SHORT COMMUNICATION

Aotus infulatus Monkey is Susceptible to *Plasmodium falciparum* Infection and May Constitute an Alternative Experimental Model for Malaria

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Aotus *is one of the WHO-recommended primate models for studies in malaria, and several species can be infected with* Plasmodium falciparum *or* P. vivax. *Here we describe the successful infection of the species* A. infulatus *from eastern Amazon with blood stages of* P. falciparum. *Both intact and splenectomized animals were susceptible to infection; the intact ones were able to keep parasitemias at lower levels for several days, but developed complications such as severe anemia; splenectomized monkeys developed higher parasitemias but no major complications. We conclude that* A. infulatus *is susceptible to* P. falciparum *infection and may represent an alternative model for studies in malaria.*

Key words: *Aotus infulatus* - *Plasmodium falciparum* - malaria - primate model

The neotropical non-human primate *Aotus* has long been known as a model for human malaria because it can support infection by human malaria parasites, including *Plasmodium falciparum* and *P. vivax* (Collins 1994). *A. lemurinus griseimembra* from northern Colombia is reported to be highly susceptible to both sporozoite and erythrocytic stage infection (Hershkovitz 1983, Collins et al. 1996), but the difficulties in obtaining this species (export has been prohibited) have led to the search, in countries other than Colombia, for other species within the genus *Aotus* as alternative models for malaria. Species belonging to both the Gray Neck (e.g. *A. lemurinus lemurinus* and *A. vociferans*) and the Red Neck (e.g. *A. nancymai* and *A. azarae boliviensis*) groups have been used, especially for vaccine and drug studies. *A. lemurinus lemurinus* from Panama has been recently reported as supporting sporozoite infection with the Santa Lucia strain of *P. falciparum* (Gramzinski et al. 1999). The Red Neck species *A. infulatus* is endemic in eastern Amazon (Pieczarka & Nagamachi 1988) and exclusive of the Brazilian territory; this species presents a karyotype and fur pattern similar to *A. azarae boliviensis*, but despite Pieczarka and coworkers have been proposing that both may form a single species (Pieczarka et al. 1993), the original classification by Hershkovitz (1983), based on differences in karyotype, fur pattern and geographical distribution, has been maintained to date. The susceptibility to plasmodial infection varies among species bearing different karyotypes. Splenectomized *A. azarae boliviensis* has been reported to be highly susceptible to different strains of *P. falciparum* (Collins et al. 1986) but, to our knowledge, *A. infulatus* from eastern Amazon has not been approached as a potential model for malaria. Here, we describe the successful infection of *A. infulatus* by assexual erythrocytic stages of *P. falciparum*, thus indicating that this species may be an alternative to others within the genus *Aotus* for research in malaria.

The authors dedicate this paper in honor of the Instituto Oswaldo Cruz, on the occasion of the centenary of its foundation, 25 May 1900.

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A. infulatus monkeys used in the present study were all bred in captivity and maintained in the Brazilian National Primate Center (Cenp). The colony was originally formed in 1984 with *Aotus* monkeys captured in both sides of Tocantins river, eastern Amazon, during flooding of Tucuruí dam's lake. They were classified as *A. infulatus*, and a number of them were karyotyped; they were shown to be homogeneous, presenting 2n chromosome numbers of 49 for males and 50 for females (Pieczarka & Nagamachi 1988). Most of the rescued animals were released in safe places and a number of them were taken to Cenp and gave origin to the present colony. Five monkeys (three males and two females), two of them splenectomized (one male and one female), were inoculated intravenously with $1x10^6$ *P. falciparum*-parasitized red blood cells (pRBC), having a clear predominance of ring forms and young trophozoites (FVO strain, a kind gift of Dr Socrates Herrera, Univalle, Cali, Colombia). Parasitized RBC were obtained from a donor *A. infulatus* previously inoculated with a thawed cryostabilate. Parasitemia was daily followed up by the examination of Giemsa-stained thick and thin blood films and expressed in terms of percentage of pRBC in relation to the total number of RBC. Hematocrit was evaluated each 3-4 days (pre-infection values ranged from 44% to 62%; median: 48%). Whenever necessary, monkeys were treated with a single dose of mefloquine (15 mg/kg), which, in all cases, caused immediate drop in parasitemia and a clearance of parasites within 1-4 days.

First parasites were detected in thick smears on day 4 of infection, both in the splenectomized and in the intact monkeys. Parasitemia grew then exponentially in the following days, until reaching values near 1% (around day 7 for most monkeys – Fig. 1a). From then, parasitemia kept growing although in a non-geometrical basis and was apparently assynchronous. Mature forms were commonly observed; in some cases the percentage of schizonts reached 15% of all forms in peripheral blood, and that of mature trophozoites up to 20%, though the rule was the clear predominance of ring and young trophozoites. Common features of young trophozoites were double chromatin and peripheric localization in the RBC.

