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# The Detection of Haemogregarines in Blood of Some Japanese Snakes

Akira MIYATA

*Department of Epidemiology, Institute for Tropical Medicine, Nagasaki University*

(Chief: Prof. Toshio NAKABAYASHI)

ABSTRACT: Totally 275 individuals of various snakes collected from April to September, 1967, in Nagasaki City and the vicinities were examined for finding blood-dwelling parasite. Three species of haemogregarines were detected. One of them seems to be identical with *Haemogregarina tigrinae* which has been originally described by Hoare (1918) from a Japanese snake, *Natrix tigrina* (= *Rhabdophis tigrinus tigrinus*). Detection rates obtained were: 78 out of 193 (40%) in *R.t. tigrinus*, 8 out of 43 (19%) in *Elaphe quadrivirgata*, and 1 out of 5 (20%) in *Agkistrodon halys*. The second species which had two different types of parasites was found in 3 out of 24 (13%) of *Elaphe clima corphora*, in 2 out of 43 (5%) of *E.quadrivirgata*, and in 3 out of 193 (2%) of *R.t.tigrinus*. The first species of haemogregarine was detected from snakes living near water side such as rice-field, pond, or small stream, and eating frogs and other small animals. Leech is suspicious as a vactor. The vector of the second species of haemogregarine might be different from that of first species. The third species of haemogregarine which was the smallest species among the three haemogregarines was detected from one of *R. t. tigrinus*. *Pirhemocyton* was also detected from *R.t.tigrinus* and *Natrix vibakari*.

During the period of April to September, 1967, various snakes were collected by members of the Department of Virology and the Department of Medical Zoology of the Institute to attempt to detect Japanese encephalitis virus (Mifune *et al.*, 1969), and at that time blood smears were also prepared from the snakes. Recently all the smears have been examined by the present author to find out any blood-dwelling parasites, and three species of haemogregarines have been detected. In the present paper, the brief description and the detection rate for each haemogregarine are reported.

## MATERIALS AND METHODS

A total of 275 snakes were collected in Nagasaki City and the vicinities during April to September, 1967. The scientific names of the snakes used by Yamaguchi (1966)

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Table 1. Detection rate of parasites in various snakes.

| Name of snake | No. of Exam. | Hg-1 | Hg-2 | Hg-3 | *Pirhemocyton* |
|---|---|---|---|---|---|
| *Elaphe quadrivirgata* | 43 | 8 | 2 | 0 | 0 |
| *Elaphe conspicillata* | 4 | 0 | 0 | 0 | 0 |
| *Elaphe clima corphora* | 24 | 0 | 3 | 0 | 0 |
| *Natrix vibakari* | 6 | 0 | 0 | 0 | 1 |
| *Rhabdophis tigrinus tigrinus* | 193[a,b] | 78 | 3 | 1 | 6 |
| *Agkistrodon halys* | 5 | 1 | 0 | 0 | 0 |
| Total | 275 | 87 | 8 | 1 | 7 |

a : One of them is a mixed infection case of Hg-1 and Hg-2.
b : One of them is a mixed infection case of Hg-1 and *Pirhemocyton*.

and Nakamura and Ueno (1974) were adopted in the present paper.

Family COLUBRIDAE
*Elaphe quadrivirgata* (Boie, 1826) (Japanese name: *Shimahebi*)
*Elaphe conspicillata* (Boie, 1826) (Japanese name: *Jimuguri*)
*Elaphe clima corphora* (Boie, 1826) (Japanese name: *Aodaisyo*)
*Natrix vibakari* (Boie,1826) (Japanese name: *Hibakari*)
*Rhabdophis tigrinus tigrinus* (Boie, 1826) (Japanese name: *Yamakagashi*)
Family VIPERIDAE
*Agkistrodon halys* (Pallas,1776) (Japanese name: *Mamushi*)

Thin blood smears were taken from peripheral blood of each snake by cutting off a small piece of the tail. After fixation by absolute methyl alcohol, the smears were stained in 3% of Giemsa's solulion for 30 minutes, and examined at first by an immersion lense (×100) for 200 fields, then, if being negative for parasites, the smear was checked again by a low power objective lense (×40) for 100 fields. In this study, only blood smears were taken and examination of any tissue or organ was not attempted.

## RESULTS

Three species of haemogregarines detected from totally 95 individuals of snakes examined (Table 1). The detected parasites in the erythrocyte were described below.

Haemogregarine 1 (Hg-1) (Figs. 1~7, 72, and 75~77)

