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 [Cq] 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 required for reliable SBV RNA detection in semen samples (1). The on-set 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

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LETTERS

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

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DOI: http://dx.doi.org/10.3201/eid2002.131790

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 20, No. 2, February 2014
infectious dose for SBV. Cattle might be more susceptible than IFNAR−/− mice to infection, particularly when SBV titers are low or borderline (7, 8). Therefore, we cannot exclude the possibility that the semen batches tested only in IFNAR−/− mice might be infectious for cattle or that semen samples with higher SBV titers might be infectious in the mice.

We used subcutaneous injection of SBV RNA–positive semen to demonstrate infectivity because this transmission route has a high sensitivity for proving infectivity of SBV-containing samples (7). However, the possibility of intrauterine SBV infection of dams is unknown. Oro-nasal inoculation of 2 calves did not result in SBV infection of the animals (5), which suggests that mucosal infection with SBV-containing semen is unlikely. In contrast, viremia was detected in most cows that were artificially inseminated and simultaneously inoculated in the uterus with cell culture–passaged Akabane virus, a teratogenic orthobunyavirus closely related to SBV (9). Intrauterine lesions caused by insemination or breeding might therefore increase the risk for SBV infection.

In conclusion, we demonstrated that SBV RNA–positive bovine semen could contain infectious SBV. However, the actual risk for transmission of SBV by insemination of dams with SBV-containing semen remains to be evaluated. Although SBV infection of the developing embryo is unlikely, venereal transmission would lead at worst to viremia of the dam, facilitating vector transmission. To prevent venereal SBV transmission, sensitive PCR testing of semen batches from SBV-infected bulls is the method of choice (1,10).

Acknowledgments

We thank Patrick Zitzow and Susanne Zahnow for excellent technical assistance and the staff of the Biosafety Level 3 facility at Friedrich-Loeffler-Institut for their dedicated animal care.

The animal experiments were approved by an independent ethical committee (LALLF 7221.3-1.1-004/12 and LALLF M-V TSD/7221.3-2.5-005/12). This study was supported by the German Federal Ministry of Food, Agriculture and Consumer Protection and the European Union, as outlined in Council Decision 2012/349/EU concerning a financial contribution by the Union for studies of Schmallenberg virus.

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NDM-1–producing Strains, Family Enterobacteriaceae, in Hospital, Beijing, China

To the Editor: The prevalence of New Delhi metallo-b-lactamase-1 (NDM-1)–producing strains (family Enterobacteriaceae) in China remains unclear. Recently, to clarify the prevalence of blaNDM-1 in Enterobacteriaceae strains, we carried out retrospective surveillance for blaNDM-1 among carbapenem-resistant enterobacterial strains isolated from patients at the Chinese PLA General Hospital in Beijing. This tertiary teaching hospital has 4,000 beds and 12,000 daily outpatient visits. More than 50% of patients admitted to the hospital are from areas outside Beijing. During January 2009–June 2013, a total of 8,586 enterobacterial isolates were obtained from routine clinical samples that had been passively sent to the microbiology department. Of these, 242 (2.8%) strains exhibited resistance to carbapenems.

In this study, we used PCR amplification to screen the carbapenem-resistant strains for the blaNDM-1 gene and other common resistance determinants. The MICs of various antimicrobial drugs were measured by E-test (AB bioMérieux, Solna, Sweden). S1 nuclease pulsed-field gel electrophoresis and Southern blot analysis were used to identify the sizes of blaNDM-1-carrying plasmids. The incompatibility (Inc) groups of the plasmids were detected by several multiplex and simplex PCRs. Multilocus sequence typing (MLST) was carried out for Klebsiella pneumoniae and Escherichia coli isolates, according to protocols provided on MLST websites (www.pasteur.fr/recherche/genopole/PP8/mlst/Kpneumoniae.html and http://mlst.ucc.ie/mlst/dbs/Ecoli). The transferability of plasmids was identified by conjugation experiments.

Five blaNDM-1–positive enterobacterial isolates of the following species were identified: E. coli (1 isolate in October 2010), K. pneumoniae (1 isolate in August 2012), Providencia rettgeri (1 isolate in October 2012), Enterobacter cloacae (1 isolate in November 2012), and Raoultella ornithinolytica (1 isolate in March 2013). According to the 2013 Clinical and Laboratory Standards Institute performance standard M100-S23 (www.clsi.org/), the NDM-1-producing K. pneumoniae (IR5047) isolate exhibited low-level resistance to imipenem and meropenem, whereas other isolates showed high-level resistance to carbapenems. Only E. coli and Providencia rettgeri, which carry 16S rRNA methylase genes, exhibited high-level resistance to amikacin (Table). S1 nuclease pulsed-field gel electrophoresis and Southern blot analysis showed that the blaNDM-1 gene was located on plasmids of various sizes belonging to different Inc groups. The K. pneumoniae isolate was defined as a novel ST1240 with the allelic profile 2–1–1–1–3–24, and the E. coli isolate was identified as ST167.

In China, various blaNDM-1–carrying strains of the Enterobacteriaceae family have been sporadically identified, including K. pneumoniae, K. oxytoca, Escherichia coli, Enterobacter cloacae, Enterobacter aerogenes, and Citrobacter freundii (1–4). We identified a P. rettgeri isolate and an R. ornithinolytica isolate that produced NDM-1. The blaNDM-1–positive P. rettgeri isolates have also been identified in Pakistan, India, Canada, and Mexico, whereas the NDM-1–producing R. ornithinolytica strain has only been detected in India (5–9). In this study, all 5 NDM-1–producing strains were isolated only once, and no dissemination of NDM-1–producing strains of Enterobacteriaceae has been found. Two strains (K. pneumoniae and Enterobacter cloacae) were isolated within 48 hours of the patient’s hospital admission.