ASJ: *International Journal of Health, Safety and Environments (IJHSE)*
Vol. 04 (05) 25 December, 2018, Pp. 308-314
www.academiascholarlyjournal.org/ijhse/index_ijhse.htm
ISSN: 2360-9311©Academia Scholarly Journals
Indexed In: Directory of Research Journals Indexing - http://www.drji.org
Also Available@; Internet Archive Library@Okonkwo and Okaka
Open access

Full Length Research

AN INVESTIGATION INTO PARASITES OF THE AFRICAN GRAND PANGOLINS *Manis temminckii* (AKABO in IGBO LANGAGE) AYAMELUM L.G.A. OF ANAMBRA SOUTH EASTEN NIGERIA

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Accepted December 14, 2018

A preliminary survey was conducted into the parasites of African grand pangolin *Manis temminckii* in Anambra State Nigeria. The pangolins were purchased alive in four locations in Ayamelum Local Government Area (LGA) of Anambra State, where bush meat are sold as a good source of protein and for medicinal purposes to native doctors. Six pangolins were examined for parasite infection and a prevalence of 100% with endo-parasite the cestode (*Metadavainae sp)* and the Nematode (*Parastrongyloide sp*). Overall prevalence of ectoparasite 83.33% was observed with the entire male infected and 1 female out of the 2 examined was not infected with the ectoparasite but had the cestode species and nematode parasite. The overall result of the six pangolins examined showed that the parasite recovered consisted of two species of ixodide hard tick ectoparasits *Amblyomma sp* and *Rhipicephalus sp.* The cestode *Metadavainea sp* (tapeworm) was the most prevalent with an occurrence rate of 37.82% and 22 mean parasite intensity, while the hard tick ixodide *Rhiphicephalus sp* recorded the least occurrence of 1.72% and mean parasite intensity of 1. The second endo-parasite recovered from the 6 pangolins examined was the nematode *Parastrongyloides sp* which recorded 36.39% prevalence and 21.17 mean parasite intensity. The prevalence of ecto-parasite showed that one(1) out of 2 female grand pangolins examined was entirely free from any species of the hard tick recovered in the five others, thereby presenting 15.56% parasite occurrence and 7 mean parasite intensity.

Key Words: Parasite, African grand pangolin, Anambra State, Nigeria.

INTRODUCTION

Pangolins also known as Trenggiling/scaly anteater (Ethnic Igbo Language = Akabo) are placental toothless mammals which belong to the order Pholidota. Pangolins derived from Malaya language meaning “to roll up” describes the defensive strategy employed by the animal whenever they sense danger, they are found throughout the tropical regions of Africa and Asia (Mohapatra et al., 2015). The family Manidae is extant with three general and eight species of Pangolins which include the giant

