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A Malaria Parasite (*Plasmodium* (*Garnhamella*) *coturnixae*) of the Grey Quail *Coturnix coromandelica* (*Gmelin*). Aves - Galliformes.

by

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SUMMARY 1. A new malaria parasite is fully described from black-breasted quail *Coturnix coromandelica*. It possesses elon gated gametocytes and erythrocytic schizonts with ten to fourteen merozites; it could not be placed in any of the four sub-genera of *Plasmodium* of avian origin. Therefore a new sub-genus, *Garnhamella*, was proposed (Sarkar & Ray, 1969). The species is thus called *Plasmodium* (*Garnhamella*) *coturnixae*.

2. A second species belonging to the *Haemamoeba* was also present in this quail.

3. A third species resembled *P.* (*Giovannolaia*) *circumflexum*, but certain minor differences were noted.

INTRODUCTION

During the year 1967 while studying the coccidial fauna of game birds we came across two species and a strain of *Plasmodium* in *Coturnix coromandelica*. A reference to the literature on the subject immediately indicated that so far no malarial parasite has been recorded from this migratory galliform bird. In order to study the parasites in detail, they were maintained in uninfected *Coturnix* by needle passage.

MATERIAL AND METHODS

Six specimens of *Coturnix coromandelica* were purchased from a local dealer in Calcutta. A malaria parasite was found in the blood of one bird. More quails were obtained and their blood was examined daily for at least ten days before using them for experimental work. 0.5 ml of infected blood in saline citrate obtained from the wing vein of an infected host was inoculated into clean birds either sub-cutaneously or intra-muscularly. Blood smears were stained with Leishman-Giemsa as a routine. The Giemsa solution was prepared in buffered distilled water (pH 7.2 - 7.4).

To study the tissue phases the infected birds were killed and dry and wet smears of the organs were made for subsequent staining. Pieces of organs were taken at the same time and fixed in Zenker-formol, formol-saline, alcoholic Bouin's and Serra's fluid. Dry contact

*Died 30th May 1969.

smears were stained in the same manner as blood smears, while others were stained by special methods as mentioned in the text. Sections were cut at 6 to 7 μm and stained with MacNamara's modification of Colophonium Giemsa, Feulgen's nuclear reaction, Heidenhain's iron-alum haematoxylin and Gomori's trichrome stains.

The sporogonic stages were studied in laboratory bred *Culex pipiens fatigans* and *Aedes aegypti* at room temperature (27-29 °C) after feeding the mosquitoes on an infected bird.

Injection of sporozoites as well as feeding infected mosquitoes on clean birds was employed to produce sporozoite-induced malaria in clean birds.

Figures illustrating the life cycle are all photomicrographs taken with a Leica microphotographic attachment, with Agfa Light chlorophyll or red filters.

RESULTS

Genus Plasmodium

Sub-genus *Garnhamella* Sarkar & Ray. This sub-genus was reported by Sarkar & Ray in the proceedings of the *IIIrd. International Congress on Protozoology*, Leningrad, July, 1969

(PL — 1. Figs — 1-11)

One out of six grey quails *Coturnix coromandelica* showed a natural infection with a malaria parasite.

Asexual cycle in the blood.

Schizogony cycles in the blood occurred fairly synchronously every 24 hours (as determined by examining the blood at regular six hourly intervals) with a peak in the afternoon. The incubation period of the parasite was found to be seven days after the introduction of the infected blood inoculum.

The youngest parasite was a small rounded body (1.2 μm) with a distinct vacuole and a crescentic nucleus situated at one pole or laterally or tangentially to the host cell nucleus in young erythrocytes (Pl. 1 fig. 1). The trophozoite showed no pigment and became cigar-shaped, measuring 6.0 x 2.4 μm in size with scanty cytoplasm and still maintaining a vacuole in the centre (Pl. 1. fig. 1). After further growth, the nucleus divided with the production in the schizont (size - 6.0 μm in diameter) of ten to twelve solid blocks of irregular chromatin masses, without a vacuole in the centre and little cytoplasm. The position within the cell was polar, lateral or tangential in relation to the host cell nucleus. Two unequal blocks of black pigment granules were found at the centre or towards one side of the early schizont. The nucleus of the host cell was always displaced and sometimes pyknotic.

The mature schizont (7.6 μm in diameter, Pl. 1, figs. 2, 3) contained ten or rarely up to fourteen merozoites. The merozoites were either arranged in a circular fashion with pigment in the centre in a round schizont, or irregularly with pigment granules at the periphery.

