Malaria is caused by parasites in the family Plasmodidae, genus *Plasmodium*. Twenty-three species have been described in nonhuman primates. Malaria parasites are classified based on their natural host (human, ape, monkey), on the morphology of the parasite, or on the type of cyclic fever they produce. Thus quotidian malaria have a 24hr cycle, tertian malaria have a 48hr cycle and quartan malaria have a 72 hour cycle (Figure 1, Table 1). This is determined by the time the organism parasitizes the hosts erythrocyte (red blood cell).

**HINT:** Record the animal’s temperature every 12 hours, to indicate type of cyclic fever, and thus type of *Plasmodium*.
The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female Anopheles mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. (Of note, in P. vivax and P. ovale a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes).

Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes), which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

<table>
<thead>
<tr>
<th>Natural Host (Common)</th>
<th>Natural Host (Scientific)</th>
<th>Plasmodium spp.</th>
<th>Blood cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Homo sapiens</td>
<td>P.falciparum, P.vivax, P.malariae</td>
<td>72 hr (quartan), except vivax which is 48 hr (tertiary)</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>Pan troglodytes</td>
<td>P.rodluini (similar to P.malariae and can readily be transmitted to people) P. reichenowi (similar to P.falciparum but cannot be transmitted to humans) P. schwetzi (similar to P.vivax and has been transmitted to humans)</td>
<td>72hr Quartan</td>
</tr>
<tr>
<td>Gorilla</td>
<td>Gorilla gorilla</td>
<td>P.rodluini (similar to P.malariae and can readily be transmitted to people) P. reichenowi (similar to P.falciparum) but cannot be transmitted to humans P. schwetzi (similar to P.vivax and has been transmitted to humans)</td>
<td>72hr Quartan</td>
</tr>
<tr>
<td>Drill</td>
<td>Mandrillus leucophaeus</td>
<td>P.gonderi</td>
<td>48hr tertiary</td>
</tr>
<tr>
<td>Black Mangabey</td>
<td>Cercocebus aterrimus</td>
<td>P.gonderi</td>
<td>48hr tertiary</td>
</tr>
<tr>
<td>Sooty Mangabey</td>
<td>Cercocebus atys</td>
<td>P.gonderi</td>
<td>48hr tertiary</td>
</tr>
<tr>
<td>Agile Mangabey</td>
<td>Cercocebus galeritus agilus</td>
<td>P.gonderi</td>
<td>48hr tertiary</td>
</tr>
<tr>
<td>Sooty Mangabey</td>
<td>Cercocebus galeritus</td>
<td>P.gonderi</td>
<td>48hr tertiary</td>
</tr>
</tbody>
</table>

Table 1. Plasmodium Species of African Primates
Thus most monkey malaria parasites exhibit tertian periodicity, completing one asexual cycle every 48 hours, and most ape malaria parasites exhibit quartan periodicity, completing one cycle every 72 hours.

Anecdotal evidence exists of malaria in guenons (Pruess’ guenon) and other African monkey species, but this has not been published.

*Hint: These natural infections in non human primates are unlikely to induce disease. Infection with the human strains is more likely to result in clinical disease.*

The primate malarias are generally thought to have arisen from endocellular, coccidian parasites in the intestinal tract of reptiles, birds, and amphibians. The earliest primates probably carried the ancestors of present-day malaria parasites when they began to evolve in the Lower Tertiary period. The putative ancestor of present-day primate malaria parasites is Hepatocystis, a ubiquitous parasite of African monkeys and apes. This is a well-adapted, relatively benign parasite, which produces only gametocytes in the circulation. The liver stages are macroscopic and were undoubtedly the first liver stage of primate malaria seen by man, as hunters and cooks prepared monkeys for the table.

It is interesting to note that the malaria parasites of New World monkeys, however, are thought to have come from Europe and Africa during the slave trade. There is little evidence of malaria in the pre-Columbian New World, and the two species of monkey malaria in the New World, *P. brasilianum* and *P. simium*, are closely related molecularly, and possibly identical, to the human malaria parasites, *P. malariae* and *P. vivax*. ? Is it worth mentioning *P. knowlesi* from Indonesia as this is now known to infect humans as well as macaque monkeys (24 hr schizogony)

**DIAGNOSIS**

**Equipment needs**
- Accurate thermometer
- Anaesthetic and Blood collection equipment
- A good light microscope with a 100x lens for oil immersion
- Good quality glass slides
- A heat box to protect all equipment from moisture
- Field’s stain  (Giemsa stain, or ability to make it )

Malaria should be a major differential diagnosis in any primate in Africa with an unexplained fever. Blood should always be collected in these circumstances as part of the diagnostic workup. Blood samples can be taken by earstick or tailstick using disposable lancets. It is helpful to shave the tip of the tail or ear before taking a drop of blood in a capillary tube. The remainder of this section will concentrate on the microscopic diagnosis of malaria.

