



## Research Article

# Occurrence of avian malaria parasites in bird population in Hpa-an Township Kayin State, Myanmar

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**Abstract:** The avian malaria study was conducted in Tayrok-Hla-village, Hlar-Ka-Daung, Don-Yin and Naung-Ta-Lone villages Hpa-an Township environs in Kayin State from January 2019 to September 2019 to determine bird malaria parasite positivity in wild and domestic birds. Therefore, local and migratory birds were collected for blood films. Blood slides were stained with Giemsa's stain and parasites were diagnosed by oil emersion lens. Results revealed that a total of 1616 birds were collected from above four study sites in Hpa-an Township and found that 39(35.45%) *Haemoproteus*, 63(57.27%) *Plasmodium relictum* and 8(7.27%) mixed infections were observed in 110 birds. Avian malaria parasites positive rate was found 7.43% in Don-Yin village followed by 7.18% in Tayout-Hla village and lowest was observed 6.18% positivity in Hlar-Ka-Daung village. High positivity rate of *Plasmodium relictum* was found 66.67% and (46.15%) in *Gallus sp* (Indigenous Chicken) in Naung Ta Lone and Tayout Hla villages and high positivity rate of *Haemoproteus* in bird population were found (63.64%) and 66.67% in *Meleagris gallopavo* in Hlar-Ka-Daung and Don-Yin villages. *Meleagris gallapavo* was found highest avian malaria parasite positivity 7 (28%) in Hlarka Daung, 8(26.67%) Don Yin, and *Gallus sp*. (Indigenous Chicken) was found high positivity 12(41.38%) in Tayout Hla and 7 (26.92%) in Naung Ta lone village. *Streptopelia chinensis* was found lowest positivity for avian malaria parasite in all selected areas. The study conclusion that wild *Columba livia* was found higher risk in transmission of *Plasmodium relictum* than *Meleagris gallopavo*. 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 parasites.

## INTRODUCTION

Avian malaria has become a model system for the investigation of the ecological and evolutionary dynamics of *Plasmodium* parasites, little is still known about the field prevalence, diversity and distribution of avian *Plasmodium* species within the vectors, or about the extrinsic factors affecting *Plasmodium* population dynamics in the wild [1]. *Plasmodium relictum* is a common mosquito-transmitted blood protozoan parasite of wild birds that has 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 non-invasive 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 asymptotically 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 [2]. In Myanmar earliest known studies on avian malaria reported the presence of *Plasmodium* and *Haemoproteus* in several wild birds' species [3]. In Hpa-an Township Kayin State, there are many kind of migratory and residential birds are living in urban, rural and the forested areas. Avian malaria parasite is a responsible for mass mortality, population declines and even extinctions of many bird's 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 live 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, *Plasmodium relictum*, has been introduced to the Hawaiian Islands [4]. 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. *Culex quinquefasciatus* is a nocturnal, widely distributed mosquito that has been established in the Hpa-an. Females occurs 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 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 [5] 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 [6-8]. Rainfall frequency and intensity are major factors influencing *Culex quinquefasciatus* population dynamics. Temperature is one of the main factors likely to influence the *Plasmodium relictum* life cycle and transmission rates, as it influences development of oocytes and sporozoites in the vector.

Malaria transmission becomes increasingly seasonal as elevation increases, both because numbers of mosquitoes are very low at higher elevations during the cooler winter months, because of thermal constraints on 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 [9]. The aim of the present study is to establish whether there are variations across space and time in the prevalence and diversity of avian malaria infections in local birds' populations in different areas of Hpa-an Township, Kayin State.

## MATERIALS AND METHODS

**Study areas:** The study was conducted in Hlar-Ka-Daung, Tayrok-Hla, Don-Yin and Naung-Ta-Lone village Hpa-an Township environs in Kayin State from January to September 2019. *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-an 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.

## Sample collection

Ten micro liters of blood of birds were collected from sub-clavion 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 glass slide by permanent pen and the history of birds were recorded in note book. Thin blood film slides were dried in room temperature. Temperature, humidity and rain fall were recorded. After blood taking on glass slides, all collected birds were released from cages.

## Staining procedure

Dried glass slides were fixed with 90% 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 7 water. All the washed slides were dried in room temperature for 3 hours. Dried slides were kept in slide box for diagnosis of Avian malaria parasites [10].

