EXPERT REVIEWS

Therapy of vector-borne protozoan infections in nonendemic settings

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Vector-borne protozoan infections are responsible for a wide variety of illnesses (mainly malaria, trypanosomiasis and leishmaniasis) affecting tropical and subtropical areas, but increasingly diagnosed in nonendemic settings. This article summarizes the therapeutic developments for these conditions during the past decade and focuses specifically on treatment recommendations for returning travelers and migrants. The treatment of malaria has known the most spectacular improvements. Progress in the management of leishmaniasis and trypanosomiasis has also been substantial and includes introduction of new drugs into clinical practice, combinations of existing drugs, or new laboratory tools for treatment monitoring as well as extension of treatment indications to new groups of patients. Serious gaps still exist in terms of effectiveness and tolerance. Since the research pipeline is very limited for the coming 5–10 years, optimized combinations of existing drugs need to be urgently explored.

Keywords: Chagas disease • leishmaniasis • malaria • migrant • nonendemic • protozoa • sleeping sickness • therapy • traveler • trypanosomiasis

Protozoa are a diverse group of unicellular organisms that may cause a wide variety of vector-borne or food-borne systemic and gastrointestinal infections in humans. The major vector-borne protozoan infections are malaria, leishmaniasis, human African trypanosomiasis (sleeping sickness) and American trypanosomiasis (Chagas disease). These conditions disproportionally affect the tropical and subtropical areas, where effective vectors are present almost exclusively. Epidemiology has, however, considerably evolved during the last decade. First, control programs have made substantial progresses in many endemic regions, shrinking the malaria map, for example, in all continents [1] or reducing the burden of Chagas disease in Latin America [2] or sleeping sickness in Central Africa [3]. On the other hand, the growth of international travel has resulted in increasing numbers of protozoan infections diagnosed in nonendemic settings among returning travelers or migrants [4,5]. In parallel, after decades of scientific stagnation, most protozoan infections considered as 'neglected tropical diseases' have recently attracted more attention in the global research agenda thanks to various international alliances involving the WHO, governments, academic institutions, the pharmaceutical industry and nonprofit associations such as the Drugs for Neglected Diseases Initiative (DNDI) [6,7].

The purpose of this article is to describe the developments in therapy for vector-borne protozoan infections during the last decade and to provide updated recommendations for international travelers. Therapeutic guidelines for travelers may differ somewhat from those in endemic settings, because maximal efficacy is required in this nonimmune and often older population at higher risk for complications [8], while issues related to treatment administration, laboratory monitoring or costs are less crucial than in low-resource settings. However, it must also be acknowledged that treatment recommendations are often exclusively based on studies conducted in endemic countries, since specific data on travelers are often limited to small case series.

It is beyond the scope of this article to review in detail all epidemiological, clinical and diagnostic features of the protozoan diseases under discussion. However, some basic information with therapeutic implications is summarized in Table 1 for the readers less familiar with tropical pathology. Food-borne protozoan diseases will not be addressed here.

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Table 1. Epidemiology, burden, clinical features and diagnostic methods of vector-borne protozoan infections (cont.).

Species	Epidemiology	Route of transmission	Clinical features	Diagnostic methods	Ref.
Malaria					
Plasmodium falciparum	175–630 million clinical cases a year; >1 million deaths/year mostly among children in sub-Saharan Africa Distribution in sub-Saharan Africa, Central and South America (and the Hispaniola Island), the Middle East, Indian subcontinent, Southeast Asia and Pacific islands Sensitivity to chloroquine only in Hispaniola Island, Central America west of the Panama Canal and most regions of the Middle East 1000–1200 cases reported/year in the USA; 10,000 cases a year in Europe	Bites from infected mosquitoes with nocturnal activity (*Anopheles* spp.) Congenital Transfusion related	Nonspecific systemic febrile illness, most often acute, sometimes with digestive symptoms High risk of complications such as severe anemia or end-organ dysfunction (e.g., cerebral malaria, kidney failure, liver insufficiency and respiratory distress) in nonimmune patients	Microscopic detection of parasites on stained thick or thin blood smears Immunochromatographic RDT detecting the HRP-2 antigen or a parasite LDH enzyme (pan-malaria or specific for *P. falciparum*); sensitivity above 95% for parasite count above 1000/µl Species-specific PCR in reference centers	[9–22]
Plasmodium vivax	70–391 million clinical cases a year Distribution throughout the tropics and subtropics, including North Africa, the Middle East Reduced sensitivity to chloroquine in Indonesia, Papua New Guinea and Oceania (the Solomon Islands, Vanuatu) 250–500 cases a year in the USA; 2000 cases a year in Europe	Bites from infected mosquitoes with nocturnal activity (*Anopheles* spp.) Congenital Transfusion related	Nonspecific systemic febrile illness, sometimes with a typical tertian fever pattern Risk of late relapses (due to liver hypnozoites) Risk (limited) of complications (e.g., severe anemia, respiratory distress and splenic rupture)	Microscopy RDT detecting a parasite LDH enzyme (pan-malaria or specific for *P. vivax*); sensitivity of approximately 95% for parasite count above 500/µl PCR in reference centers	[9–22]
Plasmodium ovale	No reliable data on global burden Mainly distributed in West Africa; sporadically reported in Latin America and Asia Sporadically reported in travelers	Bites from infected mosquitoes with nocturnal activity (*Anopheles* spp.) Congenital Transfusion related	Nonspecific systemic febrile illness, sometimes with a typical tertian fever pattern Risk of late relapse (due to liver hypnozoites) No complicated course reported	Microscopy RDT detecting a parasite LDH enzyme (pan-malaria only; little accuracy) PCR in reference centers	[9–22]
Plasmodium malariae	No reliable data on global burden Distribution throughout the tropics; rather uncommon Sporadically reported in travelers	Bites from infected mosquitoes with nocturnal activity (*Anopheles* spp.) Congenital Transfusion related	Nonspecific systemic febrile illness, sometimes with a typical quartan fever pattern Risk of late relapses (possibly due to erythrocytic hypnozoites) No complicated course reported	Microscopy RDT detecting a parasite LDH enzyme (pan-malaria only; little accuracy) PCR in reference centers	[9–22]

