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.

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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 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 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 assay 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 URRWXCal2. 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.

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

| Primer | Genomic region | Sequence (5′ → 3′) | Amplicon size, bp |
|--------|----------------|---------------------|------------------|
| RR-F8  | 17-kDa (Rickettsia spp.) | GGC GGY GCA TTA CTT GGT TCT CAA TTC GC | 304 |
| RR-R12 | 60-kDa heat-shock protein | GTT TTC CSC CTA TTA CAA CTG TTT GAT | |
| RR-F1  | groEL gene (Rickettsia spp.) | AAA ATG GTT GCT GAG CTT TT | 191 |
| RR-R2  | ACT TTC AAA CCA CCA GGT AAT CTA TTG AC | |
| RR-R22 | ompB gene (Rickettsia spp.) | ATG GTR TAT GGG CWA AAC CTI TCT ATA | 330 |
| RR-R25 | TAG MTT CCA AGA AGT AAC GCT GAC TTT | |
| RST-14F | 56-kDa gene (Orientia tsutsugamushi) | CCA TTT GGG GGT GAT ACA TTA GCT GCA GGT | 233 |
| RST-6R  | TCA CGA TCA GCT ATA CTA ATA GGC A | |
| OMP3   | com-1 (Coxiella burnetii) | GAA GGC CAA CAA CAA 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.
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 northeastern 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 fleas were triturated 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. rochalimae, and B. claridgeaei (data not shown). Moreover, Bartonella from 3 pools of P. irritans demonstrated only 1.8% to 2.4% divergence to B. vinsonii sub-species arupensis isolated from a human patient in Wyoming, USA. Likewise, sequences of Bartonella from...