

Shiga Toxin 2A–Encoding Bacteriophages in Enteroggregative *Escherichia coli* O104:H4 Strains

To the Editor: In 2011, enteroaggregative *Escherichia coli* (EAEC) O104:H4 strains that produce Shiga toxins (EAEC-STEC) caused an outbreak of hemorrhagic disease affecting nearly 4,000 patients in Europe (1). During 2001–2013, several countries reported infections caused by EAEC O104:H4 and EAEC-STEC O104:H4 strains (1–9). Genomic analysis of EAEC and EAEC-STEC O104:H4 strains revealed high similarity, and it has been suggested that EAEC-STEC O104:H4 strains evolved from EAEC O104:H4 strains by uptake of Shiga toxin 2 (Stx2)–producing bacteriophages (3,4).

We investigated Stx-2 subunit A (Stx-2A) bacteriophages in a group of epidemiologically unrelated EAEC-STEC O104:H4 strains isolated from animals and food in Germany (collection of the National Reference Laboratory for *Escherichia coli*). One phage genome (P13374) was sequenced (2). The Stx-2A bacteriophages were highly similar in morphologic features, restriction endonuclease profiles, chromosomal integration sites, and superinfection immunity (2,3) and showed <65% similarity to Stx phages from non-O104 strains. Major genetic differences between the bacteriophages we investigated and other Stx phages were found in the genes for DNA replication, DNA metabolism, and in the immunity region (2,3).

We identified 2 genes, *orf15* and *cI*_{P13374}, that were specific to Stx-2A bacteriophages found in EAEC-STEC O104:H4 strains (10). These genes were found in only 14 (5.8%) of 241 Stx-2A–positive non-O104 STEC strains. Viable Stx-2A bacteriophages isolated

from 4 bovine non-O104 STEC strains were similar to Stx-2A bacteriophages from EAEC-STEC O104:H4 strains for all features described above (10). Similar to P13374, one of the bovine phages (P13803) lysogenized an Stx-negative EAEC O104:H4 strain and converted it into an EAEC-STEC–producing Stx-2A bacteriophage (10).

Our results provide experimental evidence that EAEC-STEC O104:H4 have evolved by uptake of a distinct type of Stx-2A bacteriophage. Bovine STEC harboring Stx-2A bacteriophages able to transduce Stx-2A genes to EAEC O104:H4 are found worldwide, and phage-mediated transfer of Stx-2A can occur in the environment (10). Thus, the emergence of EAEC-STEC O104:H4 does not appear to be the result of introduction of the strains from areas to which they are endemic. Instead, the process may have occurred spontaneously by phage transduction, which could explain why EAEC-STEC O104:H4 infections were found at different locations and at different times. Regardless of time or place, however, these strains show characteristic differences in their prophage and plasmid profiles, which may serve as indicators of epidemiologic origin (1–4). Investigation of EAEC-STEC O104:H4 strains from sporadic cases of human infection could reveal these markers and help differentiate between strains that were introduced from other areas and strains that were newly generated by phage transduction.

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Rio Mamoré Virus and Hantavirus Pulmonary Syndrome, Brazil

To the Editor: Hantavirus pulmonary syndrome (HPS) is an acute, severe, frequently fatal disease associated with cardiopulmonary failure; it is caused by hantaviruses naturally hosted by wild rodents. Rio Mamore virus (RIOMV) was first described in 1996 in Bolivia; it was associated with the small-eared pygmy rice rat, *Oligoryzomys microtis* (1). Subsequently, 1 strain of RIOMV was isolated from *O. microtis* rats in Peru, designated HTN-007 (2); and 2 strains were recovered in the Brazilian Amazon from *O. microtis* rats (RIOMV-3) and uncharacterized species of rodents of the genus *Oligoryzomys* (RIOMV-4) (3). Recently, HPS cases associated with RIOMV have been reported: 2 cases in Peru (4) and 1 case in French Guiana (caused by a variant named Maripa virus) (5). We report isolation of a strain of RIOMV from a patient with fatal HPS in Brazil.

