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Plasmodium dissanaikei n.sp. a New Avian Malaria Parasite from the Rose-ringed Parakeet of Ceylon, *Psittacula krameri manillensis**

by

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SUMMARY A new *Plasmodium* species is described from the rose-ringed parakeet of Ceylon, *Psittacula krameri manillensis* and is named *Plasmodium* (*Novyella*) *dissanaikei* n. sp. This is the first detailed description of a *Plasmodium* species from a Psittaciforme bird.

INTRODUCTION

There are very few published records of species of *Plasmodium* in Psittaciforme birds. No detailed descriptions accompany the few that exist. Earlier records of *Plasmodium* species in Psittaciforme birds are found in reports on the deaths occurring in the London Zoological Gardens (Plimmer, 1912; Scott, 1926, 1927 and Hamerton, 1930). In many instances it was not possible to determine whether the parasite seen was a species of *Haemoproteus* or a *Plasmodium*. Ogaki (1949) in a survey of malaria parasites of birds of the Malayan peninsula found *P. circumflexum* in a parrot, *Psittinus cyanurus cyanurus*. The blue-headed parrot, *Pionus menstruus* of Panama was found infected with *P. nucleophilum*, *P. relictum* and *P. circumflexum* (Gorgas lab. 1961).

Bennett and Cheong (1965) found an unknown species of *Plasmodium* in the blood of a naturally infected kea which died in the National Zoological Gardens in Ulu Klang.

Manwell (1963) working with parakeets (budgerigars) found that they were insusceptible to a number of species of *Plasmodium*. Even after splenectomy and cortisone administration it proved impossible to reduce resistance to a degree sufficient to obtain an infection.

A new species of *Plasmodium* was discovered in the rose-ringed parakeet of Ceylon, *Psittacula krameri manillensis* and was maintained in the laboratory for three years. It is proposed to name the parasite *Plasmodium dissanaikei* n. sp. in honour of Professor A. S. Dissanaike.

Type host — The rose-ringed parakeet of Ceylon, *Psittacula krameri manillensis*.
Type locality — Ja-ela, Ceylon.
Type specimens — Deposited in the collection of Professor P.C.C. Garnham, Imperial College Field Station, Ashurst Lodge, Ascot, Berkshire, England.

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MATERIALS AND METHODS

Parakeets were obtained from dealers in St. John's Market, Colombo and Ja-ela, Ceylon. Strains of the parasite were maintained in the laboratory in ducklings obtained from a farm. Turkeys and chicks were obtained from a commercial dealer. Canaries, budgerigars and quail were obtained from a pet shop. The domestic pigeons used were bred at Winches Farm, St Albans, Herts. Mosquitoes used for transmission experiments were maintained in insectaries.

Blood films were stained for 1 hour in 10% Giemsa stain (Gurr's R66).

RESULTS

A total of 47 parakeets was examined during this study. The actual incidence of infection is not known, as many of the birds were already selected in Ceylon prior to despatch to London. Levels of parasitaemia in infected birds varied from mild to moderate with a few heavy infections. Gametocyte levels varied from 5-13% of the total parasite level. Heavily infected birds showed signs of illness, such as ruffled feathers and drooping eyelids. The birds did not eat and sat huddled in a corner of the cage. Varying degrees of anaemia were noticed.

Morphology :—Merozoites enter the red blood cell and lie anywhere in the cytoplasm of the host cell (Plate I.1).

Very soon the cytoplasm of the parasite becomes visible as small pseudopodial projections giving the parasite a fimbriated appearance. Typical ring stages are scanty. (Plate I.2). When the parasite reaches a size of 2-3 μm it lies towards the polar end of the host cell, closely attached to the host cell nucleus. Pigment becomes visible in the form of very fine yellowish brown granules (Plate I.4.)'

Further growth occurs with the parasite still attached to the host cell nucleus. Remnants of a vacuole may be seen (Plate I. 5). Pigment is more prominent but remains scattered. Nuclear division occurs when the parasite reaches a size of 4.5-5μm (Plate I.6). The nuclear masses usually move to the periphery giving the parasite a fan shaped appearance. A schizont approaching full maturity shows very little or no cytoplasm (Plate I. 8). The number of nuclear divisions in the mature schizont varies between 6-12. In a count of 100 consecutive mature schizonts the following pattern emerged.

