Ecological attributes of *Hepatozoon lacertilis* Gupta et al., 2011 susceptibility in Indian lizards, *Hemidactylus flaviviridis* (Gekkonidae) and *Calotes versicolor* (Agamidae)

Neelima Gupta1*, Manju Bhaskar1 and Dileep K. Gupta2
1Department of Animal Science, M.J.P. Rohilkhand University, Bareilly 243006. India
2Department of Zoology, Bareilly College, Bareilly 243005. India
*Corresponding author email: guptagrawal@rediffmail.com
Received 7 August 2012; received in revised form 8 January 2013; accepted 10 January 2013

Abstract. Ecological attributes of haematozoan parasites are poorly understood. In this study, we report haematozoan prevalence in two species of Indian lizards, *Hemidactylus flaviviridis* (Family: Gekkonidae) and *Calotes versicolor* (Family: Agamidae) under three macro-environmental variables: host location, weight and seasonal variations. *Hemidactylus flaviviridis* (*n* = 199) and *Calotes versicolor* (*n* = 34) were sampled from Bareilly, Chandausi and Mirzapur, Uttar Pradesh, India belonging to different weight groups [Group I (0-5 gm), Group II (5-10 gm) and Group III (10-15 gm)] and during various seasons [Summer (May-July), Rainy (August-October), Winter (November-January), Spring (February-April)] of the year. A haemogregarine, *Hepatozoon* Miller, 1908 was discovered from both host species. Test for identity of the parasites was conducted by feeding infected *Culex quinquefasciatus* (Diptera: Culicidae) on infection-free *H. flaviviridis* and *C. versicolor* and blood examinations on 22nd day (*H. flaviviridis*) and 25th day (*C. versicolor*) post feeding (pf) revealed similar haematozoan parasites and were identified as *Hepatozoon lacertilis* Gupta et al., 2011. Infectivity from different locations indicated a prevalence of 5.26% (Bareilly) and 16.36% (Mirzapur) in *H. flaviviridis* whereas infectivity was comparatively higher (19.23%) in *C. versicolor*. In different weight groups, Group III indicated highest infectivity in both lizards being 21.42% (*C. versicolor*) and 17.85% (*H. flaviviridis*). Parasites showed highest prevalence during spring season (*H. flaviviridis* : 9.52%; *C. versicolor* : 25%). Values of significance were determined by chi-square test to compare the prevalence within different variables (host location, weight and season). The study has importance for its contribution to the knowledge on the diversity of reptilian hosts infected by haemogregarines. It is the first record of *Hepatozoon* infectivity in both lizard species with respect to the three macro-environmental variables.

INTRODUCTION

Studies pertaining to lizards naturally infected with protist blood parasites have been important in the development of our understanding of host parasite evolutionary ecology. Such studies have mainly been confined to Europe (Sorci, 1995), America (Schall, 1996) and Australia (Salkeld & Schwarzkopf, 2005). Parasites can act as important selective agents affecting host population, biology, ecology and evolution (Hudson et al., 2002). Parasitic diseases can pose major threats to endangered animal population and have serious economic impact on agriculture and human public health making research into host parasite ecology critical (Galvani, 2003).

Reports on parasite occurrence with respect to the age/weight/body size of the host are not frequent. Sporadic reports occur from Spain (Amo et al., 2004) and Canada (Brown et al., 2006). On the other hand, studies on the influence of season on parasite incidence have attracted more attention as evident by reports of Cheke et al. (1976), Hatchwell et
al. (2000), Deviche et al. (2001), Schrader et al. (2003) and Wood et al. (2007). In lizards, Anjos et al. (2005) and Leinwand et al. (2005) are amongst the few workers who have reported the impact of season on parasite prevalence. Thus the environmental dynamics of *H. lacertilis* Gupta *et al.*, 2011 in two species of lizards has been investigated during the present piece of work. The parasite infectivity with respect to host location, host weight and seasonal variation is expressed herein.

