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Research Article

Prevalence of *Plasmodium relictum* in residential birds from Hpa-an Township Kayin State, Myanmar

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Abstract: The increasing emergence of wildlife diseases with potential threats to ecological systems, as well as domestic animal and human health, emphasize the importance of understanding disease dynamics and associated risks to biological conservation and human health. The avian malaria study was conducted in Ta-yrok- hla-village, Hlar–ka daung village, Don-yin village and Naung–ta-lon village Hpa-an Township environs in Kayin State from January 2017 to July 2017 to deter mine bird malaria parasite positivity in wild and domestic birds. Therefore *Gallus sp*.,(Chicken) *Meleagris gallopavo* (Turkey), *Numida meleagris* (*Helmeted guineafowl*), *Columba livia* (Rock pigeon), *Streptopelia chinensis* (Spotted Dove), *Anastomus oscitans* (Asian Operbill), *Anas sp*p (domestic duck) and *Anser anser* (domestic durk) were collected in January 2017 and July 2017. After blood taking on glass slides, all collected birds were released from cages. Blood slides were stained with Giemsa's stain and parasites were diagnosed by oil emersion lens. Results revealed that 7/60 (11.67%) of *Plasmodium relictum* was positive in wild *Columba livia* (wild pigeon) population in January and 2/70 (2.86%) of *Meleagris gallopavo* (Turkey) and 3/70(4.29%) of *Columba livia* (wild pigeon) were found *P. relictum* positive in July. When compared the positivity of *P. relictum* against total collected birds in both month was found (7/430) 1.63% positive was higher in the first survey in January than 5/510 (0.98%) positive in the second survey in July. The study demonstrated that wild *Columba livia* (wild or rock pigeon) was found higher risk in a transmission of *P. relictum* than *Meleagris gallopavo* (Turkey). The study on the distribution of host communities and their relationship to *P. relictum* in Hpa-an regions would be useful and may provide some insight into the regional distribution of bird malaria parasite.

INTRODUCTION

Plasmodium relictum is a common mosquito- transmitted blood protozoan parasite of wild birds that have a worldwide distribution. It has been reported from at least 411 avian species from 67 avian families and is considered to be relatively non-pathogenic and noninvasive under most circumstances and most geographic areas. Avian malaria is a mosquito-borne disease and distributed to endemic areas of Asia, Africa, Central and South America certain Caribbean Islands. The infection a great economic significance to the poultry industry and pathogenic to penguins, domestic poultry, ducks, canaries, falcons and pigeons, most commonly carried asymptomatically by passerine birds. Avian malaria parasites are taxonomically described more than 200 species of avian haemosporidians from hundreds of bird species and can be identified based on single cell blood films. The parasites are four distinct genera as *Plasmodium*, *Haemoproteus*, Leucocytozoon and Fallisia. These parasites can be identified based on the morphology of their blood stages and limited experimental information on their vertebrate host specificity [1]. In Myanmar earliest known studies on avian malaria reported the presence of *Plasmodium* and *Haemoproteus* in several wild birds' species [2]. In Hpa-an Township Kayin State, there are many kinds of migratory and residential birds are living in urban, rural and the forested areas. The avian malaria parasite is a responsible for mass mortality, population declines and even extinctions of many bird species. Avian malaria is a disease caused by species of protozoan parasites (*Plasmodium*) that infect birds. Related species commonly infect reptiles, birds, and mammals in tropical and temperate regions of the world. Transmitted by mosquitoes, the parasites spend part of their lives in the red blood cells of birds. Avian malaria is common in continental areas but is absent from the most isolated island archipelagos where mosquitoes do not naturally occur. More than 40 different species of avian *Plasmodium* have been described,

but only one, *P. relictum*, has been introduced to the Hawaiian Islands [3]. Because they evolved without natural exposure to avian malaria, native Hawaiian honeycreepers are extremely susceptible to this disease. Malaria currently limits the geographic distribution of native species, has population-level impacts on survivorship, and is limiting the recovery of threatened and endangered species of forest birds.

