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Full Length Research paper

An evaluation of the incidence of *Plasmodium* parasites in birds and the *in-vivo* efficacy of chloroquine in the examined birds against the *Plasmodium* parasites

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As an attempt to keep abreast of the variety of avian *Plasmodium* parasite in Kano State, a total of 218 blood films were made from wild (116) and domesticated (102) bird species collected between January and July, 2009 period. The slides were examined for the presence of *Plasmodium* parasite (parasitaemia value). Birds examined were six *Columbidae livia* (pigeons), two *Cisticola cantans* (Singing cisticola), two *Crinifer piscato* (Western grey plantain eaters), two *Lamprotornis superbus* (Buffalo weavers), five *Stigmatopelia senegalensis* (Laughing doves), twenty-six *Ploceus cucullatus* (Black-headed weavers), forty-three *Amadina fasciata* (Cut-throat finches), five *Lamprotoni caudatus* (Long-tailed glossy starlings), twenty-four *Uraeginthus bengalus* (Cordon bleu finches), fifty poultry chickens, forty-six local chickens *(Gallus gallus*), three *Nigrita Spp* (Negro finches) and four *Streptopchia decipiens* (African mourning doves). Results of the investigation showed that *Plasmodium circumflexicum*, *Haemoproteus columbae* and *Plasmodium gallinaecium* were present. The frequencies of occurrence in the birds' species were: 19.56% for local chickens, 50% for pigeons, 13.95% for Cut-throat finches, 50% for Grey plantain eaters, 33.3% for Negro finches and 0% for other birds. Overall, 6.89% of all the wild birds screened were infected as against 11.7% in domesticated birds. Domesticated birds had higher parasetaemia value (100 to 1000) cells per field than the wild birds (10 to 100) cells per field. However, the distribution of the parasites among the different species of the host birds was not statistically significant (P > 0.05). Chloroquine was found to be potent at 2.2 mg/ml concentration upon infected pigeons. A lineage of *H. columbae* named as type COLIV03 in MalAvi database was identified from the pigeons. This finding has thus called immediate massive screening of pigeons in Kano for this new variant of *Plasmodium* species with a view to elucidating its molecular and virulence nature as agent of avian malaria.

Key words: Kano, *Plasmodium*, pigeon, survey, malaria.

INTRODUCTION

Humans are not the only animals suffering from malaria resulting from *Plasmodium* infection (Jennings et al., 2006). Avian malaria is an arthropod-borne disease where protozoan blood parasites (*Plasmodium* species) are transmitted to birds by mosquitoes (Derraik and Maguire, 2005; Jennings et al., 2006). The causative agent of malaria in birds was discovered in 1885, only five years after it was first recognized in man (Carlton, 1938). Although, malaria parasites have been reported from many species of birds throughout the world, very little is known of their prevalence among the avifauna or the effects on the avian population (Carlton, 1938). In its broadest sense malaria, which originally meant "bad air", may today be interpreted as applying to the disease case used by the blood-inhabiting protozoa of the family *Plasmodium*. At least three genera of *Plasmodium* have been reported from birds: *Lecocytozoon, Heamoproteus* and *Plasmodium.* All these forms live within red blood cells. Carlton (1938) reported that mosquito transmission was first worked out with birds in India by Ross in 1898. Host susceptibility to avian malaria varies, and some widespread species, such as the *Passer domesticus*

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(exotic sparrow), *Turdus philomelos* (song thrush) and *T. merula* (black bird) may be asymptomatic carriers (Laird, 1950a, b). This research was carried out in Kano State. This is because no such study was ever carried out in the State and the need to explore the possibility of the infection in the area was long overdue. The study was therefore aimed at determining the presence or otherwise of *Plasmodium* parasites in birds, the assessment of the parasitaemia value and evaluating the *in-vivo* efficacy of chloroquine in the examined birds against the *Plasmodium* parasites.

