

ity with *R. sibirica* subsp. *mongolotimonae* when compared with those in the GenBank database.

Infections caused by *R. sibirica* subsp. *mongolotimonae* have been reported as lymphangitis-associated rickettsiosis (4). Our case-patient had the clinical symptoms reported for this disease: fever, maculopapular rash, eschar, enlarged satellite lymph nodes, and lymphangitis. Seasonal occurrence of this disease in the spring is common and has been reported in 9 of 12 cases, including the case-patient reported here (2–6). A total of 75% of these *R. sibirica* subsp. *mongolotimonae* infections occurred in southern France; other cases have been recently reported in Greece (5), Portugal (6), and South Africa (7). However, the vector of *R. sibirica* subsp. *mongolotimonae* has not been identified (7). This rickettsia has been isolated from *Hyalomma asiaticum* ticks in Inner Mongolia, from *H. truncatum* in Niger (8), and from *H. anatolicum excavatum* in Greece (5). *Hyalomma* spp. ticks are suspected of being the vector and are widespread in Africa, southeastern Europe (including France), and Asia.

Rickettsiosis caused by *R. rickettsii* and *R. conorii* during pregnancy has been reported without risk for vertical transmission (9). First-line antimicrobial drugs used to treat rickettsial disease are cyclines and quinolones, but they are contraindicated during pregnancy. Chloramphenicol is an alternative drug for pregnant women but it is not available in France. Macrolides (azithromycin, clarithromycin, and josamycin) are effective against rickettsial disease and can be used safely during pregnancy.

No ocular complications were reported in the 11 previous cases of rickettsiosis caused by *R. subsp. mongolotimonae*. However, ocular lesions, including optic disk staining, white retinal lesions, retinal hemorrhages, multiple hypofluorescent choroidal dots, mild vitritis, and retinal vasculitis, have been described in patients

with rickettsiosis caused by *R. conorii*, *R. rickettsii*, and *R. typhi* (10). Most of these posterior segment manifestations are usually asymptomatic in patients with acute Mediterranean spotted fever (10) and can be easily overlooked. Retinal vasculitis was reported in 45%–55% of the patients, but retinal artery occlusion secondary to vasculitis has been described in only 2 cases of infection with *R. conorii* and *R. rickettsii* (10) without details of clinical symptoms. Because ocular involvement could be asymptomatic and easily overlooked, an ophthalmic evaluation should be conducted when rickettsiosis is suspected.

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Rickettsia felis in Fleas, France

To the Editor: *Rickettsia felis* belongs to the spotted fever group of rickettsia. The pathogenic role of this intracellular Proteobacteria in humans has been reported in patients from the United States (Texas) (1), Mexico (2), Germany (3), Brazil, and France (4). *R. felis* is widely distributed, is associated with blood-sucking arthropods, and has been isolated from fleas in several countries (5).

To obtain new information about the distribution of *R. felis* in France and potential vectors/ reservoirs of this emerging pathogen, 550 fleas were collected from 82 dogs and 91 cats in 7 widely distributed locations in France (Bordeaux, Toulouse, Cosnes-Cours sur Loire, Dijon, Moulins, Limoges, and Aix-en-Provence). Specimens were collected by combing, recorded, and stored at –20°C. Samples were shipped on dry ice to the entomologic laboratory of the Institute of Comparative Tropical Medicine and Parasitology in Munich, Germany, and species identification was performed by using

light microscopy and following the determination key of Hopkins and Rothschild (6). Because infestation levels varied (1–150 fleas/animal), we randomly analyzed 1–8 fleas (mean 3.4) from each host animal.

We homogenized fleas individually in 80 μ L of phosphate-buffered saline by using 5-mm steel beads in a RETSCH Tissue Lyser Mixer Mill 300 (QIAGEN, Hilden, Germany). A total of 100 μ L of ATL buffer and 20 μ L of proteinase K (QIAGEN) were added, and the homogenate was incubated at 56°C in a thermomixer (Eppendorf, Hamburg, Germany) until the tissues were lysed. DNA was extracted from each flea by using a QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions (tissue protocol) and stored at –20°C until used in a PCR.