## Diagnosis of parasite

Diagnosis of malaria parasites in thin blood films were done under oil emersion lens (100x) of compound Olympus microscope. *Plasmodium relictum* and *Haemoproteus* parasites were identified morphologically according to Valkiunas et al., 2018 [11]. Parasites were count against 200 WBC.

## Data analysis

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

## RESULTS

Table 1. shows that a total of 404 birds consist of 8 species were collected from Hlar Ka Daung village and found that 28% of percentage of *Meleagris gallopavo* was highest positivity with birth malaria followed by *Gallus sp.* (Indigenous Chicken) 24% lowest was observed *Anas sp.* 4%. Among the positive birds, *Plasmodium relictum* found 56% positivity and *Haemoproteus* was found 44% of positivity. In the *Haemoproteus* positive birds group, *Meleagris gallopavo* was found to be highest positivity 63.64% positive than other birds. Six species of collected birds were found *Plasmodium relictum* positivity., the highest positivity was found *Gallus sp.* (Indigenous Chicken) and *Numida meleagris* 28.57% each and followed by *Gallus sp.* (Domestic Chicken) 21.43%.

**Table 1.** Avian malaria parasites positivity in blood films collected from some migratory and residential birds in Hlar-Ka-Daung village

No.	Bird species	No. of examined	No. of Infected	<i>Haemoproteus</i>	<i>Plasmodium relictum</i>
1	<i>Columba livia</i>	52	2 (8%)	1(9.09%)	1 (7.14%)
2	<i>Streptopelia chinensis</i>	46	2 (8%)	1 (9.09%)	1 (7.14%)
3	<i>Meleagris gallopavo</i>	46	7 (28%)	7(63.64%)	
4	<i>Gallus sp.</i> (Indigenous Chicken)	52	6 (24%)	2(18.18%)	4 (28.57%)
5	<i>Gallus sp.</i> (Domestic Chicken)	52	3 (12%)	-	3 (21.43%)
6	<i>Anas sp.</i>	52	1 (4%)	-	1 ((7.14%)
7	<i>Anser anser</i>	52	-	-	
8	<i>Numida meleagis</i>	52	4 (16%)		4 (28.57%)
	<b>Total</b>	404	25 (100%)	11 (44%)	14(56%)

Table 2. shows that a total of 404 birds from Tayout Hla Ywar were collected and blood films were examined for bird malaria parasites results found that 29 collected birds consist of 6 species of birds were positive with bird malaria parasites i.e. 12 (41.38% *Haemoproteus*, 13(44.83%) *Plasmodium relictum* and 4 (13.79%) were mixed. Of this 41.67% of *Gallus sp.* (Indigenous Chicken) were positive with *Haemoproteus* followed by *Meleagris gallopavo* 25%, lowest was found *Columba livia* and *Anas sp.*8.33%. *Plasmodium relictum* was found highest positivity (41.67%) in *Gallus sp.* (Indigenous Chicken) followed by (30.77%) in *Columba livia*. *Streptopelia chinensis* was not found bird malaria positivity. Mixed infection was found in *Columba livia* 3 (75%) and *Gallus sp.* (Indigenous Chicken) 1 (25%).

**Table 2.** Avian malaria parasites positivity in blood films collected from some migratory and residential birds in Tayout-Hla village

No.	Bird species	No. of examined	No. of Infected	<i>Haemoproteus</i>	<i>Plasmodium relictum</i>	mixed
1	<i>Columba livia</i>	52	8 (27.59%)	1(8.33%)	4 (30.77%)	3 (75%)
2	<i>Streptopelia chinensis</i>	46	-	-		
3	<i>Meleagris gallopavo</i>	46	3 (10.35%)	3 (25%)		
4	<i>Gallus sp.</i> (Indigenous Chicken)	52	12 (41.38%)	5 (41.67%)	6 (46.15%)	1 (25%)
5	<i>Gallus sp.</i> (Domestic Chicken)	52	3 (10.35%)	2 (16.67%)	1 (7.69%)	
6	<i>Anas sp.</i>	52	2 (6.90%)	1 (8.33%)	1 (7.69%)	
7	<i>Anser anser</i>	52	-	-		
8	<i>Numida meleagis</i>	52	1 (3.45%)		1 (7.69%)	
	<b>Total</b>	404	29 (100%)	12 (41.38%)	13(44.83%)	4 (13.79%)

