

was found to be slightly different from other HEVs and included a putative ORF (ORF4) of 552 nt that overlapped with ORF1 (online Technical Appendix Figure). A similar pattern of genome organization was observed for both FRHEVs.

Phylogenetic analysis of the complete genomes clearly showed that FRHEV was separated from genotype 1–4 HEVs and clustered with rat HEV (Figure). Similar phylogenetic clustering was observed when nucleotide and deduced amino acid sequences of ORF1, ORF2, and ORF3 were analyzed separately. The phylogenetic distance between rat HEV and FRHEV is larger than the distance between genotype 1 and genotype 2 HEV.

In recent years, an increasing number of sporadic cases of hepatitis E have been reported (1,9). Several observations suggest that autochthonous cases are caused by zoonotic spread of infection from wild or domestic animals (1,3,9). In addition, IgG anti-HEV seropositivity in the United States has been associated with several factors, including having a pet at home (10). Further studies are needed to identify the zoonotic potential of FRHEV.

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## References

- Aggarwal R, Jameel S, Hepatitis E. Hepatology. 2011;54:2218–26. <http://dx.doi.org/10.1002/hep.24674>
- Boccia D, Guthmann JP, Klovstad H, Hamid N, Tatay M, Ciglenecki I, et al. High mortality associated with an outbreak of hepatitis E among displaced persons in Darfur, Sudan. Clin Infect Dis. 2006;42:1679–84. <http://dx.doi.org/10.1086/504322>
- Meng XJ. Hepatitis E virus: animal reservoirs and zoonotic risk. Vet Microbiol. 2010;140:256–65. <http://dx.doi.org/10.1016/j.vetmic.2009.03.017>
- Johne R, Heckel G, Plenge-Bönig A, Kindler E, Maresch C, Reetz J, et al. Novel hepatitis E virus genotype in Norway rats, Germany. Emerg Infect Dis. 2010;16:1452–5. <http://dx.doi.org/10.3201/eid1609.100444>
- Batts W, Yun S, Hedrick R, Winton J. A novel member of the family *Hepeviridae* from cutthroat trout (*Oncorhynchus clarkii*). Virus Res. 2011;158:116–23. <http://dx.doi.org/10.1016/j.virusres.2011.03.019>
- Provacia LB, Smits SL, Martina BE, Raj VS, Doel PV, Amerongen GV, et al. Enteric coronavirus in ferrets, the Netherlands. Emerg Infect Dis. 2011;17:1570–1.
- van Leeuwen M, Williams MM, Koraka P, Simon JH, Smits SL, Osterhaus AD. Human picobirnaviruses identified by molecular screening of diarrhea samples. J Clin Microbiol. 2010;48:1787–94. <http://dx.doi.org/10.1128/JCM.02452-09>
- Victoria JG, Kapoor A, Dupuis K, Schnurr DP, Delwart EL. Rapid identification of known and new RNA viruses from animal tissues. PLoS Pathog. 2008;4:e1000163. <http://dx.doi.org/10.1371/journal.ppat.1000163>
- Rutjes SA, Lodder WJ, Lodder-Verschoor F, van den Berg HH, Vennema H, Duizer E, et al. Sources of hepatitis E virus genotype 3 in the Netherlands. Emerg Infect Dis. 2009;15:381–7. <http://dx.doi.org/10.3201/eid1503.071472>
- Kuniholm MH, Purcell RH, McQuillan GM, Engle RE, Wasley A, Nelson KE. Epidemiology of hepatitis E virus in the United States: results from the third national health and nutrition examination survey, 1988–1994. J Infect Dis. 2009;200:48–56. <http://dx.doi.org/10.1086/599319>

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## Epidemic *Clostridium* *difficile* Ribotype 027 in Chile

**To the Editor:** The increased severity of *Clostridium difficile* infection is primarily attributed to the appearance of an epidemic strain characterized as PCR ribotype 027 (1). The only report that identified epidemic *C. difficile* ribotype 027 in an American country outside of North America comes from Costa Rica, raising the possibility that strains 027 might also be present in other countries of Latin America (2). Several studies between 2001 and 2009 have been conducted in South American countries to detect the incidence of *C. difficile* infection in hospitalized patients, but they did not identify which *C. difficile* strains were causing these infections (3).

During an epidemiologic screening of patients with *C. difficile* infection in a university hospital in Chile, we analyzed all stool samples of patients with suspected *C. difficile* infection during a 5-month period (June–November 2011). Two cases of *C. difficile* infection were associated with ribotype 027.

