On the Occurrence of a Haemogregarinae (Apicomplexa) Parasite from Freshwater Turtles of South 24 Parganas, West Bengal, India

Hindistan (Batı Bengal)'daki Tatlısu Kaplumbağalarında Hemogregarin (Apicomplexa) Parazitin Bulunuşu

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ABSTRACT

Objective: The aim of this study was to investigate Haemogregarine parasites (Apicomplexa: Haemogregarinidae) of freshwater turtles (*Lissemys punctata andersoni, Geoclemys hamiltonii*) of India.
Methods: Turtles were collected by net from two ponds of South 24 Parganas, West Bengal, India. A small amount of blood was taken from the subcarapacial vein puncture site. The blood smears were prepared and air dried and fixed in absolute methyl alcohol. The slides were stained with Giemsa.
Results: Haemogregarine parasites were recorded from the erythrocytes of turtles. Multiple stages of intraerythrocytic gametocytes (microgametocytes, macrogametocytes, early schizonts and mature schizonts) were observed in blood films.
Conclusion: It was found that only twenty out of the twenty five turtles (80%) were infected with the parasite. The prevalence rate was higher in larger turtles in comparison to smaller ones. It was also found that female turtles had a higher prevalence of infection than males.
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Key Words: Haemogregarine parasite, freshwater, turtle, *Lissemys punctata andersoni, Geoclemys hamiltonii*, India
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ÖZET

Amaç: Bu çalışmanın amacı Hindistan'daki tatlısu kaplumbağalarını (*Lissemys punctata andersoni, Geoclemys hamiltonii*) Hemogregarin parazitler (Apicomplexa: Haemogregarinidae) açısından araştırmaktır.
Yöntemler: Kaplumbağalar Hindistan'ın Batı Bengal bölgesindeki iki tatlısu kaynağından ağ ile toplanmıştır. Karapaks altındandaki toplardamardan az miktarda kan alınmıştır. Kan yayma preparatları hava yardımıyla kurutulmuş ve absolü metal alkolde tespit edilmiştir. Preparatlar Giemsa'yla boyanmıştır.
Bulgular: Kaplumbağaların eritrositlerinde Hemogregarin parazit saptanmıştır. Kan yayma preparatlarında eritrosit içi gametositlerin çeşitli safhaları (mikrogametositler, makrogametositler, genç şizontlar ve olgun şizontlar) gözlemlenmiştir.
Sonuç: İncelenen 25 kaplumbağanın 20'si (%80) parazitle enfektedir. Parazitin yaygınlık oranı büyük kaplumbağalarda küçüklere oranla daha fazladır. Dişi kaplumbağaların erkek kaplumbağalardan daha yüksek enfeksiyon yaygınlığına sahip olduğu da tespit edilmiştir.
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Anahtar Sözcükler: Hemogregarin parazit, tatlısu, kaplumbağa, *Lissemys punctata andersoni, Geoclemys hamiltonii*, Hindistan
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INTRODUCTION

Turtles are primitive animals and parasites of turtles bear a great importance from an evolutionary point of view. Freshwater turtles are generally prone to various intraerythrocytic blood parasites infection (1-3). In 2009, Telford established that intracellular erythrocytes are common and widely distributed in freshwater turtle (4). These parasites are found in the cytoplasm of host erythrocytes, are banana shaped and are not necessarily host-specific (5).

Haemogregarines were first described by Danilewsky in 1885 from a European tortoise, *Emys orbicularis* (Linn) (6). The detailed account of the structure and life cycle of Haemogregarine parasite in the same tortoise was described by Reichnow in 1910 (7). In India, chelonian Haemogregarine was first observed by Simond in 1901 who described two Haemogregarines, *Haemogregarina laverani* and *Haemogregarina mesnili* (8); they worked on *Emyda granosa* and *Emys tectum,* respectively. Many scientists like Wang and Hopkings (9), Marquard (10), Strohlein and Christensen (11), McAuliffe (12), Acholonu (13), Edney (14) and Siddal and Desser (15), have worked a lot on Haemogregarine infection of turtles. The parasite takes up over half of the volume of an infected erythrocyte and destroys the host's blood cell causing anaemia (16, 17). According to Sheldon and Verhulst (18), the host develops an immune defence against the parasite. Haemogregarines have an indirect life cycle involving definitive invertebrate hosts and vertebrate hosts such as lizards, turtle, snake or frogs (19-21).

