



Investigation of Avian Malaria Parasites of Domestic Birds in Nsukka Local Government Area of Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study assessed the presence of avian malaria parasites in thin blood smears stained with Giemsa. A total of 109 poultry birds were examined out of which 49 were chicken, 20 were pigeons, 20 were turkeys and 20 were ducks. The poultry subjects comprised 45 males and 56 females. Malaria parasites were observed in 18 (16.5%) of these animals. Three species of *Plasmodium* were observed among the poultry subjects, which are: *Plasmodium gallinaceum* in the chicken, *Plasmodium relictum* in the pigeon and *Plasmodium durae* both in the turkey and the duck. Pigeon had the highest rate of parasitaemia 6 (30%), followed by turkey 5 (25%), duck 3 (15%) and the least in the chicken 4 (8.2%). Out of the 45 male and 56 female birds examined, infection rates were 8 (17.8%) and 10 (17.9%) respectively. Both adult and young birds had the same infection rate 9 (8.3%). Infection rates did not show any significant difference ($P < 0.05$) between age and sex of the poultry subjects. Of the 109 birds sampled, 48 (44%) were sampled in Obukpa and 61 (56%) in Nsukka town out of which 10 (20.8%) were infected in Obukpa and 8 (13.1%) in Nsukka.

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1. INTRODUCTION

Avian malaria is a protozoan disease caused by members of the genus *Plasmodium*. The disease is transmitted by Culicidae – *Culex* and *Aedes*, though species of *Mansonia* and *Anopheles* also transmit them. Recent studies have focused on avian blood parasites as a model system for host-parasite interactions in an evolutionary and ecological context [1,2] and [3]. Relative to the published information on wild birds, a much larger literature exists on these parasites in poultry. Extensive laboratory studies have been conducted describing their pathologies [4].

Avian malaria is caused by parasites of the *Plasmodium* genus. Mosquitoes transmit these parasites to the birds. Avian malaria characteristically develop in Culicidae – *Culex* and *Aedes*, though species of *Mansonia* and *Anopheles* also transmit them [5,6]. There are several species of *Plasmodium* that infects birds. Some 65 *Plasmodium* spp have been isolated from over 1,000 different species of birds [7]. Few of the *Plasmodium* spp which have been identified appear to be natural parasites of domestic poultry. The few *Plasmodium* spp include: *Plasmodium gallinaceum*, *Plasmodium juxtannucleare*, *Plasmodium durae* and *Plasmodium relictum*. They are known to cause up to 90% mortality in poultry [8].

Plasmodium spp that are pathogenic to poultry are found mainly in Africa, Asia and South America. Avian malaria produces a wide range of effects in avian hosts, from no apparent clinical signs to severe anaemia and death. Mosquitoes serve as vectors of the parasites by biting an infected bird and transmitting it to an uninfected bird.

According to Dipeolu et al. [9] *Plasmodium* spp was observed in a survey carried out in turkeys and ducks in Northern Nigeria. Earlier studies showed *Leucocytozoon* spp infected 55 of 163 (34%) examined chickens in Ibadan but no *Plasmodium* spp was found [10]. A survey on wild birds around Kainji Lake in Nigeria by Huff [11] found blood parasites in 58.1% out of the 210 birds. These parasites include species of *Plasmodium*, *Leucocytozoon* and *Haemoproteus*. Since avian malaria is extremely pathogenic for poultry birds especially for young birds, very heavy losses have been reported from various

parts of the world with death rate reaching up to 90% in some flocks [12].

Generally, species of *Plasmodium* infecting birds include: *P. accipiteris*, *P. alloelongatum*, *P. anasum*, *P. ashfordi*, *P. bambusicolai*, *P. bigueti*, *P. biziurae*, *P. buteonis*, *P. cathemerium*, *P. circumflexum*, *P. coggeshalli*, *P. corradettii*, *P. coturnix*, *P. dissanaikei*, *P. durae*, *P. elongatum*, *P. fallax*, *P. forresteri*, *P. gabaldoni*, *P. gallinaceum*, *P. garnhami*, *P. giovannolai*, *P. globularis*, *P. griffithsi*, *P. gundersi*, *P. Guangdong*, *P. lucens*, *P. hegneri*, *P. hermani*, *P. hexamerium*, *P. huffi*, *P. jiangi*, *P. juxtannucleare*, *P. kempfi*, *P. lophurae*, *P. lutzi*, *P. matutinum*, *P. megalobularis*, *P. multivacuolaris*, *P. nucleophilum*, *P. panernai*, *P. parahexamerium*, *P. paranucleophilum*, *P. parvulum*, *P. pediocetti*, *P. paddae*, *P. pinotti*, *P. polare*, *P. relictum*, *P. rouxi*, *P. tenue*, *P. tejeraei*, *P. tumbayensis* and *P. vaughani* [13].

