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Effects of seasonal and site variations on the prevalence of parasites on Black-crowned waxbill (*Estrilda nonnula*) in Dschang and its environs, Cameroon

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ABSTRACT

This study was carried out to evaluate the prevalence of ectoparasites and haemoparasites in Black-crowned waxbill (*Estrilda nonnula*) in the town of Dschang and its environs. From June 2011 to March 2012, 52 birds were captured by various techniques (sticky surface, traditional trap and nest capture) at three sites. Examination of feathers showed that 65.4% of these birds carried at least one genus of ectoparasites. A total of five genera were detected with a prevalence and average intensities of (53.8% and 1.6 ± 0.9) for *Menopon* sp, (46.2% and 4.5 ± 2.3) for *Columbicola sp*, (23.1% and 1.9 ± 1.3) for *Liperus sp*, (13.5% and 1.0 ± 0.0) for *Menacanthus sp* and (11.5% and 1.7 ± 0.5) for *Goniocotes sp*. Microscopic examination of thin blood smears revealed that 10 (19.2%) of the sampled birds were infected with at least one genus of haemoparasites with a prevalence of 15.4% for *Plasmodium sp* and/or *Haemoproteus sp* and 7.7% for *Leucocytozoon sp*. Capture sites did not influence the parasitic load. Rainy season favoured infestation with ectoparasites. Males were more infested with ectoparasites than females but not for haemoparasites. Prevalence of haemoparasites varied significantly with age and weight groups. Close interactions of Black-crowned waxbills with humans can constitute health risks given the presence of parasites observed.

1. INTRODUCTION

Interest in the study of avian parasites has intensified over the past few years. However, the parasites of African birds are not well known although all species of birds are likely to be infested by parasites with a prevalence estimated at 68% [1]. In fact, birds serve as host for most parasites, amongst which are helminths [2]; ectoparasites [3], [4]; protozoa [5]; haemoparasites [6] and microorganisms which cause various diseases such as avian malaria, ornithosis or psittacosis and bird flu [7]. Birds have the particularity of being able to fly over long distances. As such, in just a few weeks, billions of birds migrate every year from one continent to another to their wintering sites or breeding sites depending on the season [8]. During these movements, they carry with them a whole lot of pathogens that can lead to the spread of diseases in areas which hitherto were not affected. It is for this reason that the medical and veterinary world today has revamped interest on diseases associated with wild birds, much more so for pet/caged species amongst which is the Black-crowned waxbill (*Estrilda nonnula*). This study thus seeks to contribute to the knowledge of parasites of Black-crowned waxbill (which is locally caught as cage birds) and to assess its epidemiological factors in relation to public health. The main objective of the study was thus to evaluate the prevalence of infestation of ectoparasites and infection with haemoparasites in Black-crowned waxbill *i*n the town of Dschang and its environs. More specifically, it was aimed at making an inventory of ectoparasites and haemoparasites of this bird within the Dschang metropolis and to assess the influence of some environmental and inherent factors specific to the birds on the prevalence of parasites.

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2. MATERIAL AND METHODS

2.1 Study area

This study was carried out in Dschang (5°20-7°00 N and 10°30-12°00 E with an average altitude of 1500 m), the headquarter of Menoua Division in the West Region of Cameroon from June 2011 to March 2012. Birds were captured at three sites, two within the Dschang metropolis and one at the outskirt of Dschang. These sites show different urbanization characteristics (with the University Campus and Ngui as urban sites while Tsenfem is rural and about 5km from the town of Dschang). The vegetation within the urban habitat consisted primarily of scattered pine trees and hedge rows with ground level vegetation of mixed grasses. The rural habitat consisted of fruit trees mainly *Psidium guajava* and *Persa americana*, alongside other trees such as *Dracaena Sp*,

Pine trees and conifers as well as small holder plantations of *Eucalytus saligna* and *Grevillea robusta* around the fields and houses. The climate consist of two major seasons: the rainy season starts from mid March to October/November with peak precipitation in August while the dry season runs from December to mid March. Data collected from the Agricultural Research Institute for Development (IRAD) indicated that mean annual temperature ranges from 21.3°C in August to 29°C in March, and relative humidity (RH) ranges between 75–80% within the study area.

