

cattle sera (1,3), we cannot exclude the possibility that the S-1-HUN-like kobuvirus can cause viremia (and generalized infection) in swine. S-1-HUN-like virus may typically cause asymptomatic infections in swine. However, epidemiologic and molecular studies are required regarding the importance of this virus as a causative agent of some diseases of domestic pigs and related animals. Sequence analysis of the complete nucleotide and amino acid sequences of coding (L, P1, P2, and P3: 7,467 nt) and noncoding regions and the genetic organization strain indicate that S-1-HUN is a typical kobuvirus. Phylogenetic analysis shows that S-1-HUN strain is genetically included in the genus *Kobuvirus* but is distinct from Aichi and bovine kobuviruses. Porcine kobuvirus strain S-1-HUN is a candidate for a new, third species of the genus *Kobuvirus*.

This work was supported by grants from the Hungarian Scientific Research Fund (OTKA, F048433), and the Enteric Virus Emergence, New Tools (EVENT, SP22-CT-2004-502571) funded by the European Union.

**Gábor Reuter, Ákos Boldizsár,
István Kiss,
and Péter Pankovics**

Author affiliations: ÁNTSZ Regional Institute of State Public Health Service, Pécs, Hungary (G. Reuter, Á. Boldizsár, P. Pankovics); and Veterinary Diagnostic Directorate, Debrecen, Hungary (I. Kiss)

DOI: 10.3201/eid1412.080797

References

1. Online ICTV. Discussion forum of the International Committee on Taxonomy of Viruses, ICTV 2008 Official Taxonomy [cited 2008 Oct 22]. Available from <http://talk.ictvonline.org>
2. Yamashita T, Kobayashi S, Sakae K, Nakata S, Chiba S, Ishihara Y, et al. Isolation of cytopathic small round viruses with BS-C-1 cells from patients with gastroenteritis. *J Infect Dis*. 1991;164:954–7.
3. Yamashita T, Ito M, Kabashima Y, Tsuzuki H, Fujiura A, Sakae K. Isolation and characterization of a new species of kobuvirus associated with cattle. *J Gen Virol*. 2003;84:3069–77. DOI: 10.1099/vir.0.19266-0
4. Pham NT, Khamrin P, Nguyen TA, Kanti DS, Phan TG, Okitsu S, et al. Isolation and molecular characterization of Aichi viruses from fecal specimens collected in Japan, Bangladesh, Thailand, and Vietnam. *J Clin Microbiol*. 2007;45:2287–8. DOI: 10.1128/JCM.00525-07
5. Oh DY, Silva PA, Huroeder B, Deidrich S, Cardoso DD, Schreier E. Molecular characterization of the first Aichi viruses isolated in Europe and in South America. *Arch Virol*. 2006;151:1199–206. DOI: 10.1007/s00705-005-0706-7
6. Ambert-Balay K, Lorrot M, Bon F, Giraudon H, Kaplon J, Wolfer M, et al. Prevalence and genetic diversity of Aichi virus strains in stool samples from community and hospitalized patients. *J Clin Microbiol*. 2008;46:1252–8. DOI: 10.1128/JCM.02140-07
7. Khamrin P, Maneekarn N, Peerakome S, Okitsu S, Mizuguchi M, Ushijama H. Bovine kobuviruses from cattle with diarrhea. *Emerg Infect Dis*. 2008;14:985–6.
8. Jiang X, Huang PW, Zhong WM, Farkas T, Cubitt DW, Matson DO. Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. *J Virol Methods*. 1999;83:145–54. DOI: 10.1016/S0166-0934(99)00114-7
9. Reuter G, Krisztalovics K, Vennema H, Koopmans M, Szűcs Gy. Evidence of the etiological predominance of norovirus in gastroenteritis outbreaks—emerging new variant and recombinant noroviruses in Hungary. *J Med Virol*. 2005;76:598–607. DOI: 10.1002/jmv.20403
10. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol Biol Evol*. 2007;24:1596–9. DOI: 10.1093/molbev/msm092

Address for correspondence: Gábor Reuter, Regional Laboratory of Virology, National Reference Laboratory of Gastroenteric Viruses, ÁNTSZ Regional Institute of State, Public Health Service, Szabadság út 7, H-7623 Pécs, Hungary; email: reuter.gabor@baranya.antsz.hu

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

Human Case of *Rickettsia felis* Infection, Taiwan

To the Editor: *Rickettsia felis*, the etiologic agent of flea-borne spotted fever, is carried by fleas worldwide (1). In the past decade, several human cases of *R. felis* infection have been reported (1–3). Clinical symptoms and biological data for *R. felis* infections are similar to those for murine typhus and other rickettsial diseases, which makes clinical diagnosis difficult (2). Patients with *R. felis* infections may have common clinical manifestations, such as fever, headache, myalgia, macular rash, and elevated levels of liver enzymes (4,5).

