Morphological study on parasitic fauna of four species of lizards in Mizoram, India

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ABSTRACT

This study revealed the parasitic fauna of different species of lizards from different parts of Mizoram, India. Four different species of lizards, namely House lizard (Hemidactylus flaviviridis, Gekkonidae), Monitor lizard (Varanus indicus, Varanidae), Gecko (Gekko gecko, Gekkonidae) and Chameleon (Chamaeleo zeylanicus, Chameleonidae) were surveyed from different parts of Mizoram, India in an attempt to investigate the prevalence of parasites. Faecal samples were examined by conventional sedimentation and floatation techniques. Lizards that were run over by car or sacrificed underwent post-mortem examination. Blood samples were also examined for the presence of any haemoprotozoan infection. Light microscopy (LM) and scanning electron microscopy (SEM) were applied for detailed morphological study. The ecto-parasites were identified on the basis of morphology. A total of 69 lizards were examined and found to be infected with three species of nematode (Pharyngodon sp., Rhabdias sp. and Strongyle), one species of trematode (Postorchigenes sp.), three species of tissue protozoa (Eimeria spp., Isospora spp. and Cyclospora spp.) and two species of haemoprotozoa (Trypanosoma sp. and Lankesterella sp.). Only one species of tick belonging to the genus Amblyomma was found on the body surface of monitor lizard. The study clearly indicates that lizards can harbour different parasites which can affect the health status of these reptiles.

INTRODUCTION

In recent times, many reptiles are being collected from the wild and kept as home pets. Reptiles are also used as animal models in biomedical research for studies like cardiovascular physiology, environmental toxicology, evolutionary and reproductive biology, vector borne diseases and others (Greenberg et al., 1989; Driscoll and Henderson, 2008). Reptiles in the wild are naturally infected with a wide range of parasites. Parasitic infections are frequently encountered in captive bred reptiles also. The incidence of parasites in reptiles including lizard has been reported by several studies (Scullion and Scullion, 2009; Majlathova et al., 2010; Rataj et al., 2011). Lizards are frequently found in human
dwellings in tropical countries including India. Several factors undermine the immune response of reptiles which predispose the opportunity for pathogenic organisms to cause infections and resultant diseases. Reptiles may also act as carriers for different pathogens which are transmitted to other animals and even to humans (Rataj et al., 2011; Papini et al., 2011). Mizoram is one of the global hot spots in India for reptilian diversity. The major reptilian families in Mizoram include Gekkonidae, Varanidae and Chameleonidae. Little is known about the parasitic fauna of lizards in this part of India. The prevalence and the intensity of parasitic infections act as a source of information regarding the status and the possible impact of parasites in the lizard population in natural conditions. Therefore, this present study was intended to investigate the prevalence and intensity of parasites in three groups of lizards. It also provides detailed light microscopy (LM) and scanning electron microscopy (SEM) study of few parasites of lizards.

**MATERIALS AND METHODS**

**Study area and lizard collection**

Different areas of Aizawl district of Mizoram were selected as parts of the study area. The lizards were captured using fish nets, by hand or by noosing. The survey was carried out from September 2016 to February 2017. Some lizards killed accidentally were also treated as sample survey. A total of 69 lizards belonging to the family Gekkonidae (*H. flaviviridis* and *G. gecko*), Varanidae (*V. indicus*) and Chameleonidae (*C. zeylanicus*) were examined.

**Collection and examination of faecal samples**

Faeces (n=185) were examined fresh. Direct smear, floatation and sedimentation techniques were employed following standard procedure (Souza et al., 2014; Wolf et al., 2014). Faecal sample of dead lizards were also examined.

**Examination of blood samples**

A total of 69 blood samples from each adult lizard specimen were collected by puncturing of the caudal vein with disposable sterile syringes. Thin blood smears were prepared and quickly air dried. In case of field samples away from laboratory, slides were stored and brought to the laboratory in plastic slide boxes. Slides were stained with Giemsa stain for 45 min and examined under oil immersion at ×1000 magnification.

**Post mortem examination**

Twenty dead lizards were brought to the laboratory during the whole period of survey. Post mortem was done systematically and thoroughly. Any parasite found was washed several times with 0.85% normal saline solution (NSS) before transferring to 70% alcohol.

**Examination of skin for ectoparasites**

Skins of both live and dead lizards were carefully examined for any ecto-parasites. Live ticks were directly dropped into 10% alcohol. For permanent preparation of slides, bodies of ticks were perforated with entomological pins and boiled with 70% potassium hydroxide solution for 5-10 min. The treated samples were put in 70% alcohol for 5 min and 90% alcohol 5 min. Finally the specimens were kept in carbolic acid for 10 min before mounting in D.P.X. mountant. *Amblyomma* sp. were identified on the keys provided by Soulsby (1982).