Number of merozoites/schizont	6	7	8	9	10	11	12
Number of schizonts	25	0	64	3	5	0	3

When a schizont ruptures there is a tendency for the merozoites to remain within the host cell (Plate I. 10). A few circular refractile globules are visible, scattered irregularly in the cytoplasm of the growing trophozoites (Plate I. 5). Nuclear displacement occurs in heavy infections (Plate I. 9). The parasite does not preferentially enter reticulocytes. There is no enlargement of the host cell or change in colour or shape.

Gametocytes :—The gametocytes are irregular elongate bodies showing the usual staining reactions. Developing gametocytes possess a " tail-like " process and measure 5.5-7 μm (Plate I. 11, 12). Mature gametocytes measure 13.2-14.4 μm and extend from pole to pole showing a slight tendency to curve round the nucleus of the host cell. The medial border is very irregular. The gametocytes do not displace the nucleus of the host cell. (Plate I. 14, 16)

The cytoplasm of the mature female gametocyte stains a deep blue colour, with a few vacuoles occasionally seen. The nucleus is central in position and compact. Pigment is yellowish brown in colour and plentiful (Plate I. 16). The male gametocyte has a pinkish tint and a delicate appearance. The nucleus of the parasite appears to be in strands or whorls extending more than half the length of the parasite. Pigment may be clumped or diffuse. (Plate I.14)

Morphology in experimental hosts :—Strains of the parasite were maintained in ducklings. A change in morphology of the parasite was observed after about 6-7 blood passages. The parasite becomes larger and nuclear attachment less marked. Multiple infections are more frequently seen and higher parasitaemias are observed. Gametocyte production ceases after about the seventh passage.

The parasite is not infective to chicks, budgerigars or quail but is mildly infective to domestic pigeons and canaries. Turkeys are moderately susceptible to infection.

Exoerythrocytic stages :—Rare exoerythrocytic schizonts were found in impression smears of the liver of a turkey which was treated with quinine, 16 days after infection. The cells infected were probably lymphocytes.

Sporogony :—Attempts to obtain sporogonic development using the following species and strains of mosquitoes were unsuccessful: *Aedes aegypti* var *queenslandensis*, *Aedes togoi* (London School of Hygiene and Tropical Medicine strain) *Culex gelidus* (American strain) *Culex pipiens pipiens* (Mill Hill strain) and *Culex pipiens fatigans* (Lagos, Venezuelan and. Rangoon strains).

Ookinetes :—The ookinetes are usually banana shaped. At times small vacuoles are found. The nucleus is central in position and stains deeply. In some of the smears, especially those made after 30 hours, elongate distorted ookinetes are seen.

DISCUSSION

Plasmodium dissanaikei n. sp. is placed in the subgenus *Novyella* because it has schizonts with scanty cytoplasm and eight merozoites. The gametocytes are elongate. Rare exoerythrocytic schizonts were found in lymphoid macrophage cells.

The distinguishing features of the parasite are the presence of refractile blue globules, one to six in number in the growing trophozoites; the tendency for the merozoites to be held within the host cell for a time after rupture of the schizont; the polar position of the

parasite and close apposition to the nucleus of the host cell and the irregular ragged appearance of the gametocytes.

P. dissanaikei must be distinguished from *P. vaughani*, *P. nucleophilum* and *P. hexamerium* to which it bears a close resemblance. *P. dissanaikei* is larger in all stages than *P. vaughani* and the number of merozoites is 8 in *P. dissanaikei* and 4 in *P. vaughani* though occasionally it may be 8 (Novy and MacNeal, 1904).

Manwell (1935) described the schizonts of *P. nucleophilum* as containing 4-9 merozoites. The large trophozoites were closely applied to the host cell nucleus. The pigment was black. Mature schizonts were hard to find as the erythrocytes were soon destroyed and when seen the host cell nucleus was found to be displaced and frequently in the process of dissolution. The gametocytes were elongate and closely applied to the host cell. *P. dissanaikei* resembles *P. nucleophilum* in the displacement of the host cell nucleus, shape of the gametocytes and close association of the parasite to the host cell nucleus. However, *P. dissanaikei* does not cause dissolution of the host cell nucleus or destruction of the red cell. The pigment is yellow brown in colour.

The characteristic number of merozoites in the schizont of *P. hexamerium* is six, whereas in *P. dissanaikei* it is eight. Also *P. hexamerium* has less affinity for the host cell nucleus (Huff, 1935) than *P. dissanaikei*.