pangolin, tree pangolin or long-tailed pangolin, Chinese pangolin, Indian parigolins, Malayan Pangolin, Philippine pangolin and the ground pangolins. All pangolins have long claws, ability to curl into a ball and are primarily nocturnal animals (Gaudin, 2009). Four species which are Temminck's ground pangolin (*Manis temminckii*) giant ground pangolin (*Manis gigantea*), *Smutsia gigeantea* or long-tailed pangolin (*Manis tetradactyla*) and tree pangolin (*Phataginus tricuspis)* occur in Africa (Gaudin et al., 2009). Pangolins had been classified as critically endangered species such *Manis javanica* and *Nanis pentadactyla* while *Manis culionensis* and *Manis crassican* data and Africa Pangolins are classified as vulnerable by the International Union for Conservation of Nature(IUCN) (Challender et al., 2014). African tree pangolins and giant grand pangolin *Manis gigantean* are characterized by their eponymous scales terminating into three cups. They weigh between 4.5kg and 14kg and are measured 31cm to 45 cm in length, their average body temperature ranges, from 32.6^0c to 33.6^0c (Rahm,1956). Colorations varies from light to yellowish brown, through olive to dark brown depending on the age and species, however the number of scales remain constant throughout life (Craig, 2012). Breeding in pangolins is continuous and the gestation period is approximately 150 days, after which the female give birth to a single young, which is carried by the female on her tail (Kingdom and Hoffmann, 2013). Pangolins feed exclusively on ants, termites and also soft bodied invertebrates, which included bee larvae, flies, worms, earthworms and crickets (Shi and Wang, 1985). Pangolins harbour a few organisms, both parasitic and mutuality, that inhabitant on and in them. There were reports of six new parasitic species described from pangolins, including two protozoan parasites, *Eimeria tenggilingi* and *Eimeria nkaka* three helminth parasites *Dipetalonema fausi, Leipernema leiperi,* and *Prolospiruru hamospiculala* (Esslinger, 1966; Singh, 1976; Else and Colley, 1976). A total of 34 genera of helminthes, 8 genera of ticks, 2 genera of mites and 7 genera of bacteria were check listed in a review by Mohapatra et al., (2015). Out of eight pangolin species, parasites were reported from seven species and no parasite was reported from the species *Manis culionensis.* The helminthes parasites recorded from pangolins so far, belong to eight genera which viz., *Cylicospirura, Leipernema, Mainstrongyloid, Necator, Strongyloides, Trichochenia, Ancylostma* and *Gendrespirrura* occuring in the intestine and stomach, small intestine and large intestine and three genera including *Dipetalonama, Brugis* and Microfilaria were blood parasites (Naidu and Naidu, 1981; Heath and Vanderlip, 1988). Ticks are also known to parasitize pangolins and they have been found underneath the scales, 8 genera and 20 species have been reported from six pangolin species namely *Manis crassicaudata, Manis pentadactyla, Manis javanica, Manis termminckii/Smutsia temmicki, Smutsia gigantea.* Pangolins have been found in some part of Africa to be of great medicinal value, some parts of the animal such as the scale, the blood, the tail head, claws and whole body are used to prepare charms (Soewu, 2008). Durojaye and Ibukun (2009) reported that pregnant female pangolins are used to treat barrenness, prevent miscarriage, and to remove fibroid tissue, antenatal care and ejection of prolonged pregnancy in women. Pangolins are considered as bush meat delicacy despite being used as traditional medicine purpose (Soewu, 2008).

Tree and grand pangolins are listed as vulnerable under criteria A4d because the species had already beginning to decline and has continued to decline by at least 40% over a 21 years period due to the impact of hunting, poaching and increased demand from the international market (IUCN, 2014).

Lack of adequate information on parasites of African grand pangolins *Manis termminckii/Smutsia temmickii,* pangolins which is always at close interaction with human and also used for both medicinal purpose and source of food especially in Anambra State, again little information available about the biology, ecology, diseases and parasite of pangolin both in captivity as well as wild is very scarce. Therefore, it is necessary to collect more data and carry out more survey research on these animals, so as to promote their conservation knowledge. This study is aimed at investigating the prevalence and intensity of ecto-parasities and gastro-intestinal parasite of African grand pangolin and provides information on the possibility of zoonotic occurrence of the parasite infection between pangolins and human.