MATERIALS AND METHODS

Description of the study area

The study area was the environs of Kano State. The State lies between latitude 13^{o}N in the North and 11^{o}N in the South and longitude 8^{o}E in the West and 10^{o}E in the East. The state capital is located on latitude 12^{o}N and longitude 8.3 ^{o}E. It is within the semi-arid Sudan savannah zone of West Africa about 840 km from the edge of the Sahara desert. It has a mean height of about 472.45m above sea level and has two seasonal periods categorized on the basis of moisture as dry and rainy seasons. The temperature of Kano usually ranges between a maximum of 33^{o}C and a minimum of 15.85^{o}C although, sometimes during the harmattan it falls down to as low as 10^{o}C. The average rainfall is between 63.3 $\pm$ 48.2mm in May and 133.4 $\pm$ 59 in August the wettest month. The soil in most part of Kano State is light or moderately leached, yellowish brown and sandy just like most other savanna parts of Northern Nigeria. The soil fertility supported agriculture. The natural vegetation as earlier mentioned is semi-arid Sudan savanna, which is sandwiched by the Sahel savanna in the north and the Guinea savanna in the south. The savanna has been described as the zone that provides opportunity for optimal human attainment. This is because it is rich in faunal and floral resources, and as such suitable for both cereal agriculture and livestock rearing (Shekarau, 2010).

Sample collection sites

Eight local government areas (LGAs) in Kano State were chosen for this study in order to diversify the sampling area. They included Nassarawa, Kano Municipal, Wudil, Bebeji, Doguwa and Gwale. Amongst the areas chosen were places where there are water bodies, a lot of trees and animals, which provide conducive breeding spaces for both wild birds and mosquitoes. Such areas included the Botanic garden in Bayero University, Kano (Gwale LGA), Zoological garden (Kano Municipal LGA), Tiga (Bebeji LGA) and Falgore forest reserve (Doguwa LGA). Market places are chosen in order to have bird's representation of locally kept birds such as pigeons and chickens. Poultry farm was taken into consideration in the study so that the poultry birds kept within the State would have a good representation too. No specificity was given to particular bird specie but rather any bird was observed in the study to get a general over view of the infection in all birds' species.

Collection and handling of birds species used for the study

The wild birds were captured using birds trap and the domesticated birds were with hand.

The wild birds were collected from the following areas:

1. Botanical Garden of Biological Science Department, Bayero University, Kano.
2. Falgore Forest along Jos Road, Kano – Doguwa LGA.
3. Zoological Garden, Kano State – Kano Municipal.
4. Getso Town – Gwarzo LGA.
5. Tiga Dam - Bebeji Local Government.

The domesticated birds were collected at:

1. Farida Farms, Hotoro GRA - Poultry Chickens – Nassarawa LGA
2. Wudil Local Market – Local Chickens – Mudil LGA
3. Sharada Local Market – Piegions – Kano Municipal

The birds were handled carefully in cages of appropriate size and were fed adequately. The birds were identified by Suleiman Abubakar Fagge; Director of Wild Life Services, Zoological Garden, Kano and the parasites were confirmed by Veterinary Dr. Yahuza Aliyu of the Epidemiology unit, Kano State Ministry of Agriculture and Natural Resources.

Collection of blood from the study birds

A total of 218 blood smears of different species of birds were collected from different areas in Kano State. They include 102 domestic and 116 wild birds. The domestic birds were divided into 50 hybrid (exotic) chickens, 46 local chickens and 6 pigeons. Blood samples were taken from brachial vein of the avian hosts using the procedure described by Rukhsana (2005) and Chesebrough (2000). The brachial vein area was cleansed with Swab moistened with 70% v/v alcohol, and it was allowed to dry. Using a sterile lancet, the area was pricked and squeezed gently to obtain a large drop of blood. The blood was collected using heparinized capillary tube. Using a microscopic slide, a small drop of blood was dropped at the center of the slide and a larger drop on another microscopic slide. The thin film was spread using a smooth edge slide spreader (slide). Without delay, the large drop was spread, to make a thick smear. The area of about 15 × 15 mm was covered. It was mixed evenly to avoid red cells forming marked rouleaux during staining. Using a grease pencil the slide was labeled with number for identification. The blood was left to air dry with the slide in a horizontal position and it was placed in a separate box covered with a lid to protect it from insects and dust and it was kept in a warm sunny place in order for the film to dry quickly it was then removed from the sun immediately it was dried (Chesebrough, 2000).