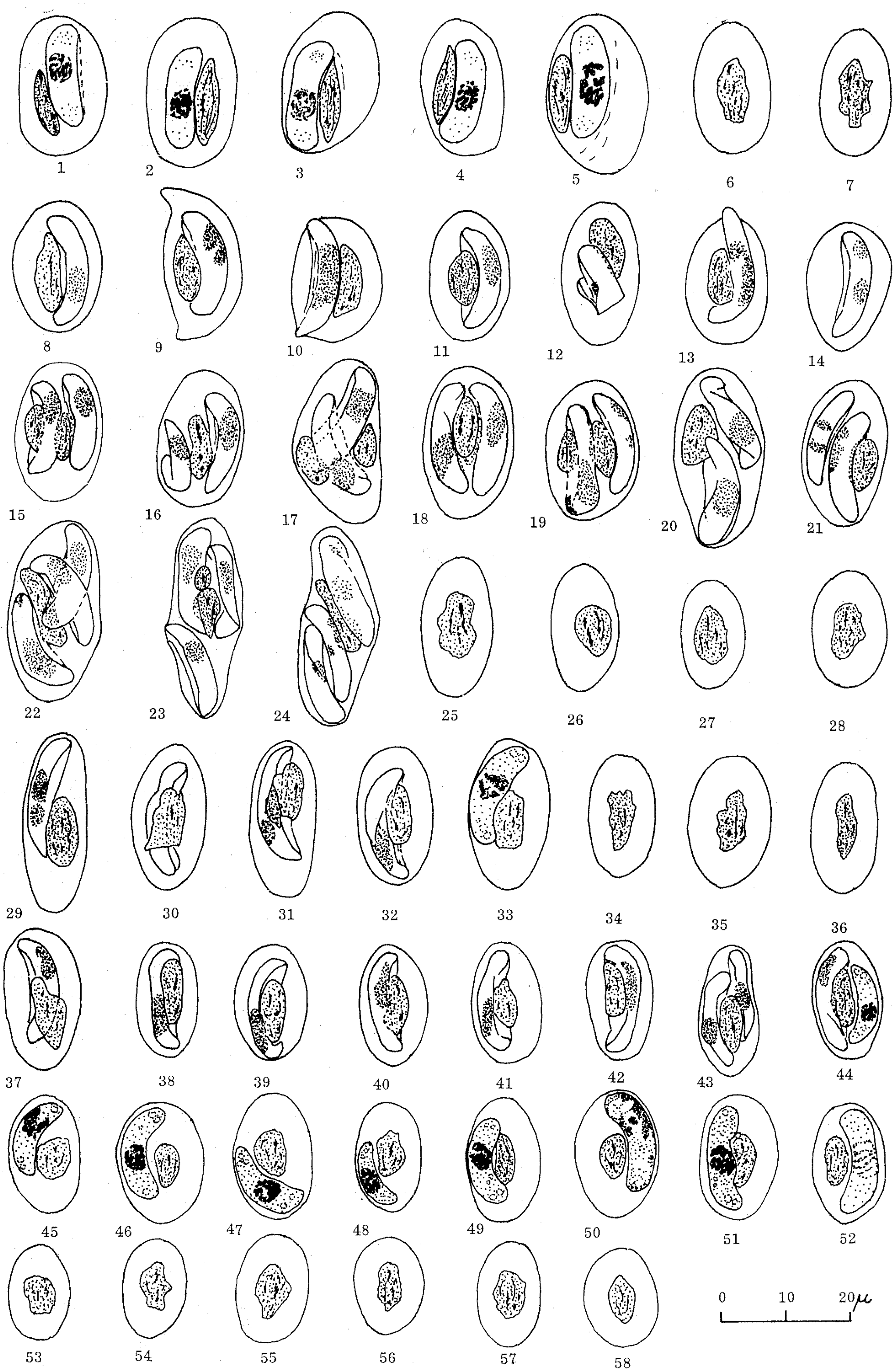
This is a species of haemogregarine of sausage-shaped body with rounded ends, and average size is 15.1 microns in the length and 4.7 microns in the breadth (Table 2). The cytoplasm of the haemogregarine was usually stained with blue and vacuole or granule was not seen in the parasite. The nucleus was stained with dark purple. The parasitized erythrocytes increased in the size and more lightly stained in colour than uninfected red cells. The host cell nucleus was pushed a way to one side by the parasite, and usually distorted and elongtaed parallel to the long axis of the haemogregarine. Only this stage could be detected and schizogonic cycle was completely absent from the peripheral blood. The parasite is called tentatively Hg-1 in this paper. The haemogregarine was detected from 78 (40%) of *R. t. tigrinus*, from 8 (19%) of *E. quadri virgata*, and from 1 (20%) of *A. halys*. Twenty four individuals of *E. clima corphora* were also examined, but the parasite was never found, although the second parasite (Hg-2) mentioned below was detected from 3 (13%) of the snake. The parasitemia of Hg-1 was quite low as compared with the Hg-2 (Table 6), and in 7 cases 4~8 infected erythrocytes were counted in 1000 erythrocytes. In the other 80 cases the number of infected erythrocytes was less than one in 1000 erythrocytes.

Table 2. Size in microns of erythrocytic stages of Hg-1 and of infected and uninfected erythrocytes of a snake, ***Rhabdophis tigrinus tigrinus*** (snake no. 1967−293)

| | Parasite | Infected erythrocyte | Uninfected erythrocyte |
|---|---|---|---|
| | 15×5 | 23×16 | 19×12 |
| | 15×5 | 21×14 | 17×11 |
| | 15×4 | 23×15 | 16×12 |
| | 15×4 | 21×15 | 20×12 |
| | 15×5 | 20×19 | 18×11 |
| | 15×4 | 22×12 | 18×12 |
| | 15×5 | 22×14 | 19×13 |
| | 15×5 | 21×13 | 19×13 |
| | 15×5 | 23×13 | 18×13 |
| | 16×5 | 24×13 | 20×9 |
| | 15×5 | 22×13 | 18×10 |
| | 15×4 | 23×14 | 18×11 |
| | 14×5 | 22×14 | 16×12 |
| | 15×5 | 23×15 | 18×10 |
| | 16×5 | 25×14 | 20×13 |
| Maximum | 16×5 | 25×19 | 20×13 |
| Minimum | 14×4 | 20×12 | 16×9 |
| Average | 15.1×4.7 | 22.3×14.3 | 18.3×11.6 |