**Hints on slides**
USE NEW SLIDES each time or acid washed grease free slides. You can use 1N HCL/H2SO4. It is very difficult to get a uniform thickness of smear on greasy slides and so distribution of white blood cells is not uniform. This leads to inaccurate differential count. Staining quality is also not good on greasy slides. Smears should be dried quickly to prevent red blood cell (RBC) distortion. In rainy season slides should be kept in an incubator, or at least in a box with a light to keep them dry (or the addition of a bag of silica gel). If slides are not completely dry, water particles are seen after staining which may be falsely interpreted as hypochromia (Figure 2). At the same time, inclusion bodies are missed e.g., malarial parasites, Howell-Jolly bodies etc.

![Figure 2. Water particles in poorly dried smears](image)

Preparing Blood Smears

If you are using venous blood, blood smears should be prepared as soon as possible after collection (EDTA is a better anticoagulant to use than heparin as the latter may affect the quality/colour of the stain) (delay can result in changes in parasite morphology and staining characteristics). The thin smear should be fixed as soon as it is dry; the thick smear must be allowed to dry thoroughly before staining. Thick smears should be examined for at least 5 minutes, corresponding to 100 oil immersion fields; thin films must be examined for 15 minutes before a monkey can be declared negative for malaria parasites. In doubtful cases, repeated blood smears can be made daily, taking into consideration that anesthetising a monkey to make a blood smear may be more stressful than the infection.

Thick films

Thick blood films consist of a layer of red blood cells piled thickly and irregularly to produce approximately a x30 concentration than in an equal area of a thin blood film. Due to this concentration effect thick films increase the sensitivity of parasite detection but are not usually adequate for species identification for which a thin film is usually required. Thick films should be made quickly, because undue delay may lead to fibrin formation or promote the auto-agglutination of red cells in anaemic blood. A thick blood film is not fixed and the aqueous stains used simultaneously lyse the red blood cells, a feature essential for later examination.
Prepare at least 2 films per patient!

- Place a small drop (around 5ul) of blood in the centre of the pre-cleaned slide.
- Using the corner of another slide or an applicator stick, spread the drop in a circular pattern until it is the area of around 2.0 cm$^2$.
- A thick smear of proper density is one which, if placed (wet) over newsprint, allows you to barely read the words.
- Keep the slides horizontal, on an even surface, and allow the smears to dry thoroughly (protect from dust and insects!). Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. The risk is increased in smears made with anticoagulated blood. At room temperature and high humidity drying can take several hours. Ideally leave films to dry for 15 mins at room temperature (20C) then for 5 minutes at around 35C (incubator). Drying may be accelerated by placing the nearly dry slide upright on a hotplate using a heat setting that one can comfortably touch OR by using a fan or hair dryer (use cool & not too fast a setting). Protect thick smears from hot environments to prevent heat-fixing the smear.

Thin films

Thin films consist of blood spread in a layer such that the thickness decreases progressively toward the feathered edge, ideally the smear should be thin enough so that a large area of the slide should be a monolayer with the cells not touching one another.

Prepare at least 2 films per patient!

- Place a small drop of blood on the pre-cleaned, labelled slide, near its frosted end
- Bring another slide backwards into the drop of blood at an angle of about 30-45° (steeper for anaemic blood) allowing the drop to spread along the contact line of the 2 slides then quickly push the spreader (upper) slide forwards using a steady movement to produce a film which has 2 straight sides, does not reach the end of the slide and has a feathered edge.
- A good thin film is achieved by using the correct amount of blood and spreading technique
- Allow the thin smears to air dry – this may be hastened by **blowing gently on the film**. (Thin films should dry much faster than thick films, if not they are probably too thick.)
- If unable to stain immediately the thin films should be fixed by dipping them in absolute methanol and air drying upright.