Body (rectal) temperature was followed from day 2 to 10 (Fig. 1b). Pre-infection temperatures ranged from 38.5°C to 40°C; in the first days, there was considerable variation in individual temperatures but, from day 7 on, all monkeys (both splenectomized and intact) showed a synchronous variation in rectal temperature: at each two days, temperatures peaked at 41-41.5°C, decreasing to nearly 40°C on the day after. This observation suggests that *P. falciparum* in this model probably keeps a periodicity similar to that observed in humans.

In the absence of previous data of infection with malaria parasites in this particular species of *Aotus*, we evaluated both intact and splenectomized monkeys. In the two splenectomized ones, parasitemia reached more than 5% on day 10; one monkey was treated on this day and the other, left untreated for longer, supported well high parasite load (parasitemia of 26% on the day of treatment – day 16) without developing signs of clinical disease or of severe anemia (hematocrit of 35% on day 18).

One of the three intact monkeys also reached a 5% parasitemia on day 10 and was treated. The second one showed an apparent control of parasite growth, with a 2% parasitemia on day 10, but presented clinical signs of disease (prostration) on this day and was treated as well. The third one showed no clinical disease and controlled well the parasitemia, which was relatively stable around 2% up to day 17; on day 18, parasitemia rose to 5.5%, and the monkey was treated. This intact animal showed signs of weakness and, despite keeping low parasitemia, developed severe anemia by day 18 (13% hematocrit). After having cleared the parasitemia, this animal received a blood transfusion but died a few days later. The three monkeys treated on day 10 showed, at that moment, the following

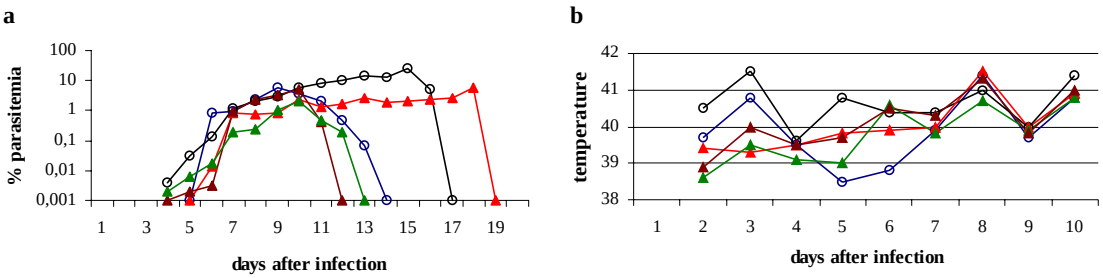

Fig. 1: course of parasitemia (a) and body temperature (b) in *Aotus infulatus* monkeys inoculated with $1x10^6$ *Plasmodium falciparum*-infected erythrocytes. Open circles: splenectomized; triangles: intact monkeys. In all cases, drop of parasitemia was caused by treatment with mefloquine.

hematocrits: 37% (splenectomized), 38% and 46% (intact).

The fact that the intact monkey (in contrast to the splenectomized one) left untreated developed severe anemia in spite of having low parasitemia may represent an additional evidence that spleen controls parasite growth probably by destroying large numbers of RBC, and that other mechanisms such as bone marrow depression, which may depend on the presence of the spleen, might also take place (Ferreira-da-Cruz & Daniel-Ribeiro 1996). Despite this possible deleterious effect, the spleen plays a crucial role in immunity against malaria and a decision for working with splenectomized animals should be carefully considered in any experimental procedure, since the use of intact animals may provide more reliable data in models that are, by definition, artificial. In any case, each infected individual must be closely followed up hematologically and treated before developing life-threatening severe anemia.

In the present work, both intact and splenectomized *A. infulatus* were shown to be susceptible to *P. falciparum* infection, though at different degrees (splenectomized animals had higher susceptibility, as expected). Accordingly, splenectomized *A. azarae boliviensis*, which presents a karyotype similar to *A. infulatus*, is also highly susceptible to *P. falciparum* infection (Collins et al. 1986). Taken together, these data indicate that *A. infulatus* can be a suitable model for *P. falciparum* malaria, at least for erythrocytic stages. However, testing a larger number of animals with different inocula is necessary to determine more precisely several parameters, such as: pre-patent periods, course of infection and its pathological consequences. Other approaches should include: definition of the susceptibility of this species to other *P. falciparum* isolates as well as to *P. vivax*, and attempts to infect *A. infulatus* with sporozoites. The establishment of *A. infulatus* as a reliable model for studies in malaria would be welcome in view of the numerous restrictions imposed for malaria vaccine and drug development and testing worldwide.

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