

indicates translocation within the United States of reassortant BTV-2.

How this virus spread to California is not known, and its distribution in the United States is uncertain because there is no comprehensive national BTV surveillance program. However, BTV-2 was not detected previously in California, suggesting that this serotype was recently introduced into the region or that it is uncommon. Identification of this novel BTV serotype in western North America emphasizes the need for ongoing entomologic and livestock surveillance, particularly in light of recent changes in the global distribution and nature of BTV infection (4,6,8).

**N. James Maclachlan,
William C. Wilson,
Beate M. Crossley,
Christie E. Mayo,
Dane C. Jaspersen,
Richard E. Breitmeyer, and
Annette M. Whiteford**

Author affiliations: University of California, Davis, California, USA (N.J. Maclachlan, B.M. Crossley, C.E. Mayo, R.E. Breitmeyer); US Department of Agriculture–Agricultural Research Service, Manhattan, Kansas, USA (W.C. Wilson, D.C. Jaspersen); and California Department of Food and Agriculture, Sacramento, California, USA (A.M. Whiteford)

DOI: <http://dx.doi.org/10.3201/eid1904.120347>

References

1. Maclachlan NJ, Drew CP, Darpel KE, Worwa G. The pathology and pathogenesis of bluetongue. *J Comp Pathol*. 2009;141:1–16. <http://dx.doi.org/10.1016/j.jcpa.2009.04.003>
2. Maan S, Maan NS, Nomikou K, Batten C, Antony F, Belaganahalli MN, et al. Novel bluetongue virus serotype from Kuwait. *Emerg Infect Dis*. 2011;17:886–9. <http://dx.doi.org/10.3201/eid1705.101742>
3. Gibbs EP, Greiner EC. The epidemiology of bluetongue. *Comp Immunol Microbiol Infect Dis*. 1994;17:207–20. [http://dx.doi.org/10.1016/0147-9571\(94\)90044-2](http://dx.doi.org/10.1016/0147-9571(94)90044-2)
4. Maclachlan NJ. Bluetongue: History, global epidemiology, and pathogenesis. *Prev Vet Med*. 2011;102:107–11. <http://dx.doi.org/10.1016/j.prevetmed.2011.04.005>
5. Tabachnick WJ. *Culicoides* and the global epidemiology of bluetongue virus infection. *Vet Ital*. 2004;40:144–50.
6. Johnson DJ. Identification of new United States bluetongue types. *Proceedings of the United States Animal Health Association*. 2011;111:209–10.
7. Saegerman C, Berkvens D, Mellor PS. Bluetongue epidemiology in the European Union. *Emerg Infect Dis*. 2008;14:539–44. <http://dx.doi.org/10.3201/eid1404.071441>
8. Purse BV, Brown HE, Harrup L, Mertens PP, Rogers DJ. Invasion of bluetongue and other orbivirus infections into Europe: the role of biological and climatic processes. *Rev Sci Tech*. 2008;27:427–42.
9. Osburn BI, McGowan B, Heron B, Loomis E, Bushnell R, Stott JL, et al. Epizootiologic study of bluetongue: virologic and serologic results. *Am J Vet Res*. 1981;42:884–7.
10. Mayo CE, Barker CM, Mullens BA, Gerry AC, Mertens PP, Maan S, et al. The combination of abundance and infection rates of *Culicoides sonorensis* estimates risk of subsequent bluetongue virus infection of sentinel cattle on California dairy farms. *Vet Parasitol*. 2012;187:295–301. <http://dx.doi.org/10.1016/j.vetpar.2012.01.004>

Address for correspondence: N. James Maclachlan, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA; email: njmaclachlan@ucdavis.edu

Hepatitis E Virus Genotype 3 Strains in Domestic Pigs, Cameroon

To the Editor: Hepatitis E virus (HEV) is a positive-stranded, non-enveloped RNA virus of the family *Hepeviridae* that is considered to be the main causative agent of enterically transmitted acute hepatitis (1). HEV is classified into 4 genotypes (1). HEV genotypes 1 and 2 cause large waterborne epidemics of acute hepatitis in developing countries, especially in Africa and Asia (1).

In contrast, HEV genotypes 3 and 4 are increasingly identified as causative agents of acute viral hepatitis in industrialized countries (1). Genotypes 1 and 2 are found only in humans, whereas genotypes 3 and 4 are associated with food-borne zoonotic transmission from domestic pigs, wild boar, and deer (1).