The isolation of the Hawaiian Islands led to a unique evolution and speciation of the avifauna, which flourished in a predator and disease-free environment until the human colonization of the islands 1,800 years ago [4]. During their evolution, many native Hawaiian forest birds lost or failed to develop resistance to mosquito-borne diseases and now are highly sensitive to the recently introduced avian malaria. This is manifested by very high mortality for some native species, 90% for the I'iwi (*Vestiaria coccinea*) or 75% for the Maui ‘alauahio (*Paroreomyza montana*) after exposure to a single infected mosquito [5]. The species causing avian malaria on the Hawaiian archipelago is *Plasmodium relictum*, transmitted mostly by the mosquito *Culex quinquefasciatus*. Other common mosquitoes, including *Aedes albopictus* and *Wyeomyia mitchelli*, are refractory to infection [6, 7]. *Culex quinquefasciatus* is a nocturnal, widely distributed mosquito that has been established in the Hpa-an. Females occur in warm and temperate areas, feed blood mostly on birds, animal, and human and do not hibernate. Thus, *Culex quinquefasciatus* continue to feed in all season in the night from 6:00 pm to 6:00 am, and they breed in polluted water in gutters, creeks, discarded water storage containers, and they breed in polluted water wells [8] in year-round in Kayin State. On Hawaii Island, it is principally found in anthropogenic larval habitats when available. On Hawaii Island, Maui, and Kauai, larvae were observed in rock pools in the bed of stream drainages and stream margins. However, larvae were also found in fern tree cavities and ground pools on Hawaii Island [9-11]. Even if the low temperatures slow down larval development and reduce adult survival [12]. Indeed, cold temperatures reduced female survival to two to six weeks at a constant temperature of 5° C [13] The incubation period is between three to five days, and larval development between 10 to 12 days with a developmental threshold of 9.5°-10.4° C, but low temperature increased both incubation and development to up to 28 days at 17° C [13, 14]. This species is known to exist in various habitats: canals, marshes, ornamental ponds, swimming pools, temporary pools, puddles, or ditches [15, 16]. Rainfall frequency and intensity are major factors influencing *Cx. quinquefasciatus* population dynamics. Temperature is one of the main factors likely to influence the *P. relictum* life cycle and transmission rates, as it influences the development of oocytes and sporozoites in the vector. La Pointe et al., [17] revealed that those particular conditions explain the historically low malaria prevalence on the Alakai Plateau where the best conditions for *P. relictum* transmission (higher temperature and lower rainfall frequency) occurred from July to September. Temperature increases and precipitation declines due to climate change over the last decade may have extended the favorable period for *P. relictum* transmission by allowing mosquitoes to better hatch and survive throughout the year, and *P. relictum* to complete its life cycle, in high altitude areas on Kauai [11]. This possibility is one focus of this study. Also, climate change may have allowed mosquitoes from lower altitudes to migrate to upper elevations throughout the year and to survive longer on the Plateau, increasing the transmission of *P. relictum* to forest birds.

The increasing emergence of wildlife diseases with potential threats to ecological systems, as well as domestic animal and human health, emphasize the importance of understanding disease dynamics and associated risks to biological conservation and human health. As invasive species, human development, and climate change alter host or vector communities and habitats, this information is increasingly essential for long-range conservation planning for species that face significant climate [18] and environmental change, particularly for threatened or endangered species. However, determining wildlife disease dynamics including rates of infection, drivers of transmission, vector feeding preferences, and host mortality presents significant challenges [19, 20]. The importance of these epizootiological parameters for understanding host-pathogen dynamics, population effects, and on host-pathogen evolution have long been recognized, but are infrequently addressed [21-24].

Malaria transmission becomes increasingly seasonal as elevation increases, both because numbers of mosquitoes are very low at higher elevations during the cooler winter months and because of thermal constraints on the development of the parasite in the mosquito vector. Malaria transmission at elevations between 900 and 1500 m typically occurs during the warmest time of the year between September and December when mosquito populations reach their peak. This period follows the nesting season for most native species and the abundance of recently fledged, susceptible juvenile birds coupled with increasing mosquito populations can lead to epidemic outbreaks that may continue to the onset of colder winter temperatures in January. Transmission may occur throughout the year at lower elevations if suitable reservoir hosts and susceptible, uninfected birds are present [5]. In Myanmar, there is very rear information about bird malaria and protozoan parasite of *Plasmodium relictum* in the bird population. Therefore the study planned to determine the prevalence of bird malaria in local bird population in Hpa-an Township, Kayin State.

MATERIALS AND METHODS

Study design

Descriptive study design was done to detect bird malaria parasites positivity in some migratory and residential birds from Hpa-an Township.

