

was  $64.3 \pm 17.3$  years. Three (14.3%) of these patients were white, and 18 (85.7%) were Asian/Pacific Islander. *Mycobacterium tuberculosis* and NTM were identified in samples from 3 (14.3%) and 18 (85.7%) of the 21 patients, respectively. Of the 18 patients with NTM-positive samples, 4 (22.2%) had definite NTM lung disease (all of these patients were Asian/Pacific Islander); 2 (11.1%) had probable NTM lung disease; and 12 (66.7%) had possible NTM lung disease. *M. chelonae* (identified by DNA sequencing) was the causative agent for most of the definite cases ( $n = 3$ , 75%), and the largest proportion of possible cases was caused by *M. avian* complex bacteria ( $n = 5$ , 41.7%).

Our finding that 22.2% (4/18) of the patients in Honolulu with NTM-positive clinical samples during June–December 2011 received a definite diagnosis of NTM lung disease is slightly higher than but consistent with reports from other regions, which show that 9.8%–17.0% of such patients receive a definite NTM disease diagnosis (4,5). For unclear reasons, the number of NTM disease cases appears to be highest in Asian/Pacific Islander populations. Determining the reason(s) for this discrepancy should be the subject of future research efforts.

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## Rickettsial Infections in Monkeys, Malaysia

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**To the Editor:** The cynomolgus monkey (*Macaca fascicularis*), also known as the long-tailed macaque or crab-eating monkey, is commonly found in the Southeast Asia region (1). The macaque has been associated with several bacterial infections, such as those caused by hemotropic *Mycoplasma* and *Bartonella quintana* (2). As a result of rapid deforestation and changes in land use patterns, cynomolgus monkeys live in close proximity to human-populated areas (1). Human–macaque conflict may increase the risk for zoonoses.

Little is known about rickettsial and anaplasma infections in cynomolgus monkeys in Malaysia. Although *Rickettsia* spp. RF2125 and Rf31 have been identified from cat fleas in Malaysia (3), the presence of *Anaplasma bovis* in monkeys is not known.

*Rickettsia felis*, a member of the spotted fever group rickettsiae, is an emergent fleaborne human pathogen distributed worldwide (4). The obligate intracellular bacterium has been identified from cats, dogs, opossums, and the ectoparasites of various mammalian hosts. Several uncultured rickettsiae genetically closely related to the *R. felis*-type strain URWXCAl2 (referred to as *R. felis*-like organisms and including *Rickettsia* spp. RF2125, Rf31, *Candidatus Rickettsia aseboensis*, and others) have also been identified from various arthropods and fecal samples of primates (5). *A. bovis* is a gram-negative, pleomorphic, tickborne intracellular bacterium that infects a wide range of mammal species in many geographic regions (6).

To learn more about these infections in monkeys, we examined blood samples from 50 cynomolgus monkeys caught by the Department of Wildlife and National Parks at 12 residential areas in Peninsular Malaysia during a population management and wildlife disease surveillance program (January 2012–December 2013). Most monkeys (14 male, 36 female) were adults and were active and healthy. DNA was extracted from 200  $\mu$ L of each blood sample by using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). We performed PCRs selective for the rickettsial citrate synthase gene (*gltA*) by using primers CS-78 and CS-323 and for the 135-kDa outer membrane protein B gene (*ompB*) by using primers 120-M59 and 120-807 (7). As positive controls, we

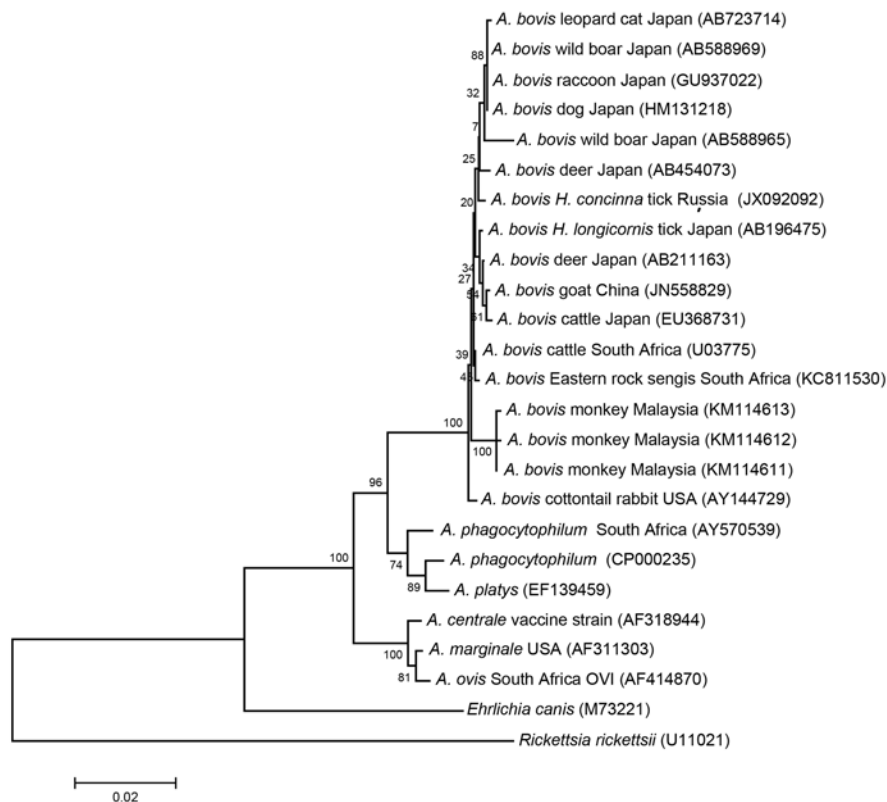
used cloned PCR4-TOPO TA plasmids (Invitrogen, Carlsbad, CA, USA) with amplified *gltA* fragment from *R. honeii* (strain TT118) and *ompB* fragment from a rickettsial endosymbiont (98% similarity to *R. raoultii*) of a tick sample. Amplification of anaplasma DNA was performed by using a group-specific primer pair (EHR 16SD/EHR 16SR) (8). As a positive control for the PCR, we used an *A. marginale*-infected cattle blood sample. The full-length sequences of the *Anaplasma* 16S rRNA gene were obtained by amplification with primers ATT062F and ATT062R (9). Sequence determination of the amplicons was performed by using forward and reverse primers of respective PCRs on an ABI PRISM 377 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). To search for homologous sequences in the GenBank database, we performed a BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) analysis and constructed a dendrogram based on 16S rDNA sequences of *A. bovis* (10).

