Review Article

Prevalence of *Plasmodium* Species among Humans and Monkeys at Mole National Park in Northern Ghana

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Abstract

Malaria is one of the most severe public health problems in Ghana. In developing countries such as Ghana, with high of prevalence of malaria, the procedures for diagnoses and detection is limited in technological depth and for that reason most of these parasites; *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium knowlesi* are not routinely screened for; as such this study seeks to determine the prevalence of *Plasmodium* species among humans and monkeys at Mole National Park in Northern Ghana.

A total sample size of 217 comprising 214 human subjects and 3 baboons were recruited in this study conducted at Mole National Park. Data including; age, marital status, gender, occupation, knowledge on malaria and educational background information were collected. Blood samples were taken from both humans and baboons for the malaria testing using RDT, Microscopy and PCR to detect *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium knowlesi*.

The mean age of the study participants was 24.2±15.2. All the 3 baboon subjects were negative on RDT but one (1) out of the three was positive on microscopy. That notwithstanding, the same microscopy positive baboon subject was Pan-*Plasmodium* positive on PCR but was negative for the tested *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium malariae*.

The study recorded prevalence of *plasmodium* species using RDT and microscopy respectively to be 22.9% and 18.2%. The prevalence of *Plasmodium falciparum*, *Plasmodium ovale* and

 Plasmodium malariae among the human participants from this study were 39.9%, 12.6% and 18.6% respectively. Mixed infections were identified in 7.5% of the human participants where 4.2% was *Plasmodium falciparum*/*Plasmodium ovale*, 2.8% was falciparum/malariae mixed infection and 0.5% malariae/ovale mixed infection.

Sociodemographics, knowledge on malaria and interaction with monkeys of study participants did not show any significant association with malaria infection.

Prevalence of malaria parasites was high among the participant, which poses threat to reduction of malaria in the country.

Keywords: Malaria; Ghana; Outpatient departments; Monkeys; *Plasmodium ovale*

Introduction

Malaria infection in humans is caused by five *plasmodium* species: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium knowlesi*. Among the five human *plasmodium* parasites, *Plasmodium falciparum* causes more than 90% of the world recorded malaria morbidity and mortality four of the *plasmodium* species: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium malariae* are transmitted from one person to another by the bite of an infected female anopheline mosquito [1]. The fifth malaria parasite, *Plasmodium knowlesi* is a simian malaria parasite predominantly found in Southeast Asia. Zoonotic transmission of *Plasmodium knowlesi* malaria occurs following the bite of an infected female

anopheles mosquito (*Anopheles Leucosphyrus* and *Anopheles latens*) which results in severe diseases, high mortality in infant, children and in naïve adults Wesolowski et al., [2].

Ghana is a malaria endemic country with the entire population being at risk of malaria infection. Children below 5 years of age as well as pregnant women are at a higher risk of malaria infection due to lowered immunity. Presumptively, malaria cases reported at the Outpatient Departments (OPD) was 37.5% in 2015 [3]. Over the years, malaria morbidity and mortality have been reported to be declining in Ghana due to the implementation of various policies by the Ministry of Health and the Ghana Health Service including the use of Sulfadoxine-Pyrimethamine (IPTp-SP) as an intermittent preventive treatment in pregnant women, the free distribution of

insecticide treated net and diagnostic based approach with RDT, microscopy or PCR analysis before patient treatment [4,5].

In Ghana, *Plasmodium falciparum* causes about 90% of all malaria infection followed by *Plasmodium malariae* which causes less than 10% and more rarely *Plasmodium ovale* which causes about 0.15%. *Plasmodium vivax* and *Plasmodium knowlesi* have been reported to be absent in the country [3]. Anopheline gambiae species complex and Anopheline funestus are the major types of female anopheline mosquitoes that transmit the *plasmodium* parasites. *Anopheles melas* is found in the mangrove swamps of the southwest while Anoheline arabiensis in savannah areas of northern Ghana [3]. Despite the availability of effective antimalarial agents Hommerich et al., [4], failure to make the correct diagnosis has resulted in inappropriate therapy leading to preventable deaths.

The use of molecular tools for the detection of *plasmodium* parasite has deepened the epidemiology of malaria and highlighted *plasmodium* parasite infection in humans. A retrospective study conducted by using Polymerase Chain Reaction (PCR) on already examined *Plasmodium malariae* slide showed that 97% of the slides tested positive for *Plasmodium knowlesi* with only one being positive for *Plasmodium malariae*. The use of molecular biological technique has significantly impacted and facilitated the identification of infections caused by *Plasmodium falciparum*, other *plasmodium* species and mixed infections.

Background

Globally, malaria remains a significant infectious disease causing about 584,000 deaths and leads to an estimated 198 million cases in humans annually [6]. Statistics from 2015 to 2017 shows no significant reduction in global malaria cases. 219 million cases and 435,000 related deaths were recorded in 2017 about half of the world populations live in malaria risk areas. The annual malaria associated mortality approaches 1 million globally, with two (2) children dying from the disease every minute [7].

The protozoan parasite belonging to the genus *Plasmodium* causes malaria in humans. More than 150 *plasmodium* species have been identified to cause malaria in mammals, birds and reptiles [8]. Malaria parasite tends to be host specific regardless of having large number of hosts.

The natural host for the four (4) *Plasmodium* species; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* is human whereas long-tailed macaques (Macaca fascicularis) and pig-tailed macaque (*Macaca nemestrina*) are hosts for five (5) *Plasmodium* species: *Plasmodium knowlesi*, *Plasmodium fieldi*, *Plasmodium coatneyi*, *Plasmodium cynomolgi*, and *Plasmodium inui*.

Even though the four species of *Plasmodium* parasite exploit humans as intermediate hosts, at least more than twenty-six additional Non-Human Primate (NHP) parasites exist outside *Homo sapiens* hosts [9]. The principal record of NHP malaria parasite was reported by in an orangutan, Pongo pygmaeus. *Plasmodium* pitheci species was described a few years later, alongside *Plasmodium inui* and *Plasmodium cynomolgi* that infect sympatric monkey species, Macaca fascicularis and *Macaca nemestrina*, in Borneo [10].

Primate malaria discoveries continued as parasitologists investigated an increasing diversity of apes, monkeys, and lemurs from across the world. The infection of human with monkey malaria in 1965 by Chin et al., [11] initiated a resurgence of NHP malaria research focused on Southeast Asia, resulting in nine newly reported malaria species in Asian apes and monkeys a review paper by Vythilingam, [12] reported that is first discovered malaria parasite in long-tailed macaques. The parasite was again identified by Knowles and Das Gupta in long-tailed monkey that was migrated from Singapore to Calcutta [13].

Epidemiology of *Plasmodium* Species

Geographic distribution

Malaria is a mosquito borne disease in humans and animals following the bite of infected anopheline mosquito [14]. Parasitic protozoan of the genus *Plasmodium* causes malaria in human [15]. The mosquitoes, which act as vector for this disease, are female Anopheles funestus, Anopheles moucheti, Anopheles gambiae and Anopheles arabiensis [1].

Plasmodium knowlesi has been reported to be absent in Africa and for that matter Ghana as there are no investigations that have proof of endemic transmission but recently, increase in deforestation has cause many monkeys to have close contact with humans. In wild life reserved areas such as Mole National Park in Ghana, monkeys especially the baboon usually find food in neighboring human habitat which may lead to possible zoonotic transmission.

