

Antibodies to *B. melitensis* were detected by agglutination by using the Rose Bengal and *Brucella* Wright tests (both from BioRad, Hercules, CA, USA). Of the 63 patients, 9 had a positive antibody response against a tested antigen (Table): 1 to phase I *C. burnetii* and 8 to *Bartonella* spp. (IgG ≥ 200). Of these, 7 had a 1-fold dilution higher titer to *B. quintana* than to *B. henselae*, including 1 with a low-level cross-reaction with *C. burnetii*, and 1 with identical titers to both. For all 8 patients, Western blot results were consistent with *Bartonella* endocarditis. For 7, cross-adsorption identified *B. quintana* as the causative species; for the other, the infecting *Bartonella* species remained undetermined because adsorption with *B. quintana* and *B. henselae* antigens removed all antibodies. Serologic results for *B. melitensis* were negative for all patients.

B. quintana is mostly associated with human body lice but has also been found in fleas (9). The predisposing factors for *B. quintana* endocarditis are homelessness, alcoholism, and exposure to body lice (10). For our patients, the common predisposing factors were poor hygiene and low socioeconomic status, which may expose them to ectoparasites including lice and fleas. In contrast with previous study findings, *B. quintana* infectious endocarditis developed on pre-existing valvular lesions in all patients (10). This finding may reflect a different clinical evolution than in Europe, where studies have suggested that *B. quintana* infectious endocarditis followed chronic bacteremia in patients who did not have previous valvular defects (10).

In summary, prevalence of negative blood culture among patients with infectious endocarditis was high (72%). The most commonly associated risk factor was rheumatic heart disease (Table). *C. burnetii* and *Bartonella* spp. were responsible for 8% of all infectious endocarditis cases and 14% of blood culture-negative cases. No

case of infectious endocarditis caused by *B. melitensis* was identified.

Our preliminary study suggests that zoonotic agents, especially *Bartonella* spp., are prevalent causative organisms of blood culture-negative endocarditis in India. We recommend serologic screening for antibodies to zoonotic microorganisms as diagnostic tools for this disease in India.

**Nandhakumar Balakrishnan,*
Thangam Menon,*
Pierre-Edouard Fournier,†
and Didier Raoult†**

*University of Madras, Taramani, Chennai, India; and †Universite de la Mediterranee, Marseille, France

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References

1. Brouqui P, Raoult D. New insight into the diagnosis of fastidious bacterial endocarditis. *FEMS Immunol Med Microbiol*. 2006;47:1–13. DOI: 10.1111/j.1574-695X.2006.00054.x
2. Koegelenberg CF, Doubell AF, Orth H, Reuter H. Infective endocarditis in the Western Cape Province of South Africa: a three-year prospective study. *QJM*. 2003;96:217–25. DOI: 10.1093/qjmed/hcg028
3. Benslimani A, Fenollar F, Lepidi H, Raoult D. Bacterial zoonoses and infective endocarditis, Algeria. *Emerg Infect Dis*. 2005;11:216–24.
4. Tariq M, Alam M, Munir G, Khan MA, Smego RA Jr. Infective endocarditis: a five-year experience at a tertiary care hospital in Pakistan. *Int J Infect Dis*. 2004;8:163–70. DOI: 10.1016/j.ijid.2004.02.001
5. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis*. 2000;30:633–8. DOI: 10.1086/313753
6. Dupont HT, Thirion X, Raoult D. Q fever serology: cutoff determination for microimmunofluorescence. *Clin Diagn Lab Immunol*. 1994;1:189–96.
7. Fournier PE, Mainardi JL, Raoult D. Value of microimmunofluorescence for diagnosis and follow-up of *Bartonella* endocarditis. *Clin Diagn Lab Immunol*. 2002;9:795–801. DOI: 10.1128/CDLI.9.4.795-801.2002
8. Houpikian P, Raoult D. Western immunoblotting for *Bartonella* endocarditis. *Clin Diagn Lab Immunol*. 2003;10:95–102. DOI: 10.1128/CDLI.10.1.95-102.2003
9. Marie JL, Fournier PE, Rolain JM, Briolant S, Davoust B, Raoult D. Molecular detection of *Bartonella quintana*, *B. elizabethae*, *B. koehlerae*, *B. doshiae*, *B. taylorii*, and *Rickettsia felis* in rodent fleas collected in Kabul, Afghanistan. *Am J Trop Med Hyg*. 2006;74:436–9.
10. Fournier PE, Lelievre H, Eykyn SJ, Mainardi JL, Marrie TJ, Brunel F, et al. Epidemiologic and clinical characteristics of *Bartonella quintana* and *Bartonella henselae* endocarditis: a study of 48 patients. *Medicine (Baltimore)*. 2001;80:245–51. DOI: 10.1097/00005792-200107000-00003