Preparing thick and thin blood films

The thick and thin blood films were made on different slides that have frosted ends for easy labeling (Chesebrough, 2000).

Standardizing the amount of blood used and area covered by the thick film

To ensure good staining, standardization and reproducible results, the amount of blood used particularly in thick film was kept as constant as possible and the blood was spread evenly over the specified area of the slide (Chesebrough, 2000).

Staining of slides

Using absolute methanol (methyl alcohol), the blood films were fixed by spreading on the microscopic slide (Chesebrough, 2000).

Table 1. Varieties of birds screened for *Plasmodium* species from some parts of Kano.

Birds species	EXC	LC	PG	WGPE	AMD	LD	LTGS	BFW	CTF	CB	NF	SC	BHW
Location													
Botanical garden BUK				1	3	2	2	2					
Falgore forest				1		1	2			24			
Kano Zoo garden					1	2			19		3		
Getso town									24			2	
Tiga town													26
Wudil LGA		46											
Nassarawa LGA	50												
Kano Municipal			6										
Total	50	46	6	2	4	5	4	2	43	24	3	2	26

EXC – exotic chickens; LC- local chickens; PG – pigeons; WGPE – Western Grey plantain eater; AMD – African mourning dove; LD – laughing dove; LTGS – long tailed glossy starling; BFW – Buffalo weaver; CTF – cut throat finches; CB – Cordon Bleu; NG – Negro Finches; SC – Singing Cisticola; BHW – black headed weaver.

The slides were placed horizontally on a staining rack. A small drop of absolute methanol was added to the thin film. The absolute methanol was not used on the thick film in order to prevent lysis of the red cells and make the thick film unreadable. Malaria parasite in thick and thin blood films were stained at the pH 7.1 - 7.2 using Giemsa stain (Chesebrough, 2000). Immediately before use, Giemsa stain was diluted at 10% solution for 10 minutes. 45 ml of buffered water, pH 7.1 to 7.2 was measured in a 50 ml cylinder and 5 ml of Giemsa stain was added to 50 ml mark then it was mixed gently. The slides were placed on a staining rack. It was immersed in a staining trough. Thick blood films were allowed to dry thoroughly while the thin blood films were fixed using methanol for 2 min. The diluted stains were poured on the staining trough for 10% solution for 10 min. The stain from the staining container and slide were washed with clean water to avoid the films being covered with a fine deposit stain. The back of each slide was cleaned and placed on a draining rack to air dry.

Microscopic estimation of parasitaemia

The blood films were examined microscopically using the 100 oil-immersion objectives. After the thick film was completely air dried, a drop of oil immersion was applied to the area of the film that appears mauve colored. The oil was spread to cover the area. This is to enable the film to be examined first at low magnification. An area that is not too thick is selected and it was examined under the ×100 oil-immersion objective. The parasites and pigments were examined. The parasites were identified and approximate number of parasites; trophozoites of plasmodium parasite, was reported (Cheesbrough, 2000). When the thin film was completely dried, a drop of oil immersion was dropped to the lower third of the film. It was examined under 40 objectives to check the staining, morphology and distribution of the cells and to detect malaria schizonts, gametocytes and trophozoites (Chesebrough, 2000).

Parasite identification using PCR technique

The specimen was placed on a filter paper and was kept in the laboratory to dry. It was covered with wire gauze to avoid tampering by flies. The specimen was forwarded to Professor Staffan Bensch of Lund University Sweden for molecular typing (Wiersch et al., 2005).

Statistical analysis of the data

Data for number and variety of birds recruited for the study were presented in frequency distribution table as percentages and means. Variation in occurrence of infection and its rates between wild birds and domesticated ones are tested by Chi-Square tool at 5% level of probability (Mukhtar, 2005).

RESULTS

Variety of birds collected and screened for Plasmodium species

Table 1 shows the variety of birds screened. It includes the following: Pigeons (2.75%), Western grey plantain eaters (0.91%), African mourning dove (1.83%), Laughing dove (2.29%), Long-tailed glossy starling (2.29%), Buffalo weaver (0.91%), Cordon bleu (11.01%), Cut-throat finches (19.72%), Singing cisticola (0.91%), Black-headed weaver (11.93%), Local and exotic chickens (21.10%) as well as Negro finches (1.38%).