Reservoir hosts

Humans are the natural reservoir host of the four common malaria parasites: *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium vivax* while distinctive hosts of *Plasmodium knowlesi* identified were long-tailed (Macaca fascicularis) and pig-tailed (*Macaca nemestrina*) macaques from Singapore [13]. Zoonotic transmission occurs in humans living close to these macaques as reported in Southeast Asia [2,16-19].

Morbidity and mortality

As indicated by World Health Organization (WHO), malaria accounts for about 219 million cases and an expected 1 million deaths per annum a 2014 report by WHO Africa indicates that in every minute a child living in Africa dies from malaria infection [6]. In 2015, Ghana recorded about 10 million suspected malaria cases with 31% being children under 5 years of age [3]. Within that same period, 7% of all mortality were attributed to malaria with 48.4% deaths being children under 5 years. Meanwhile mortality have declined significantly from 12.3% since 2011 to 4.2% in 2016 [3] due to improved health systems, financial aids from international organizations and strategic plans such as the Presidents Malaria Initiative and National Malaria Control Program [3].

Transmission

Vectors and mode of transmission: Humans becomes infected with malaria parasite following the bite of infected female anopheline mosquitoes. More than 30 species of the mosquito vectors have been described to transmit malaria parasite [1]. These mosquitoes usually bite at dusk and at night which is the most active feeding times for the vectors the mosquito is infected following the bite of

an infected human where it sucks the male and female gametocytes. The gametocytes continue the sexual phase of the cycle and the sporozoites fill the salivary glands of the infested mosquito humans can also be infected by contaminated blood in rare cases. Vertical transmission of *plasmodium* species can also occur during delivery (congenital malaria). Blood transfusion, organ transplant or shared use of needles can be a risk of malaria transmission since malaria parasite normally feed on red blood cells. [20].

Hypothetically, four modes of malaria transmission have been identified: from an infected monkey to another monkey, from an infected monkey to human, from an infected human to another human and from an infected human back to monkey.

Risk factors for *Plasmodium* species transmission: Genetic factors of an individual from birth pose a risk of malaria infection. Two genetic factors have been linked with the red blood cell, which are of epidemiological importance. People with sickle cell traits are relatively protected from falciparum malaria infection. The sickle cell traits are commonly found in Africa where malaria is endemic and is thought to provide protection from malaria infection [21]. Duffy antigen has been reported to be the invasion point for *Plasmodium vivax* and *Plasmodium knowlesi* in humans the presence of the Duffy antigen on the surface of the erythrocyte increases the susceptibility to vivax and knowlesi malaria and it is believed that Duffy antigen acts as a receptor for attachment of *plasmodium* merozoites majority of Africans especially West Africans are Duffy negative and therefore are resistant to *Plasmodium vivax* and *Plasmodium knowlesi* infection [21]. Previous repeated malaria infection may lead to the development of acquired immunity against malaria and subsequent malaria infection may not develop severe complications and may lack typical malaria symptoms [21]. Pregnancy increases susceptibility to malaria. Pregnant women who have had immunity against malaria may lose this immunity especially in their first or second pregnancy [21].

Human behavior may pose a risk to malaria infection. Forest exposures, poor rural population in endemic areas, uneducated individuals, travelers from non-endemic area to endemic area are factors associated with malaria infection. Human activities may create site for mosquito breeding such as standing waters in irrigation ditches, burrow pits, etc [21].

For all vector-borne infections, the transmission is highly based on the bionomics of the vector in question. For example, because *Anopheline arabiensis* is an outdoor resting dominant vector species Hay et al., [22] people who work outdoors are at risk of contracting malaria by this vector.

Life Cycle of *Plasmodium* Species

Malaria parasites life cycles in humans and other primates begin when an infected female anopheline mosquito inoculates sporozoites into the bloodstream of its host while feeding on the blood [8].

Sporozoite in the skin

When an infected female anopheline mosquito bite humans or primates, sporozoites are introduced with the saliva into the vascular tissues of the skin. Within one minute, the motile sporozoites pass through the capillary wall and enter the blood stream [23]. Whiles in circulation the sporozoites moves quickly to invade the parenchymal cells of the liver within 40 minutes [24].

Liver stage malaria

After a successful invasion into the hepatocyte, a single parasite undergoes mitosis to produce 10,000 to 20,000 merozoites within 7-10 days period [25]. The enlarged hepatocyte called schizonts burst to release its content (merozoites) into circulation [23]. The liver phase of the life cycle is not associated with clinical signs and symptoms of malaria, yet permits the parasite to replicate [23].

Asexual erythrocytic cycle

When the merozoites are out from hepatocytes, they invade Red Blood Cells (RBCs) and enter into the asexual erythrocytic cycle. At this point, the parasites (trophozoites) grow and utilize the RBC contents as food. The merozoites grows and develop into a ring or early trophozoite within the erythrocyte. The early or ring trophozoites now develop into a mature trophozoite that undergoes asexual multiplication to form numerous merozoites in the erythrocyte which is now called schizont. When the erythrocytic schizont ruptures, it releases the merozoites which then invade more erythrocytes, thereby completing the erythrocytic cycle. Some of the parasites also differentiates into male and female gametocytes, which are taken up by female anopheline mosquito during blood meal.

Transmission back to mosquitoes

Once the gametocyte is inside the mosquito midgut, the temperature increases and pH changes, initiating gametogenesis and fertilization leading to the formation of motile diploid ookinetes that leave the blood meal bolus and cross the midgut epithelium to become a sessile oocyst. More than 10-14 days old sporozoite develops inside the oocyst by means of mitosis, and these escape through an enzymatic process into the mosquito body cavity. The sporozoites circulate by means of the haemolymph and attaches onto the basal lamina of the mosquito salivary gland, prepared for presentation into the next host [23] (Figure 1).

Pathogenesis, Pathology and Symptomatology of *Plasmodium* Species

Pathogenesis

The pathogenesis of malaria infection is highly associated with the hemolysis of infected and uninfected red blood cell. The formation of malaria pigment occurs when the parasite releases its antigenic substance into the host, which elicits an immune response. Cytoadherence is an important virulent factor adapted by *plasmodium* parasite to cause severe complications. The process of cytoadherence is as a result of the expression on the surface of the parasitized red cell of strain specific-ligands, which stick to a particular receptor complex on the endothelial cell. Anemia is inversely proportional to the number of red blood cells in individuals with recurrent episodes of malaria infection. This indicates that non-infected red blood cell may become sensitized and destroyed [24].