**MATERIALS AND METHODS**

**Study sites**

Three study sites, Bareilly, Mirzapur and Chandausi were selected from Uttar Pradesh state of India. Out of these, Bareilly is a corporate A class city, Mirzapur is a district and B class city and Chandausi is a rural area of Moradabad district of Uttar Pradesh, India.

**Bareilly** (longitude 28.35°N, latitude 79.42°E) is one of the six Counter Magnet city of Uttar Pradesh lying on the bank of river Ramganga, 100 km away from the lower Himalayan range. It has a semi-arid climate with high variation between summer and winter temperatures. Extreme temperatures range from 4°C to 47°C, the annual mean temperature being 25°C (77°F). The average annual rainfall is approximately 714 mm (28.1 inches).

**Mirzapur** (longitude 24.42°N, latitude 83.01°E) River Ganges flows close to this city also. The temperature ranges from 4.8°C to 45.0°C.

**Chandausi** (longitude 28.36°N, latitude 78.61°E) is a rural area of Moradabad district in the state of Uttar Pradesh, India. It is famous for the cultivation of *Mentha piperata* (mint) which is aromatic. Distillation of mint oil is a cottage industry due to which a continuous aroma of mint is present in the atmosphere.

**Experimental Protocol**

*Hemidactylus flaviviridis*, (Family : Gekkonidae) (n=199) and *C. versicolor* (Family : Agamidae) (n=34) were captured with the help of hand net from walls and hidden areas of various localities of Bareilly (n= 103), Chandausi (n= 49) and Mirzapur (n= 81). They were maintained in the Animal House of Department of Animal Science, kept separately in specially designed cages under proper conditions of light and air and fed with field mosquitoes and other insects. Blood was collected from toe snips or tail puncture in heparinised tubes and one drop of saline was mixed, covered with coverslip and examined microscopically to observe live parasites. Infected RBC’s showing slow gliding movement in fresh blood indicated the presence of parasites. However, their presence was confirmed by preparing permanent stained (Giemsa-eosin-methylene blue + buffer pH 6.6) blood smears and examined under the microscope for proper identification of parasites. The parasites were drawn to scale by camera lucida and photographed under LEICA DMLB photoautomat and OLYMPUS trinocular (CX-21 TR) microscope with digital photographic attachment under 1000 magnification. To observe the effect of host weight on haemogregarine infectivity, three weight groups in *H. flaviviridis* and *C. versicolor* were made : Group I (0 – 5gm), Group II (5 – 10gm) and Group III (10 – 15gm) and seasonal impact was observed. In order to observe the effect of different seasons on the prevalence of blood parasites, the whole year was divided into Summer (May-July), Rainy (August-October), Winter (November-January) and Spring (February-April) seasons and infectivity recorded therein.

**Statistical analysis**: Statistical analysis to compare the prevalences within different variables (host location, weight and season) was done by Chi square test. The values at P<0.001 were considered to be significant.

**Test for identity of parasites from Hemidactylus flaviviridis and Calotes versicolor**

Mosquitoes (*Culex quinquefasciatus*, Diptera:Culicidae), the vectors of *Hepatozoon* were blood fed on infected *H. flaviviridis* and introduced in a cage containing 10 specimens each of *H. flaviviridis* and *C. versicolor* prior to confirming that they were infection-free.
Blood from each host species were sampled every alternate day for the presence of blood parasites. When present, they were critically examined morphologically, drawn to scale, measured and diagnosed.

RESULTS AND DISCUSSION

Test for identity of parasites from *Hemidactylus flaviviridis* and *Calotes versicolor*

On the 22nd day (*H. flaviviridis*) and 25th day (*C. versicolor*) pf of mosquitoes, the blood samples revealed haemogregarines in 3 and 2 samples respectively under low (Fig. 1) and high (Fig. 2) infectivity. A comparison of the parasites from the two host genera indicated that the parasites were similar to each other morphometrically and thus are identified as *H. lacertilis* described by the authors. The holotypes and paratypes are deposited in the Zoological Survey of India, Northern Regional Station, Dehradun, India [registration nos. Holotype ZSI/NRS/IV.383, Paratype SI/NRS/IV.384 (Gupta *et al.*, 2011)]

Prevalence of the parasite was calculated with reference to different ecological variables: host location, weight of the host and season in which the hosts were collected and their value of significance determined.