PCR amplification of rickettsial DNA was performed by using previously described oligonucleotide primer pairs Rp CS.877p/Rp CS.1258n targeting the citrate synthase (*gltA*) gene and, for the positive samples, Rr 190.70p/Rr 190.602n targeting the outer membrane protein A (*ompA*) gene (7). Amplification was conducted in 50- μ L volumes that contained 5 μ L of DNA, 30 μ L of distilled water, 10 μ L of 5 \times Taq buffer (Roche, Mannheim, Germany), 3 μ L of 25 mmol/L MgCl₂ (Roche), 1 μ L of 10 mmol/L deoxynucleotide triphosphates (Roche), 0.25 μ L of each primer (100 μ M), and

0.5 μ L (5 U/mL) of Taq polymerase (Roche). Conditions for the *gltA* and *ompA* PCRs were as described by Bertolotti et al. (8). Negative and positive controls were included in all PCRs. All PCR products were separated by electrophoresis on 1.5% agarose gels at 100 V for 60 min and examined under UV light. For both genes, positive samples were purified by using the QIAquick PCR Purification Kit (QIAGEN) and sent for sequencing to the MWG Biotech Company (Martinsried, Germany). Sequences were compared with those of previously characterized rickettsia in GenBank by using basic local alignment search tool (BLAST) (www.ncbi.nlm.nih.gov) analysis.

Five species of fleas were identified: *Ctenocephalides felis* (500, 224 from dogs and 276 from cats), *C. canis* (37 from dogs), *Pulex irritans* (11 from dogs), *Spilopsyllus cuniculi* (1 from a cat), and *Archaeopsylla erinacei* (1 from a cat). Five dogs had mixed populations of fleas; 3 of these had *P. irritans* and *C. felis*, and 2 had *C. felis* and *C. canis*. One cat had *P. irritans* and *C. felis*, and another cat had *S. cuniculi* and *C. felis*. A total of 52 (19%) of the 272 fleas from dogs and 44 (16%) of the 278 fleas from cats were positive for both the *gltA* and *ompA* genes. Positive samples were obtained from all locations. Prevalence ranged from 6% (Dijon) to 43% (Toulouse) for dogs and from 3%

(Moulins) to 37% (Bordeaux) for cats (Table). Of 550 fleas, 96 were positive for both genes (*gltA* and *ompA*) and 3 of 5 species of fleas were infected: 10 with *C. canis*, 85 with *C. felis*, and 1 with *A. erinacei*. All sequences matched *gltA* and *ompA* genes from *R. felis* (similarity 99%–100%).

Our investigation provides new information about distribution of *R. felis* and widespread flea infection with *R. felis* in France. A total of 88% of infected fleas were *C. felis*, but we found infected *C. canis* in Bordeaux and Toulouse and infected *A. erinacei* in Limoges. We report the presence in France of *R. felis* in *C. canis* and *A. erinacei* in France. *R. felis* in dog fleas in Uruguay and in hedgehog fleas in Algeria has been reported (9,10). Our findings indicate that these 2 flea species may be vectors of human *R. felis* rickettsiosis in France.

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Table. Prevalence of *Rickettsia felis* in fleas from dogs and cats, France*

Locality	Animal	No. animals	No. fleas	Flea species	No. (%) <i>gltA</i> + <i>ompA</i> +
Aix-en-Provence	Dog	6	20	<i>Ctenocephalides felis</i> ,† <i>C. canis</i> ,†	6 (30)
				<i>Pulex irritans</i>	
Bordeaux	Dog	14	67	<i>C. felis</i> ,† <i>C. canis</i> , <i>P. irritans</i>	8 (12)
	Cat	11	38	<i>C. felis</i> †	14 (37)
Cosnes-Cours sur Loire	Dog	15	44	<i>C. felis</i> ,† <i>C. canis</i>	7 (16)
	Cat	17	50	<i>C. felis</i> †	3 (6)
Dijon	Dog	6	18	<i>C. felis</i> ,† <i>C. canis</i>	1 (17)
	Cat	1	3	<i>C. felis</i> †	1 (33)
Limoges	Dog	15	45	<i>C. felis</i> †	7 (16)
	Cat	21	61	<i>C. felis</i> ,† <i>Archaeopsylla erinacei</i> †	11 (18)
Moulins	Dog	12	36	<i>C. felis</i> ,† <i>C. canis</i>	5 (14)
	Cat	22	65	<i>C. felis</i> ,† <i>Spilopsyllus cuniculi</i>	2 (3)
Toulouse	Dog	14	42	<i>C. felis</i> ,† <i>C. canis</i> †	18 (43)
	Cat	19	61	<i>C. felis</i> †	13 (21)