Table 3. shows that a total of 404 local birds consist of 8 species of bloods slides were examined for bird malaria parasites and found that 7.42%(30 birds) were positive for bird malaria. Of these 30% positive for *Haemoproteus* 60% positive for *P. relictum* and 10% positive for mixed (*Haemoproteus+ Plasmodium relictum*). *Meleagris gallopavo* was found 66.67% positivity for *Haemoproteus* and *Columba livia* was found (38.89%) positivity followed by *Gallus sp.* (Indigenous Chicken) (27.78%) and mixed infection was found 2(66.67%) in *Numida meleagis* and 1(33.33%) in *Gallus sp.* (Indigenous Chicken) collected from Don Yin village.

**Table 3.** Avian malaria parasites positivity in blood films collected from some migratory and residential birds in Don-Yin

No.	Bird species	No. of examined	No. of Infected	<i>Haemoproteus</i>	<i>Plasmodium relictum</i>	mixed
1	<i>Columba livia</i>	52	7 (23.33%)	0	7 (38.89%)	
2	<i>Streptopelia chinensis</i>	46	0	0		
3	<i>Meleagris gallopavo</i>	46	8 (26.67%)	6 (66.67%)	2 (11.11%)	
4	<i>Gallus sp.</i> (Indigenous Chicken)	52	6 (20.00%)	0	5 (27.78%)	1(33.33%)
5	<i>Gallus sp.</i> (Domestic Chicken)	52	3 (10.00%)	1 (11.11%)	2 (11.11%)	
6	<i>Anas sp.</i>	52	1 (3.33%)	1 (11.11%)		
7	<i>Anser anser</i>	52	2 (6.67%)	1 (11.11%)	1 (5.56%)	
8	<i>Numida meleagris</i>	52	3 (10.00%)		1 (5.50%)	2 (66.67%)
	<b>Total</b>	<b>404</b>	<b>30 (100%)</b>	<b>9 (30%)</b>	<b>18 (60%)</b>	<b>3 (10%)</b>

Table 4 shows that 6.44% of collected birds were positive for bird malaria, of this *Gallus sp.* (Indigenous Chicken) was found the highest positivity of 7 (26.92%) followed by *Meleagris gallopavo* 6(23.08%) and lowest was observed *Anas sp.* 1(3.85%). *Haemoproteus* positivity was found 2 (28.57%) in *Meleagris gallopavo* and *Plasmodium relictum* was found highest positivity 6 (66.67%) for *Gallus sp.* (Indigenous Chicken) followed by 4 (22.22%) for *Meleagris gallopavo* and lowest was observed 1 (5.56%) for *Gallus sp.* (Domestic Chicken) and only 1(100%) in *Numida meleagris* was found mixed infection in Naung Ta Lone village.

**Table 4.** Avian malaria parasites positivity in blood films collected from some migratory and residential birds in Naung-Ta-Lone

No.	Bird species	No. of examined	No. of Infected	<i>Haemoproteus</i>	<i>Plasmodium relictum</i>	mixed
1	<i>Columba livia</i>	52	2(7.69%)	0	2 (11.11%)	
2	<i>Streptopelia chinensis</i>	46	3(11.54%)	1(14.29%)	2 (11.11%)	
3	<i>Meleagris gallopavo</i>	46	6(23.08%)	2 (28.57%)	4 (22.22%)	
4	<i>Gallus sp.</i> (Indigenous Chicken)	52	7 (26.92%)	1 (14.29%)	6 (66.67%)	
5	<i>Gallus sp.</i> (Domestic Chicken)	52	2 (7.69%)	1 (14.29%)	1(5.56%)	
6	<i>Anas sp.</i>	52	1 (3.85%)	1 (14.29%)	0	
7	<i>Anser anser</i>	52	2 (7.69%)	1 (14.29%)	1(5.56%)	
8	<i>Numida meleagris</i>	52	3 (13.04%)	0	2 (11.11%)	1(100%)
	<b>Total</b>	<b>404</b>	<b>26(100%)</b>	<b>7 (26.92%)</b>	<b>18 (69.23%)</b>	<b>1 (3.85%)</b>

