

should be initiated immediately because  $\beta$ -lactam antimicrobial drugs are inefficient for the treatment of rickettsioses (9).

This research was funded by the Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE), Marseille, France.

D.R. is a cofounder of the biotechnology company Inodiag ([www.inodiag.com](http://www.inodiag.com)) and is the inventor of a serologic diagnostic method for which the patent is held by Aix-Marseille University; this method was not used in this study. All other authors have no conflicts of interest.

**Julie Solary,  
Cristina Socolovschi,  
Camille Aubry,  
Philippe Brouqui, Didier Raoult,  
and Philippe Parola**

Author affiliations: Aix-Marseille Université, Marseille, France

DOI: <http://dx.doi.org/10.3201/eid2002.130575>

## References

1. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev*. 2013;26:657–702. <http://dx.doi.org/10.1128/CMR.00032-13>
2. Wang JM, Hudson BJ, Watts MR, Karagiannis T, Fisher NJ, Anderson C et al. Diagnosis of Queensland tick typhus and African tick bite fever by PCR of lesion swabs. *Emerg Infect Dis*. 2009;15:963–5. <http://dx.doi.org/10.3201/eid1506.080855>
3. Mouffok N, Socolovschi C, Benabdellah A, Renvoise A, Parola P, Raoult D. PCR diagnosis of rickettsiosis on eschar swabs, Algeria. *Emerg Infect Dis*. 2011;17:1968–9. <http://dx.doi.org/10.3201/eid1710.110332>
4. Socolovschi C, Renzulli A, Brouqui P, Parola P, Raoult D. The use of eschar swabs for the diagnosis of African tick-bite fever. *Ticks Tick-borne Dis*. 2012;3:361–3.
5. Fournier PE, Gouriet F, Brouqui P, Lucht F, Raoult D. Lymphangitis-associated rickettsiosis, a new rickettsiosis caused by *Rickettsia sibirica mongolotimonae*: seven new cases and review of the literature. *Clin Infect Dis*. 2005;40:1435–44. <http://dx.doi.org/10.1086/429625>
6. Ramos JM, Jado I, Padilla S, Masia M, Anda P, Gutierrez F. Human infection with *Rickettsia sibirica mongolotimonae*, Spain, 2007–2011. *Emerg Infect Dis*. 2013;19:267–9. <http://dx.doi.org/10.3201/eid1902.111706>
7. Edouard S, Parola P, Socolovschi C, Davoust B, La Scola B, Raoult D. Clustered cases of *Rickettsia sibirica mongolotimonae* infection, France. *Emerg Infect Dis*. 2013;19:337–8. <http://dx.doi.org/10.3201/eid1902.120863>
8. Parola P, Socolovschi C, Jeanjean L, Bitam I, Fournier PE, Sotto A, et al. Warmer weather linked to tick attack and emergence of severe rickettsioses. *PLoS Negl Trop Dis*. 2008;2:e338. <http://dx.doi.org/10.1371/journal.pntd.0000338>
9. Botelho-Nevers E, Socolovschi C, Raoult D, Parola P. Treatment of *Rickettsia* spp. infections: a review. *Expert Rev Anti Infect Ther*. 2012;10:1425–37. <http://dx.doi.org/10.1586/eri.12.139>

Address for correspondence: Philippe Parola, Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes (URMITE), WHO Collaborative Center for Rickettsioses and Other Arthropod-borne Bacterial Diseases, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France; email: [philippe.parola@univ-amu.fr](mailto:philippe.parola@univ-amu.fr)

## Pandemic *Vibrio parahaemolyticus*, Maryland, USA, 2012

**To the Editor:** Since 1996, an increasing number of infections caused by *Vibrio parahaemolyticus* strains belonging to a pandemic clonal complex (CC), CC3, typically O3:K6, have been observed worldwide (1–3); most of these strains are sequence type (ST) 3. In the summer of 1998, outbreaks linked to O3:K6 occurred in Galveston Bay, Texas, and Oyster Bay, New York, USA; the illnesses were associated with oyster consumption (4). Strains belonging to CC36 are the leading cause of *V. parahaemolyticus* infections in the United States. These

strains are endemic to the West Coast (2) and have been historically linked to outbreak-associated *V. parahaemolyticus* infections caused by consumption of raw oysters harvested from the region (5).

