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A Survey on the Ectoparasites and Haemoparasites of Bats Trapped in Ogbunike Caves, Anambra State, Nigeria

O. P. Okeke[1*], J. J. Okeke[1], E. V. Orjiagu[1], B. U. Ononye[1], N. Udeh[1], I. M. Ike[1] and C. A. Imakwu[2]

[1]Department of Zoology, Nnamdi Azikiwe University, Awka, Nigeria.
[2]Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors OPO and JJO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BUO and EVO managed the analyses of the study. Authors NU, IMI and CAI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The survey of ectoparasites and haemoparasites of bats was carried out with 41 samples from Ogbunike Caves in Anambra State, Nigeria. The removal of Ectoparasites from the body of bats was carried out by brushing of the fur from the different region into a clean white calico material. To avoid damage to the morphology, the parasites brushed out were collected from the white calico material for examination using a pair of blunt forceps. The ectoparasites identified were *Cimocidae* (Bat bugs), *Nycteribiidae* (Bat flies), *Ixoodes verertiliomis* (Bat tick), *Leptotrombidium* spp. (Chigger mite larva) and Macronyssus *spp.* The haemoparasites were identified in the laboratory through parasitological diagnosis using Giemsa stained thin and thick films. The blood samples were subjected to a saline wet mount as well as microhaemocrit concentration technique. The haemoparasites identified were *Plasmodium* and *Trypanosoma* species. Statistical analysis using

**Corresponding author: E-mail: obyjesus2014@gmail.com;*

chi-square test showed a significant difference ($P < 0.05$) in prevalence of ectoparasite and haemoparasites between male and female bats. It is recommended that Cave visitors and tourists should cover themselves properly before entering the Caves.

Keywords: Ectoparasites; Haemoparasites; Bats; Ogbunike Caves; Nigeria.

1. INTRODUCTION

Ectoparasites associated with bats includes but not limited to Siphonaptera (fleas), Diptera (flies), Acaris (ticks and mites) and Hemiptera (bugs) [1]. The most studied of the bat parasites are the bat flies – Diptera: Streblidae and Nycteribiidae [2]. Apart from sucking the blood of their host in a parasite host relationship, ectoparasites are known to transmit haemoparasites and other disease-causing agents like *Trypanosoma* spp. and *Bartonella* spp. to their host [3]. Bats are also known to harbour a number of blood parasites such as *Bartonella*, Piroplasms (Babesia), Trypanosomes and Microfilaria. They also host a group of haemosorina (Apicomplexa) like *Plasmodium* spp., *Hepatocystis* spp., *Polychromophilus* spp and *Nyciteria* spp. [4].

Bats are said to be carriers of zoonotic disease agents such as the Ebola virus, Nipah virus [5], and Hendra virus [6] which are harmful to other animals, especially humans. Despite the multiple pathogens (virus, bacteria, fungi and protozoa) that Bats host, they rarely show clinical manifestation of diseases [7] because they are believed to have evolved to keep most pathogens in check [8,9].

Bats provide humans with some benefits at the cost of some threats. They provide important ecosystem services such as insect control, pollination and seed dispersal [10]. Bats dung has been mined as guano from caves and as fertilizers; they are sometimes numerous enough to serve as tourist attractions (nps.gov/subject/bats/benefits-of-bats.htm) and are used as food across Asia, Africa and the Pacific. The survey of ectoparasites and haemoparasites of bats was carried out with 41 samples from Ogbunike Cave in Anambra State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out in Ogbunike caves in Oyi Local Government Area of Anambra State, Nigeria. It falls within the geographical coordinates of $6^{o}\,10^{1}\,0$ North, $6^{o}\,54^{1}\,0$ East and it is situated in a valley with tropical rain forest behind the Ogba' hills in which lies St, Monica's College, Ogbunike.

2.2 Study Animals

The study was carried out with 41 bats captured in Ogbunike Caves, Anambra State, Nigeria. The bats were collected with a locally made broom with fresh palm fronds and perforated bags. Inside the Caves, the bats were gently swept into the bags with the brooms as they stayed in large families which made collection easier.

Note that fresh palm fronds were used in making the broom to avoid injuring and aggravating the bats. After removal of ectoparasite and bleeding, the bats were immediately released at the place of capture.

2.3 Survey of Ectoparasites

The ectoparasite study was done using hand brush, white calico material, hand lens, microscope, a pair of blunt forceps, torch light to illuminate and improve vision, specimen bottles and 70% alcohol for preservation of parasites. Each Bat's fur, ear, face, wing and tail membranes were visually examined for the presence of ectoparasites which were extracted with the blunt forceps. The fur was brushed onto the white calico to disrupt ectoparasites that may have hidden; this is to confirm that no parasites were present as in Okeke et al. [11]. With the aid of the hand lens and a pair of blunt forceps the parasites were sorted and kept in a labeled plastic vial half filled with 70% alcohol for preservation.