Reportable rickettsioses in Taiwan include scrub typhus, epidemic typhus, and murine typhus. Although there are no known human cases of infections caused by spotted fever group (SFG) rickettsiae in Taiwan, novel strains of SFG rickettsiae have been isolated as recently described (6,7). In addition, evidence for *R. felis* infections in cat and cat flea populations has been identified by using immunofluorescence assay (IFA), PCR, and organism isolation (K.-H. Tsai et al., unpub. data). We report an indigenous human case of *R. felis* infection in Taiwan.

In January 2005, a 27-year-old woman living in Fongshan City, Kaohsiung County, in southern Taiwan was admitted to Kaohsiung Medical University Hospital with a 4-day history of intermittent fever (37.8°C–38.0°C), chills, headache, and fatigue. Associated symptoms were frequent micturition and a burning sensation upon voiding. The patient was admitted with a possible urinary tract infection; urinalysis showed pyuria (leukocyte count 25–50/high-power field), compatible with the clinical diagnosis. During the 6-day hospital stay, the patient received daily intravenous first-generation cephalosporin (cefazolin); gentamicin was given only on the

first 3 days. She was discharged with a prescription for oral antimicrobial drugs (cephradine 500 mg every 6 h) to be taken for 7 days. Micturition-associated symptoms subsided after treatment.

The patient also had headaches and glove-and-stocking numbness in both hands because of fever, but denied any associated rash and arthralgia. Although the patient did not recall any arthropod bites, she had noticed some stray dogs and cats nearby and rodents in the neighborhood surrounding her house. Because of acute polyneuropathy-like symptoms and exposure history, we prescribed oral doxycycline (100 mg every 12 hours) for 5 days as empirical therapy on the second day at the hospital, suspecting a zoonosis such as rickettsioses, Q fever, or leptospirosis. Headache and glove-and-stocking numbness subsided. Her blood sugar level and thyroid function were within normal limits. Chest radiograph, liver function, renal function, and levels of electrolytes were all normal.

Whole blood counts were normal, although differential counts demonstrated a left shift (polymorphonuclear leukocytes 80.7%, reference range 37%–75%). C-reactive protein level was 66.7 µg/mL, (reference <5 µg/mL) upon admission. Blood culture and urine culture were negative for bacteria.

Additionally, painful vesicles on the external genitalia appeared on the fourth day postadmission, and valacyclovir was administered for 5 days

because of suspected infection with herpes simplex virus. The lesion subsided after valacyclovir treatment and the patient was discharged in good condition.

Patient whole blood specimens were collected on days 4 and 16 after the onset of fever and sent to the Taiwan Centers for Disease Control for laboratory diagnosis of rickettsial infection. For molecular diagnosis, DNA from the acute-phase blood sample (day 4) was analyzed by using the SYBR green-based real-time PCR specific for 17-kDa antigen, 60-kDa heat-shock protein (*groEL*) gene, and outer membrane protein B (*ompB*) gene for typhus group and SFG rickettsiae and primers listed in the Table. Nucleotide sequences of real-time PCR products demonstrated 100% identity with 17-kDa antigen, *groEL*, and *ompB* genes of *R. felis* URRWXC12. Real-time PCR results were negative for *Orientia tsutsugamushi* and *Coxiella burnetii* (8).

For serologic diagnosis, serum samples were tested for rickettsial-specific antibodies by IFA using whole cell antigens of *R. felis* isolated from the cat flea. The patient's serum (days 4 and 16) had immunoglobulin (Ig) G, IgA, and IgM titers of 160 and 2,560, respectively. The serum sample collected from *R. felis*-infected cat served as the positive control. Test results were negative for *R. typhi*, *R. conorii*, *R. rickettsii*, *R. japonica*, *O. tsutsugamushi*, and *C. burnetii*.

