EXPERIMENTAL INFECTION OF THE OLIVE BABOON (PAPIO ANUBIS) WITH PLASMODIUM KNOWLESI: SEVERE DISEASE ACCOMPANIED BY CEREBRAL INVOLVEMENT

HASTINGS OZWARA, JAN A. M. LANGERMANNS, JENNEBY MAAMUN, IDLE O. FARAH, DORCASS.YOLE, JASON M. MWENDA, HORST WEILER, AND ALAN W. THOMAS

Institute of Primate Research, National Museums of Kenya, Nairobi, Kenya; Departments of Parasitology and Animal Science, Biomedical Primate Research Center, Rijswijk, The Netherlands

Abstract. Experimental systems that model some of the complex interactions between parasite and host can be extremely valuable in identifying and developing new prophylactics and therapeutics against human diseases. Because primates have similar immune systems to humans, we have characterized a baboon model for understanding host response to *Plasmodium knowlesi*. Ten intact olive baboons (Papio anubis) of either sex were experimentally infected with *P. knowlesi* H strain erythrocytic parasites. The infection in these baboons was either acute or chronic. Animals with acute infection developed multiple system organ dysfunction and cerebral involvement. In chronically infected animals, only the spleen was moderately enlarged. The *P. knowlesi* parasitemia profile in baboons and rhesus monkeys was comparable. However, some clinical symptoms of the baboons and *P. falciparum*-infected humans were similar. These studies demonstrate for the first time that *P. anubis* is a suitable host for *P. knowlesi* for studying clinical symptoms and pathology.

INTRODUCTION

The evaluation of new therapeutics and prophylactics for use in humans often requires testing in animal models that develop a disease comparable to that in humans. In certain basic and applied studies, primates are the only animal models susceptible to the human disease under study. Similarities in biologic mechanisms between humans and nonhuman primates underlie the value of nonhuman primates as the final host for studying the safety and efficacy of drugs and vaccines developed in studies with other laboratory animals and systems. Nonhuman primates are widely used in malaria drug and vaccine development.

The two major human malaria parasites (*Plasmodium falciparum* and *P. vivax*) have a very restricted host range, which limits research on parasite biology especially at the host-parasite interface. However, experimental systems have been used to model some of the complex interactions between parasite and host. There are three *Plasmodium* groups that are mainly used in experimental studies on host-parasite interactions, i.e., rodent, avian, and primate. Rodent malaria parasites are used to study parasite biology. However, these parasites are phylogenetically distant from human *Plasmodium*, and do not easily allow investigations of natural host-parasite interactions. Although *P. gallinaceum* and *P. lophurae*, the most widely used avian malaria parasites are closely related to *P. falciparum*, their development in nucleated cells and the wide phylogenetic distance between birds and humans limits their applicability to study important questions on host-parasite interaction relevant to human malaria. Simian *Plasmodium* such as *P. knowlesi* have a comparable phylogeny and host-parasite relationships to human malaria parasites. The parasites are used to identify, develop, and evaluate vaccine and drug candidates and to characterize host responses.

*Plasmodium knowlesi* malaria infection has been described in *Macaca fascicularis* (natural host), as well as experimentally induced in a number of other nonhuman primates such as *M. mulatta*, *M. radiata*, *M. assamensis*, *Presbytis entellus*, *Callithrix jacchus*, *Aotus trivigatus*, *Saimiri sciureus*, and baboons. In baboons, the infection has been induced in *Papio cynocephalus*, *P. doguera*, *P. jubilaeus*, and *P. papio*. The parasite also infects humans and clusters phylogenetically with *P. vivax*. In most experimental models, *P. knowlesi* infection is acute, whereas in their natural host *M. fascicularis*, it generally induces a chronic infection. The availability of both natural and artificial hosts combined with the close relationship between primates and man make *P. knowlesi* infection in nonhuman primates attractive to study host-parasite interaction in detail.

