

performed, HU/5862/Osaka/JP distinctly belonged to genotype 1. Therefore, sapovirus classification based on capsid sequence is questionable. We suggest that sapovirus classification should rely not only on capsid sequence but also on polymerase sequence.

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## Postmortem Confirmation of Human Rabies Source

**To the Editor:** Rabies is a fatal encephalitis caused by a neurotropic RNA virus of the family *Rhabdoviridae*, genus *Lyssavirus*. The predominant rabies virus reservoir hosts are bats and carnivores. Among these, rabid dogs represent a substantial public health problem, particularly in developing countries (1).

Laboratory diagnosis of rabies is essential to guide control programs, epidemiologic surveys, and prophylactic measures (2). Among the laboratory tests recommended by the World Health Organization (WHO), the fluorescent antibody test (FAT) is the accepted standard for rabies diagnosis (1). Although rabies virus antigens can be detected in decomposed samples, FAT is less effective when such samples are tested. In those cases, polymerase chain reaction (PCR) can provide better results (3). Since the degree of decomposition at which FAT starts to become ineffective is unknown (4), when smears from decomposed samples are made for FAT, a suspension of the same brain tissues should be made in the appropriate diluents for the mouse

inoculation test (MIT), cell culture, or reverse transcription–polymerase chain reaction (RT-PCR) (2). However, if all test results are negative, rabies cannot be ruled out because of the condition of the sample.

On February 28, in the city of Carbonita, Minas Gerais State, in southeastern Brazil, a 62-year-old man was bitten by a bat on the right ankle. Approximately 50 days later, his leg began to feel numb, and he experienced a continuous headache, pain at the site of the bite, convulsions, frequent urge to clear his throat, hiccups, nausea, difficulty in swallowing, dry lips, slightly elevated body temperature (37°C–37.5°C), paralysis of superior and inferior left limbs, shaking, and hallucinations. On May 4, 16 days after clinical manifestations began, the patient died; the cause of death was registered as a cerebral vascular accident. One month later, the body was exhumed to obtain a sample from the central nervous system (CNS), which was sent to Instituto Pasteur, São Paulo, registered as sample 5341 M/04 and tested by FAT, MIT, and RT-PCR.

In total, 8 smears were prepared from the sample to be analyzed by FAT according to the method of Dean et al. (5) with fluorescein isothiocyanate–labeled polyclonal antinucleocapsid antibodies. MIT was carried out as described by Koprowski (6) with 7 mice. For RT-PCR, RNA was extracted from the CNS sample with TRIzol, according to the manufacturer's instructions (Invitrogen, Rockville, MD, USA). RT-PCR was carried out with modifications as described by Orciari et al. (7), with primers 504 (sense) and 304 (antisense), aiming at the amplification of a 249-bp fragment of rabies virus nucleoprotein (N) gene, by using Superscript II (Invitrogen) and Taq DNA-polymerase (Invitrogen).

Fluorescent inclusions were observed in 6 of the 8 slides prepared for the FAT. The RT-PCR of the RNA

sample resulted in amplicons of the correct size (249 bp), as did the positive control sample, CVS strain rabies virus. No bands were observed in the reaction corresponding to the negative/reagent control (ultra-pure water). The MIT results were negative. Because the virus could not be isolated, antigenic typing with monoclonal antibodies could not be performed.

The fragment obtained in the RT-PCR was bidirectionally sequenced with DYEnamic ET Dye Terminator (Amersham Biosciences, Piscataway, NJ, USA) in a MegaBACE DNA sequencer (Amersham Biosciences) and resulted in a 165-nucleotide sequence. The final sequence was aligned with homologous sequences from GenBank by using the ClustalW (available from <http://www.ebi.ac.uk/clustalw>) and Bioedit software (Isis Pharmaceuticals, Carlsbad, CA, USA). The phylogenetic tree was

produced by using the neighbor-joining DNA-distance method and the Kimura 2-parameter model with 1,000 bootstrap replicates in Mega 2.1 (version 2.1) (available from <http://www.megasoftware.net/>). The sequence was segregated in the variant 3 cluster (*Desmodus rotundus*-related variants), which suggests that *D. rotundus* is the most probable source of infection (Figure). The sequence was assigned GenBank accession no. DQ177278.