2.2 Material and procedures

The birds were captured using several techniques.

2.2.1.1 Use of sticky surfaces

This technique is suitable for the capture of small birds. A metal sheet or plywood approximately 30 cm x 30 cm is smeared with superglue and some rice or corn flour put on it for use as bait. The sheet is placed at the entrance of Black-crown waxbill nests to avoid the capture of non-target birds [9].

2.2.1.2 Use of traditional traps

These traps were made with paste of red earth in a 30 cm x 30 cm tray and nodes were made from the hair of a horse and fixed into the earth. The earth is left to dry and solidify after which rice or corn flour is used as bait and deposited in the nodes which slither to capture the Black-crown waxbill by the leg. The traps are placed in the feeding ground of the bird.

2.2.1.3 Night capture in the nest

This technique involves the identification and location of Black-crown waxbills nests during the day in the study area (with the exception of active nests) and then return in the evening between 18h30-20h to hand capture it.

The captured birds were transported in cages to the laboratory. Prior to sacrificing the bird, the following data were obtained and recorded:

- Sex of birds, which was determined based on their breeding plumage
- Age, using the maturity of beak to divide into adult and young
- Weight, using a Jeulin brand balance with capacity of 200g and sensitivity 0.01
- Site of capture and season during which birds were captured.

Cotton soaked in chloroform was placed in a bowl and the bird placed in it and closed. Five to ten minutes later, the bird was removed, and its feathers examined to take off ectopararasites [10]. Apart from feathers, the skin and the epithelial cells of the legs were also examined. After harvest, these ectoparasites were directly conserved in 70% ethanol in a glass bottle [4]. The specimens were not stained, but mounted on slides directly from the alcohol, and covered with a cover slide. The preparation was mounted on a lens and placed on an electric microscope for identification to the level of the genus using a combination of morphological characteristics [5] and photographs of species already described. Blood samples were taken from veins, wings or legs of live birds. A drop of blood was put at the edge of a slide well cleaned with alcohol. A second slide was applied on top at an angle of 45° such that the blood running at the edge was touched first. The slide was then pulled away along the first slide toward the opposite edges producing a thin film of blood which reduced the thickness and increased the distance of the initial drop [11]. The smears were dried in air. Identification of haemoparasites was based on the morphology, height and the pigmentation of endoerythrocytic parasite [5], [6] and photographs of already described species. The separation of genus *Plasmodium* and *Haemoproteus* was not possible since the smears showed only gametocytes, so the two genera were grouped in one category called "*Plasmodium*/Haemoproteus sp".

2.3 Statistical analysis

Given that the work consisted of assessing the prevalence of parasites with respect to age, sex, weight, season, location of the parasites and the site of capture, the Chi-square test at threshold probability of 5% was applied and the intimacy of link between two quantitative variables calculated by the coefficient of correlation using the software SPPS, version 12.8. Prevalence is the ratio of the number of hosts infected by one or more individuals of a parasite species to the number of hosts examined for this species and the average intensity was taken as the ratio of the total number of parasites of a species recorded in a sample to the number of hosts infected with this parasite [12].

3. RESULT AND DISCUSSION

3.1 Ectoparasites

A total of five genera of ectoparasites were collected from 34 of the 52 birds sampled. This gave a prevalence of 65.4% and an overall average intensity of 5.7±2.9 (Table 1). *Menopon sp* had the highest prevalence of 53.8% with most of the individuals collected from the feathers. The highest average intensity was observed for the genus *Columbicola sp* (4.5±2.3) (Table 1).

Table. 1: Prevalence and average intensity by genus of ectoparasite on Black-crown waxbill in Dschang and its environs (I= Number of infected individuals, E= Number of individual examined).