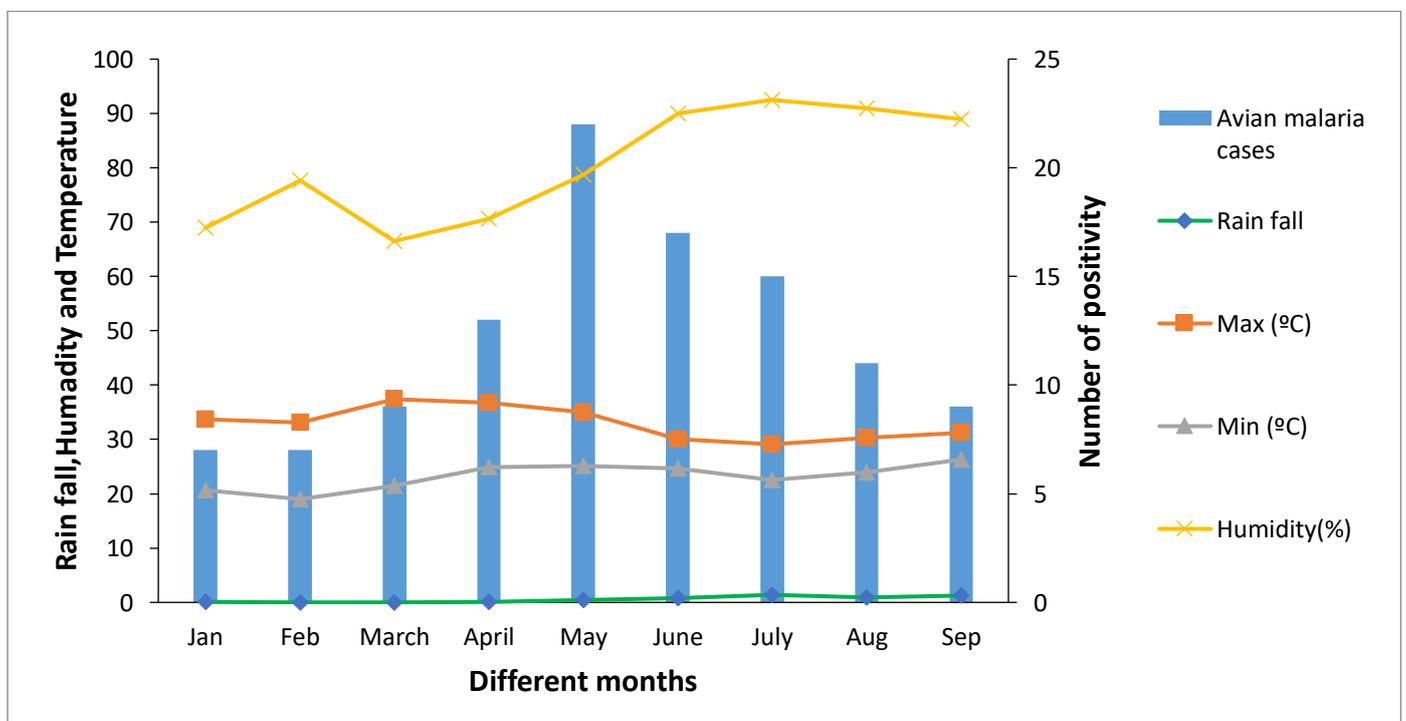
Table (5). Shows that a total of 1616 birds blood films were collected from different areas of Hpa-an Township for the detection of bird malaria parasites and found that 6.81% of birds were positive for malaria parasite. Of this 2.41%, *Haemoproteus*, 3.90% *P. relictum* and mixed 0.49% were positivity in bird population. *Gallus sp.* (Indigenous Chicken) was found highest positivity rate 31(28.18%) followed by 24(21.18%) in *Meleagris gallopavo* and lowest was observed 4(3.64%) in *Anser anser*. Positivity of malaria parasite species in bird's population were found *Haemoproteus* 35.45%, *Plasmodium relictum* 57.27% and mixed 7.27% positivity. In *Haemoproteus* positivity, highest positivity was found *Meleagris gallopavo* birds 18(46.15%) followed by *Gallus sp.* (Indigenous Chicken) 8(20.51%) and lowest was found. In *Plasmodium relictum* positive group, the highest positivity was found *Gallus sp.* (Indigenous Chicken) 21(33.33%) followed by *Numida meleagris*

8((12.70%) and lowest was observed *Anas sp.* and *Anser anser* 2(3.17%). Mixed infection was found *Numida meleagis* 3(37.50%), *Columba livia* 3(37.50%) and *Gallus sp.* (Indigenous Chicken) 2(25.00%).