The lack of diagnosis or delay in diagnosis can increase the number of persons potentially exposed to rabies virus infection by contact with the patient or even by organ transplantations (8). Moreover, an early diagnosis can decrease the cost of treatment by eliminating the use of ineffective drugs and unnecessary diagnostic tests (2), as well as allowing potentially useful emerging therapeutic strategies to be used (9).

Before this report, no reference of a rabies diagnosis by FAT or RT-PCR had been reported from a human exhumed 30 days postmortem. The RT-PCR results agree with those obtained by David et al. (10) from a decomposed sample of animal origin after 36 days.

These facts demonstrate that rabies should be considered in cases of encephalitis with the classic clinical signs and symptoms as well as the paralytic form of disease (paresis and paralysis). Rabies should be suspected when early clinical symptoms, for example, itching and paresthesia, are demonstrated at the local site of infection. In addition, the laboratory investigation showed that molecular methods such as RT-PCR and sequencing were sensitive assays for nucleic acid detection and determination of the rabies virus variant in this unusual case from an exhumed human.

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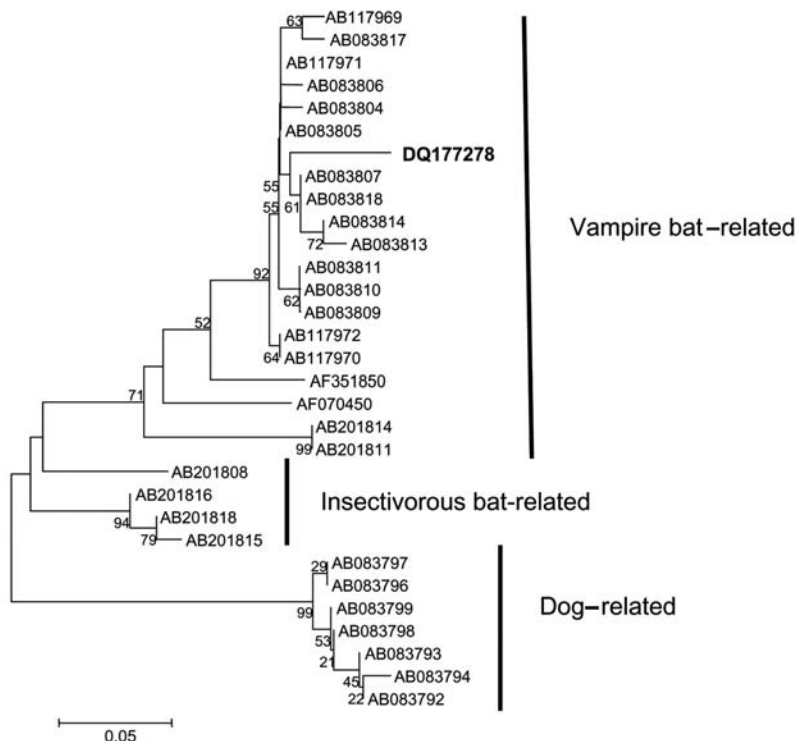


Figure. Neighbor-joining phylogenetic tree to a stretch of the 3' end of the N gene of rabies virus variants related to vampire bats, insectivorous bats, and dogs. Strain DQ177278 is shown in bold. The bar indicates the genetic distance scale. Numbers at each node indicate 1,000 replicates of bootstrap values.