In addition to these 4 genotypes, HEV-related viruses were detected in avian, rodent, and bat hosts, which formed novel genera within the family *Hepeviridae* (2). In Africa, HEV genotype 1 and 2 strains have been identified during HEV epidemics (3–5). An HEV genotype 3 strain was detected in 1 of 40 fecal samples from domestic pigs in Kinshasa, Democratic Republic of the Congo, and it was suggested that this strain was imported from Belgium to the Democratic Republic of the Congo by animal trade (6). Therefore, we investigated whether HEV strains of genotype 3 or 4 are circulating among domestic pigs in Cameroon.

During February–March 2012, a total of 345 liver samples were collected from domestic pigs (age range 6 months–3 years) in abattoirs in Douala and Yaoundé, Cameroon, and in slaughter slaps (areas) in Bamenda, Cameroon. Pigs were mainly of the local breed. In addition, pigs originating from extensive cross-breeding (local X landrace and local X Duroc) were sampled. Liver samples were collected during post-mortem inspection.

Viral RNA was extracted from liver samples by using the RTP DNA/RNA Virus Mini Kit II (STRATEC Molecular, Berlin, Germany) according to the manufacturer's instructions. Extracted RNA was analyzed for HEV RNA by using 2 nested reverse transcription PCRs (RT-PCRs) specific for open reading frame 1 (ORF 1) and ORF 2 of HEV (7,8). Nested RT-PCRs and direct sequencing of amplicons were performed as described (9). RNA of

HEV strain Hamburg-HB (GenBank accession no. JN986840) was used as a positive control for nested RT-PCRs.

HEV RNA was detected in 2 samples from female pigs in Yaoundé (2/139) and 1 sample from a male pig in Bamenda (1/39). All 167 samples from Douala were negative for HEV RNA. The sample from Bamenda showed a positive result for the nested RT-PCR specific for HEV ORF 1. Genetic distances calculated with partial nucleotide sequences of ORF 1 (280 nt) and ORF 2 (373 nt) between strain Yaounde56 and the most closely related HEV genotype 3 strains

from Japan (JSWINE150-Aom04R; GenBank accession no. AB221520) and Mongolia (swMN06-A1354; GenBank accession no. AB290105) were 90% and 91%, respectively.

At the amino acid level, the partial RNA-dependent RNA polymerase sequence (ORF 1) and the partial capsid protein sequence (ORF 2) of strain Yaounde56 were identical to those of HEV genotype 3 strains HEV/Gt3/HSD40/2009 (GenBank accession no. AFO71833) from Germany and swJ12-1 (GenBank accession no. BAC66273) from Japan. Thus, all mutations were silent.

In agreement with distance analysis, phylogenetic reconstruction using partial nucleotide sequences of ORF 2 (278 nt) showed a close relationship of strains Yaounde56 and Yaounde94 with HEV genotype 3 strains (Figure). However, the HEV strains from Cameroon do not cluster with the classified HEV genotype 3 subtype reference strains (10) in the phylogenetic tree (Figure). These strains cluster within a clade of subtype undefined strains and are most closely related to strain swMN06-A1354 from Mongolia (Figure).

Because the pig production cycle is shorter than that for cattle, pig production is a major economic activity in Cameroon. Most pigs in Cameroon are local raised, and extensive cross-breeding is used. The infection rate of pigs with HEV genotype 3 strains from Cameroon is lower than that of pigs from Europe. Thus, HEV genotype 3 seems to have an extensive distribution that includes Africa. Future studies should investigate how HEV genotype 3 strains contribute to sporadic HEV cases in Cameroon.

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

**Vanessa S. de Paula,
Matthias Wiele,
Afegehi H. Mbunkah,
Achukwi M. Daniel,
Manchang T. Kingsley,¹ and
Jonas Schmidt-Chanasit¹**

Author affiliations: Fundação Oswaldo Cruz, Rio de Janeiro, Brazil (V.S. de Paula); Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (V. Salet de Paula, M. Wiele, J. Schmidt-Chanasit); Centre Pasteur, Yaoundé, Cameroon (A.H. Mbunkah); and Institute of Agricultural Research for Development, Ngaoundere, Cameroon (A.M. Daniel, M.T. Kingsley)

DOI: <http://dx.doi.org/10.3201/eid1904.121634>

¹These authors contributed equally to this article.