Incidence of malaria parasite in the variety of birds screened

Tables 2 to 5 show the incidence of avian plasmodia in wild varieties of birds caught around Kano State. Cut-throat finches were found at Getso rural area of Gwarzo LGA. Eighty percent of the five catch were infected with *Plasmodium circumflexicum* at a parasitaemia value of 1 to 10 per microscopic field of their blood. The remaining 20% showed parasitic load of 10 to 100 per microscopic field. At Falgore forest reserve in Tudun Wada LGA, Grey plantain eater was identified to possess a *Plasmodium* species whose identification was terminated at the genus level. Around Kano municipal local government area (precisely in Zoological garden), Negro finch and Cut-throat

Table 2. Density and suspected species of *Plasmodium* found in wild birds.

S/No.	Bird species	Site	Slide Code	Suspected *plasmodium* species	Degree of parasitaemia
1.	Cut throat finch	Getso	Getso 13	*Plasmodium circumflexicum*	+
2	Cut throat finch	Getso	Getso 13	*Plasmodium circumflexicum*	+
3	Cut throat Finch	Getso	Getso 10	*Plasmodium circumflexicum*	+
4	Cut throat finch	Getso	Getso 9	*Plasmodium circumflexicum*	+
5	Cut throat finch	Getso	Getso3	*Plasmodium circumflexicum*	++
6	Grey plantain eater	Falgore	Falgore A	*Plasmodium Spp.*	+
7	Negro finch	KMC	Zoo 10	*Plasmodium circumflexicum*	+
8	Cut throat finch	KMC	Zoo 6	*Plasmodium circumflexicum*	+

+ = 1-10 per 100 high power field; ++ = 11-100 per 100 high power field.

Table 3. Density and suspected species of *Plasmodium* in domesticated birds.

S/No.	Bird species	Site	Slide code	Suspected *plasmodium* species	Degree of parasitaemia
1	Pigeon	KMC	P1	*Haemoproteus columbae*	+
2	Pigeon	KMC	P2	*Haemoproteus columbae*	++
3	Pigeon	KMC	P3	*Haemoproteus columbae*	++
4	Local chicken	Wudil	LDP	*Plasmodium gallinaecium*	+++
5	Local chicken	Wudil	LDO	*Plasmodium gallinaecium*	++
6	Local chicken	Wudil	LDN	*Plasmodium gallinaecium*	+++
7	Local chicken	Wudil	LDM	*Plasmodium gallinaecium*	+
8	Local chicken	Wudil	LDS	*Plasmodium gallinaecium*	+++
9	Local chicken	Wudil	LDQ	*Plasmodium gallinaecium*	+++
10	Local chicken	Wudil	LDA	*Plasmodium gallinaecium*	+
11	Local chicken	Wudil	LDC	*Plasmodium gallinaecium*	+++
12	Local chicken	Wudil	LDE	*Plasmodium gallinaecium*	++

+ = 1 to 10 per 100 high power fields; ++ = 11 to 100 per 100 high power fields; +++ = 1 to 10 in very high power field; ++++ = More than ten in very high power field.

Table 4. Occurrence of *Plasmodium* parasites in domesticated birds in Kano.

S/No.	Bird type	Locality	No. examined	No. infected	Frequency (%)
1	Local chickens	Wudil LGA	46	9	19.56
2	Poultry chickens	Nassarawa LGA	50	NPF	0.0
3	Pigeons	KMC	6	3	50.0
	Total		102	12	12.24

NPF = No parasite found.

finch were found to be infected with *P. circumflexicum* at 1 to 10 parasitic densities per microscopic field. The varieties of domesticated birds showing the infection with the parasites were pigeons 25% and local chickens 75% found from Kano municipal and Wudil LGAs respectively (Table 3). Pigeons were heavily infected with *H. columbae* with a density of 10 to 1000 per microscopic field. The infected local chickens showed also 10 to 1000 parasites per microscopic field and the best identified were *P. gallenaecium*. Based on the PCR analysis (Table 6), one new lineage of *H. columbae* namely COLIV03 was identified at Lund University of Sweden by Prof. Staffan Bensch. In terms of frequency of occurrence (Table 4), local chickens had 19.56% as obtained from Wudil. Exotic poultry chickens from Nassarawa LGA had zero percent incidence of infection.

