

Hematozoa of Snakes in Queen Saovabha Memorial Institute

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ABSTRACT

During December 1997 to March 2001, 275 blood samples of snakes from eighteen species of four families, including Pythonidae, Colubridae, Elapidae and Viperidae, at Queen Saovabha Memorial Institute were examined for blood parasites. 111 samples (40.4%) were positive for hematozoa infections. The single infection (38.2%) were following genera: *Hepatozoon* (32.4%), *Haemogregarina* (4.0%) and trypanosome (1.8%). The incidence rates of mixed infections between *Hepatozoon* and *Haemogregarina* and between *Hepatozoon* and trypanosome were 0.4% and 1.8% respectively. *Hepatozoon* infections were found in mangrove snake (*Boiga dendrophila melanota*), mask-faced water snake (*Homalopsis buccata*), rainbow water snake (*Enhydryn enhydryn*), copper head racer snake (*Elaphe radiata*), common rat snake (*Ptyas mucosus*), Siamese spitting cobra (*Naja siamensis*), golden spitting cobra (*Naja sumatrana*), king cobra (*Ophiophagus hannah*), banded krait (*Bungarus fasciatus*), Malayan krait (*Bungarus candidus*), mangrove pit viper (*Trimeresurus purpureomaculatus*) and Siamese russell's viper (*Daboia russellii siamensis*). There were three types of gamont of *Hepatozoon* sp., including small gamont ($3.0 \times 10.9 \mu\text{m}$, n=120), medium gamont ($3.3 \times 14.8 \mu\text{m}$, n=60) and the large gamont ($3.9 \times 15.0 \mu\text{m}$, n=140). *Haemogregarina* infections were found in Burmese python (*Python molurus bivittatus*), mangrove snake, mask-faced water snake and rainbow water snake. The gamonts of *Haemogregarina* sp. were very large ($7.6 \times 15.2 \mu\text{m}$, n=40). Trypanosomes were found only in mask-faced water snake and rainbow water snake. They were large flagellate hematozoa with 10-20 μm body width ($14.1 \pm 3.0 \mu\text{m}$, n=120).

Key words: *Haemogregarina*, hematozoa, *Hepatozoon*, Queen Saovabha Memorial Institute, snake, trypanosome

INTRODUCTION

There are 163 species of snake in Thailand. The 48 venomous species of snake are in the family Elapidae and Viperidae (Jintakune and Chanhom, 1996). The number of poisonous snakes for antiserum production in Queen Saovabha Memorial Institute (QSMI) was decreasing. Establishing of snake farm in QSMI was started in 1994.

Hematological examinations were performed in these snakes for general health examination.

Hematological examination has been used in the lower vertebrates during the last 20 years (Frye, 1991). There were a number of hemoparasites in reptiles (Campbell, 1996). The major hematozoas found in reptiles were trypanosomes, *Plasmodium* and haemogregarines. The *Hepatozoon*, *Haemogregarina* and *Karyolysus* were difficult to

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differentiate when found in the blood so were grouped as haemogregarines. In general, *Haemogregarina* is found in aquatic reptiles, *Hepatozoon* is occurred in terrestrial snakes and *Karyolysus* is existed in old world lizards and possibly tree snakes (Campbell, 1996).

In this study, the percentage of hematozoa infection in snakes from QSMI and morphological features of the hematozoa are described.

MATERIALS AND METHODS

During December 1997 to March 2001, 275 blood samples of snakes from eighteen species in Queen Saovabha Memorial Institute were collected from the ventral caudal vein using a 22-gauge needle. Some snakes were captured from different locations in Thailand, some were bred in the Institute. Blood samples were anticoagulated with ethylene diaminetetraacetic acid (EDTA) except for samples from copper head racer snakes were anticoagulated with heparin sodium because EDTA causes hemolysis in this snake. Blood smears were prepared immediately and air dried. Then they were fixed in absolute methanol and stained with Wright-Giemsa for determination of differential leukocyte counts and hematozoa examination. Buffy coat examination for moving blood parasite was done on microhematocrit tubes prepared for hematocrit determination under 100X magnification (Schalm *et al.*, 1975)

Grading of *Hepatozoon* sp. infection was quantitated by the number of infected erythrocytes per 200X microscope field. The degree of infection was shown as 1+ to 4+, in which 1+ is equal to 1 to 20 infected erythrocytes; whereas 2+, 3+ and 4+ are equal to 21 to 40; 41 to 60; and more than 60 infected erythrocytes per field, respectively. Hematozoa body width and length were measured with eyepiece micrometer of Olympus®, microscope Bx50 (Japan) at 1,000X field. Means and other statistic data were generated for each hematozoa.