*C. difficile* was isolated from stool samples according to published protocols (4). Briefly, stool samples were spread onto taurocholate-cefoxitin-cycloserine fructose (Merck, Rahway, NJ, USA) agar plates and incubated for 96 hours at 37°C in a Bactron III-2 anaerobic workstation (SHEL LAB, Cornelius, OR, USA). Plates were examined for the characteristic *p*-cresol odor unique to *C. difficile* culture (5). The aminopeptidase test (6) was also used to differentiate *C. difficile* strains. Suspected colonies were further analyzed by PCR to amplify *tcdA*, and *tcdB* genes (7). The presence of binary toxin gene (*cdtB*) and deletion in the negative regulator of the pathogenicity locus, *tcdC*, were determined by using Cepheid GeneXpert PCR (Cepheid, Sunnyvale, CA, USA). We used *C. difficile* ribotype 027 strain R20291 as a reference strain for comparative purposes. PCR ribotyping was performed as described (8). The specific ribotype 027 of each of the clinical isolates was determined by visual analysis and with the GelCompar II v6.5 software (Applied Maths, St-Martens-Latem, Belgium).

Case-patient 1 was a 60-year-old man with a history of coronary disease who required a coronary artery bypass graft because of 3-vessel coronary disease. Forty-eight hours after receiving 3 doses of cefazoline to prevent surgical wound infection, he exhibited severe and diffuse abdominal pain with frequent loose stools (8 bowel movements/day), fever (up to 39°C), and hemodynamic compromise, which required high doses of vasopressors. Stool samples were positive for *C. difficile* by ELISA, and the patient received intravenous metronidazole and oral vancomycin. However, because of the severity of the course of the disease, he underwent an urgent total colectomy with terminal ileostomy. The patient showed progressive improvement, and he was discharged

11 days after surgery. No relapse of *C. difficile* infection was reported in this patient in the next 5 months. Isolation of toxigenic culture and PCR demonstrated that the bacterial pathogen causing the diarrhea was *C. difficile* ribotype 027 (i.e., strain PUC51) (Figure).

Case-patient 2 was a 46-year-old man with a history of ischemic stroke with hemiparesis of the left side who had experienced a urinary tract infection that had been treated with ciprofloxacin 2 months earlier. Four weeks before admission, he had frequent loose stools with no fever and diffuse abdominal pain after meals. On admission, a computed tomographic scan and angiograph of the abdomen showed pancolitis with colonic wall thickening and scant ascites, suggestive of an inflammatory

or infectious cause, without vascular compromise. However, ELISA of stool samples was negative for *C. difficile* toxin. Treatment with ceftriaxone reduced his symptoms, and he was discharged. Seven days after discharge, he had intense diffuse abdominal pain, with frequent loose stools and fever up to 38.9°C, and was again admitted to the hospital. A new computed tomographic scan of the abdomen showed no change; however, an ELISA of a new stool sample for *C. difficile* toxin was positive, and the patient was given oral vancomycin. No relapse of *C. difficile* infection was observed within 3 months of observation. Toxigenic culture from stool samples and PCR identified the *C. difficile* isolate as ribotype 027 (i.e., strain PUC47) (Figure).

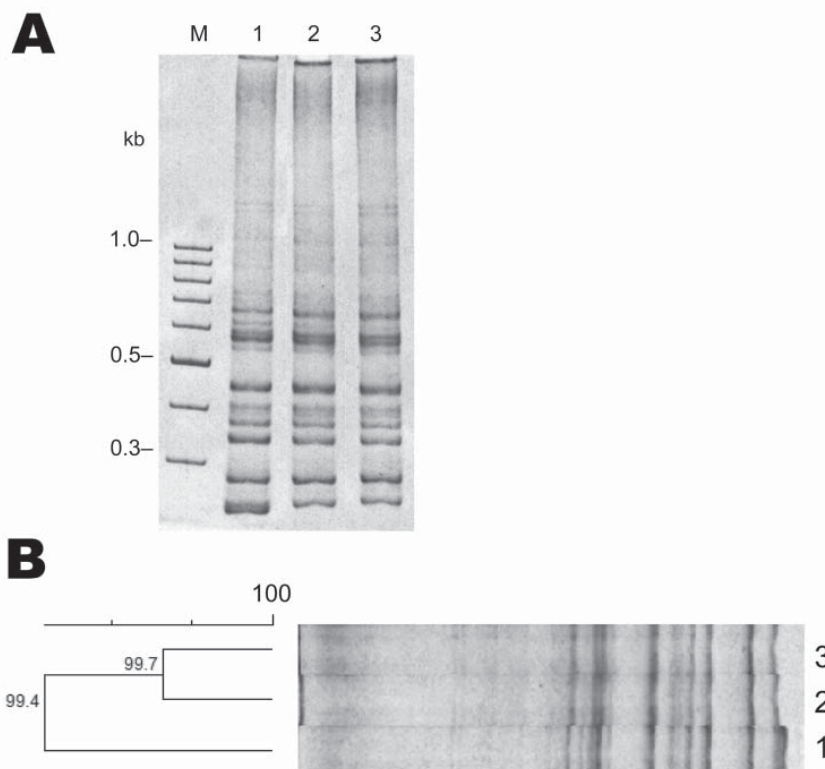


Figure. Results of PCR ribotyping of *Clostridium difficile* 027 strains from Chile. M indicates the 100-bp DNA ladder; lane 2, R20291; lane 2, PUC47; lane 3, PUC51. A) PCR ribotyping of *C. difficile* isolates. PCR results show that the band pattern of the ribosomal intergenic regions of strains PUC47 and PUC51 are similar to those of the reference (epidemic) strain R20291. B) Cluster analysis of strains PUC47, PUC51, and the epidemic strain 027 R20291 shows >99% similarity and that they belong to the same epidemic clade. Scale bar indicates percent identity.

