

Efficacy of Artemisinin-lumefantrine for the treatment of uncomplicated malaria after more than a decade of its use in Kenya

Gabriel M. Kishoyian (✉ gkishoyian@gmail.com)

Kenya Medical Training College

Eliud N. M. Njagi

Kenyatta University

George O. Orinda

Kenyatta University

Francis T. Kimani

kenya medical research institute

Kevin Thiongo

Kenya Medical Research Institute

Damaris Matoke-Muhia

Kenya Medical Research Institute

Research

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Abstract

Background: *Plasmodium falciparum* resistance to antimalarial drugs remains to be a major threat to the control of malaria globally. After the deployment of artemisinin-based combination therapy (ACT), there have been reports of reduced sensitivity of the drug to parasite clearance. In Kenya, artemisinin-lumefantrine (AL) is the recommended first-line drug in the treatment of uncomplicated malaria. This study sought to assess the efficacy of AL after its reintroduction in Kenya, a decade later. In this study, we assessed clinical and parasitological responses of children under five years in May and November 2015 in Chulaimbo sub-County, Kisumu, Kenya.

Method: Patients of ≥ 6 and ≤ 60 months of age with confirmed *Plasmodium falciparum* mono-infection were enrolled in the study. The patients were treated with a standard dose of AL and followed up for 28 days. During which period we monitored treatment responses and follow-up adherence.

Results: Of the 90 patients enrolled, fourteen (14) were lost to follow-up, with 76 completing the study period. Seventy-five patients 75 (98.7%) cleared, parasitemia within 48 hours while one (1.3%) cleared on day 3. There was 100% clinical and parasitological parasite clearance.

Conclusion: Artemisinin lumefantrine was found to be highly efficacious to *plasmodium falciparum* parasites in children aged ≥ 6 and ≤ 60 months. Based on this, the drug can be used to treat uncomplicated malaria in the study population. However, there is need for continued monitoring of its effectiveness in children and adults to counter the threat of resistance.

Introduction

In spite of the a

In spite of the tremendous decline of malaria burden over the past decade, the disease still remains a major public health concern globally with sub-Saharan Africa bearing the greatest burden [1]. In 2018, approximately 219 million malaria cases and 446,000 deaths were reported worldwide. Of this 92% of cases and 93% of deaths were from sub-Saharan Africa, with children under five years of age and expectant mothers being affected the most [1]. There are 5 species of malaria parasites that infect humans: *Plasmodium falciparum*, *P. ovale*, *P. malariae*, *P. vivax* and *P. knowlesi* with *P. falciparum* being the most life-threatening, and responsible for the majority of morbidity and mortality [1].

World Health Organization Global Malaria Programme (WHO/GMP) recommends three primary malaria interventions comprising of; 1) Prompt diagnosis & treatment with effective medicines; 2) Insecticide-treated nets (ITNs) distribution to achieve full coverage of populations at risk of malaria; and 3) Indoor Residual Spraying (IRS) as a major means to reduce and eliminate malaria transmission [2, 3]. Since the initiation of the Roll Back Malaria (RBM) Project, over a decade ago there has been increased Long Lasting Insecticide Treated Nets (LLINs) coverage and intense case management in most countries where malaria is endemic and success has been reported due to the decline in Malaria transmission [4].

Despite the success of the current tools there is a need for more stringent measures of monitoring their efficiency and implementation of novel strategies. Regrettably, although the malaria vaccine known by its scientific name Mosquirix has offered modest protection against malaria in children in various countries such as Malawi, Ghana, and Kenya, the vaccine may not be available in the next few years and no other highly effective vaccine is on the horizon [5]. This calls for the integration of preventive chemotherapy and case treatment into main stream malaria interventions [6]. Early case detection and prompt treatment is the mainstay to minimize malaria-related morbidity and mortality with appropriate use of antimalarial drugs remaining the cornerstone of malaria control [1]. However, it's being threatened by the parasite resistance to anti-malarial drugs that have been reported in Southeast Asia with potential spread to Africa.

