Novel Bunyavirus in Domestic and Captive Farmed Animals, Minnesota, USA

To the Editor: Xing et al. (*1*) conclude that evidence of infection with a severe fever with thrombocytopenia syndrome (SFTS)–like virus or Heartland-like virus (HRTV) was found in many captive large mammals from much of Minnesota, raising the specter of widespread distribution of a novel pathogen. Although it is likely that HRTV can be found beyond the areas in northwestern Missouri, where it was discovered (*2*), we contend that this conclusion is not substantiated by the data presented by Xing et al., which were generated by an assay that was developed to diagnose SFTS virus infections in China (*1*,*3*). The study used an ELISA developed for an SFTS virus recombinant nucleocapsid protein that detects SFTS-reactive antibodies (*3*). The conclusions reached by Xing et al. are based on the assumption that the SFTS assay developed in China will cross-react with HRTV antibodies (*1*). This assumption remains unsupported because the SFTS assay has not been evaluated for cross-reaction with antibodies to other non-SFTS members of the genus *Phlebovirus* (*1*,*3*). In addition, it is well recognized that serologic tests, like the ELISA, are often group reactive (*4*), requiring neutralization tests to confirm antibody presence and provide specificity. Alternative explanations include the possibility that positive results from testing by Xing et al. may have been caused by cross-reaction with antibodies directed against other known tick-associated phleboviruses endemic to North America, such as Lone Star virus (*5*), which is not known to be pathogenic. In the absence of confirmatory data generated by an independent method, the report by Xing et al. (*1*) should be considered speculative. Reports suggesting substantial expansion in the geographic range of a pathogenic organism should be based on rigorously validated laboratory methods.

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In Response: We welcome the critiques of Nasci et al. (*1*), who may have misinterpreted the point of our Dispatch. Regarding identification of Heartland virus (HLV) in farm animals, in our article (*2*), we stated that "the viruses detected in this region are most likely HLV or close relatives of HLV," which indicates that the exact identification of the viruses in the animals in Minnesota will not be confirmed until the viruses are isolated and/or the genomic sequence data are available.

The underlying data were obtained with an ELISA specific to severe fever with thrombocytopenia syndrome virus (SFTSV). The conclusion was based on our knowledge, at the time our manuscript was submitted, that in North America no other known phleboviruses of this expanded Uukuniemi group that contains SFTSV and HLV were reported to be cross-reactive with SFTSV. When tested by using our reagent, SFTSV was not cross-reactive with Rift Valley fever virus. Related phleboviruses of this group (e.g., Bhanja, Palma, Forecariah, and Kismayo viruses) have not been reported in North America (*3*). Phleboviruses of this group, such as Murre virus and RML-105355 virus, and Sunday Canyon virus, were isolated in Alaska and Texas, respectively, but are not cross-reactive with SFTSV (*4*).

Other bunyaviruses in North America (e.g., Cache Valley virus and California serogroup viruses) are distantly related and have ≈11% amino acid sequence homology to SFTSV. The recently characterized Lone Star virus appears to be the closest relative to SFTSV and HLV and may cross-react with SFTSV and HLV, as also suggested by Nasci et al., but this virus is apparently known only from 1 isolate obtained in 1967 (*5*). These data suggest that SFTSV is not serologically cross-reactive with the known Uukuniemi group viruses that are currently being transmitted in North America. Our report shows that tickborne

phleboviruses, which are closely related to SFTSV and HLV, may be more generally distributed in the midwestern United States and emphasizes the need to substantiate our serologic evidence with virus isolation and genomic characterization, which are underway.

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Infectious Schmallenberg Virus from Bovine Semen, Germany

To the Editor: The teratogenic Schmallenberg virus (SBV) (genus *Orthobunyavirus*) was detected in bovine semen in a recent German field study (*1*). Vector-borne transmission by *Culicoides* spp. biting midges is most common (*2*), but venereal transmission of SBV might contribute to the spread of this virus to previously unaffected regions. We investigated the infectivity of SBV RNA–positive semen by experimental subcutaneous injection of cattle and interferon α/β receptor–deficient ($IFNAR^{-/-}$) mice (*3*).

Commercially produced semen straws with egg yolk–based diluent were used for the injection of 6- to 9-month-old heifers. The straws originated from 6 semen batches (quantification cycle [C_q] values 26.4–36.4) collected from 6 bulls (designated A–C and E–G) during August and September 2012 (*1*). To increase the probability of SBV infection of injected cattle, 5 straws of semen (≈220 μL each) from 1 batch from an individual bull were pooled and diluted in minimal essential medium with antibiotics to 4 mL. Six cattle (C1–6) were subcutaneously inoculated, each with a pool from 1 of the 6 bulls. To investigate the infectivity of a single insemination dose (1 straw), 5 cattle (C7–C11) were subcutaneously injected with single straws from bull F that had been confirmed to contain infectious SBV. Serum samples were obtained on several days (Figure), and clinical signs and rectal body temperatures for the injected cattle were monitored daily.

In addition, 20 SBV RNA–positive semen batches (C_q 25.9 to 36.5) collected from 11 bulls (A–K) during August–November 2012 (*1*) were subcutaneously injected into 40 $IFNAR^{-/-}$ mice (4–6 weeks old). For each batch, 2 mice were each injected with half of a semen straw (80–120 μL). All mice were monitored clinically and weighed daily. Samples of serum, liver, and spleen were harvested immediately after euthanasia at 22 days postinjection (dpi).

All serum samples and organ homogenates were tested for SBV RNA by using small segment–specific quantitative reverse transcription PCR (*4*). Serum samples were tested for SBV-specific antibodies by using the ID Screen Schmallenberg Virus Competition ELISA (IDvet, Montpellier, France), according to the manufacturer's instructions; selected serum samples were also tested by neutralization test against an original SBV isolate from Germany, as described (*5*).

SBV infection was confirmed in 5 of 11 injected cattle: C3, C5, and C9–C11. SBV RNA (C_q 25.0–29.3) was first detected in serum at 3 to 6 dpi and persisted for 2–4 days. Seroconversion occurred at 8–12 dpi (Figure). None of the SBV-infected animals showed obvious clinical signs or fever; this finding is in accordance with reports of subclinical SBV infection in adult cattle (*5*–*7*). Samples from the other 6 cattle and all $IFNAR^{-/-}$ mice had negative results (data not shown).

The 2 infectious semen batches contained moderate (C_q 26.4) or low (C_q 34.2) viral loads of SBV RNA, indicating that a high sensitivity is required for reliable SBV RNA detection in semen samples (*1*). The onset of SBV infection in the 3 animals injected with single semen straws ranged from 3 to 5 dpi, and not every straw was infectious, although biologic and technical replicates of straws from 1 semen batch showed similar PCR results (data not shown) (*1*). Possible explanations for differences in the infectivity of individual straws are that the viral RNA load of an SBV-containing straw does not necessarily correlate with infectivity or that the infectivity of 1 straw is lower than the minimal cattle