Pathology

Hyperparasitaemia: Hyperparasitaemia remains one of the significant virulence features of *Plasmodium* species. Investigations by Chaudhury and Venkataramana [26], Cox-Singh et al., [17] recorded parasitemia of over 700,000 per μL of blood on account of the short
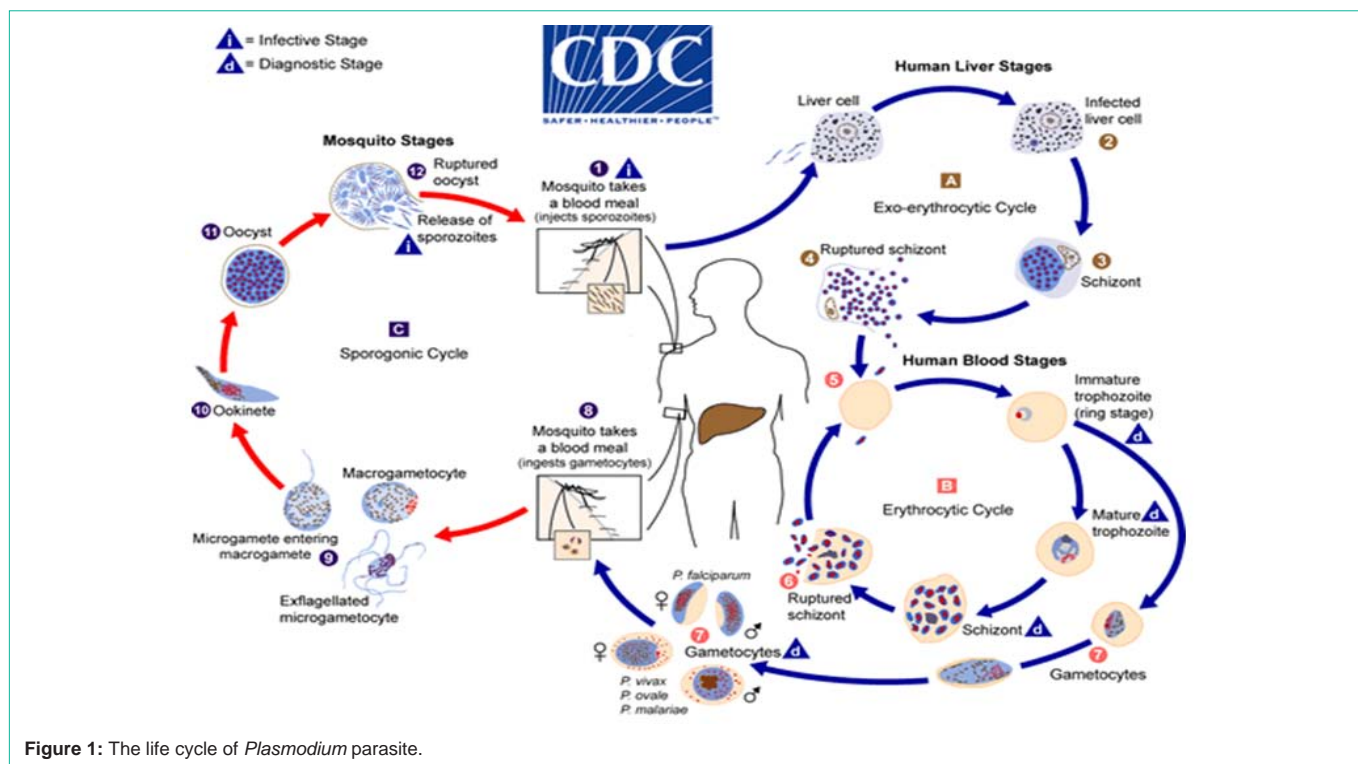
Figure 1: The life cycle of *Plasmodium* parasite.

erythrocytic cycle of some of the parasites particularly *Plasmodium knowlesi*. This parasitemia can build daily if uncontrolled.

Parasite sequestration: Parasite sequestration is an important factor in the development of cerebral malaria in falciparum infection and in a post mortem brain section of a fatal case of *Plasmodium knowlesi* reported by Cox-Singh et al., [27], sequestration of parasitized RBCs was evidenced. In another ex-vivo investigation by Chaudhury and Venkataramana [26], parasitized erythrocytes exhibited cyto-adherence to the receptors of the human endothelial cells. Their findings point to a conceivable sequestration of *Plasmodium* species infected RBCs in the capillaries.

Symptomatology

Fever, chills and rigors accompanied by cerebral headache, myalgia, poor appetite, cough, abdominal pain and diarrhea are the major symptoms of malaria [26,28]. An experiment conducted by Chin et al., [11] shows that patients injected with infected blood through intravenous or intramuscular route, developed clinical signs after an average of 8 days (3-14 days).

Plasmodium Species Diagnosis and Treatment

Clinical diagnosis

The most widely recognized research discoveries reported for 107 prospectively studied malaria patients were tachypnea, fever, and tachycardia [28]. In two prospective study of 107 and 130 patient by Barber et al., [29]; Daneshvar et al., [28], hepatosplenomegaly was reported for in 24 to 40% and 15 to 26% of cases, respectively. Clinical indications of severe complications including low oxygen saturation, tachypnea, chest crackle (indicating acute respiratory distress or co-existing pneumonia), hypotension, and jaundice have been recorded [17,28,30].

Laboratory finding

Thrombocytopenia: The most commonly reported blood abnormality which appears to be almost universal in all malaria infection is thrombocytopenia in a study by Daneshvar et al., [28] at the Kapit hospital, 98% of 107 patients presented with thrombocytopenia, and 2% of patients also became thrombocytopenic within 24 hour of admission. In spite of the incredibly high extent of patients with thrombocytopenia, and a 3rd of these patients being severely thrombocytopenic (50,000 platelets per µL blood), bleeding complications were infrequently observed a Comparative studies carried out by Barber et al, shows that the severity and recurrence of thrombocytopenia were higher with knowlesi malaria than with falciparum, ovale, malariae and vivax malaria infection Barber et al., [29]. Willmann et al., [31] also described laboratory markers of severity in malaria infection and discovered thrombocytopenia (45,000 platelets per ul blood) to be associated with complication having sensitivity and positive predictive value of 71% and 22% respectively [31].

Severe Anemia: Severe malarial anemia is commonly accompanied with chronic and recurrent infections of malaria and can results in hypohaemoglobinaemia < 5g/dl (normal values are between 10-15g/dl for humans).

Low hemoglobin levels occurring during malarial infections can be attributed to sequestration of RBC during parasite multiplication as well as removal of infected RBCs as part of immune - mediated clearance mechanism. Also, hyperactivity of the immune system in the spleen could result in premature removal of uninfected RBC. The development of new RBCs in the process of erythropoiesis replaces the loss RBCs. However, haemozoin and anti-malarials produced by

these *plasmodium* species alter the normal hematopoiesis Lamb [23] which can result in severe anemia.

Renal complications: Renal complication may develop during malaria infection and it is generally oliguric (<400ml/day) or anuric (<50ml/day), uncommonly nonoliguric and occasionally may need dialysis. In acute cases, tubular necrosis may result secondary to renal ischemia. 'Blackwater fever' resulting in hemoglobinuria may be temporal and unassociated with renal failure [32].

Metabolic acidosis: Metabolic acidosis is significantly associated with severe malaria infection. It is facilitated by the lack of RBC in circulation especially in patients with SMA as a result of hypoxemia and anaerobic metabolism. Also, in severe malaria, hypervolemia can enhance metabolic acidosis [23].

Pulmonary complications: Tachypnea and dyspnea are the characteristic symptoms of pulmonary edema accompanied by hypoxemia and respiratory failure due to intubation. Acute Respiratory Distress Syndrome (ARDS) and increase in pulmonary capillary permeability may develop as a result of pulmonary edema. A 5.6% and 10.7% frequencies were recorded on pulmonary edema for 107 and 130 knowlesi malaria cases studied respectively [28,29]. ARDS was seen in 52% cases with a crude mortality rate of 37% in severe cases of knowlesi malaria reported by [33]. Frequently associated complications were reported to be present (median 3), and in four patients, ARDS developed following admission, a pattern similar to that seen with falciparum malaria [33]. Statistical study undertaken by Barber et al., [29]; Daneshvar et al., [28] showed positive and independent association with hyperparasitaemia, neutrophilia and anemia at admission.

