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
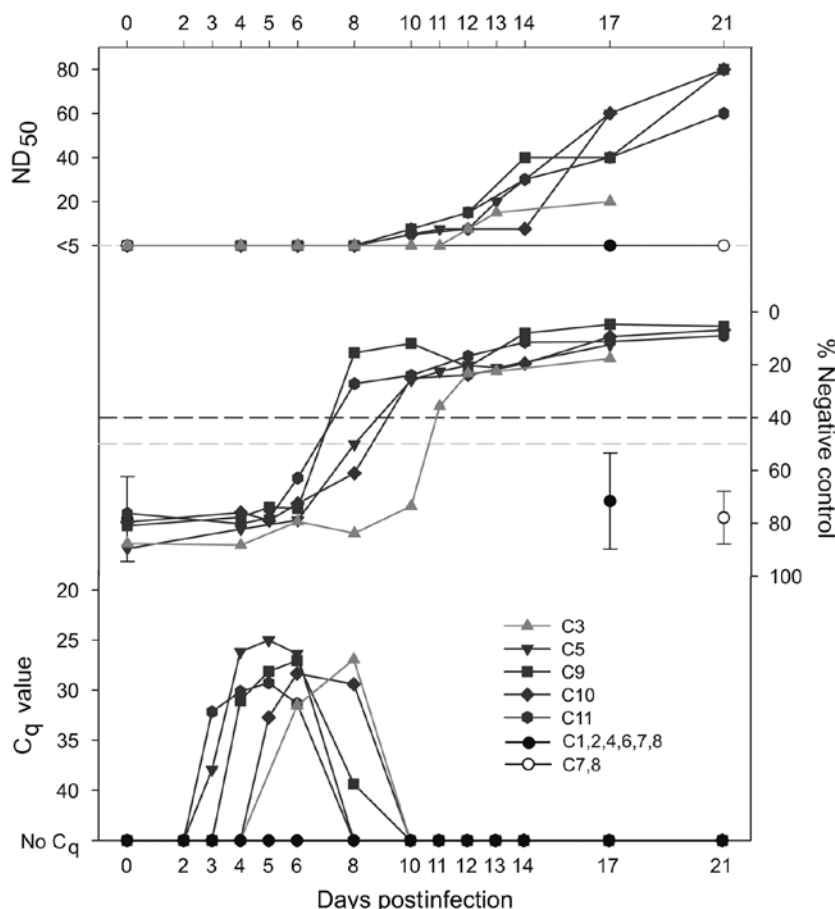
Figure. Detection of Schmallenberg virus (SBV) RNA and antibodies in serum of 5 of 11 cattle (C) injected with SBV RNA–positive bovine semen (quantification cycle [C_q] values 26.4–36.4). Infectivity was measured by using reverse transcription PCR (C_q), competitive ELISA (% negative control), and serum neutralization test (ND_{50}, serum dilution that caused virus neutralization in 50% of the replicates). RNAemia and seroconversion occurred in C3, injected with pooled semen from bull C (C_q 34.2), and in C5 and C9–C11, injected with pooled or single semen straws from bull F (C_q 26.4). The onset of infection in cattle injected with semen from bull F varied between 3 and 5 days postinfection. Black lines indicate virologic and serologic results for cattle that remained uninfected. Dotted lines indicate positive (top) and negative (bottom) cutoff values; whiskers indicate SD of the mean.

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. Oronasal inoculation of 2 calves did not result in SBV infection of the animals (5), which suggests that mucosal in utero 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).

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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 bla_{NDM-1} in *Enterobacteriaceae* strains, we carried out retrospective surveillance for bla_{NDM-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 bla_{NDM-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 bla_{NDM-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/PF8/mlst/Kpneumoniae.html and http://mlst.ucc.ie/mlst/dbs/Ecoli). The transferability of plasmids was identified by conjugation experiments.

Five bla_{NDM-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 bla_{NDM-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 2–1-1–1-1–3-24, and the *E. coli* isolate was identified as ST167.

In China, various bla_{NDM-1}-carrying strains of the *Enterobacteriaceae* 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 bla_{NDM-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,