**HINT:** Under field conditions, where slides are scarce, you can prepare both a thick and a thin smear on the same slide. This works adequately if one makes sure that of the two smears, only the thin smear is fixed.
METHOD OF PREPARING A THIN BLOOD FILM

1. Transfer a small drop of blood to one end of a clean microscope slide.
2. Allow the blood to run along the back edge of another slide, the 'spreading slide'.
3. Push the spreading slide along the blood slide, gently, evenly and quickly.
4. Label the blood slide with the name (or identification) of the patient and the date.

METHOD OF PREPARING A THICK BLOOD FILM

1. Transfer two or three drops of blood to the centre of a clean microscope slide.
2. Spread the blood rapidly and evenly into an area about 1.5cm in diameter, using a needle or the corner of another slide.
3. Label the slide.
STAINING BLOOD FILMS

Stain only one set of smears, and leave the duplicates unstained. The latter will prove useful if a problem occurs during the staining and/or if you wish later to send the smears to a reference laboratory. All Romanowsky stains (Giems, Leishman’s, Field’s, Wright-Giemsa) will stain blood parasites but some give better results than others.

Wright (Wright-Giemsa) stain

Used in hematology, this stain is not optimal for blood parasites. It can be used if rapid results are needed, but should be followed up when possible with a confirmatory Giemsa stain, so that Schüffner’s dots can be demonstrated.

Field’s stain

This Romanowsky stain may be used for the detection and identification of blood parasites. It has the advantage of speed- a thick film is stained in 18 seconds and a thin in 24 seconds!

Field’s stain may be purchased as a commercial “compound” powder, as a liquid ready to use stain or alternatively the constituent dyes may be purchased to prepare one’s own stain. The advantage of the commercial compound powder is that it only requires the addition of the correct quantity of distilled (or filtered/boiled/cooled water). If the stain is prepared from base powders then these must be dissolved in phosphate buffer.

A  Methylene Blue 0.4g
   Azur B (l) 0.25g

B  Eosin (yellowish, water sol,)0.5g

250ml phosphate buffer            250 ml phosphate buffer

STOCK Phosphate buffer:

1.  Di sodium hydrogen phosphate Na2HPO4 (anhydrous) 28.39 g
2.  Sodium dihydrogen phosphate NaH2PO4.H2O 27.6g

Dissolve each salt TO 1 Litre of distilled water (place chemicals in 1L flask and add water slowly to dissolve, make up to 1L mark, these stock buffers will last for approximately 6 months. For use :

Stock 1: 360ml, Stock 2:140ml, plus 500ml distilled water makes 1L at pH7.2

Field’s A: Add eosin to 250ml of buffer, mix well, leave for 24hrs then filter. Ready to use no further diluting.

Field’s B: Grind Azur B in a mortar with a few ml of buffer taken from the remaining 250ml. Place methylene blue in a suitable container and add about 200ml of the phosphate buffer, add the liquefied Azur stain to the methylene
blue mix, rinse mortar with remainder of buffer and add to stain. Mix well, leave overnight and filter next day (Whatman’s no. 1 paper).

**Using commercial compound powder (tcsbiosciences.co.uk)**

Add 2g of Fields A (HD1410) to 80ml of DW, mix. Add 1 g of Fields B compound (Cat. No. HD 1415) to 80ml of distilled water, mix. The 80ml volume will just fit into standard Coplin jars stain and is ready for use. These jars of stain may last for 1 month or more depending upon how frequently the stain is used.

**Staining Method Thick Films**

Lysis and staining of a thick blood film occur simultaneously.

1. Dip thick film in solution A for 5 seconds, drain. (avoiding agitation of the stain, because this will disperse bacteria concentrated toward the bottom of the container, and cause them to adhere to the blood film).
2. Wash gently in a jar of water for 5 seconds, drain.
3. Dip in solution B for 3 seconds, drain.
4. Wash again in the jar of water for 5 seconds.
5. Stand the slide nearly upright to allow the haemoglobin to drain out while the slide dries.

The times given are approximate, and vary with batches and age of stain and the thickness of the films. The film is usually of varying thickness, and different parts take up the stain at different rates. The optimum staining of parasites, usually near the edges of the film, occurs where the nuclei of the leucocytes are stained a rich purple colour.

An oxidation scum will appear on the surface if the stain is used infrequently, but this scum can be removed by drawing filter paper across the surface. Ideally, the stains should be filtered daily and renewed weekly in laboratories in warm climates.

**Reverse Field’s stain for thin films**

1. Fixed thin film by dipping in methanol (or industrial methylated spirit) for 8 seconds (8 “dips”- lifting slide out of stain after each dip)
2. Dip into Field’s B (eosin) for 8 seconds.
3. Wash for 8 dips in a jar of tap water, agitating well.
4. Dip into Field’s A for 8 dips
5. Wash for 8 seconds in a second jar of tap water.
6. Dry upright.

