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Concurrent infection of *Pseudomonas* and *Haemogregarina* Sp. in fresh water turtle (*Lissemys Punctata*)

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Abstract

Indian flap-shelled fresh water turtles (*Lissemys punctata*) were presented to Veterinary Clinical Complex, Veterinary College, Bidar with the history of inappetence, respiratory distress and skin and shell infection. On clinical examination turtles were lethargic, inactive with foamy nasal discharge. White necrotic spots and haemorrhagic ulcerative lesions were seen on both carapace and plastron. An attempt was initiated for symptomatic treatment; however both the turtles succumbed to death without responding to the treatment. On post-mortem examination, along with ulcerative lesions on both carapace and plastron, froth mixed with debris filled nares, trachea and lungs. Isolation and identification revealed the presence of *Pseudomonas aeruginosa* from skin swab, tracheal swab and lung samples, whereas blood smears and lung impression cytology showed to harbour intra-erythrocytic *Haemogregarina* sp. Hence, the concurrent infection of these two opportunistic pathogens might have caused severe infection and led to death of fresh water turtles in captivity.

Keywords: Indian flap-shelled fresh water turtles, *Pseudomonas aeruginosa*, *Haemogregarina* sp. and concurrent infection

Introduction

Aquatic turtles in captivity are under great deal of stress and succumb to bacterial, viral, fungal and protozoan infections. Turtles being affected either of these agents alone or concurrent infection may end up with fatal condition. *Pseudomonas* sp. are commonly found in natural sources of water and associated with septicemia in aquatic animals [1]. These bacteria are considered opportunistic pathogens, causing disease when the host is under stress. A number of aquatic animals including fish, frogs and turtles are reported to be susceptible to *Pseudomonas* spp. with moderate to high losses [2]. Freshwater turtles are also generally prone to various intra-erythrocytic haemoparasitic infections like *haemogregarina* sp. (Apicomplexa: Adeleina). These parasites are found in the cytoplasm of host erythrocytes, are banana shaped and are not necessarily host specific [3,4]

In India, Indian flap-shelled fresh water turtles (*Lissemys punctata*) are common in lakes ponds and rivers and they are well adapted morphologically and behaviorally to Indian conditions [3,5]. Often they are exploited for profit as a food source and also as pets in captivity. Due to which Indian flap-shelled fresh water turtles are under huge stress and become susceptible to various pathogens. There are few reports on pathogens involved in the occurrence of disease in fresh water turtles across different states of India.

Therefore, this paper deals about the clinical implication and diagnosis of concurrent infection of *pseudomonas* sp. and *haemogregarina* sp. in Indian flap-shelled fresh water turtles.

Materials and Methods

Two Indian flap-shelled fresh water turtles (*Lissemys punctata*) were brought to Veterinary Clinical Complex, Veterinary College, Bidar, Karnataka, with a history of inappetence, respiratory distress and skin and shell infection. On clinical examination, the turtles were suspected for bacterial infection. Swabs from the lesions and little quantity of blood by pricking the dorsal coccygeal vein were collected. An attempt was made to isolate the bacteria and also screened the blood smear for any haemoparasites.

Then the samples of an aliquots and blood smears were prepared and submitted to Central Institute of Brakish water aquaculture (CIBA), Chennai, India for further confirmation. Meanwhile turtles were symptomatically treated by frequent dipping in KMnO₄ (3times/day for 15-20 min) and painted the lesions with povidone iodine solution and kept for drying. To reduce the infection parenteral antibiotic enrofloxacin (5mg/kg, IM) was administered [6]

Necropsy was performed immediately and gross findings were recorded. Ancillary tests were performed to identify the etiological agents. Blood smear, lung, liver, spleen, and kidney impression smears were prepared on a clean microscopic slide, air dried and fixed with methanol and stained with Giemsa's and Gram's stain and examined. Both morphological analysis and marphometric analysis were carried out to identify intra-erythrocytic parasite sp. and its load [7].

