Curr Infect Dis Rep (2015) 17: 8
DOI 10.1007/s11908-015-0469-6

Plasmodium knowlesi malaria: Overview Focussing on Travel-Associated Infections

Jakob P. Cramer

Published online: 29 March 2015

Abstract In 2004, *Plasmodium knowlesi* was first recognised as a relevant cause of human malaria in Southeast Asia. Since then, *P. knowlesi* has been described from all Southeast Asian countries except Laos and has become well-established as the fifth human malaria parasite and the first significant zoonotic *Plasmodium* species. As countries endemic for *P. knowlesi* malaria are among the most popular and most highly visited international destinations, travel medicine experts should be aware about disease and risks including prophylactic and therapeutic measures. Between 2005 and 2012, 15 cases of *P. knowlesi* malaria have been recognised and published in international travellers. Male gender and travel to rural/forested areas with contact to wild monkeys are risk factors for *P. knowlesi* infection. The present review gives an overview on current literature on the *P. knowlesi* parasite and summarises recent findings related to epidemiology, diagnostics, treatment and prophylaxis focussing on travellers.

Keywords *Plasmodium knowlesi* · Monkey · Simian · Macaques · Zoonotic · Malaria · Traveller

Introduction

Over 120 *Plasmodium* species generally causing host-specific infections in mammals, birds and reptiles have been described [1]. The recognition of *Plasmodium knowlesi* as a protozoan parasite causing relevant malaria morbidity and mortality in humans has added two new aspects to our knowledge on malaria: a fifth species was added to the list of human malaria parasites and it has been established that human malaria is not necessarily caused by exclusively human-specific parasites.

P. knowlesi has first been studied in detail as a malaria parasite in monkeys in India in the early 1930s by Robert Knowles, Biraj Mohan Das Gupta and others. While long-tailed and pig-tailed macaques were found to be the natural hosts, it was soon observed that the parasite was also able to cause clinical disease in humans under experimental conditions [2, 3]. The first published natural infection in a traveller occurred in a US army member in 1965 after having spent 4 weeks in Peninsular Malaysia [4]. Yet, zoonotic malaria was considered as very rare until about 10 years ago. In 2004, a relevant proportion of clinical malaria had been attributed to *P. knowlesi* in Malaysian Borneo by recognising discrepancies between microscopic and molecular-genetic findings in human malaria cases [5]. Since then, *P. knowlesi* has been isolated from malaria patients in nearly all Southeast Asian countries including Malaysia, Thailand, the Philippines, Myanmar, Singapore, Vietnam, Indonesia, Brunei and Cambodia as illustrated in Fig. 1 [6–9••]. In some areas of Malaysian Borneo, *P. knowlesi* accounts for the majority of hospitalised malaria cases and the species has been shown to cause a high proportion of severe disease [10••].

As *P. knowlesi*-endemic countries attract a major proportion of international travel, this review provides an overview on this *Plasmodium* species and summarises clinical evidence focussing on international travellers.

Parasite and Epidemiology

Of the five human *Plasmodium* species, *P. knowlesi* replicates most quickly with a life cycle of about 24 h. The parasite is phylogenetically closely related to *Plasmodium vivax* [11]. Both *P. knowlesi* and *P. vivax* use the Duffy red blood cell

This article is part of the Topical Collection on *Tropical, Travel and Emerging Infections*

J. P. Cramer (✉)
Bernhard Nocht Institute for Tropical Medicine,
Bernhard-Nocht-Strasse 74, 20359 Hamburg, Germany
e-mail: cramer@bni-hamburg.de

