R. japonica is the specific pathogen of Japanese spotted fever, which has been found mainly in southwestern Japan (*5*). The present strain, closely related to *R. japonica,* is likely to have been isolated from *H. hystricis* in Thailand because *R. japonica* frequently has been isolated, or detected by PCR, from the same tick species in Japan (*6*). Such tick species–specificity of SFGR should be considered when speculating on any geopathologic relationships of rickettsioses among different SFGR-endemic areas. Previous reports on spotted fever–positive results of human serosurveys (*7,8*) and on a clinical case (*9*) in northern Thailand may provide epidemiologic background. In Asia, multiple species of rickettsiea (e.g., *R. japonica, R. heilongjiangensis, R. honei*) are the causative agents of spotted fever rickettsioses, so the agent closely related to *R. japonica* could cause spotted fever in Thailand. Additionally, *R. japonica* has been found in Korea (*10*), and our current study indicates that *R. japonica* and its genetic variants are widely distributed in Far Eastern countries, including Japan (Grant-in-Aid for International Cooperative Research, unpub. data). Therefore, the epidemiology and genetic variation of SFGR throughout Asia should be examined by molecular studies.

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References

1. Gaywee J, Sunyakumthorn P, Rodkvamtook W, Ruang-areerate T, Mason CJ, Sirisopana N. Human infection with *Rickettsia* sp. related to *R. japonica*, Thailand. Emerg Infect Dis. 2007;13:671–3.
2. Fujita H, Takada N, Chaithong U. Preliminary report of rickettsial strains of spotted fever group isolated from ticks of China, Nepal and Thailand. Annual Report of Ohara General Hospital. 2002;44:15–8.
3. Zhang JZ, Fan MY, Wu YM, Fournier PE, Roux V, Raoult D. Genetic classification of "*Rickettsia heilongjiangii*" and "*Rickettsia hulinii*," two Chinese spotted fever group rickettsiae. J Clin Microbiol. 2000;38:3498–501.
4. Kollars TM Jr, Tippayachai B, Bodhidatta D. Short report: Thai tick typhus, *Rickettsia honei*, and a unique *Rickettsia* detected in *Ixodes granulates* (Ixodidae: Acari) from Thailand. Am J Trop Med Hyg. 2001;65:535–7.
5. Mahara F. Rickettsioses in Japan and the Far East. Ann N Y Acad Sci. 2006;1078:60–73. DOI: 10.1196/annals.1374.007
6. Fujita H, Takada N. Diversity of genus *Rickettsia* detected from ticks in Japan. Acari and emerging/reemerging infectious diseases [in Japanese]. Tokyo: Zenkoku Noson Kyoiku Kyokai Publishing; 2007. p. 129–39.
7. Takada N, Fujita H, Yano Y, Huang W-H, Khamboonruang C. Serosurveys of spotted fever and murine typhus in local residents of Taiwan and Thailand compared with Japan. Southeast Asian J Trop Med Public Health. 1993;24:354–6.
8. Parola P, Miller RS, McDaniel P, Telford SR, Rolain J-M, Wongsrichanalai C, et al. Emerging rickettsioses of the Thai-Myanmar border. Emerg Infect Dis. 2003;9:592–5.
9. Sirisanthana T, Pinyopornpanit V, Sirisanthana V, Strickman D, Kelly DJ, Dasch GA.. First cases of spotted fever group rickettsiosis in Thailand. Am J Trop Med Hyg. 1994;50:682–6.
10. Lee J-H, Park H-S, Jung K-D, Jang W-J, Koh S-E, Kang S-S, et al. Identification of the spotted fever group rickettsiae detected from *Haemaphysalis longicornis* in Korea. Microbiol Immunol. 2003;47:301–4.

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Segniliparus rugosus Infection, Australia

To the Editor: Recently, a female teenager with cystic fibrosis who resided in tropical north Queensland, Australia, was found to be infected with *Segniliparus rugosus*. She was homozygous for the deltaF508 mutation, had well-preserved lung function, and regularly played competitive sports. Unlike many cystic fibrosis patients, she did not have a history of chronic *Pseudomonas aeruginosa* infections, but *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* had been previously isolated from her sputum. In May 2007, she described reduced exercise tolerance and increased cough with excess sputum production. Lung function testing showed modest spirometric decline. A computed tomographic scan of the chest showed significant mucus plugging and bronchiectasis, uncommon without previous *P. aeruginosa* infection. Sputum was 3+ smear positive for acid-fast bacilli (AFB), and *S. rugosus* was isolated from liquid culture. Empiric antimicrobial drug therapy was changed to rifabutin and co-trimoxazole because these drugs have been effective in previous cases (*1*). Clinically, the patient showed response to the treatment. After 12 months of treatment, her sputum was still 3+ positive for AFB, and *S. rugosus* was again found in culture. She was referred to a pediatric teaching hospital in Brisbane with worsening respiratory symptoms precipitated by influenza B infection. Antimicrobial drug therapy with intravenous imipenem, oral moxifloxacin, and co-trimoxazole for 2 weeks resulted in clinical improvement but little reduction in smear positivity.