In August 2012, a *V. parahaemolyticus* outbreak involving 6 persons occurred in Maryland, USA. The patients (members of 2 dining parties) had eaten in the same restaurant on the same day; raw and cooked seafood was served at the restaurant. Party A comprised 4 diners, of whom 2 had laboratory-confirmed illness and 2 were probable case-patients. Party B comprised 2 diners, of whom 1 had laboratory-confirmed illness and 1 was a probable case-patient. Probable case-patients were epidemiologically linked to confirmed case-patients, but *V. parahaemolyticus* was not detected in their stool samples. The epidemiologic investigation did not conclusively identify the specific food responsible for the outbreak. The affected diners had not eaten oysters, lobster, or mussels, but they had eaten cooked clams, fish, crab, and shrimp. Because the patients had not eaten oysters, a traceback investigation was not conducted. The outbreak possibly was caused by cross-contamination during food preparation. No other cases were reported from this restaurant or the surrounding area.

*V. parahaemolyticus* was isolated from stool samples of 3 of the patients. The isolates were characterized by real-time PCR for virulence-related genes (*tdh* and *trh*). All 3 isolates were *tdh* positive and lacked the *trh* gene. Pulsed-field gel electrophoresis (PFGE) was run, using *SfiI* and *NotI*; the resulting K16S12.0138 (*SfiI*) and K16N11.0143 (*NotI*) patterns were indistinguishable. The PFGE pattern combination was queried against combination entries made in PulseNet ([www.cdc.gov/pulsenet/](http://www.cdc.gov/pulsenet/)) during February 4, 2010–April 16, 2013, and found to be indistinguishable from other clinical entries (online Technical

Appendix 1 Table, [wwwnc.cdc.gov/EID/article/20/4/13-0818-Techapp1.pdf](http://wwwnc.cdc.gov/EID/article/20/4/13-0818-Techapp1.pdf)). This PFGE pattern combination has been seen 25 times; all patterns were for strains from humans (N. Facundo, pers. comm.). In 2012, this PFGE pattern combination was observed in 3 US states—California (6 cases), Arizona (6 cases), and Texas (5 cases)—but those isolates were not further tested (S.G. Stroika, pers. comm.), suggesting that other cases of pandemic *V. parahaemolyticus* infections have occurred in the United States but were not identified as being caused by pandemic clones.

The whole genomes of the 3 Maryland strains were sequenced by using the Ion Torrent personal genome machine (Life Technologies, Grand Island, NY, USA); in silico multilocus sequence typing (MLST) (2) showed that the isolates were all ST3, the most common ST belonging to CC3. Bioinformatic analysis of the whole genomes was conducted with the Bacterial Isolate Genome Sequence Database (6) genome comparator tool available within the *V. parahaemolyticus* MLST database (<http://pubmlst.org/vparahaemolyticus>) (7,8). Results confirmed that these outbreak isolates were linked to the O3:K6 pandemic clone of *V. parahaemolyticus* (Figure). We identified 2,613 variable loci in this analysis by using as reference genome the prototype pandemic *V. parahaemolyticus* clonal strain RIMD221633 (available from GenBank, [www.ncbi.nlm.nih.gov/genome/?term=vibrio+parahaemolyticus](http://www.ncbi.nlm.nih.gov/genome/?term=vibrio+parahaemolyticus)) (10). Differences in variable loci and the absence of certain genes indicated that, although indistinguishable by MLST and PFGE, these strains are easily differentiated from RIMD2210633 (online Technical Appendix 1). The draft genome sequences for the 3 strains are available at the *V. parahaemolyticus* MLST database (identification nos. 1187 [Vp16MD], 1188 [Vp17MD], and 1189 [Vp18MD]).