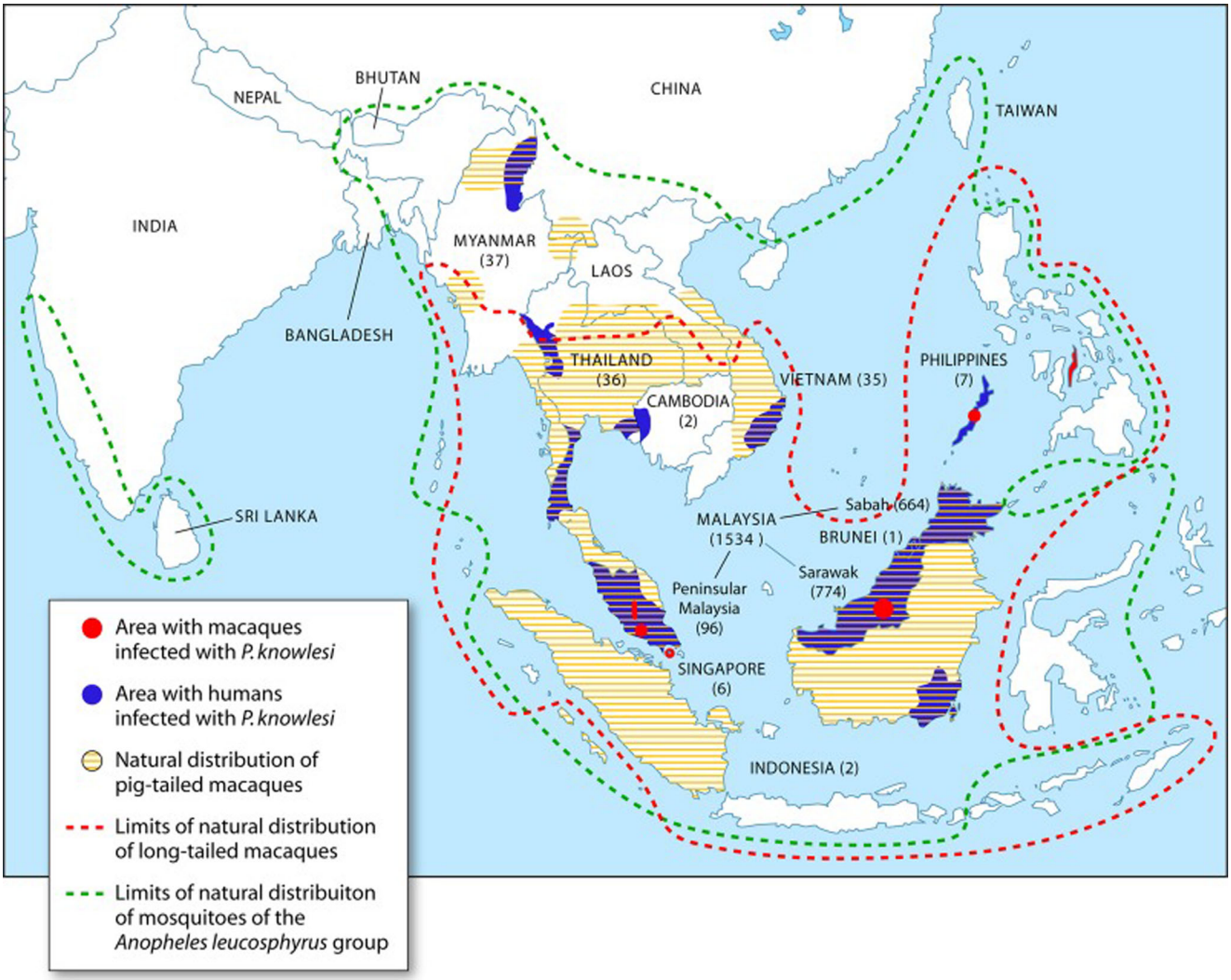

Fig. 1 Areas with reported *Plasmodium knowlesi* infections in humans and macaques as well as natural distribution of macaques being the natural host as well as of the responsible vector belonging to the *Anopheles leucosphyrus* group: Areas overlap [9••]. *Numbers in parentheses* indicate reported *P. knowlesi* cases in travellers to respective countries. Comment: instead of summarising all *P. knowlesi* cases recorded in that area, I suggest to provide numbers of *P. knowlesi* infections reported in international travellers, which would be as follows: Malaysian Borneo=5, Peninsular Malaysia=3, Thailand=3, Philippines=2, Indonesia=1, Brunei=1

antigen as a receptor for cell invasion [12]. The rapid replication cycle leads to a quotidian fever after average prepatent and incubation times of 7, 2 and 11 days, respectively [1, 13]. Most human cases initially present with low parasite levels but the short replication time may rapidly lead to hyperparasitaemia [14]. Spontaneous remissions without antimalarial therapy have been described [13, 15]. As *P. knowlesi*, in contrast to phylogenetically related *P. vivax*, does not produce hypnozoites, relapses do not occur but reinfections with a different strain are possible [16, 17].

After first recognising *P. knowlesi* as a zoonotic malaria parasite contributing to a significant proportion of human malaria in 2004, *P. knowlesi* infections have been observed in every country in Southeast Asia except in Laos. *P. knowlesi* malaria appears to be more frequent in adults than in children as well as in males than in females which may reflect specific risk behaviour [9••]. Precise data on incidence and prevalence in Southeast Asia are lacking as it is morphologically difficult to differentiate *P. knowlesi* from *Plasmodium malariae* and *Plasmodium falciparum* by microscopy. In some areas of Malaysian Borneo where molecular genetic typing methods have been applied, all locally acquired malaria cases were attributable to *P. knowlesi* [9••]. In a recent study assessing parasites species in 453 samples positive for malaria parasites in 22 hospitals all over Malaysia, *P. knowlesi* was identified in 56 % of the samples followed by *P. vivax* (29 %), *P. falciparum* (11 %), *Plasmodium ovale* (<1 %) and *P. malariae* (<1 %) [18].

The natural hosts of *P. knowlesi* are long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaques which are widely distributed throughout Southeast Asia. In some areas like Malaysian Borneo, up to 87 % of wild macaques have been shown to be infected with *P. knowlesi* [9••].

The main transmission cycle is largely confined to monkey-to-monkey transmission by forest-dwelling zoophilic anopheline mosquitoes of the *Anopheles leucosphyrus* group [9••]. In Malaysian Borneo, the area with the most intense *P. knowlesi* transmission, several *Anopheles* species competent for monkey-to-human transmission have been identified which predominantly feed between 7 and 10 p.m. [19–21]. Interestingly, *P. knowlesi* alone but also together with *P. vivax* and/or *P. falciparum* has been isolated from saliva glands of *Anopheles dirus* in southern Vietnam [22]. As *A. dirus* is the only known vector for human malaria transmission in this part of Vietnam, this observation indicates the potential for human-to-human transmission at least in some *P. knowlesi*-endemic areas [22].

As the habitats of the natural host and, correspondingly, of the *Anopheles leucosphyrus* group responsible for *P. knowlesi* transmission are located in forested areas, the main risk for human infections is confined to those entering forests for professional (e.g. farming, hunting, woodwork) or leisure (e.g. camping) activities [5, 14, 22]. As international travellers also enter these areas, an increasing number of *P. knowlesi* malaria cases have been published in the literature since 2005 (see Tables 1 and 2).

Diagnostics

Under the microscope, early trophozoites from *P. knowlesi* and *P. falciparum* are indistinguishable. Both parasite species can reach very high parasite loads; multiple infected red blood cells are characteristic. The more mature forms of *P. knowlesi* blood stage parasites including mature trophozoites and schizonts as well as gametocytes are similar to those of *P. malariae*. In fact, most *P. knowlesi* infections have been erroneously identified as *P. malariae* infections in routine microscopy [23–25]. Hence, blood microscopy is suitable for diagnosing plasmodial infection in general but it is of limited value in species differentiation in *P. knowlesi*-endemic areas. In contrast to *P. malariae* infection, *P. knowlesi* malaria can be life-threatening. Therefore, WHO recommends reporting *P. malariae*-positive blood films as *P. malariae*/*P. knowlesi* in areas endemic to the latter species [26].

Because currently used rapid diagnostic tests (RDTs) have been developed before the detection of *P. knowlesi* as a relevant cause of human malaria in Southeast Asia, their diagnostic specificity and sensitivity regarding this zoonotic *Plasmodium* species has not systematically been assessed. Results from tourists are inconclusive: RDTs containing pan-*Plasmodium* antibodies may react positive but sensitivity is lowest in *P. knowlesi* infections compared to other *Plasmodium* species. Pan-*Plasmodium* lactate dehydrogenase (*p*LDH) seems to have a slightly higher sensitivity as pan-*Plasmodium* aldolase (*p*ALD) [27, 28•]. On the other hand, species-specific monoclonal antibodies for example against histidine-rich protein 2 (*pf*HRP-2) or lactate dehydrogenase of *P. falciparum* (*pf*LDH) or *P. vivax* (*pv*LDH) do not reliably react [9••, 28•]. In areas where *P. vivax*, *P. falciparum* and *P. knowlesi* co-exist, RDTs containing *pf*LDH or *pv*LDH may react positive due to the fact that *P. vivax*- and *P. falciparum*-LDH share 97 and >90 % homology with *P. knowlesi*-LDH [29]. In a recent study, the effectiveness of three commonly used RDTs was assessed in patients with *P. knowlesi* infection [30]. Using fresh blood samples from patients with microscopically or PCR-confirmed infections, OptiMAL-IT showed the highest sensitivity of 71 % (95 % confidence interval (CI) 54–88 %—yielding predominantly *P. falciparum*-positive results) compared to BinaxNOW® Malaria and Paramax-3 RDT with a sensitivity of 29 % (95 % CI 12–46 %) and 40 % (95 % CI 21–59 %), respectively. Even the combination of two RDTs containing *p*LDH/*pf*LDH (OptiMAL-IT) and *p*LDH/*pf*HRP (CareStart) did not increase the sensitivity which was 25 % (95 % CI 19–32 %) but increased the specificity to 97 % (95 % CI 92.00 %) [31]. The sensitivity seems to be higher in severe malaria cases possibly due to higher parasite levels. To date, no *P. knowlesi*-specific antigen suitable for RDTs in endemic areas has been identified [25]. All in all, the additive value of RDTs in diagnosing *P. knowlesi* infections remains uncertain—in particular, in very low parasite levels.