RESULTS

There were two kinds of blood parasites moving in the plasma above the buffy coat, including trypanosome and *Hepatozoon* sp. According to blood smear examination, there were three hematozoas in the snakes; *Hepatozoon* sp., *Haemogregarina* sp. and trypanosome. There were no hematozoa in the 5 studied species including reticulated python, fishing salt-water snake, monocled cobra, Malayan pit viper and white-lipped green pit viper. The percentage of hematozoa infection in the snakes is summarized in Table 1. The highest rate of infection was seen in the banded krait. The grading of *Hepatozoon* sp. infected erythrocytes in three species of snake with high percentage of infection is shown in Table 2. The highest rate of parasitemia was seen in the mangrove snake.

Hepatozoon sp.

Among the twelve species of snake which were infected with *Hepatozoon* sp., there were three distinct forms of intraerythrocyte gamont (Table 3). The small gamonts were averaged 3.0 ± 0.3 by 10.9 ± 0.6 μm . They were oval, slightly curved with round ends; round to oval band nucleus, nearly centrally located. Some hepatozoas had blue inclusions and small granules in the cytoplasm (Figure 1). The parasitized erythrocytes were slightly enlarged and had some halo-like appearances were seen in the cytoplasm. The small gamonts were found in seven species of snakes, including mask-face water snake, copper head racer snake, common rat snake (Figure 2), Siamese spitting cobra, king cobra (Figure 3) banded krait and Malayan krait. In heavier infections, two gamonts may infect the same erythrocyte (Figure 4).

The medium gamonts were averaged 3.3 ± 0.6 by 14.8 ± 0.9 μm . Gamonts were slender, stained dense basophilia at the periphery with bipolar stretching (Figure 5). The infected erythrocyte

were intact and with no hemolysis. The medium gamonts were found in mask-faced water snakes and rainbow water snakes.

The large gamonts, averaged 3.9 ± 0.6 by

$15.0 \pm 1.1 \mu\text{m}$, were found in mangrove snakes, golden spitting cobras, king cobras, mangrove pit vipers (Figure 6) and common rat snakes. Gamonts were oval, slightly curve with smooth round ends

Table 1 Percentage of hematozoa infection (number) in 257 snakes.

Snake species (common name)	Number	Hepatozoon	Haemogregarina	Trypanosome
<i>Python reticulatus</i> (Reticulated python)	16	0	0	0
<i>Python molurus bivittatus</i> (Burmese python)	14	0	14.3 (2)	0
<i>Boiga dendrophila melanota</i> (Mangrove snake)	17	72.3 (34)	2.1 (1) ¹	0
<i>Homalopsis buccata</i> (Mask-faced water snake)	47	10.6 (5)	17.0 (8)	14.9 (7) ²
<i>Enhydryis enhydryis</i> (Rainbow water snake)	13	7.7 (1)	7.7 (1)	23.1 (3) ¹
<i>Elaphe radiata</i> (Copper head racer snake)	12	8.3 (1)	0	0
<i>Cerberus rhynchops</i> (Fishing salt-water snake)	1	0	0	0
<i>Ptyas mucosus</i> (Common rat snake)	5	80.0 (4)	0	0
<i>Naja kaouthia</i> (Monocled cobra)	17	0	0	0
<i>Naja siamensis</i> (Siamese spitting cobra)	12	41.5 (5)	0	0
<i>Naja sumatrana</i> (Golden spitting cobra)	6	50.0 (3)	0	0
<i>Ophiophagus hannah</i> (King cobra)	22	68.2 (15)	0	0
<i>Bungarus fasciatus</i> (Banded krait)	16	93.8 (15)	0	0
<i>Bungarus candidus</i> (Malayan krait)	6	16.7 (1)	0	0
<i>Calloselasma rhodostoma</i> (Malayan krait)	6	0	0	0
<i>Trimeresurus albolabris</i> (White-lipped green pit viper)	4	0	0	0
<i>Trimeresurus purpureomaculatus</i> (Mangrove pit viper)	6	66.7 (4)	0	0
<i>Daboia russellii siamensis</i> (Siamese russell's viper)	25	4.0 (1)	0	0
Total	275	32.4 (89)	4.4 (12)	3.6 (10)

¹ One snake was co-infected with *Hepatozoon* sp.

