transfusion-associated cases were reported throughout the year, underscoring the need for constant awareness. The 2 cases of probable congenital infection highlight the need to consider Babesia infection for newborns who have compatible clinical manifestations, especially if the mother had risk factors for infection.

Prompt identification of babesiosis is essential to prevent disease transmission from infected blood donors to recipients. Although we modified New Jersey surveillance to include transfusion as a risk factor, collaboration with stakeholders (including blood centers) will further facilitate case detection and confirmation and identification of infected donors. Including babesiosis on the list of nationally notifiable diseases will improve national disease reporting and clarify the geographic distribution and incidence of tickborne and possible transfusion-associated cases. With increasing public awareness and screening, public health professionals and stakeholders might consider dedicating public health resources for babesiosis surveillance.

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Antibodies against West Nile and Shuni Viruses in Veterinarians, South Africa

To the Editor: Many arboviruses are zoonotic; humans acquire infection from the bites of arthropod vectors or through exposure to the tissues and body fluids of infected animals. West Nile virus (WNV), a widely endemic zoonotic agent in South Africa, occurs wherever the principal vector (Culex univittatus mosquitoes) and avian hosts are present (1). Serosurveys based on hemagglutination inhibition and neutralization assays conducted during 1950–1970 indicated that 17%–20% of long-term rural residents in the Karoo, 4%–8% in the Highveld, and 1%–3% in the Natal and the Eastern Cape areas had antibodies against WNV (1). Most human infections tend to be sporadic and are characterized by mild febrile illness (2); however, severe disease has been documented (3). WNV has caused severe neurologic disease of horses in South Africa (4), and zoonotic transmission was recorded in a veterinary student who performed a necropsy on an infected horse (5).

Shuni virus (SHUV) (genus Orthobunyavirus, family Bunyaviridae) was first isolated in Nigeria in 1966 during surveys of livestock, Culicoides midges, and mosquitoes. SHUV also once was isolated from a febrile child (6,7). SHUV recently was identified as a previously undetected cause of neurologic disease in horses in southern Africa (8) and is thus of interest in comparison to WNV.

To determine the potential for human infections, we tested veterinarians as a high-risk group for evidence of infection with these 2 viruses. Veterinarians with regular exposure to horses, livestock, or wildlife—and thus to vectors because of an

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outdoor lifestyle—were invited to donate blood samples at specialist veterinary conferences in South Africa in 2011 and 2012.

The Kunjin MRM61C strain of WNV (9) and SHUV isolate SAE 18/09 (8) were cultured and harvested when the cytopathic effect (CPE) reached 80%. Stock virus was titrated in 100-mL volumes in 6 replicate wells per serial 10-fold dilution (10⁻¹ to 10⁻⁹) in Leibowitz medium with 5% fetal calf serum (Invitrogen, Carlsbad, CA, USA), with 100 mL of medium added in place of test serum dilution, and 25 mL of Vero cells (8 × 10⁵ cells/mL) added per well. Plates were incubated at 37°C; CPE was monitored; and 50% tissue culture infectious dose per milliliter endpoints were calculated. For neutralization tests, serum were inactivated at 56°C for 30 min; duplicate 100-mL volumes of doubling dilutions were prepared in Leibowitz medium (1:10–1:640) and incubated with equal volumes of medium containing a calculated 100 50% tissue culture infectious dose virus at 37°C for 45 min, and 25 mL of Vero cells (8 × 10⁵ cells/mL) were added per well. Medium was added in place of virus to replicate test serum controls at a 1:10 dilution to monitor for toxicity of the serum, and the virus used in the test was back titrated. Tests were monitored for 10 days. Neutralization titers were expressed as the reciprocal of the serum dilution that inhibited ≥75% of CPE in both replicates, and only titers ≥20 were recorded as positive for virus antibodies.

Serum samples were received from 123 veterinarians in South Africa and 4 from neighboring countries. Ten (7.9%) serum samples tested positive for antibody to WNV and 5 (3.9%) for antibody to SHUV; all positive serum samples (titers 20–80) were from South African veterinarians. Prevalence of WNV antibody in men (5/81 [6.2%]) and women (5/46 [10.9%]) did not differ significantly. The veterinarians ranged in age from 23 to 71 years and had practiced an average of 22 years; the prevalence of WNV antibody was similar in age groups 23–50 years (6/74 [8.1%]) and 51–71 years (4/53 [7.5%]).