In June 2011, a 28-year-old man was admitted to the Tropical Medicine Foundation Dr. Heitor Vieira Dourado, Amazonas State, with a 4-day febrile illness that included nonproductive cough, myalgia, and headache. Laboratory

testing revealed hematocrit within reference range (43.9%), thrombocytopenia (27,000 cells/mm³), elevated levels of liver enzymes (alanine transaminase 347 IU/L, aspartate transaminase 139 IU/L), creatinine (1.2 mg/dL), and urea (40 mg/dL). Laboratory testing ruled out malaria, leptospirosis, and dengue. About 24 hours after hospitalization, the patient experienced hypotension, progressive dyspnea, and acute respiratory distress. Thoracic radiographs revealed bilateral diffuse alveolar pulmonary infiltrates. Despite empirical treatment with antimicrobial drugs, mechanical ventilation, and inotropic therapy, the patient's clinical condition deteriorated and he died on day 6 after illness onset.

The patient, who had no history of travel, resided on a submerged region in the western floodplain of the Solimões-Amazon River, Amazonas, a state with low population density (6.2 persons/square mile), in a rural area of Careiro da Várzea Municipality (3°11'53"S, 59°52'18"W), where access is possible only by boat. He had a history of contact with rodents not only at home but also in the boat he used. A serum sample collected on day 6 after illness onset was evaluated for hantavirus by serologic and PCR testing. ELISA result was positive for IgM and IgG against recombinant nucleocapsid protein (N) of the Juquitiba virus (6). Viral genome was detected by reverse transcription PCR, and the complete genomic small segment sequence, designated LH60_11/Hu (GenBank accession no. KF584259), was determined (7). This sequence was compared with a reference panel of sequences that covered the diversity of most hantaviruses in South America and was subjected to phylogenetic analysis by MrBayes software version 3.1.2 (8). Nucleotide and amino acid sequence similarities between all taxa for the partial N gene were calculated by using MegAlign version 5.05 (DNASTAR, Inc., Madison, WI, USA). The best-fit evolution-

ary model general time reversible + Γ + proportion invariant was determined by using MEGA version 5.2.2 (<http://www.megasoftware.net>), and the dataset compiled only 905 nt of the N gene to include sequences of Anajatuba and Rio Mearim viruses from Brazil for comparison.

Bayesian analysis indicated that strain LH60_11/Hu is closely associated with rodent-derived RIOMV-3/Olm strain (Itacoatiara, Amazonas State) and in a sister relationship with RIOMV-4/Olsp strain (Alto Paraíso, Rondônia State) from Brazil (Figure). Analysis of the partial sequence revealed 86.6%–95.4% of genetic identity with the strains recovered from rodents and 83.4% with the Maripa virus strain from humans. The sequence from the human patient in Peru was not available for comparison.

In July, the patient's house and environment were investigated; accumulation of garbage and other waste in homes that were still flooded was observed. We obtained and tested serum samples from 15 healthy residents (10 female, 5 male) with a recent history of acute fever; IgG against hantavirus was detected in samples from 3 women (17, 25, and 57 years of age).

This case report describes RIOMV as a highly pathogenic agent of HPS in Brazil. The location of the patient with this fulminant case of HPS, Careiro da Várzea, borders the Municipality of Itacoatiara, where RIOMV-3–infected *O. microtis* rats and the first HPS case in Amazonas State, with no etiologic identification so far, have been reported (3,9). Careiro da Várzea is part of an area in which grain production is expanding, an activity that attracts rodents to human dwellings, especially those in lowland regions that are constantly flooded.

The close association between the sequences from the human and the *O. microtis* rat (>98% aa identity) suggests that the patient might have been infected as a consequence of close physical contact with an RIOMV-