² Four snakes were co-infected with *Hepatozoon* sp.

Table 2 Grading percentage of *Hepatozoon* sp. infected erythrocytes in three species of snake with high percentage of infection.

Snakes species	Total snakes	+	(n)	2+	(n)	3+	(n)	4+	(n)
<i>Boiga dendrophila melanota</i>	47	59.6	(28)	6.4	(3)	2.1	(1)	4.3	(2)
<i>Bungarus fasciatus</i>	16	68.9	(11)	25	(4)	-		-	
<i>Ophiophagus hannah</i>	22	59.1	(13)	9.1	(2)	-		-	

and had oval nucleus, located from central to the subterminal; and its cytoplasm contained small granules. The host cell nucleus was usually intact except in some infections in mangrove snakes (Figure 7) which caused hypochromic. In heavy infections, more than one gamonts were found (Figure 8) and likely appeared as vermicules in the plasma leaving the parasitophorous vacuole membrane in the hemolytic erythrocyte (Figure 9). Vermicules were detected by moving in the plasma under light microscope examination. In some infections, there were stout microgamonts (6x14 μm , $n = 20$) with the smaller number than those of the macrogamonts.

Usually there was one type of gamont in each infection but small and large gamonts were detected in the same infection in the common rat snake, king cobra and banded krait (Figure 10).

***Haemogregarina* sp.**

Intraerythrocyte gamonts of *Haemogregarina* sp. were found in mask-faced water snakes and rainbow water snakes. The gamonts were larger than those of hepatozoa gamonts (Table 3) that made host cells enlarged and laterally displaced of the nuclei. They had big oval nuclei, that were as wide as the body, and cytoplasm filled with red fine granules and vacuolated blue granules (Figure 11). In some gamonts, a long tail at one end of the gamont was observed (Figure 11, arrowhead).

Trypanosome

Trypomastigote forms of trypanosome was found in mask-faced water snakes and rainbow water snakes. They were large, broad body width (Table 3), light blue cytoplasm, well defined undulating membrane, free flagellum and kinetoplast (Figure 12).

DISCUSSION

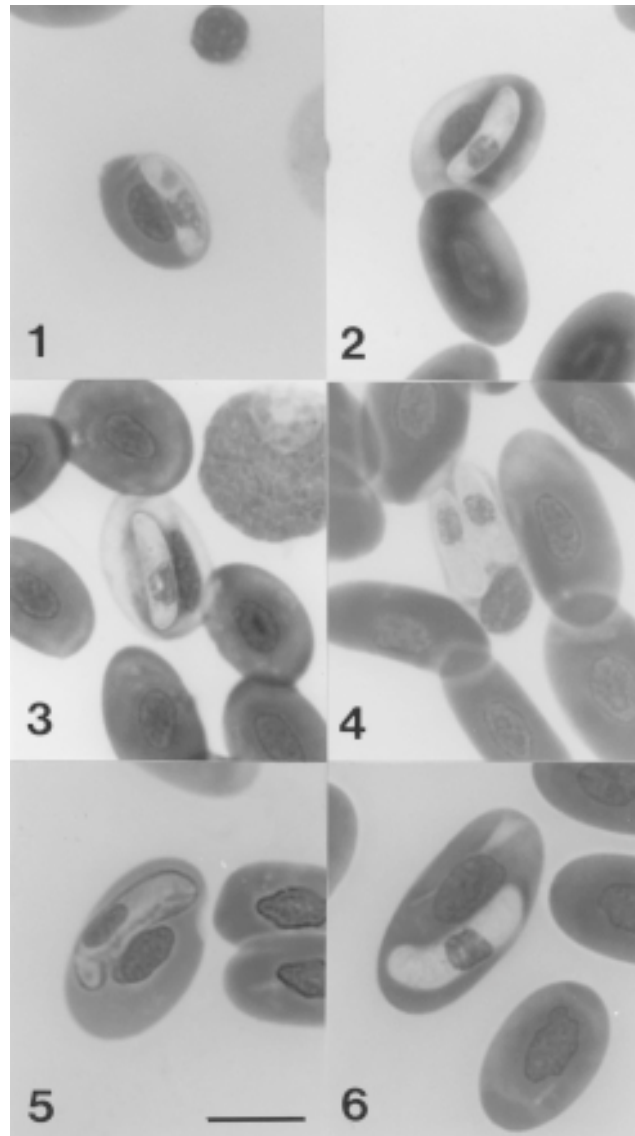
There were three types of hematozoa in thirteen of the eighteen species of snakes in this study. The total rate of hematozoa infection was very high (40.4%) with *Hepatozoon* and *Haemogregarina* infections being 35%. So the haemogregarines were the most common blood parasites of snakes in Thailand which is agreed with other reports (Smith, 1996; Wozniak *et al.*, 1998).