CATT: Card agglutination test for trypanosomiasis; CSF: Cerebrospinal fluid; HRP: Histidine-rich protein; IHA: Indirect hemagglutination; IIF: Indirect immunofluorescence; LDH: Lactate dehydrogenase; RDT: Rapid diagnostic test.

Table 1. Epidemiology, burden, clinical features and diagnostic methods of vector-borne protozoan infections (cont.).

Species	Epidemiology	Route of transmission	Clinical features	Diagnostic methods	Ref.
Malaria (cont.)					
Plasmodium knowlesi	Recently reported in Southeast Asia and Indonesia Some cases reported in travelers since 2007	Bites from infected mosquitoes with nocturnal activity (*Anopheles* spp.) Congenital Transfusion related	Nonspecific systemic febrile illness, with quotidian pattern Risk (not yet well quantified) of end-organ complication	Microscopy RDT detecting a parasite LDH enzyme (pan-malaria only; performance unknown) PCR in reference centers	[9–22]
Leishmaniasis					
Cutaneous leishmaniasis	1.5 million new cases/year 90% of all cases occurring in Iran, Syria, Saudi Arabia, Afghanistan, Algeria, Peru and Brazil Distribution of leishmaniasis species is as follows: • In the Old World: *L. tropica* complex ('urban'), *L. major* complex ('rural') and *L. aethiopica* complex; also *L. infantum* and *L. donovani* • In the New World: *L. mexicana* complex (e.g., *L. mexicana*, *L. amazonensis* and *L. venezuelensis*); *L. (Viannia) braziliensis* complex (*L. braziliensis*, *L. peruviana*); *L. (Viannia) guyanensis* complex (*L. guyanensis, L. panamensis*) Also *L. infantum/chagasi*	Bites of infected female *Phlebotoma* spp. (Old World) or *Lutzomia* spp. (New World) Reservoirs of *L. tropica* are humans; reservoirs of *L. major, L. aethiopica*, *L. mexicana* and *L. (Viannia) braziliensis* are rodents	Incubation of 1–8 months usually in the Old World; up to 2–3 years in the New World Ulcerous lesion (dry for *L. tropica*, wet for *L. major*) on exposed areas of the body; sometimes nodular or diffuse lesions or lymphatic dissemination	Giemsa staining of skin biopsy or dermal scrapings (amastigotes) Species-specific PCR in reference centers (Culture on Novy, McNeal and Nicolle medium in reference centers)	[125–137]
Mucocutaneous leishmaniasis	Almost exclusively endemic in Central and South America. Burden less quantified Mucocutaneous leishmaniasis is mainly due to two species (*L. braziliensis* and *L. panamensis*) but occasionally due to other species (such as *L. guyanensis*, *L. amazonensis* or *L. infantum/chagasi*)	Bites of infected female *Lutzomia* spp.	Metastatic development in 2–4% of the patients, with an incubation of months to years (up to 20 years) after the primary skin lesion has resolved Mucosal ulcerations leading to perforation of the nasal septum or palate	Giemsa staining of skin biopsy or dermal scrapings (little sensitivity) Species-specific PCR	[125–137]

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Table 1. Epidemiology, burden, clinical features and diagnostic methods of vector-borne protozoan infections (cont.).

