A Pictorial Guide to Rodent Malaria Parasites

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This book is meant as a training guide for laboratory personnel working with rodent malaria parasites. It contains photos of the blood stages of all 4 rodent malaria species, and mosquito stages of *Plasmodium chabaudi* and *Plasmodium yoelii*.

Origins of rodent malaria parasites:
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The brief descriptions of the rodent malaria parasites at the beginning of each section are taken from the Sanger Institute website.
*Plasmodium chabaudi*

*P. chabaudi* was first isolated from the blood of a shiny thicket rat in the Central African Republic in Africa, by I. Landau and A. Chabaud in 1965. Two subspecies have been defined, *P. chabaudi chabaudi* and *P. chabaudi adami*. The parasite is readily grown in laboratory mice and rats, where it shows a preference for mature red blood cells are synchronous with a periodicity of 24 hours. The parasite may be transmitted in the laboratory by *Anopheles stephensi* mosquitoes.

The following photographs show *P. chabaudi* strain CB parasites in mouse blood. All photos are at x100 magnification, although some have been digitally enlarged.

*P. chabaudi* day 2 post-innocation (pi). Infected red blood cells (RBCs) are marked with black arrows. These are early "ring-stage" trophozoites, and consist of a dark purple nucleus and lighter, blue coloured cytoplasm, often forming a ring around the nucleus. The darker coloured objects (one is marked with a white arrow) are platelets.

*P. chabaudi* day 2 pi. A ring stage trophozoite is shown, exhibiting the characteristic signet ring arrangement of nucleus and cytoplasm.
*P. chabaudi* day 3 pi. Note increased number of parasites. Only ring stage trophozoites visible. *P. chabaudi* infections are synchronous.
*P. chabaudi* day 4 pi. the RBC marked with an arrow contains two parasites
*P. chabaudi* day 5 pi. There are numerous multi-infected RBCs. Note how all parasites are at the same stage of development (i.e. ring stage trophozoites).

*P. chabaudi* day 5 pi. An RBC containing multiple parasites, next to a singly infected cell. The dark photo on the right shows the same field under polarised light. Note the absence of malaria pigment (which should glow under polarised light) in these trophozoites.
*P. chabaudi* day 6 pi. A high parasitaemia and many multi-invaded cells.

*P. chabaudi* day 6 pi. Female gametocyte (blue coloured cytoplasm). Under polarised light (right hand frame) the large amount of pigment (haemozoin) glows brightly.
*P. chabaudi* day 6 pi. Another female gametocyte. Notice the large vacuole and grainy pigmented cytoplasm.

*P. chabaudi* day 6 pi. The frame on the right shows the same field under polarised light. Notice the absence of glowing pigment, and compare to *Plasmodium yoelii* and *Plasmodium vinckei*.
*P. chabaudi* day 7 pi. Note high parasitaemia. The blood cell marked with an arrow shows a crenulated appearance.
Plasmodium yoelii – avirulent strains.

P. yoelii has been isolated from the blood of shiny thicket rats from the Central African Republic, from Brazzaville and from Western Nigeria. Three subspecies are recognized, P. yoelii yoelii, P. yoelii killicki and P. yoelii nigeriensis. The parasite is readily grown in laboratory mice and rats, where it shows a preference for immature red blood cells. Infections are asynchronous with a periodicity of 22-25 hours. The parasite may be transmitted in the laboratory by Anopheles stephensi mosquitoes.

P. yoelii 17x day 2 pi. Ring stage trophozoite. P. yoelii has a reticulocyte invasion preference, and only very rarely is seen in normocytes. Reticulocytes can be identified in Giemsa’s solution-stained blood by their darker blue colour and larger size in comparison to normocytes. However, this is an imperfect and difficult way to distinguish between reticulocytes and normocytes, and should not be relied upon for accuracy. An alternative method is to stain with both Geimsa’s Solution and Cresyl Blue.
P. yoelii 17x day 3 pi. 2 infected RBCs (both reticulocytes). Multi-inaded cells are common with this species, as the number of reticulocytes available for parasite invasion is low.

P. yoelii 17x day 3 pi. A reticulocyte containing 4 parasites.
*P. yoelii* 33x day 4 pi. These three parasites are at different stages of development, the earliest on the left. *P. yoelii* is asynchronous.

*P. yoelii* 17x day 6 pi. As the infection continues, all available reticulocytes get used up, and most contain more than one parasite.
*P. yoelii* 17x day 7 pi. A schizont producing about 9 merozoites is marked.

*P. yoelii* 17x day 7 pi. Ring stage trophozoites in reticulocytes.
P. yoelii gametocytes and schizonts
a) 17X day 7 male
b) 33X day 4 male
c) 33X day 4 male
d) 33X day 4 male
e) 33X day 4 female (top) and a schizont and a trophozoite in the same cell (bottom)
f) 33X day 4 schizont containing numerous merozoites
g) 33X day 11 female gametocyte
Plasmodium yoelii YM - a virulent strain

P. yoelii parasites usually only invade reticulocytes. YM is an exception, however, that invades both reticulocytes and normocytes. Parasitaemias get very high very quickly, and infected mice usually die by day 5 post-inoculation.

P. yoelii YM day 2 PI. Developing trophozoite.

P. yoelii YM day 3 PI. Notice normocyte invasion, and the multi-invaded reticulocyte.
*P. yoelii* YM day 4 PI. Notice high parasitaemia, and asynchronous development.