2. METHODOLOGY

2.1 Study Area

The study area covered includes Obukpa and Nsukka towns both of which are found in Nsukka Local Government Area of Enugu State, Nigeria. Nsukka area is a region of high-lying hills with a topography that promotes an even distribution of birds especially the domestic ones. The Town comprises many villages, and falls within latitudes 05°- 51°N and longitude of 07°- 27°E. The climate of Nsukka area is a tropical wet and dry type. The annual rainfall for Nsukka area varies from 97.30 cm to 206 cm.

A typical compound is made up of houses. The houses in some areas are built with mud and thatched, others are built with cement blocks and corrugated zinc sheets. Two types of farmlands exist, nearby farmlands usually located close to the compound and farmed year after year and outfield farms usually located some kilometers away from the home. The nearby farms are heavily manured with house-hold refuse and animal droppings.

The livestock are allowed to wander about the bushes, farmlands and refuse dumps, eating anything from human excreta to drinking and bathing in mud water found along the roads or within the compounds [14].

2.2 Poultry Subjects Age and Sex Determination

The animals examined were poultry birds reared both in intensive and extensive care management system. These include: the duck (*Anas spp*), chicken (*Gallus domesticus*), pigeon (*Columba livia*) and turkey (*Meleagris spp*). Birds sampled were mostly local breeds especially the domestic chicken. The age (between 2 -4 months) and sex of the poultry subjects were obtained from the owners.

2.3 Method of Sample Collection

The collection of blood samples was conducted within August to October, 2009. There were difficulties in getting the natives convinced that the blood was not for some ritual practices and that the quantity of blood required was not enough to kill their birds. In some difficult situations payment were made to induce the co-operation of the poultry owners in Nsukka town.

Blood samples were collected from a total of 109 domestic birds which include 20 samples of Pigeon (*Columba livia*), 49 samples of *Gallus domesticus*, 20 samples of Turkey (*Meleagris spp*) and 20 samples of Duck (*Anas spp*) from Nsukka Local Government Area. The birds constitute species chosen randomly from free ranging forms to different poultry farms and the various market places from Nsukka Local Government Area.

In this work, blood samples were collected through the wing web vein of the birds with the aid of syringes. Blood samples were collected through the wing web vein of the birds through the following procedures [9]:

- 1) Firstly, some feathers were pinched off from the wing web of the bird to get a clear vision of the vein.
- 2) The vein was dampened with the thumb.
- 3) Carefully, the syringe was inserted into the vein in a direction from outside the wing to the body of the bird because blood flows towards the heart.
- 4) The syringe was drawn gently to collect 1ml of blood and immediately transferred to the EDTA specimen bottles to avoid clotting before reaching the laboratory for diagnosis.
- 5) Immediately the syringe was out, a cotton wool soaked in methylated spirit was used

to gently massage the vein to avoid bleeding.

Ten blood samples were collected on daily basis in the morning hours and returned to the laboratory for its proper diagnosis not later than 6 hours after collection.

2.4 Diagnosis

Identification of malaria parasites in blood samples generally was done through making of thin blood films. These films were afterwards fixed and stained (stains include: Giemsa stain, Field's stains and Leishman's stain) [11]. The thin film technique caused very little distortion of the parasite and permits species identification.

2.4.1 Making of thin blood smear

The technique used in making the thin blood smear on a slide is as follows:

- 1) A completely clean (grease-free) and scratch free slide was placed on a firm laboratory table.
- 2) With the aid of a dropper, a single drop of blood was collected from the EDTA bottle and dropped in the middle of the slide.
- 3) Using another clean slide as "a spreader", the small drop of blood was touched with the spreader so that the spreader makes an angle of about 45° with the slide carrying the drop.
- 4) In one continuous motion, the spreader was smoothly pushed forward along the slide, keeping the spreader at the angle of 45°, making sure that the spreader is in even contact with the surface of the slide all the time the blood is being spread.
- 5) The blood film was allowed to air-dry thoroughly with the slide in a horizontal position.

