INSIGHTS INTO PARASITIC FAUNA AMONG RESCUED SNAKE SPECIES: A STUDY FROM TAMIL NADU, INDIA

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.

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 called farmer’s friend as they kill the vermins like rats and protect agricultural crops. In addition, the venom of some snakes is used to prepare antivenom to save lives of human and animals (Yimming et al., 2016). Reptiles are hosts for a wide variety of protozoan and metazoan parasites (Wilson and Carpenter, 1996). Reptiles including snakes act as an 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, (Rataj et al., 2011; Vimalraj et al., 2015). They are also affected by external parasites, most probably ticks like Amblyomma sp., mites like Ophionyssus natricis (Pietzsch et al., 2006) and
biting flies (Zhang et al., 2014) and they transmit some haemoproteans 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 (Sulekha et al., 2018). 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 Ophodascaris robertsi is commonly seen in snake was found in human brain and it was removed successfully (Mehrab et al., 2023). 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.

**MATERIALS AND METHODS**

**Study area**

Thanjavur (N-10° 47' 13.1964", E-79° 8' 16.1700") and Pudukkottai (N-10°25'48.00", E-79°19'12.00") forest divisions of Tamil Nadu, India, constituted the study area for the investigation of parasite 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 Pudukkottai 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.

**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.

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 (1982). 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.

RESULT

Ectoparasites

Out of 16 snakes examined, 14 snakes showed ixodid ticks belonging to one genera were identified (Table. 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.

<table>
<thead>
<tr>
<th>Table. 1 – Ticks sp. identified</th>
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<tbody>
<tr>
<td><strong>Amblyomma gervaisi</strong> (Fig. 1)</td>
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<tr>
<td><strong>Amblyomma pattoni</strong> (Fig. 2)</td>
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<td><strong>Amblyomma cordiferum</strong> (Fig. 3)</td>
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<th>Table. 2 List of ectoparasites</th>
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<tr>
<td>Ectoparasites</td>
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<tr>
<td>Rat snake (<em>Ptyas mucosa</em>) (n = 11)</td>
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</table>
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 (Table 3 & 4). The faecal analysis revealed that sample from Rat snake indicated presence of mixed infections (strongyles and *Oxyurid* sp., eggs).

<table>
<thead>
<tr>
<th>Helminths</th>
<th>Species of snakes (n=16)</th>
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<tr>
<td></td>
<td>Rat snake (<em>Ptyas mucosa</em>) (n = 11)</td>
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<tr>
<td>Strongylid egg and larva</td>
<td>9</td>
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<tr>
<td><em>Oxyurid</em> sp., egg</td>
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<td><em>Pentastome</em> sp., egg</td>
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<tr>
<td>Strongyle + <em>Oxyurid</em> sp., egg</td>
<td>1</td>
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<td>Absence of endoparasites</td>
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Protozoal infection

Out of 16 snakes blood sampled, 10 were positive for the intra-erythocytic 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. Floatation method showed unsporulated coccidia *Eimeria* sp., characterized by oval shaped unsporulated oocysts (Table. 5).

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<tr>
<th>Table. 5 List of Protozoal infection</th>
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<tr>
<td><strong>Protozoal infection</strong></td>
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<td></td>
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<tr>
<td>Blood Protozoa</td>
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<td><em>Haemoproteus</em> sp., (Fig. 8)</td>
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<td><em>Hepatozoon</em> sp., (Fig. 9)</td>
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<tr>
<td>Intestinal Protozoa</td>
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<tr>
<td><em>Eimeria</em> sp., (Fig. 10)</td>
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<tr>
<td><em>Cryptosporidium</em> sp., (Fig. 11)</td>
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</table>

DISCUSSION

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 Guglielmone and Robbins (2018), 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 (Soundararajan et al., 2013; Catherine et al., 2017). 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, 2020), 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 (2003) and corroborated by Norval et al. (2008) 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 (Ghosh and Nagar, 2014). Amblyomma gervaisi ticks have been encountered on wild and captive snakes in southern Asia, including Oriental Rat snakes (Ptyas mucosa), Indian Cobras (Naja naja), King Cobras (Ophiophagus hannah), Indian Rock Pythons (Python molurus) and reticulated Pythons (Malayopython reticulatus) (Rajesh et al., 2015). 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, 1971) and they may transmit haemoproteozon diseases such Aeromonas septicaemia, Haemoproteus sp., leading to snake mortality (Rosenthal, 1997). Pandit et al. (2011) reported that among the 30 snake 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.

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 (Silva et al., 2001). 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., 2013; Hallinger et al., 2020), 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. (2011) 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 (Soulsby, 1982). Trematodes have been identified in all reptile groups, but only a few of them have been linked to disease. In snakes, they can be found in the digestive, respiratory and urinary tracts (Halán and Kottferová, 2021) our study may associate with the trematode found in the gastro-intestinal tract. Pentastomids are primarily discovered in carnivorous reptiles (90%) but can also infect toads, birds, and mammals. Adult pentatsomes resemble worms and are primarily found in reptiles' respiratory tracts (Self, 1969). Sulekha et al. (2018) documented the presence of strongyle larvae in king cobras, a finding that aligns with our research, in which we have identified the strongyle larval in Indian cobra and rat snake.

**Protozoal infection**

Veeraselvam et al. (2018) confirmed the presence of *Haemoproteus* sp. infection in a rescued rat snake from the Thanjvaur region of Tamil Nadu, similarly our study reports *Haemoproteus* sp. in rat snakes, Indian cobra and green vine snake. Zhang et al. (2014) 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., (2023) 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. 2004) and molecular techniques (Harris et al., 2011) 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 (Han et al., 2015). The detrimental potential of *Hepatozoon* spp. in snakes remains controversial (Brown et al., 2006). 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 (Tomé et al., 2012). *Cryptosporidium serpentis* infection in snakes causes chronic hypertrophic gastritis with inappetence, postprandial regurgitation, lethargy and gastric swelling (Lobão et al., 2023) . The prevalence of cryptosporidiosisis in snakes ranges from 4% to 73% in the USA and Brazil with higher affinity towards captive snakes than wild snakes (Hellebuyck et al., 2007), 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., 2000) and has been documented in the present study. Paperna and Landsberg
(1989) proposed 2 new genera, *Choleoeimeria* and *Acroeimeria* infecting reptiles, based mainly on the location of the endogenous stages and form of the oocysts.

**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.

**REFERENCES**


FIGURES

**Fig. 1. Amblyomma gervaisi** – Male - Gross  
**Fig. 2. Amblyomma pattoni** – Male - Gross  
**Fig. 3. Amblyomma cordiferum** – Female - Gross
Fig. 4. Oxyurid ova x400

Fig. 5. Strongylid egg x100

Fig. 6. Ova of Pentastome sp., x100

Fig. 7. Strongylid larvae x100

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