



Research Article

Tick Infestation of Pangolin (*Phataginus tricuspis*) in Omo Forest Reserve Ogun State Nigeria

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Abstract

Seven pangolins (*Phataginus tricuspis*) were trapped using active tracking at nights (from 21:00-03:00 hrs.) and straight line transects (1.8 km) method on foot with spotlights. Forceps were used to randomly remove adult ticks from the skin of each pangolin. The tick samples were preserved with 5% formalin in 25 ml capacity universal tubes. Blood samples were collected into EDTA sample bottles for hematology through caudal venipuncture. The study identified *Aponomma* sp. of ticks from the samples collected. The mean values of PCV, Hb, RBC, WBC and Total Protein were 36.33 ± 10.00 , 12.17 ± 3.32 , 5.82 ± 1.68 , 46.33 ± 13.31 and 8.14 ± 0.17 respectively, while the mean values of the morphometric parameters of the sampled pangolin species were 1.47 ± 0.65 , 76.71 ± 14.86 and 48.86 ± 12.80 for body weight (kg), total length (cm) and tail length (cm) respectively. The results showed that pangolins in the Omo Forest Reserve were infested with the tick species *Aponomma* sp., however, hematological values obtained were within the normal range for healthy pangolins.

Keywords: Pangolin; Ticks; Hematology; Omo forest reserve

Introduction

Pangolins are the only known mammals with large protective keratin scales covering their skin [1,2]. Over the past decade, about one million pangolins are believed to have been illegally trafficked, making it the most trafficked animal in the world [3]. They are listed as endangered on the International Union for Conservation of Nature [4] Red List of threatened species.

Pangolins which are primarily nocturnal mammals are also referred to as scaly anteaters of the order Pholidota, and the genus *Manis*. The scales cover the dorsal and lateral surface forming an overlapping hard plate of armor [5] which afford them protection from most predators and renders them easily distinguishable from other mammals. When threatened, a pangolin rolls into a tight ball with its vulnerable head and soft underbelly covered by the broad, muscular tail, thus presenting the attacker with a nearly impenetrable barrier of armor [6]. Of the eight species of pangolins, four are native to Africa, three of which are found

in West Africa: African White-bellied Pangolin or tree pangolin *Phataginus tricuspis*, long-tailed pangolin *Uromanis tetradactyla* and giant ground pangolin *Smutsia gigantea*. The fourth African species, Temminck's ground pangolin *Smutsia temminckii* is found in north, east and southern Africa [7]. Though performing similar ecological functions as the armadillos and anteaters of the order Xenarthra, they are taxonomically distinct [8]. These animals find refuge in burrows, feeding only on termites and ants [9]. All pangolin species are listed on Appendix I of Convention for the International Trade in Endangered Species (CITES) [10] while all four species are listed in class B of the 1968 African Convention on Nature and Natural Resources [11]. The African White-bellied pangolin which is native (not endemic) to the Omo Forest Reserve is not just endangered but facing continuing decline [12].

Ticks are blood-sucking arthropods found in virtually all terrestrial regions of the planet. Globally, there are approximately 900 species, comprising of the Ixodid (hard ticks) and the Argasid (soft ticks). Approximately 200 Ixodid and 40 Argasid species are present in the Afro-tropical region but only a small number

are of veterinary and medical importance [13]. Many of the ticks and tick-borne diseases occur usually in specific geographical areas but with globalization and Climate Change, their range may assume international proportions [13]. Ticks are among the most important vectors of human and animal diseases caused by protozoa, rickettsiae, bacteria, viruses and helminthes of vertebrates. They rank second only to mosquitoes as vectors of life threatening or debilitating human and animal diseases while they are also known to transmit a greater variety of infectious agents than any other arthropod group [14]. They are also important as pests and parasites affecting humans, livestock and wildlife [13].

Sound knowledge of the parasites of pangolins is crucial for an evaluation of their health status. Owing to the dearth of such information on the pangolins in Nigeria, this work identified the tick species isolated from the trapped pangolin species (*Phataginus tricuspis*) sampled in Omo Forest Reserve with their associated hematological profile.