Ethical approval

The present study was approved by the Ethical Review Committee of Department of Medical Research.

Study areas

The study was conducted in Ta-yrok- hla-village, Hlar –ka daung village, Don-yin village and Naung –ta-lon village Hpa-an Township environs in Kayin State from January 2017 to July 2017. *Gallus sp*.,(Chicken) *Meleagris gallopavo* (Turkey), *Numida meleagris* (*Helmeted guineafowl*) , *Columba livia* (Rock pigeon), *Streptopelia chinensis* (Spotted Dove),

Anastomus oscitans (Asian Operbill), *Anas spp* (domestic duck) and *Anser anser* (domestic duck) were collected by hunters. In Hpa-a Township, Wild pigeons were caught using bird catch net by hunters and caught pigeons were kept in pigeon cages. After blood taking, all pigeons were released from cages. Ducks, Chickens, Poultry chickens, Geese and Turkeys blood, were collected from poultry by the permission of authorities.

Blood slides preparation

Ten microliters of the blood of birds were collected from the subclavion vein with EDTA capillary tubes. Thin films were drowning on grease free glass slides individually. Date, bird name, and place were noted on the left side of a glass slide by permanent pen, and the history of birds was recorded in a note book. Thin films were dried in room temperature. Temperature, humidity and rain fall were recorded.

Staining procedure

Dried glass slides were fixed with Methanol and dried in room temperature. Methanol-fixed blood slides were stained with 10% Giemsa's stain for 10 minutes. After 10 minutes stained slides were washed in pH seven water. All the washed slides were dried in room temperature for 3 hours. Dried slides were kept in slide box and parasites were detected by compound microscope.

Diagnosis of parasite

Diagnosis of malaria parasites in thin blood films was made under oil emersion lens (100x) of compound Olympus microscope. Parasites were count against 200 WBC.

Data analysis

Data of birds were analyzed by Microsoft Excel software. Parasite positivity was performed in percentage.

RESULTS

A total of 940 migratory and residential birds were collected from Hpa-an Township. All collected birds were consisted of Class Ave and found 4 orders as *Galliformes, Columbiformes, Ciconiiformes, Anseriformes* and 5 families as *Gallus sp*, *Numididae, Columbidae, Ciconiidae, Anatidae* and 8 species during the study periods. Collected species were *Gallus sp, Meleagris gallopavo, Numida meleagis, Columba livia, Streptopelia chinensis, Anastomus oscitans, Anas sp.* and *Anser anser.* Detail classification of collected birds was shown in Table (1). A total of 4 Orders consist of 5 families, and 8 kinds of birds were collected from studied areas of in Hpa-an Township.

Table 1. Occurrence of avian malaria parasites from some migratory and residential birds in Hpa-an (First study period (or) First-time survey in January 2017)

Sr. No.	Scientific name	Condition	Number of examined (%)	Number of infected	Parasite positivity (%)	
					P. relictum	*Haemoproteus*
1	*Gallus sp*.	Captive	20 (4.65%)		0	0%
		Free range	20 (4.65%)		0	0%
		Semi-captive	20 (4.65%)		0	0%
2	*Meleagis gallopavo*	Semicaptive	60 (13.95%)		0	0%
3	*Numida meleagris*	Semicaptive	60 (13.95%)		0	0%
4	*Columba livia*	Wild	60 (13.95%)	7	7 (11.67%)	0%
5	*Streptopelia chinensis*	Wild	60 (13.95%)		0	0%
6	*Anastomus oscitans*	Wild	10 (2.33%)		0	0%
7	*Anas sp.*	Semi-captive	60 (13.95%)		0	0%
8	*Anser anser*	Semicaptive	60 (13.95%)		0	0%
Total			430 (100%)	7	7 (1.63%)	0%

Table (1) shows that in the first study period (January) a total of 430 birds were collected , *Gallus sp*. captive chicken 20 (4.65%), free range chicken 20 (4.65%), semi captive chicken 20(4.65%), *Meleagis gallopavo* (Turkey) 60 (13.95%), *Numida meleagris* (*Helmeted guineafowl*) 60(13.95%), *Columba livia* (Rock pigeon) 60(13.95%), *Streptopelia chinensis* (Spotted Dove) 60(13.95%), *Anastomus oscitans* (Asian openbill)10(2.33%), *Anas sp* (Domestic duck) 60(13.95%) and *Anser anser* (Gooes) 60(13.95%) were caught by different methods. Of this 7/60 (11.67%) of *P. relictum* was positive in only one species of *Columba livia* (Rock pigeon) population. Within all collected birds 430, the positivity rate was found 1.63% of *P. relictum* in the first study period (January). Other species of *Gallus sp*., *Meleagis gallopavo*, *Numida meleagris*, *Streptopelia chinensis*, *Anastomus oscitans*, *Anas sp*. and Anser *anser* were found no parasite positivity.