Table 3. Size in microns of erythrocytic stages of Hg-2 and of infected and uninfected erythrocytes of a snake *Elaphe quadrivirgata* (snake no. 1967−48) (Showing the both large and small types of parasite and their infected erythrocytes respectively)

| | Large parasite | Infected erythrocyte | Small parasite | Infected erythrocyte | Uninfected erythrocyte |
|---|---|---|---|---|---|
| | 15×4 | 19×10 | 11×3 | 17×12 | 17×12 |
| | 16×3 | 18×10 | 13×3 | 22×13 | 15×10 |
| | 15×3 | 16×9 | 12×3 | 17×13 | 15×11 |
| | 16×3 | 18×11 | 11×3 | 17×12 | 15×11 |
| | 17×3 | 17×9 | 13×3 | 19×13 | 16×11 |
| | 17×2 | 18×13 | 11×3 | 17×11 | 17×11 |
| | 16×3 | 20×9 | 11×4 | 17×11 | 14×10 |
| | 17×3 | 17×11 | 12×4 | 18×12 | 17×9 |
| | 16×3 | 19×12 | 11×3 | 17×11 | 16×12 |
| | 15×3 | 21×10 | 10×2 | 16×10 | 15×11 |
| | 16×3 | 18×8 | 10×2 | 17×12 | 16×10 |
| | 16×2 | 16×9 | 10×2 | 17×12 | 16×10 |
| | 15×2 | 20×11 | 10×2 | 14×11 | 16×11 |
| | 17×3 | 17×10 | 11×3 | 18×13 | 18×11 |
| | 15×3 | 18×11 | 11×2 | 17×12 | 16×9 |
| Maximum | 17×4 | 21×13 | 13×4 | 22×10 | 18×12 |
| Minimum | 15×2 | 16×9 | 10×2 | 16×10 | 14×9 |
| Average | 15.9×2.9 | 18.1×10.2 | 11.1×2.8 | 17.3×11.9 | 15.9×10.6 |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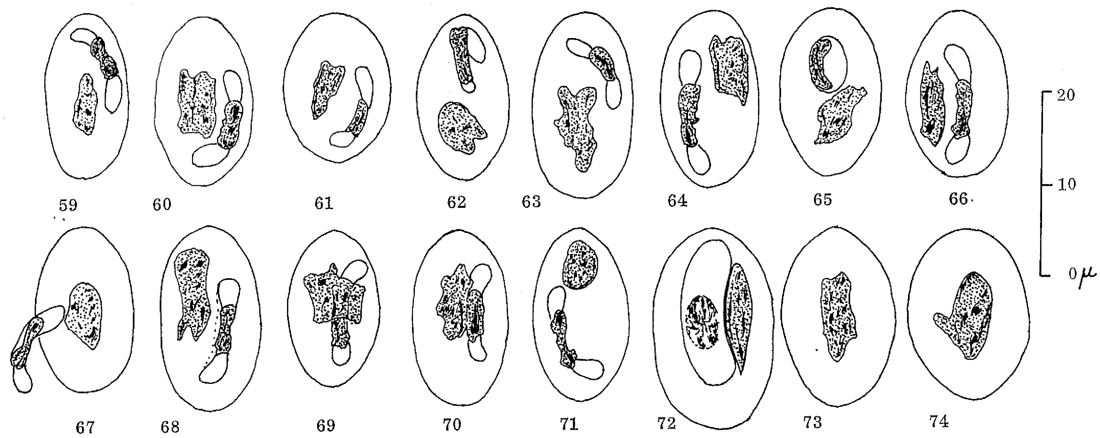


Figs. 59～74. Haemogregarines (Hg-1 & Hg-3) from *Rhabdophis tigrinus tigrinus* (snake no. 1967－151) (59～71. Hg-3 infected erythrocyte. 72. Hg-1 infected erythrocyte. 73～74. Uninfected erythrocyte.)

Haemogregarine 2 (Hg-2) (Figs. 8～58 and 78～82)

From 8 snakes another species of haemogregarine was detected, and two different types of parasites were found in the same blood smear. Large type of parasite: One of the types is a large parasite with banana-shaped body and is enclosed within a thin sheath. The size is 15.9 microns in the length and 2.9 microns in the breadth (Table 3). The nucleus and the cytoplasm of the parasite were stained with dark purple and pale colour respecetively, and granule or vacuole was not found. The infecded erythrocytes were hypertrophied, but the colour of the cell was not lightly but darkly stained as compared with uninfected one. The large type is usually bent in one side, and the parasite hugs the nucleus of host cell as shown in Figs. 29～32, 37～44, and 80. A similar figure of *Haemogregarina najae* (?) was shown by Ball (1967). The shape of the nucleus is an indeterminate form when compared with the case of Hg-1 which usually has a typical spindle form. In the case of a snake (snake no. 1967－22), sometimes the nucleus of the host cell was divided into two separate parts (Figs. 15, 17, and 19) or disappeared (Fig. 14). Double or even triple infected erythrocytes were also observed. The parasite

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Figs. 1～ 7. Haemogregarine (Hg-1) from *Rhabdophis tigrinus tigrinus* (snake no. 1967－293) (1～5. Infected erythrocyte. 6～7. Uninfected erythrocyte.)

Figs. 8～28. Haemogregarine (Hg-2) from *Elaphe quadrivirgata* (snake no. 1967－22) (8～14. Mono-infected erythrocyte. 15～21. Double-infected erythrocyte. 22～24. Triple-infected erythrocyte. 25～28. Uninfected erythrocyte.)