**Preparation of sample for SEM**

At first, any unwanted materials such as faecal debris, mucus, blood or other body fluids which would hamper microscopical observation were carefully removed by washing with nuclease free water (NFW) with the help of a fine camel brush. For SEM, specimens were washed 4-5 times with 0.2 M Cacodylate buffer (pH - 7.3) and fixed in 2.5% Glutaraldehyde at 4°C for 24 h. The fixed samples were washed in phosphate buffer saline (pH - 7.2) for three times and then in double distilled water followed by acetone dehydration. After acetone dehydration, the specimens were dried with liquid carbon dioxide at its critical point (that is, 3.5°C at 11 psi). The specimens were then immersed in tetra methyl saline (TMS) for 5-10 min for two changes at 4°C. Then they are brought to room temperature (25-26°C) to dry. The samples were mounted on aluminium stubs. The parasitic specimens were then gold coated in a sputter coat and finally examined under SEM [(JSM-6360-JEOL)] at the North Eastern Hill University (NEHU), Shillong, Meghalaya, India in Sophisticated Analytical Instrument Facilities (SAIF) Laboratory (Patra et al., 2016).

**RESULTS AND DISCUSSION**

A total of 69 lizards from three families (Gekkonidae, Varanidae and Chameleonidae) were examined during the study period and the result of the faecal sample and blood samples is shown in Table 1 in proper percentile form. Forty three lizards were found infected with three
Table 1. Species of parasites infecting lizards from Mizoram, India.

<table>
<thead>
<tr>
<th>Type of parasite</th>
<th>House lizards</th>
<th>Chameleon</th>
<th>Gecko</th>
<th>Monitor lizard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(H. flaviviridis) (37)</td>
<td>(C. zeylanicus) (14)</td>
<td>(G. gecko) (15)</td>
<td>(V. indicus) (3)</td>
</tr>
<tr>
<td><strong>Endo-parasites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharyngodon sp.</td>
<td>20 (54.05%)</td>
<td>05 (35.71%)</td>
<td>04 (26.67%)</td>
<td>-</td>
</tr>
<tr>
<td>Rhabdias sp.</td>
<td>02 (5.40%)</td>
<td>03 (21.43%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Strongyle</td>
<td>05 (13.51%)</td>
<td>02 (14.28%)</td>
<td>02 (13.33%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Trematodes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postorchigenes sp.</td>
<td>01 (2.70%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Intestinal Protozoa</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Eimeria sp.</td>
<td>04 (10.81%)</td>
<td>02 (14.28%)</td>
<td>03 (20%)</td>
<td>02 (66.67%)</td>
</tr>
<tr>
<td>Isospora sp.</td>
<td>01 (2.70%)</td>
<td>01 (7.14%)</td>
<td>01 (6.67%)</td>
<td>-</td>
</tr>
<tr>
<td>Cyclospora sp.</td>
<td>-</td>
<td>-</td>
<td>03 (20%)</td>
<td>-</td>
</tr>
<tr>
<td>Balantidium sp.</td>
<td>-</td>
<td>-</td>
<td>01 (6.67%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Haemoproteozoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lankesterella sp.</td>
<td>02 (5.40%)</td>
<td>-</td>
<td>01 (6.67%)</td>
<td>-</td>
</tr>
<tr>
<td>Trypanosoma sp.</td>
<td>02 (5.40%)</td>
<td>01 (7.14%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ecto-parasites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amblyomma sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>01 (33.33%)</td>
</tr>
</tbody>
</table>

Different species of nematodes (Pharyngodon sp., Rhabdias sp., Strongyle). One lizard was found infected with a species of trematode (Postorchigenes sp.) and eighteen lizards were found infected with three species of tissue protozoa (Eimeria sp., Isospora sp. and Cyclospora sp.). Six lizards were also found positive for two species of haemoproteozans namely Trypanosoma sp. and Lankesterella sp. Only one monitor lizard was found infested with Amblyomma sp.

Out of 69 lizards examined, the highest infection rate by nematode was found in house lizards by Pharyngodon sp. (54.05%) followed by chameleon (35.71%) and gecko (26.67%). The infection rate by Rhabdias sp. was less (5.40%) in house and garden lizards and mild in chameleon (21.43%). The lizards (house lizards, chameleon and gecko) were found infected with strongyles in more or less similar rate (13.51, 14.28% and 13.33% respectively). No infection was found in monitor lizard by any of the nematode parasites.

Only one (2.70%) lizard was found infected with Postorchigenes sp. Infection with intestinal protozoa was found significantly higher by Eimeria sp in all the groups of lizards. It was found highest in monitor lizard (66.67%) followed by gecko (20%), chameleon (14.28%) and house lizards (10.81%). Mild infection with Isospora sp was recorded in different groups of lizards. Cyclospora sp was found predominantly in gecko (20%). Balantidium sp oocyst was detected only in one gecko (6.67%).