Address for correspondence: Didier Raoult, Unite des Rickettsies, IFR 48 CNRS, UMR 6020 Universite de la Mediterranee, Faculté de Médecine, 27 blvd Jean Moulin, 13385 Marseille Cedex 05, France; email: didier.raoult@gmail.com

Acute Gastroenteritis Caused by GI/2 Sapovirus, Taiwan, 2007

To the Editor: Sapovirus is an etiologic agent of human gastroenteritis. Although many of the previously reported cases were of mild, sporadic infections in young children (1–3), several recent sapovirus-associated gastroenteritis outbreaks have affected adults, which suggests that the virus's virulence, prevalence, or both, may be increasing (4–6). In this study, we describe a sapovirus-associated outbreak of gastroenteritis that occurred during May 4–8, 2007, and involved college students in northern Taiwan.

A total of 55 students had clinical symptoms of gastroenteritis, including diarrhea (45), vomiting (22), abdominal cramps (17), and fever (2). The clinical symptoms continued for up to 10 days (mean 4.7 days). Stool

specimens were collected from 8 of 55 students on May 8 (Table). Initially, the specimens were screened for bacteria, rotavirus, and norovirus, but all specimens were negative for these pathogens. The 8 stool specimens were then examined by electron microscopy (EM), and 1 was positive for calicivirus-like particles.

To confirm the EM results, we performed reverse transcription-PCR (RT-PCR), real-time RT-PCR, and sequence analysis as previously described (7). Briefly, purified RNA (10 μ L) was reverse transcribed by using SuperScript III reverse transcriptase according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). PCR was carried out by using the SV-F11 and SV-R1 primer set directed against the conserved N terminal capsid region (8). The PCR products were analyzed with 2% agarose gel electrophoresis and visualized after ethidium bromide staining. The PCR-generated amplicons (\approx 780 bp) were excised from the gel and purified by the QIAquick gel extraction kit (QIAGEN, Hilden, Germany).

Nucleotide sequences were prepared with the terminator cycle sequence kit (version 3.1) and determined with the ABI 3130 sequencer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were aligned by using ClustalX (www.clustal.org), and the distances were calculated by using the Kimura 2-parameter method. A phylogenetic tree was generated by the neighbor-

joining method as described previously (1,8).

Of the 8 specimens, 7 were positive by RT-PCR and real-time RT-PCR (Table). SaV124F, SaV1F, SaV5F, and SaV1245R primers as well as SaV124TP and SaV5TP minor-groove binding probes were used for real-time RT-PCR diagnosis, which targets the sapovirus RdRp-capsid junction region as described (7). The number of sapovirus cDNA copies ranged from 2.86×10^7 to 1.72×10^{10} copies/g of stool specimen; mean was 2.71×10^9 copies/g of stool specimen (Table). Sequence analysis of the 7 positive specimens showed 100% nucleotide identity (nt 5098–5878), indicating that the outbreak was caused by 1 sapovirus strain.

To better classify the sapovirus, we reamplified the 3' end of the genome from 1 positive specimen and sequenced \approx 2,400 nt (nt 5074–3') (Hu/SaV/9–5/Taipei/07/TW; GenBank accession no. EU124657). PCR was performed with SV-F13, SV-F14, and TX30SXN primers as described (1). Database searches found a closely matching sapovirus sequence (99%) that was detected in a patient with gastroenteritis in Japan, in 2004 (Chiba041413 strain; GenBank accession no. AB258427). The next closely matching sequence was detected in an outbreak of gastroenteritis among adults in the United States in 1994 (Parkville strain; HCU73124) (6). Phylogenetic analysis clustered these 3 sapovirus sequences into genogroup I/genotype 2 (GI/2) (online Appendix

Figure, available from www.cdc.gov/content/EID/14/7/1169-appG.htm).