Figs. 29～36. Haemogregarine (Hg-2) from *Elaphe clima corphora* (snake no. 1967－131) (29～32. Large type of parasite. 33. Small type of parasite. 34～36. Uninfected erythrocyte.)

Figs. 37～58. Haemogregarine (Hg-2) from *Elaphe quadrivirgata* (snake no. 1967－48) (37～42. Large type of parasite. 43. Double-infected erythrocyte(large type of parasite.). 44. Double-infected erythrocyte (large and small types of parasite). 45～52. Small type of parasite. 53～58. Uninfected erythrocyte.)

Table 4. Size in microns of erythrocytic stages of Hg-2 and of mono-infected, double-infected and uninfected erythrocytes of a snake, *Elaphe quadrivirgata* (snake no. 1967—22)

| | Parasite | Mono-infected erytbrocyte | Double inf. erythro. | Uninfected erythrocyte |
|---|---|---|---|---|
| | 16×3 | 17×12 | 22×15 | 15×10 |
| | 16×3 | 18×13 | 21×13 | 18×12 |
| | 18×4 | 18×12 | 22×15 | 17×12 |
| | 16×4 | 18×12 | 23×13 | 18×11 |
| | 16×4 | 19×13 | 23×14 | 17×10 |
| | 14×3 | 18×13 | 21×15 | 17×10 |
| | 17×4 | 18×13 | 20×14 | 18×10 |
| | 15×4 | 18×12 | 23×14 | 17×11 |
| | 16×4 | 20×13 | 19×14 | 16×11 |
| | 15×3 | 21×13 | 23×15 | 16×10 |
| | 15×4 | 18×13 | 22×14 | 16×10 |
| | 16×4 | 19×13 | 20×15 | 17×11 |
| | 16×4 | 20×12 | 22×15 | 16×11 |
| | 16×5 | 20×14 | 24×12 | 16×10 |
| | 16×4 | 18×13 | 24×16 | 16× 9 |
| Maximum | 18×5 | 21×14 | 24×16 | 18×12 |
| Minimum | 15×3 | 17×12 | 19×13 | 15× 9 |
| Average | 15.9×3.8 | 18.7×12.7 | 21.9×14.3 | 16.7×10.5 |

Table 5. Size in microns of erythrocytic stages of Hg-3 and of infected and unifected erythrocytes of a snake, *Rhabdophis tigrinus tigrinus* (snake no. 1967—151) (This is a mixed-infection case with Hg-1, which is excluded in the measurement.)

| | Parasite | Infected erythrocyte | Uninfected erythrocyte |
|---|---|---|---|
| | 14×2 | 17×10 | 18×10 |
| | 13×2 | 18×10 | 17× 9 |
| | 13×2 | 20×12 | 17×11 |
| | 11×2 | 16×10 | 18×12 |
| | 13×2 | 20×12 | 21×11 |
| | 12×2 | 19× 9 | 20×12 |
| | 13×3 | 20×11 | 18×11 |
| | 11×3 | 17× 9 | 20×11 |
| | 13×2 | 18×12 | 18×11 |
| | 13×2 | 18×13 | 16×10 |
| | 14×2 | 18× 9 | 20×10 |
| | 12×2 | 18×12 | 18×12 |
| | 12×2 | 19×10 | 18×11 |
| | 13×2 | 18×12 | 21×13 |
| | 13×2 | 17× 9 | 18×11 |
| Maximum | 14×3 | 20×13 | 21×13 |
| Minimum | 11×2 | 16× 9 | 16× 9 |
| Average | 12.7×2.1 | 18.2×10.7 | 18.5×11.0 |

is broader than those of other cases (Table 4), but this means the difference in drying and staininig conditions. Usually the marginal parts of a smear are dried up more rapidly than the center of the smear, and in the marginal parts the parasite is seen broader than that in the center. In the case of snake no. 1967—22, the smear dried rapidly up because it was very thin.

Small type of parasite: Small type of parasite was always accompanied with the large type. From all 8 cases the large type was detected, but the small type which was not found alone was seen in 4 cases. This means that those two types are not different species but the two stages of one species. The small type of the parasite has a sausage-shaped body without seath (Figs. 45~52 and 81 ~82). Typical vacuoles were seen in the both ends of the cytoplasm in which many fine granules were also found. The colours of the cytoplasm and the nucleus are light blue and dark purple respectively, and the size of the parasite is 11.1 microns in the length and 2.8 microns in the breadth (Table 3). The infected erythrocytes are hypertrophied, but the colour is not different from uninfected ones.

The haemogregarine including two types is called Hg-2 in the present paper. Hg-2 was detected from 3 out of 193 individuals of *R. t. tigrinus* (detection rate 2%), 3 out of 43 *E. quadrivirgata* (5%), and 3 out of 24 *E. clima corphora* (13%). Except for 3 cases of *E. clima corphora*, in which the infected erythrocytes were less than one in 1000 erythrocytes, the parasitemia of Hg-2 was very high (Table 6) as compared with Hg-1 or Hg-3, although the detection rate of Hg-2 in this snake was the highest among the three haemogregarines. Schizogonic and divided stages were not seen in the peripheral blood.