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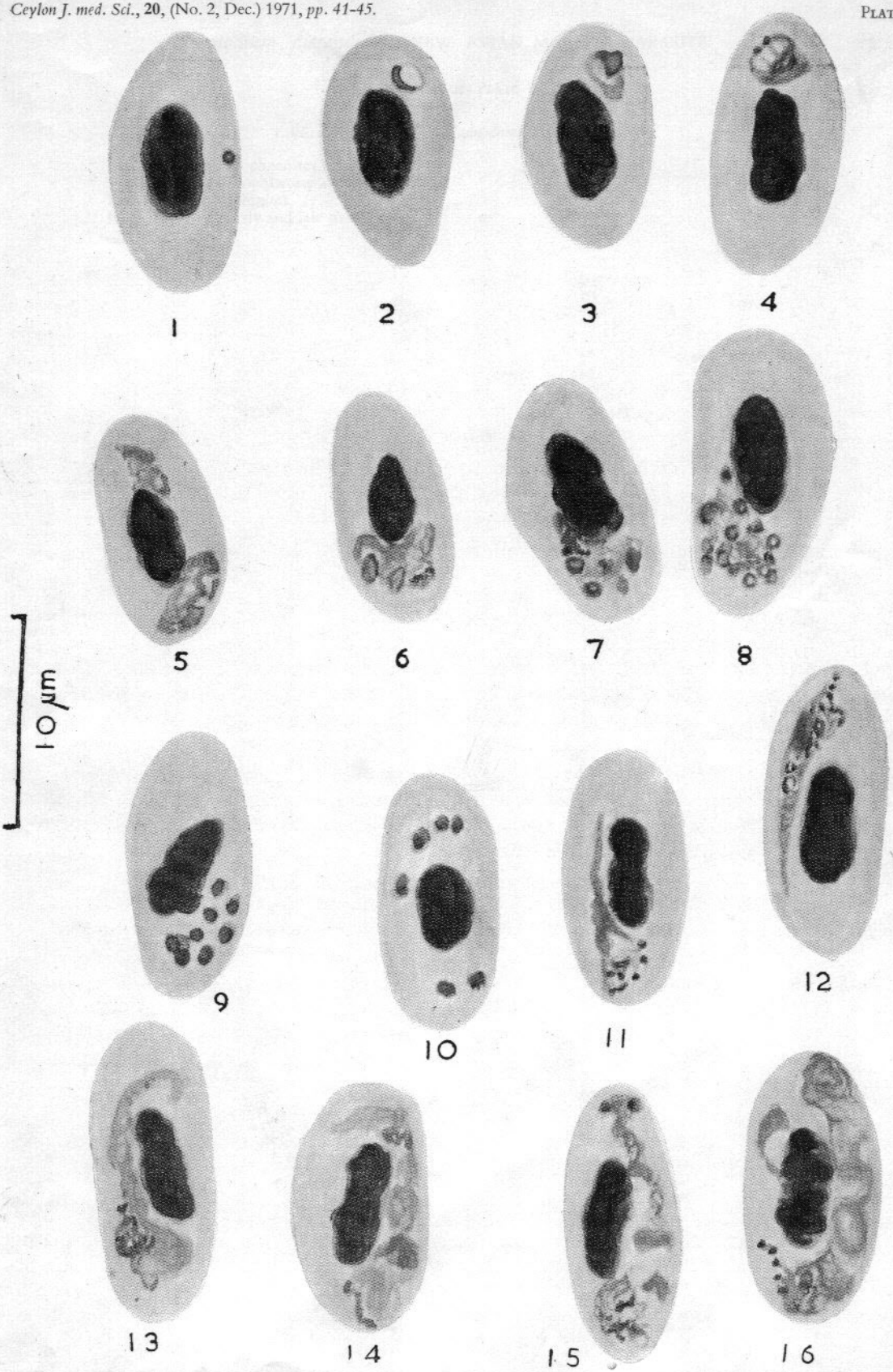
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EXPLANATION OF PLATE I

Erythrocytic stages of *Plasmodium dissanaikei*

Figs. 1 - 4 Trophozoites
Fig. 5 Trophozoite and early schizont
Figs. 6 - 16 Schizont
Figs. 11 - 16 Early and late gametocytes