The initial AFB smear-positive sputum specimen underwent routine decontamination with sodium hydroxide and neutralization and was inoculated into radiometric 12B vials (Bec-

ton Dickinson, Sparks, MD, USA). The culture was positive after 6 days' incubation and was referred to the state reference laboratory in Brisbane. The culture was smeared and stained with the Ziehl-Neelsen method, which showed short, pale AFB.

A crude boil DNA preparation was made from the positive culture, and an in-house multiplex PCR to identify *Mycobacterium* spp. was performed (*2*). No PCR products were detected, and the *Mycobacterium* genus-specific band was absent. Similarly, no *Mycobacterium* genus band was detected by using a PCR-hybridization method (Common Mycobacteria line probe assay, Hain Lifesciences GmbH, Nehren, Germany) (*3*). DNA sequencing was performed on the *Segniliparus rugosus* strain NQ1 (GenBank accession no. FJ593188), and a 1,250-bp fragment of the ribosomal 16S loci was obtained. A BLAST search (GenBank) was then performed on the sequence information obtained. The DNA sequence gave a 100% match to that of *Segniliparus rugosus* (GenBank accession no. AY608920) (*4*).

Drug susceptibility testing (DST) was attempted by using disk diffusion with Mueller-Hinton plates but was unsuccessful because of insufficient growth and indistinct margins. A commercially available microbroth dilution assay was used to determine the MICs for 12 antimicrobial drugs. The Sensititre Broth MIC Method for Rapidly Growing Mycobacteria, Nocar-

dia, and Other Aerobic Actinomycetes (Trek Diagnostic Systems, Cleveland, OH, USA) was used according to manufacturer's instructions (*5*). However, problems still occurred because of inadequate growth of the organism using the testing media approved for mycobacteria (*6,7*). Superior results were obtained when the cation-adjusted Mueller-Hinton broth with TES buffer (Trek Diagnostic Systems), which is used routinely in this assay, was substituted with an internally prepared Middlebrook 7H9 broth (Becton Dickinson) (*1*). DST results are similar to those previously reported for other strains of *S. rugosus* (Table) (*1*).

The strain had rough, wrinkled colonial morphology on both blood agar and 7H11 plates. Growth was optimal at 35°C on 7H11 media. Arylsulfatase activity was weakly positive at 3 days and positive at 14 days. Growth on Lowenstein-Jensen (LJ) medium and on LJ with 5% sodium chloride medium occurred at 6–7 days. The strain was negative for both nitrate reductase and tween hydrolysis. Mycolic acid high-pressure liquid chromotography was performed (*8*), and the pattern obtained matched that of type strain CDC 945 (AY608920) previously reported (*4*). The pattern had a double cluster of adjoining eluting peaks with the last peak co-eluting with the internal high standard.

Only 3 other cases of *Segniliparus* spp. infection (none from Australia)

have been reported. More remains to be learned about the efects of lung infections with *Segniliparus* spp. in cystic fibrosis patients. Although this patient improved clinically after treatment with antimicrobial drugs, she is still infected and will likely remain chronically infected. Because this genus is acid fast by the Ziehl-Neelsen method, laboratory workers and clinicians must be aware that AFB seen in pulmonary specimens from cystic fibrosis patients are not necessarily from the genus *Mycobacterium* and may be from the genus *Segniliparus*.

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References

1. Butler WR, Sheils CA, Brown-Elliott BA, Charles NA, Colin AA, Gant MJ, et al. First isolations of *Segniliparus rugosus* from patients with cystic fibrosis. J Clin Microbiol. 2007;45:3449–52. DOI: 10.1128/JCM.00765-07
2. Wilton S, Cousins D. Detection and identification of multiple mycobacterial pathogens by DNA amplification in a single tube. PCR Methods Appl. 1992;1:269–73.
3. Richter E, Rusch-Gerdes S, Hillemann D. Evaluation of the genotype mycobacterium assay for identification of mycobacterial species from cultures. J Clin Microbiol. 2006;44:1769–75. DOI: 10.1128/JCM.44.5.1769-1775.2006

Table. Antimicrobial susceptibility patterns for *Segniliparus rugosus* strain in a cystic fibrosis patient, Australia

