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Malaria parasites of rodents of the Congo (Brazzaville) : *Plasmodium chabaudi adami* subsp. nov. and *Plasmodium vinckei lentum* Landau, Michel, Adam and Boulard, 1970

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Summary.

Descriptions are given of the blood forms, sporogonic stages and enzyme forms of *Plasmodium chabaudi adami* subsp.-nov. and *P. vinckei lentum,* malaria parasites of the thicket-rat *Thamnomys rutilans* of the Brazzaville region. The two species differ from each other in both morphological and enzymic characters. *P.c. adami* and *P.v. lentum* differ from the other subspecies of *P. chabaudi* and *P. vinckei* principally by their enzyme forms.

Résumé.

Le paludisme des rongeurs du Congo (Brazzaville) : *Plasmodium chabaudi adami* subsp. nov. et *Plasmodium vinckei lentum* Landau, Michel, Adam et Boulard, 1970.

Nous décrirons l'infection sanguine, la sporogonie et les formes enzymiques de *Plasmodium chabaudi adami* subsp. nov. et de *P. vinckei lentum,* parasites du Rongeur *Thamnomys rutilans* de la région de Brazzaville. Les deux espèces se distinguent par les caractères morphologiques et enzymiques. *P.c. adami* et *P.v. lentum* se distinguent essentiellement des autres sous-espèces de *P. chabaudi* et *P. vinckei* par les formes enzymiques.

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In a recent paper (Carter and Walliker, 1975) we showed that three species of malaria parasites occurred in thicket-rats (*Thamnomys rutilans*) of the Central African Republic (C.A.R.). One species, *Plasmodium yoelii yoelii*, developed mainly in reticulocytes, while the other two, *P. chabaudi* and *P. vinckei petteri* invaded mature erythrocytes. Each species possessed distinctive morphological characteristics and enzyme forms. Mixed infections of more than one species were common in wild caught rodents, but pure-line infections of each could be established by cloning blood forms in laboratory mice.

TABLE I. — Enzyme forms of *P.c. adami* and *P.v. lentum* isolates examined in this study. Isolates were made from specimens of *T. rutilans* captured in degraded forest gallery near the village of N'ganga Lingolo, 15 km west of Brazzaville.

Species	Isolate	Date of Isolation	GPI	Enzyme forms		GDH
				6 PGD	LDH	
P. chabaudi adami	556 KA	1970	8	2	8	5
	408 XZ	1972	8	2	10	5
P. vinckei lentum	170 L	1966	6	5	7	6
	483 L	1968	6	5	7	6
	194 ZZ	1970	6	5	7	6
	408 XZ	1972	11	5	9	6

We describe here a similar situation among the malaria parasites of *T. rutilans* of the Congo (Brazzaville). Until now, only two species have been described, *P.y. killicki* Landau, Michel and Adam, 1968 and *P.y. lentum* Landau, Michel, Adam and Boulard, 1970, which show similar host-cell preferences to the corresponding species of the C.A.R. After examining parasites derived from naturally infected thicket-rats of the Brazzaville region, we have concluded that a third species is present in these animals which, like *P.v. lentum*, develops in mature erythrocytes. We recognise this parasite as a subspecies of *P. chabaudi* for which the name *P.c. adami* is proposed, in tribute to Dr. J. P. Adam, who has carried out numerous studies on the blood protozoa of this region. The parasite of the C.A.R. thus becomes the nominate subspecies *P.c. chabaudi*.

In this paper we describe the morphology and enzyme characteristics of *P.c. adami*. For comparison we also describe *P.v. lentum;* detailed descriptions of the blood forms of this species have not been published previously. Our observations are taken from five isolates from thicket-rats captured near Brazzaville between 1966 and 1972 (*Table I*), which were sent to our laboratory with the kind co-operation of M^me^ I. Landau. One of these isolates contained only *P.c. adami*, three contained only *P.v. lentum*, and the fifth (isolate 408 XZ) contained a mixture of the two. By cloning blood forms of this isolate by dilution, we were able to establish separate infections of each species in mice.