2.4.2 Fixation of thin blood film

Absolute methanol (methyl alcohol) was used to fix thin blood films. The alcohol must be free from water otherwise it would not fix the cells properly. I always made sure the stock bottle of alcohol was kept tightly stoppered.

The technique of fixing the thin film is as follows:

- 1) The slide was placed horizontally on a level laboratory bench.

- 2) Three drops of absolute methanol was applied to cover the thin film.
- 3) The film was allowed to fix for 1 – 2 minutes. The alcohol was tipped off and the film allowed to dry.

2.4.3 Staining of thin blood film

After the fixed thin film had dried thoroughly, the following procedures were undertaken.

- 1) The slides were placed back to back in a staining dish.
- 2) 3% Giemsa in buffered water, pH 7.2 was prepared in sufficient quantity to fill the dishes. The stain was well mixed.
- 3) The stain was poured gently into the dish, until the slides were totally covered.
- 4) It was allowed to stain for 30-45 minutes out of sunlight.
- 5) The whole dish was gently immersed in a vessel filled with clean water.
- 6) The remaining stain was poured off gently and rinsed again in clean water for a few seconds. The water was poured off.
- 7) Finally, the slides were removed one by one and placed in a slide rack to drain and dry, film side downwards.

2.5 Identification of Malaria Parasites

Prepared slides were viewed under oil immersion microscopy. Three components of malaria parasites might be seen. These are blue-staining cytoplasm, red or purple chromatin, and brown or black pigment granules or rod. Except for the early (young) ring stages, all the three components were able to be seen. Observation of the 3 components is important in order to distinguish malaria parasites from host cells like white blood cells, and artefacts that may appear on the slide during preparation.

The appearance of the parasite and the appearance of the red blood cells containing the parasites were checked.

2.6 Statistical Analysis

The relationship between the distribution of infection and sex was tested with the chi (χ^2) to show any significant difference ($P < 0.05$).

3. RESULTS

One hundred and nine (109) Giemsa stained microscopic slides from 109 poultry birds were

examined under oil immersion microscopy. Ten to fifty microscopic fields were examined for all the cases of samples collected irrespective of the degree of parasitaemia. Forty-nine, twenty, twenty and twenty samples were collected from Chicken, Pigeon, Turkey and Ducks respectively. Out of the 109 poultry birds used, 18 were infected in the ratios of 4:6:5:3 in chicken, pigeon, turkey and duck respectively. Four species of *Plasmodium* were observed. They include: *Plasmodium gallinaceum* in chicken, *Plasmodium relictum* in the pigeon, and *Plasmodium durae* both in turkey and duck.

Table 1. Prevalence of malaria parasites among the poultry subjects

Birds	Number examined	Number infected	% infected
Chicken	49	4	8.2
Pigeon	20	6	30
Turkey	20	5	25
Duck	20	3	15
Total	109	18	16.5

Altogether 109 birds of four species were examined for the prevalence of *Plasmodium* parasites. An overall prevalence of 16.5% was obtained. The highest infection rate occurred in the Pigeon (*Columba livia*) where 6 (30%) out of the 20 Pigeons harboured *Plasmodium* parasites. The least infection rate was observed to have occurred in the Chicken (*Gallus spp*) where 4 (8.2%) out of the 49 Chicken sampled had *Plasmodium* parasites.

Table 2 shows the sex distribution of malaria parasites among the poultry subjects. Generally, the female birds had a higher rate of parasitaemia 10 (17.9%) than males 8 (17.8%). There was no significant difference (0.05) infection rate observed between the male and the female bird. The female chicken (*Gallus spp*) had a higher infection rate 3 (9.7%) than the males 1 (6.7%). In the Pigeon (*Columba spp*) the males had a higher infection rate 4 (33.3%). The turkey (*Meleagridisspp*) and the chicken (*Gallus spp*) were observed to have the highest infection rate occurring among the females, 3 (27.3%) and 3 (11.5%) respectively. Among the Duck (*Anasspp*) the females also had a higher infection rate 2 (18.2) more than the males 1 (11.1%).