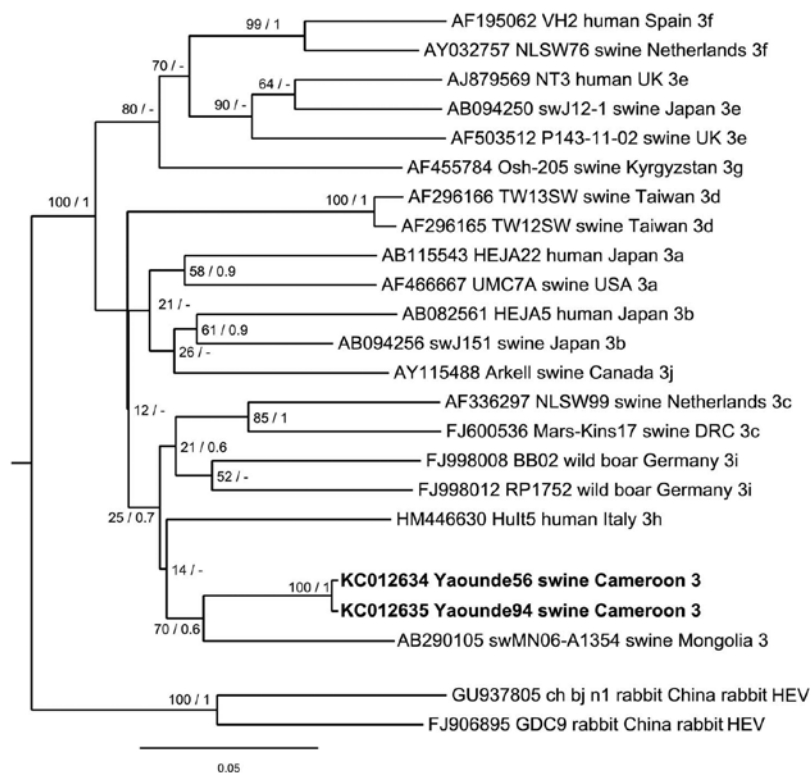


Figure. Phylogenetic analysis of hepatitis E virus (HEV) strains, Cameroon. The Bayesian phylogenetic tree was constructed by using partial nucleotide sequence of open reading frame 2 (278 nt) of HEV. For each sequence used, the GenBank accession number, strain designation, source of isolation, country of isolation, and HEV subtype are shown. Multiple nucleotide sequence alignment was analyzed by using the Markov Chain Monte Carlo method implemented in the program MrBayes version 3.0 (<http://mrbayes.sourceforge.net/>) and applying the general time-reversible substitution model. Posterior probabilities are shown at the nodes of the tree to the right of the slash if >0.5. Bootstrap values calculated from 10,000 replicates are indicated at the nodes of the tree to the left of the slash. Alignment was analyzed by using the neighbor-joining method and resulted in same tree topology (not shown). Newly described HEV sequences are shown in **boldface**. Scale bar indicates evolutionary distance. UK, United Kingdom; USA, United States; DRC, Democratic Republic of Congo.

References

1. Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, et al. Hepatitis E. *Lancet*. 2012;379:2477–88. [http://dx.doi.org/10.1016/S0140-6736\(11\)61849-7](http://dx.doi.org/10.1016/S0140-6736(11)61849-7)
2. Drexler JF, Seelen A, Corman VM, Fumie Tateno A, Cottontail V, Melim Zerbinati R, et al. Bats worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the family *Hepeviridae*. *J Virol*. 2012;86:9134–47. <http://dx.doi.org/10.1128/JVI.00800-12>
3. He J, Binn LN, Tsarev SA, Hayes CG, Frean JA, Isaacson M, et al. Molecular characterization of a hepatitis E virus isolate from Namibia. *J Biomed Sci*. 2000;7:334–8. <http://dx.doi.org/10.1007/BF02253253>
4. van Cuyck H, Juge F, Roques P. Phylogenetic analysis of the first complete hepatitis E virus (HEV) genome from Africa. *FEMS Immunol Med Microbiol*. 2003;39:133–9. [http://dx.doi.org/10.1016/S0928-8244\(03\)00241-4](http://dx.doi.org/10.1016/S0928-8244(03)00241-4)
5. Nicand E, Armstrong GL, Enouf V, Guthmann JP, Guerin JP, Caron M, et al. Genetic heterogeneity of hepatitis E virus in Darfur, Sudan, and neighboring Chad. *J Med Virol*. 2005;77:519–21. <http://dx.doi.org/10.1002/jmv.20487>
6. Kaba M, Colson P, Musongela JP, Tshilolo L, Davoust B. Detection of hepatitis E virus of genotype 3 in a farm pig in Kinshasa (Democratic Republic of the Congo). *Infect Genet Evol*. 2010;10:154–7. <http://dx.doi.org/10.1016/j.meegid.2009.09.011>
7. Johne R, Plenge-Bönig A, Hess M, Ulrich RG, Reetz J, Schielke A. Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad spectrum RT-PCR. *J Gen Virol*. 2010;91:750–8. <http://dx.doi.org/10.1099/vir.0.016584-0>
8. Mizuo H, Suzuki K, Takikawa Y, Sugai Y, Tokita H, Akahane Y, et al. Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. *J Clin Microbiol*. 2002;40:3209–18. <http://dx.doi.org/10.1128/JCM.40.9.3209-3218.2002>
9. Pfefferle S, Frickmann H, Gabriel M, Schmitz N, Günther S, Schmidt-Chanasit J. Fatal course of an autochthonous hepatitis E virus infection in a patient with leukemia in Germany. *Infection*. 2012;40:451–4. <http://dx.doi.org/10.1007/s15010-011-0220-7>
10. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol*. 2006;16:5–36. <http://dx.doi.org/10.1002/rmv.482>