Sapovirus was reported in Japan in water samples (untreated wastewater, treated wastewater, and a river) and in clam samples intended for human consumption (1). Apart from these 2 environmental studies, little is known about reservoir of sapovirus or its route of infection in the natural environment. The source of contamination in this current outbreak was not determined; however, none of the food handlers associated with the college reported symptoms of gastroenteritis. However, in a recent molecular epidemiologic study in Japan, a large number of symptomatic and asymptomatic food handlers were found to be infected with noroviruses (9). Several seroprevalence studies also indicated high prevalence rates of antibodies to sapovirus in adults and children (10). All of these findings highlight the need to collect stool specimens from asymptomatic persons and indicate possible "silent" transmission through an asymptomatic route. Symptoms of sapovirus infection are thought to be milder than symptoms of norovirus infections. However, in this study approximately one third (17) of the 55 students reported symptoms of abdominal pain and 22 (40%) reported symptoms of vomiting. Many of the earlier sapovirus studies described sapovirus GI/1 infections in young Japanese children (1), which indicated that infecting virus had a different genotype than the virus detected in this study (GI/2).

Table. Clinical symptoms and laboratory diagnosis results for sapovirus-related outbreak among college students, northern Taiwan, May 2007*†

Specimen no.	Patient sex/age, y	Date of illness onset	EM results	RT-PCR results	Copies cDNA/g of stool‡	Symptom			
						Fever	Diarrhea	Vomiting	Abdominal pain
1	F/20	May 5	–	+	1.69×10^8	–	+	+	+
2	F/26	May 5	–	+	6.19×10^8	–	+	+	–
3	M/19	May 6	–	+	2.32×10^8	–	+	–	+
4	M/18	May 6	–	+	3.24×10^8	–	+	+	+
5	F/21	May 7	+	+	1.72×10^{10}	–	+	–	–
6	F/18	May 4	–	–	–	+	+	+	–
7	M/19	May 7	–	+	4.28×10^8	–	+	+	+
8	F/20	May 6	–	+	2.86×10^7	–	+	–	+

*EM, electron microscopy; RT-PCR, reverse transcription-PCR; –, negative; +, positive.

†All specimens were collected May 8.

‡cDNA copies were determined by real-time PCR.

In addition, the viral load in this study appeared to be comparatively high. These results suggest that some sapovirus genotypes are more virulent than others. Similar findings were obtained with norovirus infections around the world; strains belonging to norovirus GII/4 were the most prevalent in many countries. Although several recombinant sapovirus strains have been identified and found to be the cause of increased numbers of infections in some countries (1,5), they were not observed in this study. Increased sapovirus surveillance and reporting are needed to shed some more light on this poorly understood virus.

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**Fang-Tzy Wu,* Tomoichiro Oka,†
Naokazu Takeda,†
Kazuhiko Katayama,†
Grant S. Hansman,†
Chih-Hsin Muo,*
Shy-Yuan Liang,*
Ching-Hsiang Hung,*
Donald Dah-Shyong Jiang,*
Jui Hsin Chang,*
Jyh-Yuan Yang,* Ho-Sheng Wu,*
and Chen-Fu Yang***

*Centers for Disease Control, Taipei, Taiwan, Republic of China; and †National Institute of Infectious Diseases, Tokyo, Japan

References

1. Hansman GS, Oka T, Katayama K, Takeda N. Human sapoviruses: genetic diversity, recombination, and classification. *Rev Med Virol.* 2007;17:133–41. DOI: 10.1002/rmv.533
2. Khamrin P, Maneekarn N, Peerakome S, Tonusin S, Malasao R, Mizuguchi M, et al. Genetic diversity of noroviruses and sapoviruses in children hospitalized with acute gastroenteritis in Chiang Mai, Thailand. *J Med Virol.* 2007;79:1921–6. DOI: 10.1002/jmv.21004

