



Insights into Parasitic Fauna among Rescued Snake Species: A Study from Tamil Nadu, India

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Authors' contributions

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

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ABSTRACT

The study presents an investigation into parasitic fauna inhabiting various snake species from Tamil Nadu, India, focusing on ectoparasites and endoparasites. Over an 18-month period of study, sixteen snakes comprising Rat snakes, Indian cobra and Green vine snake were meticulously examined. Ectoparasite assessment unveiled ixodid ticks infestation, predominantly featuring *Amblyomma gervaisi*, alongside *Amblyomma pattoni* and *Amblyomma cordiferum*. Among the endoparasites identified, helminthic species included strongyles, *Oxyurid* sp. and Pentastome sp., with instances of mixed infections observed. Protozoal infections were detected through blood and faecal analysis, with the detection of *Haemoproteus* sp., *Hepatozoon* sp., *Cryptosporidium* sp. and *Eimeria* sp. These findings emphasize the necessity for comprehensive monitoring of parasitic infections in snakes, not only for conservation endeavours but also for mitigating potential public health risks associated with zoonotic diseases and biological balance.

Keywords: *Amblyomma*; *Cryptosporidium*; *Eimeria*; *Haemoproteus*; *helminths*; *snakes*.

1. INTRODUCTION

Snakes are elongated, limbless, carnivorous reptiles, grouped under the order Squamata. It is present all over the continent except at Antarctica. There are almost 4000 species of snakes around the world and out of these 600 species are venomous. Snakes act as predator and as well as prey to maintain a balanced ecosystem. Snakes are helpful to farming community as they kill the vermins like rats destroying the crop production and plays a significant role in food web. In addition, the venom of some snakes is used to prepare antivenom to save lives of human and animals [1]. Reptiles are hosts for a wide variety of protozoan and metazoan parasites [2]. Reptiles including snakes act as a intermediary or definitive hosts for many nematodes such as *Angusticaecum* sp, *Porocephalus crotali*, *Capillaria* sp., *Ophidascaris* sp. and as well as for protozoa such as *Eimeria* sp., *Isospora* sp., *Caryospora* sp., *Tyzzeria* sp, [3,4]. They are also affected by external parasites, most probably ticks like *Amblyomma* sp., mites like *Ophionyssus natricis* (Pietzsch et al., [5] and biting flies Zhang et al., [6] and they transmit some haemoproteozoans notably *Hemoproteus* sp., and *Hepatozoon* sp., to snakes. Mostly endo parasites from snakes are identified through necropsies but effective diagnosis can be achieved by detecting parasites from faecal droppings. Even though, the latter method is non - invasive it may be useful for conservation projects [7]. The protozoa *Cryptosporidium* from snake faeces has a zoonotic potential to cause watery diarrhoea in humans with immunodeficiency and in aged persons. A nematode named *Ophidascaris robertsi* is commonly seen in snake was found in human

brain and it was removed successfully [8]. The diagnosis of the parasitic infection should be effectively made to supplement the conservation activities with the elimination of zoonotic diseases. This study deals with parasitic fauna from different species of snakes from Tamil Nadu, India.

2. MATERIALS AND METHODS

2.1 Study Area

Thanjavur (N-10° 47' 13.1964", E-79° 8' 16.1700") and Pudukottai (N-10°25'48.00", E-79°19'12.00") forest divisions of Tamil Nadu, India, constituted the study area for the investigation of parasitic fauna in snakes. These forest divisions, characterized by diverse ecosystems and varying environmental conditions, provide an ideal setting for survival of various reptilian species in both rural and urban areas. The authorities of the Thanjavur and Pudukottai divisions of the Forest departments and non-governmental organization called Endangered Wildlife Environment Trust (EWET) frequently report many rescued snake species to the Veterinary Clinical Complex, Veterinary College and Research Institute in Orathanadu, Tamil Nadu veterinary and Animal Sciences University for treatment and health check-up before releasing them back into the forest. This study involves the rescued snakes from the households and roadsides of the study area.

2.2 Collection of Samples

A total of 16 snakes which included, 11 Rat snakes (*Ptyas mucosa*), 4 Indian cobra (*Naja naja*) and 1 green vine snakes (*Oxybelis fulgidus*) were reported for treatment over a

period of 18 months (August 2022 – January 2024), and were included in the present study. All snakes were carefully restrained and its head, body, and tail (ventral and dorsal sides) were carefully examined for the ticks in between the scales and were collected in 70% ethanol. Droppings and cloacal swab were also collected in 70% ethanol for screening endoparasites. Whole blood from ventral tail vein was collected in a blood vial containing heparin as an anti-coagulant for haematological analysis and screening haemoparasites.