Molecular typing analysis showed that both case-patients had a monoclonal infection caused by *C. difficile* ribotype 027. Both isolates had *tcdA*, *tcdB*, and *cdtB* and had a deletion in *tcdC* (data not shown).

In summary, the described severe cases of *C. difficile* infection in Chile were caused by epidemic *C. difficile* ribotype 027. One of these case-patients required urgent colectomy. These results demonstrate that epidemic *C. difficile* 027 strains are present in South America, highlighting the need for enhanced screening for this ribotype in other regions of the continent.

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#### References

1. McDonald LC, Killgore GE, Thompson A, Owens RC Jr, Kazakova SV, Sambol SP, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N Engl J Med*. 2005;353:2433–41. <http://dx.doi.org/10.1056/NEJMoa051590>
2. Quesada-Gómez C, Rodríguez C, Gamboa-Coronado Mdel M, Rodríguez-Cavallini E, Du T, Mulvey MR, et al. Emergence of *Clostridium difficile* NAP1 in Latin America. *J Clin Microbiol*. 2010;48:669–70. <http://dx.doi.org/10.1128/JCM.02196-09>
3. Balassiano IT, Yates EA, Domingues RM, Ferreira EO. *Clostridium difficile*: a problem of concern in developed countries and still a mystery in Latin America. *J Med Microbiol*. 2012;61:169–79. <http://dx.doi.org/10.1099/jmm.0.037077-0>
4. Wilson KH, Kennedy MJ, Fekety FR. Use of sodium taurocholate to enhance spore recovery on a medium selective for *Clostridium difficile*. *J Clin Microbiol*. 1982;15:443–6.
5. Dawson LF, Donahue EH, Cartman ST, Barton RH, Bundy J, McNerney R, et al. The analysis of para-cresol production and tolerance in *Clostridium difficile* 027 and 012 strains. *BMC Microbiol*. 2011;11:86. <http://dx.doi.org/10.1186/1471-2180-11-86>
6. Fedorko DP, Williams EC. Use of cycloserine-cefoxitin-fructose agar and L-proline-aminopeptidase (PRO Discs) in the rapid identification of *Clostridium difficile*. *J Clin Microbiol*. 1997;35:1258–9.
7. Rupnik M. *Clostridium difficile* toxinotyping. *Methods Mol Biol*. 2010;646:67–76. [http://dx.doi.org/10.1007/978-1-60327-365-7\\_5](http://dx.doi.org/10.1007/978-1-60327-365-7_5)
8. Bidet P, Barbut F, Lalande V, Burgoffer B, Petit JC. Development of a new PCR-ribotyping method for *Clostridium difficile* based on ribosomal RNA gene sequencing. *FEMS Microbiol Lett*. 1999;175:261–6. <http://dx.doi.org/10.1111/j.1574-6968.1999.tb13629.x>

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## Zoonotic Pathogens among White-Tailed Deer, Northern Mexico, 2004–2009

**To the Editor:** Intense wildlife management for hunting affects risks associated with zoonotic pathogens (1). White-tailed deer (*Odocoileus virginianus*) are increasingly managed by fencing, feeding, watering, and translocation to increase incomes from hunting in northern Mexico (2). These deer also play a major role in dissemination and reintroduction of pathogens and vectors from Mexico into the United States (3,4). White-tailed deer are suitable reservoir hosts for *Mycobacterium bovis* (1), and an *M. bovis*-positive white-tailed deer was recently found in Tamaulipas in northeastern Mexico (2). Brucellosis is widespread in many animal hosts in Latin America (5) and thus of interest in white-tailed deer. Another major zoonosis, sometimes linked to raw deer meat consumption, is hepatitis E, which is caused by genotypes of hepatitis E virus (HEV) (6). HEV is increasingly prevalent in red deer (*Cervus elaphus*) (7), but its prevalence in white-tailed deer is unknown.

The objective of this study was to determine the prevalence of zoonotic pathogens in white-tailed deer in northern Mexico. This study was conducted under a scientific collecting permit issued by the Mexican Division of Animal and Wildlife Health and on 8 ranches in 3 states in northern Mexico (≈26–28°N, 99–100°W).

Serum samples (n = 347) were collected during 2004–2009 in a cross-sectional survey for antibodies against HEV, *Brucella* spp., and mycobacteria. Deer were opportunistically sampled during live-capture operations as described by Cantú et al. (8). Bleeding was performed by using jugular venipuncture and vacuum tubes