Clinical Manifestations of *Plasmodium berghei*ANKA Infection in Juvenile Mice: A Short Case Report

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Abstract— Malaria is an important health and development challenge in Africa, Animalmodels most particularly mice, have long been employedto study malaria pathogenesis. Clinical manifestations due to Plasmodium bergheiANKA infection in juvenile mice as a model for understanding the complications ofcongenital malaria in neonates.Forty-five juvenile mice (5-7 days old) were acquired from University College Hospital, Ibadan and injected with 2 x 10⁷ (0.2ml) Plasmodium berghei ANKA parasitized red blood cells (PRBCs). Mice were transported to the study site, kept in well ventilated cages and fed daily with a balanced ration. Every day after post-P. berghei infection, mice were monitored for mortality. Clinical manifestations ofexperimental cerebral malaria (ECM) was assessed and confirmed if at leastruffled fur, hunching, wobbly gait, limb paralysis, convulsions, or coma was observed. Each sign was given a score of 1. Animals with scores ≥4 were considered to have severe ECM.20 (44%) micewerelost due to natural cause (i.e. stress) at day 2 of the experiment. Between day 4 and 9, 25 (56%) of the studymice presented clinical signs of ECM which includes; ruffled fur 25(100%), hunching 21 (84%), wobbly gait 17 (68%), limb paralysis 20 (80%), convulsions 25 (100%) and subsequently died. Survival rate and severity of ECM in the mice differs, 22 (88.0%) had severe ECM and 3(12.0%) had mild ECM.This study has shown that parasite establishment and malaria complications can manifest as early as 4 days'postP. berghei infection in 5-7 days old mice.
Keywords— Malaria, Neonates, Juvenile, Mice, ECM.

I. INTRODUCTION

Malaria is a parasitic disease caused by protozoan parasite from the genus *Plasmodium (P.)* with female *Anopheles* mosquito acting as the biological vector. Four species of *Plasmodium* are known to infect human, namely *P. Ovale, P. Vivax, P. Malariae*and *P. falciparum*, with thelatest being the most virulent and lethal (WHO 2016). Undoubtedly, malaria is still considered as the most serious tropical disease troubling mankind throughout the world with the greatest burden in sub-Saharan African countries. In 2015, there were 214 million cases and 438,000 deaths from this disease, with pregnant women and children under age-five been the most at-risk (WHO, 2015). Children under five are particularly susceptible to malaria illness, infection and death, and about 306,000 of them were killed globally by the disease in 2015 (WHO, 2015).

In non-immune individual, symptoms appear 7 days or more (usually 10–15 days) after the infective mosquito bite. The first symptoms – fever, headache, chills and vomiting – may be mild and difficult to recognize as malaria. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness, often leading to death. Children with severe malaria frequently develop one or more of the following symptoms: severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ involvement is also frequent (WHO 2015).

Experimental animal models have been used in understanding of human disease pathogenesis and development of new drug compounds and vaccines (Drulhe*et al.,* 2002), due to the impossibility of some research procedures on humans for practical or ethical reasons (Basir*et al.,* 2012). So far, Malaria models have been developed in monkey, rats and mice to understand certain aspects of *Plasmodium* pathogenicity in humans. Notably, the mouse model has been extensively studied due to the numerous similarities between the definedmalaria antigens in murine and human parasites and also between the murine andhuman immune response pathway. The many species and strains of *Plasmodium* available and the very large selection of inbred and outbreed laboratory rodents has created a great number of mouse-parasite combinations that can beused experimentally (Hunt and Grau, 2003; Combes *et al.,* 2005)

In this study, we used *P. berghei*ANKA infection in juvenile mice as a model to investigate the complications of untreated

Plasmodium infection. We describe clinical manifestations due to *Plasmodium berghei*ANKA infection in juvenile mice as a model for understanding the complications of congenital malaria in neonates.

II.   METHODOLOGY

2.1 Study Area

This study was conducted at the Zoology Laboratory, Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

2.1.1 Animals

Juvenile mice (5-7days) old were used in this study. The mice were obtained from the University College Hospital, Ibadan and transported to the study site, where they were maintained at a constant room temperature (25-27°C) in well ventilated cages with unlimited access to food and water. Animals were tagged with a permanent ink conspicuously on the tail for ease of identification.

