cattle and sheep, and absence of a live or inactivated vaccine against this serotype could lead to its reemergence and to severe economic losses. In the absence of an appropriate vaccine and control strategy, the virus could potentially spread to neighboring countries and pose an additional risk to Europe.

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Rabies Virus RNA in Naturally Infected Vampire Bats, Northeastern Brazil

To the Editor: Rabies is a major zoonotic disease and causes ≥55,000 human deaths annually worldwide (*1*). The predominant infection route for humans is by canids but zoonotic transmission from bats has been reported (*2*,*3*). Of >1,000 bat species, the only 3 species that feed on blood (*Desmodus rotundus*, *Diphylla ecaudata*, and *Diaemus youngi*) are found exclusively in Latin America (*4*). Rabies outbreaks caused by *D. rotundus* vampire bats have resulted in human deaths in Latin America and estimated livestock losses of $6 million annually (*4*).

To study rabies virus (RABV) prevalence and transmission in bat populations, we sampled 199 suborder Microchiroptera bats (mostly from families Phyllostomidae [86.4%] and Molossidae [11.1%]) in Bahia, northeastern Brazil, during 2008–2010. Areas where vampire bat activity or rabid livestock were reported were visited by members of the Bahia State Agency for Agriculture and Livestock Defence to identify bat roosts. All sampling was approved by the Brazilian Institute of the Environment and Natural Renewable Resources.

Bats were caught at roosts by using mist nets, killed with ether, and transported on ice to our laboratory. In accordance with rabies control program policies in Brazil, only vampire bats that were physically impaired (e.g., poor flight ability) or found dead could be sampled.

Thirty milligrams of brain or medulla oblongata per animal was homogenized and purified by using the RNEasy Kit (QIAGEN, Hilden, Germany). RNA was detected by using nested reverse transcription–PCR (RT-PCR) specific for viral nucleoprotein gene (*5*). RABV RNA was detected in 8 (27.6%) of 29 *D. rotundus* bats.

The 8 bats originated from 6 of 9 sampled roosts located in an area of ≈7,200 km^2. Nucleotide sequencing of PCR amplicons confirmed close phylogenetic relationships with vampire bat RABV (GenBank accession nos. HM171529–HM171536), which is consistent with reported absence of other *Lyssavirus* species in the Americas (*4*). Conventional RABV diagnostic tests (direct immunofluorescent test and infection of suckling mice) confirmed presence of RABV in central nervous system specimens from all 8 bats.

Viruses were quantified by using strain-specific real-time RT-PCR with the OneStep RT-PCR Kit (QIAGEN) and primers BRDesrot-Fwd, 5′-CGTACTGATGTGGAAGGGAATTG-3′; BRDesrot-Probe, 5′-FAMACAAGGGACCCTACTGTTTCAGAGCATGC-3′-Black Hole Quencher 1; and BRDesrot-Rev, 5′-AAACTCAAGAGAAGGCCAACCA-3′. Absolute quantification was performed by using in vitro–transcribed cRNA for the specific region.

Muscle, interscapular brown fat, tongue, and reproductive, thoracic, abdominal, and retroperitoneal organs from all 8 RABV-positive bats were tested. RNA concentrations were consistently highest in central nervous system specimens (median

$10^{10.91}$ genome copies/g tissue) (online Appendix Figure, www.cdc.gov/EID/content/16/12/2004-appF.htm). Tongue specimens (containing salivary glands) also showed high concentrations (median $10^{8.66}$ copies/g tissue). High concentrations in heart and lung were compatible with anterograde virus secretion through the vegetative system, similar to that in other mammals (*6*). Not all spleen samples were RABV positive, which suggested no specific involvement of RABV with the lymphatic system. Two of 5 female RABV-positive vampire bats were pregnant at the time of sampling. Virus was detected in both placentas and in 3 of 4 uterus specimens tested (median $10^{6.55}$ copies/g tissue). However, the 2 fetuses were too immature for analysis. Testicle specimens were available for 2 of 3 male bats; 1 bat was positive ($10^{6.76}$ copies/g tissue).

Modes of RABV transmission and pathobiology in bat populations are unclear. RABV infection at high doses leads to death. High seroprevalence rates in populations of apparently healthy animals suggest that bats may be capable of controlling natural infection, in contrast to other mammals (*6*). Nevertheless, these findings and those of another study (*7*) demonstrated that bats may also die from natural RABV infection. However, because only moribund vampire bats were sampled in our study, the proportion of bats that die of natural infection is unknown.

Although regurgitation of blood for feeding offspring or roost mates was suggested to be a route of infection in vampire bats (*8*), the organ distribution of RABV in our study suggests secretion from salivary glands after spread from the central nervous system, which is compatible with virus transmission in other mammals. Our finding is also consistent with those of a study on tissue distribution of European bat Lyssavirus 2 in *Myotis daubentonii* bats (*9*).

Increased virus concentrations in placentas and reproductive organs suggest vertical transmission, supporting previous findings of RABV in reproductive organs of a deceased *Eidolon helvum* bat (*7*). However, whether similar observations can be made in healthy bats is unknown. Sporadic detection of virus and low virus concentrations in bladder and intestine make RABV transmission by excreta less likely (*7*,*9*). Whether RABV infection was the primary cause of disease in our RABV-positive bats is unknown. Distribution of RABV in organs of moribund vampire bats was similar to that observed in autopsy specimens from humans (*10*). Thus, if we assume that the patterns of organ distribution we observed are representative for free-ranging vampire bats, transmission patterns may be similar to those seen in other mammals.