Hypoglycemia: Low blood sugar is a characteristic symptom in patients with severe malaria. Hypoglycemia may be induced by quinine or quinine-induced hyperinsulinemia; however, it may be found in patients with normal insulin levels [32].

Laboratory Diagnosis

Microscopy

Microscopy still remains an easy, economical and standard method for the diagnosis of malaria using thick and thin blood film stained with Giemsa, Wright's, or Field's stain. An ideal sample for malaria microscopy is blood obtained from the earlobe or finger. The reason being that, the density of developed trophozoites or schizonts is greater in blood from this capillary-rich area. Blood obtained by venipuncture collected in heparin or sequestrine (EDTA) anticoagulant-coated tubes should be immediately used to prevent modification in the morphology of White Blood Cells (WBC) and malaria parasites the four main *Plasmodium* species that cause malaria in humans, are identified widely by trained microscopists microscopic diagnosis of *Plasmodium knowlesi* is inadequate and unreliable due to morphological similarities it shares with *Plasmodium falciparum* and *Plasmodium malariae* [34]. In view of the possibly fatal result in *Plasmodium knowlesi* compared with the benign nature of *Plasmodium malariae*, it was recommended at a WHO consultation meeting on *Plasmodium knowlesi* that, microscopists should report all *Plasmodium malariae* positive outcomes as *Plasmodium malariae* / *Plasmodium knowlesi*.

Antigen detection test

Several Rapid Diagnostic Tests (RDTs) are available, which are fast, reliable and simple to use and can detect *Plasmodium falciparum* and non-falciparum infections or both [18]. RDTs are based on the detection of antigens derived from malaria patients in lysed blood, using immunochromatographic methods all the current commercially available RDTs were developed before it was discovered that *Plasmodium knowlesi* is a significant cause of human malaria.

Polymerase chain reaction (PCR)

Currently, molecular diagnostic assays specific for multicopy genes (such as small subunit rRNA or mitochondrial cytochrome b are actually considered the gold standard to detect unequivocally *Plasmodium* species malaria in humans [35].

Of the various gene targets which have been tried, the 18s Small-Subunit Ribonucleic Acid (SSUrRNA) has been used in most studies because of its genetic variation and hence for species differentiation [36]. The PCR can be used with the DNA from various samples like whole blood with anticoagulant, dried blood spots on filter paper and even scrapings from stained blood slides [17,26]. The conventional nested PCR is considered as "molecular gold standard" for malaria species detection [26]. However, this technique requires 5-6 separate PCR reactions to detect the 5 malaria parasites. This necessitates the use of multiple reagents and disposables increasing the chances of cross contamination. Moreover, it is cumbersome, time consuming and labour intensive. In view of the limitation of nested PCR, real time PCR has been employed for *Plasmodium* species detection. It is highly sensitive and can detect even very low parasitaemia of 1-2 parasites/ μL. Using a genome-mining approach, researchers from USA developed a new single-step PCR assay that targets a multicopy sequences for *Plasmodium* species and was apparently able to detect 1 parasite/μL with a specificity of 100% [37].

Loop mediated isothermal application (LAMP): A highly sensitive assays based on loop-mediated isothermal amplification (LAMP) which uses primers that detect the species-specific gene of *Plasmodium* species have been developed [38-40]. LAMP is highly sensitive, specific, rapid and does not require sophisticated instrumentation. However its assays need to be validated in large studies on the field [41].

In spite of these superior performances, the molecular detection methods are slower and more expensive than microscopy, and hence unlikely to replace routine microscopy in rural setting.

Treatment

Malaria is preventable and treatable but can be fatal if it's left untreated. Quick and complete removal of the *plasmodium* parasite from the patient's blood is the primary aim of treatment therapies. This is to prevent the development of uncomplicated malaria to severe infection that may lead to malaria related anemia and to reduce mortality. In the view of public health, treatment is intended to lessen the transmission of the infection to others, by reducing the infectious reservoir and to prevent the development and spread of resistance to antimalarial medicines [1]. Majority of the drugs used for the treatment of malaria are active against trophozoite stage of the parasite in the blood including chloroquine, atovaquone-

proguanil, artemether-lumefantrine, mefloquine, quinine, quinidine, doxycycline (used in combination with quinine), clindamycin (used in combination with quinine), artesunate. In addition, primaquine is active against the dormant parasite liver forms (hypnozoites) and prevents relapses. Primaquine should not be taken by pregnant women or by people who are G6PD (glucose-6 phosphate dehydrogenase) deficient. Patients should not take primaquine until a screening test has excluded G6PD deficiency [21].

Prevention and Control

Vector control is the ultimate intervention program to lessen the burden of malaria transmission at the community level. Personal protection against the bites of mosquitoes is the ultimate means of preventing malaria infection. Insecticide-Treated Mosquito Nets (ITNs) and Long-Lasting Insecticidal Nets (LLINs) are the two main effective vector control programmes established by the public health distribution program. WHO has recommended the distribution of nets to high risk persons and malaria endemic areas. The provision of free LLINs to individuals has been the effective means for everyone to sleeps under a LLIN every night. To effectively prevent malaria infection, Indoor Residual Spraying (IRS) with insecticides is an efficient way to minimize the spread of malaria. The potency of indoor spraying is 3-6 months, based on the surface on which it is sprayed and the type of insecticide used. Dicholoro Diphenyl Trichloroethane (DDT) can be effective for 9-12 months in some cases. Longer-lasting forms of existing Indoor Residual Spaying (IRS) insecticides, as well as new classes of insecticides for use in IRS programmes, are under development.

Malaria in Baboons

Initially discovered *Plasmodium* species in the blood of monkeys in Africa. Leveran also discovered similar malaria parasite in an Africa monkey and named it after Koch as *Plasmodium*. Following the discovery of malaria parasite in the blood of monkeys by Koch, malaria parasites have been reported as occurring in the monkeys of the New World, baboons, orang-utan, chimpanzee and gorilla [42].

In May 1922, microscopic analysis of a blood sample from a sick baboon found in Accra, Gold Coast, West Africa revealed the presence of hyperparasitemia in the baboon with *plasmodium* parasite [43].

According to the human-like structure of the baboon placenta and the cyto-adherent property of *Plasmodium knowlesi* have informed the choice for baboon-*Plasmodium knowlesi* model. The baboon model of placental malaria is therefore useful in understanding pathophysiology of malaria infection in humans.

Challenges in the Diagnosis of *Plasmodium* Species Infection

Timely diagnosis and treatment are crucial for reducing the risk of malaria complication [2,44]. In view of this, proper management and control strategies are imperative for the accurate identification for the *Plasmodium* species. Microscopy remains the ultimate standard method for diagnosing malaria infection in clinical practice and research but there are limitations in sensitivity and specificity [45].

