# Haematology and plasma chemistry of Bornean river turtles suffering from shell necrosis and haemogregarine parasites

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**ABSTRACT**: Nine Bornean river turtles (*Orlitia borneensis*, Gray, 1873) suffering from lethargy, ulcerations and caseous necrosis of the plastron were evaluated for haematology and plasma chemistry. Intra-erythrocytic haemogregarine parasites were associated with anaemia, low haemoglobin, basophilia, eosinophilia, heterophilia and azurophilia. After eight months of treatment consisting of antibiotics, debridement and scrubbing of lesions with enilconazole or povidone iodine, rehydration, deworming and tube feeding, lymphocytes, basophils, eosinophils, heterophils and azurophils returned to the normal ranges. Haematocrit, RBC and haemoglobin concentration were under the normal ranges in 24 months. A progressive decrease in haemogregarine parasitaemia was also seen, however, haematologic changes could not be definitely attributed to these parasites.

**Keywords**: reptiles; *Orlitia borneensis*; blood profile; blood parasites

The Bornean river turtle (or Malaysian giant turtle) *Orlitia borneensis* (Gray, 1873) is a large semiaquatic turtle living in the rivers and lakes of Malaysia, Sumatra and Borneo. Hundreds of these turtles were confiscated in 2001 in Macau and suitable candidates taken to various zoos in Europe and USA. The turtles were in poor health condition, many had ingested fish hooks and one group had chronic ulcerative shell necrosis (Knotek et al., 2003). Clinical signs consisted of necrotic ulcerations of the shell and skin, general weakness and anorexia. Gram-negative bacteria as well as yeast elements were identified cytologically at the sites of deep shell lesions. The aim of this study was to compare haematology and the plasma chemistry profile in Bornean river turtles suffering from ulcerative shell necrosis with the data obtained from the animals after eight months of intensive treatment and after complete shell healing.

## MATERIAL AND METHODS

Nine Bornean river turtles (*Orlitia borneensis*, Gray, 1873) were included in this study (Table 1). Turtles were sexed by prolapsing the hemipenis from the cloaca, weighed (electric scale Tonava TH 20, Czech Republic) and measured (length and width of plastron, Table 1) upon admission to the zoo. Females were evaluated for the presence of eggs and none of them had eggs as controlled by manual control and/or radiographical imaging. Faecal exams (faecal flotation method) were done twice within the first week and they revealed the presence of parasite eggs (ascarids). Patients were therefore dewormed with mebendazole (25 mg/kg b.w., PO, 2× in 14 days).

Blood (2–3 ml) was collected from the coccygeal vein using of 23G × 1 Luer needles (Redrobe and McDonald, 1999) at the beginning of the treat-

Table 1. *Orlitia borneensis* turtles included in this study

| Turtle | Sex | Plastron length × width (cm) |
|---|---|---|
| 1 | M | 46.0 × 26.5 |
| 2 | F | 41.5 × 26.5 |
| 3 | F | 42.0 × 27.0 |
| 4 | F | 37.0 × 21.5 |
| 5 | F | 42.0 × 28.0 |
| 6 | F | 37.5 × 27.0 |
| 7 | F | 38.0 × 24.0 |
| 8 | F | 35.0 × 23.0 |
| 9 | F | 42.0 × 26.0 |

ments, after 8 and 32 months. Whole blood was placed in heparinized tubes (Leciva inj., Prague), centrifuged immediately, and plasma was removed and frozen (–20°C). Plasma was analysed within 24 hours using an automated analyser (CobasMira, Roche) for total protein (TP), glucose, uric acid, blood urea nitrogen (BUN), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), phosphorus (P). Plasma calcium concentrations were analysed with an Atomspec analyser (Hilger 1550). Haematocrit was measured by microhaematocrit tubes. Haemoglobin (Hb) was determined by the cyanmethaemoglobin method, and total red and white cell counts were determined according to Natt and Herrick (1952). Blood smears were prepared immediately: they were air-dried and stained on a cover slip using May-Grünwald and Giemsa-Romanowski stain. Two hundred leukocytes were counted for each smear and classified as heterophils, eosinophils, basophils, lymphocytes, azurophils and monocytes (Mader, 2000). Five thousand red cells on each smear were examined for the presence of intra- and extra-cellular parasites. Statistical comparison of differences in haemograms and plasma chemical values of turtles before and after treatment was performed by the *t*–test (STAT Plus software manual 1.01; Matouskova et al., 1992).