The morphology of the blood forms was examined in thin blood films taken from laboratory mice and thicket-rats (*Grammomys surdaster*) and stained with

Giemsa's stain. Sporogonic stages were examined in *Anopheles stephensi* maintained at 24-26°C. Sporozoites were measured from midline drawings made with the aid of a camera lucida. For enzyme analysis, blood forms were subjected to starch gel electrophoresis, and the electrophoretic forms of glucose phosphate isomerase (GPI), 6-phosphogluconate dehydrogenase (6PGD) and lactate dehydrogenase (LDH) characterised by comparison with the enzyme forms of other rodent malaria species. The conditions of electrophoresis were similar to those used previously (Carter, 1973; Carter and Walliker, 1975). An additional enzyme studied was NADP-dependent glutamate dehydrogenase (GDH) (Carter, 1974 *a*), for which a citrate phosphate buffer at pH 7.0 was used.

The system of numbering enzyme forms is based on that used previously (Carter, 1973). Recent refinements in the technique have led to improved resolution of enzyme forms, with a consequent revision in the numbering (Carter, 1974 *b*); the numbering used in the present study is based on the revised system.

Plasmodium chabaudi adami *subsp. nov.*

A description of the blood-forms, sporogony and enzyme forms.

P.c. adami gives rise to a synchronous infection in mice and thicket-rats, with a periodicity of 24 hours. Maximum numbers of schizonts appear around midnight. Of the two parasite lines studied, one, derived from isolate 408 XZ, gave rise to virulent infections in mice. The parasitaemia rose to 80 % or higher, and mice usually died within seven days of injection of blood forms. The other line, derived from isolate 556 KA, produced a mild infection in mice. Parasitaemias rarely exceeded 10 % and no deaths were recorded.

During the early stages of blood infection, parasites are found mainly in mature erythrocytes. There does not, however, appear to be a preference for this cell-type, as immature cells are also invaded in approximate proportion to their prevalence in the blood.

Morphology of the blood forms.

The blood forms are similar to those of *P.c. chabaudi* of the C.A.R. (Carter and Walliker, 1975).

Ring forms typically possess a thin circle of pale, blue cytoplasm around a central vacuole; their diameter may be up to 1/3 of that of the host-cell. Both single and double chromatin « dot » nuclei are seen. As trophozoites develop, the vacuole is gradually lost, and the parasite becomes amoeboid with a ragged outline (*fig.* 1 *a*). The cytoplasm becomes moderately dense blue, and may contain several small vacuoles. Pigment is inconspicuous in young trophozoites, but fine gra-

nules are laid down in mature forms. Trophozoites remain small, seldom exceeding 1/2 the diameter of their host cell. Occasionally, reddening of the infected cell is seen as trophozoites mature. No enlargement of the host-cell occurs.

As schizogony commences, trophozoites become condensed, and nuclei appear as deep red dots. The mature schizont (*fig.* 1 *b*) remains small, possessing 4-10 merozoites (average 6). A single lump of pigment remains at the centre or edge of the schizont. The host cell membrane may become ragged as schizonts mature, but usually remains intact as merozoites separate.

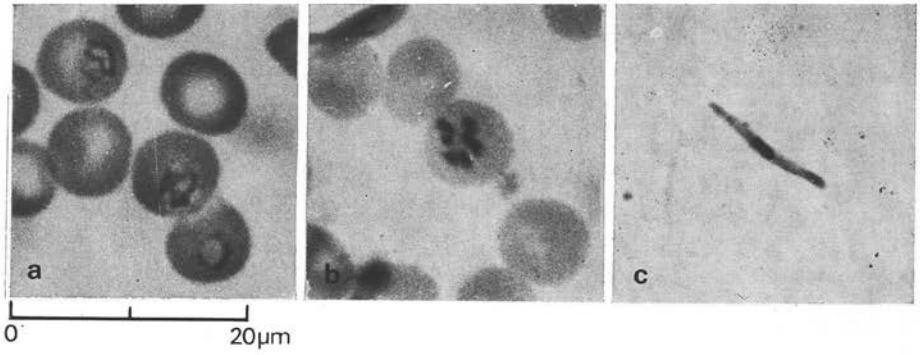

FIG. 1. — *Plasmodium chabaudi adami*. a. Trophozoites ; b. Schizont ; c. Sporozoite.