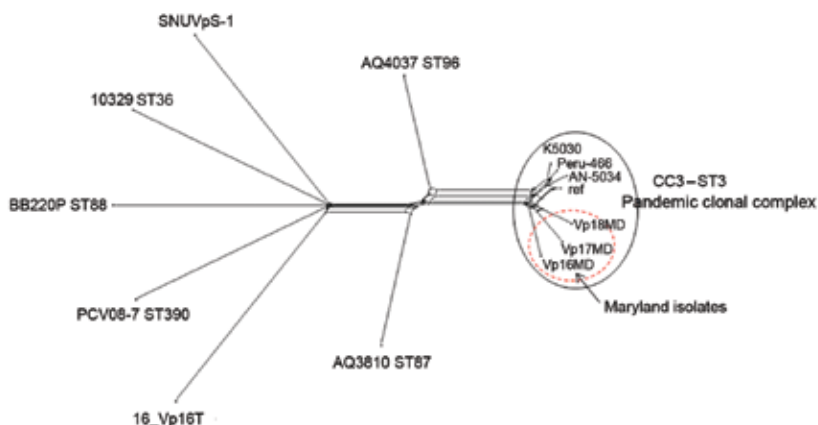


Figure. Neighbor-Net graph generated with the Bacterial Isolate Genome Sequence Database genome (BIGSdb) comparator tool implemented within the *Vibrio parahaemolyticus* MLST database (<http://pubmlst.org/vparahaemolyticus>) (7,8) using 2,613 variable loci. These loci were identified by using as a reference (ref) the *V. parahaemolyticus* strain RIMD2210633 chromosome I (3,080 genes) and conducting a whole-genome MLST (wgMLST) for *V. parahaemolyticus* genomes available through GenBank (AN-5034 O4:K68 ST3, Peru-466 ST3, K5030 ST3, 16\_Vp16T, AQ3810 ST87, AQ4037 ST96, PCV08-7 ST390, BB220P ST88, and SNUVpS-1) and 3 Maryland outbreak strains (Vp16MD, Vp17MD, and Vp18MD). This typing showed that these 3 strains belonged to the pandemic CC3. A similar graph was obtained by using chromosome II of the same strain as reference (data not shown). In brief, the BIGSdb genome comparator tool performs wgMLST, which produces a color-coded wgMLST output (online Technical Appendix 2, [wwwnc.cdc.gov/EID/article/20/4/13-0818-Techapp2.xlsx](http://wwwnc.cdc.gov/EID/article/20/4/13-0818-Techapp2.xlsx)) that facilitates comparison among isolates. This loci output is further categorized into loci that are 1) variable among all isolates, 2) identical among all isolates, 3) missing in all isolates, and 4) incomplete because of being located at the ends of contigs. The variable loci among all isolates are the loci used for assessing relationships and producing a distance matrix based on the number of variable alleles; the strains are resolved into a network by using the NeighborNet algorithm (9). MLST, multilocus sequence typing; CC, clonal complex; ST, sequence type.

*V. parahaemolyticus* strains belonging to the pandemic CC have caused thousands of infections and a *V. parahaemolyticus* pandemic (3). Foodborne illnesses caused by pandemic *V. parahaemolyticus* are uncommonly reported in the United States. In Maryland, 12 and 21 cases of *V. parahaemolyticus*-associated gastroenteritis were reported in 2012 and 2013, respectively. We report that the pandemic CC was still causing US outbreaks as recently as August 2012. It is possible that complete availability of PFGE patterns during the outbreaks (online Technical Appendix 1) could have provided additional insight into the scope of the outbreak and implicated food sources. The application of rapid, whole-genome sequencing

technology aided our discovery that the Maryland outbreak strains were part of the pandemic CC and likely related to *V. parahaemolyticus* strains that shared common PFGE patterns and that were reported as the cause of illnesses in several states around the same time as the Maryland outbreak.

The presence of this virulent *V. parahaemolyticus* strain in Maryland is an ongoing public health concern, requiring continued microbiological surveillance. This pandemic strain also indicates the need for establishing a *V. parahaemolyticus* genome database that is accessible worldwide. Such a database would enable improved tracking and faster responses to emergent and dangerous pandemic clonal strains.

**Julie Haendiges, Marvin Rock,  
Robert A. Myers,  
Eric W. Brown, Peter Evans,  
and Narjol Gonzalez-Escalona**

Author affiliations: Department of Health and Mental Hygiene, Baltimore, Maryland, USA (J. Haendiges, M. Rock, R.A. Myers); and Food and Drug Administration, College Park, Maryland, USA (E.W. Brown, P. Evans, N. Gonzalez-Escalona)