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Wildlife-associated *Cryptosporidium fayeri* in Human, Australia

To the Editor: Molecular tools are essential for *Cryptosporidium* spp. identification, taxonomy, and epidemiology because of morphologic similarities between species within this genus. Molecular analyses have now identified 22 *Cryptosporidium* spp. and >40 cryptic species (i.e., genotypes) across all vertebrate classes (*1*). The myriad of potential *Cryptosporidium* spp. hosts, in conjunction with the robustness of the infectious stage (oocyst), means diverse *Cryptosporidium* spp. constantly circulate through the environment. This circulation increases the potential for disease from a diversity of contamination sources.

Human cryptosporidiosis is a global problem causing illness in young, elderly, immunocompromised, and immunocompetent persons in both industrialized and developing nations. The 2 most common etiologic agents, responsible for 90% of reported human infections, are *C. hominis* and *C. parvum* (*2*,*3*). Additional species identified as human pathogens are *C. meleagridis*, *C. canis*, *C. felis*, and the *Cryptosporidium* rabbit genotype (*4*). Each of these species was once thought to be specific for turkeys, dogs, cats, and rabbits, respectively. Incidental findings of *C. muris*, *C. andersoni*, *C. suis*, *C. hominis* monkey genotype, *C. parvum* mouse genotype, and *Cryptosporidium* cervine (W4), chipmunk I (W17), skunk, and horse genotypes have also been reported in humans (*4*). The pathogenicity of these zoonotic species and genotypes to humans remains unclear.

In July 2009, a 29-year-old woman who sought care because of prolonged gastrointestinal illness had a fecal test positive for *Cryptosporidium* spp. by the Remel ProSpecT *Giardia*/*Cryptosporidium* microplate assay (Thermo Fisher Scientific, Lenexa, KS, USA). Oocysts were purified from the specimen (*5*) and stained with the *Cryptosporidium* spp.–specific antibody CRY104 labeled with fluorescein isothiocyanate (Biotech Frontiers, North Ryde, Australia) for enumeration. A parasite load of 1.34 × 10^6 oocysts/g feces was determined by using epifluorescence microscopy at 400× magnification.

To identify *Cryptosporidium* spp., DNA was extracted (*5*), and a diagnostic fragment of the small subunit (SSU) rRNA) was amplified (*6*). Clones were screened to identify species and determine the possibility of mixed infection. Plasmids from 50 clones were recovered and digested with the enzyme *SspI* (New England Biolabs, Beverly, MA, USA) (*6*). Two different restriction profiles were visualized. The sequence from each of the restriction types was determined; profile 1 contained *SspI* fragment sizes of 33, 109, 247, and 441 bp; profile 2 had fragments of 33, 254, and 540bp. A BLAST search (www.ncbi.nlm.nih.gov/blast) confirmed the sequences as *C. fayeri* type 1 and type 2. These 2 sequences correspond to known heterogeneity within the SSU rRNA of *C. fayeri* (*7*).

The identification of *C. fayeri* by SSU rRNA was confirmed by the sequence of the actin gene (*8*), showing 99.8% similarity to *C. fayeri* (GenBank accession no. AF112570). Further analysis at the 60-kDa glycoprotein (gp60) locus was used to determine the *Cryptosporidium* subtype family (*5*). The MQ1022 gp60 sequence was 98% similar to *C. fayeri* subtype family IVa (*9*). Analysis of the microsatellite region further characterized isolate MQ1022 to *C. fayeri* subtype IVaA9G4T1R1. The nucleotide sequences generated in this study were submitted to GenBank under accession nos. HQ008932–HQ008934.

Because the patient was imunocompetent, the disease was believed to be self-limiting, and she was lost to follow-up. The patient resided in a national forest on the east coast of New South Wales, Australia, an area where marsupials are abundant. She had frequent contact with partially domesticated marsupials. Notably, *C. fayeri* has been identified in 6 Australian marsupial species. Identification of *C. fayeri* in a human patient is a concern for water catchment authorities in the Sydney region. The main water supply for Sydney, Warragamba Dam, covers 9,050 km^2 and is surrounded by national forest inhabited by diverse and abundant marsupials. A previous study that investigated *Cryptosporidium* spp. in a wild eastern gray kangaroo (*Macropus giganteus*) population reported a prevalence of 6.7% (*10*). Oocyst shedding ranged from 20/g feces to 2.0 × 10^6/g feces (*10*). Subtype IVaA9G4T1R1 identified from the patient in this study has been characterized from eastern gray kangaroos in Warragamba Dam (*9*). Throughout the year, large groups of eastern gray kangaroos graze within riparian zones in the catchment. Such close proximity to the water presents a high possibility that the dam's water is contaminated with oocysts from these animals.

The *Cryptosporidium* genus is diverse, both in species and suitable hosts. The mechanisms of host specificity remain unknown, but the frequency of *Cryptosporidium* spp. crossing the host barrier and becoming