A series of PCR-based molecular-genetic detection methods has been established but these are usually not broadly available for routine diagnostics in particular in resource-poor settings. Even in Western countries, PCR-based malaria diagnostics are limited to specialised parasitology laboratories. Nested PCR assays are widely used to ascertain *Plasmodium* species but this approach is labour-intense, involving a PCR each for all five parasite species. Furthermore, previously used nested PCR assay developed for the detection of *P. knowlesi* have been modified due to their false-positivity in *P. vivax* infections indicating cross-reactivity and limited species-specificity of previously used molecular-genetic methods [32, 33]. Very sensitive real-time multiplex PCR assays offer a more rapid diagnostic approach but also require more sophisticated laboratory equipment. A single-step hexaplex PCR system targeting the 18S ssu-rRNA of all five human *Plasmodium* species that overcomes some of the obstacles of the previous two methods has recently been described [34].

Due to these diagnostic difficulties related to microscopy and RDTs as well as the limited availability of molecular-genetic methods beyond research laboratories in endemic areas it can be anticipated that the current knowledge on *P. knowlesi* epidemiology is still incomplete. The lack of specific detection methods in *P. knowlesi*-endemic tourist destinations and in community hospital settings in the traveller's home country should not delay rapid initiation of antiparasitic treatment given the fact that *P. knowlesi* replicates quickly and infection may progress to life-threatening disease.

Table 1 Overview on epidemiologic characteristics of cases of imported *Plasmodium knowlesi* malaria in intercontinental travellers

No.	Year	Age (years)	Sex	Country of origin	Destination country (most likely place of infections)	Travel duration	Risk behaviour (visiting areas of high risk like forest, jungle, outdoor activity, etc.)	Chemoprophylaxis	Reference
1	1965	37	Male	USA	Peninsular Malaysia	4 weeks	No information	None	[4]
2	2005	60	Male	Taiwan	Philippines (Palawan Island)	2 weeks	Bird watching, ecotourism Palawan Island	No information	[49]
3	2006	35	Male	Sweden	Malaysian Borneo (Sarawak)	2 weeks	Trekking in the jungle of the Bario Highlands (altitudes 800–1400 m)	None	[51]
4	2007	53	Male	Finland	Peninsular Malaysia	4 weeks	Kuala Lumpur (2 weeks) with day trips to surrounding rural areas, northwestern coast and 5 days in jungle ~80 km south of Ipoh, 7 days Langkawi Beach	None	[52]
5	2008	50	Female	USA	Philippines (Island of Palawan)	13 days	Visiting friends and relatives in her country of origin: cabin lodge at the edge of a forested area (habitat for long-tailed macaques)	none	[53]
6	2008/2009	39	Male	Spain	Thailand (Bangkok), Indonesia (Banda Ace, Pulau Weh), Malaysia (Kuala Lumpur), Vietnam (Hanoi)	6 months	Reported contact with simians as well as travelling to rural areas	Mefloquine, changed to atovaquone-proguanil (80 % compliance)	[15]
7	2009	38	Male	The Netherlands	Malaysian Borneo (Kapit, Sarawak)	3 months	Hunting wild animals in surrounding jungles	No information	[54]
8	2010	39	Male	Australia	Indonesian Borneo (Kalimantan)	18 months	Had spent an average of 10 days per month working adjacent to a forest area	None	[36]
9	2010	40	Male	New Zealand	Malaysian Borneo (Sabah, Sarawak)	Travelled regularly for 6 weeks since years	Helicopter pilot, works in the forested areas of Borneo	None	[55]
10	2010	45	Male	France	Thailand (West coast)	3 months	West coast near the town of Ranong. For the last month, he stayed on Ko Payam directly on the beach near a forest (noted many monkeys)	None	[56]
11	2011	32	Female	The Netherlands	Malaysian Borneo (Sarawak)	3 weeks	2-day jungle trek during a 1-week vacation	None	[57]
12	2012	35	Male	Japan	Peninsular Malaysia (Temengor)	2 months	Entomological and botanical field investigations in Temengor (4 weeks), Johor (2 weeks) and Kuala Lumpur (1 week). In Temengor, he stayed in a tent near a forested areas, saw wild monkeys	None	[50]
13	2014	33	Female	Scotland	Malaysian Borneo	10 days		Atovaquone-proguanil for the first 4 days in the endemic country	[58]
14	2013	54	Male	Germany	Thailand (Ranong)	4 weeks	Stayed nearly all the time at Phuket visiting his Thai wife	None	[59]
15	2013	55	Female	Germany	Thailand	No information	Stay in forested Khao Sok National Park inhabited by monkeys	None	[60]

Table 2 Overview on clinical signs, course and outcome of imported *Plasmodium knowlesi* malaria in international travellers