MATERIAL AND METHODS

Animals used in this study were purchased alive

between April 2018 and July 2018 from Oye Ifite Ogwari market, Nkwo Omasi and Eke umueje respectively in Ayamelu Local Government of Anambra State. Anambra State lie between longitudes $06^0$00N and D$6^0$20N and latitude 06·33 and D7°00E it is bounded in the west by Delta and Edo States in the South by River, Imo and Abia State in east by Enugu State and in the North by Kogi State. Ninety eight percent of it populace is of Igbo ethnic extraction while 2% belong to the Igala tribe the area consist of tropical rain forest with the temperature ranging between 26°C to 35°C. Animal specimens were transported live to the laboratory for examination. Laboratory equipment used in the experiment include, binocular compound microscope, dissecting microscope, specimen bottles (universal containers), petri-dishes, dissecting set, disposable hand gloves hand lens, slides and cover glasses, synthetics face mask, pipette, reagents and stains, graduated cylinders, digital camera, disinfectant, towels and distilled water. A total of 6 animals was obtained randomly from different hunters is different locations. The sex of the animals was determined by the observation of the external appearance of genital organs and animal graded as male or female. Comprehensive critical physical examination was conducted on various parts of the body beneath the scale of the animal. The ecto-parasites under the scale collected using hand (covered with disposable hand gloves) and a pair of forceps which was dipped in alcohol. The ticks were then transferred to specimen bottles and identified with similar morphological characteristics separated according to species and preserved for further studies. Dissection was done by following the procedures by Rowett (1962), by placing the pangolin on a board with the arms fully extended and securely pinned to the dissecting board, a skin incision was made directly on the ventral midlines and the animal cut open with the abdominal cavity in full view. The lungs heart and the liver were examined *in situ* and then the colour and the presence of necrotic areas, nodules, cyst or abscess were noted. The alimentary canal was stretched out on the dissecting table and separated into regions, which included the esophagus, the stomach, the small intestine, the large intestine and the rectum. The different regions of the alimentary canal were then placed in different pre-labeled petri-dishes containing normal saline, and finally the various parts were then examined for the presence of helminthes parasites.

Identification of parasites

Ticks were identified using, Tick and Domestic Animals in Africa; Guide to identification of species by Walker et al., (2003) recovered nematodes were identified using descriptions key by Smyth (1976). Cestodes specimens in this study were identified with keys to cestode parasites of vertebrates by Khalil et al., (1994). Parasites were measured using a calibrated microscope eye piece. Length, width esophageal length and tail length of the nematodes were recorded accordingly.

Statistical Analysis

Prevalence of parasite was calculated by the number of host species infected with a particular parasites divided by the number of hosts species examined and this was expressed in percentages while the mean intensity of the parasite was calculated by the total number particular parasite specimen divided by the total member of hosts infected with the parasite.

RESULT

The Overall Prevalence of Ecto-parasite is 83.33%

Six adult pangolins four males and two females with their lengths varying between 71.05 to 90.20cms were studied. Overall prevalence of ecto-parasite 83.33% was observed with the entire male infected and 1 female out of the 2 examined was not infected with the ecto-parasite but had the cestode species and nematode parasite. The overall result of the six pangolins examined showed that the parasite recovered consisted of two species of ixodide hard tick ectoparasits *Amblyomma sp* and *Rhipicephalus sp.* The cestode *Metadavainea sp* (tapeworm) was the most prevalent with an occurrence rate of 37.82% and 22 mean parasite intensity, while the hard tick ixodide *Rhiphicephalus* recorded the least occurrence of 1.72% and mean parasite intensity of 1. The second endo-parasite recovered from the 6 pangolins examined was the nematode *Parastrongyloides sp* which recorded 36.39% prevalence and 21.17 mean parasite intensity (Table 1).

The four male pangolin examined had 100% infection rate of endo-parasite *Parastrongyloides sp*

Table 1. Parasites Found and Their Percentage Occurrence.

Species of parasite	Site of infection	Number of parasite Found	Percentage Occurrences[%]	Mean Parasite Intensity
Cestode *Metadavainea SP*	Intestine	132	37.82	22
Nematode *Parastrongylodies SP*	Intestine	127	36.39	21.17
Arthropod[Tick] *Amblyomma SP* *Rhipicephalus SP*		1846	24.67 1.72	14.00 1
TOTAL		349	100%	

Table 2. Prevalence of Endo-parasite with Respect to Host Sex.

S/N	Sex	Number of host Examine	Number of host Infected	Prevalence of infection	Number of Parasite Recorded	Mean Parasite intensity
1	Male	4	4	100%	192(74.13%)	48
2	Female	2	2	100%	67(25.87%)	33.5
3	TOTAL				259	

Table 3. Prevalence of Ecto-parasite with Respect to Host Sex.

