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Prevalence and intensity of *Haemogregarina stepanowi* (Apicomplexa: Haemogregarinidae) in two species of freshwater turtles (*Mauremys caspica* and *Emys orbicularis*) in Iran

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Abstract

Prevalence and intensity of the intracellular Haemogregarine parasites (*Haemogregarina stepanowi*) was investigated in two species of freshwater turtles in Iran. Blood smears were collected from the European pond turtle (*Emys orbicularis*) and the Caspian pond turtle (*Mauremys caspica*), air dried and stained by Giemsa. Prevalence and mean intensity of parasitemia in turtles of each locality were between 75-100% and 0.07-0.2 per 10^4 red blood cells respectively. The mean intensity of parasitemia in *M. caspica* was higher than *E. orbicularis*. There was no significant difference in prevalence and intensity of parasitemia between male and female in these two species of turtles ($p>0.05$). However, adults had significantly ($p<0.05$) higher prevalence and intensity than juveniles. Additional studies with more localities are needed to better understand the ecology and epidemiology of this parasite in turtles.

Keywords: Haemogregarine parasites, *Emys orbicularis*, *Mauremys caspica*, Iran

1. Introduction

Haemogregarine parasites (Order Eucoccidiorida, suborder Adeleorina) are the most widely distributed blood parasites of turtles world-wide [1]. Family Haemogregarinidae in freshwater tortoise with heteroxenous life-cycles, gamonts within erythrocytes, most with merogony in erythrocytes, are not necessarily host-specific [2]. The sporozoites of these parasites are within invertebrate host, merozoites are thought to be transmitted to turtles by leeches [3, 4] and in general, larger turtles are better target to leech attachment because of their larger surface area [5]. *Haemogregarina* species are most frequently reported from turtles living within or nearby freshwater habitats among aquatic turtles [1]. *Haemogregarina stepanowi* Danilewsky, 1885, is Haemogregarine parasitizing the European pond turtle (*Emys orbicularis*). Recently in some studies prevalence and intensity of Haemogregarine parasites have been investigated in freshwater turtles including Romania [6], USA [7] and India [8]. In most cases, the level of infection was less than 2%. In a study of Haemogregarine on *Emys orbicularis* and *Mauremys caspica* in Iran it was shown that *H. stepanowi* is the only blood parasite in these turtles [9].

Three species of freshwater turtles, the Caspian pond turtle (*M. caspica*), the European pond turtle (*E. orbicularis*), and the Euphrates soft shell turtle (*R. euphraticus*) occur in Iran. However, the turtles of Iran have received scant scientific attention specially about diversity, distribution and abundance of protistan (protozoan) apicomplexan parasites. In this study, prevalence and intensity of *H. stepanowi* (family: Haemogregarinidae) in two species of turtles (*M. caspica* and *E. orbicularis*) were studied. We also aimed to define the effect of age and sex on the prevalence and intensity of these parasites.

2. Materials and Methods

A total of 27 turtles of two species were collected from 4 localities in Iran during period June-September 2013 (Fig.1). All work was carried out in spring-summer time, when turtles are most active. Turtles (*E. orbicularis* and *M. caspica*) were captured by fishing net. For each locality, coordinates and altitude have been recorded (Table 1). All specimens were identified to species [10], sexed, and their age was estimated based on the number of growth rings on their carapace [11].

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Blood samples of each specimen were collected from the dorsal coccygeal vein by insulin syringes. Blood smears on microscopic slides were prepared for each individual. They were air dried in the field, then fixed in absolute methanol and stained by Giemsa for 15-20 minutes in laboratory.

Stained blood smears were examined under Olympus BX-53 light microscope using 100 \times magnification lens equipped by immersion oil (Fig. 2). Digital camera and imaging software

(Quick Photo Camera 2.3) was used to examine slides and estimating calculation. Parasite species and their characteristics were identified according to Telford [1]. The intensity of parasitaemia was estimated for 10⁴ red blood cells (RBC). Prevalence was estimated as the percentage of infected tortoises. Single factor analysis of variance (ANOVA) was used to test for significant differences for effect of age, sexual prevalence and intensity of host parasites.

