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1 **A novel agent of human anaplasmosis in Amazon rainforest**

2

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27 **Abstract**

28 We report a case of unusual human anaplasmosis from remote rainforest areas of French
29 Guiana. Molecular typing showed that the pathogen is a novel *Anaplasma* species, different to
30 all known species, and more genetically related to recently described *Anaplasma* spp. causing
31 infections in rainforest wild fauna of Brazil.

32 Anaplasmosis are emerging tick-borne zoonoses caused by intracellular bacteria of the
33 *Anaplasma* genus. Eight *Anaplasma* species and several candidate species are described, and
34 at least five species can be transmitted to humans (1,2). Of particular concern, the agent of
35 human granulocytic anaplasmosis, *A. phagocytophilum*, has a specific tropism to
36 polymorphonuclear neutrophils (1,3). Another species, *A. capra*, recently described from
37 asymptomatic goats, is now recognized as an agent of human intraerythrocytic anaplasmosis
38 in China (4). The three other species detected in humans are major veterinary agents
39 sporadically identified in few patients worldwide: *A. ovis* and *A. bovis* in erythrocytes, and *A.*
40 *platys* in platelets (1,5). Human anaplasmosis are consistently associated with people living in
41 rural areas in habitats favorable to ticks or working closely with domestic animals (1,6).
42 However, recent surveys report the presence of overlooked *Anaplasma* species of
43 undetermined zoonotic potential in wild fauna (1,2).

44

45 **The study**

46 We assess the presence of *Anaplasma* in blood samples of clandestine gold miners working in
47 rainforest of French Guiana, South America. This 83,000 km² territory, located between
48 Suriname and Brazil, is one of the regions of highest biodiversity in the world, with over 280
49 species of wild mammals (7). Human population of French Guiana (284,000 inhabitants) is
50 concentrated principally in a handful of towns spread along the coastline and the two main
51 rivers (8). The interior is largely uninhabited and covered by dense rainforest where illegal
52 gold mining camps are located (9,10).

53

54 We examined 363 archived DNA extracts obtained from human blood samples. We had
55 primarily collected these samples in 2019 as part of a malaria survey in remote mining camps
56 in French Guiana (Malakit) (See methodology in (11)). To characterize the whole bacterial

57 diversity, DNA blood samples were typed using a high-throughput bacterial 16S rDNA (*rrs*)
58 sequencing approach (bacterial barcoding, as described in (12)). Bacteria were characterized
59 as operational taxonomic unit (OTU) and amplicon sequence variants (ASV), and
60 taxonomically assigned using the Silva database (<https://www.arb-silva.de/>).

61

62 Examination of OTUs and ASVs revealed the presence of *Anaplasma* sequences in one DNA
63 sample. No OTU and ASV assigned to the *Anaplasma* genus or to the Anaplasmataceae
64 family was detected in the 362 other samples. We further conducted two independent
65 *Anaplasma*-specific PCR assays targeting a region of the 16S rDNA gene (544 bp) and the
66 23S–5S (ITS2) intergenic region (423 bp) following Calchi 2020 (13), and obtained
67 amplicons of correct sizes for the positive sample. The Sanger sequencing of amplicons
68 obtained with each pair of primers each confirmed the presence of *Anaplasma*. These
69 sequences have been deposited on GenBank (accession numbers: *///pending///*).

70

71 We used BLAST (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) to compare these 16S rDNA
72 and ITS2 nucleotide sequences with *Anaplasma* sequences available in GenBank. None of the
73 nucleotide sequences observed in this study is 100% identical to known *Anaplasma*
74 sequences. The 16S rRNA sequence showed highest identities with *Anaplasma* found in wild
75 fauna of Brazil, including an *Anaplasma* sp. detected in *Amblyomma coelebs* ticks collected
76 on South American coatis, *Nasua nasua* (99.8%; GenBank MT019560), another *Anaplasma*
77 sp. of black rats, *Rattus rattus* (99.8%; GenBank KY391803), and *Candidatus Anaplasma*
78 *amazonensis* (13) of brown-throated sloths, *Bradypus variegatus*, and of two-toed sloths,
79 *Choloepus didactylus* (99.1%; GenBankMT199827). All other *Anaplasma* species showed
80 identities <99%. The ITS2 sequences showed highest nucleotide identity with *Cand.*
81 *Anaplasma amazonensis* of Brazilian brown-throated sloths and two-toed sloths (96.8%;

82 GenBankMT267354), and lower identities with other *Anaplasma* species or strains (<92%).
83 On account of these distinct genetic traits, we propose the designation *Cand. Anaplasma*
84 *sparouinii* for this novel bacterium. The specific name refers to the Sparouine River where the
85 infected patient lived.

