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, endoparasites, and protozoal infections. 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 come under the order Squamata. It is present all over the continent except at Antarctica. There are almost 4000 species of snakes’ lives 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 vermin like rats and protecting 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 haemoprotozoan notably Hemoproteus sp., and Hepatozoon sp., to snakes. Mostly endo parasites from snakes 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. The protozoa Cryptosporidium from snake faeces has a zoonotic potential to cause watery diarrhoea in humans with immunodeficiency and 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 Pudukottai (N-10°25'48.00", E-79°19'12.00") forest divisions of Tamil Nadu, India, constitute 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 in the rescued snakes from the households and road sides.

Collection of samples

A total of 16 snakes which include, 11 Rat snakes (Ptyas mucosa), 4 Indian cobra (Naja naja) and 1 green vine snakes (Oxybelis fulgidus) which were reported for treatment over a period of 18 months (August 2022 – January 2024) 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). Two snakes were free from any tick infestation. 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>Amblyomma gervaisi (Fig. 1)</td>
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<td>Amblyomma pattoni (Fig. 2)</td>
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<td>Amblyomma cordiferum (Fig. 3)</td>
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<th>Table. 2 List of ectoparasites</th>
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<tr>
<td>Ectoparasites</td>
</tr>
<tr>
<td>Rat snake (Ptyas mucosa) (n = 11)</td>
</tr>
<tr>
<td>Amblyomma gervaisi</td>
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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>Endoparasites</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><em>Strongyle</em> egg</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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<td><em>Strongyle</em> larva</td>
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<tr>
<td><em>Strongyle</em> + <em>Oxyurid</em> sp., egg</td>
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<tr>
<td>Absence of endoparasites</td>
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**Table 3 – Morphology of endoparasites identified**

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

**Table 4 – List of endoparasites**

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Commented [L11]: Strongyloid

Commented [L12]: Is the same parasite that Strongyle, but different stage (egg or larva.) Just add this to the line of Strongyle (in total 8, not seven if you agree with this consideration)
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).

<table>
<thead>
<tr>
<th>Table 5 List of Protozoal infection</th>
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<tr>
<td><strong>Protozoal infection</strong></td>
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<tr>
<td>Blood Protozoa</td>
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<tr>
<td><em>Haemoproteus</em> sp., (Fig. 9)</td>
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<td><em>Hepatozoon</em> sp., (Fig. 10)</td>
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<td>No blood protozoa</td>
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<tr>
<td>Intestinal Protozoa</td>
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<tr>
<td><em>Eimeria</em> sp., (Fig. 11)</td>
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<tr>
<td><em>Cryptosporidium</em> sp., (Fig. 12)</td>
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<tr>
<td>Absence of intestinal protozoa</td>
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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. clveolatum*, *A. crenatum*, *A. gervaisi*, *A. hebraeum*, *A. helvolum*, *A. integrum*, *A. nitidum*, *A. pattoni*, *A. supinoi*, and *A. supinoi*).

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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 bring about blood-borne diseases such Aeromonas septicaemia, Haemoproteus sp., leading to snake mortality (Rosenthal, 1997). Pandit et al. (2011) reported that among the 30 snakes species investigated, majorly 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) in which our study accounts for 70% (9 out of 16) in snakes. Kalicephalus sp. is the most important strongylid snake genus belong to Diaphanocephalidae family are 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), 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, we had encountered oxyurids in rat snake and Indian cobra. Trematode infections in
captive 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 Thanjavur 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) molecularly reported the presence of Haemoproteus sp., 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. 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 (Hellebuyck et al., 2007), where our study represents 2 out of 4 Indian cobras and 4 out of 11 rat snakes were 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.,
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.

**REFERENCES**


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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.** Strongyle egg x100

**Fig. 6.** Ova of Pentastome sp., x100
Fig. 7. Larvae of Strongyle 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