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Short communication

Effects of artesunate treatment on *Plasmodium gallinaceum* transmission in the vectors *Aedes aegypti* and *Culex quinquefasciatus*

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ABSTRACT

In the absence of vaccines, chemotherapy is an effective and economical way for controlling malaria. Development of anti-malarial drugs that target pathogenic blood stage parasites and gametocytes is preferable for the treatment as it can alleviate the host's morbidity and mortality and block transmission of the *Plasmodium* parasite. Recently, our laboratory has developed an *in vivo* transmission blocking assay that involves administration of 7 consecutive daily doses of a test compound into domestic chickens (*Gallus gallus domesticus*) infected with the avian malaria parasite *Plasmodium gallinaceum* with 10% parasitaemia and 1% gametocytaemia. To compromise the cost and time for artesunate (ATN) treatment, this study aimed to investigate effects of a 5-day consecutive administration of 10 milligrams per kilogram (mg/kg) ATN on *P. gallinaceum* infection in chickens and transmission to two natural vectors, *Aedes aegypti* and *Culex quinquefasciatus*. Our study showed that the treatment with 10 mg/kg ATN for 7 days, but not 5 days, completely eliminated blood stage infections, prevented recrudescence and blocked gametocyte production and transmission of *P. gallinaceum* to its vectors, thereby confirming the potent schizontocidal and gametocytocidal activities of ATN. This regimen should be further evaluated in field trials.

1. Introduction

Transmission of a malaria parasite from a vertebrate host to a mosquito vector is an obligatory step in the malaria parasite's life cycle. The malaria parasite of birds and mammals all share the same complex life cycle in the insect vectors (McGhee, 1988). Thus, the malaria parasites of experimental animals, such as avian malaria parasites, are considered to be alternative research tools for investigating mechanisms of malaria transmission and developing platforms for testing transmission blocking agents (Gerberg, 1971; Gwadz et al., 1983). *Plasmodium gallinaceum* is the malaria parasite of domestic chicken (*Gallus gallus domesticus*) and is the major cause of avian malaria in

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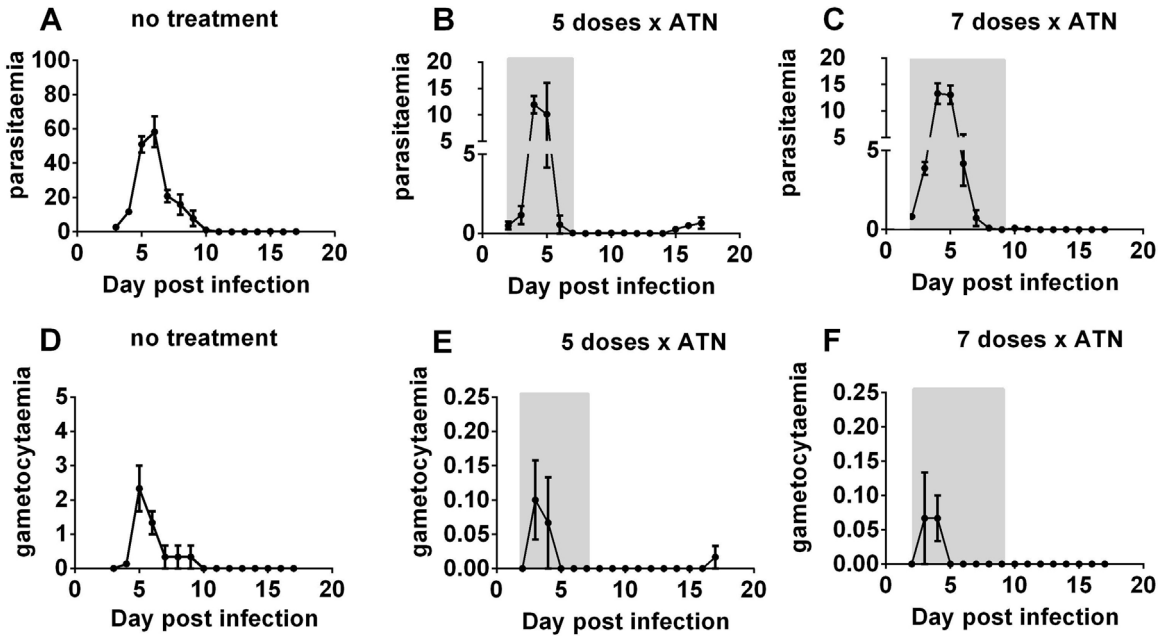