**Table 5.** Prevalence of bird malaria parasite in residential birds from Hpa-an Township

No.	Bird species	No. of examined	No. of Infected	<i>Haemoproteus</i>	<i>Plasmodium relictum</i>	Remarks
1	<i>Columba livia</i>	208	19(17.27%)	2 (5.13%)	14(4.76%)	3(37.50%)
2	<i>Streptopelia chinensis</i>	184	5(4.55%)	2 (5.13%)	3(4.76%)	0(0.0%)
3	<i>Meleagris gallopavo</i>	184	24(21.18%)	18(46.15%)	6(9.52%)	0(0.0%)
4	<i>Gallus sp.</i> (Indigenous Chicken)	208	31(28.18%)	8(20.51%)	21(33.33%)	2(25.00%)
5	<i>Gallus sp.</i> (Domestic Chicken)	208	11(10.00%)	4(10.26%)	7(11.11%)	0(0.0%)
6	<i>Anas sp.</i>	208	5(4.55%)	3(7.69%)	2(3.17%)	0(0.0%)
7	<i>Anser anser</i>	208	4(3.64%)	2(5.13%)	2(3.17%)	0(0.0%)
8	<i>Numida meleagis</i>	208	11(10.00%)	0	8((12.70%)	3(37.50%)
<b>Total</b>		1616	110(100%)	39(35.45%)	63(57.27%)	8(7.27%)
<b>% Positivity</b>			6.81%	2.41%	3.90%	0.49%

Fig. 1 shows that rain fall, maximum temperature and humidity were not significantly correlated between monthly avian malaria cases i.e.,  $R=0.2911$ ,  $-0.0977$  and  $0.3227$ . Only minimum temperature was found correlation between monthly avian malaria cases i.e.,  $R=0.562$ . Although when compared with compound data of rain fall, maximum temperature, minimum temperature and humidity with monthly avian malaria cases, the multiple correlation was found moderately correlated i.e.=  $0.638$  (rain fall, maximum temperature, minimum temperature and humidity SPSS version 23). Monthly avian malaria positivity was found highest in May followed by June and lowest was observed in February.



**Figure 1.** Meteorological data correlation with avian malaria cases

## DISCUSSION

There are different kinds of protozoan parasite which cause malaria in different specific hosts such as rodent malaria, bird malaria, monkey malaria and human malaria etc. The term malaria has confusingly been used for either all *Haemosporidians* 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 [11]. Thirty-eight morphologically different avian *Plasmodium* species have been described. The parasite species that has received the most attention is *Plasmodium relictum*. This parasite is not only well known from avian malaria in Hawaii [12] and avian malaria outbreaks in Zoos all around the World [13-15] but it has also been used as a model species to study human malaria during the end of the 19<sup>th</sup> and beginning of the 20<sup>th</sup> century [16].

Unfortunately, there is little information available on the vector species of avian malaria. However, since much experimental work has been carried out with *Plasmodium 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* [17-18]. The life cycles of the different species of avian malaria parasite are generally similar. The aim of this study was to detect transmission of bird malaria parasite it means that *Plasmodium relictum* in the migratory and residential birds population in Hpa-a Township Kayin State.

In Myanmar there was no history of bird's malaria previously. Although in 2007, Farah and his associate revealed that *Plasmodium* and *Haemoproteus* were found in several wild bird species [2]. In the present study a total of 1616 birds were collected from Hlar-Ka-Daung, Tayout-Hla, Don-Yin and Naung-Ta-Lone village in Hpa-an Township and found that 110 birds were avian malaria parasite positive, of this 39(35.45%) *Haemoproteus*, 63(57.27%) *Plasmodium relictum* and 8(7.27%) mixed infections were observed. A same study was done in Myanmar by Farah et al., [2] mentioned that 335 birds blood samples were tested and the prevalence of infection was 122 (37.3%). Of these, 113 individuals were positive for *Haemoproteus spp.* (45 [40%]) and *Plasmodium spp.* (68 [60%]) were agreed with the present positivity ratio of *Haemoproteus spp.* and *Plasmodium spp.* In the life birds, 93.19% were healthy birds (active) and 6.80 % were sick birds from the field areas. However, the 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 [12,19].