Absence of rash, eschar, and unawareness of arthropod bite may be easily overlooked in some patients

with rickettsial infections. In this case, suspicion of rickettsial infection was based on exposure history and acute polyneuropathy, which responded quickly to doxycycline treatment. There are limited reports of rickettsioses with polyneuropathy, and none for cases of *R. felis* infection (9,10). It was hard to tell whether the urinary tract and herpes simplex virus infections were associated with an *R. felis* infection, but it is quite rare for 3 different infections to occur in a patient at the same time as isolated entities. The finding of a human case of infection and the existence of *R. felis* in cat fleas highlights the need for further studies on flea-borne rickettsioses in Taiwan.

**Kun-Hsien Tsai, Hsiu-Ying Lu,
Jih-Jin Tsai, Sheng-Kai Yu,
Jyh-Hsiung Huang,
and Pei-Yun Shu**

Author affiliations: Centers for Disease Control, Taipei, Taiwan, Republic of China (K.-H. Tsai, H.-Y. Lu, S.-K. Yu, J.-H. Huang, P.-Y. Shu); and Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China (J.-J. Tsai)

DOI: 10.3201/eid1412.080525

References

1. Pérez-Osorio CE, Zavala-Velázquez JE, Aris León JJ, Zavala-Castro JE. *Rickettsia felis* as emergent global threat for humans. *Emerg Infect Dis*. 2008;14:1019–23. DOI: 10.3201/eid1407.071656
2. Pérez-Arellano JL, Fenollar F, Angel-Moreno A, Bolaños M, Hernández M, Santana E, et al. Human *Rickettsia felis* infection, Canary Islands, Spain. *Emerg Infect Dis*. 2005;11:1961–4.

Table. Primer sequences for the SYBR-based real-time PCR assay*

Primer	Genomic region	Sequence (5' → 3')	Amplicon size, bp
RR-F8	17-kDa (<i>Rickettsia</i> spp.)	GGC GGY GCA TTA CTT GGT TCT CAA TTC GC	304
RR-R12		GTT TTC CSC CTA TTA CAA CTG TTT GAG T	
RR-F1	<i>groEL</i> gene (<i>Rickettsia</i> spp.)	AAA ATG GTT GCT GAG CTT GAA AAT CCT TT	191
RR-R2		ACT TTC AAA CCA CCA CGT AAT CTA TTG AC	
RR-F22	<i>ompB</i> gene (<i>Rickettsia</i> spp.)	ATG GTR TAT GGG CWA AAC CTT TCT ATA	330
RR-R25		TAG MMT CGA AGA AGT AAC GCT GAC TTT	
RST-14F	56-kDa gene (<i>Orientia tsutsugamushi</i>)	CCA TTT GGT GGT ACA TTA GCT GCA GGT	233
RST-6R		TCA CGA TCA GCT ATA CTT ATA GGC A	
OMP3	<i>com-1</i> (<i>Coxiella burnetii</i>)	GAA GCG CAA CAA GAA GAA CAC	438
OMP4		TTG GAA GTT ATC ACG CAG TTG	

**groEL*, 60-kDa heat-shock protein; *ompB*, outer membrane protein B; *com-1*, 27-kDa outer membrane protein.

3. Parola P, Miller RS, McDaniel P, Telford SR, Rolain JM, Wongsrichanalai C, et al. Emerging rickettsioses of the Thai-Myanmar border. *Emerg Infect Dis*. 2003;9:592-5.
4. Schriefer ME, Sacchi JB, Dumler JS, Bullen MG, Azad AF. Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. *J Clin Microbiol*. 1994;32:949-54.
5. Zavala-Velázquez JE, Ruiz-Sosa JA, Sánchez-Elias RA, Becerra-Carmona G, Walker DH. *Rickettsia felis* rickettsiosis in Yucatán. *Lancet*. 2000;356:1079-80. DOI: 10.1016/S0140-6736(00)02735-5
6. Tsai KH, Wang HC, Chen CH, Huang JH, Lu HY, Su CL, et al. Isolation and identification of a novel spotted fever rickettsiae, strain IG-1, from *Ixodes granulatus* ticks collected on Orchid Island (Lanyu), Taiwan. *Am J Trop Med Hyg*. 2008;79:256-61.
7. Tsui PY, Tsai KH, Weng MH, Hung YW, Liu YT, Hu KY, et al. Molecular detection and characterization of spotted fever group rickettsiae in Taiwan. *Am J Trop Med Hyg*. 2007;77:883-90.
8. Zhang GQ, Hotta A, Mizutani M, Ho T, Yamaguchi T, Fukushi H, et al. Direct identification of *Coxiella burnetii* plasmids in human sera by nested PCR. *J Clin Microbiol*. 1998;36:2210-3.
9. Raoult D, Weiller JP, Chagnon A, Chaudet H, Gallais H, Casanova P. Mediterranean spotted fever: clinical, laboratory and epidemiological features of 199 cases. *Am J Trop Med Hyg*. 1986;35:845-50.
10. Font-Creus B, Bella-Cueto F, Espejo-Arenas E, Vidal-Sanahuja R, Muñoz-Espin T, Nolla-Salas M, et al. Mediterranean spotted fever: a cooperative study of 227 cases. *Rev Infect Dis*. 1985;7:635-42.