Species	Epidemiology	Route of transmission	Clinical features	Diagnostic methods	Ref.
Leishmaniasis (cont.)					
Visceral leishmaniasis	500,000 new cases/year; >50,000 deaths/year 90% of all cases occurring in India, Bangladesh, Nepal, Ethiopia, Sudan and Brazil *L. donovani* complex, with *L. donovani* (Indian subcontinent and East Africa), *L. infantum/chagasi* (Mediterranean Basin and South America) and *L. archibaldi* (East Africa)	Bites of infected female *Phlebotoma* spp. (Old World) or *Lutzomia* spp. (New World) Vertical transmission Transfusion related Reservoirs of *L. donovani* are humans; reservoirs of *L. infantum/chagasi* and *L. archibaldi* are dogs and wild canidae	Subacute febrile illness and wasting syndrome, associated with hepatosplenomegaly and pancytopenia Post-Kala-Azar dermal leishmaniasis (only *L. donovani*)	Giemsa staining of lymph node, bone marrow or splenic aspiration (sensitivities of 50–60, 50–80 and >90%, respectively) Antibody-detection tests (direct agglutination tests, ELISA, immunochromatographic strip test based on rK39); variable sensitivities Antigen-detection test (urinary latex agglutination; sensitivity: 30–100%) PCR-based assays	[125–137]
Sleeping sickness					
Trypanosoma brucei gambiense	50,000–70,000 estimated new cases/year; 12,000 new cases reported in 2006 Foci in rural areas of West and Central Africa; most prevalent in the Democratic Republic of Congo, Northern Angola and Southern Sudan Rarely observed in travelers	Bites of tsetse flies of the genus *Glossina* (*G. palpalis* complex) Transfusion related Congenital	Trypanosomal inoculation chancre (rarely seen) First (early) hematolymphatic stage (months to years): intermittent fever, headache, pruritus, lymphadenopathy; sometimes peri(myo)carditis (mostly subclinical) Second (late) meningoencephalitic stage (months to years): sleep disturbances and chronic neuropsychiatric disorders CSF examination for second stage diagnosis: presence of trypanosomes; presence of more than five white blood cells per µL or protein level above 370 mg/dl. Controversy exists for patients with 6–20 white blood cells in CSF	CATT: sensitivity: 87–98%; specificity: 93–95% Microscopy: blood, lymph node aspirate or CSF: very low sensitivity Concentration methods (microhematocrit centrifugation technique, qualitative buffy-coat analysis, mini-anion-exchange centrifugation technique): sensitivity above 50% Species- and subspecies-specific PCR in reference centers	[184–191]

CATT: Card agglutination test for trypanosomiasis; CSF: Cerebrospinal fluid; HRP: Histidine-rich protein; IHA: Indirect hemagglutination; IIF: Indirect immunofluorescence; LDH: Lactate dehydrogenase; RDT: Rapid diagnostic test.

Table 1. Epidemiology, burden, clinical features and diagnostic methods of vector-borne protozoan infections (cont.).

Species	Epidemiology	Route of transmission	Clinical features	Diagnostic methods	Ref.
Sleeping sickness (cont.)					
Trypanosoma brucei rhodesiense	Foci in rural areas of East and Southern Africa (~1500 cases/year?) Sporadically observed in travelers	Bites of tsetse flies of the genus *Glossina* (*G. morsitans* complex)	Trypanosomal inoculation chancre (20%) First (early) hematolymphatic stage (weeks to months): high fever, lymphadenopathy, hepatosplenomegaly; often multiorgan failure Second (late) meningoencephalitic stage (weeks to months): acute/subacute neuropsychiatric disorders CSF examination for second stage diagnosis: same criteria as for *T. b. gambiense*	No accurate serology Blood (and CSF) microscopy: high load of circulating parasites Species- and subspecies-specific PCR in reference centers	[184–191]
Chagas disease					
Trypanosoma cruzi	Approximately 8 million chronic cases and 40,000 new cases in 2005 (compared with 18 million chronic cases and 700,000 new cases in 1985) Mainly in rural areas from Mexico to Argentina; highly endemic in Bolivia, Northern Argentina, Western Paraguay (>5% prevalence) Estimated number of infected Latin American migrants: 30–300,000 cases in the USA; 30,000 in Europe (more than half in Spain only); approximately 1% diagnosed Very rare in travelers	Contact with skin, mucous tissues or food with contaminated feces of triatomine bugs (vectors) Congenital transmission (1–5% of pregnancies in infected women) Blood or organ donation	Acute phase: asymptomatic or self-limiting febrile illness (sometimes chagoma and Romana's sign); 10% complicated course (myocarditis, encephalitis) Long period of latency (10–30 years) Chronic phase (30–40% of infections) with late complications: cardiopathy (20–30%) or digestive megasyndromes (5–10%) Risk of reactivation in immunocompromised patients (with CNS involvement) Congenital Chagas (10–30% symptomatic newborns; 10% mortality)	• Acute phase and reactivation: parasitological detection in blood smears or by concentration; PCR on blood (reference centers) • Chronic phase: – Serology: requires at least two positive tests with two different techniques (e.g., ELISA, IHA, IIF) – Xenodiagnosis (endemic zone): cumbersome – PCR on blood (reference centers): sensitivity below 80% • Congenital: parasitology or PCR; serology (patient must be >9 months of age)	[213–223]

CATT: Card agglutination test for trypanosomiasis; CSF: Cerebrospinal fluid; HRP: Histidine-rich protein; IHA: Indirect hemagglutination; IIF: Indirect immunofluorescence; LDH: Lactate dehydrogenase; RDT: Rapid diagnostic test.