At the height of infection a moderate parasitaemia occurred ; the erythrocyte usually contained a single parasite but the occurrence of multiple infection was not uncommon (Pl. 1, fig. 3.). The parasitaemia was low or moderate at first, rose to a peak and later became chronic, persisting for 3 or 4 months. Towards the final stage the parasites were not visible in the peripheral circulation and the birds were immune to fresh infection.

Gametocytes

Gametocytes usually appeared in moderate numbers in mature erythrocytes in the blood two weeks after the initiation of infection. They were elongate with one pole narrower than the other and the margins were irregular or slightly crenated. They lay parallel to the long axis of the erythrocytes without coming in contact with either the nuclear margin or the wall of the host cell, but the host cell nucleus was appreciably displaced (Pl. 1, figs. 5, 6, 7). The young forms could be distinguished from the asexual stages by their prominent cytoplasm and absence of vacuoles.

The macrogametocytes showed the usual difference in staining reaction, with basophilic, vacuolated blue cytoplasm and a banded nucleus. Small black granular pigment granules (15-20) were usually aggregated at either pole of the female gametocyte (Pl. 1 figs. 5, 7).

Microgametocytes (Pl. 1, fig. 6) were slightly wider than the macrogametocytes, with alveolar cytoplasm and diffuse filamentous chromatin that occupied 3/4th of the entire length of the parasite. Four to five coarse, black pigment granules were usually placed at either pole.

Exo-erythrocytic Schizogony

The primary tissue phases of the parasite were not encountered owing to failure to establish infection through the mosquito. However secondary exo-erythrocytic schizogony could easily be produced by intramuscular inoculation of infected blood. This phase of development appeared to start 3 or 4 weeks after infection, when the infection became chronic.

The earliest exo-erythrocytic schizonts were found in macrophage cells of the bone-marrow, liver, spleen, kidney and in lungs but not in the brain. The host cell later became greatly enlarged with a thin rim of vacuolated pale blue cytoplasm and its nucleus appeared as a thin strip of chromatin pushed to one side ; the cytoplasm was occupied by the parasite.

Mature exo-erythrocytic schizonts usually became detached from the host cell.

The mature exo-erythrocytic schizont measured 18 - 22 μm in diameter and showed round nuclei with little cytoplasm around them and one or more residual bodies (Pl. 1, fig. 4). The number of merozoites exceeded 100.

Mosquito stages

The natural vector of this parasite is not known but in the laboratory both *Aedes aegypti* and *Culex.p. fatigans* produced ookinetes and oocysts after an infected blood meal. The formation of sporozoites was completed only in two specimens of *Aedes aegypti* and one of *Culex.p.fatigans.* (out of 11 batches of *Aedes of* 40 each and 13 batches of *Culex* of 50 each at 30° C).

Gametes

Exflagellation of the male gametes was found to occur in the stomach of both *Aedes* and *Culex* after the intake of the infected blood meal (Pl 1, fig. 8.) No exflagellation occurred at room temperature (30° C) or at 4° C., when examined in a sealed cover slip preparation for 15 hours at half an hour intervals. However gametocytes were sometimes seen to free themselves from the erythrocytes after 4 to 5 hours in these conditions.

The male gametes, 8 in number, were filamentous bodies measuring 5-6 μm long and 0.5 μm broad. Usually they were arranged in a circular fashion around the parent body.

Zygote and Ookinete

Zygotes were present in moderate numbers in the stomach of both species of mosquitoes, 14 hours after the infective blood meal. They were oval, rounded or irregular bodies measuring 5-6 μm in diameter with blue cytoplasm and eight chromatin dots arranged at the periphery of the nucleus. A few black pigment granules were scattered in the cytoplasm.

Ookinetes were seen from 16 to 30 hours after the infective blood meal, but their number in any single smear was smaller than the number of zygotes.

The sausage-shaped ookinete measured 13-17 μm in length and 3-4 μm in breadth (15.6 x 3.6 μm) with one pole broader than the other ; the cytoplasm stained pale blue with Leishman Giemsa (Pl. 1. fig. 9). Eight chromatin dots were clearly seen in the nucleus. Black pigment granules 10 to 15 in number, were either scattered or aggregated at the narrow pole.

Oocyst

The earliest oocyst with 6 to 8 nuclei was observed 72 hours after the infective meal in both species of mosquitoes. The size of the oocysts appeared to increase progressively as evidenced by the study of smear preparations at different intervals ; 12 μm on the 3rd day, 32.4 μm on the 5th day (Pl. 1, fig. 10). and 36.0 μm on the 6th day. No further increse in size was noted on the 7th or 8th day.

The number of oocysts in both species usually varied from four to ten but two specimens of *Aedes aegypti* dissected on the 6th day after infection showed 50 to 70 oocysts respectively.