In the early 1940s, chloroquine (CQ) was the drug of choice for the treatment of malaria in many countries having been confirmed as an anti-malarial drug with a quick metabolism, good curative effect including affordable cost [7-10]. The first case of *P. falciparum* resistance to CQ was noted in the Thai-Cambodia border in Southeast Asia in 1957 and in 1959, the resistance observed in the Venezuela-Cambodia border in Northern South America and finally span to other countries around the world [11-12]. In Africa, resistance to chloroquine (CQ) led to its withdrawal as an antimalarial drug and replaced with sulfadoxine-pyrimethamine (SP) in the early 1980s [13]. Malawi was the first country in the continent to cease the administration of CQ in malaria chemotherapy in the year 1993 [14]. In Tanzania, CQ was used as a first-line malaria treatment drug since the 1970s but due to high levels of resistance, it was replaced with SP in the year 2001. This was short-lived, as resistance emerged soon after, thus necessitating the adoption of AL in 2006 [14].

In Cambodia ACTs was introduced in the year 2000 in form of artesunate with mefloquine (AM) [15]. Artemisinin resistance which is the cornerstone of ACTs was reported in western Cambodia and subsequently spreading several neighboring countries in the Greater Mekong sub region of Southeast Asia in recent years [16-20]. The World Health Organization recommends that malaria-endemic countries should monitor the efficacy of nationally recommended ACT to guide national treatment guidelines [11]. The Artemisinin-based combination reduces both malaria-associated morbidity and mortality as well as the transmission of *P. falciparum* by acting on gametocytes, decreasing the likelihood of drug resistance development [11, 21]. To date, approximately 40 countries in Africa and six in South America are using AL as their first or second-line treatments [2, 22].

The following artemisinin-based combinations are recommended; artemether-lumefantrine (AL), artesunate-amodiaquine (AS+AQ), artesunate-mefloquine (AS+MQ), artesunate-sulfadoxine/pyrimethamine (AS+SP), and dihydroartemisinin-piperaquine (DP) with AL being the most widely used combination and is currently the first-line antimalarial drug in most malaria endemic countries in the WHO African region [2, 19, 22]. AL which is a co-formulation Artemether and lumefantrine which, is an aryl related alcohol linked to quinine, mefloquine and halofantrine that is commercially accessible in fixed-dose combination. In this combination, artemether, existence as a first acting drug, rapidly lower the parasite biomass and reinstate the clinical symptoms, while long-acting lumefantrine

counteracts recrudescence. This dual effect eventually reduces the selective pressure on the parasite to develop resistance [23].

In Tanzania for example, AL was introduced as its first line for the treatment of uncomplicated malaria caused by *P. falciparum* in 2006 and it remained the drug of choice [24-25]. Several studies conducted in Uganda [26], Burundi [27], Rwanda [28] and other African countries have observed that AL including other artemisinin-based combinations, for example, ASAQ and DP which are first or second-line treatments in other African countries have extreme therapeutic efficacy [3]. However, among these studies, there are no reports of clinically significant artemisinin resistance in Africa, and the laboratory correlates of resistance in Southeast Asia including the delayed parasites clearance are rare in Africa [29-31].

