese sika deer *(Cervus nippon pseudaxis*)	1/1	2014	12 y		SPBB
Vigogna (*Vicugna vicugna)*	1/1	2013	4 y		MJP
Waterbuck (*Kobus ellipsiprymnus ellipsiprymnus*)	1/4	2011, 2014	7 y	6 mo, 4 y, ND	SPBB
Watusi (*Bos taurus taurus watusi*)	0/1	2011		1 y	SPBB
West Caucasian tur (*Capra caucasica caucasica*)	2/2	2014	10 y, 14 y		MJP
Yak (*Bos grunniens grunniens*)	4/5	2012, 2013, 2014	2 y, 3 y, 11 y, ND	1 y	SPBB (4), MJP (1)

*MJP, Ménagerie du Jardin des Plantes (Muséum National d'Histoire Naturelle, Paris, France); ND, not determined; SPBB, Safaripark Beekse Bergen (Hilvarenbeek, the Netherlands).
† Animals sampled more than once.

blue wildebeest (*Connochaetes taurinus taurinus*) collected in September 2011 in SPBB was positive (quantitation cycle value = 30), whereas the other samples were negative. We also performed several in-house conventional reverse transcription PCR targeting the small, large, and medium segments on the positive sample, which enabled us to retrieve a 2,866-bp partial sequence from the medium segment (deposited in GenBank under accession no. KR828816) and a 1,374-bp partial sequence from the L segment (deposited in GenBank under accession no. KR828815). Genetic analyses based on BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed that the large and medium partial sequences had 100% and 99.79% identity, respectively, with SBV sequences from cows (GenBank accession nos. KM047418 and KP731872, respectively).

Subcutaneous inoculation of serum to adult IFNAR$^{-/-}$ mice, which have been reported to be susceptible to SBV infection (*9*,*10*), did not trigger any clinical sign or seroconversion. No genome could be amplified from their blood.

According to the medical records of SPBB, no clinical signs possibly related to an SBV infection were observed in the ruminants during the period studied. Abortions were reported in MJP in 2 bharals in 2011 and 2012 and in 1 West Caucasian tur (*Capra caucasica caucasica*) in 2013, but no correlation could be drawn between these abortions and the SBV serologic results.

This study demonstrates the circulation of SBV in 18 wild and exotic ruminant species kept in captivity in the Netherlands and in France during 2011–2015. Exposure to the virus may occur even in an urban area (such as central Paris). We report evidence of SBV viremia in a blue wildebeest that was seronegative by ELISA and VNT when the serum was collected. SBV RNA has previously been found in an elk (*6*), but the duration of viremia was not determined. Further investigations are required to determine whether zoo ruminants may play a role in dissemination of SBV.

Acknowledgments

We are grateful to Dylan Duby and Claire Réjaud. We thank Manjula Deville and Marc Chodkiewicz for editing the manuscript.

This study was supported and financed by the Muséum National d'Histoire Naturelle (grant identification: ATM—Collections vivantes 2014 and 2015).

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Fatal Case of West Nile Neuroinvasive Disease in Bulgaria

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DOI: http://dx.doi.org/10.3201/eid2212.151968

To the Editor: West Nile virus (WNV) is a mosquitoborne flavivirus. Approximately 80% of human infections are asymptomatic, 10%–20% are characterized by an acute febrile illness, and <1% by involvement of the central nervous system (West Nile neuroinvasive disease) (*1*). Sporadic human cases and small outbreaks of West Nile fever were reported in Europe until the mid-1990s (*2*), when the first large outbreak occurred in Romania in 1996 (*3*).

Since then, and especially in recent years, sporadic human cases and outbreaks have been reported in other countries in Europe and neighboring countries on the Balkan Peninsula (*2*). A large outbreak of WNV lineage 2 infection occurred in Greece in 2010 (*4*). Outbreaks have also been reported in other countries in Europe, which showed spread of WNV lineage 2 (*5*–*8*). Some probable cases of West Nile fever were reported to the Bulgarian Ministry of Health on the basic of serologic test results.

We report a case of fatal West Nile neuroinvasive disease in a man in Bulgaria. This case was confirmed by detection of specific antibodies against WNV and sequencing of the full virus genome.

A 69-year-old man was admitted to the Emergency Center, Military Medical Academy (Sofia, Bulgaria), on August 27, 2015, because of fever, headache, hand tremor, muscle weakness and disability of lower extremities, nausea, and vomiting. These signs and symptoms developed 3 days before hospitalization. The patient reported being bitten by insects through the summer. He also had concomitant cardiovascular disease. In the 24-hour period after hospitalization, a consciousness disorder and deterioration of the extremities' weakness developed, and the patient had a Glasgow come score ≤8.

The patient was transferred to Department of Intensive Care. Neurologic examination showed neck stiffness, positive bilateral symptoms of Kernig and Brudzinski, right facial paralysis, and areflexia of the lower extremities. The patient underwent intubation, and despite complex medical therapy, a cardiopulmonary disorder developed, and he died 14 days after admission.

Laboratory test results at admission were within reference ranges. Lumbar puncture was performed, and cerebrospinal fluid (CSF) testing showed a clear color, leukocytes 39 $\times 10^6$ cells/L (reference range 0–5 $\times 10^6$ cells/L), polymorphonucleocytes 2% (0%–6%), lymphocytes 93%