Methodology

Study Area

This work was conducted at the Omo Forest Reserve, located north of Sunmoge, between latitudes 6° 35' to 7° 05' N and 4° 19' to 4° 40' E in the Ijebu area of Ogun State, South-Western Nigeria. The reserve covers an area of roughly 130,500 hectares. It was legally constituted as a forest reserve as part of a bigger Shasha Forest Reserve [15]. Shasha was later split into Omo, Oluwa and Shasha Forest Reserves. The terrain of the Omo Forest Reserve is undulating and the maximum elevation of 150 m above sea level is towards the west while the lowest parts of the reserve are in the south where the River Omo joins River Oni before flowing into the Lekki Peninsular on the Atlantic coast. The mean annual rainfall ranges from 1600 to 2000 mm with two annual peaks in June and September, while November and February being the driest months [16]. Some of the abundant plant species in the forest on ferruginous soils include *Hunteria umbellata*, *Lannea welwitschii*, *Terminalia superba* and *Triplochiton scleroxylon*. *Bridelia atroviridis*, *Celtis milbraedii*, *Discoglypsemna caloneura*, *Erythrophloeum ivorense*, *Khaya ivorensis*, *Mitragyna ciliata*, *Pausynstalia macroceras*, *P. talbotii*, *Scottellia coriacea* and they are characteristic of the wet sub- group [17]. The Omo Forest Reserve also serves as a refuge

for animals some of which are endemic and endangered such as Forest Elephants (*Loxodonta cycotis*), Yellow-Backed Duiker (*Cephalophus silvicultor*), Chimpanzee (*Pan troglodytes elliotis*), White-throated Monkey (*Cercopithecus erythrogaster*), and Black Duiker (*Cephalophus niger*). Some reptiles like the African dwarf crocodile (*Osteolaemus tetraspis*); Nile crocodile (*Crocodylus niloticus*), Monitor lizard (*Varanus niloticus*), rock python (*Python sebae*) and African black-necked spitting cobra (*Naja nigricollis*) have also been sighted.

Sampling Procedure, Sample Collection and Laboratory Procedure

Samples were collected from seven pangolin all of the same species (*Phataginus tricuspis*). Pangolin specimens were found during active tracking at nights (from 21:00-03:00 hrs.) using straight line transects (1.8 km) method on foot with spotlights. Five (5) to eight (8) adult ticks were removed from each of the host skins using a medium size with blunt points and serrated inner-surfaced steel forceps whilst retaining their good condition for identification. The forceps were used to grip the tick firmly over its scutum and mouth parts as closely to the host skin as possible and then pulled strongly and directly out from the skin of the pangolin. Tick samples were collected in a 25 ml capacity glass tubes with thick walls and metal screw caps (Universal tubes); some were preserved in 5% formalin and identified to species level using entomology field guides [13,18]. Seven of the ticks (one from each sampled pangolin) were dissected under a microscope to confirm and identify if they have any protozoan parasite in their gut. Body sites where the ticks were collected were noted and recorded. GPS location of individual sampled pangolins (Figure 1) were taken and morphometric parameters taken and recorded. Seven (7) ml of blood was collected by caudal veni-puncture using 23-G 1 inch sterile needle and 10 ml syringe by mild physical restrain of the animal. Then 4 ml of blood from each individual pangolin sample was collected into sampling vials containing Ethylene-Diamine-Tetra Acetic Acid (EDTA) for hematology. The blood samples collected were stored in a cold box and taken to the laboratory for analysis. After sampling, animals were released near their capture site. Data collection was conducted for a period of 12 weeks between February 2017 and May 2017 in order to cover a wet and dry season [19].