In Kenya, CQ resistant *P. falciparum* was reported in 1977, whereby by 1998 resistant levels had reached 70% [7]. Like other sub-Saharan countries, Kenya replaced CQ with SP in 1999 as the official first-line in the treatment of uncomplicated malaria [14]. As a result of widespread increasing reports of SP efficacy being compromised in Kenya especially at the coast [32, 34], prompted another policy change in the treatment of malaria by introducing Coartem TM, an artemether-lumefantrine in government hospitals in the year 2006. A number of studies have observed that artemisinin-based combination therapy (ACT) is still efficacious for the treatment of uncomplicated falciparum malaria especially in Africa, and is the recommended antimalarial drug for the treatment of uncomplicated malaria caused by *P. falciparum* [10, 34-35]. However, as with other drugs, the curative effect of ACT has declined gradually along with its use within vivo *P. falciparum* susceptibility studies showing reduction over time [36]. Due to the threat of potential artemisinin drug resistance, the WHO recommends regular surveillance to monitor the performance of antimalarial drugs in malaria-endemic countries [2]. The study was carried out with the primary objective of assessing the clinical efficacy of AL with a six-dose regimen for the treatment of uncomplicated *P. falciparum* malaria using the WHO therapeutic efficacy protocols after its introduction in Kenya over a decade.

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Materials And Methods

Study area

We conducted the study in Chulaimbo Sub County Hospital, Lake Region of Nyanza Kisumu County, Kenya. The altitude level of the area is 1,131 m above sea level with yearly rainfall between 1200 mm and 1300 mm. The humidity ranges of 50% and 68% with temperatures ranging between 20°C and 35°C. Chulaimbo Sub County is a malaria-endemic zone with stable *P. falciparum* transmission [37]. Malaria infection accounts for 38% of all outpatient hospital visits and 40% of all admissions being children below five years and expectant mothers [7].

Study population

Children aged ≥ 6 and ≤ 60 months visiting the outpatient clinic of Chulaimbo Sub County Hospital with signs of uncomplicated malaria were recruited for the study in the month of May 2015 to November 2015. Inclusion criteria were children who were residents of the area, with a body weight of ≥ 5 kg, history of fever in the previous 24 hours or fever with temperature $\geq 37.5^{\circ}\text{C}$, infected only with *P. falciparum* and parasitemia in the range of 2,000 to 200,000 asexual parasites per microliter of blood, no general danger signs of severe and complicated malaria (prostration, breathing difficulties, severe anemia, convulsions and inability to drink or vomiting). Written consent was obtained from the parent or guardian of the child before recruitment into the study. Patients were excluded from the study if they were below 6 months and above 60 months, body weight less than 5 kg with a history of fever for more than 24 hours with a temperature of above 37.5°C , multiple infections, any signs describe above of complicated malaria, severe malnutrition (MUAC $<12\text{cm}$), hemoglobin $<10\text{gms/dl}$, infection with other diseases for example pneumonia, on antibiotics and treatment with antimalarial for the last 28 days. Also, the inability to take the drug orally, having taken antimalarial chemotherapy in the past two weeks, evidence of liver disease or acute infection other than malaria and unwilling to participate were excluded from the study.

Sampling design

This was a single-arm prospective in vivo study intended to assess the therapeutic efficacy of artemether-lumefantrine resistant after treatment of uncomplicated malaria.

Sample size determination

The sample size was calculated using [38] whereby 90 patients were recruited for the study.

Sample collection

Approximately, 0.05 ml of blood from a finger prick was collected; thick and thin smears were prepared on two different slides. One of the slides was stained with 10% Giemsa for 10-15 min and examined by microscopy to detect the presence of the malaria parasites and density estimated. The second thin film blood slide was stained with 3% Giemsa for 30-45 min and used to determine the species and presence of gametocytes. Parasitemia was measured by counting the number of asexual parasites against 200 leucocytes in thick blood films. Parasite density per μL of blood calculated by multiplying the total count by 40, assuming that $1\mu\text{L}$ of blood had a mean count of 8000 leucocytes [39]. The blood slides were declared negative when the examination of 100 high power fields did not show the existence of any malaria parasite. For quality control, each slide was re-examined by a second laboratory technologist, and those with discrepant results were re-examined by a third technologist. Final parasitemia was calculated from the average between the two readings.