2.1.2 Parasite and infection procedures

Plasmodiumberghei ANKA strain (chloroquine sensitive), was obtained from the University College Hospital (UCH), Ibadan. Serial passage of the parasite was initiated by intraperitoneal (i.p) injection of 0.2ml blood containing the parasiteinto a normal mouse (donor mouse). Subsequent passage for maintaining the parasites were carried out by i.p inoculation of study mice with 0.2 ml blood, diluted to contain 2×10^7 parasitized red blood cells (PRBC) from the donor mouse to study mice.

2.1.3 Routine observation of infected mice.

Every day after post-*P. berghei* infection, infected mice were routinely monitoredfor unusual symptoms and eventual mortality (3 times daily). For each of the tagged experimental mouse, clinical symptoms were recorded.

2.2 Data Analysis

Data obtained were entered using Microsoft Excel 2007 and described using Statistical package for Social Sciences, SPSS Version 20.0 (SPSS Inc. Chicago, Illinois, USA).

III.   RESULTS

3.1 Incidence of death and clinical manifestations in the experimental mice

A total of 45 (100%) mice were monitored over the study period, 20 (44.4%) were lost on Day 2 of the experiment. By day 4, 10 (22.2%) had convulsion, and an additional 10 (22.2%) had ruffled fur, hunching, limb paralysis by day 6. On the last day of the experiment, additional 5(11.1%) of mice had convulsion (Table 1).

Table.1: Incidence of clinical manifestations

Days	No of mice lost (%)	Clinical symptoms Before death	Probable cause of death
1	0 (0)	-	NA
2	20 (44.4)	-	Stress
3	-	-	-
4	10 (22.2)	Convulsion	*Plasmodium berghei ANKA*
5	-	-	-
6	10 (22.2)	Ruffled fur Hunching limb paralysis Convulsion	*Plasmodium berghei ANKA*
7	-	-	-
8	-	-	-
9	5 (11.1)	Convulsion	*Plasmodium berghei ANKA*
Total	45 (100%)		

NA: not applicable

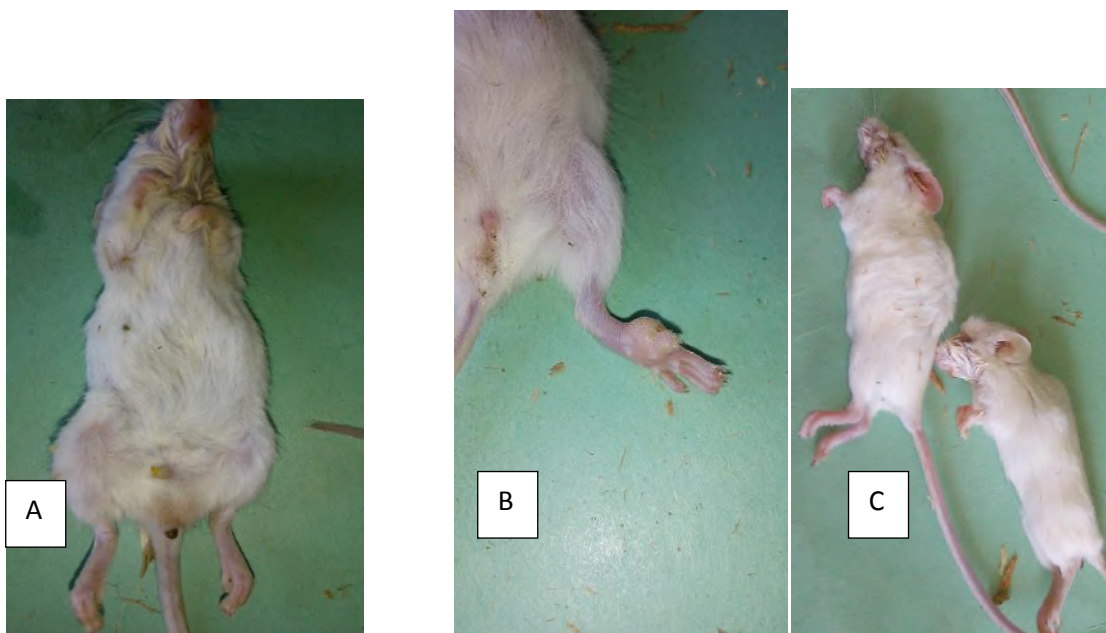### 3.2   Prevalence of Clinical Manifestation in Experimental Mice

Of the 25 mice closely monitored for clinical manifestations, 21(84.0%) had hunched back, 25 (100%) had ruffled fur, 17(68.0%) had wobbly gait, 20(80.0%) had paralyzed limb and 25 (100%) had convulsion (Table 2).