Haemogregarine 3 (Hg-3) (Figs. 59～71 and 83～85)

An apparently distinct haemogregarine from Hg-1 and Hg-2 was found from a snake, *R. t. tigrinus* (snake no. 1967-151). The haemogregarine which was called Hg-3 in this paper was smaller than other two haemogregarines, and the size was 12.7 microns in the length and 2.1 microns in the breadth (Table 5). The most important difference of the haemogregarine from other two species is that the infected erythrocytes are not

Table 6. Parasitemia in 1000 erythrocytes of snakes infected each haemogregarine

| Species of parasite | Species of snake | Snake no. | Infection rate in 1000 erythrocytes |
|---|---|---|---|
| Hg-1[a] | *Rhabdophis tigrinus tigrinus* | 1967 − 134 | 4 |
| | 〃 | 1967 − 167 | 8 |
| | 〃 | 1967 − 197 | 4 |
| | 〃 | 1967 − 239 | 4 |
| | 〃 | 1967 − 259 | 4 |
| | 〃 | 1967 − 277 | 5 |
| | 〃 | 1967 − 291 | 7 |
| Hg-2 | *Elaphe quadrivirgata* | 1967 − 22 | 50[b] |
| | 〃 | 1967 − 48 | 15[c] |
| | *Elaphe chima corphora* | 1967 − 6 | < 1 |
| | 〃 | 1967 − 110 | < 1 |
| | 〃 | 1967 − 131 | < 1 |
| | *Rhabdophis tigrinus tigrinus* | 1967 − 24 | 48 |
| | 〃 | 1967 − 29 | 61 |
| | 〃 | 1967 − 42 | 61 |
| Hg-3 | *Rhabdophis tigrinus tigrinus* | 1967 − 151 | 8[d] |

a : In the other 80 cases the number of infected erythrocytes was less than one in 1000 erythrocytes. Double-infected erythrocytes were rarely seen in some blood smears.

b : About 75% of them was mono-infected erythrocytes and the rest 25% was double-infected ones. Triple-infected erythrocytes were very rare.

c : About 70% of them was infected with large type of parasite and the rest 30% was infected small one.

d : Double-infected erythrocytes were rarely seen.

hypertrophied, and the parasite without sheath usually lies in the separate position from the nucleus of the host erythrocyte. A large and dark purple nucleus situates in the middle of the parasite, which has a pale cytoplasm without granule or vacuole. The parasitemia was comparatively high and 8 infected erythrocytes were counted among 1000 erythrocytes. A mixed infection of Hg-1 was also detected from the same blood smear but parasitemia for Hg-1 was less than 0.1%.

*Pirhemocyton* (Figs. 75~76)

Very small red dots were detected in 6 out of 193 blood smears collected from *R. t. tigrinus* and some of them looked like just the ring form of malaria parasites. "Albuminous bodies" were also found in some cases, and according to Wenyon (1926) the morphological feature was very similar to *Pirhemocyton tarentolae* Chaton and Blanc, 1914. The organism was also detected in 1 out of 6 blood smears in tbe case of *N. vibakari*.

Hg-1 was detected throughout the survey area, while Hg-2 was endemic in limited places. The most abundant place for Hg-2 was Matsushima, a small island situated 35 km north-west from Nagasaki City, and the natural environment still remains undestroyed. Six out of 8 cases of Hg-2 were detected from the snakes collected in the island. Other cases were obtained from Nagasaki City and from Kayaki 10 km south-west from the city. Hg-3 was found in the snake taken from Kayaki.

## Discussion

Snake haemogregarines had been recorded by several Japanese workers, particularly Toshioka (1970) described 7 types of haemogregarines based on morphology and host-parasite relationship. His C- and G-types detected from *Rhabdophis tigrinus tigrinus* and *Elaphe quadrivirgata* respectively are apparently identical with Hg-1, and his D-type from *Dinodon semicarinatus* also might belong to Hg-1. His A- and B-types detected from Japanese and Korean specimens of *Agkistrodon halys* respectively might be identical with Hg-2, although due to his description the infected erythrocytes did not increase in the size. His E-type (from *Trimeresurus okinawaensis*) and F-type (from *T. tokaraensis*) are also simiar to Hg-2, and E-type has two different forms as same as Hg-2. According to his personal comminucation (under date of 16, June, 1974), he believed that snake haemogregarines recorded in Japan mignt be divided into two species, which might correspond to Hg-1 and Hg-2. In addition to these two, Hg-3 is distinguishable morphologically, then 3 species of snake haemogregarines have been known in Japan. Uegaki (1928) detected a haemogregarine from *R. t. tigrinus* and *E. quadrivirgata*, and he showed its schizogonic stages. This had karyolystic action to host cell nucleus, and sometimes the nucleus was divided into two or three parts. This haemogregarine is similar to Hg-2, but the figures of erythrocytic stages shown by Uegaki are different from those of Hg-2. He recorded the other haemogregarine and its schizogonic stages from *Elaphe clima corphora*, and the haemogregarine seems to be identical with Hg-2. The present author could not understand why Uegaki could not find Hg-1 from *R. t. tigrinus* and *E. quadri-*

*virgata*, whereas he examined almost 150 snakes in Kyushu.

Hg-1 found from about 40% of *R. t. tigrinus* might be identified as *Haemogregarina tigrinae*. According to Wenyon (1926), this species seems to be originally described by Hoare in 1918 as a new species from a Japanese snake *Natrix tigrina* Boie, which is now known as *R. t. tigrinns*. However, the Hoare's original description has not been available for the present author up to date. This Hg-1 was also detected in blood smears from *E. quadrivirgata* and *A. halys*. These three species of snakes were collected in similar ecological condition, for instance, near water side such as rice field, pond, or small stream. Their major baits might be frogs and other small animals living in those places. On the other hand, Hg-1 was never found in *E. clima corphora* examined. The second species (Hg-2) appears to prefer rather this snake, it is of interest to note that this snake is usually found in houses or the garden, and eats birds or rodents, particularly house rats. Hg-2 was also detected from *R. t. tigrinus* and *E. quadrivirgata*.