According to Barber et al., [29], Lee et al., [34], the use of microscopy for the detection of *plasmodium* parasite cannot accurately and reliably distinguish *Plasmodium knowlesi* from *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium vivax*. The developmental stages of *Plasmodium knowlesi* thus the early trophozoite stage shares similar features such as double chromatin dots, multiple-infected erythrocytes and appliqué forms with *Plasmodium falciparum* and *Plasmodium malariae* [34]. The advent of RDTs has enhanced the diagnosis of human malaria. The advantages range from being rapid; user friendly to its cost effectiveness According to Moody one of the greatest advantages of RDTs is its ability to detect parasitemia above 100 parasites per μL of blood. However, *Plasmodium knowlesi* specific RDTs are not commercially available and the existing formats of RDTs are found to perform sub optimally when used in *Plasmodium knowlesi* endemic regions [46]. When the sensitivity of pan malarial marker pALD for *Plasmodium* species was compared, the sensitivity of *Plasmodium knowlesi* was found to be lower than that of *Plasmodium vivax* and *Plasmodium falciparum*.

Taking into account the many challenges associated with microscopy of *Plasmodium* species and antigen detection tests, molecular diagnostic tools are currently the only reliable option for the definitive diagnosis of *Plasmodium* species malaria infections. Molecular technique is the accurate method that has made the discovery of *Plasmodium knowlesi* malaria possible.

The genomic targets of *plasmodium* being studied currently are the 18S Small-Subunit rRNA (ssu- rRNA), the circumsporozoite surface protein gene, a nuclear gene encoding a cysteine protease and the cytochrome [47]. The 18s ssu-rRNA is the mostly studied genetic targets for species characterization of the different plasmodia [37].

Studies by Eede et al., [48], Imwong et al., [49] Sulistyaningsih et al.,[50] shows that the *Plasmodium knowlesi* -primers developed by Balbir Singh and co-workers, demonstrate cross- hybridization with *Plasmodium vivax*-sequences giving false positive results. A real-time PCR give an optimal performance by binding specifically with a 30-base pair of a variable region of *Plasmodium knowlesi* which showed a 100% specificity and a high analytical sensitivity (detecting 3 parasites/L of blood) [51]. However, they are expensive to run and require a substantial initial financial investment. The availability of this technique is found in referral laboratories in developing countries and diagnostic laboratory in developed countries. PCR and other molecular methods such as Loop Mediated Isothermal Amplification (LAMP) will not replace microscopy in rural areas due to their relatively high cost.

Study Area

The study was conducted at the Mole National Park, which is the largest wildlife reserve in Ghana. The park hosts different species including fauna, grassland, trees, herbaceous plants, shrubs and over 93 mammal species, covering an area of 4840 km² and lies between 9°42'N 1°50'W/9.700 °N 1.833°W. About 600 inhabitants of Mole include employees of the National Wildlife Division of the forestry commission of Ghana who reside in the park with their families. Baboons, Patas, Green vervet are the most common monkeys that come to the community every day for leftover food. The coexistence of these primates with the community makes zoonotic transmission a possibility.

Study site

Public health laboratory: The Public Health Laboratory (PHL) is located in the Tamale Metropolitan area and has WHO accredited malaria microscopists who assisted in the examination of the smears.

West African Centre for Cell Biology and Infectious Pathogens (WACCBIP): The West African Centre for Cell Biology and Infectious Pathogens (WACCBIP) is located at the University of Ghana, Legon, Accra. The institution is well equipped with modern and standard molecular assays for the conduct of this study.

Study Design

A cross-sectional study design was conducted from 29th January to 20th May 2019 at the Mole National Park.

Sample Size

Humans

The minimum sample size of humans that were screened for *Plasmodium* species was determined by the Yamane's formula.

$$n_{Yamane} = \frac{N}{1 + Ne^2}$$

Where, n = the sample size

N = the size of the population e = the error 5% point

By using Yamane's formula, a minimum sample size of 240 for humans was calculated from a population of 600 (according to National Population census, 2010).

Monkeys

Three monkeys (Olive Baboon) were sampled from Mole National Park for the identification of *Plasmodium* species.

Data Collection

A structured questionnaire was used for participant data collection. The socio-economic data collected include age, gender, marital status, occupation and educational information. Their knowledge on malaria and their interaction with the monkeys were also sampled.

Blood Sample Collection

Human sampling

An informed consent form was read and signed by the participant if satisfactory. Questionnaires were administered to the consented participant to fill. The informed consent was read and interpreted to participants who could not read. The questionnaires were then filled based on answers given by the participant. Consent was sought from parents or guardians for participants below 18 years of age. Tourniquet was applied to the upper arm of the participants to make the veins more prominent. The puncture site was cleansed with 70% ethanol and allow to air dry.

Blood samples were obtained by venipuncture. Approximately three milliliters (3 ml) of venous blood sample was drawn from human participants with a sterile dry plastic 5ml syringe and needle into a standard Ethylene-Diamine-Tetra acetic acid (EDTA) vacutainer tube. The tourniquet was released and a piece of dry cotton was pressed on the puncture site until bleeding stops. A volume of 75μL of the blood was spotted on a 3mm thick Whatmann filter

Paper, allowed to dry and sealed in clean zip-lock transparent plastic bag with desiccant.

RDT for falciparum malaria

The Rapid Diagnostic Test (RDT) for malaria, CareStart HRP-2 (AccessBiolnc., New Jersey, USA) was used to test for *Plasmodium falciparum* parasite in all the samples. The RDT test was conducted base on manufacturer's instructions by dropping 5μL of blood into a sample well (labeled "S") on the cassette and 2 drops of the buffer dropped into the buffer well (labeled "A" on the cassette). The cassette was placed on a horizontal surface for 20 minutes. A positive result was indicated by the appearance of both the test (labeled "T") and control (labeled "C") bands and a negative result indicated by only the control band. All samples were labeled using unique codes generated at the point of collection from Mole National Park.

Capture and Immobilization of Non-Human Primates (NHPs)

Three ml of intravenous blood samples were taken from the femoral vein of the olive baboon (Papio anubis) species after they were carefully trailed and darted with a Dan Inject Dart Gun for immobilization using Ketamine 10% in combination with medetomidine as a tranquilizer.

Sampling from NHP

A volume of 75μL was spotted on a 3mm thick Whatmann filter paper, allowed to dry and sealed in clean zip-lock transparent plastic bag with desiccant. The Rapid Diagnostic Test (RDT) for malaria, CareStart HRP-2 (AccessBiolnc., New Jersey, USA) was used to test for *Plasmodium falciparum* parasite in all the samples. The RDT test was done following manufacturer's instructions as previously described.

Sample storage and transport

The dry blood spot from both Humans and Baboons were stored at room temperature and transported to the West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), University of Ghana for molecular detection of *Plasmodium* species.

Sample processing and microscopic analysis

A 2μL and 6μL of whole blood were used to prepare thin and thick films respectively on the same slide and labeled appropriately. The dried thin film was immediately fixed in absolute methanol while the thick film was allowed to air dry. The thick and thin films were stained with Giemsa stain (1:9 dilutions with buffer) and examined with immersion oil (x100) objective. For the thick film, at least 100 fields were observed for trophozoites, schizonts and gametocytes and were counted against at least 200 - 500 white blood cells. Thin film was observed for speciation of organism against white blood cell count and parasitized red blood cell. Stained slides were examined by 2 qualified microscopists from the Public Health Laboratory. The results were recorded in a Microsoft excel Spreadsheet.