We have recently developed protocols for long term in vitro culture and genetic modification of *P. knowlesi*. These are powerful tools for understanding parasite biology, especially gene function. This has been facilitated further by the recent sequencing of its genome (http://www.sanger.ac.uk/Projects/Protozoa/) and that of *P. falciparum*. The function(s) of attractive drug and vaccine candidates can be determined using homologous *P. knowlesi* genes. However, versatile in vivo systems are required to determine host-parasite interaction of the genetically modified parasites. The baboon is attractive because it is a well characterized and frequently used primate in biomedical research. At the moment, the patterns and mechanisms of *P. knowlesi* infection in baboons are relatively unknown.

In this study, we determined the disease profile and pathology of the *P. knowlesi* H strain infection in the olive baboon as an experimental host system for understanding parasite biology of *P. knowlesi*, especially host-parasite interaction.

MATERIALS AND METHODS

Parasites. *Plasmodium knowlesi* H strain parasites for inducing baboon infection were retrieved from liquid nitrogen and cultured overnight. The original parasite inoculum was clone PkI(A+), previously cloned by micromanipulation and passaged in rhesus monkeys.

Animals. Adult *Papio anubis* (weight range = 11–21 kg) of either sex and originating from the Kajiado district of Kenya were used. Prior to the experiment, all animals were screened and determined to be free of infection with *Plasmodium* by a Giemsa-stained thick blood smear film. Ten baboons were inoculated intravenously with 1 × 10⁴ to 1 × 10⁶ *P. knowlesi*.
blood stage parasites. As controls for clinical symptoms, four uninfected animals were housed under similar conditions. All the animals were fed on a standard diet for baboons and water was provided *ad libitum*. The Institutional Animal Care and Use and the Scientific Review Committees of the Institute of Primate Research approved the baboon experiments. All experiments were performed in a biocontainment facility.

For comparative purposes, historical data were collected from six rhesus monkeys that had been infected with $1 \times 10^5$ *P. knowlesi* H strain parasites of the same stock. These experiments were done at the Biomedical Primate Research Center after approval by the Institutional Animal Care and Use Committee (the DEC).

**Animal observation and sampling.** Baboon health was monitored on a daily basis. Animals were assessed for 1) general agility, 2) playing habit, and 3) appetite (by weighing leftover food). Agility and playing habits of the monkeys were assessed by an attendant familiar with normal behavior of the particular animals. Animals were weighed on a weekly basis. Parasitemia was determined microscopically on finger prick thin blood smears stained with Giemsa and plotted as the number of infected cells in $1 \times 10^4$ erythrocytes.

**Clinical chemistry and hematology.** Venous EDTA blood was obtained from infected baboons after sedation with ketamine. Blood was analyzed in a Coulter counter (Beckman-Coulter, Mijdrecht, The Netherlands) to determine hematoctrit and hemoglobin changes. Serum was collected before and during peak infection, and used to analyze blood creatinine, bilirubin and urea. Commercial kits for Creatinine (Biotech Laboratories, Ipswich, United Kingdom), total bilirubin (Biotech Laboratories), and urea (Randox Laboratories, Antrim, United Kingdom) were used according to manufacture’s instructions.

**Pathology.** After the duty veterinarian had certified baboons with severe malaria as lethargic or comatose, they were humanely killed and autopsies performed immediately. Two animals died unexpectedly and were immediately presented for necropsy. Animals with chronic infection were killed at four weeks post-infection. Gross organ and pathophysiologic derangements were observed during necropsy. Brain, liver, lung, kidney, spleen, and lymph node specimens were collected and immediately fixed in formalin. Sections of 5-μm thickness were prepared from paraffin-embedded specimens, stained in hematoxylin and eosin and analyzed for pathologic changes. Erythrocyte aggregation in the brain was quantified by examining 100 small blood vessels and expressed as percentages, as described by Pongponratn and others.29