**Giemsa stain**

Recommended for detection and identification of blood parasites. I’ve provided the formula below for reference, but commercial preparations of this stain are available. However, the advantage of preparing your own is that the formula gives more consistent results, and doesn’t expire.
Stock 100× Giemsa
Buffer preparation
0.67 M
Na$_2$HPO$_4$ 59.24 g
NaH$_2$PO$_4$H$_2$O 36.38 g
Deionized water 1000.00 ml
Autoclave or filter-sterilize (0.2 μm pore). Sterile buffer is stable at room temperature for one year.

Working Giemsa
Buffer preparation
0.0067M, pH 7.2
Stock Giemsa Buffer 10.0 ml
Deionized water 990.0 ml
Check pH before use. Should be 7.2. Stable at room temperature for one month.

Triton X-100 5%
preparation
Deionized water (warmed to 56°C) 95.0 ml
Triton X-100 5.0 ml
Prewarm the deionized water and slowly add the Triton X-100, swirling to mix.

Stock Giemsa stain preparation
Glass beads, 3.0 mm 30.0 ml
Absolute methanol, acetone-free 270.0 ml
Giemsa stain powder (certified) 3.0 g
Glycerol 140.0 ml
- Put into a 500 ml brown bottle the glass beads and the other ingredients, in the order listed. Screw cap tightly. Use glassware that is clean and dry
- Place the bottles at an angle on a shaker; shake moderately for 30 to 60 minutes daily, for at least 14 days
- Kept tightly stoppered and free of moisture, stock Giemsa stain is stable at room temperature indefinitely (stock stain improves with age)
- Just before use, shake the bottle. Filter a small amount of this stock stain through Whatman #1 filter paper into a test tube. Pipet from this tube to prepare the working Giemsa stain

Working Giemsa stain (2.5%): make fresh for each batch of smears
Working Giemsa buffer 39 ml
Giemsa Stain Stock 1 ml
5% Triton X-100 2 drops
STAINING PROCEDURE

- Prepare fresh working Giemsa stain in a staining jar, according to the directions above. (The 40 ml fills adequately a standing Coplin jar; for other size jars, adapt volume but do not change proportions).
- Pour 40 ml of working Giemsa buffer into a second staining jar. Add 2 drops of Triton X-100. Adapt volume to jar size.
- The thin smear is fixed in absolute methanol for 2 minutes; the thick smear is left unfixed.
- Place slides into the working Giemsa stain (2.5%) for 45-60 minutes at a pH of 7.25.
- Remove thin smear slides and rinse by dipping 3-4 times in the Giemsa buffer. Thick smears should be left in buffer for 5 minutes.
- Dry the slides upright in a rack.

If using Giemsa stain, Giemsa improved staining solution (Gurr’s R66) works well for malaria parasites. Using R66 at a dilution of 1:10 in buffered water pH7.2 staining time is about 15 minutes. Slides should be rinsed in buffered water for optimum staining. The main drawbacks of using Giemsa is that the stain must be diluted daily before use and any excess stain discarded at the end of the day.

An easy way of making buffered water is by using commercial buffer tablets: 1 tablet to 1 litre water (VWR- cat no. 1.09468.0100, Merck buffer tablets ph7.2, pack 100 tabs)

Over staining (figure 3) can leave stain particles on slides which may be sometimes mistaken as platelets.

MICROSCOPIC EXAMINATION OF FILMS

Examining thick films

Since the erythrocytes have been lysed and the parasites are more concentrated, the thick smear should be used to screen for parasites and for detecting mixed infections.

- First examine the entire smear at a low magnification (10× or 20× objective lens), to detect large parasites such as microfilaria. If working in an area where trypanosomes may be present these may be seen more easily using a x40 objective).
Then examine the smear using the 100× oil immersion objective lens. If using Field’s stain select an area where the nuclei of the white blood cells (WBCs) are stained purple- in this area parasite cytoplasm will stain blue and nuclear material (chromatin) a dark red. Ideally examine an area with sufficient number of WBC’s, field (10-20 WBCs/field).

If parasites are seen, make a note of the stages seen (mainly rings, etc) and look for the presence of Schuffner’s dots (seen as a halo of pink around the parasites) to help make a tentative species determination. Examine the thin smear to determine species of malaria. With practice it may be possible to determine species from the thick film.