All nine turtles included in this study suffered from necrotic ulcerative shell necrosis with severe lesions on the plastron and carapace (Table 2, Figures 1, 2). Surgical intervention therefore consisted of the debridement of necrotic tissue from the plastron and carapace followed by scrubbing with enilconazole or povidone iodine. Three to four turtles were placed in a plastic basin (2.5 m × 1.7 m × 0.6 m, air temperature 28–35°C, water temperature 22–27°C) with salt water (0.025% NaCl solution, 0.5 kg NaCl/1700 l) for 6 hours and then kept out of water on the clean floor (to minimise the risk of mycotic infection). During the first month antibiotics were injected daily (from 14 to 30 days, according to the healing of plastron and carapace injuries).

The supportive treatment protocol for necrotic ulcerative shell necrosis was based on the administration of fluid (20–230 ml/kg b.w., ICC), systemic antibiotic treatment (enrofloxacin 5–10 mg/kg b.w.,

Table 2. Health condition of *Orlitia borneensis* turtles included in this study

| Turtle | Shell lesions | | Weight (kg) | | Comment 7. 12. 2004 | |
|---|---|---|---|---|---|---|
| | 15. 4. 2002 | 7. 12. 2004 | 15. 4. 2002 | 7. 12. 2004 | | |
| 1 | severe | severe | 9.2 | 9.8 | died (24. 4. 2003) | chronic liver + renal disease[b] |
| 2 | severe | mild[a] | 7.4 | 11.1 | good health condition | quarantine aquarium |
| 3 | severe | none | 9.0 | 12.3 | good health condition | exhibition aquarium |
| 4 | severe | moderate | 5.6 | 7.1 | good health condition | quarantine aquarium |
| 5 | severe | mild | 8.4 | 11.9 | good health condition | quarantine aquarium |
| 6 | severe | mild[a] | 6.1 | 7.9 | good health condition | quarantine aquarium |
| 7 | severe | none | 6.5 | 8.4 | good health condition | quarantine aquarium |
| 8 | severe | severe | 5.5 | 6.0 | died (13. 5. 2003) | chronic liver + renal disease[b] |
| 9 | severe | none | 8.4 | 11.5 | good health condition | exhibition aquarium |

[a]small white dots of depigmented shell; [b]post-mortem examination

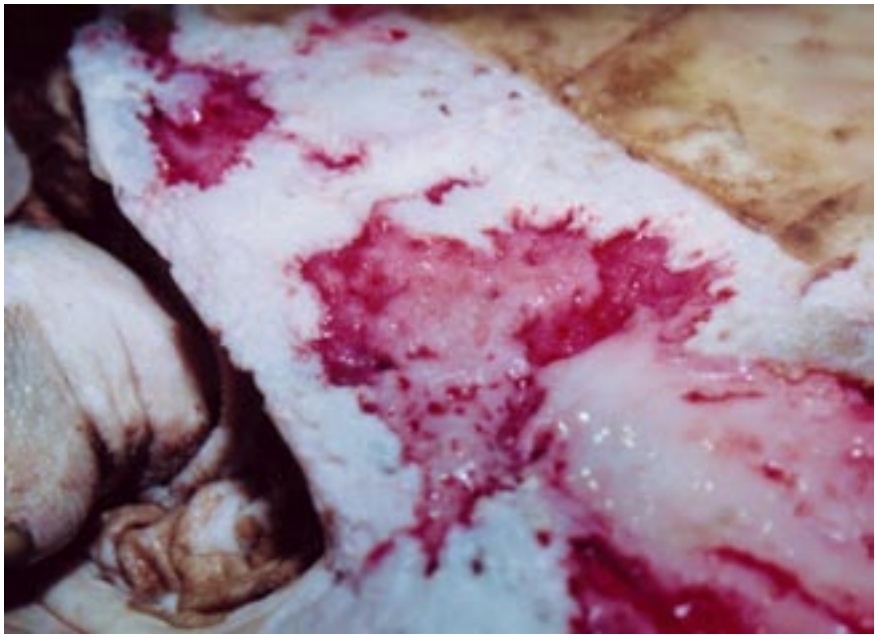

Figure 1. Deep necrosis of the plastron in *Orlitia borneensis*

SC to the front legs) and tube feeding (a semi-fluid mixture was composed of bananas, fishes, earthworms, insects and fruits, supplemented with calcium). A balanced diet (grapes, bananas, fishes, rats, earthworms, insects, gelatine with pieces of fruits) supplemented with calcium was provided *ad libitum*. In the subsequent 31 months, the health status of each turtle was evaluated by clinical examination (once a week).