Address for correspondence: Jonas Schmidt-Chanasit, Bernhard Nocht Institute for

Tropical Medicine, World Health Organization Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Bernhard-Nocht-Strasse 74, 20359 Hamburg, Germany; email: jonassi@gmx.de

Novel Respiratory Syncytial Virus Subtype ON1 among Children, Cape Town, South Africa, 2012

To the Editor: Human respiratory syncytial virus (RSV) is a common cause of severe acute lower respiratory tract infection in young children, accounting for ≈160,000 deaths/year worldwide (1,2). As part of an RSV nosocomial transmission study, we detected RSV genotype ON1, which was identified during November 2010–February 2011 as a novel genotype in Ontario, Canada, in samples from children in a tertiary pediatric hospital in Cape Town, South Africa during 2012. The genotype described in Canada was characterized by a 72-nt sequence duplication within the second variable domain of the envelope glycoprotein. The 72-nt duplication within the second variable domain in ON1 was the largest sequence duplication described in this virus (3).

RSV is divided into 2 genetically distinct groups, RSV A and B, based on the viral envelope glycoprotein nucleotide sequences (4). Sequence variability in the C-terminal variable domain of the glycoprotein gene is commonly used to determine RSV phylogeny (3,5). To date, 11 RSV A (ON1, GA1–GA7, SAA1, NA1, and NA2) and 17 RSV B (GB1–GB4, SAB1–SAB3, and BA1–BA10) genotypes have been identified (3,6).

As part of the aforementioned molecular epidemiology study surveying RSV infection in a pediatric hospital, (University of Cape Town research ethics study no. 305/2012), we sequenced the RSV glycoprotein second variable domain of nucleic acid extracts derived from RSV-positive respiratory secretion samples from 160 young children hospitalized for treatment of respiratory tract infections. The techniques used have been described (7). During January–April, in an area where NA1 was the dominant circulating RSV genotype, 119 (74%) of 160 RSV isolates were RSV A. We noted the presence, albeit at a low incidence, of the novel ON1 genotype cluster (8 viral isolates) (online Technical Appendix Figure, wwwnc.cdc.gov/EID/pdfs/12-1465-Techapp.pdf) in specimens collected during February–April.

Children in the RSV ON1-infected cohort were brought to health care facilities during February 24–April 25, 2012 (Figure and online Technical Appendix Figure), where they received a diagnosis of bronchiolitis or bronchiopneumonia (online Technical Appendix Table). With the exception of 1 patient, child 8, who had been hospitalized before onset of this illness, all ON1 isolates were community acquired. Seven of the 8 ON1 isolates were obtained from infants <4 months of age (median 7 weeks), who were younger than the 152 children who were not infected with the ON1 genotype (median age 3.5 months). The RSV ON1-infected children lived within a 2.5-km radius of one another (online Technical Appendix Table). The children who were not infected with RSV ON1 lived in a much wider geographic area; >90% lived within an 18-km radius of one another. These spatial associations with disease prevalence suggested that the ON1-infected children represented a localized cluster of transmission.

None of the children were infected with HIV, although 3 had antenatal