Published in final edited form as:

J Med Primatol. 2014 June ; 43(3): 206–208. doi:10.1111/jmp.12105.

Persistent babesiosis in a Rhesus macaque (*Macaca mulatta*) infected with a simian-human immunodeficiency virus

David X. Liu¹, Amy Gill, Patricia J. Holman, Peter J. Didier, James L. Blanchard, Ronald S. Veazey, and Andrew A. Lackner

Divisions of Comparative Pathology (Liu, Gill, Didier, Veazey, Lackner) and Veterinary Medicine (Blanchard) Tulane National Primate Research Center, Covington, LA, and the Department of Veterinary Pathobiology (Holman), College of Veterinary Medicine, Texas A&M University, College Station, TX.

Abstract

A rhesus macaque developed persistent babesiosis following inoculation with a simian-human immunodeficiency virus. Blood smears demonstrated intraerythrocytic piroplasms and rare Maltese cross forms. *Babesia microti*-like protozoa were confirmed by PCR and gene sequence. With using nonhuman primates as models for human diseases, infection and complications from *Babesia* should be monitored.

Keywords

Babesia microti-like; simian-human immunodeficiency virus (SHIV); nonhuman primate

A 7-year-old, female rhesus macaque (*Macaca mulatta*) was originally imported from China through a vendor. Tests for herpesvirus papio 2, simian immunodeficiency virus, simian type D retrovirus, simian T-cell lymphotropic/leukemia virus types 1, and *Mycobacterium tuberculosis* were negative. All clinical data and physical observations were unremarkable during the 3-months quarantine. The animal was assigned to a human immunodeficiency viral vaccine study, which received prior approval from the institutional animal care and use committee (IACUC) of the TNPRC in Covington, LA. This study was conducted within the guidelines for ethical use of animals in United States Public Health Service policy as outlined in the Guide for the Care and Use of Laboratory Animals.

The animal was first challenged intravaginally with simian-human immunodeficiency virus (SHIV-RT) 8 hours after vaginal administration of a proprietary candidate microbicide. The animal was clinically and hematologically normal for the following 7 months. The animal was then inoculated intravaginally a second time with SHIV-RT. Two weeks later, the animal developed mild to severe anemia, fever, and anorexia. The number of red blood cell counts (RBCs) and lymphocytes decreased gradually, and reached their lowest levels (RBCs: $3.24 \times 10^6/\text{UL}$, reference interval [RI] $4.98\text{--}6.42 \times 10^6/\text{UL}$; Hgb: 8.6 g/dl, RI 11.7 –

¹Corresponding Author: David X. Liu, Tulane National Primate Research Center, 18733 Three Rivers Road, Covington, LA 70433. dliu1@tulane.edu.

14.7 g/dl; HCT: 28.6%, RI 37.2-47.1%; and total lymphocytes: $1.01 \times 10^3/\text{UL}$, RI 2.3-13 $\times 10^3/\text{UL}$) 15 months after the 2nd SHIV inoculation (Fig. 1). At that time, Wrights-stained blood films were performed and parasitized erythrocytes were noted. The parasitemia was persistent but varied from greater than infection of 50% RBCs at the peak lymphopenia to less than 5% at the end of the study 38 months after the 2nd SHIV inoculation. SHIV infection was detected in the lung, spleen, lymph nodes and spleen by in situ hybridization.

Peripheral blood smears (Wright's stain) revealed intraerythrocytic protozoa of varying morphology. In the severely anemic stage, greater than 50% of RBCs contained 1 or more protozoa. The most common features were 1-4 μm in diameter piroplasms in a signet ring, or pyriform structure that usually had 1 or 2 round dark nuclei. Trophozoites in dots (early stage) or signet ring form (developing stage) usually had large, round, dark nucleus with scant bluish cytoplasm. Piroplasms with paired ring forms (Merozoites from binary fission) often had a small nucleus and larger vacuole and more bluish cytoplasm. Some large merozoites displayed an irregular ring form with 3 or more variable-sized granules of chromatin arranged along the peripheral cytoplasm. The rare tetrad forms of merozoites (Maltese cross) were also observed (Fig. 2). Occasionally low numbers of extraerythrocytic piroplasms (merozoites released from lysed RBCs) were noted. The infected RBCs had no intracytoplasmic pigment. RBCs had mild to moderate anisocytosis, acanthocytosis, macrocytosis, polychromasia, and rare nucleated RBCs. The numbers of platelets and the morphology of leukocytes appeared normal. During recovery from anemia, the parasitemia decreased to less than 5%. The piroplasm was identified by PCR and subsequent 18S ribosomal RNA gene sequencing [3] as a rhesus monkey *B. microti*-like parasite [7], showing 100% identity to Genbank Accession No. EU168705.1. (The sequence was deposited in GenBank with Accession No. KC904078).