Treatment, clinical monitoring and follow-up

Treatment with AL was done for 3 days based on patient weight. A fixed-dose combination of 20 mg of artemether and 120 mg lumefantrine per tablet, translating to one, two or three tablets per patient depending on the individual weight respectively was administered. A full course of AL consisted of 6 doses given twice daily (8 hours apart on day 0 and 12-hour on days 1 and 2). Patients were observed for 20 minutes to make sure they did not vomit. If vomiting occurred, a repeat dose was given after vomiting stopped. Any patient who persistently vomited was withdrawn and treated with parenteral artesunate or parenteral quinine according to the national guidelines for the management of severe malaria [40]. Besides, paracetamol was given to all patients with body temperature $\geq 38^{\circ}\text{C}$. Patients were admitted at the health facilities for close monitoring of response to treatment, therefore drugs were administered orally at the health facility under direct observation of a study nurse.

On completion of the dose, after three days, the patients were allowed to go home and follow-up visits were done on day 7, 14, 21 and 28 or at any time the patient felt unwell. Parents/guardians were informed and encouraged to bring their children back to the clinic whenever there were unwell without waiting for scheduled visits. Parents who did not show up during their scheduled visit by mid-day of day they were visited at home by a member of a study team and asked to come to the health facility. If a patient could not be traced for scheduled follow-up, he/she was classified as lost to follow-up. During the visits, both clinical and parasitological assessment were performed. Patients with recurrent infections occurring on day 7 and afterward were treated with artesunate or quinine (tablets, injection/intravenous) based on clinical presentation according to WHO protocol [41].

Treatment outcome classification

The clinical outcome was corrected with the parasitological cure on day 28 based on WHO protocol and the second part included parasitemia on day 3 after treatment [41]. Treatment outcome was classified as either early treatment failure (ETF) which refer to danger signs or severe malaria on day 1, 2 or 3, with the manifestation of parasitemia or parasitemia on day 2 being higher than on day 0, regardless of axillary temperature or parasitemia on day 3 with axillary temperature $\geq 37.5^{\circ}\text{C}$ and parasitemia on day 3 $\geq 25\%$ of count on day 0. Late clinical failure (LCF) that include danger signs or severe malaria with the manifestation of parasitemia on any day from day 4 to day 28 among patients who did not meet any of the criteria of early treatment failure; and presence of parasitemia on any day from day 4 to day 28 with axillary temperature $\geq 37.5^{\circ}\text{C}$ in patients who never met criteria of early treatment failure or late clinical failure. Late parasitological failure (LPF); this refers to parasitemia on any day from day 7 to day 28 with axillary temperature $< 37.5^{\circ}\text{C}$ in patients who never met any of the norms of early treatment failure or late clinical failure and adequate clinical and parasitological response (ACPR) which includes parasitemia on day 28, irrespective of axillary temperature, in patients who never met criteria of early treatment failure, late clinical failure or late parasitological failure.

DNA extraction using chelex method

DNA extraction was done on dried blood spot as described by [42]. Briefly, each dried filter paper was cut into small pieces and soaked in Saponin-phosphate buffered saline (PBS) overnight at 4°C. This was followed by washing with 1 x PBS and incubated for 30 minutes. The brown solution from the tube was discarded and 50 microliters of the stock 20% solution and 150 microliters of DNase free water were added followed by vigorous vortexing. The tubes were then heated at 100°C and centrifuged at 10,000 g for two minutes. Lastly, the supernatant was transferred to a new tube, spun again and a final transfer done. The DNA product was then stored at -20°C.

Amplification of Pf18sRNA gene

Plasmodium falciparum species were screened by amplifying the 18s RNA gene. Primers pairs designed by [43] of 18R-18F were used; (5'-CTGAGTCGAATGAACTAGCT-3') and (5'-CCATTTTACTCGCAATAACG-3') respectively. The PCR reaction, 1x of PCR buffer included MgCl₂, 400nM, 200nM of primers, 1U of Taq Polymerase and 1µL of DNA template was used. PCR was then run with the initial denaturation set at 94°C for 3 minutes, followed by denaturation at 94°C for 1 minute, annealing at 55°C for 2 minutes and extension at 72°C for 2 minutes. The final extension was set at 72°C for 10 minutes with a total of 30 cycles and finally halting the reaction at 4°C.