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## Potential for Zoonotic Transmission of *Brachyspira pilosicoli*

**To the Editor:** Anaerobic intestinal spirochetes of the genus *Brachyspira* colonize the large intestine (1). Most *Brachyspira* species have a restricted host range, whereas *Brachyspira* (formerly *Serpulina*) *pilosicoli* colonizes a variety of animal and bird species and humans. *B. pilosicoli* is an important colonic pathogen of pigs and chickens (2). It occurs at high prevalence rates in humans in developing countries and

in male homosexuals and HIV-positive persons in industrialized countries (3). Its potential as a human pathogen was emphasized after its identification in the bloodstream of a series of debilitated persons (4).

*B. pilosicoli* isolates from humans and other species have been used experimentally to colonize chicks, piglets, and mice (5–7). While these results indicate that the *B. pilosicoli* strains used lacked host-species specificity, few data exist on whether natural zoonotic spread of *B. pilosicoli* strains occurs. In 1 study that used pulsed-field gel electrophoresis (PFGE) to type isolates from Papua New Guinea, 2 dogs were colonized with *B. pilosicoli* isolates with the same PFGE types as those from villagers. However, the higher prevalence of colonization with *B. pilosicoli* in humans than dogs suggested that the dogs were infected with human isolates, probably through consumption of human feces (8).

Multilocus enzyme electrophoresis (MLEE) has been used to study variation in *B. pilosicoli* isolates; most studies have focused on isolates from only 1 or 2 host species (8–10). Generally, *B. pilosicoli* isolates are diverse, and a lack of linkage disequilibrium in the MLEE data for human isolates suggests that the species is recombinant (8).

We used MLEE to investigate relationships between 107 *B. pilosicoli* isolates of diverse geographic and host-species origins and the *B. aalborgi* type strain (NCTC 11492<sup>T</sup>). Isolates were selected on the basis of their diverse origins and availability in the Murdoch University culture collection. They originated from feces of 34 pigs, 19 chickens, 13 ducks, 1 rhea, 25 humans, and 4 dogs; from 7 human blood samples; and from 4 water sources frequented by waterfowl. Isolates originated from Australia, Canada, France, Italy, the Netherlands, Oman, Papua New Guinea, the United Kingdom, and the United States.

The MLEE method used was as previously described (8–10); the electrophoretic mobility of 15 constitutive enzymes was analyzed. Variations in electrophoretic mobility were interpreted as representing products of different alleles at each enzyme locus. Isolates with identical enzymatic profiles at 15 loci were grouped into an electrophoretic type (ET). Genetic distance between ETs was calculated as the proportions of loci at which dissimilar alleles occurred. PHYLIP version 3.51c (Phylogeny Inference Package, University of Washington, Seattle, WA, USA) was used to analyze data and generate a dendrogram by using the unweighted pair-group method with arithmetic mean clustering fusion strategy. Genetic diversity (h) was calculated for the number of ETs as  $(1 - \sum p_i^2)/(n/n - 1)$ , where  $p_i$  is the frequency of the indicated allele and  $n$  is the number of ETs.

*B. pilosicoli* isolates were divided into 80 ETs (mean 1.35 isolates per ET) (Figure). *B. aalborgi* NTCC 11492<sup>T</sup> was distinct in ET81. The *B. pilosicoli* isolates were diverse, with an h value of 0.41. Generally, they did not cluster according to host species of origin, and isolates from a given species were distributed throughout the dendrogram. Isolates from birds were more diverse than those from humans and pigs. Eight ETs contained multiple isolates, in each case from the same host species (either chickens or pigs). In 4 cases these originated from different countries: ET47 contained 2 Australian porcine isolates and 2 from the United States; ET53 contained 2 Australian porcine isolates and Scottish porcine type strain P43/6/78<sup>T</sup>; ET54 contained 2 Australian and 2 Canadian porcine isolates; ET65 contained 1 Dutch and 1 US chicken isolate.

Although human isolates did not share an ET with isolates from other species, they were frequently closely related, differing in 1 allele. This occurred with US and Australian pig