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Multiple Serotypes of Bluetongue Virus in Sheep and Cattle, Israel

To the Editor: In September 2008, the Israeli Veterinary Field Services were notified of uncharacteristic disease on a dairy farm near the border with Lebanon in Rosh Ha Nikra, (online Appendix Figure, www.cdc.gov/EID/content/16/12/2003-appF.htm). In November, blood samples were obtained from 5 cows, 4 of which were recovering from signs of infection with bluetongue virus (BTV). Virus isolation was conducted at the Kimron Veterinary Institute, Bet Dagan, Israel. One isolate (ISR2008/03) was sent to the World Animal Health Organisation Bluetongue Reference Laboratory at the Institute for Animal Health (IAH), Pirbright, UK, for further characterization. BTV-16 was identified by using serotype-specific real-time reverse transcription–PCR (RT-PCR) for genome segment 2 (Seg-2). BTV-16 has been detected in Israel and is considered endemic, along with BTV serotypes 2, 4, 6, and 10 (*1*).

Ten additional blood samples and 1 spleen sample subsequently obtained from affected cattle on the farm were sent to IAH. All samples were tested for BTV by serogroup-specific real-time RT-PCR specific for Seg-1. Six samples (including 1 from the spleen) were positive for BTV. Serotype-specific real-time RT-PCR showed that 2 blood samples contained BTV-16 and 1 blood sample contained BTV-4 and BTV-16. The amount of BTV RNA in the remaining 3 RT-PCR–positive samples was low, and attempts to identify serotype were unsuccessful. Virus from the spleen was isolated in an insect cell line (KC cells from *Culicoides sonorensis* midge embryos, CRL 1660; American Type Culture Collection, Manassas, VA, USA), and the virus was serotyped as BTV-8 by RT-PCR.

BTV-4 was isolated from bovine blood obtained in October 2008 from a farm in Zde Eliahu, 100 km east of Rosh Ha Nikra. However, this animal was co-infected with BTV-24, which has been found at numerous sites in Israel (online Appendix Figure). BTV-24 was isolated at IAH from samples obtained from sheep and cattle showing clinical signs of disease. BTV-4, BTV-16, and BTV-24 all reemerged in Israel during 2009, the mortality rate was up to 80% on 1 sheep farm infected with BTV-24 (*2*). An outbreak in Hatzafon in November 2009 was confirmed as BTV-5 by serotype-specific real-time RT-PCR.

To determine origins of BTV strains causing these outbreaks, we sequenced Seg-2 of the BTV-4 (Zde Eliahu) and BTV-8 and BTV-16 (ISR2008/02, ISR2008/13, and ISR2008/03) isolates from Israel. BTV-16 ISR2008/03 had >99% nt sequence identity (2,935 bp) with BTV-16 (GRE1999/13) isolated in Greece in 1999 but was distinct from BTV-16 (OMN2009/02) recently isolated in Oman. BTV-8 isolate ISR2008/13 had >99% nt sequence identity (2,939 bp) with the northern European strain of BTV-8 (NET2006/04). This finding indicates that the BTV-8 isolate from Israel (ISR2008/13) belongs to the same lineage as BTV-8 from northern Europe (NET2006/04) and may have been introduced into Israel during importation of BTV-8–positive animals from northern Europe.

BTV-4 isolate ISR2008/02 had >99% nt sequence identity (2,926 bp) with BTV-4 (DQ191279) isolated in Israel in 2001, which suggests that this serotype has either continued to circulate or has reemerged. BTV-24 (ISR2008/05) belongs to a western topotype. However, few nucleotide sequences are available for comparison of BTV-24 Seg-2 regions. BTV-5 has not been isolated; therefore, no sequence data are available.

Although BTV-2, BTV-4, BTV-6, BTV-10, and BTV-16 are considered endemic to Israel, clinical signs of disease are uncommon. We report clinical signs of infection in cattle in Israel caused by BTV-8 and BTV-24. We also report active circulation of 5 BTV serotypes (BTV-4, BVT-5, BTV-8, BTV-16, and BTV-24) during 2008–2009. Multiple serotypes were isolated on 3 farms containing sheep that had clinical signs of BT (farm 1: BTV-4 and BTV-24, farm 2: BTV-8 and BTV-24, and farm 3: BTV-4, BTV-8, and BTV-24). BTV-4, BTV-8, and BTV-16 were also isolated from cattle at Rosh Ha Nikra. Identification of multiple cocirculating BTV serotypes increases the likelihood of genome segment reassortment, which could potentially lead to increased virulence. Whole genome sequencing of isolates from these farms is in progress to determine whether any of these isolates are reassortants, as has been observed in Italy (*3*)

Our study indicates that BTV-8 strains from Israel and northern Europe (*4*–*6*) are closely related and share a recent common origin. The strain from Israel may represent an extension of the outbreak in Europe. Use of inactivated virus vaccines has dramatically decreased the number of cases caused by virulent BTV-8 in Europe (*7*), which suggests that a similar campaign might be effective in Israel. However, the BTV-24 strain from Israel appears to be highly virulent in

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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DOI: 10.3201/eid1612.100239

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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