DISCUSSION

Among the 116 wild species of birds observed it was found that only eight wild birds from three different species

Table 5. Occurrence of *Plasmodium* parasites found in wild birds in Kano.

S/No.	Bird species	Locality	Number examined	Number infected	Percentage frequency
1	Cut throat finches	Getso, Zoo	43	6	13.95
2	Grey plantain eater	Falgore, BUK	2	1	50.0
3	Negro finches	Zoo	3	1	33.33
4	Laughing dove	BUK, Falgore	5	0	0.0
5	African mourning dove	BUK, Zoo	4	0	0
6	Long tailed glossy starling	BUK, Falgore, Zoo	5	0	0.0
7	Buffalo weaver	BUK	2	0	0.0
8	Singing cisticola	Getso	2	0	0.0
9	Cordon blue finches	Falgore, BUK	24	0	0.0
10	Black headed weaver	Tiga	26	0	0.0
Total			116	8	9.28

Table 6. Results of *Plasmodium* species based on PCR method analysis.

S/No.	Bird speci	Locality	Filter paper code	Result	Plasmodium specie	Remarks
1.	Pigeon	Tarauni market	PE	Positive	*Haemoproteus columbae*	Identified as Existing Lineage in the Mal Avi Data Base HAECOL1
2.	Pigeon	Tarauni market	PF	Positive	*Haemoproteus columbae*	Identified as new Lineage in the Mal Avi Data Base. It is termed COLIV03
3.	Pigeon	Tarauni market	PD	Negative	-	
4.	Local chicken	Tarauni market	CC	Negative	-	
5.	Local chicken	Tarauni market	CD	Negative	-	
6.	Local chicken	Tarauni market	CE	Negative	-	
7.	Cut throat finch	Zoological garden	W3	Negative	-	
8.	Cut throat finch	Zoological garden	W4	Negative	-	
9.	Cut throat finch	Zoological garden	W10	Negative	-	

were actually infected with the *Plasmodium* species. The dominant bird specie infected was the Cut-throat finches (*Amadina fasciata*). About seven were infected and six out of the seven were sampled from Getso town of Gwarzo LGA. Only one was sampled from Zoological garden in Kumbotso LGA indicating its scarcity in the urban settlement. In the domesticated birds, pigeons and local chickens were the most infected birds' species (Figure 1). This may be as a result of a prolonged evolutionary association of the host with the parasite. Furthermore, the domestication and lack of proper care by the keepers of the birds might have exposed them to the parasite in spite of the fact that the birds are known to have high disease resistance (Wikipaedia, 2008; Valkiunas et al., 2009b). Less care was given to them and this might have made them prone to infection by plasmodiasis of birds. Another factor could be lack of proper education or enlightenment on how to keep the poultry chickens on the part of the local keepers (Baker, 1976).

The goal of this study was the identification of plasmodium parasites in birds using microscopy. The parasites were seen and the level of infection evaluated in the wild and the domesticated birds. Identification of the genus *Plasmodium* was done microscopically by the use of Giemsa staining technique. For more elaborate study to identify the species of the *Plasmodium* found in different types of birds' species a more effective method must be used which is called molecular typing. Molecular Typing using Polymerase Chain Reaction Mechanism, which amplifies DNA and the particular specie is identified as noted by Valkiunas et al. (2005, 2009a). Because of high sensitivity PCR may detect very small numbers of sporozoites in birds. Standard microscopy protocols are insufficiently sensitive to detect such light parasetaemias of both sporozoites and gametophytes; this could explain some discrepancies between levels of infection prevalence recorded using these two methods in parallel (Valkiunas et al., 2005).