The movement of the vermicule in the plasma was called traveler by Sambon and Seligmann (1908) and Plimmer (1912). The vermicule was identical to type 4 haemogregarines identified by Hull and Camin (1960) or identical to E-type haemogregarines identified by Toshioka (1970). The size of the vermicule in this study was different from the vermicule of *Hepatozoon rarefaciens* in indigo snake, *Drymarchon corais* (Frye, 1991).

In this study, the hepatozoa were classified by size into three types; small, medium and large gamonts. The small gamonts was identical with type 1 haemogregarines identified by Hull and Camin (1960). The three types of gamont in this study were different from the gamont found in the southern water snake, *Nerodia fasciata pictiventris*

Table 3 Measurement (mean $\pm$ SD) of the hematozoa size in snake.

	<i>Hepatozoon</i>			<i>Haemogregarina</i>	Trypanosome
	Small	Medium	Large		
Number	120	60	140	40	30
Body width (μm)	3.0 ± 0.3	3.3 ± 0.6	3.9 ± 0.6	7.6 ± 1.2	14.1 ± 3.0
Body length (μm)	10.9 ± 0.6	14.8 ± 0.9	15.0 ± 1.1	15.2 ± 2.6	ND



- Figure 1** Small gamont ($3 \times 11 \mu\text{m}$) of *Hepatozoon* in $9 \times 13 \mu\text{m}$ erythrocyte of the Siamese spitting cobra. Note the prominent nucleus and blue inclusion in the cytoplasm of the hepatozoa.
- Figure 2** Small gamont ($3 \times 12 \mu\text{m}$) of *Hepatozoon* in $10 \times 16 \mu\text{m}$ erythrocyte of the common rat snake. Note the halo appearance of the erythrocyte cytoplasm.
- Figure 3** Small gamont ($3 \times 11 \mu\text{m}$) of *Hepatozoon* sp. in the king cobra. Note the displacement of intact nucleus of the erythrocyte.
- Figure 4** Two small intraerythrocyte gamonts of *Hepatozoon* are packed in the erythrocyte of the banded krait. Note the displacement of the host nucleus.
- Figure 5** Medium gamont of *Hepatozoon* sp. in the mask-faced water snake. Note the dense basophilia at the peripheral of the gamonts.
- Figure 6** Large gamont ($4 \times 16 \mu\text{m}$) of *Hepatozoon* sp. in $12 \times 23 \mu\text{m}$ erythrocyte of the mangrove pit viper.

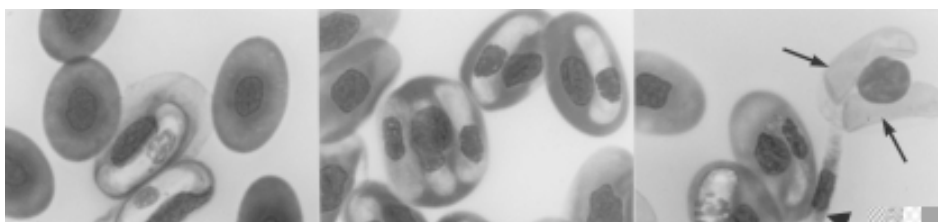


Figure 7 Two large gamonts of *Hepatozoon* sp. infected two erythrocyte of the mangrove snake. Note the halo appearance of the erythrocyte cytoplasm and bipolar stretching of the hepatozoa.

Figure 8 Three large gamonts infecting an erythrocyte of the mangrove snake with 4+ infection.

Figure 9 An exerythrocytic or vermicule form of a *Hepatozoon* (arrowhead) in the mangrove snake. Note the stout microgametocyte ($5 \times 13 \mu\text{m}$) and the slender macrogametocyte ($3 \times 17 \mu\text{m}$) in different erythrocytes and the parasitophorous vacuole membrane (arrows) left in the hemolytic erythrocyte.