Wintrobe (22) established that Haemogregarines cause a depletion of haematocrit levels. As a result, infected turtles may have reduced concentrations of haemoglobin and the capacity for oxygen transportation to muscle tissue (23, 24). The parasites are mechanically transmitted by either mite or tick or by arthropod or leech. According to Paperna (25) and Siddal and Desser (26) the Haemogregarine infections are thought to be transmitted by leeches as the vector. Readel et al. (27) reported that because of the large surface area, the hosts are more prone to leech attachment.

Kntotkova et al. (28) established that heavy infection with Haemogregarines causes poor health in turtle populations. Paperna (25) and Siddal and Desser (26) reported that bottom-dwelling turtles are more prone to infection than basking turtles. Ryan and Lambert (29) and Strohlein and Christensen (30) also observed the highest infection of Haemogregarines in bottom-dwelling turtle. Moreover, McCoy et al. (31) reported that female hosts are more susceptible to infection than males.

The aim of this study was to investigate Haemogregarine parasites (Apicomplexa: Haemogregarinidae) of freshwater turtles (*Lissemys punctata andersoni*, *Geoclemys hamiltonii*) in (West Bengal) India.

METHODS

Turtles were captured from two ponds located in the South 24 Parganas district of West Bengal, India, using nets. Two species were collected from the pond for examination and brought to the laboratory alive. The length of carapace (CL) of each individual was recorded. A small amount of blood was taken from subcarapacial vein puncture site. The blood smears were prepared and then air dried and fixed in absolute methyl alcohol. Then, the slides were stained with Giemsa (pH 6-8) for 45 minutes and dried again. The dried slides were mounted in DPX. Finally, all the turtles were left on the sand to recover for about 2-3 hours before being transferred back to the water.

RESULTS

The slides were examined under the phase contrast microscope for identification of the parasite. The Haemogregarine parasites stained light blue with Giemsa. Due to the presence of parasites, the shape of the erythrocytes was changed; the red blood cells became elongated in order to accommodate the parasite, and the nucleus of the erythrocyte became displaced to one pole. The cells looks like banana-shaped. Multiple stages of intraerythrocytic Haemogregarine gametocytes were observed in blood films including microgametocytes, macrogametocytes, early schizonts and mature schizonts. The microgametocytes were kidney-bean shaped with a light cytoplasm and disperse nucleus. (Fig. 1A, B). The average area of microgametocytes was found to be 20.896±0.42 μm^2. The macrogametocytes were elongated, slightly curved with granulated deep stain cytoplasm and condensed nucleus (Fig. 1C, D). The average area of macrogametocytes was found to be 24.907±0.64 μm^2. Intraerythrocytic early schizonts were oval- to bean-shaped with four nuclei (Fig. 1E, F), and an average area of 35.078±0.63 μm^2. The mature schizonts were bean-shaped and contained eight nuclei (Figure 1G, H). The average area of mature schizonts was found to be 29.168±0.31 μm^2 (Table 1).

A total of twenty five turtles were collected and examined for Haemogregarine parasites. The *Lissemys punctata andersoni* and *Geoclemys hamiltonii* were infected with a prevalence rate of 93.33% and 60.0%, respectively (Table 2). There were significant differences in relation to body size of the host. The prevalence rate was higher in larger turtles in comparison to smaller ones (Table 3). It was found that only twelve out of thirteen (92.31%) females and eight out of twelve (66.66%) males were infected with Haemogregarine parasites (Table 4). Therefore, it appeared that female turtles were more susceptible to infection.