The prevalence of avian malaria in bird species in different sites of Hpa-an Township were found that the highest avian malaria parasites positive rate was 7.43% in Don-Yin village followed by 7.18% in Tayout-Hla village and lowest was observed 6.18% positivity in Hlar-Ka-Daung village. Highest number of malaria parasite positive in different sites was found 30 in Don-Yin followed by 29 Tayout-Hla villages. A study in same areas 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 *Plasmodium relictum* positive in July [20]. Although high positivity rate of *Plasmodium relictum* was found in 66.67% and (46.15%) in *Gallus sp* (Indigenous Chicken) in Naung-Ta-Lone and Tayout-Hla villages followed by (38.89%) in *Columba livia* in Don-Yin village as well as highest mixed positive rate was found 4(13.79%) in Tayout-Hla village. High positivity rate of *Haemoproteus* in bird population were found (63.64%) and 66.67% in *Meleagris gallopavo* in Hlar-Ka-Daung and Don-Yin villages, followed by 41.67% in *Gallus sp* (Indigenous Chicken) in Tayout-Hla village. *Meleagris gallopavo* was found highest avian malaria parasite positivity in Hlar-ka-Daung 7 (28%), Don-Yin 8(26.67%), and *Gallus sp.* (Indigenous Chicken) was found high positivity 12(41.38%) in Tayout-Hla and 7 (26.92%) in Naung-Ta-lone villages. *Streptopelia chinensis* was found lowest positivity for avian malaria parasite in all selected areas. Parasite diversity was highest in Myanmar followed by India and South Korea. Parasite prevalence was differed among regions but not among host families and four lineages of *Plasmodium* and one of *Haemoproteus* shared between Myanmar and India and only one lineage of *Plasmodium* shared between Myanmar and South Korea [2]. A study mentioned that in Hawaii, overall prevalence of malaria based on parasitemia and serology at first capture ranged from 2.6% (27/1,046) in Iiwi to 40% (37/2,116) in Apapane and 39% (2,072/ 5,353) in Hawaii Amakihi [21].

Avian malaria positivity (both *Haemoproteus* and *Plasmodium relictum*), was found highest in May followed by June and lowest was observed in February. Other researcher revealed that *Columba livia* in same study places was found higher positivity of *Plasmodium relictum* in July followed by January. 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 [2]. 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 bird's population in these regions. Only minimum temperature was found 0.562 correlations against malaria positive cases. Although when compared with compound data of rain fall, maximum temperature, minimum temperature and humidity with monthly avian malaria cases, the multiple correlation (rain fall, maximum temperature, minimum temperature and humidity versus positivity) was found moderately correlated (i.e. R= 0.638, SPSS version 23). It means that all the bacteriological conditions directly affected for the malaria positivity.

Birds' malaria is mostly transmitted by *Culex* mosquitoes. 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 [17,22]. Other researchers revealed that *Culex quinquefasciatus* larvae can have a slow but complete development at 14.2°C

annual average temperature, and populations can persist if seasonal average is above 13.2° C [23]. 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 [24]. 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 [25]. Although in the present study minimum temperature range between 19°C and 26.3°C was correlated with the monthly malaria positivity and also multiple correlation was highly correlated with avian malaria cases.

## CONCLUSION

Prevalence varied between selected sites but not at the host family level. An in-depth study on the distribution of local and migratory birds, vector communities and their relationship to bird malaria parasites in these regions would be useful and may provide some insight into the regional distribution of specific avian malaria parasites family. The study of avian malaria parasites will contribute much to our knowledge and can be used as a model for other emerging infectious diseases. The endemic Hpa-an bird population will teach us how the birds evolve to gain resistance and adapt to their new pathogen.

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## CONFLICT OF INTEREST

The author declared no potential conflicts of interest concerning the research, authorship, and publication of this article.

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