Table 2. Sex distribution of malaria parasites among the subjects

Birds	Number Sampled		Number Infected		Infection rate %	
	Male	Female	Male	Female	Male	Female
Chicken	15	31	1	3	6.7	9.7
Pigeon	12	8	4	2	33.3	25
Turkey	9	11	2	3	11.1	27.3
Duck	9	11	1	2	11.1	18.2
Total	45	56	8	10	17.8	17.9

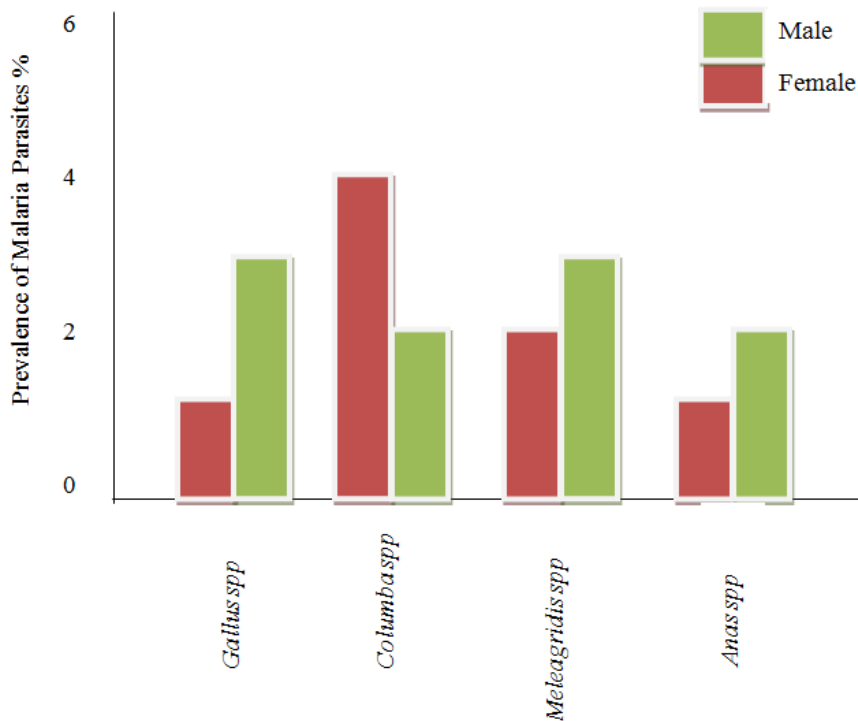


Fig. 1. Sex distribution of malaria parasite among the poultry subjects

Table 3. Age-specific distribution of malaria parasites among the poultry subjects

Birds	Number Sampled		Number Infected		Infection rate %	
	Young	Adult	Young	Adult	Young	Adult
Chicken	25	24	3	1	6.1	2.0
Pigeon	11	9	3	3	15	15
Turkey	7	13	1	4	5	20
Duck	12	8	2	1	10	5
Total	55	54	9	9	8.3	8.3

From the result it is seen that the highest infection rate occurred in the females except in the pigeon and duck where the males were more infected.

Among the chicken (*Gallus spp*) the young birds had a higher infection rate 3 (6.1%) than the adults 1 (2.0%). The infection rate in the Pigeon (*Columba spp*) recorded an equal infection rate

among both the young and the adult 3 (15%). Turkey (*Meleagris spp*) had a higher infection rate occurring in the adult 4 (20%) than the young 1 (5%). The infection rate observed in the Duck (*Anas spp*) indicated that the young had a higher infection rate 2(10%) than the adult 1(5%). However, infection rate does not show any significant difference ($P < 0.05$) between the young and the adult.