DISCUSSSION

The different developmental stages found in this study agree with the findings of Paperna et al. (32). The morphological characteristics of gametocytes have been rejected by many authors (33, 34) as valid criteria for differentiating genera of Haemogregarines because: (i) the gametocytes of two or more species may be present in the same vertebrate host (35), (ii) confusion may arise between mature and immature gametocytes of the same species, which could also be considered different species (34), and (iii) macro- and microgametocytes of the same species might be considered different species (35, 36). Therefore, the developmental stages found in vertebrate hosts (intermediate host) and invertebrate hosts (definitive host) involved in the life-cycle, and the biogeography and ecology of the vertebrate hosts, have been used to differentiate genera of these blood parasites. On the basis of some characteristics of the develop-

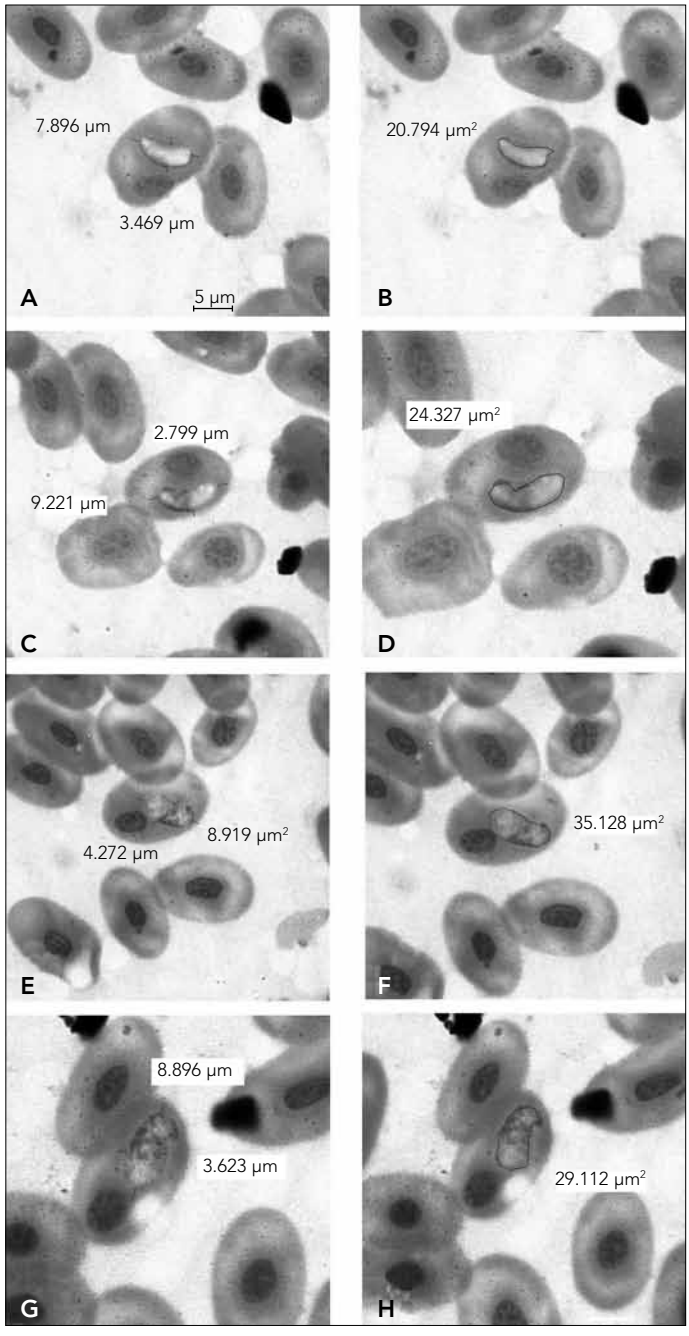

Figure 1. Multiple stages of intraerythrocytic Haemogregarine gametocytes. A, B. Microgametes, C, D. Macrogametes, E, F. Early schizonts, G, H. Mature schizonts

mental stages, it is confirmed that the organism belongs to the genera Haemogregarina.