Since first described in a *Macaca irus* in 1934, *Babesia* have been reported in *Cercopithecus* monkeys, *Macaca mulatta*, African baboon (*Papio*), and pigtail macaques (*Macaca fascicularis*). Severe babesiosis is typically associated with splenectomy, anti-lymphocyte antibody therapy, and type D retrovirus infections [1]. *B. microti*-like organisms have been widely detected in wild rodents, Japanese macaques, free-range baboons and African green monkeys in Asia and African [2, 6]. These monkeys usually are asymptomatic although parasitemia can reach 0.3% in blood smears [2]. The animal in this current report was inoculated twice with SHIV. The vaginal administration of the test microbicide before the first challenge may have prevented SHIV infection in this animal, but the animal developed immunodeficiency after the 2nd SHIV inoculation without microbicide protecting. A similar vaginal microbicide has been demonstrated 24 h of protection from SHIV-RT vaginal infection [4]. The mild to severe babesiosis persisted for more than 2.5 years until the end of study. Persistent and relapsing babesiosis has been reported in HIV-infected patients and in other immunocompromised patients despite drug treatment. HIV-infected patients usually have chronic anemia, pancytopenia, and low CD4⁺ cell counts [5]. The lymphopenia in this animal was likely due to low CD4⁺T- cell counts [4], but these data were not available. HIV-infected patients with *babesia* commonly have a history of overseas travel or exposure to ticks in the woods of the northeastern of the United States [8]. Although the source of

Babesia in this case was uncertain, it is possible that this animal was infected with *B. microti*-like parasites while in China.

Due to the ability to remain latent in immune competent hosts but cause severe disease in immunosuppressed patients, *Babesia* has emerged as a growing public health concern. Babesiosis in this case suggests that *Babesia*, like other opportunistic infectious agents, could complicate studies when nonhuman primates are chosen as an animal model.

Acknowledgments

We thank Dr. Melissa Robbiani for the animal source. This work was supported by the National Center for Research Resources, and the Office Research Infrastructure Programs (ORIP) of the National Institutes of Health through grant no. OD011104-51 and Texas A&M AgriLife Research Project 08987.

References

1. Emerson CL, Tsai CC, Holland CJ, Ralston P, Diluzio ME. Recrudescence of *Entopolooides macaci* Mayer, 1933 (Babesiidae) infection secondary to stress in long-tailed macaques (*Macaca fascicularis*). *Lab Anim Sci*. 1990; 40:169–171. [PubMed: 2157097]
2. Hirata H, Kawai S, Maeda M, Jinnai M, Fujisawa K, Katakai Y, Hikosaka K, Tanabe K, Yasutomi Y, Ishihara C. Identification and phylogenetic analysis of Japanese Macaque *Babesia*-1 (JM-1) detected from a Japanese Macaque (*Macaca fuscata fuscata*). *Am J Trop Med Hyg*. 2011; 85:635–638. [PubMed: 21976563]
3. Holman PJ, Carroll JE, Pugh R, Davis DS. Molecular detection of *Babesia bovis* and *Babesia bigemina* in white-tailed deer (*Odocoileus virginianus*) from Tom Green County in central Texas. *Vet Parasitol*. 2011; 177:298–304. [PubMed: 21194841]
4. Kenney J, Singer R, Derby N, Aravantinou M, Abraham CJ, Menon R, Seidor S, Zhang S, Gettie A, Blanchard J, Piatak M Jr, Lifson JD, Fernandez-Romero JA, Zydowsky TM, Robbiani M. A single dose of a MIV-150/Zinc acetate gel provides 24 h of protection against vaginal simian human immunodeficiency virus reverse transcriptase infection, with more limited protection rectally 8–24 h after gel use. *AIDS Res Hum Retroviruses*. 2012; 28:1476–1484. [PubMed: 22737981]
5. Krause PJ, Gewurz BE, Hill D, Marty FM, Vannier E, Foppa IM, Furman RR, Neuhaus E, Skowron G, Gupta S, McCalla C, Pesanti EL, Young M, Heiman D, Hsue G, Gelfand JA, Wormser GP, Dickason J, Bia FJ, Hartman B, Telford SR 3rd, Christianson D, Dardick K, Coleman M, Girotto JE, Spielman A. Persistent and relapsing babesiosis in immunocompromised patients. *Clin Infect Dis*. 2008; 46:370–376. [PubMed: 18181735]
6. Maamun JM, Suleman MA, Akinyi M, Ozwara H, Kariuki T, Carlsson HE. Prevalence of *Babesia microti* in free-ranging baboons and African green monkeys. *J Parasitol*. 2011; 97:63–67. [PubMed: 21348608]
7. Voorberg-vd Wel A, Kocken CH, Zeeman AM, Thomas AW. Detection of new *Babesia microti*-like parasites in a rhesus monkey (*Macaca mulatta*) with a suppressed *Plasmodium cynomolgi* infection. *Am J Trop Med Hyg*. 2008; 78:643–645. [PubMed: 18385363]
8. Vyas JM, Telford SR, Robbins GK. Treatment of refractory *Babesia microti* infection with atovaquone-proguanil in an HIV-infected patient: case report. *Clin Infect Dis*. 2007; 45:1588–1590. [PubMed: 18190320]

Figure 1.
Hematology of a rhesus macaque with babesiosis.

Figure 2.

Peripheral blood smears (Wright's stain) 15 months after 2nd SHIV inoculation. About 50% of erythrocytes contain 1 or more different stages of piroplasms. Short thick arrows: single nucleus ring form trophozoite; >: Early dot form trophozoites; >>: Ring form merozoite with paired nuclei; >>>: Ring form merozoites with 3 to 4 nuclei; Long thin arrow: rare tetrad forms of merozoite (Maltese cross). 1,000× magnification.