Ectoparasite	I/E	Prevalence (%)	Average Intensity
Menopon sp	28/52	53.8	1.6 ± 0.9
Menacanthus sp	7/52	13.5	1.0 ± 0.0
Columbicola sp	24/52	46.2	4.5 ± 2.3
Liperus sp	12/52	23.1	1.9 ± 1.8
Goniocotes sp	6/52	11.5	1.7 ± 0.5
Total	34/52	65.5	5.7 ± 2.9

The total prevalence of 65.4 % of ectoparasites infestation encountered on Black-crowned waxbill in Dschang is high compared with result of birds obtained in Germany [4]. They obtained 11.4% as prevalence of mites on birds of several species with different modes of life (solitary and colonies). This should be

obvious as the tropics are noted for high species richness and abundance. However our prevalence is lower when compared with the works *on* poultry birds in Nairobi [13], and on chickens of Zimbabwe [14] both of which had similar results of 95.8%. Poultry birds are usually confined and thus more prone to the spread of parasites compared to the wild Black-crowned waxbills in our study.

Younger Black-crowned waxbills carry significantly higher prevalence of ectoparasites than the adults (Table 2). Contrary to the prevalence of ectoparasites on Village weavers which did not vary with respect to age in the town of Dschang [15], young Black-crowned waxbills were significantly more infested than adults. This observation could be due to the fact that after hatching, the small birds spend much time in the nest, which favours the exchange of parasites with their milieu of life and with their parents. Moreover they have not yet acquired habits of grooming against ectoparasites [16].

Table. 2: Prevalence of ectoparasites with respect to age, sex, weight and location of parasite on Black-crown waxbill in Dschang and its environs.

Variables	Groups	N	Prevalence of ectoparasites (%)	Chi Square	Value of p	<0,05 = *
Age	Young	20	80	2.87	0.047	*
	Adult	32	56.4			
Sex	Male	21	74.6	7.32	0.006	*
	Female	31	59.4			
Weight	[5-7[(g)	32	74.6	44.33	0.062	NS
	[7-10[(g)	20	55			
Location of parasite	Wing	52	63.5	9.86	0.032	*
	Tail	52	13.5			
	Head	52	34.6			
	Chest	52	11.5			

NS = non significant * = significant N= number examined

The wings and the head are more infested (Table 2) and harbour all the genera of ectoparasites found in the Black-crowned waxbills. Wings and head are sites of predilection of parasites in birds [14]. Black-crowned waxbills are not excluded in this observation. A greater proportion of ectoparasites were found on these parts of the body. This can be explained by the fact that the wings are covered by many feathers which serve as hideouts for ectoparasites, making grooming difficult and painful for the bird. On the head grooming is difficult if not impossible. Males tend to be infested by all the genera of ectoparasites and also have a significantly higher parasitic load than the females (Table 2). This is unexpected as the birds live as couples thus both sexes are expected to be exposed to the same risks. This result is thus contrary to that for free range chickens in Kenya [14]. Black-crowned waxbills of smaller weight [5-7[(g), had a relatively high infestation of ectoparasites but the difference was not significant (Table 2) meaning that infestation by ectoparasites is irrespective of the weight of the bird. This observation was also reported for chickens in Ibadan [17]. However, contrary results were observed with Swallows in the Town of Dschang [18] which could be attributed to the fact that birds of smaller weight are generally younger and still lack the ability to groom. Prevalence of ectoparasite varied significantly with season, with Black-crowned waxbills harbouring more parasites during the raining season (Table 3). Capture sites in general did not influence the parasitic load ($p > 0.05$) (Table 3). In the rainy season, the birds presented a relatively higher infestation of ectoparasites. This observation could be due to the fact that this period corresponds to the nesting period of the Black–crowned waxbill; they live in groups of great numbers as couples which favours the survival, transmission and proliferation of ectoparasites between them [19]. Between the sites, the differences in the prevalence were not significant which perhaps is due to the fact that the three sites were not geographically isolated from each other though site selection was based on urban and rural areas.