Results

Demographic characteristics

A total of 76 *P. falciparum* positive samples from children five (5) years and below were analyzed in this study. The mean age and standard deviation were 32 months and SD ± 11.232 while the weight mean in Kg and the standard deviation was 14.07 and ± 2.970 respectively. The minimum and maximum age were 12 and 58 months, while weight was 8 and 20 respectively. According to gender, we had 43 (56.6%) males and 33 (43.4%) females. The geometric mean of microscopic parasite count on day 0 (before treatment) was 120,595 parasites/µL while the standard deviation was 163,395.1 (95% CI: 82319.4-15887.89). On day 1, the parasite clearance rate had a mean and standard deviation of 3508.93 and 11,783 respectively (95% CI: 797.78-6220.67), on day 2, the parasite clearance had a mean and standard deviation of 24 and 170.85 (95% CI -15-63.31) (Table 1).

Table 1: Profile of children on therapeutic in the study population

Characteristics	Artemether-lumefantrine
	n=76
Male n (%)	43 (56.6%)
Female n (%)	33 (43.4%)
Mean age in months	32
Standard deviation	11.232
Range in age	12-58
Mean weight in Kg	14.07
Standard deviation	2.970
Range	8-20
Temperature mean on day 0	38.12±1.08
Temperature on day 3	36.5±0.62
Parasitemia (per μ L) on day 0 geometric mean	120,595
Range	880-832,000

Therapeutic efficacy outcomes

Of the 90 patients who participated in the study as shown in Table 2, 14 (15.6%) were lost (four, two, five and three) and were excluded on day 7, 14, 21 and 28 leaving 76 (84.4%) completing the follow-up to day 28. Within 48 hours (2days) of treatment, 75 (98.7%) had cleared parasitemia and 1 (1.3%) had parasitemia but cleared on day 3 as shown in Figure 2 with no recrudescence infection observed in the study while the mean body temperature on day 0 was 38.12± while on day 3 it subsided to 36.5±0.62 (Figure 3).

Table 2: Therapeutic efficacy of AL before and after PCR correction

Variables	Frequency
Parasitemia on day 3	0 (0.0%)
ETF	0 (0.0%)
ETF	0 (0.0%)
LCF	0 (0.0%)
LPF	0(0.0%)
ACPR	76(100%)
Initial no. of samples	90
lost to follow up	14
Total baseline	76

Discussion

The instant and extensive increase of anti-malarial drug resistance are hindering the progress of malaria control [12, 44]. In this study, we confirm a high cure rate and efficacy (100% ACPR) of the nationally recommended AL for the treatment of uncomplicated malaria since its introduction in Kenya over a decade ago. Also, the clearance of parasitemia was attained on day 3 an indicative of an extraordinary parasite clearance rate. However, though the 100% adequate clinical and parasitological response was observed in the present study, the results were comparable with studies in Papua New Guinea which confirms a high efficacy rate 97.8% [45]. These studies agree with the observation in Ethiopia, which shows a high therapeutic cure rate of above 98% [5-6]. The high cure rate of AL, especially in children less than five years is a good sign since treatment failure manifest easily in this age group because of low immunity [46].

In this study, AL has shown high efficacy similar to one carried in Tanzania and other parts of Africa primarily supporting the high efficacy rates of AL despite its use in the continent for over ten years [19, 47]. The elevated parasite clearance rates may possibly be described by the steadfast act of artemether to clear parasites biomass leading to a quick resolution of clinical manifestation. An indication of suspected Artemisinin resistance according to the WHO is the presence of delayed parasite clearance showing a slope half-life >5hours or day 3 positive rate <10% [11]. In the current study, immediate clearance of parasites after AL dose indicate the absence of Artemisinin resistance. This outcome correlate to a number of studies undertaken earlier in Kenya, Uganda, Somalia, Mali, and several other African countries indicating that Artemisinin resistance has not appeared in the African continent [19, 34]. The component drug, lumefantrine, is a gradually acting drug with a long half-life time ranging from 4-7 days [48]. This leads to successive accumulation of the drug after the completion of the full dose sufficient enough for the elimination of residual parasites and possibly prevention of new infection [6].