Antimicrobial agent	MIC, µg/mL
Amikacin	>128
Amoxicillin/clavulanic acid (ratio 2:1)	64/32
Cefoxitin	64
Ceftriaxone	>64
Ciprofloxacin	4
Clarithromycin	16
Gatifloxacin	0.5
Imipenem	1
Linezolid	64
Minocycline	>32
Tobramycin	>64
Trimethoprim/sulfamethoxazole	2/38

4. Butler WR, Floyd MM, Brown J, Toney SR, Daneshvar M, Cooksey RC, et al. Novel mycolic acid–containing bacteria in the family *Segniliparaceae* fam. nov., including the genus *Segniliparus* gen. nov., with descriptions of *Segniliparus rotundus* sp. nov., and *Segniliparus rugosus* sp. nov. Int J Syst Evol Microbiol. 2005;55:1615–24.

5. Brown-Elliott B, Beierle K, McGlasson M, Wallace R. MICs of non-tuberculous mycobacteria using the TREK Vizion system compared to manual readings. In: 18th European Congress of Clinical Microbiology and Infectious Diseases; Barcelona, Spain; 2008 April 19–22. Abstract R2410.

6. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial testing; sixteenth informational supplement. CLSI14 document M100–S16. Wayne (PA): The Institute; 2006.

7. National Committee on Clinical and Laboratory Standards (NCCLS). Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard. NCCLS document M24–A. Wayne (PA): The Committee; 2003.

8. Butler WR, Guthertz LS. Mycolic acid analysis by high performance liquid chromatography for identification of mycobacterium species. Clin Microbiol Rev. 2001;14:704–26. DOI: 10.1128/CMR.14.4.704-726.2001

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Multigenotype Q Fever Outbreak, the Netherlands

To the Editor: Q fever is a zoonosis caused by *Coxiella burnetii* (*1*). An ongoing Q fever outbreak has occurred in the Netherlands since 2007; incidence rates have increased >50-fold compared with the baseline rate (*2*). The source of this outbreak is unknown. Identifying the source of an infection is complicated because of difficulties in obtaining sufficient clinical and/or environmental samples for testing. Molecular diagnosis of Q fever has focused on the use of serum samples. Up-to-date genotyping of *C. burnetii* has depended on cultivation and enrichment of the isolate before analysis (*3*). We report multiple-locus variable-number tandem repeat analysis (MLVA) typing of *C. burnetii* for a variety of human and animal clinical samples obtained from different locations in the Netherlands (Table).

Severe pneumonia developed in patient 1 after close contact with sheep (ewes) and intimate cuddling with a newborn lamb. Patients 2 and 3 (a dairy goat farmer and his wife from another village) tested positive for Q fever after a large part of their goat herd aborted offspring. The farmer had no clinical symptoms; his wife had mild symptoms that disappeared spontaneously within 2 days. No samples from any of the goats were available. Two additional patients were tested, 1 of which lived in the same village as patients 2 and 3.

Swab specimens from all sheep and lambs tested in the first case yielded identical MLVA genotypes. The same genotype was also found in patient 1 but not in the other examined samples, implicating sheep as the origin of patient 1's infection. Although patients 2 and 3 lived together, the genotype found in patient 2 differed from the (partial) genotype found in patient 3. Yet another genotype was found in a patient from the same village (patient 4). However, an identical genotype found in patient 2 was found in a patient from a distant village (patient 5). The village had only 1 goat farm, and if this herd of goats was the source of infection for the farmer, his wife, and patient 4, it would have contained >1 genotype. At least 1 of the obtained genotypes has spread over a wider surface area in the Netherlands.

Our results show that the unprecedented, ongoing Q fever outbreak in the Netherlands involves multiple genotypes of *C. burnetii*. Because most of the genotypes differ only by a single repeat difference, they might represent microvariants of a hypervirulent strain that has been introduced in the Dutch animal population. MLVA schemes with up to 17 markers have been previously reported (*3*). In this "proof of concept" (applying direct genotyping of *C. burnetii* on clinical samples), we focused on the 3 shortest repeat units because we believed that these units might have the highest à priori chance of successful amplification in clinical samples (especially in serum/plasma). Similar genotypes as those reported here were found in the MLVA database (http://mlva.u-psud.fr), but these similarities need confirmation by using more markers. Although using only 3 markers may lead to poor discriminatory power, we were still able to distinguish 4 different genotypes in a relatively small collection of serum samples. We are currently exploring the use of additional MLVA markers.

Our results also show a poor correlation between DNA load and clinical symptoms. Multiple human and animal clinical samples, including serum and plasma, throat or genital swabs, or sputum and urine, may be useful for direct genotyping and outbreak source tracking.