For the exotic chickens, no single one was found to be infected with the parasite. This might be as a result of good care attributed to the standards (their housing, feeding, medication and appropriate knowledge in keeping the birds) provided in keeping the birds. More so,

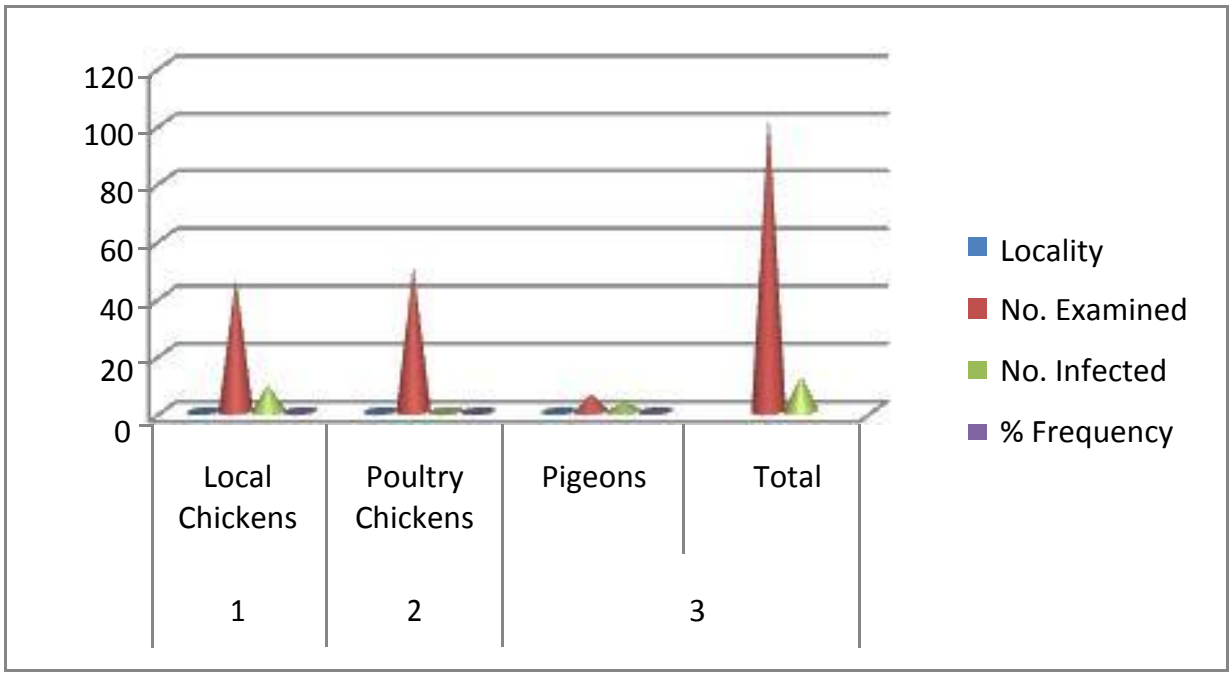

Figure 1. Domesticated birds examined against the infected birds.