Gametocytes are present throughout the infection, but are particularly numerous after the parasitaemia peak. They occupy the entire volume of the host cell, but no host cell enlargement occurs. In macrogametocytes, the cytoplasm is very pale blue, and contains numerous golden brown pigment granules. The nucleus is small, sometimes elongated, and usually lies in a small region of clear cytoplasm. Microgametocytes possess pink cytoplasm with abundant pigment granules. The large nuclear region is distinguished from the cytoplasm by an absence of pigment.

Sporogony.

The sporogonic stages of cloned *P.c. adami* derived from isolate 408 XZ were studied in *A. stephensi* maintained at 24-26°C. Mature oocysts first appeared on the ninth day of infection. The average diameter of 15 mature oocysts was 51 μm (range 45-64 μm).

Sporozoites (*fig. 1 c*) first appeared in the salivary glands on the eleventh day. They were slightly curved in shape with bluntly pointed ends. In fresh preparations seen under phase-contrast microscopy, the average length of 50 sporozoites was 13.37 μm (S.D. ± 1.75 μm). In smears fixed in methanol and stained with Giemsa's stain, the average length of 75 sporozoites was 11.68 μm (S.D. ± 1.66 μm).

There was no significant difference between the lengths of sporozoites from rupturing oocysts and from salivary glands.

Enzyme forms (*Table I*).

Both the mild and virulent lines of *P.c. adami* were characterised by enzyme forms GPI-8, 6PGD-2 and GDH-5. The lines differed in LDH type, the mild line, derived from isolate 556 KA possessing LDH-8, and the virulent line from isolate 408 XZ, possessing LDH-10.

Plasmodium vinckei lentum

Landau, Michel, Adam and Boulard, 1970

A description of the blood-forms, sporogony and enzyme forms.

P.v. lentum gives rise to infections in mice and thicket-rats with a periodicity of 24 hours. Maximum numbers of schizonts are seen around midnight, although the infection is less synchronous than *P.c. adami*, small numbers of schizonts being present at other times of the day. Parasitaemias may rise to 60 % or higher, but infections are not usually lethal. The blood forms show a marked preference for mature erythrocytes, as the number of ring forms seen in immature cells is disproportionately low even at high parasitaemias.

Morphology of the blood-forms.

Ring forms are similar to those of *P.c. adami*, consisting of a thin circle of blue cytoplasm surrounding a central vacuole. Single and double chromatin « dot » nuclei are common. Multiple infections of erythrocytes are rare.

As trophozoites develop, the parasite outline may appear irregular at first, but mature trophozoites are usually round or oval in shape (*fig. 2 a*). The cytoplasm becomes pale in colour, and dense speckled pigment of golden brown colour is laid down. A vacuole, or two or three smaller vacuoles, may be present. The nucleus enlarges slightly, and may lie in a clear region of cytoplasm. The mature trophozoite is larger than that of *P.c. adami*, occupying up to 3/4 of its host-cell volume. The host-cell membrane frequently appears crenated.

When schizogony commences, the parasites appear more deeply stained. The pigment granules form a single mass which is normally centrally placed. Mature schizonts (*fig. 2 b*) possess 6-16 merozoites (average 10). As the schizont matures, the host-cell membrane usually breaks down.

Gametocytes possess similar morphology to those of *P.c. adami*. Macrogametocytes, which may be difficult to distinguish from large trophozoites, contain pale blue cytoplasm with abundant pigment granules, and a small slightly elongate nucleus lying in a clear region of cytoplasm. Microgametocytes are pink with

conspicuous pigment in the cytoplasm, the large nuclear region being recognisable by an absence of pigment.

Sporogony.

The sporogony of cloned *P.v. lentum* derived from isolate, 408 XZ was studied in *A. stephensi.* Matue oocysts first appeared on the ninth day of infection. The average diameter of 15 mature oocysts was 45 μm (range 38-53 μm).