Quantification

Parasite Density against Relative White Blood Cell (WBC) Count and relative Red Blood Cell count

Thick film: Thick film microscopic reporting of malaria parasite was done by counting 8000 WBC per uL of blood

$$Parasites\big/\mu l = \frac{Parasite\ count}{WBC\ Count} \times WBC\ count$$

Thin film: Thin film microscopic reporting of malaria parasite was done by counting 5,000,000 Red Blood Cell (RBCs) perµL of blood

$$Parasites\big/\mu l = \frac{Parasite\ count}{WBC\ Count} \times RBC\ count$$

Species Identification by Nested Polymerase Chain Reaction (PCR)

DNA Extraction

The Chelex DNA extraction method was used to extract DNA from the dry blood spot as described by [21]. DBS containing 75µL of blood were excised into 2 mL Eppendorf tube and soaked overnight in 1 mL of 0.5% saponin in 1X Phosphate Buffered Saline (PBS). The Eppendorf tubes were centrifuged at 14000 rpm for 2 minutes, Saponin suctioned, and the contents of the Eppendorf tubes were washed with 1 mL of 1X PBS and suctioned. The washing step was repeated until no haem or red colour was unseen on the filter papers. A volume of 50µL of 20% chelex and 100µL nuclease free grade water were added to the tubes, and incubated in a boiling water bath for 20 minutes. The tubes were centrifuged at 14000 rpm for 2 minutes and 100µL of the resulting supernatant was pipetted into a 96 well plate and stored at -20ºC until used.

Plasmodium parasite detection by nested PCR

Malaria parasites were detected using nested PCR. Speciation and the identification of the *plasmodium* species were based on the amplification of the small subunit ribosomal genes with the second round PCR primers and amplicons sizes [52]. A 2µL of DNA was used in a 15µL reaction with outer forward primer rPlu6 and reverse primer Plasmo 2 for the first round PCR reaction using the Applied Biosystems Proflex PCR system of Thermo Fisher Scientific. The first-round PCR product was amplified in 15µL solution containing 7.5µL 2X Hotstar DreamTaq master mix, 0.3µL of 10µM rPlu6 Primer, 0.3µL of 10µM Plasmo2 Primer, 4.9µL of nuclease free grade Water and 2µL of genomic DNA. The PCR was performed following the standard thermycling conditions involving an initial denaturation period of 95ºC for 2 minutes, denaturation for 30 seconds, annealing at 58ºC for 30 seconds, extension at 72ºC for 60 seconds and final extension of 72ºC for 5 minutes. This first-round PCR was run for 35 cycles. For the second round PCR, 1µL of the first-round PCR product and species-specific primer for the four human malaria species were mixed in a 2 ml Eppendorf reaction tube. A total of 35 PCR cycles (95ºC for 2 minutes, 95ºC for 15 seconds, 58ºC for minutes, 72ºC for 30 seconds and 72ºC for 5 minutes) were performed. The PCR product were resolved in 1.5% agarose gel electrophoresis and viewed under florescence light using the Amersham Imager 600, (GE Healthcare UK Limited Amersham Place Little Chalfont Buckinghamshire HP7 9NA United Kingdom).

## Parasite Diagnosis by RDT, Microscopy and PCR for Human Samples

RDT and Microscopy

A total of 214 samples were tested with RDT and microscopy and confirmed by PCR analysis. Of the 214 samples tested by RDT, 22.9% (49/214) were positive for HRP2 antigens. On the other hand, 18.2%

Table 1: Prevalence of malaria by RDT and microscopy.

	RDT (%)	Microscopy (%)
Positive	49 (22.9)	39 (18.2)
Negatives	165 (77.1)	175 (81.8)

RDT and Microscopy results of human samples.
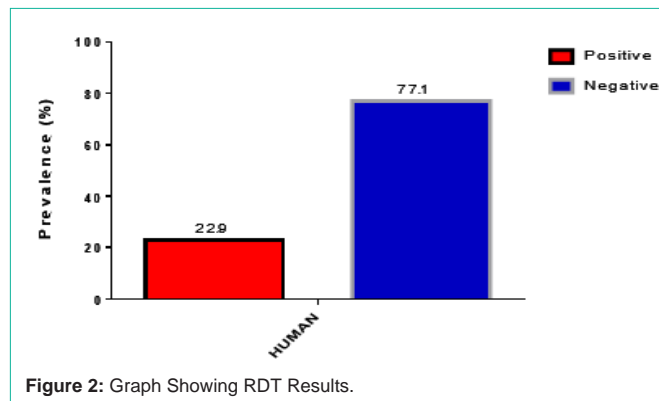
Figure 2: Graph Showing RDT Results.

Table 2: *Plasmodium* parasite identification by PCR analysis.

	PCR n=198 (%)		
	Pf	**Po**	**Pm**
POSITIVE	79 (39.9)	25 (12.6)	31 (18.6)
NEGATIVE	119 (60.1)	173 (87.4)	167 (96.3)

Pf: *Plasmodium falciparum*; Po: *Plasmodium ovale*; Pm: *Plasmodium malariae*.

Table 3: Mixed infection detected by PCR analysis.

PCR (%)	
Pf+Po	9 (4.2)
Pf+Pm	6 (2.8)
Pm+Po	1 (0.5)
Total	16 (7.5)

Pf: *Plasmodium falciparum*; Po: *Plasmodium Ovale*; Pm: *Plasmodium Malariae*.

Table 4: *Plasmodium* parasite detection of the diagnostic tools of NHP.

	RDT (n)	MCROSCOPY(n)	PCR (n)			
		Parasitemia Parasite/µL	Genus-specific *Plasmodium*	Pf	Po	Pm
Positive	0	3080	1	0	0	0
Negative	3		2	3	3	3

(39/214) were positive for malaria by blood films. Table 1, Figure 2 and 3 presents the results of RDT and microscopy analysis.

Species identification by species-specific PCR for human samples

Species-specific PCR based on ribosomal RNA (rRNA) small subunit (SSU) detected 39.9% (79/198) of test PCR samples to be *Plasmodium falciparum* mono-infection, 12.6% (25/198) *Plasmodium ovale* mono-infection and 18.6% (36/198) were *Plasmodium malariae* mono- infection. Mixed infections were also observed in 16 samples (Table 3). There were 4.2% *Plasmodium falciparum/plasmodium ovale* coinfection, 2.8% falciparum/malariae coinfection and 0.5% malariae/ovale coinfection. The prevalence of *plasmodium* parasite by PCR analysis is presented in Table 2 and Figure 4.

Outcome of the NHP Sampling

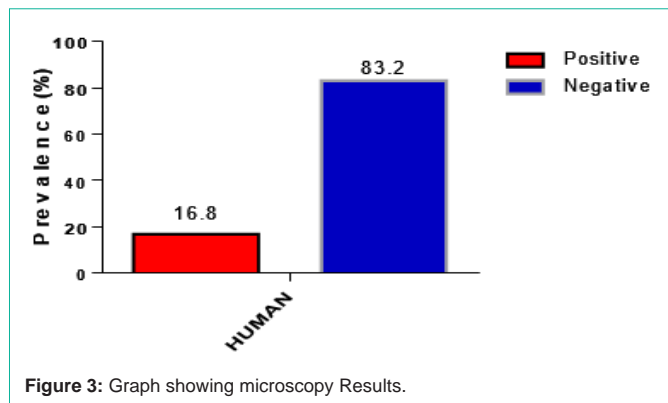All the three baboon monkeys that were captured for the malaria
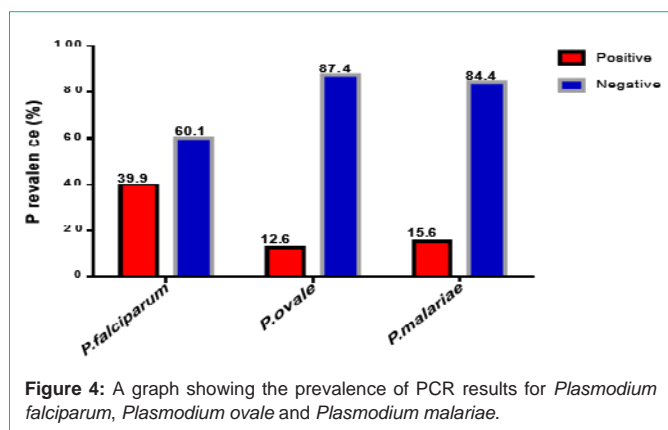
Figure 3: Graph showing microscopy Results.