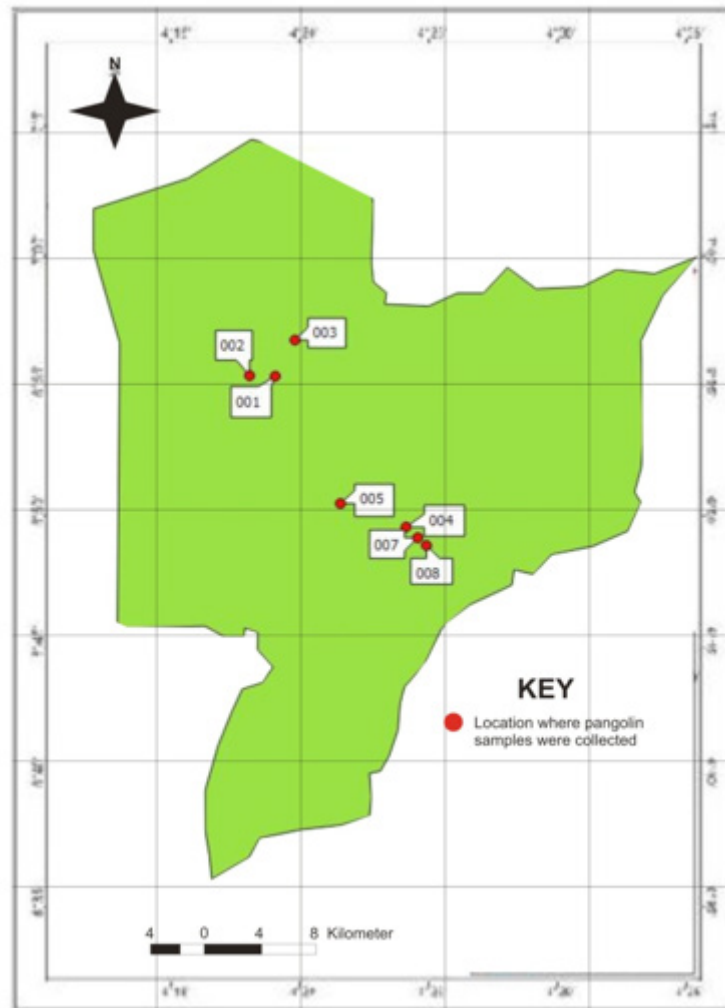


Figure 1: GPS location of pangolin sampled in Omo Forest Reserve.

Statistical Analysis

Data on hematology was subjected to descriptive analysis (expressed in Mean \pm Standard Deviation) while data on morphometric parameters were presented descriptively and subjected to correlation analysis.

Results

Morphometric data of Sampled Pangolins

The pangolins' body weight is directly proportional to tail and body length. Morphological data shows that there is a correlation between body weight as well as body and tail length as shown in Table 1. Only one female pangolin was sampled 007 this could be attributed to the fact that the sampling period coincided with the onset of the rains when most female were either littering or nursing their young in burrows making it impossible for them to leave the burrow.

S/N	GPS coordinates	Pangolin species	Sex	Weight (Kg)	Total length (cm)	Tail length (cm)
001	N6.91828 E4.31842	<i>Phataginus tricuspis</i>	M	0.7	58	34
002	N6.91824 E4.30427	<i>Phataginus tricuspis</i>	M	0.8	56	35
003	N6.94044 E4.35266	<i>Phataginus tricuspis</i>	M	2.3	84	50
004	N6.82485 E.39944	<i>Phataginus tricuspis</i>	M	2.0	95	69
005	N6.83947 E4.35900	<i>Phataginus tricuspis</i>	M	2.0	89	61
007	N6.81827 E4.40653	<i>Phataginus tricuspis</i>	F	1.5	79	48
008	N6.8109 E4.4128	<i>Phataginus tricuspis</i>	M	1.0	76	45

Table 1: Morphometric parameters of sampled pangolin *Manis* species in Omo Forest Reserve, Ogun State, Nigeria.

Identification of Tick Species on the Pangolin Samples

All seven pangolin samples were infested with ticks (total of 37 adult ticks) giving a 100% prevalence of infestation. Upon laboratory examination of the 27 tick species, 18 tick species were males while 9 were females. Ticks were found all over the pangolins sampled implying that they did not have a preferred site for attachment on the animal body. The ticks were identified as *Aponomma sp* (as shown in Figure 2) based on vital parasitological keys, which include presence of ornamental plates, festoons and absence of eyes. Other characteristics of the tick species include long mouthparts, conscutum and scutum ornate or inornate, conscutum circular to laterally oval, presence of a festoon which may be inconspicuous in some species and absence of adanal plates on males (Table 2).

S/N	No. of adult ticks collected	Tick identification			Age		<i>Aponomma</i> sex ratio	
		<i>Amblyomma</i>	<i>Hyalomma</i>	<i>Aponomma</i>		M	F	
001	5	-	-	-	-	-	-	
002	5	-	-	-	-	-	-	
003	6	-	-	6	Ad	6	-	
004	8	-	-	8	Ad	3	5	
005	1	-	-	1	Ad	-	1	
007	8	-	-	8	Ad	8	-	
008	4	-	-	4	Ad	1	3	
Total	37			27		18	9	

Note: Ad: Adult

Table 2: Tick samples collected from pangolin (*Phataginus tricuspis*) samples in Omo Forest Reserve, Ogun State, Nigeria.