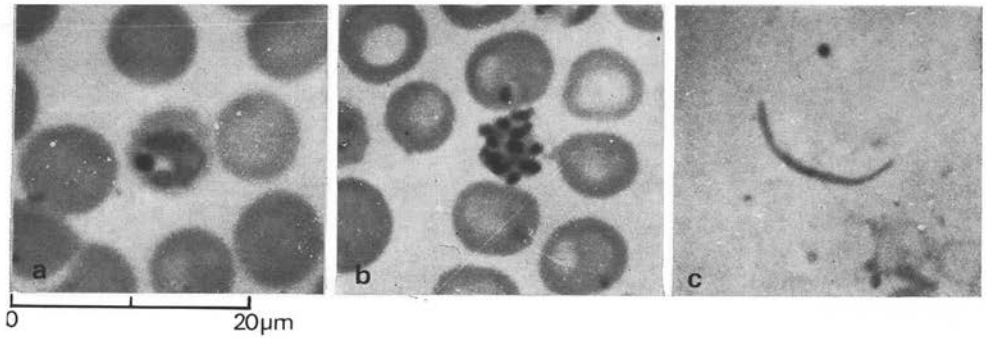

FIG. 2. — *Plasmodium vinckei lentum.* a. Trophozoite ; b. Schizont ; c. Sporozoite.

Sporozoites (*fig. 2 c*) were of slender shape with pointed ends. They first appeared in the salivary glands on the eleventh day of infection. In fresh preparations examined by phase-contrast microscopy, the average length of 50 sporozoites was 18.48 μm (S.D. ± 2.47 μm). In methanol-fixed and Giemsa-stained smears, the average length of 75 sporozoites was 15.26 μm (S.D. ± 1.38 μm). There was no significant difference between the lengths of sporozoites from rupturing oocysts and from salivary glands.

Enzyme forms (*Table I*).

Parasite lines derived from the four isolates of *P.v. lentum* were examined for enzyme forms. Each line was characterised by 6PGD-5 and GDH-6. Three lines possessed GPI-6 and LDH-7, while the fourth, derived from isolate 408 XZ, GPI-11 and LDH-9.

Discussion

Our observations show that there are two species of *Plasmodium* which infect mature erythrocytes of *T. rutilans* of the Congo (Brazzaville), *P. chabaudi adami* and *P. vinckei lentum.* The two species can be readily distinguished from a third

species *P. yoelii killicki*, which preferentially invades reticulocytes of these animals. The situation is similar to that seen in the C.A.R. where *T. rutilans* is infected by *P.c. chabaudi, P.v. petteri* and *P.y. yoelii* (Carter and Walliker, 1975).

Two subspecies of *P. chabaudi* and four of *P. vinckei* have now been described from African rodents, which are listed in Table II. *P. chabaudi* differs from *P. vinckei* (1) by its smaller and more irregularly shaped trophozoites which lack conspicuous pigment, (2) by its smaller schizonts which produce fewer merozoites, (3) by the shape and shorter length of its sporozoites [with the possible exception of *P.v. vinckei* wich may have sporozoites of comparable size (Killick-Kendrick, 1975)] and (4), most clearly, by its characteristic enzyme forms, none of which are shared by *P. vinckei*.

The two *P. chabaudi* subspecies are almost identical in the morphology of their blood forms and sporozoites. The line of *P.c. adami* derived from isolate 556 KA, however, is less virulent than any *P.c. chabaudi;* it is not known whether this is an innate characteristic of this line, or whether it is due to contamination by another organism such as *Eperythrozoon coccoides* (Ott, Astin and Stauber, 1967). The character which most clearly differentiates the two subspecies is their enzyme patterns. *P.c. chabaudi* and *P.c. adami* possess 6PGD-2 and GDH-5 in common, but differ in their forms of GPI and LDH; *P.c. chabaudi* possesses GPI-4 and LDH-2, -3, -4 and -5, while *P.c. adami* lines are characterised by GPI-8 and LDH-8 and -10. The two forms of LDH found in *P.c. adami* probably represent an example of enzyme polymorphism within the subspecies. If more isolates of *P.c. adami* become available for examination, it is possible that other variant forms of their enzymes will be found; in *P.c. chabaudi*, two forms of 6PGD and four forms of LDH were found among 15 isolates examined (Carter and Walliker, 1975).