**gltA*, citrate synthase A; *ompA*, outer membrane protein A.

†Species positive for *gltA* and *ompA*.

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Novel Nonstructural Protein 4 Genetic Group in Rotavirus of Porcine Origin

To the Editor: Infection with group A rotavirus is the main cause of acute gastroenteritis in infants and young children worldwide and in young animals of many species, including piglets. In recent years, several epidemiologic studies designed to monitor the appearance of novel or atypical rotavirus antigenic types have provided evidence for the increasing antigenic diversity of group A rotaviruses (1–3). In addition to the 2 rotavirus classification systems, VP7 (G) and VP4 (P) genes, the virus can also be classified on the basis of the nonstructural glycoprotein 4 (NSP4)-encoding gene. Sequence analyses of the NSP4 gene indicated the presence of at least 5 distinct genetic groups among human and animal rotaviruses, termed A to E (1,4,5). Among human rotaviruses, the diversity of NSP4 genes has been restricted mainly to genetic groups A and B; only a few human strains possess genetic group C. Conversely, all 5 NSP4 genetic groups (A–E) have been identified in rotaviruses of animal origins. To our knowledge, porcine rotaviruses (PoRVs) have been reported to belong only to NSP4 genetic group B (1).

During an epidemiologic survey of PoRV from June 2000 through July 2001, a total of 175 fecal specimens were collected from diarrheic piglets from 6 different farms in Chiang Mai Province, Thailand. Of these, 39 (22.3%) specimens were positive for group A rotavirus (6). A novel and unusual PoRV CMP034 strain was isolated from a 7-week-old piglet during this survey. Molecular genetic characterization showed that the CMP034 strain carried a novel P[27] genotype with a new lineage of G2-like rotavirus genotype (7). We performed a molecular analysis of the NSP4 gene of

this strain in comparison with those of other NSP4 gene sequences available in the GenBank database.

The full-length of NSP4 gene was amplified by NSP4–1a and NSP4–2b primer pairs (8). The PCR amplicon was sequenced in both directions by using the BigDye Terminator Cycle Sequencing kit (PerkinElmer-Applied Biosystems, Inc., Foster City, CA, USA) on an automated sequencer (ABI 3100; PerkinElmer-Applied Biosystems, Inc.). The sequence of CMP034 was compared with those of reference strains available in the National Center for Biotechnology Information GenBank database by using BLAST (www.ncbi.nlm.nih.gov/blast). The NSP4 nucleotide sequence of the CMP034 strain was deposited in GenBank under accession no. DQ534017.

The complete NSP4 nucleotide sequence of PoRV CMP034 strain was 750 bp and contained a single long open reading frame coding for a protein of 175 aa. Comparative analysis of the CMP034 NSP4 sequence with those of the 5 representative established genetic groups (A–E) showed the highest sequence identity, at 92.6% nt and 96.9% aa levels, with 1 PoRV strain, P21–5 (9). However, CMP034 and P21–5 shared a low degree of sequence identity with other NSP4 genetic groups. The NSP4 sequence identities of the CMP034 and P21–5 strains ranged from 74% to 78% nt and 75%–79% aa levels with those of genetic group A; 77%–86% nt and 79%–86% aa levels with genetic group B; 69%–73% nt and 75%–78% aa levels with genetic group C; 62%–65% nt and 55%–60% aa levels with genetic group D; and only 43%–50% nt and 29%–33% aa levels with genetic group E. The phylogenetic tree confirmed that PoRV strains CMP034 and P21–5 were located exclusively in a separated branch, which was distantly related to the other 5 known NSP4 genetic groups (Figure). However, a bootstrap support for the separation of