Malaria

Malaria is caused by one or more of the five plasmodia (subphylum sporozoa) that infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* [9] and the recently recognized zoonotic *Plasmodium knowlesi* [10]. Malaria usually presents as an acute nonspecific febrile illness but, if left untreated, fatal complications may arise, particularly in children or pregnant women in endemic areas as well as in nonimmune travelers [9]. A severe course occurs almost exclusively in infections due to *P. falciparum* [11], but has also been reported in *P. vivax* [12,13] and *P. knowlesi* malaria [14]. Approximately 500 million clinical cases of malaria occur worldwide every year [15], including more than 10,000 cases diagnosed in Europe and the USA [16,17]. Malaria remains the leading etiology of fever in patients presenting with fever in travel clinics and the almost exclusive tropical cause of death in nonendemic settings [4,18,19]. During the last decade, antigenic rapid diagnostic tests and PCR-based assays have complemented the traditional but labor- and expertise-demanding microscopy technique [20–22].

Therapeutic progress up to 2000

For more than 300 years, quinine, an inhibitor of parasitic heme polymerization, was the only specific treatment for malaria. Quinine, first extracted from the bark of the South American tree *Cinchona ledgeriana*, was successfully synthesized in 1944, and converted to its stereoisomer quinidine later on. Attempts to synthesize quinine led to the discovery of chloroquine in 1934. The straightforward production of chloroquine at low cost brought great hope of global malaria eradication in the 1950s. However, resistant strains of *P. falciparum* emerged in the 1970s on the Thai–Cambodia and on the Venezuela–Colombia borders. Resistance to chloroquine gradually spread to other endemic regions in the following decades, sparing only a few areas (Table 1). World War II and the Vietnam War stimulated the development of new quinoline derivatives (primaquine, amodiaquine, mefloquine, halofantrine and lumefantrine), antifolate drugs (proguanil, pyrimethamine and the fixed-dose combination [FDC] sulfadoxine–pyrimethamine [SP]) and the sesquiterpene peroxide artemisinin (known as Qinghaosu in Chinese medicine). Artemisinin was first extracted from the leaves of the wormwood *Artemisia annua* but more potent derivatives (dihydroartemisinin, artemether and artesunate) were rapidly synthesized. Finally in the 1990s, the antimalarial activity of atovaquone, a mitochondrial electron transport inhibitor, was demonstrated [23].

Drug-resistant *P. falciparum* malaria (defined as resistance to chloroquine and SP) emerged and spread in the 1970s; multidrug resistance (defined as resistance against >two antimalarial compounds of different chemical classes) developed in the 1980s on the Thai–Cambodian border when treatment failures with mefloquine were also observed [23]. Artemisinin derivatives were then deployed in Southeast Asia, but combination with another animalarial drug was rapidly advocated [24,25]. The combination artesunate–mefloquine became the first-line treatment for uncomplicated *P. falciparum* malaria in Thailand in 1995 and in Cambodia in 2000 [26].

Developments since 2000

Uncomplicated *P. falciparum* malaria

The recognition of atovaquone as a potent antimalarial drug led to the production of the FDC atovaquone–proguanil (AP; Malarone® [GlaxoSmithKline BV]) in 1999. Both drugs have synergistic blood-stage activity *in vitro* and *in vivo*. Several randomized controlled trials (RCTs), with various comparative regimens and on all continents, have demonstrated a 28-day cure rate of 94–100% in (drug-resistant) uncomplicated *P. falciparum* malaria and a rate of treatment-limiting adverse events below 1% [27]. In the only RCT conducted in a nonendemic setting, all 21 adults included in the AP arm were cured, with no notable side effects [28]. Observational studies have largely confirmed these findings later on in travelers [29,30]. When atovaquone was initially used alone, resistance appeared rapidly [31]. Since 2002, approximately 30 cases of genetically confirmed clinical resistance to AP have been reported in travelers returning from West, Central and East Africa [32–41], as well as from Comoros [42], South America [43] and India [44]. Clinical failure in travelers presented mostly as late recrudescence and was associated with single point mutations in the parasite cytochrome b gene, exclusively found in strains previously exposed to atovaquone [38,39,45,46]. Although such mutations have been observed in up to 5% of *P. falciparum* isolates unexposed to atovaquone in one study in Nigeria [47], prevalence of molecular markers of resistance remained below 1% in all surveys conducted in pooled traveler samples and in various endemic sites to date [46,48–51].

Owing to its cost, AP has remained almost exclusively limited to travel medicine. Fortunately for low-resource settings, artemisinin derivatives were confirmed as safe and highly active agents against all asexual and sexual parasite blood stages [52–54]. Studies in the 1990s had shown, however, that when used in monotherapy, artemisinin derivatives with their short half-life had to be administered for at least 7 days to prevent recrudescence, underlying the need to combine them with a partner drug with longer antimalarial activity [55]. Artemisinin-based combination therapy (ACT) has become the first-line treatment for uncomplicated *P. falciparum* malaria in all endemic areas with drug resistance. All available ACTs have been thoroughly reviewed recently [56,57], five of which are currently recommended by the WHO in a 3-day regimen [11]: artesunate–SP; artesunate–amodiaquine; artesunate–mefloquine; artemether–lumefantrine (AL) in a six-dose regimen [58]; and dihydroartemisinin–piperaquine. Treatment efficacy is usually above 95% for the three latter combinations and above 90% for the two former, at least in regions where 28-day cure rates with the partner drugs alone (either SP or amodiaquine) is still above 80% [56]. No single ACT regimen is clearly superior to the other ACT regimens, provided that the partner drug still has some efficacy in the region it is used [59]. All but one (artesunate–SP) recommended ACTs exist now in FDCs and pediatric formulations are increasingly available [57]. Once daily dosage is now promoted to improve adherence [57]. Dihydroartemisinin–piperaquine was the first once daily artemisinin-based FDC implemented in some Asian countries [60–62]. A new fixed dose artesunate–amodiaquine combination was effective in once daily dosage [63], as well as the

new combination artesunate–pyronaridine [64]. Another new artemisinin–naphthoquine combination, tested in Papua New Guinea as a single-dose administration, provided promising preliminary results [65].