Table. 3: Prevalence of ectoparasites on Black-crown waxbill with respect to site and season in Dschang and its environs

Variables	Groups	N	Prevalence of ectoparasites (%)	Chi Square	Value of p	<0.05 = *
Site	Campus	20	60	30.82	0.720	NS
	Ngui	17	76.5			
	Tsenfen	15	60			
Season	Dry	23	60.8	4.6	0.028	*
	Wet	29	69.1			

NS = non significant * = significant

3.2 Haemoparasites

As a whole, microscopic examination of blood smears revealed that 10 birds on 52 (19.2%) carried at least one genus of haemoparasites. *Plasmodium*/*Haemoproteus* sp had a prevalence of 15.4% while that of *Leucocytozoon* sp was 7.7%. Pictures 1, 2 and 3 represent respectively the gametocytes of *Plasmodium*/*Haemoproteus* sp, the gametocytes of *Leucocytozoon* sp and schizonts of *Plasmodium* sp in a blood smear.

Three genera of haemoparasites were identified after a blood smear examination giving a prevalence of 19.2%. This prevalence is relatively high compared to the 15.8% obtained with birds of the Order Passeriformes from the Atlantic forest of Brazil [20]. However, they are comparable to those observed in birds from the equatorial region of West Africa where only 3 countries (Cameroon, Equatorial Guinea and Ivory Coast) were involved [21] but lower than that observed for birds in Burkina Faso (46.5%) [22], and for Village weavers from the town of Dschang (66.1%) [15]. The prevalence of *Leucocytozoon* sp. obtained in Black-crowned waxbill (7.7%) is concurrent to that of birds from the equatorial region of West Africa earlier reported [21]. This low prevalence could be linked to unfavourable climatic conditions for the vector transmission of this parasite in West Cameroon [23].

Prevalence of infection with haemoparasites in adults was significantly higher ($P < 0.05$) (Table 4). Adults are more frequently infected with haemoparasites (31.3%) against 10% with respect to the young. Similar observations were reported with wild birds of Seychelles [24]. This result could be explained by the frequent displacement of adults, thereby exposing them to transmission and eventual high infection. Moreover, most of the adults generally sleep in nests on conifers with dense foliage and near water courses where there are many arthropods, some of which are the heamoparasite vectors while most of the young sleep in trees at times far away from their natural habitat [19]. Although

more females than males were infected by *Plasmodium*/ *Haemoproteus* sp, with the reverse for *Leucocytozoon* sp the difference was not significant ($p > 0.05$). The infection frequencies between male and female are similar; this could be due to the habit of the species living a gregarious life style. In fact, these birds live and fly together in the same type of habitats. In literature, few articles suggest the relationship between sex and prevalence of haemoparasites. Even though the existence of many factors notably in some species of wild birds e.g. *Carduelis chloris* has been contemplated with more mortality observed in males [25], [26].

Table. 4: Prevalence of Haemoparasites with respect to age, sex, and weight in Black-crown waxbill in Dschang and its environs.

	Plasmodium sp/Haemoproteus sp			*Leucocytozoon sp*		
	I/E	**Prev(%)**	**p**	**I/E**	**Prev(%)**	**p**
Age						
Adult	7/32	21.9	0.006	3/32	9.4	0.0047
Young	1/20	5		1/20	5.0	
Sex						
Male	1/21	4.8	NS	2/21	6.5	NS
Female	7/31	22.6		2/31	3.2	
Weight Class (g)						
[5-7[	3/32	9.4	0.012	3/32	9.3	0.002
[7-10[	5/10	25		1/20	5	

I= Number of infected individuals E= Number of individual examined
P= value of p NS = Non significant Prev = Prevalence