2.4 Haemoparasitological Examination

Blood samples were collected by pricking the cephalic vein (a prominent vein that runs along the uppermost part of the propatagium) with a sterile 5ml disposable syringe and stored in EDTA bottles and taken to the laboratory for diagnosis. The blood samples not processed

immediately were stored at a temperature of 20^0 C until used as in Okeke et al. [11] Microscopic examination of blood samples were done through saline wet mount technique, Giemsa stained thin and thick films, Delafield's' haemotoxylin stained thick films and microhaemocrit concentration method. The procedures for the above techniques were strictly adhered to in line with W.H.O [12]. The mounting, making of films, concentration of blood and principle of microscopy for each was done using a Nikon electron microscope as regards oil immersion and specified objective lens. The identification of the haemoparasites were done using W.H.O [13] Standard Operating Procedure (SOP) and manual on microscopy examination of thick and thin blood film for identification of parasites in red blood cells.

The results obtained from the ectoparasite examination were subjected to statistical analysis using Chi – square test to determine the differences in the prevalence of the haemoparasites and ectoparasites obtained.

3. RESULTS AND DISCUSSION

3.1 Results

The result of the survey revealed that ectoparasites were found on five (5) species of Bats. *Nycteris javanica* had the highest ectoparasite infestation with a prevalence of (30.77%), followed by *Nycteris tragata* (25.64%), *Rhinolophus acuminatus* (20.51%), *Rhinolophus fumigatus* (12.82%) and *Rhinolophus ziama* had the lowest infestation of (10.26%). However, there was no significant ($p>0.05$) difference in the prevalence of ectoparasite between the various species of Bats examined ($\chi^2 = 4.055$, df = 4, P = 0.39) (Table 1).

Similarly the result of the study revealed that eight (8) bats from five (5) species were infected with haemoparasites (19.51%) identified as *Plasmodium spp. Nycteris tragata* had the highest haemoparasites infection (37.50%) followed by *Nycteris javanica and Rhinolophus acuminatus* (25% respectively) while Rhinolophus *ziama had* no haemoparasites infection. Gametocyte of *Nycteris spp.* was found in *Nycteris tragata.* There was no significant difference in prevalence of haemoparasites infection between the various spices of examined bats ($\chi^2 = 2.068$, df = 4, P = 0.72) (Table 1).

Table 2 shows the prevalence of ectoparasites of bats based on sex. The females had more ectoparasites infection with prevalence of 63.00% while the males had the lowest (37 %) There was significant ($p < 0.05$) difference in the prevalence of ectoparasites between male and female bats examined.

Also, the prevalence of haemoparasites of bats based on sex shows that females had more parasitaemia (62.50%) then the males (37.50%) and the differences were not significant ($p>0.05$).

Table 1. Prevalence of ectoparasite and haemoparasites of bats with species

Species of Bats	Number examined	Number infected (%)	
		Ectoparasite	Haemoparasites
Nycteris javanica	14	12 (30.77)	2 (25.00)
Nycteris tragata	10	10 (25.64)	3 (37.50)
Rhinolophus fumigatus	5	5 (12. 82)	1 (12.50)
Rhinolophus ziama	4	4 (10.26)	0 (0.00)
Rhinolophus acuminatus	8	8 (20.51)	2 (25.00)
Total	41	39 (100)	8 (100)

Figures in parentheses = %

Table 2. Prevalence of ectoparasites and haemoparasites of bates based on sex

Sex	Number examined	Number infected	
		Ectoparasites	Haemoparasites
Male	16	10 (37.00%)	3 (37.50)
Female	17	17 (63.00%)	5 (62.50)
Total	33	27 (100%)	8 (100%)

Figures in parentheses = %

3.2 Discussion

Ectoparasites were found in all the five (5) species of the 41 bats examined, this in agreement with Whitaker and Ritzi [14]. They reported that ectoparasites are present on almost all species of mammals, including bats. The ectoparasite identified were mostly Dipterans belonging to the families Cimicidae (Bat bugs) and Nycteribiidae (Bat flies). The long-legged bat tick (Ixodes verspertilionis and Blyboroughtic (Agers verspertilionis) were seen. Chigger mite larvae (leptotrombidium spp.) were seen in the ears, face and forearms while macronyssid mites of *Macronyssus spp*. were found on the wing membranes. This location of the ectoparasites collected is in accordance with Davids Dodd's Brief Guide to Bats Ectoparasite [15].

Haemoparasites were all trophozoites of *Plasmodium ssp.* and they were found in all the species of the female bats expect in *Rhinolophus fumigatus* and *Rhinolophus ziama.* In the males, only *Nycteris tragata* and *Rhinolophus acuminatus* were infected with *Plasmodium* spp. Also, the microscopy result showed that some bats had less volume of blood and scanty red blood cells while very few had full red blood cells. Erythrocytes in bats are smaller than those of other mammals and their blood contains higher red blood cells than humans. The blood volume of bats is not especially large, it ranges from 7 t0 10ml/100g body weight therefore, for animal such as bat that needs to minimize its body weight it would not be practical to increase oxygen – carrying capacity simply by increasing blood volume [16].

Blood clumps were discovered from four bats, consequences of this are that at certain concentrations, a viral suspension may bind together (agglutinate) the red blood cells thus preventing them from settling out of suspension. This is in agreement with Virology Blog [17]. Eosinophil was also seen in the red blood cells of male *Rhinolophus acuminatus.*

4. CONCLUSION

The result of this study shows that bats play an important role in the epidemiology of certain zoonotic diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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