Table 1: Summary information from each sampling site

N	Locality	Latitude	Longitude	Species	No tortoise
1	Leil	37° 62'N	50° 87'E	<i>E.orbicularis</i>	3
2	Roodbaneh	37°15' N	50° 63'E	<i>E.orbicularis</i>	12
3	Samangan	34° 26' N	47° 33'E	<i>M. caspica</i>	7
4	Kermanshah	34° 34' N	47° 26'E	<i>M. caspica</i>	5



Fig 1: Map of Iran depicting the sampling sites

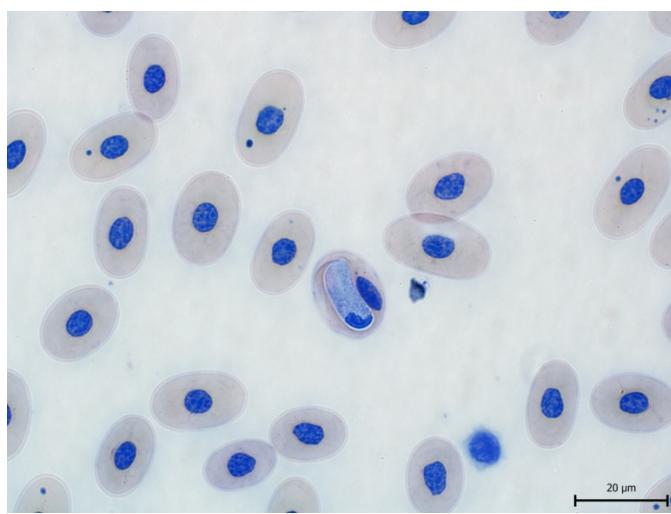


Fig 2: *Haemogregarina stepanowi* in blood cells of *Mauremys caspica*.

3. Results

Microscopic visualization confirmed the presence of *H. stepanowi* in blood smear of *E. orbicularis* and *M. caspica* (Fig.

2). A total of 11 individuals (73.3%) in *E.orbicularis* and 7 individuals (58.3%) in *M. caspica* were infected with *H. stepanowi*. The prevalence and mean intensity of parasitemia

for all sampled areas is given in Table 2.

The prevalence and intensity of parasites according to the sex and age of the host was reassessed. The prevalence of *H. stepanowi* for male and female *E. orbicularis* was 100% (n=2) and 83.33% (n=12), and in *M. caspica* was 100% (n=6) and 100% (n=4) respectively. The mean intensity of *H. stepanowi* for male and female in *E. orbicularis* and *M. caspica* is given in Table 3.

When we investigated parasite prevalence according to age, the turtles were classified in two age groups- adult (more than 3 years) and juvenile (1- 3 years). Prevalence of *H. stepanowi* in *E. orbicularis* for adult and juvenile was recorded as 91.66% (n=13) and 0% (n=2) respectively. In *M. caspica* prevalence of *H. stepanowi* for adults and juvenile was found to be 100% (n=9) and 50% (n=2) respectively. The mean intensity of *H. stepanowi* for adults and juvenile of *E. orbicularis* and *M. caspica* is given in Table 4.

Table 2: Results of parasites analyses for each locality.

No	Locality	No tortoises	prevalence	Mean parasitemia; SD; range
1	Leil	3	100	0.08± 0.37; 0.4-0.11
2	Roodbaneh	12	75	0.07± 0.09; 0.05-0.32
3	Samangan	7	87.5	0.19± 0.18; 0.01-0.53
4	Kermanshah	5	75	0.2± 0.33; 0.02-0.7

Table 3: Differences in intensity of *Haemogregarina stepanowi* in male and female of two species of freshwater turtles from Iran.

No	species	sex	No tortoises	Mean carapace length (mm)	Mean parasitemia; SD; range
1	<i>E. orbicularis</i>	male	2	120	0.17+0.21;0.02-0.32
2		female	12	133	0.05+0.03;0-0.11
3	<i>M. caspica</i>	male	6	175	0.29+0.28;0.02-0.53
4		female	4	176	0.11+0.07;0.02-0.2

Table 4: Differences in intensity of *Haemogregarina stepanowi* in different age groups (juveniles and adults) of two species of freshwater turtles from Iran.