	Disease uncomplicated/ severe	Initial parasitaemia	Thrombocytes upon first presentation	Complication or severe malaria-defining symptom/condition	Time from leaving endemic area to disease manifestation	Treatment	Outcome	Reference
1	Uncomplicated	"Many ring-form parasites"	No information	–	9 days	Chloroquine followed by primaquine	Survived	[4]
2	Uncomplicated	No information	No information	–	No information	Chloroquine	Survived	[49]
3	Uncomplicated	0.1 %	58.000/μl	–	11 days	Mefloquine	Survived	[51]
4	Uncomplicated	<0.1 %	143.000/μl	Hypoglycaemia most likely related to quinine	3 days	i.v. quinine and oral doxyccline	Survived	[52]
5	Uncomplicated	2.9 %	"Thrombocytopaenia"	–	0 days	Atovaquone-proguanil and primaquine	Survived	[53]
6	Uncomplicated	250/μl (0.003 %)	86.000/μl	–	No information	Recovered spontaneously, received chloroquine afterwards	Survived	[15]
7	Complicated	84.000/μl (2 %)	22.000/μl	Jaundice	2 days	Chloroquine	Survived	[54]
8	Uncomplicated	185/μl	106.000/μl	–	13 days	Atovaquone-proguanil	Survived	[36]
9	Uncomplicated	Not specified	71.000/μl	–	5–12	Atovaquone-proguanil changed to artemether-lumefantrine	Survived	[55]
10	Uncomplicated	0.8 %	73.000/μl	–	9 days	Chloroquine	Survived	[56]
11	Uncomplicated	0.0005 %	72.000/μl	–	3 days before leaving the endemic country	Atovaquone-proguanil	Survived	[57]
12	Uncomplicated	10.120/μl (0.2 %)	47.000/μl	–	1 day	Mefloquine	Survived	[50]
13	Uncomplicated	<1 %	241.000/μl	–	13 days	Chloroquine	Survived	[58]
14	Uncomplicated	473/μl (0.01 %)	197.000/μl	HIV test turned out positive	9 days	Atovaquone-proguanil	Survived	[59]
15	Complicated	0.2 %	27.000/μl	Acute renal failure (creatinine 3.45 mg/dl)	10 days	Intravenous artesunate followed by oral artemether-lumefantrine	Survived	[60]

Clinical Disease

Clinical signs and symptoms of acute *P. knowlesi* malaria are non-specific and frequently include daily fever and chills corresponding with the short erythrocytic parasite cycle. Accompanying symptoms may include headache, malaise, reduced appetite, abdominal pain, diarrhoea and even cough. While most patients presented to a health care facility within 4–5 days, some patients were ill for several weeks before seeking medical care [10••, 35–37]. Upon clinical examination, the most common findings include tachypnea and tachycardia in association with fever. In a quarter to a third of patients, enlarged liver and spleen have been reported [10••, 35].

Nearly all patients are thrombocytopaenic upon or shortly after hospitalisation. Nevertheless, bleeding complications are rare [35]. Pronounced thrombocytopaenia may occur at low parasite levels in *P. knowlesi* malaria. Therefore, careful examination of blood films is necessary before excluding *P. knowlesi* malaria in these patients [9••]. While anaemia occurs, severe anaemia is less frequent compared to *P. falciparum* malaria [35]. Renal impairment has been described in 6.9 and 14.5 % in two recent prospective studies on 130 and 110 patients with *P. knowlesi* malaria but is usually reversible [10••, 38••].

P. knowlesi infection can cause complicated and even life-threatening malaria. In fact, recent prospective case-control studies conducted in Malaysian Borneo indicate that up to 29 % of patients with *P. knowlesi* malaria progress to severe disease rendering the proportion of patients with severe malaria (applying severe *P. falciparum* malaria definitions) threefold higher compared to patients with *P. falciparum* malaria in endemic areas [10••, 38••]. Complications include parasite levels >100.000/μl, jaundice, acute respiratory distress, hypotension, acute renal failure, acidosis and, rarely, hypoglycaemia [10••, 35]. A parasitaemia of ≥35.000/μl or 1 % infected red blood cells and a thrombocytopaenia of ≤45.000/μl have been associated with a ten- and fivefold higher risk of developing complications, respectively, in adult patients with *P. knowlesi* malaria [38••]. Cerebral malaria, the complication associated with highest mortality in severe *P. falciparum* malaria, has not been described in *P. knowlesi* malaria. High parasite loads have been shown to be associated with complicated disease but severe disease can also occur at lower parasite levels [10••, 38••].

Treatment

No randomised clinical trial has been conducted to assess the efficacy of antimalarials in *P. knowlesi* malaria. Accordingly, none of the antimalarials has been licensed for its treatment in the Western world. Currently, available evidence, mainly obtained from case series to smaller prospective observational studies, covers a wide range of antimalarial drugs and indicates that most antimalarials are effective [9••].

Cases with uncomplicated *P. knowlesi* malaria have been successfully treated with virtually all conventional antimalarials including chloroquine, mefloquine, atovaquone-proguanil, artemether-lumefantrine, quinine and intravenous artesunate [9••], see also Table 2. A randomised study comparing artesunate-mefloquine combined therapy versus chloroquine in uncomplicated *P. knowlesi* malaria is expected to complete recruitment at the end of 2014 but it is unlikely that mefloquine will play a relevant role in treating *P. knowlesi* due to better-tolerated alternative drugs [39]. *P. knowlesi* is considered as sensitive to chloroquine [40•]. From a practical approach, however, this antimalarial does not seem to be a suitable prophylactic or therapeutic option as *P. knowlesi*-endemic areas have the highest proportion of antimalarial drug-resistant strains of other malaria parasites, in particular, *P. falciparum*, while rapid and reliable species-differentiation can not be warranted outside specialised centres. In addition, ex-vivo assessment of the sensitivity of human *P. knowlesi* isolates indicate high sensitivity to artemisinins, variable and moderate sensitivity to chloroquine, and lower sensitivity to mefloquine [41•].

The WHO favours a pragmatic approach by recommending artemisinin-based combination therapies (ACT) generalising existing evidence as well as data derived in particular from *P. falciparum* malaria to *P. knowlesi* infections [26]. German guidelines recommend an ACT or atovaquone-proguanil following the therapeutic approach for *P. falciparum* malaria [42]. The US Centers of Disease Control and Prevention (CDC) currently recommend chloroquine for treating *P. knowlesi* malaria [43]. As *P. knowlesi* infection may rapidly lead to high parasitaemia, it seems reasonable to treat promptly with a fast-acting antimalarial. Despite the fact that oral ACTs available for treatment of uncomplicated malaria in the Western world (including artemether-lumefantrine (Riamet®, Coartem®) or dihydroartemisinin-piperaquine (Eurartesim®)) are not licensed for *P. knowlesi* infection, an oral ACT seems to be the drug of choice in cases in which *P. knowlesi* malaria has to be considered in particular when the species identification can not be rapidly and reliably secured in specialised centres. This practical approach is supported by a recent publication from Malaysian Borneo showing that of six deaths related to *P. knowlesi* malaria, all had initially been reported as *P. malariae* infection by microscopy and only two received immediate parenteral antimalarial treatment [44••].