Among the Artemisinin-based combination therapies, artesunate-amodiaquine (ASAQ), artesunate-sulfadoxine-pyrimethamine (ASSP), dihydroartemisinin-piperaquine (DHA/PPQ), artesunate-mefloquine (ASMQ) and AL are the most commonly recommended ACTs for the treatment of uncomplicated falciparum malaria in African countries [11]. Several recent studies have observed that these ACTs have maintained high efficacy (cure rate $\geq 95\%$) in many of these countries, despite their use for more than a decade [49-51]. Nevertheless, a study carried out in Angola from two different regions in 2013 and 2015 showed a lower efficacy ($<90\%$ cure rate) of AL. In these studies, the administration of the evening dose was not supervised hence no confirmation that this dose was consumed by the patient. It also meant that the lower cure rate observed in the two regions of Angola in those two years was contributed as a result of a sub-therapeutic doses of the AL or might signal reduced efficacy [46]. The efficacious reported here for AL was also observed in northwest Ethiopia where there was the absence of ETF, confirming nonexistence of possible Artemisinin-resistant *P. falciparum* in the study area. Despite the study showing the absence of ETF with low recurrent malaria (1 LTF), the outcome of the Ethiopian study point to a highly therapeutic efficacy of both partners of AL [5].

Factors such as host immunity, nutritional, initial parasitemia level, pharmacokinetics, and pharmacodynamics may influence the therapeutic efficacy of a drug apart from inherent parasite susceptibility [5]. Any of the above may contribute to low efficacy of a highly efficacious drug. At the same time, resistant parasites may be cleared with the help of the immune system resulting in exaggerated efficacy of otherwise a less efficacious antimalarial drug [5]. The unfortunate emergence of Artemisinin resistance in Southeast Asia and the China-Myanmar border is a global concern on the treatment of uncomplicated malaria [52].

For example, in another clinical efficacy studies in Ethiopia observed that among the study population, there were five treatment failures, 1(1.1%) LTF and 4(4.5%) were LPF while 84 (94.4%) ACPR confirming that the treatment of uncomplicated malaria using AL has a high clearance rate similar to the current study which has observed a 100%.ACPR. These studies have shown high efficacy of AL in the treatment of uncomplicated malaria and agree with findings from other east African countries [52]. In this study, the baseline mean parasitemia was $120,595 \pm$. Parasitemia was linked to the degree of malaria severity and hence it's an important parameter to help in the decision of the type of treatment to be initiated. It is also an epidemiological implication parameter as it indicates the level of transmission in a specific area. The level of malaria endemicity in the study is characterized as holoendemic [53]. According to the health facility's official data, malaria incidence in the area is seasonal, subject to the amount and length of rainfall.

We observed that fever was associated with discomfort and was the major clinical manifestation with the mean baseline body temperature being 37.5 ± 0.62 . Artemether-lumefantrine has been reported as a fast-acting drug in case of fever in parts of sub-Saharan Africa [6, 54-55] contrary to data obtained from Southeast Asian countries [29]. Of major concern for ACTs in Africa especially in areas of intense transmission is the sluggish clearance of the parasite thus facilitating the development of resistant strains, necessitating the need for continuous surveillance of its efficacy.

Conclusion

This study concludes that the efficacy of AL for uncomplicated malaria caused by *P. falciparum* in Kenya is high despite the use of this antimalarial capabilities drug for over a decade making it a drug of choice. However, intensive and regular surveillance of ACT partner drugs needs be conducted to facilitate early detection of resistance to *P. falciparum* to inform policy makers on decisions on malaria treatment.