2.3 Processing of Samples

The ticks collected were processed in 10% potassium hydroxide, dehydrated in ascending grades of alcohol, followed by clearing for morphological identification. Concentration methods of faecal examination including sedimentation and floatation (with saturated salt solution, specific gravity 1.18) methods were used for detecting of endoparasites ova and oocysts as described by Soulsby [9]. Faecal droppings smear was subjected to modified Ziehl-Neelsen stain for screening oocysts of *Cryptosporidium* sp. Blood smears were made in

grease free, clean glass slides stained with Giemsa's stain for 40 minutes and screened for blood parasites.

3. RESULTS

3.1 Ectoparasites

Out of 16 snakes examined, 14 snakes showed ixodid ticks belonging to one genera were identified (Tables 1 & 2). Among the tick species identified on snakes, *Amblyomma gervaisi* was observed on 10 rat snakes and 2 cobras. Other tick species reported on snakes includes *Amblyomma pattoni* and *Amblyomma cordiferum*. Some snakes endured concomitant infections with these ticks.

3.2 Endoparasites

Coprological examination revealed parasitic helminths in 15 of the 16 snakes examined. The genera of helminths found in the positive snakes were strongyles, *Oxyurid* sp., *Pentastome* sp., trematode eggs and larva of strongyles (Tables 3 & 4). The faecal analysis from the Rat snake revealed the presence of mixed infections (strongyles and *Oxyurid* sp., eggs).

Table 1. Ticks sp. Identified

<i>Amblyomma gervaisi</i> (Fig. 1)	Ornate scutum with five creamy yellow spots, one at the centre and the others at the edge on male ticks, and a heart-shaped scutum with three velvety yellow spots, on female ticks.
<i>Amblyomma pattoni</i> (Fig. 2)	Inornate scutum with longirostrate mouth parts accompanied with blade like chelicerae and 3:3 hypostomal dentition.
<i>Amblyomma cordiferum</i> (Fig. 3)	Scutum covering less than half of the body. The scutum is rhomboid and has dark brown ornamentation.



Fig. 1. *Amblyomma gervaisi*– Male - Gross



Fig. 2. *Amblyomma pattoni*– Male - Gross



Fig. 3. *Amblyomma cordiferum* –Female - Gross

Table 2. List of ectoparasites

Ectoparasites	Species of Snakes (n=16)		
	Rat snake (<i>Ptyas mucosa</i>) (n = 11)	Indian cobra (<i>Naja naja</i>) (n = 4)	Green vine snake (<i>Oxybelis fulgidus</i>) (n = 1)
<i>Amblyomma gervaisi</i>	5	2	1
<i>Amblyomma pattoni</i>	1	-	-
<i>Amblyomma cordiferum</i>	1	-	-
<i>Amblyomma gervaisi</i> + <i>Amblyomma pattoni</i>	2	1	-
<i>Amblyomma gervaisi</i> + <i>Amblyomma cordiferum</i>	1	-	-
Absence of tick infestation	1	1	-

Table 3. Morphology of endoparasites identified

<i>Oxyurid</i> sp., egg (Fig. 4)	Oval ova with thick wall
Strongyle egg (Fig. 5)	Oval shaped thin-walled egg with unsegmented embryo
<i>Pentastome</i> sp., egg (Fig. 6)	Small size, oval shape, and a thick, multilayered shell
Strongyle larvae (Fig. 7)	Slender larva with rhabditiform oesopahus



**Fig. 4. *Oxyurid* ova
x400**



**Fig. 5. Strongylid egg
x400**



**Fig. 6. Ova of *Pentastome* sp.,
x400**



Fig. 7. Strongylid larvae x400

Table 4. List of endoparasites

Helminths	Species of Snakes (n=16)		
	Rat Snake (<i>Ptyas mucosa</i>) (n = 11)	Indian Cobra (<i>Naja naja</i>) (n = 4)	Green Vine Snake (<i>Oxybelis fulgidus</i>) (n = 1)
Strongylid egg and larva	9	1	-
<i>Oxyurid</i> sp., egg	-	1	-
<i>Pentastome</i> sp., egg	1	-	-
Strongyle + <i>Oxyurid</i> sp., egg	1	-	-
Absence of endoparasites	-	-	1

Table 5. List of protozoal infections

Protozoal infection	Species of snakes (n=16)		
	Rat snake (<i>Ptyas mucosa</i>) (n = 11)	Indian cobra (<i>Naja naja</i>) (n = 4)	Green vine snake (<i>Oxybelis fulgidus</i>) (n = 1)
Blood Protozoa			
<i>Haemoproteus</i> sp., (Fig. 8)	6	2	1
<i>Hepatozoon</i> sp., (Fig. 9)	4	1	-
Intestinal Protozoa			
<i>Eimeria</i> sp., (Fig. 10)	3	-	-
<i>Cryptosporidium</i> sp., (Fig. 11)	4	2	-