The rickettsial *gltA* gene was detected from 12 (24%) blood samples of mostly male monkeys from 8 locations. BLAST analysis of 210 nucleotides (GenBank accession no. KP126803) amplified from all samples demonstrated 100% sequence similarity with *Rickettsia* sp. RF2125 (accession no. AF516333), *Candidatus Rickettsia asemboensis* (accession no. JN315968), and *Rickettsia* spp. clone 4G/JP102 and 11TP21 (accession nos. JN982949 and JN982950), which had been identified from cat fleas in Southeast Asia, Africa, and Costa Rica, respectively. The rickettsial sequence also

showed 99.0% similarity (2-nt difference) with *R. felis*-type strain (accession no. CP000053). The rickettsial *ompB* gene was amplified from 4 samples, and BLAST analysis of the sequences (556–779 bp) revealed closest match to several *R. felis*-like organisms, including *Rickettsia* sp. RF2125 (100%, accession no. JX183538) and *Candidatus Rickettsia asemboensis* (99%, accession no. JN315972). BLAST analysis of the longest *ompB* sequence (accession no. KP126804) obtained in this study showed 93% similarity with that of the *R. felis*-type strain.

*Anaplasma* DNA was amplified from 5 (10%) monkeys at 2 locations by using group-specific primers. Analysis of the nearly full-length sequences of the *A. bovis* 16S rRNA gene (1,457 nt) revealed 3 sequence types (GenBank accession nos. KM114611–3) with 99.1%–99.2% homology to that of the *A. bovis* strain from cattle in South Africa (accession no. U03775). The phylogenetic tree (Figure) inferred by using various *Anaplasma* species confirms the clustering of the strains from monkeys with *A. bovis* from different animals (i.e., goats, cattle, deer, ticks, wild boars, dogs, raccoons, leopard cats, eastern rock sengis, and cottontail rabbits). Co-infection of *R. felis*-like organisms and *A. bovis* was detected in only 1 sample.

Infections caused by *R. felis*-like organisms and *A. bovis* in the cynomolgus monkeys were subclinical (i.e., monkeys showed no evident signs of infection at the time of blood sampling). The diverse range of the organisms'



**Figure.** Phylogenetic relationships among various *Anaplasma* species, based on partial sequences of the 16S rRNA gene (1,263 bp). The dendrogram was constructed by using the neighbor-joining method in MEGA6 software (10) with the maximum composite likelihood substitution model and bootstrapping with 1,000 replicates. *Rickettsia rickettsii* (U11021) was used as an outgroup. Numbers in brackets are GenBank accession numbers. Representative Malaysian *A. bovis* sequences were deposited into the GenBank database under accession nos. KM114611–3. Scale bar indicates nucleotide substitutions per site.

ectoparasite and animal hosts raises concern about their potential risk to human and animal health. Further study on the interactions between the microbes, vectors, and reservoir hosts is needed to assess their effects on public health.

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## Effect of Ciliates in Transfer of Plasmid-Mediated Quinolone-Resistance Genes in Bacteria

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**To the Editor:** Previous studies have suggested that protozoa may promote horizontal gene transfer among bacterial species (1,2). This process is largely, although not exclusively, responsible for increasing the incidence of antibiotic-resistant bacteria through various mechanisms, such as transformation by acquisition of naked DNA, transduction by acquisition of DNA through bacteriophages, and conjugation by acquisition of DNA through plasmids or conjugative transposons (3,4). Because antibiotic resistance may be mediated by horizontal gene transfer, it is necessary to understand whether protozoa, which are widely distributed in nature, facilitate the acquisition and spread of antibiotic resistance genes. The aim of this study was to explore whether the ciliated protozoan *Tetrahymena thermophila* promotes the transfer of plasmid-mediated quinolone-resistance (PMQR) genes in bacteria.

Two *qnr* gene–positive bacterial strains (*Klebsiella oxytoca* and *Escherichia coli*) were chosen as donors, and azide-resistant *E. coli* strain J53 was used as a recipient for the assessment of gene transfer frequency. The *K. oxytoca* and *E. coli* strains were previously isolated and identified from the Ter River (Ripoll, Spain) in the framework of a multidisciplinary study on antibiotic-resistant bacteria (5). Donor and recipient bacteria, previously grown in Luria-Bertani broth for 5 h at 37°C, were mixed in equal numbers (10<sup>9</sup> CFU/mL) with or without *T. thermophila* strain SB1969 (10<sup>5</sup> cells/mL) in Page’s amoeba saline for 24 h, as previously described (1). Heat-treated ciliates, exposed for 10 min at 90°C, were also tested to determine whether viable organisms are required for gene transfer. Conjugation experiments were performed at 37°C, and, after the incubation period, the cultures were treated as previously