Table.2: Prevalence of Clinical Manifestation in Experimental Mice

Clinical symptoms	NE	NP (%)
Hunching	25	21 (84.0)
Ruffled fur	25	25 (100.0)
Wobbly gait	25	17 (68.0)
Limb paralysis	25	20 (80.0)
Convulsion	25	25 (100.0)
Coma	25	-

NE: Number examined; NP: Number with clinical presentations

Plates:1
A- Experimental mice with ruffled fur
B- Experimental mice with paralyzed limb
C- Experimental mice with hunched back

3.3 Prevalence of Experimental Cerebral Malaria (ECM)

For each observed clinical manifestations, a score of one was given to the experimental mice. Animals with scores ≥ 4 were considered to have severe ECM and those with lower scores (≤ 3) had mild ECM. Of the 25 (100%) mice observed, 3 (12.0%) had Mild ECM and 22 (88.0%) had severe ECM (Table 3).

Table.3: Prevalence of Experimental Cerebral Malaria

	NE	NP (%)
Mild ECM (ECM = 1, 2, 3)	25	3 (12.0)
Severe ECM (ECM $\geq$ 4)	25	22 (88.0)

IV.   DISCUSSION

Approaches to understanding disease pathogenesis are not new, and have involved experimental infectionsin animal model and subsequent monitoring of the pathogenic processes (*Basir et al.,* 2012). In this study, we used *P. berghei*ANKA infection in juvenile mice as a model to investigate the complications of untreated *Plasmodium* infection. Data obtained substantiated that juvenile mice from UCH Ibadan are highly susceptible to *P. berghei*ANKA infection, with rapid onset of malaria symptoms beginning on the fourth-day post-inoculation.

Clinical manifestations including hunching, ruffled fur, wobbly gait, limb paralysis, convulsions and subsequent death were observed, and are reflective of the high degree of parasitaemiathat could be associated with untreated *P. berghei*ANKA infections.

In humans, *P. falciparum* have been identified as the most virulent and lethal of all *Plasmodium* species, with various complications and ultimate death. Cerebral Malaria (CM) is one of the major lethal complications of *P. falciparum*infection in human which involved an extremelycomplex multi-process and multisystem disorder presenting a wide range ofclinical features. It is characterized by a sequestrationofparasitized red blood cells (PRBC) as the main importantfeature, particularly in the deep cerebral microvasculatureand by the increased levels ofpro-inflammatory cytokines (Clarke and Rocket, 1994; Artavanis-Tsakonas*et al.,* 2002).

Using the method described by Wu *et al.* (2010), we identified clinical signs of ECM in our study, with majority of the mice presenting severe ECM before death. Although we could notfurther investigatethe pathogenesis of *P. berghei*ANKA infection in the mice, this has been adequately described by Basir*et al.* (2012) in another study.

Complications of malaria and subsequent death in mice used during this experiment issues a call for concern in humans, especially neonates exposed to *Plasmodium* parasites and left untreated. It is therefore important to intensify on efforts that prevent congenital transmission of malaria parasite between mother and child, and as well be keen about procedures of prompt diagnosis and treatment of malaria disease in children with developing immunity.

V. CONCLUSION

Clinical manifestations observed during this study are reflective of the high degree of parasitaemia that could be associated with untreated *P. berghei*ANKA infections. Such complications issue a call for concern in humans, especially neonates exposed to *Plasmodium* parasites and left untreated. It is therefore important to intensify on efforts that prevent congenital transmission of malaria parasite between mother and child, and as well be keen about procedures of prompt diagnosis and treatment of malaria diseasein children with developing immunity.

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