Fig. 1. *Effects of artesunate treatments on blood stage parasitaemia and gametocytaemia of Plasmodium gallinaceum.* On day 4 PI, the infected chickens were treated with 5 and 7 doses of 10 mg/kg ATN that was represented by area labelled in grey (B–F). Batch mates of the infected chickens receiving no drug treatment served as a control group (A and D). The infected chickens were monitored daily for parasitaemia (A–C) and gametocytaemia (D–F). Data given is the mean parasitaemia/gametocytaemia ± standard deviation.

southeast Asia and south Asia (Ruff, 1999; Valkiunas, 2005). It accounts for substantial economic loss to the poultry industry. Without appropriate treatments, avian malaria can cause severe pathology and death (Williams, 2005). To search for effective drugs that target both pathogenic blood stages and gametocytes, our laboratory has recently established an *in vivo* transmission blocking assay, in which *P. gallinaceum*-infected chickens with 10% parasitaemia and 1% gametocytaemia are treated with chemicals of interest or a vehicle (negative control) for seven days and the mosquitoes *Aedes aegypti* are then allowed to feed on chickens to determine *P. gallinaceum* infectivity (Kumnuan et al., 2013). It was shown that daily intramuscular injections of 10 mg/kg artesunate (ATN) for 7 consecutive days led to parasite clearance and the transmission inhibition in *A. aegypti*. It was also noted that the parasites disappeared from the blood, even after only 3–4 doses of the ATN treatments. Thus, in order to minimize the cost and time for treatment, our goal was to determine whether the treatment with 10 mg/kg ATN for 5 days was capable of clearing the blood stage malaria parasites and gametocytes and inhibiting transmission.

In our original *in vivo* transmission blocking assay, *A. aegypti* served as an insect vector for transmission because of the high infectivity rate (Weathersby and McCall, 1968; Garnham, 1966). It is known that mosquitoes in the genus *Culex* could also be potential vectors (Kim et al., 2009). In Thailand, *Culex quinquefasciatus* is highly prevalent in areas where *P. gallinaceum* is endemic (Thongsripong et al., 2013), and this species was also shown to be a vector for avian malaria (Vargas and Beltrán, 1941). In the present study, we carried out *in vivo* transmission blocking assays using two vector species, *A. aegypti* and *C. quinquefasciatus*, in order to determine the transmission blocking potential of ATN against the parasite. The outcome of this study will provide important information for the design of an effective regime for treatment of *P. gallianceum* in field sites.

2. Materials and methods

2.1. Malaria parasite and hosts

This study was conducted in compliance with the Mahidol University-approved animal use protocols and animal care & use regulations. The avian malaria parasite *P. gallinaceum* strain Pg01/2013MU was originally isolated from a domestic chicken in Chachoengsao, Thailand in November 2013. The parasite species was confirmed by microscopic analysis of the blood stages and by analysis of cytochrome B sequence (Hikosaka et al., 2011). Insect vectors were female mosquitoes *A. aegypti* and *C. quinquefasciatus* and avian hosts were female domestic chickens (*G. gallus domesticus*) strain CP Brown. The animals were maintained at Department of Parasitology, Faculty of Public Health, Mahidol University as previously described (Kumnuan et al., 2013).

2.2. Blood stage infections of P. gallinaceum and ATN treatment

Blood stages parasites of *P. gallinaceum* were injected intravenously into a jugular vein of experimental chickens ($n = 9$), 4 weeks of age. Each chicken received 10^6 infected red blood cells (iRBCs). Starting on day 4 post infection (PI), two groups of chickens ($n = 3$) with parasitaemia of 10% and gametocytaemia of 1% were injected intramuscularly into thigh muscles with 5 and 7 consecutive daily doses