Figure 10 Small ($5 \times 11 \mu\text{m}$) and large ($5 \times 15 \mu\text{m}$) hepatozoa infected different erythrocyte of the banded krait. Bar = $10 \mu\text{m}$

Figure 11 Two gamont of *Haemogregarina* sp. in two erythrocyte of mask-faced water snake. Note the displacement of the host nucleus and well-pigmentation of the parasite. The long tail at one end of the gamont (arrowhead) was easily observed.

Figure 12 Trypomastigote of trypanosome in the blood from the mask-faced water snake. Note the well-defined kinetoplast (arrowhead), undulating membrane and free flagellum.

(Wozniak *et al.*, 1998) or the gamont of *Hepatozoon sipedon* in the northern water snake (*Nerodia sipedon sipedon*) collected from Frontenac Country in the eastern part of Ontario (Smith *et al.*, 1994).

Among the seven species infected with the small gamont, some infected erythrocytes were intact but some were hypochromic and had displaced nuclei (Figure 1-4). Among the five species infected with the large gamont, most of the infected erythrocytes were intact (Figure 6, 8) except in

some infections of mangrove snakes (Figure 7). The different in host cell cytopathological effects may indicate a difference in the species of the hepatozoa.

Species differentiation of *Hepatozoon* sp. was usually classified by complete life cycle or parasite developmental patterns within invertebrate vectors, including oocyst and sporocysts dimensions, the number of sporocyst/oocyst, the number of sporozoite/sporocyst (Smith, 1996).

Despite the appearance and wide spread distribution of the intraerythrocytic gamonts of *Hepatozoon* sp. from many species of snakes, only 13 species of *Hepatozoon* have been described from North America, with only 4 species having complete life cycle. The description of the 4 species needs morphological and morphometric features at the oocyst stage (Smith, 1996) to identification of their species. The *Hepatozoon* infection in snakes is a 3-host life cycle which was proved by Landau *et al.* (1972). They fed mosquitoes containing oocysts of *H. domerguei* to the insectivorous lizards *Oplurus sebae* and *Lacerta muralis*. Snakes became infected after ingesting these lizards.

The gamonts of *Haemogregarina* sp. were larger than those of *Hepatozoon* sp. which appeared with well-pigmentation of the cytoplasm. Some gamonts revealed a long-tailed folding at one end. These characteristics were identical to type 3 haemogregarines identified by Hull and Camin (1960) even though they were larger in size. The well-pigmentation and vacuolated cytoplasm of the *Haemogregarina* sp. gamont was easily differentiated from those gamont of *Hepatozoon* sp. Wozniak and Telford (1991) differentiated genera by the developmental pattern in invertebrate host : *Haemogregarina* produces sporocyst within the vector gut while *Hepatozoon* forms oocysts containing sporocyst within the vector hemocoel. Siddall (1995) suggested that all haemogregarines infecting snakes, lizards, crocodilians, birds and mammals that remained in the genus *Haemogregarina* were transferred to genus *Hepatozoon*.

The study of trypanosome infections in reptiles was limited because the finding of these flagellates in the hematological examination was difficult due to low parasitemia (Chia and Miller, 1984) The size of trypomastigote in the mask-faced water snakes and rainbow water snakes was larger than those *Trypanosoma hydrae*, trypanosome in mammals or Atkinson and Valkalis found in broad-banded water snake (*Nerodia fasciata confluens*)

from Louisiana which were 1.8-2.6 μ m body width (Chia and Miller, 1984). Anyhow, trypanosome infections were only found in water snakes.

The naturally infected hematozoa in the reptiles were slightly pathogenic or non-pathogenic (Campbell, 1996; Hawkey and Dennett, 1989) but there was a report of granulomatous hepatitis associated with *Hepatozoon* sp. in the southern water snake (Wozniak *et al.*, 1998). In the case of heavy infection, the hemolysis of infected erythrocyte was observed in the blood smear (Figure 9) and hemolytic anemia may have occurred.

In conclusion, there were hematozoa in 13 species/18 species of studied snakes. Hepatozoas were the most common hematozoa of snake in Thailand. There were no hematozoa in reticulated pythons, fishing salt-water snakes, monocled cobras, Malayan pit vipers and white-lipped green pit vipers. This is the preliminary study of hematozoa of snake in Thailand.

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