For severe *P. knowlesi* malaria, retrospectively collected data indicate a superior effect on parasite clearance time as well as survival of intravenous artesunate versus quinine which is in line with existing evidence derived from large trials on severe *P. falciparum* malaria [45]. A recently published prospective study conducted in Malaysian Borneo indicates that early referral and prompt initiation of intravenous

artesunate is highly effective also in severe *P. knowlesi* malaria [10••]. Whether complications like delayed haemolysis after artesunate therapy, in particular in patients with high parasite loads, also occur in *P. knowlesi* malaria is unclear but it seems to be justified to follow-up on haemoglobin levels at 2 and 4 weeks after initiation of intravenous artesunate in particular in those patients treated with high parasite levels (e.g. >5 % infected red blood cells) [46].

P. knowlesi has so far mainly been considered a zoonotic disease and its relevance in human malaria has only recently been established. Yet, this does not exclude existing drug resistance per se as human cases may have broadly been mistaken as *P. malariae* (or *P. falciparum*) infections in the past and may have been exposed to respective antimalarials in a relevant dimension. In fact, parasite levels continued to increase despite mefloquine therapy in a recent case report which supports the observation, that antimalarial resistance can easily be induced by repeated drug exposure to *P. knowlesi* infected rhesus macaques [17, 47, 48].

P. knowlesi should be considered in all travellers returning with microscopically diagnosed *P. malariae* (or *P. falciparum*) infection and parasite levels should be assessed. However, antimalarial treatment should not be delayed until the causative species has been ascertained as *P. knowlesi* may progress to high parasitaemia quickly resulting in complicated, life-threatening disease. Despite lacking respective evidence, an ACT like artemether-lumefantrine or, alternatively, atovaquone-proguanil should be the treatment of choice. Transmission areas for *P. knowlesi* and dengue virus overlap with dengue fever being the suspected primary diagnosis in many febrile patients with thrombocytopaenia. Therefore, dengue virus infection should be considered and ruled out.

Role in Travellers/Prophylaxis

The first case of natural *P. knowlesi* infection was described in a US army surveyor in 1965 after returning from a 4-week trip to Peninsular Malaysia [4]. Since then and after *P. knowlesi* has been recognised to contribute to human malaria in Southeast Asia, 12 cases have been reported in intercontinental travellers between 2006 and 2013, see Tables 1 and 2. The UK Health Protection Agency mentions one additional case of *P. knowlesi* malaria reported in a traveller to Brunei without providing any further information [40•]. Two cases have been reported in international travellers within Asia: a Taiwanese traveller to Palawan Island, the Philippines, in 2005 and a Japanese traveller to Malaysia in 2012 [49, 50]. Except for the one case reported from Brunei, countries of infection were Malaysia (n=8 with three cases from Peninsular Malaysia and five cases from Malaysian Borneo), Thailand (n=3), the Philippines (n=2) and Indonesia (n=1). One case had travelled to several countries including Thailand, Indonesia, Malaysia and Vietnam. For epidemiologic and clinical details on all 15 informative cases in international travellers see Tables 1 and 2, respectively.

Male gender and trips to forested areas inhabited by monkeys have been described as risk factors for infection [9••]. This is in line with the characteristics of the 15 *P. knowlesi* infections reported in international travellers so far (Table 1). Eleven (73 %) travellers were male, median age was 40.4 years (range 32–60). Almost all travellers had visited rural/forested areas; several reported that they had seen wild monkeys during their journey. The travel duration ranged from 10 days to 18 months indicating that *P. knowlesi* malaria also occurs in short-term travellers. None of the travellers had taken regular/continuous malaria prophylaxis. As shown in Table 2, 85 % were thrombocytopaenic upon hospitalisation. While one patient became symptomatic 3 days before leaving the endemic country, the mean time from last potential exposure (leaving the endemic country) to disease onset was 7.4 days (range 0–13). The initial treatment in the 15 travellers with *P. knowlesi* malaria was atovaquone-proguanil (n=5), chloroquine (n=5), mefloquine (n=2), intravenous quinine (n=1) and intravenous artesunate (n=1). One patient who took irregular malaria prophylaxis recovered apparently without antimalarial therapy. One case was complicated by jaundice; in another patient, hypoglycaemia occurred most likely related to the quinine treatment and the other 13 cases were uncomplicated—all survived.

Travellers to *P. knowlesi*-endemic areas, in particular, those intending to travel to rural, forested areas should be informed about the risk as well as respective exposure prophylaxis measures including repellents, bed nets etc. Current commercially available RDTs are unreliable in detecting *P. knowlesi* infection. While epidemiologic data on risks in local populations is still incomplete, specific risks in travellers are unknown. In the author's opinion, continuous malaria prophylaxis is not generally warranted in travellers to *P. knowlesi*-endemic areas but in particular, travellers at higher risk of infection (outdoor activities, ecotourism, stay in rural/forested areas, anticipated contact with wild monkeys) should be provided with advice to seek medical advice as soon as possible after onset of fever during or after travel and should be provided with either an ACT or atovaquone-proguanil as antimalarial drug for standby emergency treatment. Recommendations for malaria prevention in travellers to *P. knowlesi*-endemic areas according to German, US and UK guidelines are summarised in Table 3.

Conclusion

P. knowlesi occurs in all Southeast Asian countries except Laos. Microscopy is useful to detect plasmodial infections but is of limited value for species differentiation. Rapid diagnostic tests are not reliable in detecting *P. knowlesi* infection because of low sensitivity. In patients returning with malaria from Southeast Asia—in particular in those with *P. falciparum*

Table 3 Recommendations for malaria prevention in selected *Plasmodium knowlesi*-endemic areas with reported cases in travellers