Determination of "No Parasites Found" (NPF): For malaria diagnosis, the World Health Organisation recommends that at least 100 fields, each containing approximately 20 WBCs, be screened before calling a thick smear negative. Assuming an average WBC count of 8,000 per microliter of blood, this gives a threshold of sensitivity of 4 parasites per microliter of blood. In non-immune human patients, symptomatic malaria can occur at lower parasite densities, and it is normal to examine 200 oil immersion fields on 2 different films before declaring no parasites found.

**Examining thin films**

Thin films are useful for species identification of parasites already detected on thick smears, screening for parasites if adequate thick smears are not available, and a rapid screen while the thick smear is still drying. If NO thick film is available it is recommended that the thin film is examined for 30 minutes before declaring no parasites found.

Some parasitised red blood cells roll to the edges and are carried to the tail of the film, so that these parts should be examined for malaria parasites, in addition to those areas of the film where the red cells are just separated. Try to avoid parts of the film where the red blood cells are piled on top of each other. As haemoglobin is retained during staining, malaria parasites appear framed by the red blood cells.

- Screen at low magnification (10× or 20× objective lens) if this has not been done on the thick smears.
- Carefully examine the smear using the 100× oil immersion objective lens.

**Quantifying parasites**

In some cases (especially malaria) quantification of parasites yields clinically useful information as the malaria parasites can be quantified against blood elements such as RBCs or WBCs. Only asexual blood stage parasites should be counted as gametocytes of all species may persist following treatment, gametocytes will die in the host within a month and cannot continue their cycle unless they are sucked up by an anopheline mosquito.
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Parasite numbers fluctuate daily for a variety of reasons. A series of counts over several days will usually indicate a general decline, stabilisation or increase. Estimates of parasitaemia are important in monitoring the response of patients to treatment. However, as parasite numbers generally rise for about 12-18 hours after the beginning of successful therapy, only a continuing rise beyond this period is of concern.

**Percentage of parasitised red cells using thin blood film**

1. The thin film should be a well-prepared and stained monolayer. Using the x 100 (oil immersion) objective find a field that contains an infected erythrocyte.
2. Reduce the field size either by using an Ehrlich's eye piece until the field of vision contains about 50 erythrocytes or by using an eyepiece graticule to “section” the field of view.
3. Using 2 hand tally counters, count the total number of cells in the field on one counter; use the other to count the number of erythrocytes infected with asexual stages of parasites.
4. Count a minimum of 1,000 erythrocytes and express the parasitaemia as a percentage. \( \% \text{ parasitemia} = \frac{\text{parasitized RBCs}}{\text{total RBCs}} \times 100 \)
5. Repeat the process in another area of the film and take the mean of the two results

NB If the parasitaemia is <1% a more accurate estimation of parasitaemia will be obtained by counting parasites on a thick blood film. Table 2 is an estimate of parasite numbers if the same microscope is used each time for examining blood films.

<table>
<thead>
<tr>
<th>Parasites observed</th>
<th>Percentage of red cells parasitized</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20 per field</td>
<td>1</td>
</tr>
<tr>
<td>1-2 per field</td>
<td>0.1</td>
</tr>
<tr>
<td>1-2 per 10 fields</td>
<td>0.01</td>
</tr>
<tr>
<td>1-2 per 100 fields</td>
<td>0.001</td>
</tr>
<tr>
<td>1-2 per 1000 fields</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Table 2. Estimates of parasitaemia from thick film using a x 100 oil immersion objective and x 10 eyepieces. Confidence limits of low counts are fairly wide.*

**Number of parasites/μl blood using thick blood film**

Malaria parasites are counted in relation to the patients' total white cell count. If the total white cell count is unavailable it is assumed to be 8,000 WBC/μl.

1. The thick film is examined using the x100 oil immersion objective and leucocytes are counted on one tally counter until 100 are recorded.
2. At the same time, using another tally counter, the total number of asexual stages of malaria parasites seen are recorded.
3. This process is repeated twice, in other areas of the film, until a total of 300 WBC’s have been recorded. Calculate the mean number of parasites per 100 WBC’s.

4. The number of parasites/µl is calculated using the formula:

$$\text{No. parasites} \times \frac{\text{WBC/µl}}{100} \text{ (or 8,000)}$$

An alternative (and less accurate) method for estimating the number of parasites in thick films is as follows:

- 1-10 per 100 high power fields +
- 11-100 per 100 high power fields ++
- 1-10 in every high power field +++
- more than 10 in every high power field ++++

Results in % parasitized RBCs and parasites per microliter blood can be interconverted if the WBC and RBC counts are known (thus ALWAYS do a Complete Blood Count (CBC)), or otherwise (less desirably) by assuming 8,000 WBCs and 4,000,000 RBCs per microliter blood.