## RESULTS

After 8 months of the treatment, 6 out of the 9 turtles were still affected with lesions on the plastron with 3 having mild lesions, 1 having moderately-severe and 2 having severe lesions. Only small white dots of depigmented shell were present in 2 turtles after 32 months (Table 2). After eight months of the treatment the number of leukocytes and lymphocytes decreased significantly ($P < 0.01$), the number of basophils, eosinophils, heterophils and azurophils returned to the normal ranges (Table 3). After 24 months the number of leukocytes increased, and after 32 months of this study plasma concentrations for total protein were very low. BUN increased significantly ($P < 0.01$), then returned back to the normal range. Plasma levels for glucose, uric acid, ALP, ALT, AST, calcium and phosphorus were within normal ranges for healthy

Figure 2. Carapace shell necrosis in *Orlitia borneensis*

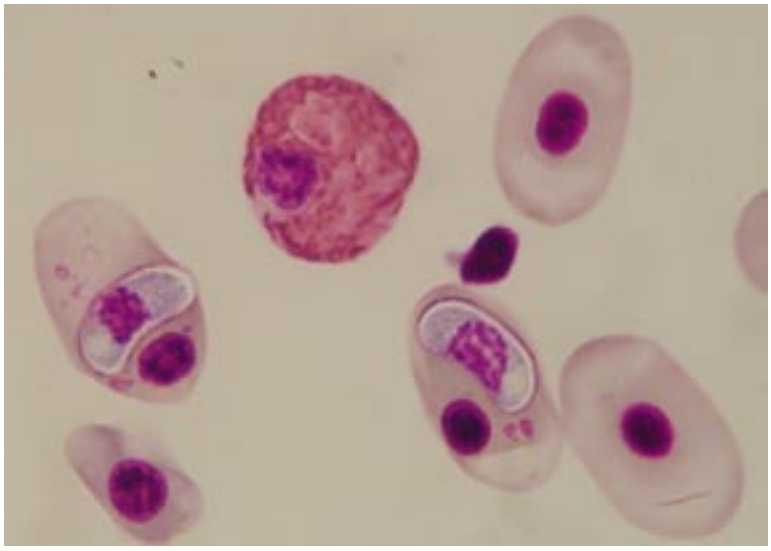

Figure 3. The banana-shaped intraerythrocytic haemogregarine gamonts

chelonians (Table 3). The banana-shaped intraerythrocytic nucleated organisms were seen in red cell cytoplasm (Desser, 1993; Desser and Bennett, 1993; Barta, 2002) in all turtles with parasitaemias ranging from 0.2 to 28.2 % (mean 59/5 000 RBC, Figure 3). Parasites were present (mean 27/5 000 RBC) in stained blood smears of five out of the nine patients. Parasites were present (mean 3/5 000 RBC)

Table 3. Haematology and plasma chemistry in *Orlitia borneensis* turtles (mean ± SD)