86

87 We conducted phylogenetic analyses based on these 16S rDNA and ITS2 nucleotide
88 sequences using the maximum-likelihood (ML) method. We obtained trees of similar
89 topologies with a robust clustering of *Cand. Anaplasma sparouinii* with some *Anaplasma*
90 associated with Brazilian wild fauna: *Cand. Anaplasma sparouinii* is phylogenetically more
91 related to *Anaplasma* sp. infections detected in ticks of coatis, black rats and, at a lesser
92 extent, to *Cand. Anaplasma amazonensis* of sloths (Figures 1A and B). Altogether, they
93 delineate a clade of neotropical *Anaplasma* divergent to all other *Anaplasma* species (Figures
94 1A and B).

95

96 The DNA sample positive for *Cand. Anaplasma sparouinii* was from a 58-years-old male who
97 had a history of post-traumatic splenectomy and malaria attacks caused by *Plasmodium vivax*.
98 This patient originated from Maranhão, Brazil, but has been working exclusively in rainforest
99 of French Guiana from the past three years. The Sparouine anaplasmosis was retrospectively
100 diagnosed in September 2021 on the basis of PCR survey of previous blood samples (October
101 2019 and May 2021) and blood smears (October 2019) as further detailed.

102

103 We primarily detected the presence of *Cand. Anaplasma sparouinii* in a blood sample
104 collected in October 2019. At this time, the patient was asymptomatic, with no fever, normal
105 blood pressure, negative tests for agents of diseases usually tested in French Guiana
106 (serologies for yellow fever, Q fever, hepatitis B and C, HIV, syphilis, molecular tests for

107 malaria and leptospirosis). He showed anemia with a hemoglobin level of 10.5 g/dL. The
108 reexamination of Giemsa-stained thin blood film taken for malaria diagnosis at that time
109 revealed the presence of intraerythrocytic bodies, which could be *Cand. Anaplasma*
110 *sparouinii*. No infection was detected in granulocytes and platelets, but around one third of
111 erythrocytes harbored one or two small, round dark purple inclusions located at their
112 periphery and which could be *Anaplasma* (Figures 2A and B). We also detected the presence
113 of Howell–Jolly bodies in erythrocytes (Figure 2B), which could be a consequence of
114 splenectomy.

115

116 Eighteen months later (May 2021), the patient was admitted in the Cayenne Hospital Center
117 with fever, myalgia, headache, epistaxis and severe anemia (Hb = 6.6 g/dL). A broad
118 microbiological investigation ruled out covid-19, dengue, chikungunya, zika, influenza,
119 malaria, HIV, HBV, HCV, and leptospirosis. The only positive test was a subnormal level of
120 anti-*Coxiella burnetii* IgM and IgG leading to the introduction of antibiotic treatment
121 (doxycycline 100 mg twice a day for 21 days and ceftriaxone 1 g per day for 5 days). The
122 anemia was considered autoimmune hemolytic due to a positive Coombs test and was thus
123 treated with prednisolone with decreasing doses from 60 mg/d to 10 mg/d for 3 months. The
124 patient recovered within three weeks with resolution of symptoms and an improvement of the
125 hemoglobin level to 9.4 g/dL at discharge. Our a posteriori *Anaplasma* PCR survey of May
126 2021 blood samples (prior to and at Day 7 of the antibiotic treatment) again revealed the
127 presence of *Cand. Anaplasma sparouinii*. No further blood sample was preserved thus the
128 disappearance of the *Anaplasma* infection at the end of the antibiotic treatment could not be
129 ascertained.

130

131 **Conclusions**

132 We characterize here *Cand. Anaplasma sparouinii* as a novel human intraerythrocytic
133 pathogen. The infection arose over at least 18 months in a patient living in rainforest and
134 potentially more susceptible due to a previous splenectomy. The phylogenetic proximity of
135 *Cand. Anaplasma sparouinii* with other *Anaplasma* associated with Amazonian wild
136 mammals highlights that a genetic cluster of *Anaplasma* is circulating in French Guiana and
137 Brazil. While these neotropical *Anaplasma* species are different to species currently
138 recognized as human pathogens, they may represent a source of novel infections to humans in
139 Amazon rainforest.

140

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147

148 **About the author**

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152

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190 **Figure 1.** *Anaplasma* phylogenies constructed using maximum-likelihood (ML) estimations
191 based on (A) 16S rDNA (485 unambiguously aligned bp; best-fit approximation for the
192 evolutionary model: HKY+I) and (B) ITS2 sequences (387 unambiguously aligned bp; best-fit
193 approximation for the evolutionary model: HKY+I) including *Cand. Anaplasma sparouinii*
194 characterized in this study. GenBank accession numbers of sequences used in analyses are
195 shown on the phylogenetic trees. Numbers at nodes indicate percentage support of 1,000
196 bootstrap replicates.

197

198 **Figure 2.** Thin blood films showing inclusions of *Cand. Anaplasma sparouinii* located at the
199 periphery of the red blood cells as small round dots of 0.3 – 0.4 μm (arrow heads). Other red
200 blood cells contain Howell-Jolly bodies of various shapes and sizes $>1 \mu\text{m}$ (arrows). Some
201 Howell-Jolly bodies are found in the background of the smear (Wright Giemsa stain, x100).