S/N	Sex	Number of host Examine	Number of host Infected	Prevalence of infection	Number of Parasite Recorded	Mean Parasite intensity
1	Male	4	4	100%	76(84.44%)	19
2	2	1	1	50%	14(15.56%)	7
3	TOTAL	8	5		90%	

which recorded 36.39% prevalence and 21.17 mean parasite intensity Table 2. *Metadavainea sp* and *parastrongyloide sp* present 74% prevalence and 48 mean parasite intensity, likewise the two female grand pangolins recorded 100% prevalence infection and 25.87% prevalence infection and 25.88% prevalence occurrence and 33.5 mean parasite intensity Table 2. The entire parasites recovered from this study are presented in Table 1, while Table 3 presents the prevalence of the ecto-parasite with respect to host sex. The prevalence of ecto-parasite showed that one(1) out of 2 female grand pangolins examined was entirely free from any species of the hard tick recovered in the five others, thereby presenting 15.56% parasite occurrence and 7 mean parasite intensity. The analysis of the stomach food items and intestine not shown revealed that pangolins feed mostly on arthropods organisms. The food items recovered from the stomach and intestine consisted of ants, insects and termites and broken down arthropod parts.

DISCUSSION

In this study two ecto-parasites and two endo-parasites were found parasitizing the African grand pangolins *Manis termminckii/Smutsia temmickii.*

The occurrence of the endo-parasite (*Parastrongyloides sp* and *Metadaviner sp*) was not unexpected since the animal anteater feeds on

arthropods most of which are known to serve as intermediate host and vectors of parasite (Parola et al., 2013; Orhierhor et al., 2017). In their study Heath and Vanderlip (1988) on, the biology of four adult captive Chinese pangolins *Manis pentadactyla* maintained at the University of California, observed the presence of *Strongyloides sp,* unidentified hookworms and filarial nematodes, and nematodes of the genus *Cylicospirura* in the intestine of the pangolin. The current study observed two different types of helminths namely; the cestode *Metadavainea sp* and nematode *Parastrongyloides sp*, disparity from Heath and Vanderlip(1998) study may be probably environment and parasitic speciation. The entire parasite recorded in the current study may be the first major documented study of parasite of pangolins in Anambra the south eastern Nigeria although similar ecto-parasites have been recorded in some study outside Nigeria. Three genera of ticks *Amblyomma, Rhipicephalus* and *Apolomma* were reported from both Asian and Africa pangolin species, *Amblyomma javanense* was observed in three Asian pangolins. *Manis crassicaudata, Manis pentadactyla* and *Manis javanica.* Similarly *Amblyomma compresum* was also recorded from three African pangolin species, *Manis temminckii, Phataginus tricuspis, Manis gigantea,* apart from pangolins *Amblyomma javanense* ticks had been reported from water-monitor lizard, python, skink, hill turtle, bat, wild boar, hyena, bear and sambar deer (Kollars and Birth prasana, 2000; Hassan et al., 2013). The soft tick *Ornithodorus moubata,* transmits *Borrelia duttoni,* the agent of African relapsing fever (Spirochaetial infection) in human and the tick has also been described to have been found in the ground temminickii pangolin (Jacobson et al., 1991). Occurrences of *Rhipicephelus lussinus* and *A. compressum* have been reported in pangolins from Zambia (Tandon, 1991) also in Congo and Liberia (Mediannikov et al., 2012). *Metadavainea sp* tape worm reported in the current study was the first cestode reported in the current study was the first cestode reported from pangolin so far in the south eastern Nigeria. Similar tape worms have been previously recorded in pangolin from other parts of Africa and the world, example Côte d'Ivoire, Rwanda and Burundi (Baer and Fain, 1955). The nematode, *Parastrongyloides sp* has also been reported as parasite of moles, shrew and some marsupials in Australia (Dorris et al., 2002). In this study both male and females pangolins did not present equal prevalence and females showed equal prevalence of infection (100%) although with slight deviation in the occurrence of ecto-parasite where one female pangolin did not have any ectoparasite, this is consistent to the observations made by Hassan et al., (2013) in the study of 16 Malayan pangolins for ecto-parasites, results showed that there was a significant difference in the pangolins infected according to sex with the males having higher prevalence (88.9%) as compared to females (42.9%). The current study is in line with Hassan et al., (2013). A study in Congo, reported that out of 12 *Amblyomma compressium* tick recovered from 3 pangolins (*Manis gigantea)* tested positive for Rickettsial DNA as determined by genus-specific qPCR. The detection of *R. africae* in *A. compressum* ticks, which has been reported as a highly specialized parasite of pangolins was consistent with previous data which showed the presence of *A. compressum* ticks from Liberia (Mediannikov et al., 2012). Also a study in southeastern Taiwan on critically endangered pangolins' reported that 13 out of the 25 pangolins captured between 2012-2014, were infected with ticks, a total of 21 ticks were collected and 3 species were identified which *Amblyomma sp* was one and four different tick borne pathogens comprising of *Escherichia sp, cythuzooa sp, Rickettia sp* and *Anaplasma sp,* were detected (Khatri-Chhetri et al., 2016). More studies is recommend as the current study contribute great to clear knowledge of the biology of pangolins so as to proffer solution and measures to apply to prevent their possible extinction. Again more research is advised in the molecular biology of the ticks recovered from pangolins to ensure that it constantly interacts with man does not result to the epidemic of typhus fever disease. The finding in this study is evident that African grand pangolins in Anambra are parasitized by varieties of ecto-parasites and endo-parasites of different kinds. A very deterrent enforceable polices should be put in place by the Government and all concerned in other to stop the hunting and killings of pangolins to prevent them from extinction.