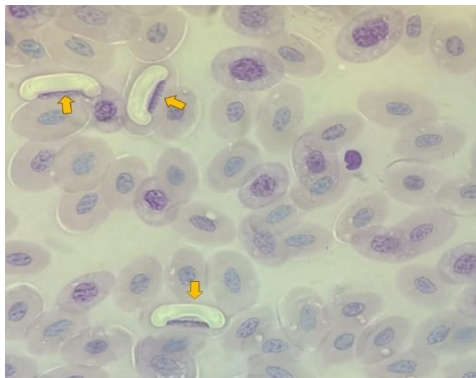


Fig. 8. Intracytoplasmic gamonts of *Haemoproteus* sp., (arrows) x1000

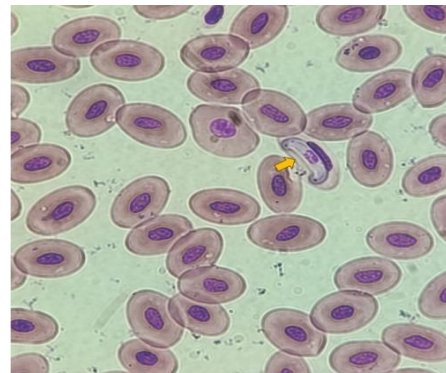


Fig. 9. Intracytoplasmic gamonts of *Hepatozoon* sp., (arrow) x1000



Fig. 10. Unsporulated oocysts of *Eimeria* sp., x400

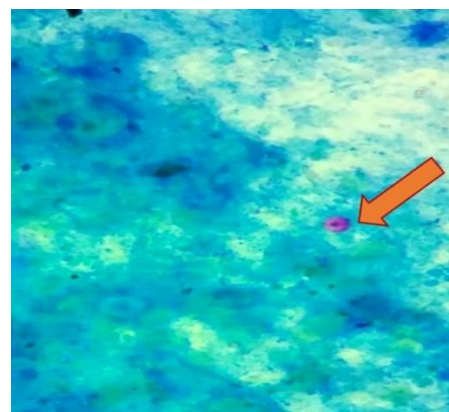


Fig. 11. Oocysts of *Cryptosporidium* sp. – Modified ZN stain x1000

3.3 Protozoal Infection

Out of 16 snakes blood sampled, 10 were positive for the intra-erythrocytic gamonts of *Haemoproteus* sp., characterized by the halter shaped gamonts over the nucleus of the RBC and 4 showed the presence of gamonts of *Hepatozoon* sp., identified by the presence of ellipsoidal shape with pleomorphic nucleus. Faecal smear examination of 6 snakes after modified ZN staining revealed the pinkish red oocysts of *Cryptosporidium* sp., against blue background. Flootation method showed unsporulated round oocysts of *Eimeria* sp., (Table 5).

4. DISCUSSION

4.1 Ectoparasites

Reports of tick parasitism on wild snakes are infrequent. This might have occurred because of difficulties with collecting samples and lack of skilled personnel to handle snakes. Ticks belonging to the *Amblyomma* (formerly *Aponomma*) genus are highly specific to reptiles (Snakes and lizards). According to Guglielmo and Robbins [10], the *Amblyomma* genus includes 138 tick species. Out of those, only ten species (*A. clypeolatum*, *A. crenatum*, *A. gervaisi*, *A. hebraeum*, *A. helvolum*, *A. integrum*, *A. nitidum*, *A. pattoni*, *A. supinoi*, and *A. testudinarium*) are found in India [11,12]. Three of these are found on snakes (*A. gervaisi*, *A. helvolum*, and *A. pattoni*). *Amblyomma* (*Aponomma*) *gervaisi* is an ectoparasite that inhabits majorly in Indian cobras, Russell's vipers, reticulated pythons, Indian rock pythons and rat snakes Soundararajan, [13] in which our study reports the presence of *A. gervaisi* and *A. pattoni* in Indian cobra, rat snakes and green vine snakes. In addition, *A. cordiferum* also have been identified in rat snakes which is an uncommon species of tick, as reported by Voltzit and Keirans [14] and corroborated by Norval et al. [15] because of the few reports from rescued snakes in Taiwan. Ticks are capable of causing dermatological problems in reptiles and spread infections that decrease normal capacity results in high mortality [16]. *Amblyomma gervaisi* ticks have been encountered on wild and captive snakes in southern Asia, including Rat snakes (*Ptyas mucosa*), Indian Cobras (*Naja naja*), King Cobras (*Ophiophagus hannah*), Indian Rock Pythons (*Python molurus*) and reticulated Pythons (*Malayopython reticulatus*) [17]. Our

study aligns with these findings, as we have also documented the presence of these ticks in rat snakes, Indian cobras and green vine snakes. Ticks are capable of transmitting infections resulting in pneumonia in snakes Marcus, [18] and they may transmit haemoprotozoan diseases such *Aeromonas septicaemia*, *Haemoproteus* sp., leading to snake mortality [19]. Pandit et al. [20] reported that among the 30 snakes species investigated, majority of ticks were only observed on the Indian rat snake and spectacled cobra. Our study is consistent with this, as we found a higher occurrence of ticks on these two particular snake species.