Reversion to chloroquine-sensitive *P. falciparum* parasites has been observed in Malawi where this drug has not been used for a long time [66]; impact on local guidelines is, however, still unclear. On the other hand, rates of treatment failure with artesunate–mefloquine have increased in the last decade both in the Pailin Province of Cambodia and in the neighboring Trat Province of Thailand [67]. Presence of *P. falciparum* strains resistant to artesunate has been formally demonstrated for the first time *in vivo* in Pailin Province, Cambodia [68] as well as in Battambang Province, Cambodia [69]. Resistance has emerged in the Greater Mekong subregion in a background of mefloquine resistance [70] and is characterized by prolonged parasite clearance times, increased 50% inhibitory concentrations of dihydroartemisin and recrudescence at day 28. Other contributing factors may have been the unregulated use of artemisinin monotherapy, the unavailability of ACT in FDC and the presence of counterfeit or substandard drugs [71,72]. Containment of extremely drug-resistant *P. falciparum* malaria [73] is now given the highest priority as no new class of drugs is likely to become available for at least a decade [67,71]. Of note, a *P. falciparum* strain with lower susceptibility to artemisinin has been identified in a traveler returning from Nigeria, who had inappropriately used artesunate as prophylaxis during travel [74].

Severe malaria

Artemisinin derivatives have the strongest antiparasitic effect of all antimalarial drugs, with parasite reduction ratios of approximately 10,000 per cycle (10–100 more potent than quinine) and complete parasite clearance from the blood after 6–8 days (3–4 cycles) [54]. In severe malaria, quinine was first compared with the oil-based artemether in the late 1990s but no survival benefit could be demonstrated. This was attributed to the slow and erratic absorption of intramuscular artemether injections [75]. It took a few more years before quinine was compared with the water-soluble artesunate in the large randomized multicenter South East Asian Quinine Artesunate Malaria Trial [76]. Mortality in artesunate recipients was 15% compared with 22% in quinine recipients, an absolute reduction of 35% ($p = 0.0002$). The beneficial effect was even more pronounced in the subgroup of severe malaria patients with parasitemia above 10% of the red blood cells (mortality of 23 vs 53%). More recently, the large multicenter African Quinine Artesunate Malaria Trial demonstrated a significant mortality reduction of 22.5% in African children with severe malaria treated with artesunate compared with quinine (8.5 vs 10.9%; $p = 0.0022$) [77]. These two pivotal studies have established definitively that parenteral artesunate should replace quinine as the treatment of choice for severe malaria in adults and children worldwide. Of note, in rural endemic areas, prereferral administration of rectal artesunate substantially reduced the risk of death in children with severe malaria, when the delay for adequate care exceeded 6 h [78].

Non-falciparum malaria

Resistance of *P. vivax* to chloroquine, which emerged in 1989 in Papua New Guinea, spread thereafter to most vivax-endemic regions. Nowadays, chloroquine fails in more than 50% of *P. vivax* malaria episodes in Eastern Indonesia [79]. Rates of resistance also appear high in Papua New Guinea, Vanuatu and the Solomon Islands but data are more limited. The risk of chloroquine failure has recently increased above 10% in some regions of Western Indonesia, Malaysia, Myanmar and Vietnam. It still appears to be low elsewhere, although cases of chloroquine-resistant *P. vivax* malaria have been sporadically confirmed in most endemic countries [67,79]. Efficacy of AP has been found to be excellent against blood-stage forms of *P. vivax* infection in Indonesia [80,81]. Artemisinin-based treatments are also effective against vivax malaria and have been adopted as first-line therapy in Indonesia, the Solomon Islands and Vanuatu where *P. falciparum* and *P. vivax* are coendemic, and where *P. vivax* is increasingly resistant to chloroquine [82]. In such settings, dihydroartemisinin–piperaquine was superior to AL in preventing recurrence at 42 days, thanks to its longer post-treatment prophylactic effect [83,84]. Activity of AP and ACT against *P. ovale* or *P. malariae* malaria has not yet been studied in detail but seems adequate [82,85,86].

