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“*Candidatus* Mycoplasma haemomacaque” and *Bartonella quintana* Bacteremia in Cynomolgus Monkeys

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Here, we report latent infections with *Bartonella quintana* and a hemotropic *Mycoplasma* sp. in a research colony of cynomolgus monkeys (*Macaca fascicularis*). Sequence alignments, evolutionary analysis, and signature nucleotide sequence motifs of the hemotropic *Mycoplasma* 16S rRNA and RNase P genes indicate the presence of a novel organism.

Hemotropic *Mycoplasma* spp. (hemoplasmas) are obligate epierythrocytic bacteria that infect numerous animal species, including *Homo sapiens*. Infections are often chronic and subclinical; however, some animals and humans develop hemolytic anemia, particularly when stressed or immunosuppressed (1, 2). Phylogenetic analyses of 16S rRNA gene sequences have defined two major subclusters of hemoplasmas, namely, the *Mycoplasma haemosuis* and *Mycoplasma haemofelis* groups (3–7).

Historically, diagnosis of hemoplasma infections has relied on cytological examination of stained blood smears. In 1994, Dillberger and colleagues described *Haemobartonella*-like parasites in five wild-caught anemic cynomolgus monkeys (*Macaca fascicularis*) that originated from the Philippines; however, the organisms were not characterized phylogenetically (8). For some animal species, the diagnostic sensitivity of a blood smear examination is very poor and unspecific (3, 4, 9). The development of molecular assays, primarily targeting the 16S rRNA and the RNase P genes of these cell wall-deficient uncultivable microbes, has resulted in the recent recognition of several novel animal hemoplasmas (4, 5, 10–13).

Bartonella spp. are facultative intracellular bacteria that also infect erythrocytes in numerous animal species, including *Homo sapiens*. Previously, *Bartonella quintana* DNA was amplified, cloned, and sequenced from lysed erythrocytes, and cultured colonies were grown from peripheral blood collected from a captive-bred cynomolgus monkey (*Macaca fascicularis*) (14). *Bartonella quintana* was subsequently isolated from 2 of 36 captive rhesus macaques in China, of which 12 of 33 were *B. quintana* seroreactive (15).

Hemotropic *Mycoplasma* and *Bartonella* organisms often cause persistent occult infection in immunocompetent hosts. The extent to which an infection with these bacteria in cynomolgus monkeys involved in a research study might influence assessments or outcomes associated with drug development studies is poorly characterized. This report describes PCR amplification and DNA sequence characterization of a novel hemotropic *Mycoplasma* sp. found in the blood of 44 of 52 cynomolgus research monkeys (*Macaca fascicularis*) and the isolation of *Bartonella quintana* from one monkey. These animals were in a chronic toxicity study, and data from the pretest phase of the study are presented. The animals were tested for *Mycoplasma* and *Bartonella* on the basis of the findings in a previous toxicity study that raised the possibility of latent infections. Based on the analysis of the 16S rRNA and RNase P gene sequences, we propose “*Candidatus* Mycoplasma haemomacaque” as the name for the novel hemotropic *Mycoplasma* sp. identified in this study.

MATERIALS AND METHODS

Blood from 52 cynomolgus monkeys (*Macaca fascicularis*) was analyzed prior to the initiation of dosing in a toxicity study for the presence of hemotropic *Mycoplasma* and *Bartonella* spp. The monkeys were considered healthy on the basis of multiple pretest physical clinical evaluations, including Coomb’s tests and microscopic blood smear evaluations.

Blood samples were collected in EDTA-containing Vacutainers and shipped overnight to Galaxy Diagnostics, Inc., to test for the presence of *Mycoplasma* spp. and *Bartonella* spp. Blood samples were analyzed for the presence of *Mycoplasma* DNA by PCR testing, targeting the 16S rRNA (a 1,200-bp fragment) and RNase P (a 160-bp fragment) genes as reported previously (16). Similarly, blood samples were analyzed for the presence of *Bartonella* spp. using the *Bartonella* alphaproteobacterial growth medium (BAPGM) enrichment culture PCR as described previously (16–18). DNAs from naive dog and human blood extracted at the same time and in the same manner were used as negative controls for the PCR testing.

Nucleotide sequence accession number. The nucleotide sequence of the partial 16S rRNA gene has been deposited in GenBank under accession no. KC512401.