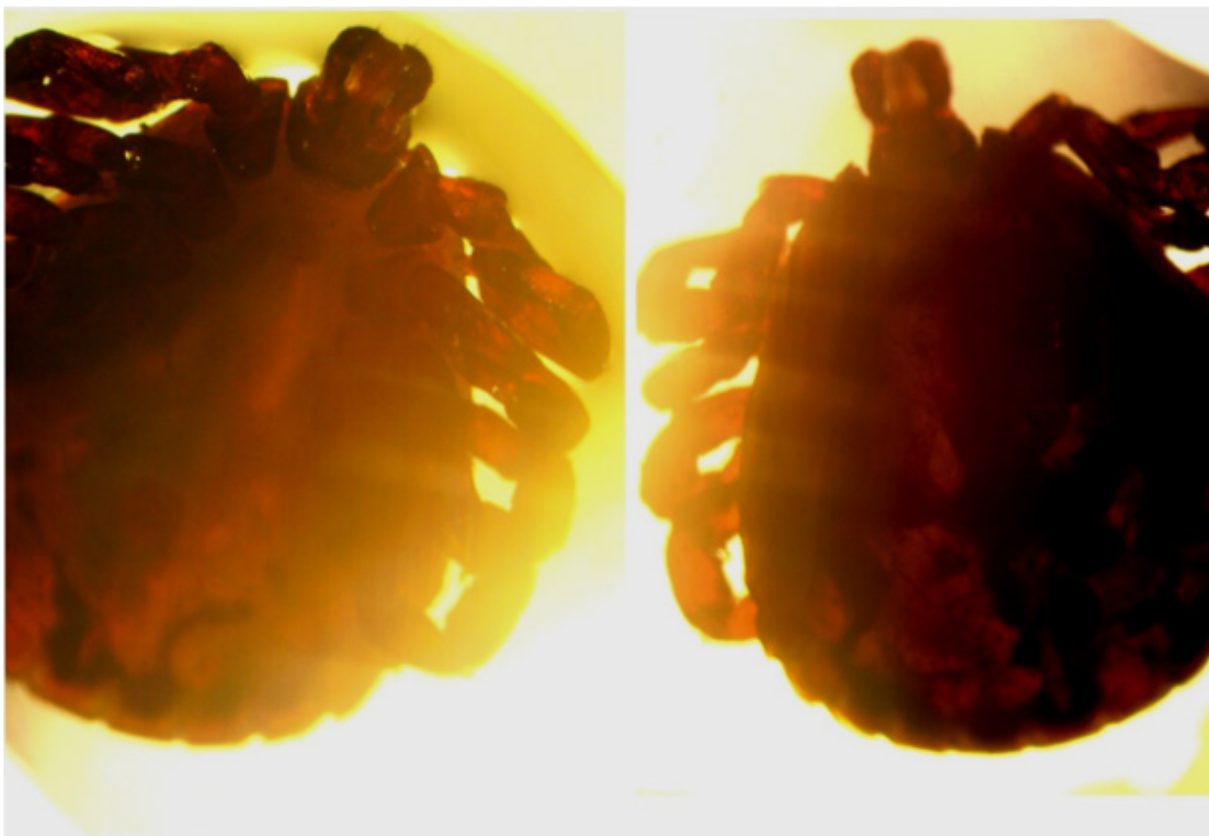


Figure 2: Ventral and Dorsal surface of male *Aponomma* sp. found during the study.

Blood smear showed that the red blood cells of the pangolin are non-nucleated like most mammalian species. Hematocrit values were collected and their Mean \pm Standard deviation recorded in table 3 below.

S. No.	Parameters	003	004	005	007	008	Mean \pm SD
1.	PCV (%)	47	-	-	27	35	36.33 \pm 10.00
2.	HB (g/dl)	15.8	-	-	9.3	11.4	12.17 \pm 3.32
3.	RBC ($10^6/\text{mm}^3$)	7.66	-	-	4.38	5.42	5.82 \pm 1.68
4.	WBC ($10^3/\text{mm}^3$).	5500	-	-	3150	5100	46.33 \pm 13.31
5.	Platelet (10^5)	504000	-	-	430000	240000	3.91 \pm 1.36
6.	Lymphocytes (%)	49	-	-	20	21	30.00 \pm 16.46
7.	Neutrophils (%)	59	-	-	74	68	67.00 \pm 7.55
8.	Monocytes (%)	2	-	-	6	9	5.67 \pm 3.51
9.	Eosinophils (%)	0	-	-	0	2	0.67 \pm 1.15
10.	Total Protein (g/dl)	8.2	8.0	8.1	8.0	8.4	8.14 \pm 0.17
11.	Albumin	3.6	3.4	3.3	3.5	3.6	3.48 \pm 0.13
12.	Globulin	4.6	4.6	4.8	4.5	4.8	4.66 \pm 0.13