3. Monica B, Ramani S, Banerjee I, Primrose B, Iturriza-Gomara M, Gallimore CI, et al. Human caliciviruses in symptomatic and asymptomatic infections in children in Vellore, South India. *J Med Virol.* 2007;79:544–51. DOI: 10.1002/jmv.20862
4. Hansman GS, Saito H, Shibata C, Ishizuka S, Oseto M, Oka T, et al. An outbreak of gastroenteritis due to sapovirus. *J Clin Microbiol.* 2007;45:1347–9. DOI: 10.1128/JCM.01854-06
5. Hansman GS, Ishida S, Yoshizumi S, Miyoshi M, Ikeda T, Oka T, et al. Recombinant sapovirus gastroenteritis, Japan. *Emerg Infect Dis.* 2007;13:786–8.
6. Noel JS, Liu BL, Humphrey CD, Rodriguez EM, Lambden PR, Clarke IN, et al. Parkville virus: a novel genetic variant of human calicivirus in the Sapporo virus clade, associated with an outbreak of gastroenteritis in adults. *J Med Virol.* 1997;52:173–8. DOI: 10.1002/(SICI)096-9071(199706)52:2<173::AID-JMV10>3.0.CO;2-M
7. Oka T, Katayama K, Hansman GS, Kageyama T, Ogawa S, Wu FT, et al. Detection of human sapovirus by real-time reverse transcription-polymerase chain reaction. *J Med Virol.* 2006;78:1347–53. DOI: 10.1002/jmv.20699
8. Okada M, Shinozaki K, Ogawa T, Kaiho I. Molecular epidemiology and phylogenetic analysis of Sapporo-like viruses. *Arch Virol.* 2002;147:1445–51. DOI: 10.1007/s00705-002-0821-7
9. Ozawa K, Oka T, Takeda N, Hansman GS. Norovirus infections in symptomatic and asymptomatic food handlers in Japan. *J Clin Microbiol.* 2007;45:3996–4005. DOI: 10.1128/JCM.01516-07
10. Farkas T, Deng X, Ruiz-Palacios G, Morrow A, Jiang X. Development of an enzyme immunoassay for detection of sapovirus-specific antibodies and its application in a study of seroprevalence in children. *J Clin Microbiol.* 2006;44:3674–9. DOI: 10.1128/JCM.01087-06

Address for correspondence: Chen-Fu Yang, 161 Kunyang St, Nan Kang, Taipei 115, Taiwan, Republic of China; email: cxy1@cdc.gov.tw



Importation of West Nile Virus Infection from Nicaragua to Spain

To the Editor: We report the case of a 51-year-old Spanish missionary who had lived Nicaragua (Managua) from 2004 to 2006. He had no other notable travel history during that period. In June 2006, he noticed malaise and nausea, followed by abrupt onset of fever (39°C), headache, cervical pain, and right hemiparesis. He was admitted to a local hospital in Nicaragua, at which time routine results of hematologic and biochemistry tests were within normal limits, except for mild neutrophilia. After cerebral magnetic resonance imaging (MRI), a diagnosis of ischemic cerebrovascular accident was made. He was treated with aspirin and ceftriaxone for an oropharyngeal infection.

Because neurologic symptoms persisted, 13 days later he was transferred to a hospital in Madrid, Spain. At that time, physical examination showed neck stiffness, a diminished level of consciousness, right flaccid hemiparesis, and facial weakness. Peripheral blood examination showed only mild neutrophilia. Cerebrospinal fluid (CSF) analysis showed a 65 mg/dL glucose level (blood glucose 140), proteins 136 g/dL, and 18 cells/mm³ (mainly lymphocytes). Serologic test results for HIV, hepatitis B virus, hepatitis C virus, syphilis, *Toxoplasma* spp., and *Brucella* spp., and CSF cultures for mycobacterial, bacterial, and fungal infections were all negative. Results of a computed tomographic scan of the brain were within normal limits. MRI showed nonspecific abnormal intensity of white matter signal. Electrophysiologic studies showed severe axonal motor neuropathy and moderate sensitive axonal neuropathy in the right upper limb. Gammaglobulin was administered intravenously for 5 days; the patient improved slightly. At