Figure 4: A graph showing the prevalence of PCR results for *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium malariae*.

Table 5: Frequency distribution of Sociodemographic Characteristics of participants.

Variables	Frequency	Percent
Gender		
Female	98	45.8
Male	116	54.2
Age		
≤ 10	25	11.7
11-20	100	46.7
21-30	22	10.3
30+	67	31.3
Education		
Uneducated	8	3.7
Educated	206	96.3
Marital Status		
Married	124	57.9
Single	90	42.1
Occupation		
Unemployed	140	65.4
Employed	74	34.6

investigation were males with an average weight of 25.4 kg. One out of the three baboon samples was positive for microscopy with parasitemia of 3080 parasite/μL of blood. Details of the monkey sampling are presented in Table 4.

Siodemographic Characteristics of Participants

The average age of the study participants was 24.2±15.2 years with 116 (54.2%) male participants and 98 (45.8%) female participants. More than half of the participants were single 124 (57.9%) while 90 (42.1%) were married. Majority of the participants were educated 206 (96.3%).

140 (65.4%) were unemployed and 74 (34.6%) were employed (Table 5). Presents the frequency distribution of the Siodemographic characteristics of the participants of this study.

Siodemographic Factors Assiated with Malaria Infection from Logistic Regression

The Siodemographic characteristics of the participant are reported in Table 6. The logistic regression model showed that the prevalence of malaria among participants had no significant difference (p>0.05) in their age, gender, educational status, marital status and cupation.

Knowledge of Participants on Malaria Assiated with Malaria Infection

The knowledge of the participants about malaria was assessed; out of the 214 participants, 207 (96.7%) have heard of malaria while 205 (95.8%) confirmed mosquito as the vector for malaria transmission. With regards to their knowledge of the four-malaria parasite:

Plasmodium falciparum, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*, 150 (70.1%) had no knowledge about the parasite while 64 (29.9%) had knowledge about the four malaria parasites. Majority of the participants 180 (84.1%) do not have knowledge about the fifth malaria parasite, *Plasmodium knowlesi*.

Their knowledge about the signs and symptoms of malaria were also assessed (multi-checked variable). Majority of the participants 177 (82.7%) responded having fever, 114 (48.6%) responded having loss of energy, 132 (53.3%) responded having headache while only 21(9.8%) responded having itches. In assessing their personal protection against malaria infection (multi- checked variables), 189 (88.3%) uses mosquito net, 70 (32.7%) uses mosquito spray, 65 (30.4%) uses mosquito coil, while 111 (57.9%) uses other forms of protection against the vector.

Majority of the participants 188 (87.9%) have mosquito net while 26 (12.1%) do not use mosquito net. More than half of the participants (60.7%) started using the mosquito net 3 months ago while 84 (39.3%) of the participants responded using the mosquito 36 month ago. Only 56 (26.2%) of the participants used mosquito net a night before filling this questionnaire. Additionally, participants interaction with monkeys were also recorded. None of the participants (100%) had monkey as a pet. All participants live or school at the tourist site (Mole national Park) (Table 7). Presents the knowledge of the participant about malaria infection.

Logistic Regression of the Knowledge of Malaria Factors Assiated with Malaria Infection

Using binary regression model, the contribution on the knowledge of malaria of the participants were examined to predict the likelihood of participants being infected with malaria parasite. The prevalence

Table 6: Factors associated with malaria infection from logistic regression.

Variables		B	S.E.	Wald	Exp (B)	95% C.I. for EXP (B)		P- Value
						Lower	Upper	
Gender	Male	-0.001	0.338	0.000	0.999	0.515	1.937	0.997
	Female	0[b]						
Age				2.05				0.562
	≤ 10	-1.056	0.847	1.554	0.348	0.066	1.83	0.213
	11-20	-0.642	0.657	0.956	0.526	0.145	1.907	0.328
	21-30	0.2	0.581	0.119	1.222	0.391	3.817	0.73
	31+	0[b]						
Education				1.282				0.864
	Primary	-0.286	0.66	0.188	0.751	0.206	2.737	0.665
	SHS	-0.586	0.554	1.117	0.557	0.188	1.65	0.291
	JHS	-0.183	0.64	0.082	0.833	0.238	2.919	0.775
	No School	-0.454	0.895	0.257	0.635	0.11	3.67	0.612
	Tertiary	0[b]						
Marital Status	Married	-0.353	0.51	0.479	0.703	0.258	1.91	0.489
	Single	0[b]						
Occupation	Employed	0.561	0.642	0.762	1.752	0.498	6.167	0.383
	Unemployed	0[b]						

P-value: Analyzed by binary logistic regression analyses and considered significant at < 0.05 (2-tailed); the reference category is: Dependent variable is PCR *Plasmodium falciparum* positive; b: Parameter considered redundant and set to zero; B: Regression coefficient; ExpB: Exponentiation of B; which is the same as AOR: Adjusted Odds Ratio; 95% CI: 95% Confidence Interval; S.E: Standard Error.

of malaria was not statistically different (p>0.05) in those who have heard of malaria, the knowledge of the transmitting vector, knowledge of the four common *plasmodium* parasite, knowledge of *Plasmodium knowlesi*, whether there had been community insecticide spraying, the use of mosquito net, the use of treated net or the use of mosquito net yesterday (Table 8). Presents the logistic regression model on the knowledge of malaria factors Assiated with malaria infection.

Discussions

The Ghana Health Service [3] annual report indicates that 25.6% of all in-patient malaria cases in the country were recorded from the Northern region of Ghana, which was the highest among other regions in the country. Ghana is divided into three malaria epidemiological zones: the Northern savannah; the tropical rainforest; and the coastal savannah and mangrove swamps. In the Northern Savannah zone, pronounced seasonal variation curs, which has a prolonged dry season from September to April. The normal duration of the intense malaria transmission season in the Northern part of the country is about seven months beginning in April/May and lasting through to September [3]. In endemic settings including the study site where humans coexist with wild animals in a typical forest, the prevalence of malaria infection could be predicted to be high in concurrent with a study conducted by Barber et al.,[29] which reported that "a greater proportion of malaria infected patients lived in or near forested areas".

From this study, RDT and microscopy reported a malaria prevalence of 22.9% and 18.2% respectively, which is slightly below the malaria prevalence published in National survey of 34.3% and 26.4% for RDT and microscopy respectively [3]. The difference in prevalence

of RDT may be attributed to false positives that were detected by RDT test kit [53]. A study done to ascertain the prevalence of falciparum and non-falciparum in symptomatic and asymptomatic participants revealed high malaria prevalence of 87.3% when microscopy was used as the test method Previous study showed higher prevalence of 59%, 21% and 39% respectively for RDT, microscopy and PCR analysis from four different hospitals at the Volta region [53].