Table (2) shows that a total of 510 migratory and residential birds were caught from Hpa-an township, of this *Gallus sp*. Captive indigenous chicken 30(5.88%) free range chicken 30(5.88%) semi-captive chicken 30(5.88%), *Meleagris gallopavo* (Tirkey semi-captive) 70 (13.73%), *Numida meleagris* (*Helmeted guineafowl* semi-captive) 70(13.73%), *Columba livia* (Rock pigeon wild) 70 (13.73%), *Streptopelia chinensis* (Spotted Dove wild) 70 (13.73%), *Anastomus oscitans* (Asian open bill wild) 20 (3.92%), *Anas sp*.

(domestic duck semi-captive) 70(13.73%) and *Anser anser* (Gooes semi-captive) and examined for bird malaria parasite. Of this 2(2.86%) of *Meleagris gallopavo* (Turkey) and 3(4.29%) of the *Columba livia* (Rock pigeon) were found *P. relictum* positive. When compared with total collected birds the positivity rate was observed 0.98%.

Table 2. Malaria parasite positivity rate in some migratory and local birds from Hpa-an (Second study period (or) Second-time survey in July2017)

Sr. No.	Scientific name	Condition	Number of examined	Number of infected	Parasite positivity (in %)	
					P. relictum	*Haemoproteus*
1	*Gallus sp.*	Captive	30 (5.88%)		0	0%
		Free range	30 (5.88%)		0	0%
		Semi-captive	30 (5.88%)		0	0%
2	*Meleagris gallopavo*	Semicaptive	70 (13.73%)	2	2 (2.86%)	0%
3	*Numida meleagris*	Semicaptive	70 (13.73%)		0	0%
4	*Columba livia*	Wild	70 (13.73%)	3	3 (4.29%)	0%
5	*Streptopelia chinensis*	Wild	70 (13.73%)		0	0%
6	*Anastomus oscitans*	Wild	20 (3.92%)		0	0%
7	*Anas sp.*	Semicaptive	70 (13.73%)		0	0%
8	*Anser anser*	Semicaptive	70 (13.73%)		0	0%
Total			510 (100%)	5	5 (0.98%)	0%

DISCUSSION

The term malaria has confusingly been used for either all Haemosporidians (Class Sporozoa, Order Haemosporida) a group of protozoans that use blood-sucking dipteran insects as vectors to complete their life cycle, or strictly parasites of the genus *Plasmodium*. Although haemosporidian parasites are genetically closely related, the life cycle, vector species, and epidemiology of parasites from different families are very different [25]. Thirty –eight morphologically different avian *Plasmodium* species have been described. The parasite species that has received the most attention is *P. relictum*. This parasite is not only well known from avian malaria in Hawaii [26], and avian malaria out breaks in Zoos all around the World [27-29], but it has also been used as a model species to study human malaria during the end of the 19th and beginning of the 20th century [30].

Unfortunately, there is little information available on the vector species of avian malaria. However, since much experimental work has been carried out with *P. relictum*, it is known that this parasite can complete its cycle in 26 different species of the *Culicidae* family, including the genera, *Aedes, Anopheles, Culex and Culiseta* [31, 32].The life cycle of the different species of avian malaria parasite is generally similar.

The detail life cycle of birds malaria parasite was described by Huijben et al., [33] The sexual reproduction phase of the parasite takes place in the mosquito midgut, where male and female gametes fuse to form a zygote which in turn, differentiates into the mobile ookinete. The ookinete penetrates the midgut to form an oocyst on the outer midgut wall of the mosquito. Within the oocyst, hundreds of sporozoites are being produced, until the oocyst eventually ruptures, resulting in a release of the sporozoites, migrate to the salivary glands of the mosquito can transmit the sporozoites while blood feeding on a bird. The time for the parasite to develop within the mosquito takes approximately seven days.

