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Supplementary Appendix

1. Life cycle, epidemiology and clinical aspects of malaria

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1. Generalised life cycle (Figure S1)[1,2]

The disease is transmitted through sporozoites injected by the female anopheline mosquito into skin capillaries, or through blood transfusion. **Sporozoites** (2) enter the liver parenchyma cells where they grow asexually by schizogony into the first generation of **pre-erythrocytic schizonts** (3). These give rise to thousands of **cryptozoites** (merozoites), which invade red blood cells by binding to 'receptors' on red cell membrane (Duffy blood group Fy^aFy^b for *P. vivax;* glycophorin B and C for *P. falciparum*), to develop into the asexual erythrocytic cycle (4). Some sporozoites *P. vivax* and *P. ovale* in the hepatocytes stay dormant (hypnozoite) to mature after an interval of weeks, months or even years into **secondary exo-erythrocytic schizonts** (3a, 3b). The successive waves of cryptozoites emerging from these give rise to relapse infections in the blood after months or years. Intraerythrocytic parasites, now named **young trophozoites**, acquire ring shapes and devour intracellular haemoglobin, excreting as they do so lumps of brownish ferrihaeme called malaria pigment or haemozoin or haematin. (***Note:*** intracellular plasmodia protect themselves against free radical oxygen species released during haemozoin production by using host cell superoxide dismutase enzyme). The morphology of later blood stages differs from one species to another. As ring shaped trophozoites grow, they assume bulky or amoeboid shapes with enlarged chromatin mass (**late trophozoites**). The trophozoite nuclei undergo segmentation

From: Peters W, Gilles HM. A colour atlas of tropical medicine and parasitology, 2nd Edition, Wolfe, 1981.

Figure S1. Life cycle of *P. vivax* and *P. ovale*.

Incubation period of malaria: usually 7-21 days (up to 9 months in *P. vivax*).

and division (schizogony or asexual erythrocytic cycle) to form multinucleated schizonts (early schizonts). Cytoplasm condenses around each doughter nucleus, as the schizont matures, to form late schizonts (**mature schizonts**) which contain well-formed merozoites. As the infected red cells disrupt, the cargo of 6 to 24 **merozoites** is now released to infect other red blood cells. Thus a new cycle of red cell infection and destruction is initiated. Some blood stages mature to form sexual forms, the macro (female) and micro (male) **gametocytes** (5). These enter the mosquito where the males exflagellate to fertilise the females (6). The ookinete thus produced forms oocysts on the outside of the midgut (7). Sporozoites develop in the oocysts (8,9). The sporozoites enter the mosquito salivary glands, where they are ready to infect a new host (2). *P. falciparum* and *P. malaria* do not form hypnozoites and stages 3a and 3b do not exist and true relapses do not occur.

Recrudescence may occur in any type of malaria infection but **true relapses** occur only in tertian fever (*P. vivax*, *P. ovale*). Relapses develop from the persistence of sporozoites in the liver cells (hypnozoites). These dormant parasites may develop into tissue schizonts (reactivate) over a period of time ranging from a few months up to five years since initial infection, and the schizonts provide a source of new merozoites to re-invade red blood cells. The mechanisms that trigger hypnozoite reactivation and relapse of malaria are unknown but in some cases this follows a viral or microbial infection. The hypnozoites are also responsible for the long incubation period of tertian malaria (up to 9 months after the bite of anopheles in an endemic area) e.g. an infection contracted in the fall does not produce initial symptoms until the following spring, 7-9 months later.

Recrudescence in the blood film may occur after an infection which was treated with anti-malarials (i.e. non-immunologically cured infection), for up to 1 year later in the case of *P. falciparum*, and decades after the initial infection in the case of *P. malariae*. ***Note:*** *P. malariae* is associated with low-grade, prolonged parasitaemia.