13.	A.G. Ratio	0.7	0.7	0.6	0.8	0.8	0.72 ± 0.08
14.	Urea (mg/dl)	12.6	17.5	15.8	13.7	21.2	16.16 ± 3.39
15.	Creatinine (mg/dl)	0.7	0.7	0.6	0.6	0.7	0.66 ± 0.05
16.	Cholesterol (mg/dl)	95	86	77	84	81	84.6 ± 6.73
17.	MCV (fl)	61.36	-	-	61.64	64.58	62.53 ± 1.78
18.	MCHC (%)	33.62	-	-	34.44	32.57	33.54 ± 0.94
19.	MCH (pg)	20.63	-	-	21.23	21.03	20.96 ± 0.31

Note: PCV: Packed Cell Volume; HB: Hemoglobin; RBC: Red blood cell; WBC: White blood cell; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin; MCH: Mean Cell Hemoglobin

Table 3: Result sheet on blood work of pangolin (*Phataginus tricuspis*) in Omo Forest Reserve, Ogun State, Nigeria.

Discussion

Pangolins have been reported to harbor different parasites ranging from protozoan to arthropods [20]. Host-parasite interactions play a vital role in population dynamics. These interactions also ensure healthy ecosystem and in stabilizing food webs [21,22]. A parasite whether ecto or endo may cause mechanical injury including inflammatory or immune responses or simply sap nutrition from the host. Of the various arthropods capable of transmitting pathogens that cause such diseases to humans, ticks, which are vectors of more kinds of pathogens than any other group of invertebrate, have become the “loaders” of pathogen carriers. The tick identified in this study is (*Aponomma sp.*). This finding is not consistent with the finding of Ugiagbe and Awharitoma [23] who reported *Amblyomma sp.* as one of the ectoparasites infesting pangolin species in a rain forest location in Ovia North East Government Area of Edo State, Nigeria. As a result, the findings in this study are new records for pangolin (*Phataginus tricuspis*) in Omo Forest Reserve, Ogun state, Nigeria.

The finding is also contrary to most publications on *Aponomma sp.* where the species is reported to infest reptiles such as Monitor lizard [*Varanus niloticus*] [24], Indian King Cobra [*Ophiophagus hannah*] [25], Rat snake [*Ptyas mucosa*] [26]. Hematological values are among the most commonly used indices in the clinical evaluation of diseases, both for animals maintained in controlled environment and for free ranging animals [27]. Red Blood Cell (RBC) values were obtained for three blood samples due to lysis of the other samples. Hematological values obtained in this study are similar to values reported by Oyewale, et al. who obtained samples from healthy tree pangolins (*Phataginus tricuspis*) but different from values got by Mohapatra, et al. who got sample from a sick Indian pangolin (*Manis crassicaudata*) [28,29].

Conclusion

The natural habitats of pangolins are rapidly deteriorating because of extensive farming, logging, and other anthropogenic activities. In addition, the illegal trading of pangolins has substantially accelerated the decline of the pangolins’ population in Nigeria. This study has been able to prove that pangolin (*Phataginus tricuspis*) in Omo Forest Reserve, Ogun State, Nigeria are infested with ticks and identified these tick species as *Aponomma sp.* It also established the baseline values for the hematological profile of sampled pangolins in Omo Forest Reserve, Nigeria. Hematological values got in the project were similar to values got by Oyewale et al. who got samples from healthy tree pangolins (*Phataginus tricuspis*) but different from values got by Mohapatra, et al., who got sample from a sick Indian pangolin (*Manis crassicaudata*) [28]. Therefore, it can be further concluded that even though the pangolin samples were infested with tick they were healthy animals thus suggesting their adaptation and resistance to such pests [30].

Recommendation

Further studies should be carried out on the tick parasites of pangolins using scanning electron microscopy and molecular analysis to determine their species; this will enhance the differentiation between species and subspecies.

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