The black pigment granules, 10 to 15 in number in a 3 day old oocyst, were usually found to be clumped in the centre.

Sporozoites

At a temperature of 30° C the salivary glands became invaded between the 8th and 9th day after the infective blood meal in both species of mosquito (Pl. 1, fig. 11). The sporozoite had a stumpy bow-shaped appearance with both ends tapering. Each sporozoite measuring 6-8 μm in length had a single nucleus with granular chromatin placed more or less at the middle of the body.

Experimental infection in other birds

The following birds were inoculated repeatedly with high doses of infected blood as well as with heavily infected bone-marrow and liver homogenates. But none of the following birds showed any evidence of infection :

(1)	Chickens R.I.R., Leghorn and Desi-fowl.	(Galliformes)
(2)	Canary	(Passeriformes)
(3)	*Peridicula argoondah*	(Galliformes)
(4)	*Turnix suscitator*	,,
(5)	*Lonchura malabarica*	(Passeriformes)
(6)	*L. punctulate*	,,
(7)	*Passer domesticus*	,,
(8)	*Anas querquedula*	(Anseriformes)
(9)	*Nettapus coromandelianus*	,,
(10)	*Anas boschas*	,,

Pathogenic effect

The pathogenic effect of this parasite on the host was first noted in a bird purchased in the month of November (23.11.66). Later a number of experimentally infected birds at the height of infection also showed similar symptoms before death.

The birds became listless and were scarcely able to stand. The feathers were partially or completely denuded in older infections. Finally the birds lay on one side with twitching of the neck. Birds with these symptoms always died.

A marked effect on the haemopoietic system was indicated by the appearance of many young or premature erythrocytes in the circulation and usually in chronic cases the blood became thin and watery.

Post-mortem examination invariably revealed an enlarged spleen (6 to 8 times normal) with haemorrhagic areas on the surface. The colour of the spleen, liver and kidney was bronze-black. Both spleen and liver showed pigment in the macrophages and Kupffer's cells. Other organs presented a blanched appearance.

DISCUSSION

According to the classification of avian malaria parasites by Corradetti, Garnham & Laird (1963) the elongated shape of the gametocyte immediately differentiated this organism from the subgenus *Haemamoeba*. Since schizogony did not occur in the primitive blood forming cells it could not be placed in the sub-genus *Huffia*. Equally it could not be placed in the sub-genus *Giovannolaia* in which the parasites have moderate to large erythrocytic schizonts, containing much cytoplasm, whereas in the parasite under description the amount of cytoplasm was very scanty or often absent in the erythrocytic schizonts. Finally the *Novyella* sub-genus includes species with small schizonts with a maximum of 8 merozoites but in the parasite described the number of merozoites was not less than ten and never exceeded fourteen. Thus it could not be placed in the sub-genus *Novyella*. It seems to occupy a place intermediate between *Novyella* and *Giovannolaia* i.e., the parasite has large schizonts with scanty cytoplasm and elongated gametocytes. The scheme of classification therefore may be further modified as follows :—

1. Round gametocytes..... *Haemamoeba*

   Elongate gametocytes.....2

2. Schizogony in primitive blood forming cells present..... *Huffia*

   Schizogony in primitive blood forming cells absent.....3

3. Erythrocytic schizonts with plentiful cytoplasm and more than 14 merozoites.... *Giovannolaia*

4. Erythrocytic schizonts with scanty cytoplasm and less than 14 merozoites.... *Garnhamella*

5. Small erythrocytic schizonts with scanty cytoplasm and with 8 or fewer nerozoites. *Novyella.*

Other species of malaria parasites found in grey quails.

A species resembling *Plasmodium* (*Haemamoeba*) *relictum* was studied from quails, and erythrocytic, exoerythrocytic and sporogonic stages were found ; the last were produced in *Culex pipiens fatigans*. This strain failed to infect canaries, sparrows and chickens and may represent a subspecies.

A third species was also encountered in the grey quail which gave rise to a high parasitaemia. Morphologically it resembled *Plasmodium* (Giovannolaia) *circumflexum*, though minor differences were observed.

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EXPLANATION OF PLATE — I

P. (*Garnhamella*) *coturnixae* Sarkar & Ray

Scale representing 10 μm

PLATE I

Fig. 1. Ring form with crescentic chromatin, a fine rim of cytoplasm and a prominent vacuole.

Fig. 2. Mature schizont, containing ten merozoites, and dark pigment touching the nuclear border. Note the displaced nucleus of the host cell.

Fig. 3. Top, immature red blood cell with eleven free merozoites and with two mature schizonts.

Fig. 4. Secondary exo-erythrocytic schizont in bone-marrow smear, showing large number of merozoites and residual bodies.