**RESULTS**

**Parasitemia.** All baboons inoculated with *P. knowlesi* H strain developed patent parasitemia by day five post-inoculation (Figure 1A). Seven animals had acute parasitemia that systematically increased to greater than 500 parasites per $1 \times 10^4$ erythrocytes, reaching as high as 4,950 parasites per $1 \times 10^4$ erythrocytes at the time of killing. All baboons with acute infection had become lethargic by day 12 post-infection (Table 1). The remaining three animals developed chronic parasitemia (Table 1) with peak levels of less than 300 parasites per $1 \times 10^4$ erythrocytes by day 16, which thereafter decreased to less than 50 parasites per $1 \times 10^4$ erythrocytes (Figure 1A). At low parasitemia, i.e., less than 300 parasites per $1 \times 10^4$ erythrocytes schizont parasite stages were rarely observed in the peripheral circulation, suggesting sequestration. At higher parasitemia, as observed in animals with severe malaria, the number of schizont stages in the peripheral circulation increased slightly, indicating reduced sequestration. The inoculum size and *in vivo* passage of parasites used to inoculate all the baboons did not determine the parasitemia and disease profile (Table 1).

Historical data on patterns of parasitemia were collected from rhesus monkeys previously infected with the same para-

![Figure 1](https://example.com/figure1.png)
sites as the baboons. All rhesus monkeys had developed patent parasitemia by day six post-inoculation (Figure 1B). The parasitemia profile in three of the monkeys (Rh2T, Rh 4086, and Rh9154) was characterized as chronic (Figure 1B), with peak parasitemia less than 700 parasites per 1 × 10⁴ erythrocytes and thereafter decreasing (Figure 1B). The other three animals (Rh C029, Rh 3015, and Rh 3337) developed acute parasitemia, i.e., levels greater than 700 parasites per 1 × 10⁴ erythrocytes (Figure 1B).

**Clinical symptoms.** Onset of patent parasitemia was followed by loss of appetite in all animals as measured by decreased food intake (Figure 2A) and marginal weight losses. Baboons with acute parasitemia developed severe clinical symptoms and were characterized as having severe malaria. These symptoms included a significant increase in body temperature as the infection progressed (Figure 2C) and remaining in a sitting position in the cage (apathy) with raised fur. In addition, they progressively became lethargic, developed dyspnea, and produced dark colored urine suggesting cholestasis. There was reduced ocular tension and skin turgor indicating dehydration. Hematocrit and hemoglobin levels showed moderate to low decreases (Figures 2B and 2D). Analysis of serum for bilirubin, creatinine, and urea to determine liver and kidney functions showed that in animals with severe malaria, there was a four-fold increase in total bilirubin (Table 2) and an increase in creatinine and blood urea, suggestive of severe hemolysis and dysfunctional kidneys (Table 2). By day 12 post-inoculation, all the baboons with severe malaria were either lethargic or comatose.

Animals with chronic parasitemia were classified as having mild malaria; they showed moderate to low level of the clinical symptoms observed in severely infected animals (Figure 2). However, they had a significant decrease in hematocrit and hemoglobin at peak parasitemia (Figure 2). There was a marginal increase in total bilirubin, creatinine and blood urea (Table 2). After day 16 post-inoculation, the behavior of these monkeys was similar to the uninfected and healthy control group.

**Gross pathology.** At necropsy, baboons with severe malaria were remarkably similar in the quality of gross appearance, varying only in the degree of manifestation of pathologic changes. As a general feature, all tissues, particularly the mesentery, were of yellow-tan appearance. Intramural vascular deposition of malaria pigment was a widespread finding. These animals presented with severe acute hemolytic anemia, severe diffuse pre-hepatic jaundice, hydropericardium, hydroperitoneum and hydrothorax.