| Variable | 15. 4. 2002 $n = 9$ | 9. 12. 2002 $n = 9$ | 7. 12. 2004 $n = 7$ |
|---|---|---|---|
| Total protein (g/l) | 21.1 ± 8.9 | 22.3 ± 17.4 | 26.1 ± 11.5 |
| Glucose (mmol/l) | 3.9 ± 0.5 | 3.7 ± 0.9 | 5.0 ± 1.0 |
| Uric acid (μmol/l) | 16.7 ± 4.5[a] | 9.2 ± 8.4[b] | 41.8 ± 19.0[c] |
| BUN (mmol/l) | 14.3 ± 3.9 | 28.8 ± 5.8 | 4.4 ± 2.9 |
| ALP (μkat/l) | 2.3 ± 0.9[b] | 2.1 ± 1.8[b] | 7.0 ± 5.4[c] |
| ALT (μkat/l) | 0.03 ± 0.02[a] | 0.08 ± 0.05[b] | 2.07 ± 0.03[c] |
| AST (μkat/l) | 0.6 ± 0.3 | 0.5 ± 0.3 | 0.6 ± 0.3 |
| Ca (mmol/l) | 1.8 ± 0.2 | 1.9 ± 0.3 | 2.1 ± 0.3 |
| P (mmol/l) | 1.2 ± 0.6 | 1.0 ± 0.4 | 1.1 ± 0.5 |
| Haemoglobin (g/l) | 19.1 ± 8.3 | 24.1 ± 14.3 | 25.5 ± 12.2 |
| PCV (l/l) | 0.15 ± 0.10 | 0.16 ± 0.10 | 0.19 ± 0.10 |
| RBC ($10^{12}$/l) | 0.26 ± 0.10 | 0.32 ± 0.20 | 0.36 ± 0.20 |
| WBC ($10^9$/l) | 14.5 ± 6.4[b] | 6.6 ± 4.0[c] | 7.4 ± 3.2[c] |
| Heterophils ($10^9$/l) | 4.3 ± 2.4 | 2.3 ± 1.9 | 3.4 ± 1.4 |
| Lymphocytes ($10^9$/l) | 5.7 ± 2.9[b] | 2.1 ± 1.9[c] | 0.7 ± 0.3[c] |
| Azurophils ($10^9$/l) | 0.9 ± 0.6 | 0.5 ± 0.3 | 0.5 ± 0.3 |
| Eosinophils ($10^9$/l) | 1.4 ± 1.1 | 0.7 ± 0.6 | 1.4 ± 0.8 |
| Basophils ($10^9$/l) | 2.2 ± 1.9 | 0.9 ± 0.5 | 1.2 ± 1.3 |
| Parasites ($n$/5 000 RBC) | mean 59 | min.–max. 1–141 | mean 27 | min.–max. 0–104 | mean 3 | min.–max. 0–8 |

[a–b]$P < 0.05$; [b–c]$P < 0.01$

in the peripheral blood of four out of the seven patients.

## DISCUSSION

Chronic diseases of turtles and tortoises due to inadequate husbandry are commonly encountered in veterinary practice (Frye, 1991; Cooper, 1992; Rossi, 1996) causing alterations of biochemical profiles (Samour et al., 1986; Marks and Citino, 1990; Knotkova et al., 2000). Animals with extensive shell defects from the confiscated contingent of turtles *Orlitia borneensis* suffered from hypoproteinaemia, anaemia and hyperuricaemia. The alterations in haematocrit, number of erythrocytes and haemoglobin concentration, basophilia, eosinophilia, heterophilia, and azurophilia can be regarded as typical of patients with ongoing infection suffering from undernutrition and chronic stress (Hawkey and Dennett, 1989; Frye, 1991). Despite of the better health status of the patients, the heterophil to lymphocyte ratio at the end of treatment was high. The reason for this phenomenon is not clear and more studies focused on the normal blood profile of Bornean river turtles are needed. The intensive therapy of Bornean river turtles *Orlitia borneensis* was aimed at suppressing mixed shell infection and improving the alimentary status of the reptiles. All injections of the antibiotic (enrofloxacin) were administered into the front legs because of a possible influence of the renal portal system on the plasma levels. However, the existence of the renal portal system in chelonians is not accepted by some authors and further studies are needed (Beck et al., 1995; Holz et al., 1997). The adjustment of some of the blood count parameters to the physiological range and a significant improvement of the shell status in all animal patients suggest that the comprehensive intensive therapy was successful. Only residual dots of depigmented shell were present in 2 out of the 9 patients after 32 months. The intensive care and nutrition led to significantly increased body weight in 7 out of the 9 turtles ($P < 0.01$). The persisting hypoproteinaemia, low haematocrit and low number of erythrocytes in peripheral blood of *Orlitia borneensis* turtles after 32 months suggest that at least some of these turtles were not in optimal condition yet despite the complete healing of deep traumatic lesions of the shell. The fact that four out of seven turtles remained parasitaemic with haemogregarines after 32 months of optimal care raises the questions to what extent their presence and/or perhaps other factors such as lack of yet undetermined essential nutrients in their diet could have been reflected in the above-mentioned alterations of the blood count. In the present study we did not focus on the restriction of the amount or eradication of blood parasites. No successful chemotherapy of haemogregarines has been reported for reptiles (Barnard and Upton, 1994; Lane and Mader, 1996). We suggest that the continually decreased number of haemogregarines present in erythrocytes of Bornean river turtles in this study resulted from their limited life cycle in artificial conditions. The other theories, like that the supportive care can increase the reptilian immune response and suppress the number of circulating parasites need to be confirmed.

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