Limitation Of The Study

The outcome of the study could not be generalized because the small sample size and study population of children at age 6 to 60 months were recruited and only those that completed the study up to day 28 were considered in the analysis. In addition, molecular markers that are implicated in AL resistance were not included.

Abbreviations

AL Artemether-lumefantrine

SP Sulfadoxine-pyrimethamine

WHO World Health Organization

ETF Early treatment failure

LCF Late clinical failure

LPF Late parasitological failure

ACPR Adequate clinical and parasitological response

Pfdhfr Plasmodium falciparum dihydrofolate reductase

Pfdhps Plasmodium falciparum dihydropteroate synthase

IPTp Intermittent and preventive treatment programme

ASAQ Artesunate-amodiaquine

ASSP Artesunate-sulfadoxine-pyrimethamine

DHA/PPQ Dihydroartemisinin-piperaquine

ASMQ Artesunate-mefloquine

PCR Polymerase chain reaction

Declarations

Acknowledgement

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Author contributions

GK was involved in study design, data collection, and analysis, interpretation of the results and drafting of the manuscript. KT and GO were involved in data collection and manuscript writing, EN FK, DMM were involved in supervision, interpretation of the results and drafting of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data is available on request by the editor of publishing journal

Ethics approval and consent to participate

The study was approved by the Scientific Ethics Research Unit of the Kenya Medical Research Institute (SSC Protocol no. 2406) and the consent was obtained from the parents of the participating children

Consent for publication

Consent was obtained from the mothers that the results can be published provided the names of the children should not appear anywhere.

Competing interest

The authors declare that they have no competing interests

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Figures

Figure 1

No Figure 1 provided.