From the above mentioned results, the host-parasite specificity in the haemogregarines was not so constant but ecological environment where the host snakes inhabited might be important for the infection of each parasite. Under an experimental condition Hg-1 might be possible to infect *E. clima corphora*, because Ball and his colleagues pointed out that at least some haemogregarines of reptiles could be transfered experimentally to foreign hosts: for example, by feeding vector-mosquitoes infected *Hepatozoon rarefaciens*, from the indigo snake, *Drymarchon corais*, to *Boa constrictor* (Ball *et al.*, 1967), and to the gopher snake, *Pituophis melanoleucus catenifer* (Chao and Ball, 1969). They were also able to transfer *Hepatozoon* sp. from *Boa constrictor* to a lizard, *Anolis carolinensis*, (Booden *et al.*, 1970), and to other lizard, *Sceloporus occidentalis*, (Oda *et al.*, 1971), by the same method of feeding infected mosquitoes.

Ball *et al.* (1958, 1967, and 1969) transfered *Haemogregarina rarefaciens* and *Haemogregarina mirabilis* into the genus *Hepatozoon* based on the studies on their life cycles. The life cycle of Hg-1 is still unknown except that the schizogonic stages are completely absent from the peripheral blood, thus this species might be placed in the genus *Hepatozoon*. In the other two species, Hg-2 and Hg-3, the schizogonic stages were also not seen on the blood smears, and Hg-2 had at least different two stages, which were reported in *Hp. rarefaciens* by Ball *et al.* (1967) and same phenomena had been known in some other snake haemogregarines. According to Mohammed and Mansour (1966) *Haemogregarina boueti* in a toad, *Bufo regularis*, also has an elongated form (gametocyte) and a broad form, which are called a large type and a small type respectively in the present paper. They also showed the figures that the host cell nucleus was divided into 2 more or less equal and regular fragments or disappeared. It is of very interest that these phenomena are also found in the present case of Hg-2. Since name of species is not clear at present without any knowledge about the life cycle, further researches are needed to determine the proper place of this haemogregarine in the systematic position of protozoa.

Vectors of Hg-1 and Hg-2 might be different from each other, because the host snakes have different ecological habitats. At present there are no direct indication but leech might be one of the most suspicious vectors of Hg-1, whereas a possibility of mosquito as a vector for the parasite also cannot be neglected. Miyagi (1972) found that *Culex infantulus* was fond of sucking blood from snakes under a laboratory condition, and this mosquito species distributes widely in Japan. The vector of Hg-2 appears to be difficult to find out, because the detection rate of the parasite is quite low.

In addition to these haemogregarines, *Pirhemocyton*, which was already reported by Telford (1972) from a Japanese lizard, *Takydromus thachydromoides*, was also detected from *R. t. tiginus* and *Natrix vibakari*. The detection of the organism was rather difficult, especially on the dirty smears taken under a limited condition of field survey. Recently Stehbens and Johnston (1966) have described the ultrastructure of *Pirhemocyton*, and they have concluded that it is not a protozoa but in all likelihood an icosohedral virus.

Lastly the present author would like to propose to be made a monograph of haemogregaines in the world by the following reasons. According to many workers, almost 300 species of haemogregarines have been named mostly without taking any notice of previously described species under the genera *Haemogregarina*, *Hepatozoon*, *Karyolysus*, *Danilewskya*, *Drepanidium*, *Pseudohaemogregarina*, *Leucocytozoon*, and others from blood of various vertebrates. From snakes about 70 named haemogregarines have been known, but most of them are very difficult to be identified because the descriptions and figures given by early European workers are not distinct and many original descriptions are also not available for Japanese workers. Koizumi(1910) studied on the life cyele of *Haemogregarina* sp. found a common Japanese tortoise, *Clemmys japonicus*, and he pointed out in the end of his paper as follows: "As admitted by some authors, it seems probable that various stages of development of one species were described by different authors under different names. The life cycle of these forms is followed in only a few forms and in the majority of reports the description is limited in a few developmental stages. Moreover some authors do not pay much attention in identifying their forms. For these reasons, there are many species having no sufficient criteria for identification." The present author quite agrees with Koizumi's opinion and hopes to be made a complete monograph of named haemogregarines with figures and descriptions, although he well knows that the monograph is not enough to solve the confused problems. Telford (1966) also mentioned as follows: "I am in complete accord with the philosophy that new species of haemogregarines should not be described solely on the basis of erythrocytic stages. Even when schizogonic stages obtained from host tissue are available, the author of a species who lacks information on the vector stages is placing himself in the position of describing a species of unknown or at least uncertain, generic affinity." His opinion is also acceptable for the present author, but if the complete life cycle of a haemogregarine is known by a worker, the species cannot be identified by him as Koizumi's *Haemogregarina* sp., although the names of already described species should not be neglected without any enough reasons. A monograph for haemogregarines of Australian reptiles was published by Mackerras (1961). Such kind of works are needed in other parts of the world.