Influence of sampling location on prevalence of parasitism

Habitat refers to the typical environment in which parasites occur. It refers to the geographical division of the external environment where a parasite is found and is an important factor which plays an important role in the availability of parasitic species. As the environmental factors have a controlling system on the availability of parasites in their host, the ecological and biological factors may affect the incidence of parasites. On the other hand, host-specificity may determine a parasite’s distribution. Habitat, life cycle and host specificity are therefore closely interrelated. Bush *et al.* (1997) referred that locality might be a spatial region from where a host is collected or it might refer to the spatial region where a substrate is examined for parasites.

Three different host locations (Bareilly, Mirzapur, Chandausi of Uttar Pradesh, India) were selected to observe the effect on the infectivity of parasites.

A haemogregarine parasite was recorded at 5.26% (Bareilly) and 16.36% (Mirzapur) infectivity in *H. flaviviridis* whereas the infectivity was higher (19.23%) in *C. versicolor*. Infection was nil at Chandausi (Table 1). The calculated value of Chi square in Mirzapur (6.80) is significant at 0.05 level of significance. ($\chi^2 > 5.99$).

The results given above indicate that the lizards of Bareilly and Mirzapur were infected with the haemogregarine, contrarily, those of Chandausi were infection-free. As per the site characteristics, rivers Ramganga and Ganges flow in the vicinity of Bareilly and Mirzapur respectively where the mosquito (vectors of haemogregarines) population is high and coming in direct contact with the lizards, they are capable of spreading the infection. On the other hand, *Mentha piperata* (mint) cultivation is very much prevalent at Chandausi and mint aroma is constantly in the atmosphere of this study site. It is well known that mint leaves act as mosquito repellants and this may account for lesser mosquitoes in this area with a probability of lesser lizard-mosquito association. The ecological distinction, environmental difference and characteristic vegetation of this site may be responsible for causing negligible infection in Chandausi-inhabiting lizards.

Different workers have studied the correlation between type of habitat and species of parasite occurrence (Ingles, 1936; Rankin, 1945). Several characteristics of the host may be responsible for the existence of these isolationist depauperate communities: ectothermy, the simplicity of the alimentary canal, low vagility, a simple diet, generalist feeding and the small number of helminth species with a direct life cycle (Aho, 1990). Weatherhead & Bennett (1991) sampled haematozoa in red winged blackbirds *Agelaius phoeniceus* in Eastern Ontario and found that parasite prevalence was significantly higher for birds sampled in lake
Table 1. Prevalence of *Hepatozoon lacertilis* Gupta et al., 2011 in reptilian hosts (*Hemidactylus flaviviridis* and *Calotes versicolor*) in relation to ecological factors

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Host Location</th>
<th>Weight (gm)</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bareilly</td>
<td>0-5</td>
<td>Spring</td>
</tr>
<tr>
<td><em>Hemidactylus flaviviridis</em></td>
<td>n = 95</td>
<td>5.26</td>
<td>9.52</td>
</tr>
<tr>
<td></td>
<td>Mirzapur</td>
<td>6-10</td>
<td>10.84</td>
</tr>
<tr>
<td></td>
<td>Chandausi</td>
<td>11-15</td>
<td>17.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n = 71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n = 32</td>
</tr>
<tr>
<td><em>Calotes versicolor</em></td>
<td>n = 08</td>
<td>Nil</td>
<td>n = 08</td>
</tr>
<tr>
<td></td>
<td>n = 26</td>
<td>19.23</td>
<td>n = 13</td>
</tr>
<tr>
<td></td>
<td>n = 0</td>
<td>Nil</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.37</td>
</tr>
<tr>
<td>Overall value of Chi square</td>
<td>11.48*</td>
<td>13.51*</td>
<td>1.76*</td>
</tr>
<tr>
<td>P value</td>
<td>0.003*</td>
<td>0.001*</td>
<td>0.623*</td>
</tr>
</tbody>
</table>