Primaquine, an 8-aminoquinoline discovered in 1950, has remained the only available therapy to date for preventing *P. vivax* or *P. ovale* relapse due to liver hypnozoites. Its use is, however, limited by the long (2-week) treatment duration and the risk of severe hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency or in the fetus. In addition, the management of *P. vivax* malaria is now further complicated by the emergence of primaquine-tolerant strains [79]. Another hypnozoiticidal drug, tafenoquine, has been developed in the last decade but is not yet licensed. Its long half-life allows a short course or even single-dose therapy, but toxicity in G6PD-deficient individuals remains an issue [87]. This problem, however, appears more limited in preliminary studies with a new effective experimental drug, elubaquine [79].

In early 2000, *P. knowlesi*, a parasite known since 1931 to infect macaque monkeys and sporadically reported in humans, was identified in half of the patients initially misdiagnosed with *P. malariae* infection in Malaysian Borneo [10]. *P. knowlesi* parasites have a 24-h (quotidian) asexual cycle and may reach rapidly potentially lethal densities, although no sequestration occurs [14]. Complicated and lethal cases were observed in 6.5 and 1.8%, respectively, of the 107 *P. knowlesi* patients included in Malaysia in the largest prospective series to date [88]. All noncomplicated cases responded promptly to chloroquine therapy. The pathogen appears to be widely distributed in Southeast Asia [89,90]. Infection with *P. knowlesi* has also been recently recognized in travelers [91–95].

Treatment recommendations for travelers

Therapeutic guidelines for malaria are proposed in Table 2. Chemoprophylaxis has been reviewed elsewhere [96].

Uncomplicated *P. falciparum* malaria

Chloroquine remains the treatment of choice for uncomplicated *P. falciparum* malaria only in travelers who have been exclusively

Table 2. Recommended treatment of malaria in travelers.

	Adults	Children	Pregnant women	Immunosuppressed patients
Plasmodium falciparum				
Uncomplicated; stay in chloroquine-sensitive area	Chloroquine sulfate 1500 mg p.o. in total (600 mg initially; 300 mg 6 h later; 300 mg on day 2 and 300 mg on day 3)	Chloroquine sulfate 25 mg/kg p.o. in total (10–5–5–5 mg/kg following the same time schedule as in adults)	Same as 'Adults'	Same as 'Adults'
Uncomplicated; stay in chloroquine-resistant area	Atovaquone–proguanil (250 mg/100 mg) 4 tablets p.o. once daily for 3 days Artemether–lumefantrine (20 mg/120 mg) 4 tablets p.o. twice daily for 3 days Quinine sulfate 500–600 mg p.o. three-times daily for 5–7 days plus doxycycline 100–200 mg p.o. once daily for 7 days or clindamycin 10 mg/kg p.o. twice daily for 5 days	Atovaquone–proguanil (250 mg/100 mg) Contraindicated below 10 kg 11–20 kg: 1 tablet/day for 3 days 21–30 kg: 2 tablets/day once daily for 3 days 31–40 kg: 3 tablets/day once daily for 3 days Same as 'Adults' above 40 kg Artemether–lumefantrine (only above 12 years and 35 kg): same as 'Adults' Quinine sulfate 10 mg/kg p.o. three-times daily for 5 days plus clindamycin 10 mg/kg twice daily for 5 days (no doxycycline)	Quinine sulfate 10 mg/kg p.o. three-times daily for 5–7 days plus clindamycin 10 mg/kg twice daily for 5 days (no doxycycline) Artemether–lumefantrine: not recommended in travel medicine; only second and third trimester in endemic settings Atovaquone–proguanil: not indicated due to limited data	Same as 'Adults'
Complicated (or inability for oral intake)	Artesunate (2.4 mg/kg iv. at diagnosis, after 12 h, 24 h and every 24 h thereafter) Quinine hydrochloride (10–20 mg/kg iv. over 4 h at diagnosis and 10 mg/kg over 4 h every 8 h thereafter) Switch to full course oral therapy whenever possible (atovaquone–proguanil, artemether–lumefantrine, quinine plus doxycycline or clindamycin)	Quinine hydrochloride (10–20 mg/kg iv.) over 4 h at diagnosis and 10 mg/kg over 4 h every 8 h thereafter (every 12 h below 4 years); switch to oral therapy whenever possible with quinine–clindamycin (no doxycycline) or atovaquone–proguanil Consider the use of artesunate iv.	Quinine iv. or artesunate iv. as indicated for 'Adults' but switch to oral quinine–clindamycin	Same as 'Adults'
Plasmodium vivax				
Uncomplicated; stay in chloroquine-sensitive area	Chloroquine sulfate 1500 mg p.o. in total (same time schedule as above) plus primaquine 30 mg p.o. once daily for 14 days (contraindicated if severe [<10%] G6PD deficiency; if mild-to-moderate: 0.75 mg/kg once a week for 8 weeks)	Chloroquine sulfate 25 mg/kg p.o. in total (same time schedule as above) plus primaquine 0.25 mg/kg p.o. once daily for 14 days	Chloroquine only (same as 'Adults') Primaquine contraindicated	Same as 'Adults'
Uncomplicated; stay in chloroquine-resistant area (mostly Indonesia, Papua New Guinea, Oceania)	Consider in first-line atovaquone–proguanil; artemether–lumefantrine or quinine plus doxycycline or clindamycin (see 'Uncomplicated *P. falciparum* – Adults') plus primaquine 30 mg p.o. once daily for 14 days	See 'Uncomplicated *P. falciparum*' plus primaquine 0.25 mg/kg p.o. once daily for 14 days		

G6PD: Glucose-6-phosphate dehydrogenase; iv.: Intravenous; p.o.: Per oral.
Data taken from [97–123].