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日本産蛇血液中に検出されたヘモグレガリン

宮田　彬（長崎大学熱帯医学研究所疫学部門）

1967年4～9月，当研究所のウイルス学および衛生動物学両部門により，日本脳炎ウイルス検出のため，長崎市附近で275匹の蛇が集められ，同時に血液の薄層ギムザ染色標本が作られた．検査の結果，次の3種のヘモグレガリンを検出した．第1の種は，ソーセージ型で，細胞質は青く，核は濃紫．空胞，顆粒，膜はなく，大きさ 15.1×4.7 μ．感染血球は膨大し，無色．赤血球の核は原虫の長軸に平行して接し，長い紡錘型になる．ヤマカガシの 40％（78/98），シマヘビの 19％（8/43），マムシの 20％（1/5）から検出された．この種は，*Haemogregarina tigrinae* Hoare（1918）であると考えられる．第2の種は2型あり，1型は長く，核は濃紫色で，細胞質は無色．薄い膜につつまれ彎曲部に赤血球の核を抱く．大きさ 15.9×2.9 μ．第2の型はソーセージ型で小さく，大きさ 11.1×2.8 μ．青い細胞質の両端に空胞や顆粒があり，膜はない．ヤマカガシから 2％（3/98），アオダイショウから 13％（3/24），シマヘビから 5％（2/43）検出された．Mohammed ら（1966）の図示したエジプトのヒキガエルの *Haemogregarina boueti* と感染血球の変化が似ており，感染血球は膨大し，濃く染まり，重複寄生では，感染血球の核が2分したり，消失する．第3の種はヤマカガシから1例検出されたもので，小さく，大きさ 12.7×2.1 μ，膜に包まれず，感染血球は膨大せず，血球の核から離れた所に虫体が位置する．いずれの種も分裂型は見られず，生活史が判明しないと属，種は決定できないが，恐らく *Hepatozoon* 属に属する．第1の種は水際の小動物を捕食する蛇類から見つかっており，媒介者はヒルではないかと考えられ，宿主特異性は認められない．近年 Stehbens ら（1966）によりウイルスの集合体であると指摘された *Pirhemocyton* がヤマカガシやヒバカリから検出された．あたかもマラリア原虫の幼若環状体のように見える場合もあるが，細胞質らしいものはなく，"Albinous body" と呼ばれる空胞が伴って見出される．

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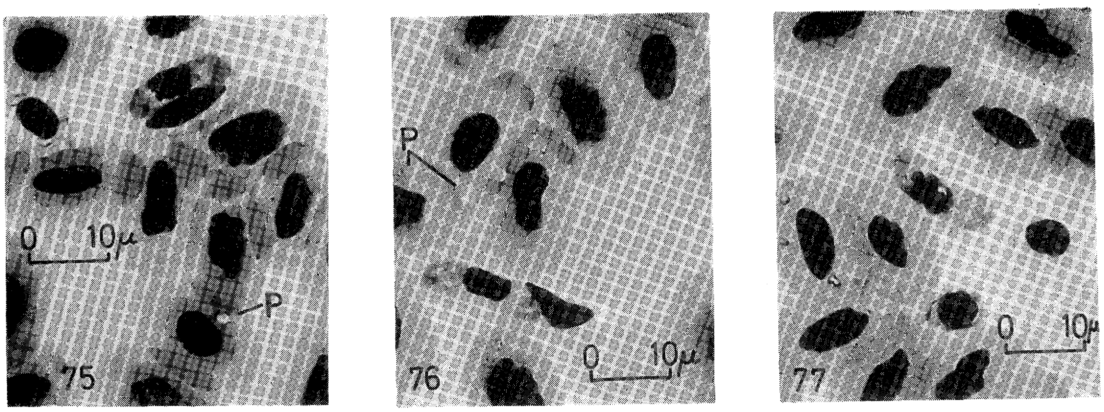
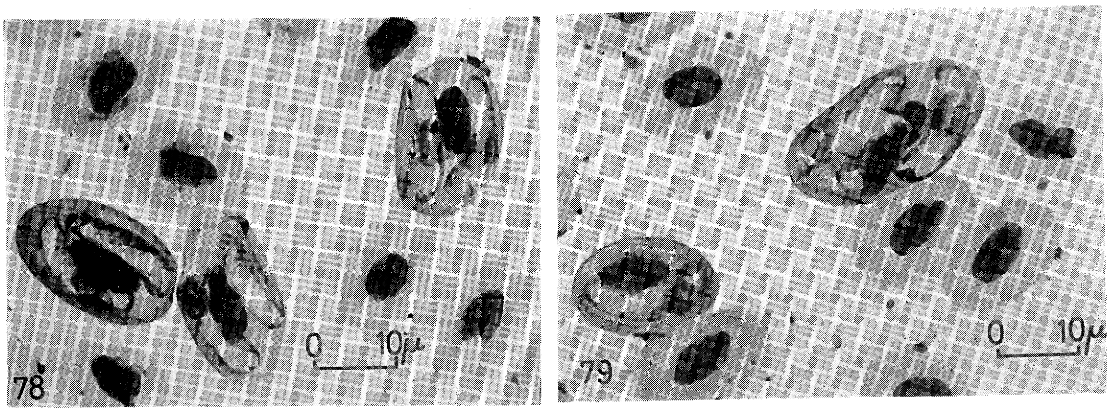