	Region	German Society of Tropical Medicine and International Health (DTG), Germany [61]	Centers of Disease Control and Prevention (CDC), USA [62]	Public Health England (PHE), U.K. [63]
Malaria prevention (riskassessment in travellers)	Malaysian Borneo	- Stand-by emergency treatment with atovaquone-proguanil or artemether-lumefantrine (low risk[a] in central parts, Sabah and Sarawak)	- Prophylaxis with atovaquone-proguanil, doxycycline or mefloquine[b] (low risk[a] in rural areas, Sabah and Sarawak provinces)	- Prophylaxis with atovaquone-proguanil, doxycycline or mefloquine[b] (high risk[a] in inland areas of eastern Sabah and in the inland, forested areas of Sarawak) - Mosquito bite avoidance only (very low risk[a] of malaria in the rest of Malaysian Borneo including the coastal areas of Sabah and Sarawak)
	Peninsular Malaysia	- Stand-by emergency treatment with atovaquone-proguanil or artemether-lumefantrine (minimal risk[a] in central parts, Johor and Pahang)	- Prophylaxis with atovaquone-proguanil, doxycycline or mefloquine[b] (low risk[a] in rural areas)	- Prophylaxis with atovaquone-proguanil, doxycycline or mefloquine[b] (risk[a] in the inland, forested areas) - Mosquito bite avoidance only (very low risk[a] in the rest of peninsular Malaysia including the Cameron highlands and the city of Kuala Lumpur)
	Thailand	- Stand-by emergency treatment with atovaquone-proguanil or artemether-lumefantrine (low risk[a] in frontier areas in the North as well as in coastal areas in the South)	- Prophylaxis with atovaquone-proguanil, doxycycline (low risk[a] in forested areas that border Burma (Myanmar), Cambodia and Laos) Rural, forested areas in districts of Phang Nga and Phuket	- Prophylaxis with atovaquone-proguanil or doxycycline (high risk[a] in the rural, forested borders of Thailand with Cambodia, Laos and Myanmar) - Mosquito bite avoidance only (very low risk[a] in the remaining areas of Thailand including Kanchanaburi, Kwai Bridge) - no risk[a] in the cities of Bangkok, Chiang Mai, Chiang Rai, Koh Phangan, Koh Samui and Pattaya
	The Philippines	- Stand-by emergency treatment with atovaquone-proguanil or artemether-lumefantrine (low risk[a] in rural areas below 600 m of Luzon, Mindoro, Palawan and Mindanao)	- Prophylaxis with atovaquone-proguanil, doxycycline or mefloquine[b] (low risk[a] in rural areas below 600 m on islands of Basilan, Luzon, Mindanao, Mindoro, Palawan, Sulu (Jolo) and Tawi-Tawi) - None[a] in urban areas	- Prophylaxis with chloroquine-proguanil (risk[a] in rural areas below 600 m and on the islands of Luzon, Mindanao, Mindoro and Palawan) - Mosquito bite avoidance only (no risk[a] in cities or on the islands of Boracay, Bohol, Catanduanes, Cebu and Leyte)
	Indonesia	- Prophylaxis with atovaquone-proguanil or doxycycline or mefloquine[b] (high risk[a] in Irian Jaya as well as on islands East of Bali) - Stand-by emergency treatment with atovaquone-proguanil or artemether-lumefantrine (in other parts of the country, occurrence of *P. knowlesi* infections in Kalimantan, Indonesian Borneo) - Large cities and tourist areas of Java and Bali: malaria-free	- Prophylaxis with atovaquone-proguanil, doxycycline or mefloquine[b] (moderate risk[a] in rural areas of Kalimantan (Borneo)), Nusa Tenggara Barat (includes the island of Lombok), Sulawesi and Sumatra - All areas of eastern Indonesia (provinces of Maluku, Maluku Utara, Nusa Tenggara Timur, Papua and Papua Barat). - None[a] in the cities of Jakarta, Ubud or resort areas of Bali and Java - Low transmission[a] in rural areas of Java including Ujung Kulong, Sukalumi and Pangadaran	- Prophylaxis with atovaquone-proguanil, doxycycline or mefloquine[b] (high risk[a] in Lombok and Irian Jaya (Papua)) - Prophylaxis with chloroquine-proguanil (risk[a] in the rest of Indonesia) - Mosquito bite avoidance only (very low risk[a] in Bali, and the cities on the islands of Java and Sumatra) - no risk[a] in the city of Jakarta
Reference		DTG. 2014	CDC. 2014	PHE, 2014

[a] *Plasmodium*-species not specified

[b] Mefloquine: special caution—travellers need to be informed on specific risks

and *P. malariae*-positive blood film readings—molecular-genetic typing should be requested. Yet, rapid initiation of antimalarial treatment should not be delayed. Patients with *P. knowlesi* malaria may proceed to high parasite levels rapidly

with the potential for life-threatening disease. Despite lacking respective evidence, an ACT seems to be the drug of choice for treating *P. knowlesi* infections. Travellers to endemic countries—in particular those intending to visit forested/rural areas or anticipate contact with wild monkeys—should be advised about risks and preventive measures including the fact that reliable diagnosis should sought rapidly while RDTs are unreliable.