The mean parasite density of the study population

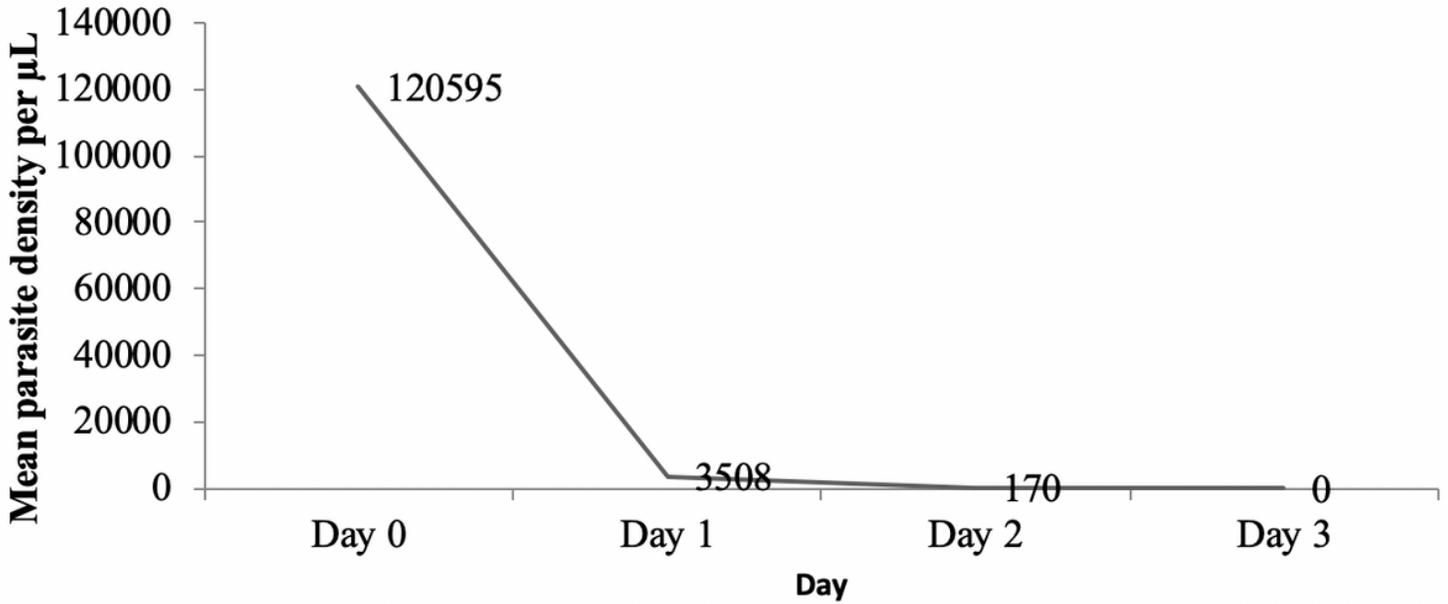


Figure 2

Presentation of mean parasite density on the first three follow-up days

The mean body temperature of the study population

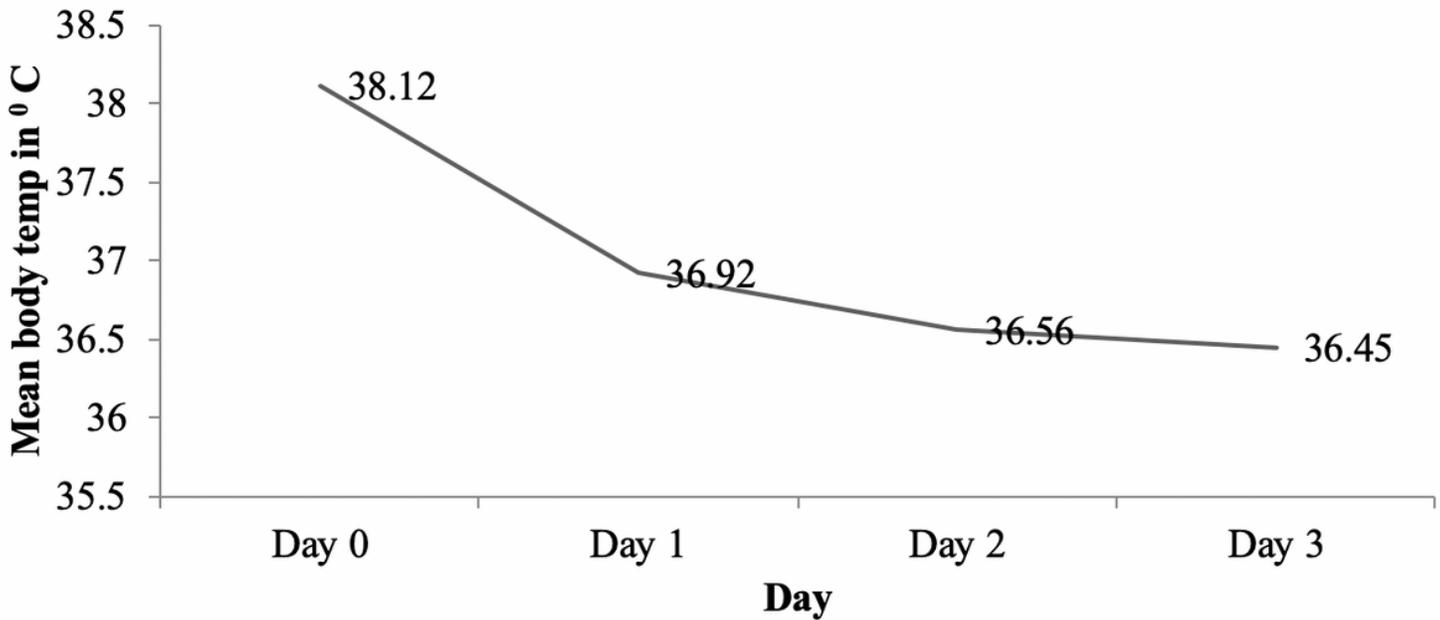


Figure 3

Presentation of mean body temperature on the first three follow-up days