DOI: <http://dx.doi.org/10.3201/eid2004.130818>

## References

- González-Escalona N, Cachicas V, Acevedo C, Rioseco ML, Vergara JA, Cabello F, et al. *Vibrio parahaemolyticus* diarrhea, Chile, 1998 and 2004. *Emerg Infect Dis.* 2005;11:129–31. <http://dx.doi.org/10.3201/eid1101.040762>
- González-Escalona N, Martínez-Urtaza J, Romero J, Espejo RT, Jaykus LA, DePaola A. Determination of molecular phylogenetics of *Vibrio parahaemolyticus* strains by multilocus sequence typing. *J Bacteriol.* 2008;190:2831–40. <http://dx.doi.org/10.1128/JB.01808-07>
- Nair GB, Ramamurthy T, Bhattacharya SK, Dutta B, Takeda Y, Sack DA. Global dissemination of *Vibrio parahaemolyticus* serotype O3:K6 and its serovariants. *Clin Microbiol Rev.* 2007;20:39–48. <http://dx.doi.org/10.1128/CMR.00025-06>
- DePaola A, Kaysner CA, Bowers J, Cook DW. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). *Appl Environ Microbiol.* 2000;66:4649–54. <http://dx.doi.org/10.1128/AEM.66.11.4649-4654.2000>
- Abbott SL, Powers C, Kaysner CA, Takeda Y, Ishibashi M, Joseph SW, et al. Emergence of a restricted bioserovar of *Vibrio parahaemolyticus* as the predominant cause of vibrio-associated gastroenteritis on the West Coast of the United States and Mexico. *J Clin Microbiol.* 1989;27:2891–3.
- Jolley KA, Maiden MC. BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics.* 2010;11:595. <http://dx.doi.org/10.1186/1471-2105-11-595>
- Jolley KA, Hill DM, Bratcher HB, Harrison OB, Feavers IM, Parkhill J, et al. Resolution of a meningococcal disease outbreak from whole-genome sequence data with rapid Web-based analysis methods. *J Clin Microbiol.* 2012;50:3046–53. <http://dx.doi.org/10.1128/JCM.01312-12>
- Jolley KA, Maiden MC. Automated extraction of typing information for bacterial pathogens from whole genome sequence data: *Neisseria meningitidis* as an exemplar. *Euro Surveill.* 2013;18:20379.
- Bryant D, Moulton V. Neighbor-net: an agglomerative method for the construction of phylogenetic networks. *Mol Biol Evol.* 2004;21:255–65. <http://dx.doi.org/10.1093/molbev/msh018>
- Makino K, Oshima K, Kurokawa K, Yokoyama K, Uda T, Tagomori K, et al. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. *Lancet.* 2003;361:743–9. [http://dx.doi.org/10.1016/S0140-6736\(03\)12659-1](http://dx.doi.org/10.1016/S0140-6736(03)12659-1)

Address for correspondence: Narjol Gonzalez-Escalona, Food and Drug Administration, Center for Food and Applied Nutrition, 5100 Paint Branch Pkwy, College Park, MD 20740, USA; email: [narjol.gonzalez-escalona@fda.hhs.gov](mailto:narjol.gonzalez-escalona@fda.hhs.gov)

## Serologic Evidence of Leptospirosis in Humans, Union of the Comoros, 2011

**To the Editor:** Leptospirosis is a worldwide bacterial zoonosis caused by infection with pathogenic *Leptospira* spp. (Spirochaetales, Leptospiraceae). Most mammals can be infected, but rats are considered the main reservoir, maintaining *Leptospira* spirochetes in the lumen of renal tubules and contaminating the environment with bacteria-infected urine. Transmission to humans is accidental, occurring through contact with animal secretions or with contaminated environmental materials.

In temperate countries, human leptospirosis is a sporadic disease; incidence is much higher in the tropics because climate and environmental conditions are conducive to the survival

of bacteria, resulting in increased exposure of humans to leptospirosis-causing pathogens (1). Among islands in the southwestern Indian Ocean, human leptospirosis is endemic to Mayotte, France, and La Réunion (2–4) and to the Seychelles, where the incidence of leptospirosis is one of the highest worldwide (5). Leptospirosis is poorly documented in other islands in the region, including Mauritius, Madagascar, and the Union of the Comoros (2,6–8). Whether the scant documentation indicates underdiagnosis or reflects local epidemiologic specificities is unknown. To improve knowledge of *Leptospira* infection in the region, we conducted a study in the Union of the Comoros to serologically assess the presence or absence of leptospirosis in humans. The Union of the Comoros consists of 3 islands: Grande-Comore, Mohéli, and Anjouan. Together with a fourth, southern island, Mayotte, these islands form the Comoros Archipelago.