ACKNOWLEDGEMENT

We are grateful to Almighty God for his life and protection. The authors are grateful to Mr. Theophilus Nweke and Vincent Ekwunife for their assistance in the collection of the Animal and to Dr.

E Uchenna Nyagu for proof reading manuscript.

REFERENCES

Baer JG and Fain A (1955). Les Cestodes de Pangolins. Extriat du bulletin dela Societe Neuchateloise des Sciences Naturelles. 78:38-52.

Challender DWS, Waterman C and Baillie JEM (2014). Scaling Up Pangolin Conservations. IUCNSSC Pangolin Specialist Group Conservation action plan. Zoological society of London, London.

Craig G (2012). Rolling with the pangolin (Manis temminckii): Interactions with other organisms. The science behind symbiosis. Chinese Medical Journal.29:191-209.

Dorris M, Yiney ME and Blater ML (2002). Molecular Phylogenetic Analysis of the Genus Strongyloides and related nematodes. International Journal Parasitology; 32:1507-1517.

Durojaye AS and Ibuukan AA (2009). Utilisation of Pangolin (Manis Sp) in traditional Youruba medicine in Ijebu province, Ogun state. Nigerian Journal of Ethnobiology and Ethnomedicine; 5:39-41.

Else JC and Colley FC (1976). Eimeria tengitings sp.n. from the Scaly ant- eater Manis javanica in Malaysia. Journal Eukaryot Microbiology; 23 (4): 487-488

Esslinger JH (1966). Dipetalonema fausti sp (Filarioidea: Onchocercidas), a filarial parasite of the scaly anteater, Manis pentadactyla from China Journal of Parasitology; 54(13): 494-497.

Gaudin IJ, Emry RJ and Wible JR (2009). The Phylogeny of living and extinct pangolins (Mamalia; Pholidata) and associated taxa: A morphology Based Analysis. Journal of Mammalian Evolution; 16:235-305.

Hassan M, Hatiz MS and Chong JL (2013). The Prevalence and Intensity of Amblyomma javanese infestation of Malaysian Pangolins Manis javanica from Peninsular Malaysia. Actatropica; 126(2):142-145.