Baboons with mild malaria had no comparable alterations indicating severe acute hemolytic crisis. However, mucous membranes were pale with yellowish tinge but not extended to the mesentery. In all infected baboons, the spleen was highly friable, hem-

<table>
<thead>
<tr>
<th>Baboon number</th>
<th>Inoculum</th>
<th>Infection profile</th>
<th>Inoculum passage in baboons†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan 1996</td>
<td>2 × 10⁶</td>
<td>Acute</td>
<td>12</td>
</tr>
<tr>
<td>Pan 2525</td>
<td>2 × 10⁵</td>
<td>Acute</td>
<td>10</td>
</tr>
<tr>
<td>Pan 2531</td>
<td>1 × 10⁶</td>
<td>Acute</td>
<td>12</td>
</tr>
<tr>
<td>Pan 2497</td>
<td>1 × 10⁶</td>
<td>Chronic</td>
<td>16</td>
</tr>
<tr>
<td>Pan 2048</td>
<td>1 × 10⁶</td>
<td>Acute</td>
<td>12</td>
</tr>
<tr>
<td>Pan 2055</td>
<td>2 × 10⁵</td>
<td>Chronic</td>
<td>16</td>
</tr>
<tr>
<td>Pan 2509</td>
<td>1 × 10⁴</td>
<td>Acute</td>
<td>11</td>
</tr>
<tr>
<td>Pan 2635</td>
<td>1 × 10⁴</td>
<td>Chronic</td>
<td>12</td>
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<tr>
<td>Pan 2550</td>
<td>1 × 10⁴</td>
<td>Acute</td>
<td>6</td>
</tr>
<tr>
<td>Pan 2451</td>
<td>1 × 10⁴</td>
<td>Acute</td>
<td>6</td>
</tr>
</tbody>
</table>

* Day post-infection when the animal profile was scored as acute or chronic.
† Parasite inoculum for the first passage was derived from rhesus monkeys and used to infect baboons after overnight in vitro culture.

### Table 2

Analysis of liver and kidney functions in baboons experimentally infected with the *Plasmodium knowlesi* H strain

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (mg/dL)</th>
<th>Total bilirubin (mg/dL)</th>
<th>Blood urea (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-infected</td>
<td>Infected</td>
<td>Pre-infected</td>
</tr>
<tr>
<td>Chronic</td>
<td>1.66 ± 0.19</td>
<td>2.80 ± 0.36</td>
<td>0.78 ± 0.16</td>
</tr>
<tr>
<td>Acute</td>
<td>1.86 ± 0.27</td>
<td>4.05 ± 1.25</td>
<td>0.58 ± 0.15</td>
</tr>
</tbody>
</table>

* Samples from infected animals were collected at peak infection. Values are the mean ± SD.
orrhagic, and pronouncedly enlarged with cut surfaces bulging out. Lungs from animals with severe malaria were distended with patchy consolidations and edematous. In animals with mild malaria, lungs had patchy consolidation and diffuse hyperemia. The liver of animals with severe malaria was enlarged, firm, and hyperemic. The lobes were distinct with rounded edges and the gall bladder was distended. Animals with mild malaria had a slightly enlarged liver with whitish streaks. Kidneys showed diffuse hyperemia and adherence of capsule in animals with severe malaria while kidneys of animals with mild malaria were without alterations. The brain of animals with severe malaria was edematous, congested on the external surfaces, and blood vessels were prominent. No pathologic changes were observed in the brain of animals with mild infection.

**Histopathology.** Histopathology showed that in animals with severe malaria, as expected from the high peripheral parasitemias, blood vessels of all diameters down to the capillaries of all tissues studied were interspersed with parasitized erythrocytes.

The brains of animals with severe malaria showed pronounced edema, multifocal neuronal degenerations, and mild gliosis (Figures 3A and C), in addition to widespread intravascular schizont-infected erythrocytes in microvessels (Figure 3C). More than 70% of brain microvessels of these animals were filled with aggregates of infected and non-infected erythrocytes which might represent sequestration (Table 3). Infiltrations of lymphocytes and phagocytic cells between endothelial cells of brain blood vessels, as observed in murine cerebral malaria30 were not encountered in baboons. The brain of baboons with mild malaria was normal (Figure 3B).

