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Presence of Antibodies to Schmallenberg Virus in a Dog in Sweden

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Schmallenberg virus (SBV) is an orthobunyavirus first discovered in November 2011 in German cattle (1). Since the first descriptions in Germany, SBV has spread all over the European continent, including Sweden (2, 3). SBV is an arthropod-borne (arbo)virus primarily spread by biting midges (*Culicoides* spp.) (2, 3), and SBV in midges has been detected in Belgium (4), Denmark (5), the Netherlands (6), and Poland (7). So far, SBV infection has been detected only in domestic and wild ruminants (2), and there is no evidence of zoonotic transmission (8, 9). To our knowledge, no investigations regarding SBV infection in other nonruminant species have been described. Here, we report the first serological evidence of SBV infection in a nonruminant species, namely, a dog.

A total of 100 serum samples that were derived from 86 female dogs that were submitted for routine analysis to the Swedish University of Agricultural Sciences were examined blindly for the presence of SBV antibodies. An initial screening for SBV antibodies was performed using a commercial competitive enzyme-linked immunosorbent assay (cELISA) according to the manufacturer's instructions (ID Screen Schmallenberg virus competition multi-species ELISA kit; IDvet, Montpellier, France). In the initial screening, two serum samples were found to be positive (23 and 20% competition). A sample was considered positive if the calculated competition percentage was ≤40% (IDvet). The samples positive in the initial screening were retested using the same SBV-cELISA with similar results (26 and 24% competition).

To confirm the presence of SBV-specific antibodies in the SBV-cELISA-positive serum samples, a serum neutralization test (SNT) was performed at the National Veterinary Institute (Statens Veterinärmedicinska Anstalt [SVA]) (Uppsala, Sweden). The virus isolate used was BH80/11-4, kindly provided by the Friedrich-Loeffler Institut in Germany and passaged in BHK-21 cells cultivated in Eagle's minimal essential medium (EMEM) (SVA, Sweden) with 2% fetal calf serum (FCS). Before the sera were analyzed, they were heated for 30 min at 56°C. The SNT was performed in a 96-well microtiter plate in which sera were 2-fold diluted in EMEM in 50-μl volumes in duplicate starting from 1:2 up to 1:256. Between 30 and 300 50% tissue culture infective doses ($TCID_{50}$) of virus in a volume of 50 μl per well was then added to each well on the microtiter plate with the exception of the first row with 1:2 serum dilutions where only medium was added (serum controls). After preincubation at 37°C for 1 h, approximately 20,000 cells in a volume of 50 μl in EMEM supplemented with 20% FCS were added to each well. The plates were then incubated for 3 or 4 days at 37°C under 5% CO_2. After incubation, the plates were examined by using a light microscope for the presence of virus-specific cytopathogenic effects (CPE). The neutralizing titer of a serum sample was determined as the highest dilution in which the cell monolayer was intact. In each run of the SNT, positive- and negative-control sera are included.

Neutralizing antibodies were found in the two samples (titers of 64 and 128), confirming the presence of SBV-specific antibodies. When we broke the code list to the serum samples, we discovered that these two serum samples came from the same 8-year-old female dog, living in central Sweden, a region where SBV-specific antibodies have been found in ruminants. The two samples were taken in January 2013 only 4 days apart, and the titer was slightly lower at the first sampling.

Our findings indicate that nonruminant species, like dogs, could be infected by and induce an antibody response to SBV. Whether the infection in this dog led to any clinical signs or whether it was a transient, recent, or subclinical infection is not known. Since SBV is an arbovirus, the likeliest route of infection is by biting midges, which are the main vectors. In Sweden, the vector season is considered to be from May to October, and the dog lived in an area where SBV has been found in ruminants. The only previous confirmed orthobunyavirus infection in a dog, leading to clinical signs, is to our knowledge a case of meningoencephalomyelitis in the United States due to LaCrosse virus (LACV) infection (10). LACV belongs to the California serogroup, whereas SBV belongs to the Simbu serogroup; thus, no cross-reactivity should take place. Antibodies to two viruses belonging to the Simbu serogroup (Peaton virus and Aino virus) have not been found in dogs (11, 12), and to our knowledge, no investigations for the most closely related viruses to SBV (Shamonda virus, Sathuperi virus, and Douglas virus) have been performed in dogs. None of these viruses, except for SBV, have been detected in Sweden.

Our observation of antibodies to SBV in a dog could have important impacts on the transmission patterns and epidemiology of disease followed by SBV infection and contribute to the overall knowledge of the nature of SBV. There are no national records of reproductive performance in Swedish dogs; hence, it is still unknown if there has been a change in the reproductive performance of the Swedish dog population since the introduction of SBV to Sweden. Whether SBV infection in dogs leads to reproductive failure and/or malformations of puppies, similar to what is seen in ruminants, warrants further investigations.

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