Table 2. Recommended treatment of malaria in travelers.

Adults	Children	Pregnant women	Immunosuppressed patients
Plasmodium vivax (cont.)			
Complicated (or inability for oral intake)	See 'Complicated *P. falciparum* – Adults' plus primaquine 30 mg p.o. once daily for 14 days	See 'Complicated *P. falciparum* – Children' plus primaquine 0.25 mg/kg p.o. once daily for 14 days	See 'Complicated *P. falciparum* – Pregnant women'	Same as 'Adults'
Plasmodium ovale			
Chloroquine sulfate 1500 mg p.o. in total (same time schedule as above) plus primaquine 30 mg p.o. once daily for 14 days	Chloroquine sulfate 25 mg/kg p.o. in total (same time schedule as above) plus primaquine 0.25 mg/kg p.o. once daily for 14 days	Chloroquine sulfate 1500 mg p.o. in total (same time schedule as above) Primaquine contraindicated	Same as 'Adults'
Plasmodium malariae			
Chloroquine sulfate 1500 mg p.o. in total (same time schedule as above)	Chloroquine sulfate 25 mg/kg p.o. in total (same time schedule as above)	Same as 'Adults'	Same as 'Adults'
Plasmodium knowlesi			
Uncomplicated	Chloroquine sulfate 1500 mg p.o. in total (same time schedule as above)	Chloroquine sulfate 25 mg/kg p.o. in total (same time schedule as above)	Same as 'Adults'	Same as 'Adults'
Complicated	See 'Complicated *P. falciparum* – Adults'	See 'Complicated *P. falciparum* – Children'	See 'Complicated *P. falciparum* – Pregnant women'	Same as 'Adults'

G6PD: Glucose-6-phosphate dehydrogenase; iv.: Intravenous; p.o.: Per oral.
Data taken from [97–123].

exposed in (the few) areas where the parasite is still fully susceptible (Table 1). For travelers returning from any other region with reported drug resistance, three different antimalarial regimens may be offered: the FDC AP, the FDC AL and the association of quinine with doxycycline or clindamycine [97–101]. There is no specific recommendation for immunosuppressed patients.

AP is administered once daily for 3 days. Absorption of atovaquone is improved if taken with food. It has been largely evaluated in children (down to 5 kg) and pediatric formulations are available. Proguanil is safe during pregnancy and no teratogenicity has been observed with atovaquone in animal studies; however, due to limited data, safety of AP in pregnancy has not been firmly established [102,103].

AL, also known as co-artemether, is the only fixed-dose ACT currently available in Europe and the USA (Riamet®, Coartem® [both Novartis AG]). For adequate absorption of lumefantrine, it should also be taken with food. Administration is normally twice daily for 3 days (six-dose regimen). Higher doses and longer courses are explored in the Greater Mekong subregion, to overcome the lower susceptibility to both drugs, but concerns have emerged on the possible risk of dose-dependant neutropenia [104]. There is no evidence of significant cardiotoxicity of AL [53,58] but the manufacturer's leaflet provides serious warnings in case of long congenital QT or concomitant use of QT-prolonging drugs. It has been approved for treatment in children and infants, and pediatric tablets are available [105]. Safety of ACTs, and particularly AL, has been established during the second and third trimester of pregnancy [103,106,107]. However, since experience is much more limited during the first trimester and concern exists from animal data about artemisinin toxicity in the first weeks of gestation [102,103], ACTs are not recommended in the first trimester in endemic settings, and during the whole pregnancy in travel medicine [301]. Of note, reduced efficacy of AL has been observed in later pregnancy because of low drug concentration [107].

The association of quinine with doxycycline has become a second choice treatment. Its 7 day administration is less convenient and often hampered by the treatment-limiting side effects of quinine (tinnitus, hearing loss, gastric discomfort and arrhythmia) [29]. Decreased susceptibility to quinine has also been reported in very heterogeneous studies in Southeast Asia and South America [67,108], as well as in travelers [109]. Quinine associated with clindamycine remains a safe therapeutic option during pregnancy [102]. Combination with azithromycin and quinine in monotherapy should be avoided in nonimmune pregnant travelers [110,111].

Ambulatory treatment of uncomplicated *P. falciparum* malaria has been proven feasible and safe in travel clinics, provided that the initial decision relies on a very careful assessment [29,112,113]. In a specialized setting,

(treatment-naive) malaria patients were managed safely outside the hospital when they did not vomit, had no criteria of severity and had a parasitemia below 1% of the red blood cells at initial contact; this policy allowed treatment of approximately 40% of all malaria cases as outpatients [29]. Some national guidelines, however, recommend admitting systematically all patients with *P. falciparum* infection for supervised initial drug administration because of the risk of rapid clinical deterioration [99,100].