In baboons with severe malaria, alveolar septa were increased in diameter due to multifocal to diffuse mononuclear cell infiltration, segmental neutrophil infiltration and interstitial edema (Figure 4A). Pigment laden desquamated alveolar macrophages were commonly observed. In addition, multifocal neutrophilic granulocytes were found within alveolar lumina. Animals with mild infection showed low congestion of the lung.

The liver of animals with severe malaria showed cloudy to hydropic swelling of hepatocytes (Figure 4B), centrilobular dissociation, and necrosis of hepatocytes. Spaces of Disse were increased in diameter. Presence of hypertrophic pigment laden Kupffer cells and pigment laden sinus lining endothelial cells was a common feature in animals with severe malaria but less pronounced in animals mild malaria.

Kidneys from animals with severe malaria were characterized by multifocal interstitial nephritis with infiltration of mononuclear cells (Figure 4C). Glomeruli often showed seg-

**TABLE 3**

<table>
<thead>
<tr>
<th>Animal</th>
<th>PAN 1996</th>
<th>PAN 2525</th>
<th>PAN 2497</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitemia profile</td>
<td>Acute</td>
<td>Acute</td>
<td>Chronic</td>
</tr>
<tr>
<td>Peripheral parasitemia*</td>
<td>4,950</td>
<td>1,710</td>
<td>20</td>
</tr>
<tr>
<td>Blood microvessels containing†</td>
<td>86</td>
<td>87</td>
<td>1</td>
</tr>
<tr>
<td>Parasitized erythrocytes</td>
<td>80</td>
<td>70</td>
<td>2</td>
</tr>
</tbody>
</table>

* Peripheral parasitemia was determined as the animal was presented for necropsy. Values are the number of parasitized erythrocytes in $1 \times 10^4$ peripheral circulation erythrocytes.

† For each baboon, 100 blood microvessels in the brain were randomly evaluated.32 Values are percentages of brain microvessels.
mental or diffuse increase in volume of mesangial matrix. Atrophic glomeruli were an occasional finding. No lesions were observed in animals with mild malaria.

In all baboons, spleens were characterized by the presence of reactive germinal centers with mantle zones and showed pronounced pigment depictions. Compared with animals with mild malaria, thickening of lienal capsules and trabeculae appeared more pronounced in animals with severe malaria.

DISCUSSION

In this report, the clinical spectrum and pathology of experimental *P. knowlesi* infection in *P. anubis* is presented for the first time. The disease profile was either severe or mild. Baboons with severe malaria developed multiple system organ dysfunction with cerebral involvement.

In baboons with severe *P. knowlesi* infection, the brain showed considerable pathology including congestion, edema, neuronal degeneration, prominence of blood vessels, mild gliosis and aggregation of infected and uninfected erythrocytes in cerebral microvessels. The presence of aggregated erythrocytes in the brain of baboons with severe malaria suggests blockade of cerebral capillaries, which is associated with cerebral malaria in humans. Cerebral malaria is a serious neurologic condition that can lead to coma and death. It is defined as an altered consciousness in a patient who has malaria parasites in the blood and in whom no other cause of altered consciousness can be found. Blockade of brain blood microvessels in *P. falciparum*-infected humans and *P. coatneyi* or *P. fragile*-infected macaques is mediated through sequestration of knob-forming, mature, parasite-infected erythrocytes. Although knob-formation has not been defined in *P. knowlesi*, sequestration of *P. knowlesi*-infected erythrocytes might be mediated by schizont-infected cell agglutination variant antigens. Further studies to elucidate cerebral phenomena in the brain of *P. knowlesi*-infected baboons are required.

In the brain microvessels of baboons with severe malaria, many parasitized erythrocytes were present, but no lymphocytes and phagocytic cells were observed in contact with parasite-infected erythrocytes. This is similar to human cerebral malaria. In contrast, numerous phagocytic cells are encountered in brain microvessels of rhesus monkeys infected with *P. knowlesi* and in rodent cerebral malaria. Together, our data suggest that the cerebral involvement in *P. knowlesi*-infected baboons resembles several aspects of human cerebral malaria.