Dipstick tests for circulating antigen (HRP2, pLDH) may give positive results for *P. cynomolgi*, *P. coatneyi*, and *P. knowlesi*, HRP2 type tests may remain positive for up to 2 weeks following successful treatment, pLDH type tests will only detect LIVE parasites. Other non-human primate malarias have not been tested. PCR is not generally useful in a clinical setting unless one is interested in specific molecular sequences. It is not more sensitive than a well-made thick smear and available primer sequences are limited to *Plasmodium* species used in malaria model work, e.g. *P. cynomolgi*, *P. knowlesi*, and *P. coatneyi*.

### HINT: A cautionary note on Babesia

Other Apicomplexan parasites that may appear in blood smears and confused with malaria parasites, are the piroplasms (family **Babesidae**). *Entopolyploides macaci* is often reported in rhesus macaques (*Macaca mulatta*), African monkeys (*Cercopithecus*), and baboons (*Papio*) but will infect a variety of non-human primates. The organisms are smaller than *Plasmodium*, markedly pleomorphic, and produce no pigment. The infections are usually benign even in splenectomized animals, and do not respond to standard antimalarial therapy. Unlike *Plasmodium*, they are tick-borne.
MICROSCOPIC IDENTIFICATION.


Cycle in Blood - The asexual blood cycle occupies 48 hours. Merozoites prefer to invade reticulocytes and the developing ring forms are often 2 - 4 per red cell. Schüffner's stippling appears in the cytoplasm with further growth of the parasite and the host cell becomes enlarged and distorted. Mature trophozoites show a deep blue staining cytoplasm, a large irregular red staining nucleus, and pigment in small aggregates. Pigment in the developing schizont is more condensed and the stippling is prominent. Older schizonts fill the red cell and show purple cytoplasm with reddish nuclei on the periphery of the parasite. The mature schizont may not fill the host cell and may contain 12 - 20 merozoites with an average of 16. The cytoplasm of the host cell is hypochromic almost to the point of being inapparent so that the schizont may appear free. Microgametocytes stain a light purplish pink, with dark pigment granules scattered in the cytoplasm. The macrogametocyte stains deep blue with scattered pigment.

Course of Infection - In sporozoite-induced infections, peak parasite counts are on the order of 190,000/mm$^3$ at day 10 of patent parasitemia. The counts then decline to a low level (about 350/mm$^3$). Animals do not generally require chemotherapy for survival.

Cycle in blood - The asexual blood cycle occupies 48 hours. The parasite closely resembles P. falciparum of man, with crescent-shaped gametocytes and usually only ring stages and gametocytes appearing in the peripheral circulation. The youngest parasites are small rings with a prominent nucleus. A consistent feature is the presence of marginal forms with single or double nuclei; accolé forms are common. The mature schizonts contain 10 - 12 merozoites, but may not be seen in the peripheral circulation, unless the animal is splenectomized. The mature macrogametocyte is crescent-shaped and somewhat slender with pale blue cytoplasm and a red-staining nucleus. The microgametocyte is more robust with bluish-red cytoplasm and a diffuse red-staining nucleus.

Course of Infection - Little is known about the early course of naturally-occurring infections. There is only meagre information about naturally-infected and blood-inoculated captive animals.

Cycle in blood - The parasite has a 48 hour asexual cycle in the chimpanzee. The earliest ring forms are relatively compact with a dark, round to oval nucleus. Growing parasites enlarge the host cell and stippling is abundant in the older trophozoite stage. The mature schizont has from 12 - 16 distinct nuclear masses and the red cell may be distinctly oval shaped. The macrogametocyte stains uniformly blue with a peripheral wine-red nucleus. The microgametocyte is brightly colored with a reddish-purple cytoplasm and a large, diffuse wine-red nucleus. The pigment is coarse, black to greenish black and the cytoplasmic edge of the parasite is crenated or lace-like.

Course of Infection - P. schwetzi appears to invoke no clinical signs, even in young chimps, nor in splenectomized older animals with high parasitemias. It generally occurs as a mixed infection with P. reichenowi and P. rodhaini.
Figure 7. Malaria in humans – please refer to general life cycle
Figure 8-11 Ring form (young trophozoites) – thin smears.