RESULTS

All animals were considered healthy on the basis of the pretest screening. In particular, there was no evidence of anemia, hyperbilirubinemia, or bilirubinuria. By targeting the 16S rRNA and RNase P genes, DNA of a novel hemotropic *Mycoplasma* sp. was amplified from 44 of 52 (84.6%) cynomolgus monkey blood samples but not from any of the negative controls tested. Sequence analyses of both genes identified a distinct genotype compared with those of sequences for other *Mycoplasma* spp. deposited in

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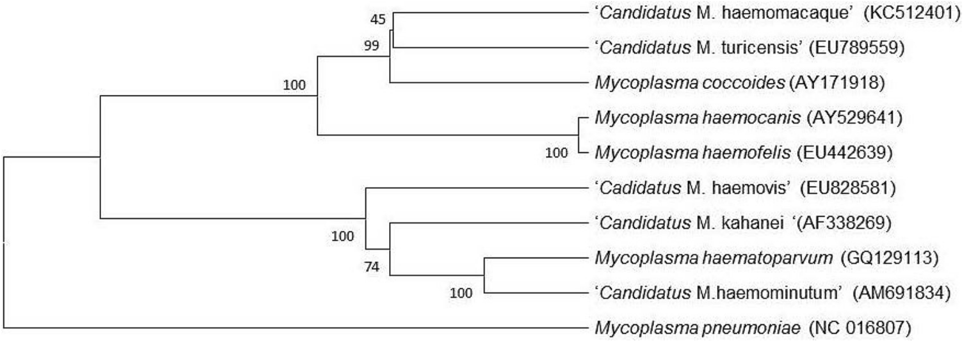

FIG 1 Phylogenetic tree based on 16S rRNA gene sequences, comparing the positions of "*Candidatus* Mycoplasma haemomacaque" and other hemotropic *Mycoplasma* spp. Bootstrap percentages are given at the nodes of the tree.

GenBank. When the 1,164-bp nucleotide sequence of the partial 16S rRNA gene was compared with those of *M. coccoides* (AY171918), "*Candidatus* Mycoplasma turicensis" (EU789559), *M. haemofelis* (EU442639), *M. haemocanis* (AY529641), "*Candidatus* Mycoplasma haemovis" (EU828581), "*Candidatus* Mycoplasma haematoparvum" (GQ129113), and "*Candidatus* Mycoplasma haemominutum" (AM691834), the novel hemoplasma from cynomolgus monkeys shared 90.9% (1,058/1,164 bp) homology with "*Candidatus* Mycoplasma turicensis," followed by 90.4% (1,052/1,164 bp) homology with *M. coccoides*. The nucleotide sequence homology was lower for *M. haemocanis* (87.3%), *M. haemofelis* (85.8%), and "*Candidatus* Mycoplasma kahanei" (79.6%) found in squirrel monkeys (19) (AF338269) and for *M. pneumoniae* (NC_016807) (76.5%). The bootstrap percentage values are given at the nodes of the phylogenetic tree shown in Fig. 1.

Similarly, when the nucleotide sequence of the partial RNase P gene obtained from *Macaca fascicularis* was compared with those of other reported *Mycoplasma* spp., *M. coccoides* (GenBank accession no. EU078619), "*Candidatus* Mycoplasma aoti" (HM123756), *M. iowae* (EU078608), *M. pirum* (EU078607), "*Candidatus* Mycoplasma turicensis" (EF212003), *M. haemofelis* (EU078617), *M. haemocanis* (AF407211), *M. haemovis* (EU078612), *M. haematoparvum* (AY380803), and "*Candidatus* Mycoplasma haemominutum" (AY150990), there was very low homology. "*Candidatus* Mycoplasma haemomacaque" shared 78% homology with "*Candidatus* Mycoplasma aoti" and 74.8% homology with *M. haemofelis* and *M. haemocanis*, followed by "*Candidatus* Mycoplasma turicensis" (72.8%), *M. coccoides* (68%), *M. pirum* (66%), *M. haematoparvum* (62%), and *M. iowae* (59.2%). Phylogenetic analysis of the partial RNase P gene, including comparisons with sequences available for hemotropic *Mycoplasma* spp., are shown in Fig. 2.

In addition to the novel *Mycoplasma* sp., the *Bartonella quintana* 16S rRNA-23S rRNA intergenic spacer region DNA was sequenced from the extracted blood, from 7- and 14-day BAPGM enrichment cultures, and from a subculture isolate (20–23) from one monkey. Sequence analysis of the *Bartonella* internal transcribed spacer (ITS) region revealed 100% homology (420/420 bp) with *Bartonella quintana* (GenBank accession no. L35100).

