Spotlight on hematology

Malaria
Acute Diagnosis and Differentiation

MQZH 2013-04

Malaria in Switzerland

Between 2006-2010, an average of approx. 180 people per year were affected with malaria in Switzerland. Of these individuals, six died from infection with *Plasmodium falciparum*, which was contracted in a sub-Saharan African country in each case.

Other than Swiss who contracted infection during vacation or while on business trips, mainly people of African or Asian origin are those who become infected with malaria parasites while visiting friends and relatives in their home countries.

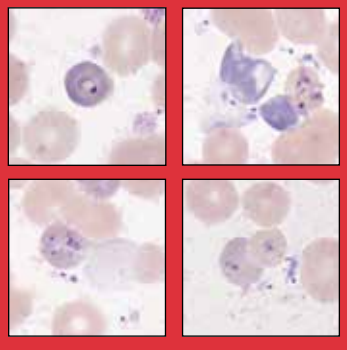
The following distribution of Plasmodia species in malaria infections is exhibited in Switzerland:

- *P. falciparum* 75%
- *P. vivax* 10%
- *P. ovale* 4%
- *P. malariae* 3%

In addition, there were multiple infections with 2-4 Plasmodia species, and/or the species could not be conclusively identified.
(Bull BAG 2011; Nr. 38: 807-811)

Possibility of confusion - Babesien

Malaria plasmodia can be mistaken for Babesia, which are also intraerythrocytic.
These are mainly transmitted to animals by ticks. An infection in humans may be potentially life-threatening, but occurs rarely and if so, mostly in immunocompromised or splenectomized patients.



Introduction

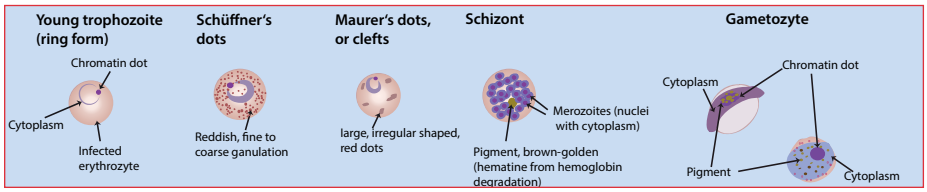
Malaria is a tropical disease. The malaria parasite, Plasmodium, is transmitted to humans via a bite of the female Anopheles mosquito, (see Focus Hematology 2007-02 „malaria"). The Plasmodium species that cause pathology in humans include *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* (5th pathogenic Plasmodium species in humans). Our survey specimen is derived from a patient infected with *Plasmodium vivax*. At the time of blood collection, the parasitemia level was 2 parts per thousand (2 of 1000 erythrocytes were infected).

Acute Diagnosis of Malaria

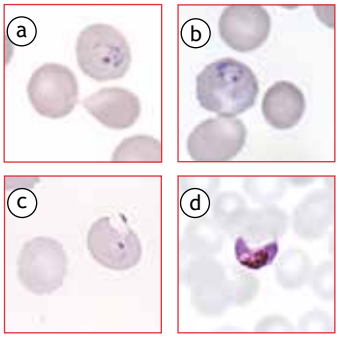
Diagnosis of malaria infection is urgent and is made by microscopic examination of peripheral blood by means of thick and thin blood smears.
Malaria is present when plasmodia are detected. Exception: plasmodia can sometimes be detected in clinically healthy subjects who live in endemic areas. False negative results can occur at very low parasitemia levels.

Method	Application	Statement	Comments
Thick blood smear	Acute diagnosis	Plasmodia detectable yes / no	6-8 fold parasite enrichment
Thin blood smear	Acute diagnosis	Plasmodia detectable yes / no Plasmodium species Parasitemia	Streak quality normal to rather thin
Immunological rapid tests	Acute diagnosis (accompanying)	Detection of plasmodium-specific antigens (not for all species) false-negative findings possible at high parasite density (prozone phenomena)	Not suitable as decision basis for patients considering emergency self-medication (handling errors, prozone phenomenon)
Serology for malaria antibodies	Not suitable for acute diagnosis		Only for retrospective questions. Titer increases only after approx. 3 weeks after infection.
PCR for pathogen-specific DNA	Not suitable for acute diagnosis. Time consuming, expensive		Only suitable for specific questions, e.g., confirmation of microscopically diagnosed species

Morphological Aspects of Plasmodia



Survey specimens: comparative images *P. vivax* versus *P. falciparum*



a) Trophozoite with slightly thickened and vacuolated cytoplasmic ring, and fine Schüffner's dots with *Plasmodium vivax*

b) Trophozoite with double nuclei and clear Schüffner's dots with *Plasmodium vivax*

c) Trophozoite with *Plasmodium falciparum*

d) Typical «banana»-shaped gametocyte with *Plasmodium falciparum*

Spotlight on hematology

Plasmodium knowlesi - the fifth Plasmodia species pathogenic in humans

Occurrence
Plasmodium knowlesi was originally identified as malaria pathogen in macaques (monkeys) in Malaysia. The pathogen is found only in Southeast Asia, in particular in Malaysia and Malaysian Borneo. Individual cases are also known from Thailand, China, the Philippines, Singapore and Central Vietnam.