4.2 Endoparasites

A study conducted in Sao Paulo reported 70.8% of rattle snakes samples were found positive for nematodes, whereas a coprological study of European reptile samples revealed a wide range of parasites, with 93.2% positive for strongyle infection [21]. The present study corroborates the observations as there was report of 70% Strongyles in snakes. *Kalicephalus* sp. is the most important strongylid snake genus belonging to Diaphanocephalidae family, commonly encountered in reptile and lizard stomachs and intestines. Many worms attached to the mucosa, causing heterophilic infiltration of the submucosa Kavitha et al., [22], Hallinger et al., [23]. Since a comprehensive assessment, including necropsy, is required to confirm the suspected *Kalicephalus* sp. infection in our study area. Nematodes, particularly oxyurids, are the most prevalent parasites observed in reptiles. Rataj et al. [3] concluded that oxyurids are a prevalent parasite in lizards, snakes, and tortoises, infecting 649 out of 949 investigated reptiles, which marginally aligns with our study, where we encountered oxyurids in rat snake and Indian cobra. Trematode infections in captivity tend to be self-limiting due to the presence of an intermediate host, which must be present for all digenetic flukes [9]. Trematodes have been identified in all reptile groups, but only a few of them have been linked to disease [24]. Pentastomids are primarily discovered in carnivorous reptiles (90%) but can also infect toads, birds, and mammals. Adult pentastomes resemble worms and are primarily found in reptiles' respiratory tracts [25]. Sulekha et al. [7] documented the presence of strongyle larvae in king cobras, a finding that aligns with our research, in which we have identified the strongyle larvae in Indian cobra and rat snake.

4.3 Protozoal Infection

Veeraselvam et al. [26] confirmed the presence of *Haemoproteus* sp. infection in a rescued rat snake from the Thanjavur region of Tamil Nadu, similarly our study reports *Haemoproteus* sp. in rat snakes, Indian cobra and green vine snake. Zhang et al. [6] concluded that *Haemoproteus* sp., can also be transmitted via biting midges and louse flies which tends to cause haemolytic anaemia when multiply in greater number, with the predisposition of stress. Tamileniyan et al., [27] reported the presence of *Haemoproteus* sp., by molecular technique in a rescued rat snake.

Hepatozoon sp. infections have been reported among several snake species worldwide using microscopy Telford et al. [28] and molecular techniques Harris et al., [29] in which our study indicates the presence of *Hepatozoon* sp., in both rat snakes and Indian cobras. *Hepatozoon*, an intracellular protozoan parasite, is commonly found in reptiles, birds and mammals with a report of 120 *Hepatozoon* species in 200 snake species [30]. The detrimental potential of *Hepatozoon* spp. in snakes remains controversial [31]. Previous research suggests that different levels of *Hepatozoon* infection can have varying impacts on snakes, ranging from minor effects on host fitness to significant influence on growth rate and reproductive output [32]. *Cryptosporidium serpentis* infection in snakes causes chronic hypertrophic gastritis with inappetence, postprandial regurgitation, lethargy and gastric swelling [33]. The prevalence of cryptosporidiosis in snakes ranges from 4% to 73% in the USA and Brazil with higher affinity towards captive snakes than wild snakes [34], whereas our study represents 2 out of 4 Indian cobras and 4 out of 11 rat snakes positive for *Cryptosporidium* sp., *Eimeria* is a genus comprising over 120 species detected in reptiles and found to be the most frequent coccidian parasites in snakes Duszynski et al., [35] and has been documented in the present study. Paperna and Landsberg [36] proposed 2 new genera, *Choleoeimeria* and *Acroeimeria* infecting reptiles, based mainly on the location of the endogenous stages and form of the oocysts.

5. CONCLUSION

Our investigation into the parasitic fauna of snakes from Tamil Nadu, India, has illuminated significant aspects of their health and ecological interactions between the parasites and snakes. Protozoal infections, specifically was identified,

underscoring the intricate interplay between snakes and these pathogens. The implications of these infections on snake health, coupled with their zoonotic potential, highlight the need for conservation. In light of our findings, we propose comprehensive measures, including tick control interventions, regular health assessments and conservation initiatives aimed at preserving snake habitats. The collaborative efforts of veterinary professionals, researchers and conservationists are imperative for advancing our understanding of snake parasitology and formulating holistic strategies for effective parasite management and snake conservation. This study contributes valuable insights towards the sustainable coexistence of snakes within their ecosystems and underscores the importance of ongoing research and conservation initiatives in the realm of herpetological health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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