The four subspecies of *P. vinckei* are also very similar in the morphology of their blood forms, although some variations in virulence occur. Lines of *P.v. petteri*, for example, are usually milder than lines of the other subspecies. The sporozoite lengths of each subspecies differ slightly from one another although there are discrepancies in the measurements recorded in different laboratories. Apart from shrinkage which may occur on fixation and staining, as noted in the present work, differences are seen even when measurements are made under similar conditions (*Table II*). A possible explanation of the differences in *P.v. lentum* measured by ourselves, by Landau *et al.* (1970) and by Killick-Kendrick (1975) is that variations in this character may occur in different parasite lines. The sporozoites which we measured were of a cloned line, derived from a different wild isolate from that used by the other authors.

As in *P. chabaudi* it is the enzyme forms which are of most value in differentiating the subspecies of *P. vinckei*. It can be seen from Table II that although the four subspecies share certain enzyme forms, each possesses its own characteristic pattern. *P.v. lentum* possesses GDH-6 in common with each of the other subspecies, GPI-6 and LDH-9 in common with *P.v. brucechwatti*, and 6PGD-5 and LDH-7 in

TABLE II. — The regions of origin, natural hosts, sporozoite lengths and enzyme-forms of *P. chabaudi* and *P. vinckei* subspecies.

	P.c. adami	*P.c. chabaudi*	*P.v. lentum*	*P.v. petteri*	*P.v. brucechwatti*	*P.v. vinckei*
Region of origin	Brazzaville	Central African Republic	Brazzaville	Central African Republic	W. Nigeria	Katanga
Natural rodent host	*T. rutilans*	*T. rutilans*	*T. rutilans*	*T. rutilans*	*T. rutilans*	*Grammomys surdaster*
Sporozoite length ..... (fixed and stained)	11.68 µm (1) (± 1.66 µm)	11.75 µm (2) (± 1.63 µm)	15.26 µm (1) (± 1.38 µm) 19.5 µm (3) (± 2.64 µm) 21.2 µm (4) (range 18-25 µm)	16.23 µm (2) (± 2.25 µm)	14.7 µm (3) (± 3.04 µm)	12.3 µm (3) (± 0.99 µm) 16 µm (5) (range 11-22 µm)
Enzyme forms.						
GPI	8	4	6,11	5,9	6	7
6 PGD	2	2, 3, 7	5	5	6	6
LDH	8,10	2, 3, 4, 5	7,9	7	9	6
GDH	5	5	6	6	6	6

(1) Original. (2) Carter and Walliker, 1975. (3) Killick-Kendrick, 1975. (4) Landau *et al.*, 1970. (5) Bafort, 1971.

common with *P.v. petteri*. In addition, it possesses a form of GPI, GPI-11, not found in any of the other three subspecies.

When we examined the malaria parasites of rodents of the C.A.R. (Carter and Walliker, 1975), it became apparent that Landau (1965) had described the blood forms of a mixed infection of *P.c. chabaudi* and *P.v. petteri* in her original account of *P. chabaudi*. It is less clear whether the parasites first recorded from the Brazzaville region by Adam *et al.* (1966) included both *P.c. adami* and *P.v. lentum*, as detailed descriptions of the blood forms were not given. They described the parasites, however, as « like *P. chabaudi* », and referred to a reddening of some infected erythrocytes, a character seen sometimes in *P.c. adami* infections, but only rarely in *P.v. lentum*. However, in a later paper (Landau *et al.*, 1970) it is probable that a pure infection of *P.v. lentum* was being described, as the sporozoites of this parasite were considerably longer than those of *P. chabaudi* (*Table II*).

With these descriptions of *P.c. adami* and *P.v. lentum*, the classification of the murine rodent plasmodia currently available in the laboratory is now largely complete. The distinction between the subspecies of each is shown most clearly by their enzyme patterns. Evidence of close relationships between the subspecies in seen in their morphological similarities and in their sharing of certain enzyme forms. It is hoped that further work on the life-cycle of each parasite, particularly on the exo-erythrocytic forms, may provide further information on evolutionary relationships within the group.

Type material of *P.c. adami* and *P.v. lentum* as described here will be deposited in the Wellcome Museum of Medical Science, London.

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