When blood feeding on a bird, the mosquito injects its saliva, containing various enzymes to enhance blood uptake and prevent clotting, together with the sporozoites invade the reticular cells of various organs, such as the spleen and tissue, such as skin tissue, where they develop into cryptozoites. The merozoites that are developing within the cryptozoites are, unlike the merozoites in human malaria unable to infect red blood cells immediately, but undergo a second exo-erythrocytic cycle. During which they invade the macrophages in many organs. Part of the resulting meta-cryptozoites stay in the primary exo-erythrocytic cycle and infect new macrophages again. The remaining parasites either (a) enter the erythrocytic cycle, where they invade the red blood cells and multiply to a schizont (b) invade a red blood cell to immediately develop into a gametocytes, (c) enter the secondary exo-erythrocytic cycle where they invade the endothelial cells of many organs (including the brain and liver) in which the phanerozoites develop, A proportion of the merozoites from the erythrocytic cycle stays in the erythrocytic cycle and re-infects new erythrocytes. The remaining of the parasites can either infect a new red blood cell to develop into a gametocyte or enter the secondary exo-erythrocytic cycle. The phanerozoites of the secondary exo-erythrocytic cycle can also develop into gametocytes or enter the erythrocytic cycle or re-infect the endothelial cells, where they can stay for the remainder of the host's life. Periodic relapses, resulting from these dormant phanerozoites often occur and are for instance related to a weakened immune system and environmental stress and often synchronized to the breeding season [32].The time to maturation of the first generation of metacryptozoites, the prepatent period, is usually less than five days for *P. relictum*. It is believed that avian malaria parasites in wilds birds are relatively harmless, whereas morbidity in captive birds can be severe frequently leading to death.

The aim of this study was to detect the transmission of birds' malaria parasite it means that *P. relictum* in the

migratory and residential birds population in Hpa-an Township Kayin State. The low malaria prevalence on the *Columba livia* was observed to be caused by a seasonal local transmission during the cold and wet period of the year between January and July. Environmental conditions such as temperature during our collections, stream flooding events, relative humidity and rainfall frequency are essential to understanding the actual status of malaria prevalence and malaria transmission in birds' population in this region. The infection rate of *P. relictum* is also essential to understanding the transmission rate to birds in the Hpa-an Township Kayin State. The results of this study indicate the prevalence and intensity of blood parasites in some migratory and residential birds from four study areas Tay- rol-hla, Hlar-ka-daung, Don-yin and Naung- ta-lon villages of Hpa-an Township, Kayin State. A total of 430 and 510 birds caught at 4 selected areas of Hpa-an Township in January and July 2017 and *Gallus sp*., *Meleagris gallopavo*, *Numida meleagris*, *Columba livia* , *Streptopelia chinensis*, *Anastomus oscitans*, *Anas sp*. and *Anser anser* native and migrant birds were caught in January and July for *P. relictum* infection using Compound microscope with Giemsa's staining method. Seven wild Columba livio in January and 2 *Meleagris gallopavo* semi-captive (Turkey) and 3 *Columba livia* wild (rock pigeon) were positive for *P. relictum* respectively in July 2017.