Compliance with Ethics Guidelines

Conflict of Interest Jakob Cramer has no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Hoffmann SL, Campbell C, White CNJ. Malaria, p. 646–675. In Gerrant RL, Walker DH, Weller PF (ed), Tropical Infectious Diseases: Principles, athogens and Practice, 3rd ed. Saunders Elsevier, Oxford, UK
2. Knowles RM, Das Gupta B. A study of monkey malaria and its experimental transmission to man. Indian Med Gaz. 1932;67:301–20.
3. Sinton JA, Mulligan HW. A critical review of the literature relating to the identification of the malarial parasites recorded from monkeys of the families Cercopithecidae and Colobidae. Rec Malar Surv India III. 1932;62.
4. Chin W, Contacos PG, Coatney GR, Kimball HR. A naturally acquited quotidian-type malaria in man transferable to monkeys. Science. 1965;149(3686):865.
5. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired Plasmodium knowlesi infections in human beings. Lancet. 2004;363(9414): 1017–24.
6. Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T, Kanbara H. Naturally acquired Plasmodium knowlesi malaria in human. Thailand Emerg Infect Dis. 2004;10(12):2211–3.
7. Luchavez J, Espino F, Curameng P, Espina R, Bell D, Chiodini P, et al. Human Infections with Plasmodium knowlesi, the Philippines. Emerg Infect Dis. 2008;14(5):811–3.
8. Van den Ende P, Van HN, Van Overmeir C, Vythilingam I, Duc TN, Hung Le X, et al. Human Plasmodium knowlesi infections in young children in central Vietnam. Malar J. 2009;8:249.
9. •• Singh B, Daneshvar C. Human infections and detection of Plasmodium knowlesi. Clin Microbiol Rev. 2013;26(2):165–84. *Very comprehensive review on Plasmodium knowlesi including epidemiology, history, parasite, clinical disease and treatment.*
10. •• Barber BE, William T, Grigg MJ, Menon J, Auburn S, Marfurt J, et al. A prospective comparative study of knowlesi, falciparum, and vivax malaria in Sabah, Malaysia: high proportion with severe disease from Plasmodium knowlesi and Plasmodium vivax but no mortality with early referral and artesunate therapy. Clin Infect Dis. 2013;56(3):383–97. *Important contribution on clinical aspects and complications related to Plasmodium knowlesi infections.*
11. Pain A, Böhme U, Berry AE, Mungall K, Finn RD, Jackson AP, et al. The genome of the simian and human malaria parasite Plasmodium knowlesi. Nature. 2008;455(7214):799–803.
12. Gaur D, Mayer DC, Miller LH. Parasite ligand-host receptor interactions during invasion of erythrocytes by Plasmodium merozoites. Int J Parasitol. 2004;34(13–14):1413–29.
13. Antinori S, Galimberti L, Milazzo L, Corbellino M. Plasmodium knowlesi: the emerging zoonotic malaria parasite. Acta Trop. 2013;125(2):191–201.
14. Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, et al. Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis. 2008;46(2):165–71.
15. Tang TT, Salas A, Ali-Tammam M, Martínez Mdel C, Lanza M, Arroyo E, et al. First case of detection of Plasmodium knowlesi in Spain by Real Time PCR in a traveller from Southeast Asia. Malar J. 2010;9:219.
16. Cogswell FB. The hypnozoite and relapse in primate malaria. Clin Microbiol Rev. 1992;5(1):26–35.
17. Lau YL, Tan LH, Chin LC, Fong MY, Noraishah MA, Rohela M. Plasmodium knowlesi reinfection in human. Emerg Infect Dis. 2011;17(7):1314–5.
18. Yusof R, Lau YL, Mahmud R, Fong MY, Jelip J, Ngian HU, et al. High proportion of knowlesi malaria in recent malaria cases in Malaysia. Malar J. 2014;13:168.
19. Vythilingam I, Tan CH, Asmad M, Chan ST, Lee KS, Singh B. Natural transmission of Plasmodium knowlesi to humans by Anopheles latens in Sarawak, Malaysia. Trans R Soc Trop Med Hyg. 2006;100(11):1087–8.
20. Tan CH, Vythilingam I, Matusop A, Chan ST, Singh B. Bionomics of Anopheles latens in Kapit, Sarawak, Malaysian Borneo in relation to the transmission of zoonotic simian malaria parasite Plasmodium knowlesi. Malar J. 2008;7:52.
21. Jiram AI, Vythilingam I, NoorAzian YM, Yusof YM, Azahari AH, Fong MY. Entomologic investigation of Plasmodium knowlesi vectors in Kuala Lipis, Pahang. Malaysia Malar J. 2012;11:213.
22. Marchand RP, Culleton R, Maeno Y, Quang NT, Nakazawa S. Co-infections of Plasmodium knowlesi, P. falciparum, and P. vivax among Humans and Anopheles dirus Mosquitoes, Southern Vietnam. Emerg Infect Dis. 2011;17(7):1232–9.
23. Lee KS, Cox-Singh J, Singh B. Morphological features and differential counts of Plasmodium knowlesi parasites in naturally acquired human infections. Malar J. 2009;8:73.
24. Barber BE, William T, Grigg MJ, Yeo TW, Anstey NM. Limitations of microscopy to differentiate Plasmodium species in a region coendemic for Plasmodium falciparum, Plasmodium vivax and Plasmodium knowlesi. Malar J. 2013;12:8.
25. Jeremiah SS, Janagond AB, Parija SC. Challenges in diagnosis of Plasmodium knowlesi infections. Trop Parasitol. 2014;4(1):25–30.
26. World Health Organization, WHO. Meeting Report: Informal consultation on the public health importance of P. knowlesi. WHO Regional Office for the Western Pacific Press, Manila, Philippines 2011. http://www.wpro.who.int/mvp/documents/docs/Pknowlesi_final_report.pdf accessed on 17.Sep.2014, accessed on 17th Sep.2014
27. Kawai S, Hirai M, Haruki K, Tanabe K, Chigusa Y. Cross-reactivity in rapid diagnostic tests between human malaria and zoonotic simian malaria parasite Plasmodium knowlesi infections. Parasitol Int. 2009;58(3):300–2.
28. • Barber BE, William T, Grigg MJ, Piera K, Yeo TW, Anstey NM. Evaluation of the sensitivity of a pLDH-based and an aldolase-based rapid diagnostic test for diagnosis of uncomplicated and severe malaria caused by PCR-confirmed Plasmodium knowlesi, Plasmodium falciparum, and Plasmodium vivax. J Clin Microbiol. 2013;51(4):1118–23. *Contributes important information on the limitations of rapid diagnostic tests in Plasmodium knowlesi infections.*