Figure 1. Photomicrograph of the blood of *Calotes versicolor* showing mild infection of *Hepatozoon lacertilis*

Figure 2. Photomicrograph of the blood of *Calotes versicolor* showing heavy infection of *Hepatozoon lacertilis*
Opinicon marshes than in beaver marshes, but overall parasite prevalence did not vary significantly with respect to habitat after second calendar year either in males or females. Studies conducted by Sanchez et al. (2007) suggest that mature forests produced nestlings in better condition but with a higher prevalence of haemoparasites than young forest, possibly because mature forests are a good habitat for both the bird and the parasite vector.

Arriero et al. (2008) assessed the habitat effects on physiological stress response in nestling blue tits and showed that newly acquired ecto and haemoparasite infections were associated with forest habitat structural characteristics, higher prevalence of fleas and blood parasites in more mature forests and higher prevalence of blow flies in degraded forests. Leech parasitism was highest on bottom walker and adults and varied throughout the year. Leech intensity was highest on large turtles and in turbid ponds.

Habitat characteristics have a huge impact on all aspects of animal life history. The differential occurrence of haemogregarines from various host locations could be due to unavailability and uninfestivity of invertebrate and vertebrate hosts.

In our studies, it was observed that host location may be an important factor for assessing the prevalence of blood parasites in reptilian hosts, the variance in infectivity may be due to different factors as each locality is typified by its own fauna and environmental conditions.

Parasitic infectivity in relation to weight of the host

Maximum prevalence recorded was 17.85% (H. flaviviridis) and 21.42% (C. versicolor) in Group III. On the other hand, Group II had less prevalence (H. flaviviridis, 10.84% C. versicolor, 12.5%). Prevalence was nil in Group I in both host species (Table 1). In H. flaviviridis, incidence is higher than expected i.e. observed and expected values differ significantly.

Parasite infectivity is often linked to host growth. Keeping this in view, very young lizards (0-5 gm) were also included as a test group to observe if they were susceptible to haemogregarine infection. The absence of infection in this group suggests that older lizards are at greater risk to haemogregarine infection as compared to the younger ones.

During the present course of study, an attempt was made to probe into the impact of the biotic factor, weight of the host on haematozoan infectivity in lizard species. Parasite abundance and diversity has been reported with respect to mammals (Watve & Sukumar, 1995) who did not find any significant correlation with host body size and parasite load and species diversity. However, Hatchwell et al. (2000) observed increase in Haemoproteus infection and decrease in Plasmodium infection in adult black birds.

These findings have been supported by the contribution of Amo et al. (2004) for haemogregarinid blood parasites in the rock lizard, Lacerta monticola, Anjos et al. (2005) for helminths in Hemidactylus mabouia and Salkeld & Schwarzkopf (2005) for Hepatozoon in Eulamprus quoyii. Brown et al. (2006) observed increase in prevalence but decrease in intensity of haemogregarines with an increase in body size of Tropidonothis marii. However, the majority of the authors have reported higher prevalence and infection intensity in adults as compared to juvenile lizards.

Our study indicates that in both lacertilians, haemogregarine prevalence was higher in the heaviest weight group as compared to the juvenile ones which may be due their older age, greater contact with
parasites during their life time as also greater contact with the vector thereby increasing the chances of infection.

**Parasitic infectivity in relation to different seasons of the host**

Seasonal variation in environmental conditions is ubiquitous and is known to play a substantial role on the occurrence and spread of parasitic diseases. Understanding seasonal pattern, parasite incidence can help to identify mechanisms such as the demography of hosts and vectors which influence parasite transmission dynamics. It was therefore proposed to examine seasonal variation of haemogregarine parasites.