For feasibility reasons, we used excess serum samples. Seventy-six samples were from healthy volunteers who gave informed consent; 318 clinical blood samples from patients had been obtained by private laboratories and by the surveillance laboratory of the National Malaria Control Programme (PNLP) during August 1–October 8, 2011. The Ministère de la Santé, de la Solidarité et de la Promotion du Genre of the Union of the Comoros, authorized the serologic investigation (authorization no. 1175/MSSPG/DNS).

We used the microscopic agglutination test (MAT) to test serum samples; the MAT was based on a panel of 15 *Leptospira* strains, enabling the screening of all recently reported serogroups for human and animal cases on neighboring Mayotte (2,4,9). A list of the tested strains follows, shown as *Genus species* Serogroup/Serovar (type strain): *L. borgpetersenii* Ballum/Castellonis (Castellon 3), *L. borgpetersenii* Sejroe/Hardjovovis (Sponselee), *L. borgpetersenii* Sejroe/Sejroe (M 84),

# Pandemic *Vibrio parahaemolyticus*, Maryland, USA, 2012

## Technical Appendix

Table. Isolates that matched PFGE patterns K16S12.0138 and K16N11.0143 combination against PulseNet entries uploaded during February 4, 2010–April 16, 2013 and associated data\*

Isolate	Source state	Source type	Patient age, y	Patient sex	Isolate date	Upload date	PFGE-Sfil-pattern	PFGE-NotI-pattern
TX__TXACB1000107	TX	Human	42	M	1/15/2010	2/4/2010	K16S12.0138	K16N11.0143
CDC__AM47597	WA	Human		M	6/11/2011	10/19/2011	K16S12.0138	K16N11.0143
CDC__AM47598	WA	Human		M	6/14/2011	10/19/2011	K16S12.0138	K16N11.0143
CDC__101215235	NY	Human	33	M	7/9/2011	8/2/2012	K16S12.0138	K16N11.0143
CDC__PI11200004 H	AZ	Human	38	F	7/11/2011	10/15/2012	K16S12.0138	K16N11.0143
CDC__PI11200004 NH	AZ	Human	38	F	7/11/2011	10/15/2012	K16S12.0138	K16N11.0143
CDC__AM47605	WA	Human		M	7/20/2011	10/19/2011	K16S12.0138	K16N11.0143
TX__TXACB1102640	TX	Human	56	M	8/8/2011	9/8/2011	K16S12.0138	K16N11.0143
CDC__AM47611	WA	Human		M	8/18/2011	11/2/2011	K16S12.0138	K16N11.0143
CDC__AM47614	WA	Human		M	8/30/2011	11/2/2011	K16S12.0138	K16N11.0143
CDC__AM47617	WA	Human		M	9/23/2011	11/2/2011	K16S12.0138	K16N11.0143
CDC__PI11272013	AZ	Human	27	M	9/25/2011	8/2/2012	K16S12.0138	K16N11.0143
CA__M12X02339	CA	Human	39	M	6/15/2012	7/23/2012	K16S12.0138	K16N11.0143
CA__M12X02915	CA	Human	39	F	7/6/2012	7/27/2012	K16S12.0138	K16N11.0143
MD__MDA12147539	MD	Human	53	M	8/21/2012	10/9/2012	K16S12.0138	K16N11.0143
MD__MDA12148581	MD	Human	49	F	8/22/2012	10/9/2012	K16S12.0138	K16N11.0143
MD__MDA12162046	MD	Human	51	NK	8/24/2012	10/9/2012	K16S12.0138	K16N11.0143
TX__TXACB1202640	TX	Human	67	F	8/20/2012	10/10/2012	K16S12.0138	K16N11.0143
CDC__PI12255002	AZ	Human		NK		11/5/2012	K16S12.0138	K16N11.0143
CDC__PI12256006	AZ	Human		NK		11/5/2012	K16S12.0138	K16N11.0143
CDC__M12X4985 G	CA	Human				1/28/2013	K16S12.0138	K16N11.0143
CDC__M12X4985 W	CA	Human				1/28/2013	K16S12.0138	K16N11.0143
CDC__M12X05012 G	CA	Human				1/28/2013	K16S12.0138	K16N11.0143
CDC__M12X05012 W	CA	Human				1/28/2013	K16S12.0138	K16N11.0143
NY__IDR1300009145	NY	Human	49	M	3/29/2013	4/16/2013	K16S12.0138	K16N11.0143

\* PFGE, pulsed-field gel electrophoresis; NK, not known.