29. Singh V, Kaushal DC, Rathaur S, Kumar N, Kaushal NA. Cloning, overexpression, purification and characterization of Plasmodium knowlesi lactate dehydrogenase. Protein Expr Purif. 2012;84(2): 195–203.
30. Foster D, Cox-Singh J, Mohamad DS, Krishna S, Chin PP, Singh B. Evaluation of three rapid diagnostic tests for the detection of human infections with Plasmodium knowlesi. Malar J. 2014;13:60.
31. Grigg MJ, William T, Barber BE, Parameswaran U, Bird E, Piera K, et al. Combining parasite lactate dehydrogenase-based and histidine-rich protein 2-based rapid tests to improve specificity for diagnosis of malaria Due to Plasmodium knowlesi and other Plasmodium species in Sabah, Malaysia. J Clin Microbiol. 2014;52(6):2053–60.
32. Imwong M, Tanomsing N, Pukrittayakamee S, Day NP, White NJ, Snounou G. Spurious amplification of a Plasmodium vivax small-subunit RNA gene by use of primers currently used to detect P. knowlesi. J Clin Microbiol. 2009;47(12):4173–5.
33. Sulistyaningsih E, Fitri LE, Löscher T, Berens-Riha N. Diagnostic difficulties with Plasmodium knowlesi infection in humans. Emerg Infect Dis. 2010;16(6):1033–4.
34. Goh XT, Lim YA, Vythilingam I, Chew CH, Lee PC, Ngui R, et al. Increased detection of Plasmodium knowlesi in Sandakan division, Sabah as revealed by PlasmoNex™. Malar J. 2013;12:264.
35. Daneshvar C, Davis TM, Cox-Singh J, Rafa'ee MZ, Zakaria SK, Divis PC, et al. Clinical and laboratory features of human Plasmodium knowlesi infection. Clin Infect Dis. 2009;49(6):852–60.
36. Figtree M, Lee R, Bain L, Kennedy T, Mackertich S, Urban M, et al. Plasmodium knowlesi in human, Indonesian Borneo. Emerg Infect Dis. 2010;16(4):672–4.
37. Kantele A, Jokiranta TS. Review of cases with the emerging fifth human malaria parasite, Plasmodium knowlesi. Clin Infect Dis. 2011;52(11):1356–62.
38.•• Willmann M, Ahmed A, Siner A, Wong IT, Woon LC, Singh B, et al. Laboratory markers of disease severity in Plasmodium knowlesi infection: a case control study. Malar J. 2012;11:363. *Contributes relevant information to clinical P. knowlesi malaria*.
39. Grigg MJ, William T, Dhanaraj P, Menon J, Barber BE, von Seidlein L, et al. A study protocol for a randomised open-label clinical trial of artesunate-mefloquine versus chloroquine in patients with non-severe Plasmodium knowlesi malaria in Sabah, Malaysia (ACT KNOW trial). BMJ Open. 2014;4(8):e006005.
40.• Public Health England. Imported malaria cases and deaths in the UK: 1994–2013. https://www.gov.uk/government/publications/imported-malaria-in-the-uk-statistics, accessed on 17th Sep. 2014 *Contributes relevant information to clinical P. knowlesi malaria*
41.• Fatih FA, Staines HM, Siner A, Ahmed MA, Woon LC, Pasini EM, et al. Susceptibility of human Plasmodium knowlesi infections to anti-malarials. Malar J. 2013;12:425. *Summarizes available information on antiparasitic treatment in P. knowlesi malaria*
42. Arbeitsgemeinschaft Wissenschaftlicher Medizinischer Fachgesellschaften, AWMF. Diagnostik und Therapie der Malaria. Version August 2014. http://www.awmf.org/uploads/tx_szleitlinien/042-001l_S1_Malaria_Diagnostik_Therapie_2014-08.pdf, accessed on 15.Sep.2014
43. Centers of Disease Control and Prevention, CDC. Guidelines for the Treatment of Malaria in the United States. Updated 1st July 2013. http://www.cdc.gov/malaria/resources/pdf/treatmenttable.pdf, accessed on 15.Sep.2014
44.•• Rajahram GS, Barber BE, William T, Menon J, Anstey NM, Yeo TW. Deaths due to Plasmodium knowlesi malaria in Sabah, Malaysia: association with reporting as Plasmodium malariae and delayed parenteral artesunate. Malar J. 2012;11:284. *This publication highlights the importance of rapid species differentiation and treatment initiation related to the difficulties of microscopical differentiation between Plasmodium knowlesi and other human plasmodium parasites*.
45. William T, Menon J, Rajahram G, Chan L, Ma G, Donaldson S, et al. Severe Plasmodium knowlesi malaria in a tertiary care hospital, Sabah, Malaysia. Emerg Infect Dis. 2011;17(7):1248–55.
46. Rolling T, Agbenyega T, Issifou S, Adegnika AA, Sylverken J, Spahlinger D, et al. Delayed hemolysis after treatment with parenteral artesunate in African children with severe malaria–a double-center prospective study. J Infect Dis. 2014;209(12):1921–8.
47. Singh J, Ray AP, Basu PC, Nair CP. Acquired resistance to proguanil in Plasmodium knowlesi. Trans R Soc Trop Med Hyg. 1952;46(6):639–49.
48. Singh J, Nair CP, Ray AP. Studies on Nuri strain of P. knowlesi. V. Acquired resistance to pyrimethamine. Indian J Malariol. 1954;8(3):187–95.
49. Kuo M-C, Chiang T-Y, Chan C-W, Tsai W-S, Ji D-D. A case reposrt of simian malaria, Plasmodium knowlesi, in a Taiwanese Traveler from Palawan Island, the Philippines. Taiwan Epidemiology Bulletin. 2009;25:178–91.
50. Tanizaki R, Ujiie M, Kato Y, Iwagami M, Hashimoto A, Kutsuna S, et al. First case of Plasmodium knowlesi infection in a Japanese traveller returning from Malaysia. Malar J. 2013;12:128.
51. Bronner U, Divis PC, Färnert A, Singh B. Swedish traveller with Plasmodium knowlesi malaria after visiting Malaysian Borneo. Malar J. 2009;8:15.
52. Kantele A, Marti H, Felger I, Müller D, Jokiranta TS. Monkey malaria in a European traveler returning from Malaysia. Emerg Infect Dis. 2008;14(9):1434–6.
53. Ennis JG, Teal AE, Habura A, Madison Antenucci S, Keithly JS, Arguin PM, et al. Simian Malaria in a U.S. traveller – New York, 2008. Morb Mortal Wkly Rep. 2009;58:229–32.
54. van Hellemond JJ, Rutten M, Koelewijn R, Zeeman AM, Verweij JJ, Wismans PJ, et al. Human Plasmodium knowlesi infection detected by rapid diagnostic tests for malaria. Emerg Infect Dis. 2009;15(9):1478–80.
55. Hoosen A, Shaw MT. Plasmodium knowlesi in a traveller returning to New Zealand. Travel Med Infect Dis. 2011;9(3):144–8.
56. Berry A, Iriart X, Wilhelm N, Valentin A, Cassaing S, Witkowski B, et al. Imported Plasmodium knowlesi malaria in a French tourist returning from Thailand. Am J Trop Med Hyg. 2011;84(4):535–8.
57. Link L, Bart A, Verhaar N, van Gool T, Pronk M, Scharnhorst V. Molecular detection of Plasmodium knowlesi in a Dutch traveler by real-time PCR. J Clin Microbiol. 2012;50(7):2523–4.
58. Cordina CJ, Culleton R, Jones BL, Smith CC, MacConnachie AA, Coyne MJ, et al. Plasmodium knowlesi: Clinical Presentation and Laboratory Diagnosis of the First Human Case in a Scottish Traveler. J Travel Med. 2014;21(5):357–60.
59. Ehrhardt J, Trein A, Kremsner P, Frank M. Plasmodium knowlesi and HIV co-infection in a German traveller to Thailand. Malar J. 2013;12:283.
60. Orth H, Jensen BO, Holtfreter MC, Kocheril SJ, Mallach S, MacKenzie C, et al. Plasmodium knowlesi infection imported to Germany, January 2013. Euro Surveill. 2013;3:18(40).
61. Deutsche Gesellschaft für Tropenmedizin und Internationale Gesundheit, DTG. http://www.dtg.org/uploads/media/DTG-Malaria_2014.pdf, accessed on 15.Sep.2014
62. Centers of Disease Control and Prevention, CDC. Malaria Information and Prophylaxis, by Country. http://www.cdc.gov/malaria/travelers/country_table/i.html, accessed on 15.Sep.2014 Public Health England. Guidelines for malaria prevention in travellers from the UK 2014. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/337761/Guidelines_for_malaria_prevention_in_travellers_UK_PC.pdf, accessed on 15.Sep.2011.
63. Public Health England. Guidelines for malaria prevention in travellers from the UK 2014. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/337761/Guidelines_for_malaria_prevention_in_travellers_UK_PC.pdf, accessed on 15. Sep.2011.