Most veterinarians came from periurban practices in Gauteng (51/123) and Western Cape Provinces (18/123); the comparatively small numbers of samples from elsewhere preclude valid comparisons with the historical surveys of rural residents. However, indications that veterinarians might be at increased risk for infection in some areas included a 23.1% (3/13) prevalence of WNV antibody in KwaZulu-Natal veterinarians and a similar prevalence of antibody in much smaller sample groups in the Free State and Northern Cape Provinces. In Gauteng, where most horses reside, 6% of veterinarians tested positive for WNV, which reflects the prevalence described for the Highveld region in the 1970s.

Four of the 5 veterinarians positive for SHUV antibody were men; 2 were in the 23–50-year age group, and 3 were in the 50–71-year age group. Of the veterinarians who tested positive, 3 were identified in Gauteng (3/51 [5.9%]) and 1 each in the Eastern Cape (1/8) and Limpopo (1/8) Provinces. No clear histories of disease compatible with the infections could be elicited from any of the veterinarians whose samples contained antibodies. Nevertheless, the 2 viruses, and related arboviruses, tended to be overlooked as animal and human pathogens in southern Africa until recently, and greater awareness is needed of their potential as zoonotic agents. Investigations of neurologic illness in humans identified several WNV cases that had been overlooked in hospitals in Gauteng (10). Similar investigations of febrile and neurologic illness in humans might shed light on the possible clinical significance of SHUV infection in humans.

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isolated in 2012, we have been reported (2,3). Single-step PCR amplification was performed by using genus-specific primers for the 17-kDa lipoprotein and the citrate synthase gene (gltA), as described previously, to obtain amplicons of 434 bp and 380–385 bp (4). PCR was positive for R. typhi, and 100 mg of oral doxycycline 2 times per day for 7 days was prescribed; the rash cleared.

We subjected 5 mL of blood to centrifugation for 1 hour at 1,000 rpm and then stored the plasma at −80°C. Blood samples from other patients were used as controls. A total of 50,000 Vero cells were grown in 8 central wells of a 24-well cell culture cluster (Corning Incorporated, Corn- ing, NY, USA) with minimal essential medium (MEM; Biowest, Nuaille, France) supplemented with 10% fetal bovine serum (Biowest, Mo, USA) and in- cubated at 37°C with 5% CO₂ for 48 hours to obtain 95% confluence. We then thawed 700 mL of the plasma in a 37°C water bath. The MEM was discarded, and the wells were refilled with 250 mL of a mixture of the plasma and fresh medium at a 1:3 ratio. The plaque was covered with paraffin and centrifuged at 700 g for 60 minutes at 22°C. The supernatant was discarded and replaced with 1 mL of MEM supplemented with 5% fetal bovine serum, 100 U penicillin, 100 µg streptomycin, and 250 ng amphotericin B (Sigma Aldrich, St. Louis, MO, USA) and incubated at 33°C with 5% CO₂.

On day 3 after sample inoculation, the antimicrobial drug–containing medium was removed and replaced with MEM without antimicrobial drug and supplemented with 5% fetal calf serum (HyClone Laboratories, Inc., South Logan, UT, USA). Medium was changed every 3 days until day 15. A cell sample from each well was tested for infection at days 9 and 15 by using Gimenez stain and PCR with 17 kDa and gltA primers.

Gimenez staining on day 15 yielded numerous red-stained bacteria in the cytoplasm of Vero cells in the 8 wells used. A single scraping of the cells from the positive wells was inoculated onto confluent layers of Vero cells, which enabled establishment of the isolate.

Three PCR amplicons of the 17kDa- and gltA-specific primers (4–6) from positive wells were fully sequenced. After removing primer sequences, we compared amplicon sequences by conducting a gapped BLAST 2.0 (http://blast.st-va.ncbi.nlm.nih.gov/Blast.cgi) search of the GenBank database; the 17-kDa (accession no. JX198507) and gltA (accession no. KC469611) gene fragment sequences showed 100% identity with R. typhi strain Wilmington (accession no. AE017197.1).

Murine typhus has been reemerging in southeastern Mexico for the past 6 years (3,7). Active epidemiologic surveillance led to early detection of human cases and opportune treatment, thereby decreasing the rate of severe illness. However, the prevalent social and cultural conditions in small villages, with close contact with domestic, peridomestic, and wild animals, facilitate the transmission of this fleaborne rickettsiosis; human infections, such as the case presented here, still occur.

We replaced shell vials with cell culture plates and isolated rickettsiae from a biological sample from a patient with acute murine typhus. The method is as simple as the shell vial centrifugation technique and is highly sensitive and easy to perform, making it an excellent choice for rickettsiae isolation when shell vials are not available.

In the United States, isolation of R. typhi from a human was last reported >50 years ago (8). The case reported here reinforces the need to extend surveillance to small towns and villages.