Only three plasmodia species out of the 5 known *plasmodium* species mentioned in this study prevailed at Mole National Park when PCR analysis was done. Majority of the malaria infection detected by PCR results were falciparum infections (39.9%) which is lower than the 90-98% which account for all malaria infections in the country [54].

Although only 0.15% of all malaria cases in Ghana are caused by *Plasmodium ovale*, 12.5% prevalence was observed in our studies [3]. The difference could be due to the sensitivity of the test method used because PCR is more sensitive than microscopy and RDT [51,53,55]. In consistence with studies done elsewhere in the country, *Plasmodium ovale* has been found to circulate in the population of the Ashanti's and in a foreigner who visited a tourist site in the northern part of the country [56]. *Plasmodium malariae* in this study showed high prevalence of 18.6% compared to the national prevalence of 2 - 9% [3]. These differences may be attributed to cohabitation of participants with NHP and climate of this forest ecological zone [57].

In most African countries and other part of South East Asia, increase in farming and forest activities have been known to increase malaria prevalence [30].

Out of the 214 samples that were tested on PCR, 7.5% were found

Table 7: Frequencies of Malaria knowledge factor of participants.

Variables	Frequency	Percentage
Heard Of Malaria		
No	7	3.3
Yes	207	96.7
Transmiting Vector		
No	9	4.2
Mosquito	205	95.8
Symptoms Of Malaria*		
Fever	177	82.7
Loss Of Energy	104	48.6
Vomiting	114	53.3
Sweating	76	35.5
Headache	132	61.7
Body Pain	113	52.8
Chill	91	42.5
Itching	21	9.8
Loss Of Appetite	115	53.7
Protection From Mosquito Bite*		
Mosquito Net	189	88.3
Mosquito Spray	70	32.7
Coil	65	30.4
Others	111	51.9
Know Of *Plasmodium* Parasite		
No	150	70.1
Yes	64	29.9
Know Of P. *Knowlesi*		
No	180	84.1
Yes	34	15.9
Malaria Kills		
No	19	8.9
Yes	195	91.1
Community Insecticde Spray		
No	174	81.3
Yes	40	18.7
Uses Mosquito Net		
No	26	12.1
Yes	188	87.9
Period Of Net Use		
36 Months Ago,	84	39.3
3 Months Ago,	130	60.7
Use Of Treated Net		
No	35	16.4
Yes	179	83.6
Used Net Yesterday		
No	158	73.8
Yes	56	26.2
Monkey As Pet		
Yes	0	0.0
No	214	100

*Multi-checked variable.

to be mixed infected. Previous studies conducted by Dinko et al., [53] at Ahafo Ano South district of Ashanti Region showed substantial mixed infections in asymptomatic school children. They detected mixed infection of *Plasmodium falciparum* with either *Plasmodium ovale* or *Plasmodium malariae* that is consistence with our study. A study by Imwong et al., [58] affirms that anopheline mosquitoes may harbor more than one malaria parasite, which can be transferred to human during blood meal resulting in mixed infections or separate insulation from different anopheline mosquitoes.

A study conducted in Mali by Dolo et al., [59] showed simultaneous infection with more than one *Plasmodium falciparum* parasite (genotype) in asymptomatic school children. At the peak of the transmission season, asymptomatic participants had six to eight different parasites in their blood at the same time. A review of mixed malaria species infections in anopheline mosquitoes showed that, Africa has contributed lesser to the diagnosis of malaria mixed infections globally [58]. This may be due to less advanced methods for *plasmodium* detection or misdiagnosis.

The persistence of the pan-*plasmodium* positive identified in the NHP in this study is a cause for concern and warrants further investigation. A laboratory-based study done to ascertain *Plasmodium knowlesi* model of placenta malaria in baboons (Papio anubis) indicated that the baboons developed acute or severe malaria after day 7 of *plasmodium* parasite inulation in view of this, the pan-*plasmodium* positive baboon will be sequenced to identify the specific *plasmodium* species present.

Binary regression model of risks factor analysis of the demographic information of having malaria in asymptomatic participants showed that age, gender, marital status, education and cupation did not cause any significant increase or decrease in risks of having malaria even though 39.9% of participant showed high falciparum infection on PCR results. A study conducted by Diallo [59] showed that being single or having no formal education had a higher odd for malaria infection. Another study done to assess demographic risk factors for malaria infection showed that male gender or forest exposures are risk factors for malaria infection [60].

In this study, knowledge of participants on malaria did not show any risk for malaria infection in contrast to a study conducted in pregnant women; knowledge on malaria and the use of mosquito net were assiated with the reduction of malaria infection [21]. Even though most participants (83.6%) have treated mosquito net, only 26.2% sleep under it a night before the demographic data was collected (Table 5). This may be attributable to the hot weather conditions at the time of this cross-sectional study (January to May) which participants complained of discomfort in sleeping under mosquito net.

Conclusion

The prevalence of *plasmodium* parasite in human samples was 22.9% and 18.2% respectively for RDT and microscopy. A prevalence of 39.9% of *Plasmodium falciparum*, 18.6% of *Plasmodium malariae* and 12.6 % of *Plasmodium ovale* were detected in human samples using PCR. One out of the 3 baboon samples were pan-*plasmodium* positive but negative for *Plasmodium falciparum*, *Plasmodium ovale* and *Plasmodium malariae* on species-specific PCR. Mixed infections were identified in 7.5% of the human participants. Siodemographic,

Table 8: Knowledge of malaria factors associated with malaria infection.

		B	Wald	S.E.	Exp(B)	95% C.I. for EXP(B)		P-VALUE
						Lower	Upper	
Heard of Malaria	Yes	-0.515	0.157	1.299	0.597	0.047	7.618	0.692
	No	0[b]						
Transmitting Vector	Yes	0.44	0.158	1.106	1.553	0.178	13.572	0.691
	No	0[b]						
Know of 4 Common Plasmodia	Yes	-0.429	1.206	0.391	0.651	0.303	1.401	0.272
	No	0[b]						
Know of *P. Knowlesi*	Yes	0.739	1.871	0.540	2.093	0.726	6.031	0.171
	No	0[b]						
Community Mosquito Insecticide Spray	Yes	0.501	1.296	0.440	1.651	0.696	3.913	0.255
	No	0[b]						
Use of Mosquito Net	Yes	0.616	1.651	0.480	1.852	0.723	4.740	0.199
	No	0[b]						
Period of Mosquito Net Use	Yes	-0.477	2.222	0.320	0.621	0.332	1.162	0.136
	No	0[b]						
Treated Net	Yes	-0.103	0.020	0.732	0.902	0.215	3.789	0.888
	No	0[b]						
Used Net Yesterday	Yes	-0.254	0.636	0.319	0.776	0.415	1.449	0.425
	No	0[b]						

P-value: Analyzed by binary logistic regression analyses and considered significant at < 0.05 (2-tailed); The reference category is: Dependent variable is PCR *Plasmodium falciparum* positive; b: Parameter considered redundant and set to zero; B: Regression coefficient; ExpB: Exponentiation of B; which is the same as AOR: Adjusted Odds Ratio; 95% CI: 95% Confidence Interval; S.E: Standard Error.

knowledge on malaria and interaction with monkeys of study participants did not show any significant association with malaria infection.

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