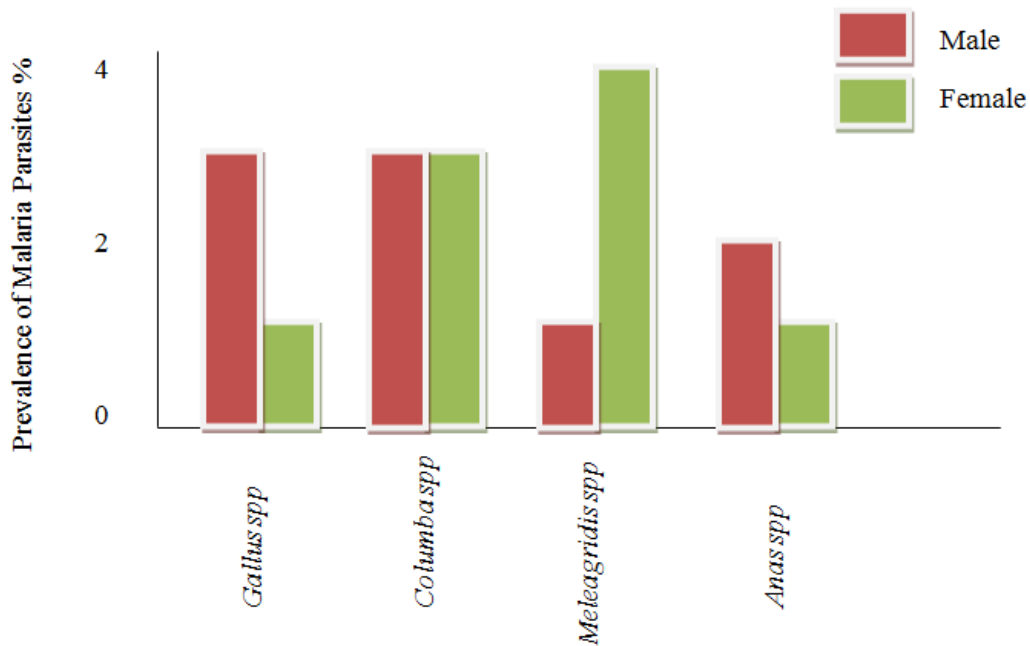


Fig. 2. Age Distribution of malaria parasites among the poultry subjects by location

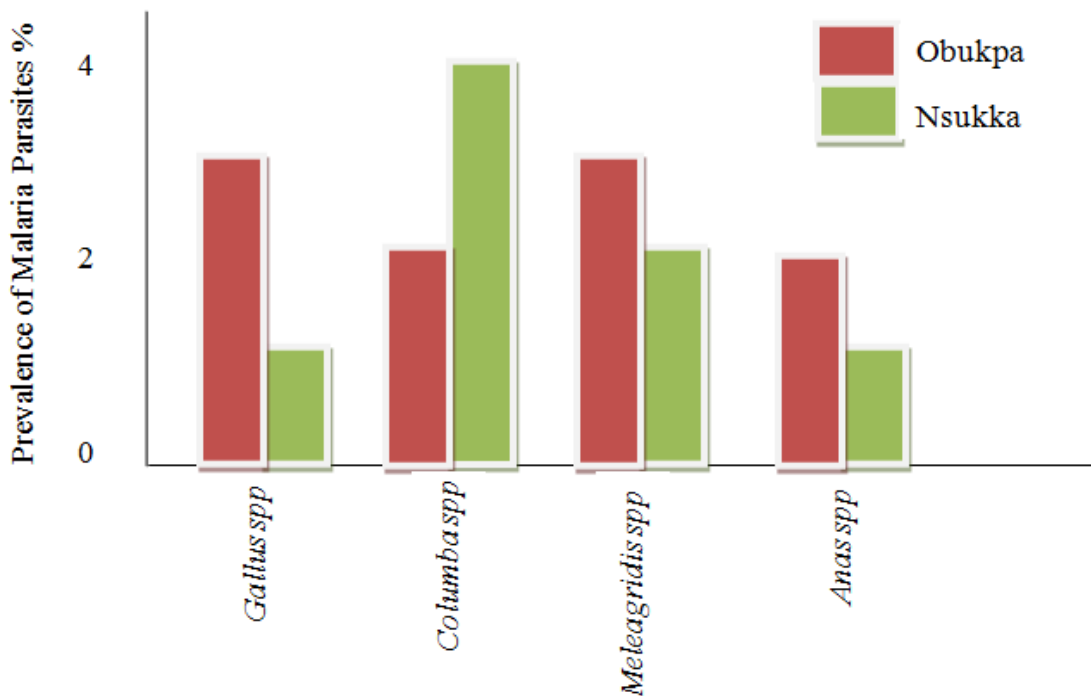


Fig. 3. Infection rates among the poultry subjects at different localities

At Obukpa, the highest rate of parasitaemia was observed in both the Chicken and the Turkey 3 (6.3%). The least infection was observed in both the Pigeon and the Duck 2 (4.2%). At Nsukka, the highest rate of parasitaemia occurred in Pigeon 4 (6.6%), while the least infection rate

occurred in both the Chicken and the Duck 1 (1.6%). The highest infection rate when looking at the different localities was found to have occurred in Obukpa 10 (20.8%) as against 8 (16.7%) in Nsukka.