*Papio anubis* developed either severe malaria or controlled the parasitemia, resulting in a mild infection. This is also seen in rhesus monkeys. The mechanisms that predispose *P. knowlesi*-infected monkeys to developing either severe or mild infection are unknown. In general, *P. knowlesi* produces a chronic self-regulating infection in the natural host *M. fascicularis*. However, Schmidt and others showed that the course of *P. knowlesi* infection can differ in *M. fascicularis* from different geographic origins. In our study, animals originated from the same area, excluding monkey origin as a factor involved in the different infection outcomes. Our study also shows that inoculum size, age, and sex were not indicative of infection outcome. In humans, basal cytokine levels at
the time of infection and host genetic factors are most likely involved in determining \textit{P. falciparum} infection outcome.\textsuperscript{46–47} The precise mechanism that predisposes a dual outcome during malaria warrants further investigation and \textit{P. knowlesi}-infected baboons might be helpful in this. Our report shows that \textit{P. anubis} is fully susceptible to experimental \textit{P. knowlesi} H strain infection since all infected animals developed patent parasitemia. The parasitemia profile observed in the baboons was comparable to those in rhesus monkeys following infection with the same parasites,\textsuperscript{20} indicating that the virulence of this strain is similar in both monkeys, although studies were not done in parallel. In contrast to rhesus monkeys, \textit{P. knowlesi}-infected baboons develop clinical symptoms at onset of patent parasitemia. \textit{Plasmodium knowlesi}–infected rhesus monkeys frequently show minor clinical symptoms until they suddenly collapse due to massive parasitemia.\textsuperscript{6,12,37,41–43} (Langermans JAM and others, unpublished data). One possible explanation is that rhesus monkeys are relatively resistant to endotoxin-like characteristics mediated by malaria parasites.\textsuperscript{6,48,49} Humans infected with \textit{P. falciparum} also frequently develop clinical symptoms at onset of parasitemia.\textsuperscript{8,33,46}

\textit{Tapio anubis} was successfully infected with an inoculum size of $1 \times 10^4$ \textit{P. knowlesi} parasites. This suggests that the infection can be initiated by mosquito bite since a single hepatoocyte infected with \textit{Plasmodium} could contain $1 \times 10^6$ merozoites. Sporozoite induced infection is necessary if the olive baboon is to be used to study \textit{P. knowlesi} liver stage analyses. Moreover, we observed that \textit{P. knowlesi} continued to produce gametocytes after four passages in baboons. The viability of the gametocytes has not yet been characterized.

Infection of olive baboons with \textit{P. knowlesi} provides an additional malaria model that allows for \textit{in vivo} analysis of mechanisms of host response during severe and mild malaria. The use of baboons to study \textit{P. knowlesi} will specifically find relevance in facilities that are not home to other hosts of \textit{P. knowlesi}. These include baboon source countries and primate research facilities with access to baboons. Baboons can also be used to analyze host-parasite interaction of transfected \textit{P. knowlesi} (Ozwara H and others, unpublished data), which is an important tool for converting genome sequence information to medical use. Overall, our findings show that \textit{P. anubis} infected with \textit{P. knowlesi} show various clinical characteristics that are also seen in human malaria including cerebral involvement.

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Authors’ addresses: Hastings Ozwara, Jenneby Maamun, Idle O. Farah, Dorcas S. Yole, and Jason M. Mwenda, Institute of Primate Research, National Museums of Kenya, PO Box 24461, Karen, Nairobi, Kenya. Jan A. M. Langermans and Alan W. Thomas, Department of Parasitology, Biomedical Primate Research Centre, PO Box 3306, 2280 GH, Rijswijk, The Netherlands. Telephone: 31-15-284-2640, Fax: 31-15-284-3986, E-mail: thomas@bprc.nl. Horst Weiler, Department of Animal Science, Biomedical Primate Research Centre, PO Box 3306, 2280 GH, Rijswijk, The Netherlands.

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