2. Distribution map of malaria (Figure S2)[1-4]

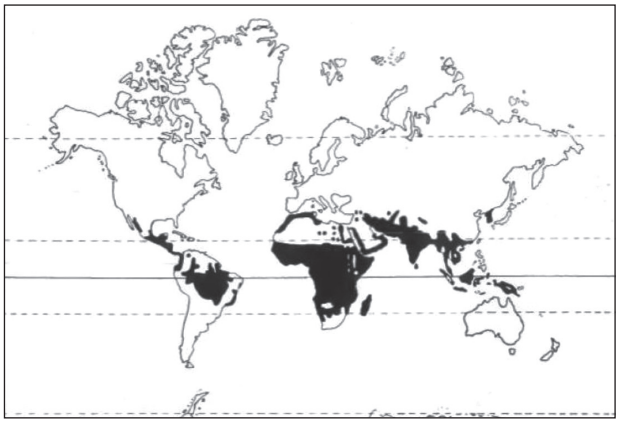
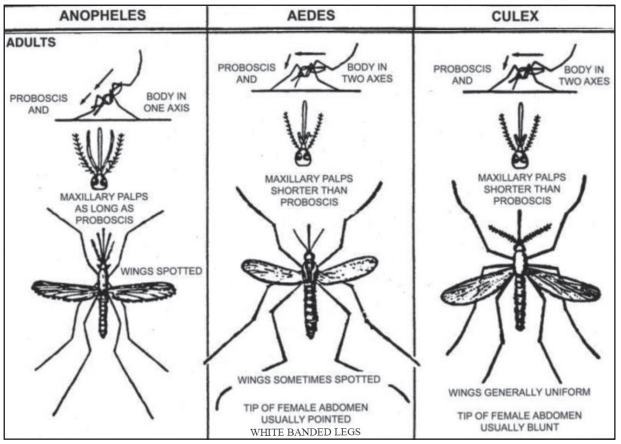


Figure S2. Distribution map of malaria.

In spite of intensive control measures over the past 50 years, malaria is still widely distributed in the tropics and subtropics (nearly half of the global population lives in endemic regions, and ~47% of the world's countries have a malaria problem). The breakdown of large scale vector control operations and the emergence of multiple drug resistance in some of the parasites has led to an increase in the incidence of malaria in some regions.

3. Characteristics of the three medically important mosquito genera (Figure S3)



Modified from: U.S. Department of Health, Education, and Welfare, Public Health Service, 1966.

Figure S3. Medically important mosquito genera.

There are 420 species of anopheles mosquito, of these 40 are malaria vectors. *Anopheles gambiae* is the most dangerous malaria vector in tropical Africa.

- *Anopheles* is active during night (nocturnal) or twilight
- *Culex* is nocturnal
- *Aedes* is active during day (diurnal). *Aedes spp.* can act as vectors for arboviruses such as dengue, chikungunya, and Zika viruses.

4. Clinical information[1-10]

- The typical clinical picture is paroxysmal rigor-fever-sweats, splenomegaly and hepatomegaly (due to hypertrophy of the reticuloendothelial system). Malaria is classified as:
  - **tertian fever** (*P. vivax*, *P. ovale*) and **malignant tertian fever** (*P. falciparum*): 48 hours between paroxysms (the asexual blood stages of *P. falciparum*, *P. vivax,* and *P. ovale* require 48 hours to complete their schizogony; fever is produced when schizonts mature i.e. at 48 hour intervals)
  - **quartan fever** (*P. malariae*): 72 hours between paroxysms

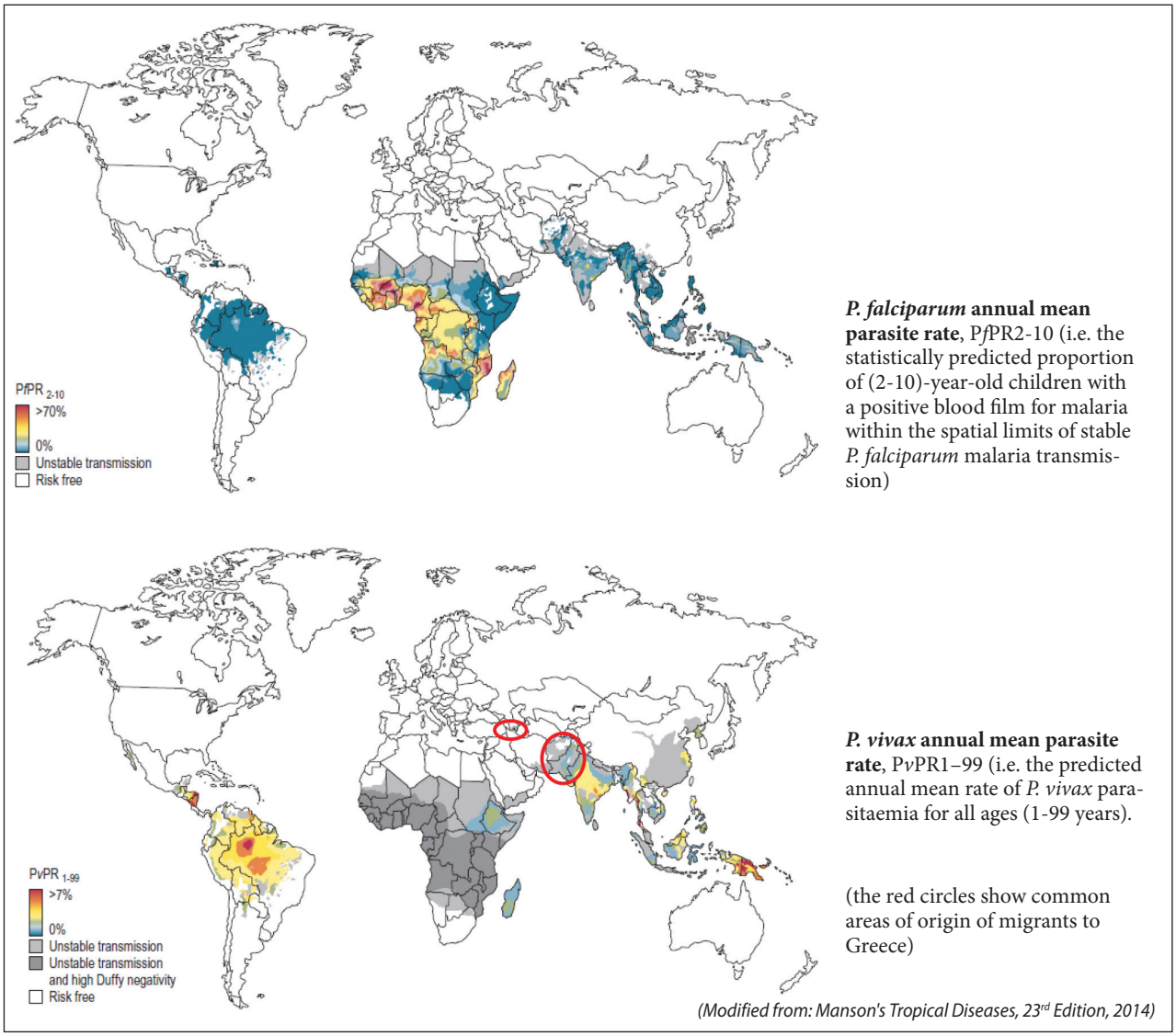
The distinction based on the pattern of fever is not practical; usually acute malaria is an undifferentiated febrile illness without a typical pattern of fever.