Fig. 5. Top—early schizont with scanty cytoplasm and bottom—female gametocyte. Note the crenated margins of the gametocyte.

Fig. 6. Male gametocyte with crenated margins.

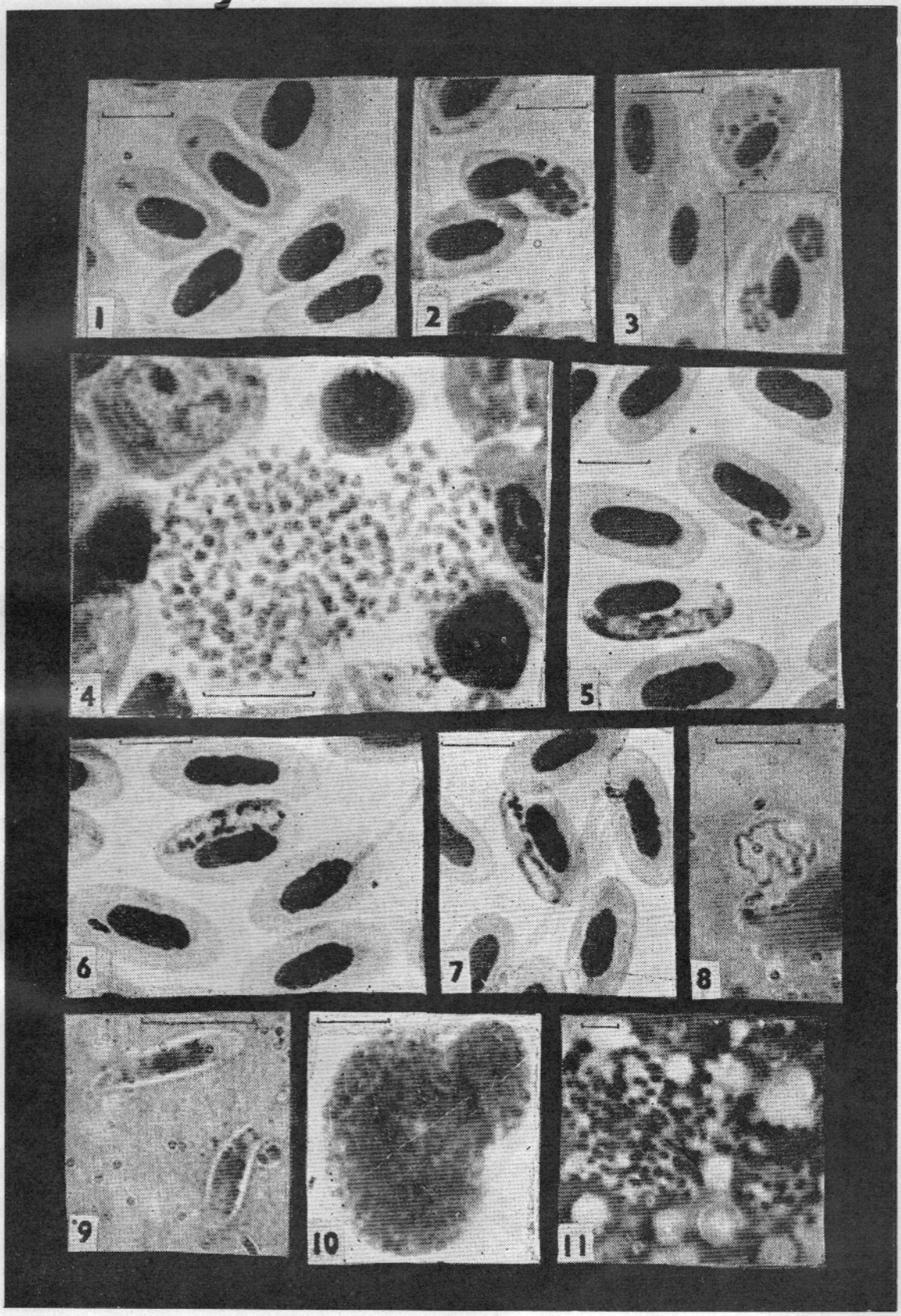
Fig. 7. Female gametocyte showing crenated margins, clear of both nuclear and cell membrane.

Fig. 8. Mosquito gut smears twelve hours after infected blood meal. Note exflagellated male gametes.

Fig. 9. Gut smear of mosquito twenty hours after the infective blood meal. Two ookinetes with each showing nucleus consisting of eight prominent chromatin granules.

Fig. 10. Oocyst seen in gut smear of mosquito at the end of 5th day.

Fig. 11. A bunch of sporozoites in the salivary gland smear at the end of 9th day after infective feed. All were stained with Leishman - Giemsa.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