*P. yoelii* YM day 4 PI. The right panel shows the same field under polarised light. The older trophozoites contain more pigment (haemozoin) and glow brightest. A schizont is marked with an arrow, showing the distribution of merozoites around a central mass of haemozoin (see below).

*P. yoelii* YM day 4 PI. Schizonts showing distribution of haemozoin and merozoites.
P. yoelii YM day 5 PI. By this stage of the infection, an uninfected RBC is rare. The dense black cells are white blood cells.
Plasmodium vinckei

*P. vinckei* was first isolated in 1952 although it was not recognized as an independent species until 1975. It is the most widely distributed of the species of murine *Plasmodium*, being found in Katanga, the Central African Republic, the Congo Republic, Nigeria, and Cameroon. Four subspecies are recognized, *P. vinckei vinckei*, *P. vinckei petteri*, *P. vinckei lentum* and *P. vinckei brucechwatti*. The parasite is readily grown in mice and laboratory-reared thicket rats, where it shows a preference for mature red blood cells. Infections are synchronous with a periodicity of 24 hours. The parasite may be transmitted in the laboratory by *Anopheles stephensi* mosquitoes over a wide temperature range.

*P. vinckei* BS day 2 pi. One ring stage trophozoite.
*P. vinckei* BS day 3 pi. Early trophozoite stage. Notice large amount of haemozoin and characteristic gold colour.

*P. vinckei* BS day 3 pi. Early trophozoite stage. Notice large amount of haemozoin and characteristic gold colour. The right frame shows the same parasite under polarised light, high-lighting the haemozoin.

*P. vinckei* BS day 3 pi. Note the normocyte preference.
*P. vinckeii* BS day 7 pi. The bottom panel shows the same field under polarised light. Trophozoites are highly pigmented, unlike *P. chabaudi*. 
*P. vinckei* BS day 9 pi. The bottom panel shows the same field under polarised light. Trophozoites are highly pigmented, unlike *P. chabaudi*. 
*P. vinckei* BS day 7. Gametocytes, female (a) and male (b, c and d)
**Plasmodium berghei**

*P. berghei* was first isolated from the blood of a thicket rat in Katanga (now Zaire) Africa, by I. H. Vinke in 1948. The parasite is readily grown in laboratory mice and rats, where it shows a preference for reticulocytes Infections are asynchronous with a periodicity of 22-25 hours. The parasite may be transmitted in the laboratory by *Anopheles stephensi* mosquitoes, but is extremely sensitive to temperatures outside the range 19-21°C.

*P. berghei* (day of infection unknown). Note asynchronicity.

*P. berghei* (day of infection unknown). Reticulocyte containing four ring stage trophozoites
*P. berghei* (day of infection unknown). The bottom panel shows the same field under polarised light. Note asynchronicity, and haemozoin content.
*P. berghei* (day of infection unknown). The bottom panel shows the same field under polarised light. Note asynchronicity, and haemozoin content.
Plasmodium falciparum

P falciparum 3D7 in human blood culture for comparison to rodent parasites
Mosquito stage parasites

The following section contains photographs of the mosquito stages of *P. chabaudi* and *P. yoelii*. All photographs show parasites from *Anopheles stephensi* mosquitoes.

*P. chabaudi* oocysts day 6 pi on mosquito mid-gut (mag X 40). Bottom panel with polarised light showing malaria pigment glowing.
*P. chabaudi* oocysts day 6 pi on mosquito mid-gut (X 100). Note haemozoin.

*P. chabaudi* oocyst day 6 pi (X 100). Note haemozoin glowing under polarised light. The oocyst is shown next to an air bubble for comparison.
*P. chabaudi*. Various oocysts, day 6 pi
P. chabaudi oocysts day 6 pi. the bottom panel shows the same field under polarised light.
*P. yoelii* oocysts (day 6 pi) on a mosquito mid-gut. They are mostly concentrated in the bottom left of the gut, and some are marked with arrows (x10).

*P. yoelii* day 8 infection. Note extremely large numbers of oocysts in this poor photo.
The following pages show oocysts of *P. yoelii* at day 8 pi:
High density of oocysts
High oocyst densities
Four oocysts squashed up next to each other.
*P. yoelii* oocysts day 6 pi (both panels)
*P. yoelii* sporozoites, liberated from oocysts by crushing the mid-gut under a cover slip (x100)

*P. yoelii* sporozoite, from dissected salivary glands (day 16 pi) stained with Giemsa’s solution

*P. yoelii* sporozoite, from dissected salivary glands (day 16 pi) stained with Giemsa’s solution
*P. chabaudi* sporozoite from dissected salivary gland (day 16 pi), stained with Giemsa’s solution

*P. yoelii* sporozoite from dissected salivary gland (day 16 pi), stained with Giemsa’s solution
Looking for exflagellation (*P. chabaudi*)

A drop of fresh, infected blood is put on a glass slide and a coverslip placed over it. At x40 magnification, the blood cells look like this:

Under polarised light, gametocytes glow…

After about 10-15 minutes, mature male gametocytes may be seen to undergo exflagellation
Male gametocyte about to exflagellate

Clustering of RBCs around gametocyte

The emerging microgametes disrupt the RBCs around the gametocyte, causing vigorous movement of the cells.