- The most common species are *P. falciparum* and *P. vivax*. **Travel history and epidemiology** offer useful clues to diagnosis:
  - tropical Africa (e.g. Cameroon) → *P. falciparum*
  - nothern Africa → *P. vivax*
  - *P. vivax* → relatively uncommon in sub-Saharan Africa
  - Middle East (e.g. Afghanistan) → *P. vivax* > *P. falciparum*
  - South-East Asia → *P. vivax* ~ *P. falciparum*
  - Central America → the usual species is *P. vivax*
  - South America → both *P. vivax* and *P. falciparum* are common species
  - *P. ovale* → is found mainly in (West) Africa
  - *P. falciparum* → maximum incidence in tropical Africa, Haiti, New Guinea, and the Western Pacific Islands

The global distribution of *Plasmodium falciparum* and *Plasmodium vivax* malaria in 2010 is shown in Figure S4

- The characteristic immunological response in malaria is **an increase in the IgG level**. Cellular immunity also plays a role (Th1 responses e.g. INFγ).

HIV infection (common in migrants from sub-Saharan Africa with malaria) and iatrogenic immunosuppression



***P. falciparum* annual mean parasite rate**, P*f*PR2-10 (i.e. the statistically predicted proportion of (2-10)-year-old children with a positive blood film for malaria within the spatial limits of stable *P. falciparum* malaria transmission)

***P. vivax* annual mean parasite rate**, P*v*PR1–99 (i.e. the predicted annual mean rate of *P. vivax* parasitaemia for all ages (1-99 years).

(the red circles show common areas of origin of migrants to Greece)

(Modified from: Manson's Tropical Diseases, 23rd Edition, 2014)

Figure S4. Global distribution of malaria (2010).

do not predispose to malaria, do not increase the severity of malaria, and they are not associated with failure of antimalarial treatment (although more delayed clearance of the parasite may be observed). Malaria is not an opportunistic infection.

Splenectomy and hyposplenism are associated with increased severity of malaria (hyposplenic individuals should avoid travelling to areas of high endemicity). Pregnancy in also associated with increased severity of malaria (danger for mother and baby).

Remember: ***malaria and babesiosis can cause extreme parasitaemia and fatal haemolytic anaemia in hyposplenic individuals.***

Individuals (>5 years old) living for a long time continuously in area of high endemicity or migrants originating from areas of high endemicity may have become **semi-immune to malaria**, and thus may have low-grade *P. falciparum* parasitaemia without fever or complications (i.e. asymptomatic infection) or an atypical clinical picture (e.g. unexpected identification of *P. falciparum* in the blood film of a migrant from Senegal without fever). In such cases, it is possible that fever may not be due to malaria, even though rare malaria parasites were found in the blood film! It has been reported that high levels of *Pf*HRP-2 protein in plasma and fundal changes (e.g. Roth spots and exudates) may differentiate between severe malaria and other serious conditions with intercurrent parasitaemia ('random' parasitaemia).