Figure 12 Ring Form (young trophozoites) – Thick smear. F
Figure 13. Mature trophozoites

Figure 14 and 15. Schizonts.

Figure 16 and 17. Gametocytes – thin smears.

Gametocytes – Thick smears. Figure 19
PATHOLOGY

Most primate malarias and infections with piroplasms are subclinical, unless the animal is immunosuppressed or splenectomized (had the spleen removed for some other disease). Experimental infections of rhesus macaques with *P. knowlesi*, *P. coatneyi*, and less often *P. cynomolgi*, may be characterized by jaundice, anorexia, listlessness, fever, anemia and splenomegaly in spleen-intact animals. Clinical signs of chills and fever are in response to toxins (*Plasmodium* GPI) exposed during the release of merozoites from the red cell. Pregnant animals may experience more severe anemia, which will have a measurable impact on the health of the fetus.

If the animal succumbs to infection, on post mortem, grossly the lungs, liver, and spleen are grey and the blood thin. Histologically, tissue macrophages are filled with malaria pigment and there are hemosiderosis and parasitized RBC's. Intravascular clotting with thrombi and parasitized erythrocytes is common. Often there is pulmonary and cerebral oedema.

TREATMENT

Most primate malarias can be treated with chloroquine at a dosage of 7 mg/kg base for 5 days (total = 35 mg/kg). This can be given as an IM injection or per os via nasogastric tube. The bitter taste of 4-aminoquinolines precludes putting it in food. Chloroquine is effective against the circulating trophozoite (feeding) stage of the parasite but will not affect hepatic stages nor circulating gametocytes. Mefloquine (Larium) can also be used, especially if an isolate is suspected to be chloroquine-resistant, at a dose of 20 mg/kg, one dose, per os. Hepatic stages of *Plasmodium* require treatment with an 8-aminoquinoline (primaquine) at a dose of 3 mg base/kg body weight per os for 7 days. This may be necessary in sporozoite-induced (i.e. natural) infections with *P. cynomolgi*, *P. simiovale* and *P. fieldi* where malarial relapse is a consideration.

Because of increased toxicity when given together, chloroquine and primaquine should be given separately. Treatment is only necessary if reinfection occurs (i.e. in endemic malarial areas).