Table 4. Distribution of the Malaria Parasites among the Poultry Subjects by Location

Location	Number Examined	Number of Chicken Infected	% Infected	Number of Pigeon infected	% Infected	Number of Turkey Infected	% Infected	Number of Duck Infected	% Infected
Obukpa	48	3	6.3	2	4.2	3	6.3	2	4.2
Nsukka	61	1	1.6	4	6.6	2	3.3	1	1.6
Total	109	4	3.7	6	5.5	5	4.6	3	2.8

Table 5. Monthly Distribution of the Malaria Parasites among the Poultry Subjects

Month	Number Examined	Number of Chicken	% Infected	Number of Pigeon infected	% Infected	Number of Turkey Infected	% Infected	Number of Duck Infected	% Infected
July	27	-	-	-	-	-	-	-	-
August	25	-	-	-	-	-	-	-	-
September	27	-	-	-	-	-	-	-	-
October	30	4	13.3	6	20	5	16.7	3	10
Total	109	4	3.7	6	5.5	5	4.6	3	2.8

4. DISCUSSION AND CONCLUSION

The bane of poultry production in developing countries is attributable to managerial and medical factors. The degree of involvement of avian malaria parasites has not been vividly brought under study. Most surveys have concentrated on gastrointestinal parasites, may be due to the fact that they have been shown to be of more economic importance. Since these infections do not occur on a single but concurrent form, the prevalence and degree of infectivity might particularly depend on those malaria parasites that are ignored.

The study with 109 blood samples collected from Nsukka area revealed a low prevalence of malaria parasites (16.5%). In the poultry subjects examined, the parasites detected include *Plasmodium gallinaceum* in Chicken, *Plasmodium relictum* in Pigeon and *Plasmodium durae* both in the Turkey and Duck. The low prevalence of malaria parasites is in accordance with such other findings by different workers. Of 110 chickens observed in Anambra, Nigeria, none was infected [15]. Earlier studies showed *Leucocytozoon spp* infected 55 of 163 (34%) examined chickens in Ibadan, Nigeria, but no *Plasmodium spp* was found [16].

Garnham [7] reported a number of *Plasmodium spp* from natural and experimental infections in Africa. *Plasmodium durae* caused clinical disease and mortality in Kenya [15] and according to Barnes [17], *Plasmodium spp* were observed in a survey carried out in turkeys and ducks in Northern Nigeria. This low prevalence of avian malaria parasite could be because most of the birds were under intensive care management system with proper hygienic conditions. The relationship between the distribution of infection and sex did not show any significant difference ($P < 0.05$) when tested with the chi (χ^2) test.

The male and the female had 8 (17.8%) and 10 (17.9%) respectively when looking at the prevalence rate. This could be attributed to the fact that both sexes actively move about in search of food and mate and therefore are equally exposed to dipteran vector. No significant difference was observed in the infection rate between the young and the adult. Both had the same infection rate 9 (8.3%).

Among the individual poultry subjects examined, the highest infection rate of malaria parasite was recorded in the Pigeon (*Columba spp*) with a prevalence rate of 6 (30%) and the lowest

infection rate was observed among the Chicken (*Gallus spp*) 4 (8.2%). This very low infection rate found among the chicken subjects could be due to the resistance acquired by the chicken since most of chicken sampled were local breed. The high rate of infection found among the Pigeon may be due to the nature of the bird. Its ability to fly exposes the pigeon to greater chances of being bitten by these mosquito flies.

A look at the distribution of the malaria parasites infection by location revealed that Obukpa town had a relatively high prevalence rate of 10 (20.8%) as compared with Nsukka 8 (13.1%). The high rate of infection may be due to the socio-economic conditions coupled with the unsanitary conditions prevailing in the town. The environment had numerous breeding grounds.

In the months of July, August and September there was no infection while in the month of October, the prevalence rate was 18 (16.5%). This high prevalence in October is probably because of balanced rainfall and temperature during the month.

In conclusion, since malaria parasites are associated with poor environmental conditions, it is then necessary that poultry farmers should keep their surrounding clean devoid of overgrown vegetations, stagnant water which may serve as breeding grounds for the mosquito vector. The government should on their own part fill the numerous pot holes in the road to avoid increase in the mosquito population.

Since these birds rarely show signs of illness except in cases of high parasitaemia, interruption of the transmission cycle would be effective in controlling the infection rate. However chemotherapy should be applied where necessary to reduce morbidity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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