Address for correspondence: Pei-Yun Shu, Vector-Borne Viral and Rickettsial Diseases Laboratory, Research and Diagnostic Center, Centers for Disease Control, Department of Health, Taipei 11561, Taiwan, Republic of China; email: pyshu@cdc.gov.tw



***Bartonella* spp. and *Rickettsia felis* in Fleas, Democratic Republic of Congo**

To the Editor: *Bartonella* and *Rickettsia* species are pathogens of humans and domestic mammals that may be transmitted by fleas and other arthropods. *Rickettsia felis* causes flea-borne spotted fever in humans who come into contact with flea-infested domestic and peridomestic animals; worldwide distribution of this pathogen in ectoparasites and mammals makes it an emerging threat to human health (1,2). Likewise, species of the genus *Bartonella* are associated with an increasing array of human diseases, including trench fever, cat-scratch disease, and endocarditis in immunocompetent patients, and bacillary angiomatosis and peliosis hepatitis in immunocompromised patients (3-5). Although *Bartonella* spp. and *R. felis* appear to be globally distributed, their presence in the Democratic Republic of Congo (DRC) has not been previously documented.

Off-host *Pulex irritans*, *Tunga penetrans*, *Ctenocephalides felis strongylus*, *Echidnophaga gallinacea*, and *Xenopsylla brasiliensis* were collected in the Ituri district of north-eastern DRC from March through April 2007, during an investigation of a plague outbreak. Our investigation area was limited to 4 villages: Djalusene and Kpandruma, which had confirmed plague patients, and Wanyale and Zaa, which had several suspect cases (6).

We collected fleas by using a kerosene lamp hung above a 45-cm diameter tray containing water (7). Captured fleas were identified using a dissecting microscope and standard morphologic keys, sorted into vials by species and locality, and preserved in 70% ethanol (7). Fleas were separated into 193 pools (2-5 fleas per pool), triturated for 10 minutes; the resultant

flea triturate was centrifuged at 3,000 rpm for 10 minutes to collect flea tissue. DNA was then obtained by using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA).

Bartonella DNA was detected by PCR amplifying a 379-bp fragment of the citrate synthase gene (*gltA*) (8). For *Rickettsia typhi* and *R. felis*, a real-time multiplex PCR assay targeting a conserved fragment of *gltA* was used (unpub. data). All assays were run in duplicate, and positive and negative controls were included in all assays. Amplicons were purified with the QIAquick PCR purification kit (QIAGEN) and sequenced in both directions by using a BigDye sequencing kit (Applied Biosystems, Foster City, CA, USA) with the same primers used for PCR amplification. Resultant sequences of *Bartonella* spp. were aligned with MegAlign by using the Clustal algorithm (DNASTAR, Inc., Madison, WI, USA), and compared with reference sequences obtained from GenBank.

Although *Yersinia* DNA and *R. typhi* were not detected, 89 of the 193 pools were PCR positive for either *Bartonella* spp. or *R. felis* (Table). Using the Microsoft Excel Add-In PooledInRate software (Redmond, WA, USA; www.cdc.gov/ncidod/dvbid/westnile/software.htm), we calculated an estimated infection rate of 10.72% (95% confidence interval [CI] 8.52-13.31) for *R. felis*, 3.66% for *Bartonella* species, and 0.91% (95% CI 0.40-1.78) for both *Bartonella* spp. and *R. felis* (Table).

Phylogenetic analysis indicated several *Bartonella* spp. in fleas that were closely aligned with pathogenic *Bartonella* spp., including *B. vinsonii*, *Candidatus B. rochalimaea*, and *B. clarridgeiae* (data not shown). Moreover, *Bartonella* from 3 pools of *P. irritans* demonstrated only 1.8% to 2.4% divergence to *B. vinsonii* subspecies *arupensis* isolated from a human patient in Wyoming, USA. Likewise, sequences of *Bartonella* from