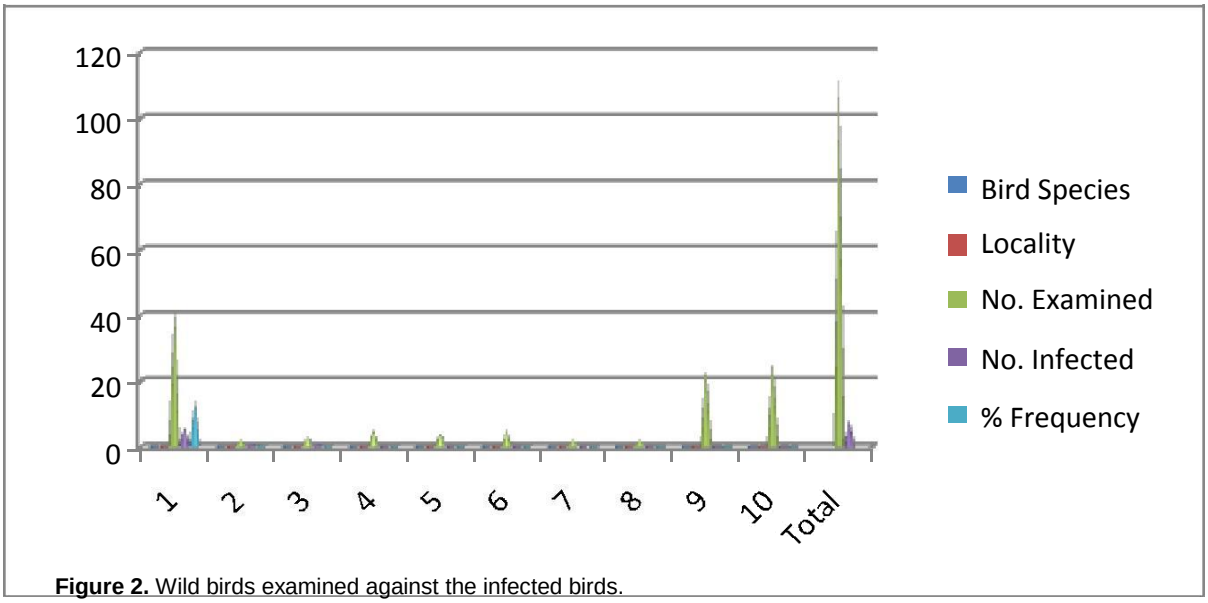

Figure 2. Wild birds examined against the infected birds.

the birds are known to have a very poor resistance for infection and are mostly kept for commercial purposes so proper care must be given to them to prevent break out of any epidemic or infection. As a result, no single bird was found to be infected with *Plasmodium*. The species of *Plasmodium* parasite were identified in both the domesticated and wild birds and with no statistically significant difference ($P > 0.05$). *Plasmodium gallinaecium* was found in the local chickens *G. gallus*, *H. columbae* was found in pigeons and *P. circumflexicum* was found in finches (Figure 2).

The parasitaemia value of the *Plasmodium* parasites in birds from the research conducted shows that Cut Throat Finches (*A. fasciata*) are more infected with the plasmodium parasite with mostly plus one degree of parasitaemia, that is, 1 to 10 parasites per 100 high power fields among the wild species of birds examined. While the domesticated birds pigeons and chickens were found to be infected more heavily than even the wild birds with degree of parasetaemia of plus two and three that is, 11 to 100 per 100 high-power field (Tables 2 and 3). The level of infection between the wild and domesticated species shows that there is signific-

ant difference ($P < 0.05$) in the parasitaemia value since most of the wild birds were in plus one degrees of infection and the domesticated are in the range of plus two and plus three parasitaemia value within the study area.

A possible new lineage identified in the present investigation

Results obtained from the molecular typing indicated a new variant of *Plasmodium* was found on certain blood samples of *Columba livia*. The results obtained on certain blood samples of *C. livia* (Pigeons) found new variant of *Plasmodium*, unknown to parasitologists before. The sample coded PE indicated perfect match to HAECOL1 (as in the MalAvi database) previously found in *C. livia* (Dranzoa et al., 1999) and identified as morphospecies *H. columbae* but sample PF was found to be a new lineage but only slightly different (4 changes) from HAECOL1. Most likely is a member of the morphospecies *H. columbae* and the lineage registered as COLIV03 in the MalAvi database.

Conclusion

The study confirms the presence of *Plasmodium* species among a variety of wild and domesticated avian species around Kano State. Infection rate was significantly higher in domesticated than the wild birds. The parasitaemia value was significantly ($P < 0.05$) higher in the local chickens than the wild birds and exotic chickens that had no parasitaemia. A new lineage of *Haemoproteus columbae* named as type COLIV03 in MalAvi data base was isolated from pigeons. This finding has thus stressed the need for active and emergent investigation for the new variant especially among the pigeons in Kano State.

RECOMMENDATION

Though bird's malaria is not well known in Nigeria, it is a well known disease of birds in many part of the world, and it has been a case study for decades in existing literatures. It is recommended that more research be carried out on this bird's disease in order to control it most especially in domesticated ones. This will prevent epidemic of the disease. In addition, obtaining a good data analysis on different States of Nigeria on bird's malaria could help in proper prevention of the disease.

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