In Myanmar, there was no history of birds malaria previously. Although in 2007, Farah and his associate revealed that *Plasmodium* and *Haemoproteus* were found in several wild bird species [2]. The present bird malaria study found to be 11.67% of *P. relictum* in *Columba livia* wild (Rock pigeon) in January 2017 and 2.86% and 4.29% of *Meleagris gallopavo* semi-captive (Turkey) and *Columba livia* wild (rock pigeon) were positive for *P. relictum* respectively in July 2017. This is the first time report of birds malaria in Hpa-an Township Kayin State. Environmental conditions such as temperature during our collections, stream flooding events, and rainfall frequency are essential to understanding the actual status of malaria prevalence and transmission on the selected areas of Hpa-an. The two lowest average temperatures were 16.1° C in January, and 23.2° C in July at the study areas in Hpa-an, but those temperatures were averaged over only ten and five days, respectively. The lowest daily temperature recorded was 13.6° C (12th of January at Hpa-an), and the highest was 30. 3° C (23rd of July 2017 at Hpa-an). Bird malaria is mostly transmitted by *Culex* mosquitoes. The species causing avian malaria on the Hawaiian archipelago is *P. relictum*, transmitted mostly by the mosquito *Culex. quinquefasciatus*. Other common mosquitoes, including *Aedes albopictus* and *Wyeomyia mitchelli*, are refractory to infection [6, 7]. Other researchers revealed that *Cx. quinquefasciatus* larvae can have a slow but complete development at 14.2°C annual average temperature, and populations can persist if the seasonal average is above 13.2° C [34]. Thus, temperatures in the Hpa-an may not be a main limiting factor for mosquito populations as the developmental threshold temperature is between 9.5° and 10.4° C [13]. However, those temperatures may be a limiting factor for *P. relictum* development in the vector. Indeed, below a daily average of 12.6° C, the development of oocysts is very slow or even non-existent [35]. The optimal range for oocyst and sporozoite development is between 17° and 25° C, leading to increased transmission rates at the highest temperatures but to incomplete or delayed development at the lowest temperatures [17]. Since 1973, some species, such as I'iwi, Akeke'e, and Akikiki, have exhibited alarming range contractions and population declines on Kauai, which are thought to be related to increased prevalence of malaria, among other threats [11, 36]. This hypothesis is supported by the increase in malaria prevalence in Kauai forest birds, observed between two sampling period (1994-1997 and 2007-2013) and by the detection of *Cx. quinquefasciatus* larvae by Atkinson et al. [11].

Samuel et al., [35] conducted multi-state capture-recapture (longitudinal) models with cumulative age-prevalence (cross-sectional) models to evaluate patterns in Apapane, Hawai'i Amakihi, and I'iwi in low, mid, and high-elevation forests on the island of Hawai'i based on four longitudinal studies of 3–7 years in length. The study mentioned that in Hawai'i overall prevalence of malaria based on parasitemia and serology at first capture ranged from 2.6% (27/1,046) in I'iwi to 40% (37/2,116) in Apapane and 39% (2,072/ 5,353) in Hawai'i Amakihi. Prevalence was strongly influenced by elevation, with the lowest prevalence of infection at high elevation (2.2% for I'iwi, 7.8% for Apapane, and 1.5% for Amakihi) followed by mid-elevation (20% for I'iwi, 60% for Apapane, and 17% for Amakihi), and low elevation studies (no captures for I'iwi, 100% for Apapane, and 85% for Amakihi). They classified >97% of the malarial infections as chronic (recovered), and the remaining birds were acutely infected but were classified as recovered.

In the present study, we have recorded the life birds which were 98% were healthy birds (active), and 2 % were sick birds from the field areas. However other researchers observations from the fields may be skewed since the observed birds are often captured with mist nets which will result in relatively healthier than sick birds being caught [26, 37]. Atkinson [5] revealed that avian malaria in Hawaiian forest birds, malaria transmission becomes increasingly seasonal as elevation increases, both because numbers of mosquitoes are very low at higher elevations during the cooler winter months and because of thermal constraints on the development of the parasite in the mosquito vector. Malaria transmission at elevations between 900 and 1500m typically occurs during the warmest time of the year between September and December when mosquito populations reach their peak. This period follows the nesting season for most native species and the abundance of recently fledged, susceptible juvenile birds coupled with increasing mosquito populations can lead to epidemic outbreaks that may continue to the onset of colder winter temperatures in January. Transmission may occur throughout the year at lower elevations if suitable reservoir hosts and susceptible, uninfected birds are present. A similar result has been found in the present study in Hpa-an Township, 1.63% of *P. relictum* positivity in the colder month of January and 0.98% positivity in birds population in a high raining month of July in Kayin State.

Other studies in Africa birds were trapped in the mist-nets set at Cape Receife, in the total of 104 birds of 29 species from 15 families were examined for haemosporidians. Parasites were found in 71 birds of 23 species. Prevalence of haematozoa in all bird species captured was 68.2%. Overall,