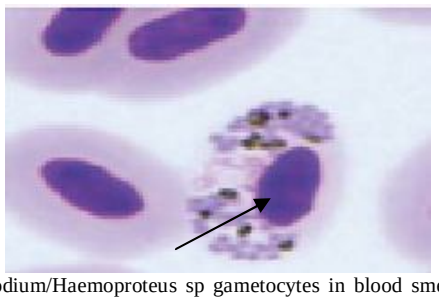
The heavier birds [7-10[(g) were more infected by *Plasmodium*/*Haemoproteus* sp than those of class [5-7[(g) which were rather more infected by *Leucocytozoon* sp (Table 4). The difference in prevalence between the weight groups was significant at threshold probability of 5%. Prevalence of haemoparasites in birds of heavy weight [7-10[g was higher in comparison with that of birds with low weight [5-7[g. This observation is unexpected, and could be linked to the fact that this class of birds contained many more adults (89.2%); the prevalence was found to be higher because of their increased travels and overexposure to transmission and possible higher infections. These observations do not allow us to know if the carrier agent preferentially attacks a host of more weight or rather if the parasite induces weight loss by its pathogenic action. In general the seasons and capture sites did not influence the parasitic load ($p > 0.05$) for *Plasmodium*/*Haemoproteus* sp but did for *Leucocytozoon* sp ($P < 0.05$) (Table 5).

Table. 5: Prevalence of Haemoparasite in Black-crown waxbill with respect to seasons and sites of capture in Dschang and its environs.

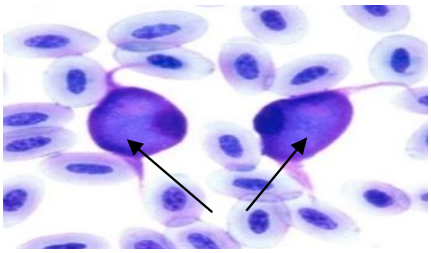
	Plasmodium sp/Haemoproteus sp			*Leucocytozoon sp*		
	I/E	Prev(%)	p	I/E	Prev(%)	p
Season						
Wet	5/29	17.2	NS	3/29	10.3	0.025
Dry	3/23	13.1		1/23	4.3	
Capture Site						
Campus C	3/20	15.0	NS	0/20	0.0	0.04
Ngui	3/17	17.6		3/17	17.6	
Tsenfem	2/15	13.3		1/15	6.7	
Total	**8/52**	**15.4**		**4/52**	**7.7**	

I= Number of infected individuals E= Number of individual examined P= value of p
NS = Non significant Prev = Prevalence

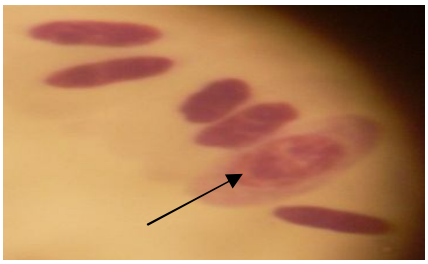
The significant difference for *Leucocytozoon* sp with season is logical but it is not entirely clear why no significant difference was observed for *Plasmodium*/*Haemoproteus* sp as this would seem illogical because during the wet season, birds nest in large numbers in wet conifers favourable for survival and transmission of parasites, their infectious agents and the proliferation of vector arthropods. This influence of season had been previously observed by [27]. Also the higher prevalence of *Leucocytozoon* sp in the rural area can be linked to the fact that, there are many conifers in the site; these trees with persistent bushy leaves are sites of preference for Black-crowned waxbill but also of arthropod vectors concerned with the transmission of haemoparasites which benefit from the humidity offered by the leaves of these trees.



Pic. 1: Plasmodium/Haemoproteus sp gametocytes in blood smear of Black-crowned waxbill.



Pic. 2: *Leucocytozoon* sp gametocyte in blood smear of Black-crowned waxbill.



Pic. 3: *Plasmodium* sp schizont in blood smear of Black-crowned waxbill.

4. CONCLUSION

It is evident from this study that Black-crowned waxbills in the town of Dschang and its environs harbour both ectoparasites and blood parasites. Consequently, close interaction of the Black-crowned waxbill with human beings can constitute health risks given the considerable number of parasites observed. Also, an environmental variable such as season influences the parasite prevalence with an increase in the rainy season while a fairly uniform distribution of parasites between capture sites implies that the risk of zoonosis is greater during the rainy season irrespective of where contact is established with the birds. It was observed that bird's own variables (age, sex, weight and location of parasites), also influenced the distribution of parasite prevalence in the sample population.

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