DISCUSSION

Infection with a novel hemotropic *Mycoplasma* sp. was documented in 44/52 (84.6%) monkeys, and *B. quintana* was isolated from 1/52 (1.9%) cynomolgus monkeys in a research colony. Analysis of the hemoplasma 16S rRNA gene sequences derived from *Macaca fascicularis* in this study identified a 92.3% similarity to *M. coccoides* and a 90.6% similarity to "*Candidatus* Mycoplasma turicensis." The RNase P sequence, used to discriminate among hemotropic *Mycoplasma* organisms, as described by Birkenheuer et al. in 2002 (5) and Tasker et al. in 2003 (3), revealed low similarities with other hemotropic *Mycoplasma* spp., including *M. haemocanis*, *M. haemofelis*, "*Candidatus* Mycoplasma turicensis," and *M. coccoides*. Based on the phylogenetic analysis of DNA sequences found in these cynomolgus monkeys compared with those of other hemoplasma and nonhemotropic *Mycoplasma*

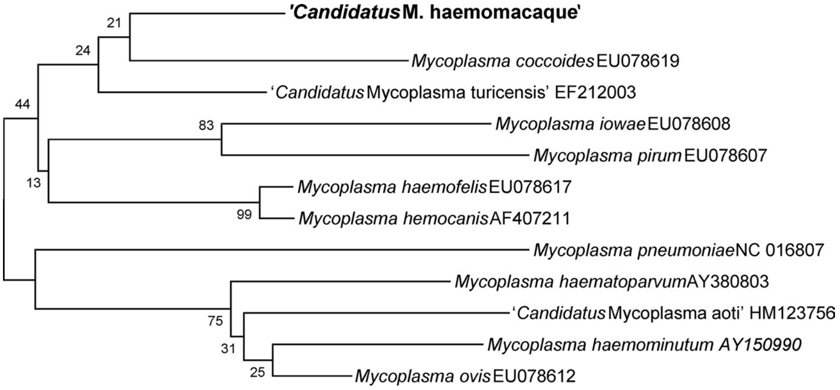

FIG 2 Phylogenetic tree based on RNase P gene sequences, comparing the positions of "*Candidatus* Mycoplasma haemomacaque" and other hemotropic *Mycoplasma* spp. Bootstrap percentages are given at the nodes of the tree.

spp., the low percentage of similarities of this bacteria supports its designation as a novel hemoplasma. Based on differences in the 16S rRNA and partial RNase P gene homologies and according to the guidelines for naming uncultivated prokaryotes (24, 25), we propose a "*Candidatus*" designation for this newly recognized macaque hemoplasma and recommend that it be named "*Candidatus* Mycoplasma haemomacaque."

This report represents the third time that *B. quintana* has been isolated from nonhuman primates raised in research facilities (14, 15). Infections resulting in chronic bacteremia have also been established experimentally in rhesus macaque monkeys (*Macaca mulatta*) inoculated with *B. quintana* isolates derived from infected humans (26, 27), which supports the fact that nonhuman primates might be able to acquire *B. quintana* from humans or from other monkeys.

Hemotropic *Mycoplasma* spp. (hemoplasmas, formerly classified as *Haemobartonella* and *Eperythrozoon* spp.) (4, 12, 28, 29) appear to have coevolved with animals, including dogs, cats, humans, alpacas, capybaras, and sea lions (1, 10, 13, 30–40). The development of molecular assays, which target primarily the 16S rRNA gene of these microbes, has resulted in the more recent recognition of several novel animal hemoplasmas (5, 12, 37, 41). Hemoplasmas are obligate epierythrocytic organisms that attach to erythrocytes, appear to be relatively nonpathogenic, and are visualized on blood smears more often during periods of stress, hard work, or concurrent infection (1, 2, 7, 10, 42, 43). In some animals, hemoplasma infection is associated with hemolytic anemia of variable severity, ranging from nonclinical hemolysis to severe anemia (7, 40, 44). There were no pretest hematological or serum biochemical abnormalities associated with the novel hemotropic *Mycoplasma* sp. or *B. quintana* in the cynomolgus monkeys in this study.

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E.B.B. is the chief scientific officer for Galaxy Diagnostics, Inc., a newly formed company that provides diagnostic testing for the detection of *Bartonella* species infection in animals and human patients. R.G.M. has led research efforts to optimize the BAPGM platform and is the scientific technical advisor for Galaxy Diagnostics. P.E.M. was a part-time research scientist at Galaxy Diagnostics. N.B., C.M.R., C.M.K., L.R., and M.W.L. have no potential conflicts of interest.

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