N	species	age	No tortoises	Mean parasitemia; SD; range
1	<i>E. orbicularis</i>	Juvenile	2	0.03±0;0
		Adult	13	0.07±0.08;0-0.32
2	<i>M. caspica</i>	Juvenile	2	0.02±0.03;0-0.05
		Adult	9	0.19±0.21;0.02-0.34

4. Discussion

Various environmental characteristics in each locality are important ecological factors that could affect the occurrence, prevalence and intensity of parasite species [12]. Differences in habitats in various localities may cause considerable changes in the availability of suitable vectors of the parasites and the relative abundance of different kinds of host and vectors [12]. No previous work has been conducted on prevalence and intensity of parasitemia of Haemogregarines in the Iranian freshwater turtles. We found no difference in prevalence of parasitemia between *E. orbicularis* and *M. caspica*, while the mean intensity of parasitemia in *M. caspica* was higher than *E. orbicularis* (Table 2). The reason for lower parasitemia in *E. orbicularis* compared to *M. caspica* is not fully known and may relate to host specificity. In turtles most of the hemogregarines are transmitted by leeches and difference in level of parasitemia between two species of turtles may be related to leeches feeding behavior in different localities [6]. Also difference in parasitemia level could be affected by basking behavior of turtle. McAuliffer [13] suggested that basking turtles have lower parasitemia compared with non basking species, while Brooks *et al.* [14] found no statistical correlations between basking time and prevalence and intensity of the infection. There is no study on basking behavior between these two species of turtles in Iran. Alternatively McCoy *et al.* [15] proposed significant differences between the total skin surface area of a turtle and leech burdens that provide more area for parasite attachment. In our study the

mean length of carapace in *M. caspica* was larger than *E. orbicularis* (Table 3). Finally the difference rate of blood parasites infection between *M. caspica* (sampled in western Iran) and *E. orbicularis* (sampled in north Iran) may be related to environmental pollution [8]. We detected 75-100% of infection in studied turtles. Our results are in conformity with the results reported by many authors in various geographical regions (in *Chelodina expansa* from Australia [16]; in *Emys orbicularis* from Romania [6]; in *Lissemys punctata* and *Geoclemys hamiltonii* from India [8]). Our results on intensity of parasitemia are consistent with other studies with values ranging between 0.05 and 3% infected erythrocytes [6, 8, 17].

Many factors may be found to affect the prevalence of infection between different turtle species, sexes and sizes [8]. In our study on prevalence and intensity of *H. stepanowi* no significant difference was detected between males and females ($p > 0.05$). However, in some studies difference between Haemogregarine level in males and females has been reported [8, 18, 19]. Females of freshwater turtles (*Lissemys punctata* and *Geoclemys hamiltonii*) were found to be more prone to the Haemogregarine infection than males [8], while in lizard of Aruba island (*Cnemidophorus arubensis*) Haemogregarine prevalence was higher in males compared to females and for adults compared to juveniles [18]. Also in Spur-thighed tortoise (*Testudo graeca*) differences in prevalence of *Hemolivia mauritanica* (apicomplexa: Haemogregarinidae) between males and females in different localities did not provide

concordant results [19]. However because of different size and behavior of the two sexes, the chances of infection may be different [8]. The prevalence of parasitemia in adults was higher than juveniles. Similarity the intensity of parasitemia in adults was significantly higher than juveniles ($P<0.05$). Higher prevalence of *Haemogregarina balli* in adults of common snapping turtle (*Chelydra serpentina*) was recorded by Siddall and Desser [4]. This can be explained by bigger size of adults as better target for leeches [15] and also turtles with longer life span were more explore for leeches while Haemogregarine infection can be long-lasting [20]. In a study on lizards infected with Haemogregarine blood parasites, Sorci [20] found the infection lasted for more than one year.

5. Conclusion

This study for the first time expands the knowledge on the distribution and epidemiology of *H. stepanowi* in turtles of Iran. However further studies with more localities will be useful to better understand the epidemiology of this parasite. Although Haemogregarines blood parasite usually have little obvious clinical syndrome [21], baseline knowledge about distribution and epidemiology of this parasite in freshwater turtles can be useful to better understanding of disease threats that may emerge in the future as a result, for example, of habitat degradation.

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