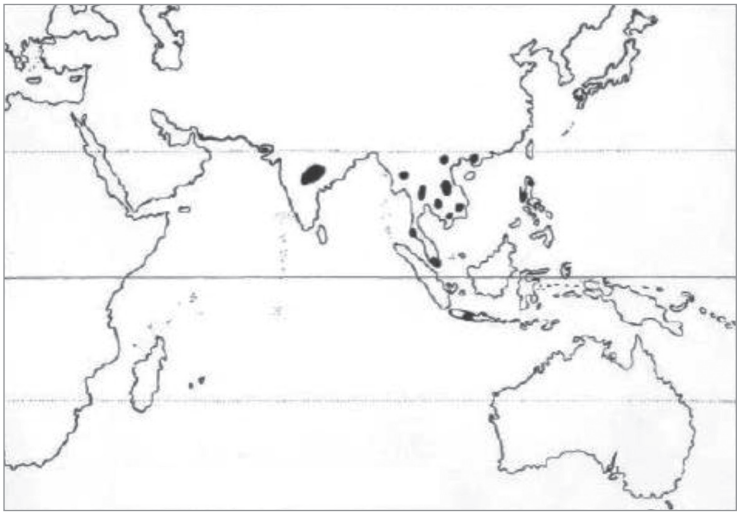
- Delays in the diagnosis of malaria often occur due to the diagnosis not being considered promptly; **non-specific laboratory clues** include elevated LDH, presence of atypical lymphocytes, elevated AST, and thrombocytopenia (present in 60–80% of cases).
- The **differential diagnosis** of fever without focal signs in returning travellers and migrants should include:

– malaria – typhoid fever – influenza – dengue	may all be accompanied by abnormal counts e.g. leukopenia, thrombocytopenia and anaemia

Typhoid fever (enteric fever) has the same geographical distribution with *P. falciparum* and, like malaria, typhoid fever can present with persistent fever, frontal headache, splenomegaly, and abdominal pain or abdominal tenderness (diffuse or right-upper quadrant); a dry cough is also common. Typhoid fever is frequently accompanied by leukopenia and eosinopenia. Generalised lymphadenopathy is uncommon in typhoid.

In the tropics, **influenza viruses** circulate throughout the year (i.e. influenza is endemic throughout the year). From a haematologist's standpoint, severe H1N1 influenza is associated with haemophagocytic syndrome.

Dengue concerns travellers to South-East Asia (e.g. Singapore, Hong Kong), India, and the Caribbean (Figure S5). Unlike malaria, dengue frequently presents with an erythematous generalised rash and a large number of atypical (reactive) lymphocytes in the blood film (ddx: infectious mononucleosis). Arthralgia, headache, and thrombocytopenia are very common; thrombocytopenia



Incubation period of dengue: 3-14 days (4-7 days in most cases).

Epidemics of dengue occur in South-East Asia, Pacific Islands and also the Caribbean. *Aedes aegypti* is the main vector. There are 4 types of flavovirus responsible for dengue (DEN 1-4).

Dengue should be differentiated from **chikungunya fever**, another common tropical viral infection with the same distribution (Caribbean countries, South-East Asia, Pacific Islands, Indian Ocean e.g. Reunion, India, tropical Africa, and South America) transmitted by *Aedes*. Incubation period is short: usually 2-12 days (2-4 days in most cases).

Fever, rash, arthralgia in a returning traveller is the classic presentation of dengue and chikungunya fever (ddx requires serology). Haematological abnormalities e.g. leukopenia, thrombocytopenia, and anaemia are common in both.

Figure S5. Distribution map of dengue.

may be severe in **dengue haemorrhagic fever (DHF)**. Unlike malaria there is no jaundice in dengue (even in DHF). In uncomplicated dengue, CRP is typically normal. In contrast, CRP may be increased in malaria. Headache (retro-orbital), generalised lymphadenopathy and/or splenomegaly may be present.

Tips:

- ***in every patient who returns from the tropics with fever, regardless of the clinical symptoms or signs you should rule out malaria (****P. falciparum* ***may manifest as acute febrile gastroenteritis, traveller's diarrhoea, atypical abdominal pain and fever, dry cough and fever, severe headache and fever, flu-like syndrome, or severe sepsis/septic shock). Arthralgia, myalgia, and nausea are very common symptoms of malaria.***
- ***malaria does not cause any lymphadenopathy or rash.***
- ***travellers who develop a fever >7-10 days after their return are unlikely to have dengue or chikungunya fever but they may have malaria.***

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