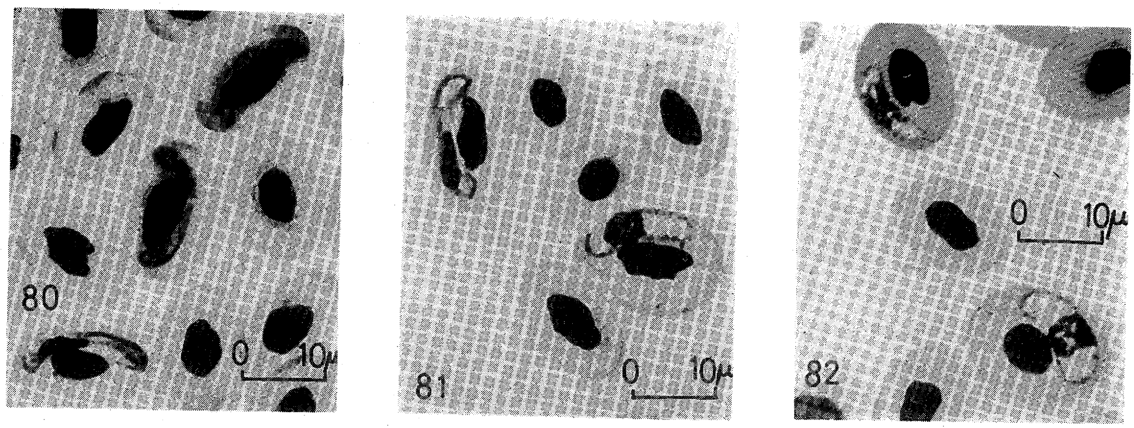



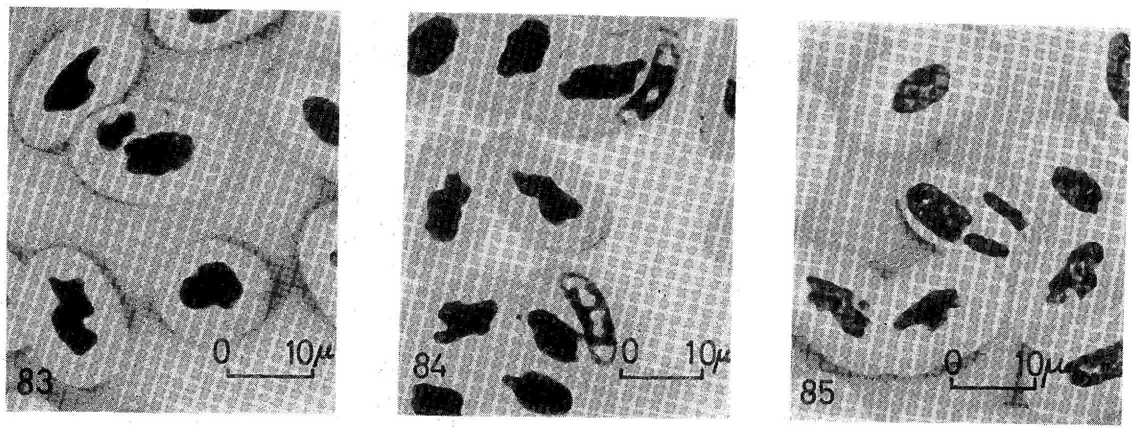

Figs. 75～77. Haemogregarine (Hg-1) from *Rhabdophis tigrinus tigrinus* (snake no. 1967–293) (In this case *Pirhemocyton* (P) was also detected.)

Figs. 78～79. Haemogregarine (Hg-2) from *Elaphe quadrivirgata* (snake no. 1967–22)

## *Note added in proof*

Recently the author has recieved the original description and some of the original blood smears of *Haemogregarina tigrinae* Hoare, 1918, by the courtesy of Dr. Cecil A. Hoare. According to his paper, *H. tigrinae* was described based on blood smears taken from *Natrix tigrina* Boie (＝*Rhabdophis tigrinus tigrinus*) by Dr. W. Roszkowski in the summer of 1917 in the vicinity of Tsuruga, Japan. The present author examined the original blood smears and found three types of haemogregarines as described by Hoare. The first one (vermicular form, Fig. 1 in Hoare's paper) is similar to Hg-3 of the present paper. The second (transitory forms) and the third (Hoare's bean-shaped form) are identical with Hg-1. Hoare's transitory forms (Figs. 2 & 3 in his paper) were detected in thick parts of the blood smears and his bean-shaped forms (Figs. 4～9 in his paper) were usually found in thin parts of the smears. The thick parts might dry up slowly as compared




Figs. 80∼82. Haemogregarine (Hg-2) from *Elaphe quadrivirgata* (snake no. 1967−48) (80. Large type of parasite. 81. Large type (left) and small type (right) of parasite. 82. Small type of parasite.)

Figs. 83∼85. Haemogregarine (Hg-3) from *Rhabdophis tigrinus tigrinus* (snake no. 1967−151) (83. Parasite situated in the center of the blood smear. 84. Parasites situated in the marginal part of the same smear (parasite becomes broader). 85. Double-infected erythrocyte (in the center of the smear).)

with the thin parts and the difference of these morphological appearences might occure under such different conditions of drying up. Hoare's description contains at least two different types each of which is identical with Hg-1 and Hg-3 respectively, and it is not clear at present whether these two types belong to two different species or to two different forms of one species. As a conclusion, however, Hg-1 should be identified as *Haemogregarina tigrinae* Hoare, 1918, because Hoare described mainly this "Hg-1" type of haemogregarine in his paper. The present author wishes to express his deepest appreciation to Dr. Cecil A. Hoare, Wellcome Museum of Medical Science, London, who kindly sent his orginal description and the smears. The original smears, which still remains the initial colour stained 56 years ago, will be deposited permanently in the Institute with the other smears used in the present study.