The parasite showed differential prevalence in different seasons being highest in spring (9.52% *H. flaviviridis*; 25.0% *C. versicolor*). Lowest prevalence in *H. flaviviridis* (4.22%) was in rainy and in *C. versicolor* (12.5%) during summers (Table 1).

The behavior of lizards is directly related to body temperature (Bhaskar, 2007). They are sluggish at low temperatures (spring-winters) when there are greater chances of blood feeding by the mosquitoes facilitating haemogregarine transmission. Contrarily, they are more active during the rainy season when they not only escape contact with the mosquitoes but other insects are also available on which they can feed instead of mosquitoes. Thus, the lizards were minimally infected during rainy season. Lizards breed during April-June (summers) when their activity again slows down and they fall prey to the mosquitoes easily thereby again elevating the infection rate.

Reports on parasite incidence with respect to different seasons are available in different groups of vertebrates. Several studies have addressed the distribution of parasites in birds. Cheke et al. (1976) recorded peak infection in British avian haemotozoa. It has also been reported that within a given species, prevalence can vary seasonally (Weatherhead & Bennett, 1992). Hatchwell et al. (2000) reported increased *Haemoproteus* infection and decreased *Plasmodium* infection in European black birds, *Turdus merula* from January to July but infection with other haematozoa showed no seasonal changes indicating that *Haemoproteus* and *Plasmodium* were negatively associated with each other with respect to seasonal changes even though infection with both genera were frequent in the studied population as also reported by Deviche et al. (2001) for the occurrence of blood parasites in Indian passerine birds. Schrader et al. (2003) recorded maximum prevalence (80%) of *Haemoproteus* in July and minimum (0%) in January. *Cryptobia tincae* occurred at the highest prevalence in spring (25.0%) as compared to autumn (16.3%) where it was lowest (Akmirza & Tepecik, 2007).

Cosgrove et al. (2008) predicted within year bimodal pattern of spring and autumn peak with winter absence of *Plasmodium* infection in avian malaria infection. While investigating the helminths of *H. mabouia*, Anjos et al. (2005) reported infection rates to be independent of season. However, Leinwand et al. (2005) reported higher prevalence of coccidian infection in lizards of Mauritius as compared to the wet season.

The present studies indicate that highest prevalence of haemogregarine in *H. flaviviridis* occurred during spring (9.52%) followed by winter (9.37%) whereas during rainy (4.22%) and summer (7.40%) seasons, infectivity was poorer. In case of *C. versicolor*, the highest infectivity was recorded in spring (25.0%) followed by winter (15.38%) and summer (12.5%) whereas it was nil in rainy season. Thus both lizard species exhibited infection peak in spring. Various reasons have been proposed for differential infection during the various seasons. As the latter acts as an important abiotic factor affecting the prevalence of parasite, seasonal variation in infection partly results from alterations in host’s susceptibility to infection that are associated with reproductive efforts (Norris et al., 1994; Deviche et al., 2001). Seasonal variations as important abiotic factor in parasite prevalence, hinders the ability of researcher to detect infection from blood smears. As the results may depend on the sampling period, the decrease in parasite prevalence during rainy season may be due to chronic infection becoming latent and
incomplete recovery from summer infection as was also opined by Schrader et al. (2003). An increase in the prevalence during spring may be attributed to a relapse of chronic infection due to physiological stress (Atkinson & Van Riper, 1991) and thus together with the latter, reproductive efforts and a number of additional factors may cause variation in parasite prevalence. It is also probable that new infection may be associated with the emergence of insect vectors. Thus, a complete understanding of association between vector abundance and prevalence of haemogregarines during different seasons is necessary.

Acknowledgements. One of us (Manju Bhaskar) expresses her thanks to the Council of Scientific and Industrial Research, New Delhi for financial help rendered by awarding a SRF fellowship.

REFERENCES