Heath ME and Vanderlip SL (1988) Biology, Husbandry and Veterinary Care of Captive Chinese Pangolins (Manies pentadactyla). Zoo Biology. 7 (4): 293-312

International Union for Conservation of Nature (2014). The IUCN Red list of threatened species. (online) Available at: www.iucnredlist.org. Accessed: 16 March, 2014.

Jacobson NHG, Newbery RE, Dewet MH, Viljoen PC and Pietersen E (1991). A contribution to the ecology of the stepp pangolin Manis temminckii in the Transvaal. International Journal of Mammalian Biology. 56:94-100.

Khalil LF, Jones A and Bray RA (1994). Keys to the Cestode Parasites of Vertebrates. International institute of Parasitology, Cab International. Pp751.

Khatri-chhetri R, His-cheih W, Chen C and Pei K (2016). Surveillance of Ticks and Associated Pathogens in tree-ranging formosan pangolins (Manis pentadactyla pendactyla). Ticks and Ticks Borne Disease. 7(6):1238-1244.

Kingdom J and Hoffmann M (2013). Mammals of Africa: Carnivores, Pangolins, Equidi and Rhinoceroses. Blooms bay Publishing, London. Pp35.

Kollars TM and Birth PR (2000). New Host and Distribution Record of Amblyomma javanensen (Acarina:Ixodidae) in Thailand .Journal Medical Entomology.37(4):640-648.

Mediannikov O, Davoust B, Socolovschi C, Tshilolo L, Roult D and Parola P (2012). Spotted Fever Group Rickettsiae in Ticks and Flea from the Democratic Republic of Congo. Ticks and Tick Borne Disease. 3(56):371-378.

Mohapatra RK, Panda S, Nair MV and Lakshini N.A (2015). Checklist of parasites and bacteria recorded from pangolins Journal of Parasitic Diseases 10:1-7

Naidu KV and Naidu KA (1981). Nematoda: Trichostrogyloidae from Pangolin in South India. Proceeding Indian Academy of Science (Animal science). 90(6): 615 -618.

Orhierhor M, Okaka CE and Okonkwo VO (2017) . A survey of the Parasites of the African White bellied Pangolin Phataginus tricuspis, in Benin City, Edo state, Nigeria. Nigerian Journal of Parasitology. 38 (2):266-270.

Parola P, Paddock CD, Socolouschi E Labruna MB, Mediannikov O, Kernift AMY, Stenos J, Bitam I, Fournier PE and Raouth D (2013). Update on tick bone Rickettioses on tick-bone Rickettsioses around the world: a geographic approach. Clinical Micro biology Review 26(4): 657-702.

Rahm U (1956). Notes on pangolin of the Ivory Coast. Journal of Mammalogy 37 (4):531-537.

Rowett HGD (1962). Guide to dissection. John Murray, London Pp 25.

Shi VQ and Wang YQ (1985). Food habits in Pangolin. Chinese Journal of Wildlife. 6:42-43.

Singh SN (1976). On a new nematode Leipenema

leiperin. sp (Strongyloidae), Parasite in the Pangolin Manis pentadactyla from Hyderabad. India Journal of Helminthology. 50:267-271.

Smyth JD (1976). Introduction to animal parasitology. Hodder and Stonghton. P10 466.

Soewu D (2008). Wild Animals in Ethnozoological Practices Among the Yoruba's of South Western Nigeria and the implications of biodiversity conservations. African Journal of Agricultural Research. 3:421-427.

Tandon SK (1991). The Ixodid tick of Zambia (Acarina: Ixodidae). A Study of Distribution, Ecology and Host Relationship Director. Zoological Survey of India (Editor). Records of Zoological Survey of India occasional paper No 134 Zoological survey of India Calcutta, Pp175P.

Walker AR, Boucittour A, Camicas JL, Estrada-Petka A, Latit AA, Pegram RG and Preston PM (2003). Ticks of domestic Animali in Africa: a Guide to identification of species. Bioscience Reports, Edinburgh scoti and, Uk Pp 227.