Infection with haemoproteozoa, Lankesterella sp. and Trypanosoma sp. were recorded as 5.4% in house lizards in blood smear examination. 6.67% geckos and 7.14% chameleons were found positive for Lankesterella sp. and Trypanosoma sp. infection respectively. Only 33.33% of the monitor lizards were found positive with ecto-parasite (Amblyomma sp.) infestation.

Based on morphometry under LM and SEM, three species of nematodes were identified as Pharyngodon sp. (Figures 1a, b, c and d), Strongyloides sp. (Figures 2a, b and c) and Strongyle species (Figures 3a, b and c). One trematode under Postorchigenes genus was found in one house lizard (Figures 4a and b). Three species of tissue protozoa were found when faecal flotation method was used (Figures 5, 6 and 7). Balantidium sp. cysts were found in four faecal samples (Figure 8). While two house lizards were found positive for Lankesterella sp. (Figure 9). One garden lizard was found infected with Trypanosoma sp. (Figure 10). Light microscopy pictures of Amblyomma sp. which was recovered from monitor lizard are shown in Figures 11a and b.

The present study underscores the prevalence and intensity of ecto- and endo-parasitic fauna of four species of lizards from different parts of Mizoram, India. Parasites occasionally cause harmful effects on lizards kept in captivity or in the wild (Ratj et al., 2011; Hedley et al., 2013). For systematic identification and classification, all morphological aspects of parasites must be studied under light microscopy. The importance of SEM study lies on its remarkable ability to provide three dimensional images with magnification that allow understanding the spatial relationship among surface structures (Hirschmann, 1983; Gibbons, 1986). Faecal flotation was found superior to other faecal examination.
Figure 1. a, *Pharyngodon* sp. (female) (X40); b, *Pharyngodon* sp. (anterior part) (X100); c, egg of *Pharyngodon* sp. (X100); d, Larval stage of *Pharyngodon* sp. (X100); e, Somatic papillae and transverse ridge of *Pharyngodon* sp (SEM).

Figure 2. a, *Rhombias* sp. (anterior end) (X100); b, *Rhombias* sp. (anterior end) (mouth part); C, posterior part of *Rhombias* sp. (SEM).
Figure 3. a, Anterior end of Strongyle (SEM) (mouth part); b, Strongyle under SEM; c, Strongyle under SEM; d, *Strongyloides* sp. egg (X100).

Figure 4. a, *Postorchigenes* sp. (SEM); b, Body surface of *Postorchigenes* sp (SEM).
Figure 5. Sporulated spp. of *Isospora* sp. (X100).

Figure 7. Unsporulated Oocysts of *Cyclospora* sp. (X400).

Figure 8. *Balantidium* cyst (X100).
Figure 9. Giemsa stained blood smear of lizard showing *Lankesterella* sp. (X1000).

Figure 10. Giemsa stained blood smear of lizard showing *Trypanosoma* sp. (X1000).

Figure 11. a, *Amblyomma* sp. *in-situ*; b, female *Amblyomma* sp. under LM (X40).
techniques for detection of helminth ova and protozoan oocysts. Eggs and oocysts were identified on the basis of morphology. The result showed higher rate of Pharyngodon sp. in different species of lizards which agreed with other works (Rataj et al., 2011). Pinworms were found in the terminal part of the intestine in lizards. Adults measured 8-10 mm in length with characteristic bulbous oesophagus. Their life cycles are direct (Klingenberg, 1993a). Lizards in captivity in endorsed spaces can re-infect themselves again and again. In 10 garden lizards’ egg, pin worms (Pharyngodon sp.) were detected. Klingenberg (1993b) pointed out that these are often found in reptile faeces, but they generally do not cause any disease in reptiles. Rhabdias nematodes were confirmed in three chameleons by faecal examination. Balantidium sp. cysts were detected only in gecko in small percentage (6.67%). Two house lizards were found positive with Postorchigenes trematode at post-mortem. Greiner and Schumacher (2000) recovered trematodes from pulmonary and biliary system. In this study, Postorchigenes was recovered from gastrointestinal tract. Cyclospora sp., Eimeria sp. and Isospora sp. oocysts were also detected in the present study. Similar findings were also seen by other workers (Klingenberg, 1993a; Mader and Lane, 1996; Klingenberg, 2000).

Blood parasites that were observed in the present investigation included Lankesterella sp. in three lizards and Trypanosoma sp. in two garden lizards. The Lankesterella sp. found in the blood film is similar to that observed by Rodrigo et al. (2016). The only tick that was recovered from monitor lizard was Amblyomma sp.

Conclusion

It is difficult to identify the parasites to species level based only on morphological characteristics. Precise identification is possible when morphological identification is combined with molecular characterization. However, the present study serves as a base to carry out future studies. It is clear that parasitic infections are quite common in lizards. Intensive farming may aggravate the impact of parasitism on the fitness of these protected animals.

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