The study revealed that the Haemogregarine infection was highest in turtles; infections were found in all two turtle species. In the present study, the overall prevalence of Haemogregarine in freshwater turtles was 80%. Many differences in the prevalence of infection were found between different turtle species, sexes and size. *Lissemys punctata andersoni* and *Geoclemys hamiltonii* were infected with a prevalence of 93.33% and 60.0%, respectively (Table 2). This agrees with the previous works (25, 26, 29, 30), which stated that bottom dwelling turtles,

Table 1. Measurement of different intraerythrocytic stages of haemogregarine parasite

Diff. stages	Length±SD (range) in µm	Width±SD (range) in µm	Area±SD (range) in µm²
Microgametocytes	7.859±0.55	3.657±0.28	20.531±0.42
Macrogametocytes	9.152±0.48	3.301±0.31	24.907±0.64
Early schizonts	8.716±0.44	4.170±0.22	35.078±0.64
Mature schizonts	8.624±0.49	3.607±0.32	29.168±0.31
SD: standard deviation			

Table 2. Showing the prevalence of Haemogregarine infection in two freshwater turtles

No.	Name of host species	No. examined	No. infection	Prevalence (%)
01	*Lissemys punctata andersoni*	15	14	93.33
02	*Geoclemys hamiltonii*	10	06	60.00

Table 3. Showing the levels of Haemogregarine parasitemia in turtle species examined in the study based on mean carapace length (cm)

No.	Name of the host species	No. infection	Mean carapace length (cm)	Ave. Parasitemia±SD (% of infected erythrocytes)
01	*Lissemys punctata andersoni*	14	29	0.91±0.12
02	*Geoclemys hamiltonii*	06	31	0.39±0.04

Table 4. Showing the prevalence of Haemogregarine infection in freshwater turtles based on sex

No.	Sex of the host species	No. examined	No. infection	Prevalence (%)
01	Male	12	08	66.66
02	Female	13	12	92.31

Lissemys punctata andersoni are more prone to the Haemogregarine infection than basking ones. There were significant differences in relation to the body size of the host; the prevalence rate was higher in larger turtles in comparison to smaller ones. Also, females were found to be more prone to infection than males, which are less affected. Since the surface area of females is large, the chance of infection is more. It can be said that all turtle species were susceptible to infection. The present study revealed that the prevalence of Haemogregarine infection was 80% in India, whereas it was 70% in North America (13), 93% in Australia (37), and 100% in Romania (38).

Thus, it appeared that the infection rate was highest in Romania in comparison to other countries, which may be due to environmental pollution.

Multiple stages of intraerythrocytic Haemogregarine gametocytes were observed in blood films. Microgametocytes, macrogametocytes and early schizonts contain four nuclei whereas the mature schizonts have eight nuclei. The study revealed that the level of Haemogregarine infection was generally low in both turtles, varying from 0.39 to 0.91% of erythrocytes containing parasites, both of which are less than 1% (Table 3). This work agrees with prior investigations made by Wang and Hopkins (9), McAuliffe (12), Storhlein and Christensen (11) and Siddal and Desser (15).

CONCLUSION

This observation corroborated with the findings of Oppliger et al. (23) and Veiga et al. (24). Due to the presence of Haemogregarine parasites, the turtles may have had reduced concentration of haemoglobin, meaning that the capacity for oxygen transportation to muscle tissue decreased. Since this is involved in various aspects of turtle physiology and behaviour, such as foraging efficiency or sprint speed, it may also affect the body condition. Therefore, it can be inferred that the Haemogregarine infection may cause damage to the blood cells which causes anaemia in the host.

Conflict of Interest
No conflict of interest was declared by the authors.

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Author Contributions
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