**“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 macaques (*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 erythrocytic 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 haemomosis* 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 lyzed 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...
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* 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.

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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, apes, and 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 erythrophagocytic 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 nonclonal 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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REFERENCES


mutagenesis strategy reveals that the Bartonella quintana variably expressed outer membrane proteins are required for bloodstream infection of the host. Infect. Immun. 76:788–795.