Plasmodium species were the most prevalent, occurring in 87.3% of infected birds (59.6% of the total sample), followed by *Haemoproteus* species in 9.9% of the infected sample. The remaining species of haemosporidians found consisted of Babesia and *Aegyptinella* species, each comprising 1.4% of the infected sample. The most heavily infected avian species was the Sombre Bulbul *Andropadus importunes*. Twenty-one of the 26 birds caught (80%) were infected with haematozoa. African Penguins in the wild have subclinical avian malaria infections [38]. Also, they often carry heavy loads of *Babesia peircei*, and when associated with avian malaria, under stressful conditions become parasitemic [39, 40], causing harmful effects on the infected individual. At the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) *B. peircei* was found in 11–15% of the penguins examined, and in 4% of free-ranging penguins [40]. Although in the present study, a total of 430 and 510 birds of 8 species were examined in January and July study periods and 7/60 (11.67%) of wild *Columba livia* were found *P. relictum* positive in first study period in January was higher than the second study period in July. The discovery of *P. juxtanuclearae* by Grim et al., [41] in African Penguin chicks on Robben Island and its presence in birds autopsied at SANCCOB, indicates that caution is necessary as to the placement of the rehabilitation center. The presence of large water-bodies situated at Cape Receife, which may encourage the breeding of mosquito vectors, could increase the risk of avian malaria infections of birds held at the Centre.

Atkinson [5] mentioned that microscopy is still considered the "gold standard" for malaria diagnosis because parasites are seen within the blood cells, but it can miss more than 70% of chronic infections because numbers of parasites are so low. PCR methods that amplify specific portions of parasite DNA are significantly more sensitive than microscopy, but are more expensive, time-consuming and may require additional sequencing steps to confirm that the products originated from parasite DNA. ELISA is more sensitive than Western Blotting but may require additional tests by Western Blotting to verify positive results. Western Blotting is the most specific serological test available for avian malaria and can be used to verify both ELISA and PCR tests. Microscopy, serology, and PCR can all play important roles in providing accurate diagnostic information about malarial infections in birds. When used alone, the tests vary in their ability to accurately detect chronic malarial infections with low intensities. Microscopy is most likely to miss chronic infections, but it is extremely accurate when parasites are detected and can be used to obtain information on the intensity of infection and host cellular responses. Serological methods are extremely sensitive for detecting older infections in recovered birds because persistent infections stimulate antibody production and cellular immunity to the parasite. They provide no information about parasite intensity or morphology, however, and are also likely to miss very early acute infections, before the host can produce antibodies to the parasite. PCR is not quite as sensitive as serological methods for detecting chronic infections, but it is much more sensitive than microscopy. When used in combination, these diagnostic methods can complete each other and provide critical information to resource managers about the prevalence and distribution of avian malaria in habitats that are being considered for restoration of threatened and endangered forest birds.

This study shows that there are important implications for the conservation of Hpa-an migratory and residential birds. As the Hpa-an area is the highest area on Kayin State, there is already an ongoing selection pressure on all birds. With the permanent presence of *Cx. quinquefasciatus* in some parts of the Hpa-an area, the selection pressure may lead some species to develop resistance to *P. relictum* , as Apapane or Hawaii Amakihi already did on other islands [42,43]. *Culex quinquefasciatus* was believed to occur in the studied areas. A larger effort to trap adults and survey larval habitat is needed to better understand the dynamics of *Cx. quinquefasciatus* on the studied areas in Hpa-an especially in winter. The topography, the meteorologically small-scale differences, and anthropogenic disturbances made it difficult to predict mosquito distribution and population dynamics [44].

Furthermore, new techniques of vector control may also be good management tools to reduce transmission, for example, the release of sterile mosquitoes and release of sterile males coated with a Densovirus [45, 46]. These are current management tools to control mosquitoes and malaria in malaria endemic areas directly. It is essential to understand the principles behind the spread of emerging infectious diseases and to study host-parasite evolution.

CONCLUSION

In conclusion, the prevalence of bird malaria parasite of *P. relictum* was highly distributed in wild *Columba livia* and low in *Meleagris gallopavo* in Hpa-an areas. The total incidence of *P. relictum* in bird populations was observed 1.63% in January and 0.98% positivity in July respectively in this study area. Prevalence varied between two study periods. The Hpa-an region harbors a diverse community of avian malaria lineages that were distributed among the bird's population. The study of avian malaria parasites will contribute much to our knowledge and practice can be used as a model for other emerging infectious diseases. The study supports the hypothesis that avian malaria has been a primary factor influencing the elevational distribution and abundance of these species, and likely limits other native and migrant bird species that are susceptible to malaria. The study on the distribution of vector communities and their relationship to *P. relictum* in Hpa-an regions would be useful and may provide some insight into the regional distribution of bird malaria parasite.

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CONFLICTS OF INTEREST

The authors have reported no conflict of interest.

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