More information is required on the possible use of new antimalarial human products in non human primates such as atovaquone and proguanil hydrochloride and artemether/lumefantrine. Widespread use of these drugs is NOT recommended, as resistance is likely to be induced more rapidly in all populations. Although research is ongoing for a malarial vaccine, this is still some years off, and potential resistance must be born in mind whatever treatment regime used.
Experimental plasmodium infection and treatment regime in a macaque monkey. This regime is typical of the disease course in humans, and possibly African primates. Information on the treatment of non-human primate piroplasms has not been reported. Human infections with *Babesia microti* have been successfully treated with a combination of clindamycin and oral quinine. Adult dosage for adults is clindamycin, 1.2 g IV b.i.d. or 600 mg orally t.i.d. plus quinine 650 mg orally t.i.d., both for 7 days. Dosages for children (more similar in body weight and surface area to non-human primates) have not been reported.
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>P.falciparum</th>
<th>P.malariae</th>
<th>P.vivax</th>
<th>P.ovale</th>
<th>Babesia sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance of infected red blood cells (size and shape)</td>
<td>Both normal</td>
<td>Normal shape. Size normal or smaller</td>
<td>1½ to 2 times larger than normal; shape normal or oval</td>
<td>As for P.vivax, but some have irregular frayed edges</td>
<td>Both normal</td>
</tr>
<tr>
<td>Schüffner's dots (eosinophilic stippling)</td>
<td>None (but occasionally comma-like red dots present = Maurer's dots)</td>
<td>None</td>
<td>Present in all stages, except early ring form</td>
<td>As for P.vivax</td>
<td>None</td>
</tr>
<tr>
<td>Red cells with multiple parasites/cell</td>
<td>Common</td>
<td>Rare</td>
<td>Occasional</td>
<td>As for P.vivax</td>
<td>Common</td>
</tr>
<tr>
<td>Stages present in peripheral blood</td>
<td>Rings and gametocytes; occasionally schizonts</td>
<td>All stages</td>
<td>All stages</td>
<td>As for P.vivax</td>
<td>Only rings and rare pear-shaped forms (‘Maltese cross’). No gametocytes</td>
</tr>
<tr>
<td>Ring form (young trophozoite)</td>
<td>Delicate, small ring; scanty cytoplasm; sometimes at the edge of red cell (accolé form)</td>
<td>Ring 1/3 diameter of cell; heavy chromatin dot; vacuole sometimes ‘filled in’</td>
<td>Ring 1/3 - 1/2 diameter of cell; heavy chromatin dot</td>
<td>As for P.vivax</td>
<td>Resembles ring of P.falciparum. Look for pear-shaped structure</td>
</tr>
<tr>
<td>Schizont</td>
<td>Occasionally in peripheral blood, 16 - 30 merozoites</td>
<td>6-12 merozoites in rosette; coarse pigment clump in centre</td>
<td>12-24 merozoites in rosette filling entire RBC; central pigment</td>
<td>8-12 merozoites in rosette</td>
<td>No schizont</td>
</tr>
<tr>
<td>Gametocyte</td>
<td>‘Crescent’ or ‘sausage’ shape are characteristic</td>
<td>Round or oval; dark coarse pigment</td>
<td>Round or oval</td>
<td>Round or oval (smaller than P.vivax)</td>
<td>No gametocyte</td>
</tr>
<tr>
<td>Main criteria</td>
<td>Delicate ring forms and crescent shaped gametocytes are the main forms in the bloodstream. Multiple infection common. Normal RBC shape/size.</td>
<td>Red cell normal or slightly smaller; trophozoites compact and intensely strained; band-form suggestive; no Schüffners dots; coarse and dark pigment.</td>
<td>Large pale RBC; presence of Schüffners dots in cytoplasm of RBC; round gametocytes; large amoeboid trophozoite with pale pigment</td>
<td>Oval RBC with fimbriated edges characteristic but not always present; generally like P.vivax</td>
<td>Ring forms very similar to P.falciparum; presence of group of 2-4 pear-shaped bodies (‘Maltese cross’) is characteristic; absence of gametocytes</td>
</tr>
</tbody>
</table>
Appearance of malaria parasites in thick blood films
<table>
<thead>
<tr>
<th></th>
<th><em>P. falciparum</em></th>
<th><em>P. vivax</em></th>
<th><em>P. ovale</em></th>
<th><em>P. malariae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Often numerous</td>
<td>Ring forms</td>
<td>Ring forms not</td>
<td>Ring forms not</td>
<td>Ring forms not</td>
</tr>
<tr>
<td>delicate rings</td>
<td>not specifically identifiable</td>
<td>specifically identifiable</td>
<td>specifically identifiable</td>
<td>specifically identifiable</td>
</tr>
<tr>
<td>Excepting</td>
<td>All stages</td>
<td>All stages may be seen</td>
<td>All stages may be seen</td>
<td>All stages may be seen</td>
</tr>
<tr>
<td>gametocytes, all</td>
<td>may be seen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parasites are at</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>the same stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of development</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ring/trophozoites)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rings may be</td>
<td>Older trophozoites</td>
<td>Parasites solid and</td>
<td>Older trophozoites</td>
<td></td>
</tr>
<tr>
<td>turned so appear</td>
<td>turned so appear</td>
<td>contain pigment</td>
<td>solid, small and</td>
<td></td>
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<tr>
<td>as exclamation</td>
<td>as exclamation</td>
<td></td>
<td>stain deeply</td>
<td></td>
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<tr>
<td>mark or propellers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gametocytes</td>
<td>Lysed RBC may</td>
<td>Lysed RBC as in *P.</td>
<td>Schizont and</td>
<td></td>
</tr>
<tr>
<td>crescent shaped</td>
<td>appear as pink</td>
<td><em>vivax</em></td>
<td>gametocyte compact</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;ghost&quot; around</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>parasites</td>
<td>parasites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If blood is</td>
<td>Parasite may</td>
<td>As in <em>P. vivax</em> –</td>
<td>Coarse pigment</td>
<td></td>
</tr>
<tr>
<td>taken several</td>
<td>be surrounded</td>
<td>Schüffner's dots</td>
<td>granules in parasite</td>
<td></td>
</tr>
<tr>
<td>hours before</td>
<td>by Schüffner's</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>films are made,</td>
<td>dots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trophozoites</td>
<td></td>
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<tr>
<td>may be very</td>
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<tr>
<td>solid and</td>
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<tr>
<td>compact,</td>
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<tr>
<td>gametocytes may</td>
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<td></td>
</tr>
<tr>
<td>round up</td>
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</table>