Results and Discussion

Two fresh water flap-shelled turtles were brought to hospital with history of being inactive, having inappetence. Clinical examination revealed respiratory distress with foamy nasal discharges, white necrotic spots all over the body and hemorrhagic, ulcerative lesions on both carapace and plastron (fig 1, 2). The microscopic examination of blood smear smears and other organ impressions showed abundant Gram negative bacteria, leukocytes and presence of intra-erythrocytic banana shaped hemoparasites. For further confirmation of the species the samples of aliquots and blood smears were submitted to CIBA, Chennai. The results confirmed the presence of concurrent infection of *Pseudomonas aeruginosa* and *Heamogregarina* sp. in the infected turtles. Many earlier workers have reported the presence of these two species in turtles and were commonly noticed as natural commensal or opportunistic pathogens [6, 7, 8]. The presence of these organisms indicates poor quality of water in the habitat and possible stress condition might have lead to severe infection in the turtles. Under stress condition the natural commensal or opportunistic pathogens may pose a potential threat to turtles [7]. In the present study both the turtles showed white necrotic spots and ulcerative lesions over both carapace and plastron. The lungs showed congestion, oedema and few petichiae (fig. 3). Respiratory distress with foamy nasal discharge may be due to *Pseudomonas aeruginosa* infection which was evident with abundant bacteria and leukocytes in the lung impression smear. Most of the RBC's in these turtles were found to harbour light blue stained unicellular, round to elongated and some as banana shaped intra-erythrocytic *Heamogregarina* spp. Parasites (fig 4). Upon morphological evaluation more than 20 per cent of the RBCs were infected with gamatocytes of different sizes and shape, pushing the nucleus to the periphery. The average, sizes of gametocytes were 7.3 to 11.7 µm (L) X 3.2 to 4.3 µm (W) by marphometrical study. The infected RBC's were bigger in size with change in the shape compared to non-infected ones. (fig 4). Presence of more number of *Haemogregarina* sp. parasites in the RBC's causes reduced concentration of haemoglobin and damage to the RBC's which further may cause anaemia in infected turtle [6]. After 24 hrs of initiation of treatment, turtles succumbed to death without responding to treatment due to concurrent bacterial infection and severe parasitaemia. On post-mortem examination, along with white necrotic ulcerative lesions on the skin and shell, respiratory tract showed hyperaemia and filled with froth and debris in nares, trachea and lungs (fig 3). *Pseudomonas aeruginosa* was isolated from the lung samples

and lung impression smears revealed the presence of Gram negative bacteria with leukocytes and *Haemogregarina* spp. in the RBC's. This confirms concurrent infection of *Pseudomonas aeruginosa* and haemoprotozoa *Haemogregarina* sp. leading to severe clinical outcome resulting in death in the Indian flap-shelled fresh water turtles.



Fig 1: Showing white necrotic spots all over the body



Fig 2: Showing hemorrhages with, ulcerative lesions on both carapace and plastron

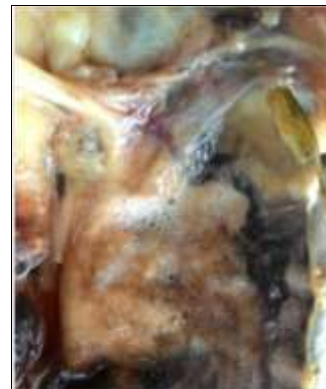


Fig 3: Showing lung congestion, edema and petichiae

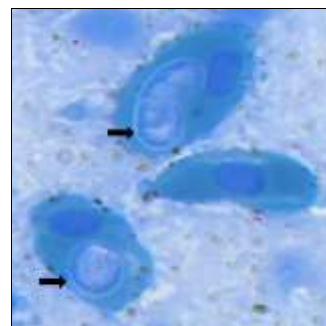


Fig 4: Showing infected RBC's with different sized gametocytes of *Heamogregarina* spp.

Conclusion

In the present study *Pseudomonas aeruginosa* and *Haemogregarina* sp. parasites were identified in the sick turtles. These are thought to be opportunistic pathogens, however they may flare up and turned to be pathogenic in conducive atmosphere. Results indicated that turtles might have been kept in contaminated water source under captivity for long time which itself might have put turtles under stressful condition. Due to which these opportunistic pathogens flared up and in concurrent infection may have caused severe clinical outcome leading to death in the turtles. Hence, turtle care requires proper housing, nutrition, health and good hygiene in captivity.

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