Recent studies after 2004 show that the transmission of Plasmodium knowlesi to humans is not uncommon in the regions of its main distribution.

Complicated course
Similar to malaria tropica elicited by Plasmodium falciparum, malaria caused by Plasmodium knowlesi may have a potentially fatal course with organ complications and cerebral involvement.

Diagnosis
Diagnosis is difficult, because on the basis of morphology only, P. knowlesi cannot be distinguished from other Plasmodium species. Young trophozoites of P. knowlesi may exhibit morphological features similar to P. falciparum (multiple infections, double nuclei, accolé form) while more mature trophozoites may present band-like forms otherwise typical of malariae P. (quartan malaria, benign malaria).

With predominant occurrence of young trophozoites, a misdiagnosis of P. falciparum infection could be made and with exclusive occurrence of mature trophozoites, schizonts and gametocytes a diagnosis of P. malariae could be made.
PCR analysis for pathogen-specific DNA can provide clarity.

An Infection with Plasmodium knowlesi can have a very aggressive clinical course, as opposed to infection with Plasmodium malariae. Infection with Plasmodium knowlesi must be considered if the patient has visited the region of Southeast Asia.

About
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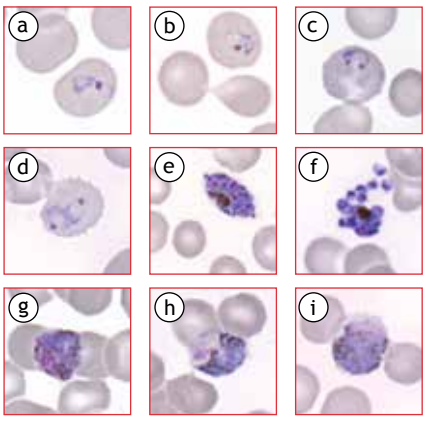
Distinguishing characteristics of different species

Species	Infected erythrocytes	Young trophozoites (ring forms)	Mature trophozoites	Schizonts	Gametocytes
Plasmodium falciparum	ERC mostly of normal size; possibly Maurer's clefts. (No Schüffner's dots)	Small, faint cytoplasm rings (approx.1/5 of the erythrocyte-diameter); 1-2 fine chromatin dots; often more rings in ERC (multiple infection) and double-chromatin dots; accolé forms.	Very rarely detectable in blood. Compact cytoplasm; dark pigment; possibly Maurer's clefts.	Very rarely detectable in blood. 8-24 merozoites; dark, stacked pigment.	Sickle-or banana-shaped parasite; chromatin stacked together (macrogametocyte) or diffusely distributed (microgametocyte); dark pigment mass.
Plasmodium vivax	ERC normal size (young trophozoites) or up to 2-fold increased (mature forms); possibly deformed; Schüffner's dots possible.	Large cytoplasm ring, possibly amoeboid form; large chromatin dot; often multiple infections, fine Schüffner's dots possible.	Large, pronounced amoeboid cytoplasm, Schüffner's dots; fine yellowish-brown pigment.	12-24 merozoites; Schüffner's dots; yellow-brown pigment flowing into each other.	Large round to oval parasite fills almost entire ERC; compact chromatin, eccentric (macrogametocyte) or diffuse (microgametocyte); scattered brown pigment.
Plasmodium ovale	ERC size normal to 1.25-fold increased with «frayed» ends (oval); Schüffner's dots possible.	Cytoplasm ring smaller than in P. vivax and thickened; large chromatin dot; often multiple infections, Schüffner's dots possible.	Cytoplasm ring compact (less amoeboid) and smaller than in P. vivax; Schüffner's dots; dark brown pigment.	6-14 merozoites with large nuclei, clustered around dark brown pigment mass; Schüffner's dots.	Compact round to oval parasite, almost fills ERC; chromatin compact, eccentric (macrogametocyte) or diffuse (microgametocyte); scattered brown pigment.
Plasmodium malariae	Possible reduced size to 3/4 of normal ERC- size, no Schüffner dots, no Maurer's clefts.	Cytoplasm ring thickened; large chromatin dot.	Compact cytoplasm ring, large chromatin dot; possibly band forms; coarse dark brown pigment.	6-12 merozoites with large nuclei; clustered around rough, dark brown pigment mass, possibly rosette shape.	Compact round to oval parasite, can almost completely fill ERC; compact chromatin, eccentric (macrogametocyte) or diffuse (microgametocyte); scattered brown pigment.
Plasmodium knowlesi	Size of ERC usually normal	Similar *P. falciparum*	Similar to *P. malariae*; frequent band forms.	Similar to *P. malariae*.	*Similar to P. malariae*.

Survey slides: Different stages of Plasmodium vivax



a-d) Trophozoites (ring forms), a / b) with slightly thickened or amoeboid cytoplasm ring, c) with double-nuclei and distinct Schüffner's dots, d) with double ring.

e-f) Schizonts with up to 20 merozoites and brown-golden pigment, f) burst schizont with release of the individual merozoites.

g-i) In Gametozytes, the parasite uses in the most cases the whole volume of the erythrocyte. The gold-brown pigment is spread over the whole cell.