Severe malaria

Although not specifically studied in nonimmune travelers, artesunate has become the treatment of choice for adults with severe malaria wherever acquired, especially those presenting with hyperparasitemia [11,99,100,114], as well as for children [115]. Intravenous administration is required until oral intake is possible and parasitemia has significantly dropped and may be followed by any of the described oral regimens. There is no need for dosage adjustment in vital organ dysfunction [11,116]. Artesunate is not licensed in Europe and the USA, because the only manufacturer (Guillin Pharmaceutical Factory, Guangxi, China) does not comply with international (but costly) good manufacturing practice (GMP) standards, with theoretical risks of inadequate quality and legal implications in case of unexpected events. It has, however, received the WHO drug prequalification attestation, an important step towards improved drug quality and the product may be obtained from IDIS Pharma in Europe. Non-GMP artesunate is, presently, almost exclusively available in reference travel centers, where its safety still needs to be closely investigated [117]. A commercial form of artesunate produced under GMP standards is awaited for 2011. Some publications have suggested combining intravenous quinine and artesunate to address the medico–legal issues [118], although no benefit of such combination has been demonstrated [119]. Quinine (or quinidine) treatment, however, remains an acceptable option for severely ill and/or vomiting malaria patients and should be administered without delay if it is the only drug immediately available [116]. Quinine dose should be reduced by one third after 48 h in case of renal failure or hepatic dysfunction. Adjunctive therapy for severe malaria has recently been reviewed [120].

Non-falciparum malaria

Chloroquine remains highly efficacious for acute attacks caused by most *P. vivax* strains and to almost all *P. ovale* and *P. malariae* strains. In the case of *P. vivax* and *P. ovale* infections, concomitant administration of primaquine is recommended for its synergistic effect and for preventing relapses [97]. Pregnancy and severe G6PD deficiency (activity below 10% of normal) must first be excluded [97]. In mild-to-moderate G6PD deficiency (>10%), primaquine dosage should be adapted (Table 2). High doses of primaquine (6 mg/kg in total administered over 14 days) retains excellent efficacy even in primaquine-tolerant *P. vivax* and *P. ovale* strains. True resistance to primaquine remains very rare [121].

Some national guidelines recommend for not using chloroquine in first-line treatment if *P. vivax* infection has been acquired in Indonesia, Papua New Guinea and the Pacific [100,101], but this remains debated [99]. In such cases, any of the three combination treatments recommended for drug-resistant *P. falciparum* malaria is effective, but must also be followed by primaquine. The same recommendation applies for mixed infections with *P. falciparum* or when the parasite identification is uncertain.

The therapeutic aspects of *P. knowlesi* malaria are not yet fully understood [122], in particular in travelers. Because severe evolution has been observed in endemic settings, experts recommend treating all cases of *P. knowlesi* malaria as they would do for severe malaria [14]. However, current evidence from endemic areas as well as from some imported cases suggests that chloroquine is a reasonable option for uncomplicated cases [91,93,123].

Leishmaniasis

Leishmaniasis is a diverse disease caused by more than 20 species of *Leishmania* that may be transmitted by approximately 30 species of phlebotomine sandflies (Table 1). Both parasite and host factors influence the clinical spectrum that may range from subclinical infection or localized self-healing skin lesions to disseminated diseases (cutaneous, mucosal or visceral). Atypical presentations are often seen in immunosuppressed individuals [124]. Leishmaniasis is widely distributed throughout the world and Epidemiology is complex [125]. Returning travelers present almost exclusively with cutaneous or mucocutaneous ('tegumentary') leishmaniasis, which is found in 3–4% of all patients consulting for skin disorders in travel clinics [5,126]. Mucosal leishmaniasis ('espundia') is not as frequent in travelers, and results mainly from an infection with *Leishmania (Viannia) braziliensis* complex in the New World (Table 1) [127–129]. In Europe, apart from the autochthonous cases seen in the Mediterranean Basin, few cases of imported visceral leishmaniasis are reported [130,131]. New diagnostic methods include immunochromatographic serology and antigenic tests designed for the field [132–134] and PCR-based assays developed in reference centers [135]. Treatment of cutaneous, mucocutaneous and visceral leishmaniasis (CL, ML and VL, respectively) has recently been reviewed [136,137].

Progress up to 2000

Pentavalent antimonial drugs (meglumine antimoniate and sodium stibogluconate) have been the mainstay of antileishmanial therapy since the 1940s. The mechanism of action is probably due to inhibition of parasite ATP synthesis. Pentavalent antimonials have been used for decades as first-line treatment both intralesionally for limited skin lesions in the Old World or parenterally for extensive CL, CL in the New World, ML and VL. Local infiltration of antimonials has long been considered as safe and efficacious in accelerating the cure of CL in the Old World. Cure rates of 70–90% have been observed for *Leishmania major* within 1 month and 75% for *Leishmania tropica* within 3 months [138], compared with time for spontaneous healing of >3 months and >6 months, respectively (Table 3) [138,139]. Systemic administration of antimonials has been associated with variable but satisfactory response rates (30–90%) in CL/ML in the New World [127,128,138]. For VL, after decades of excellent efficacy, failure to antimonials increased dramatically in the 1990s in Bihar,

India [137]. Antimonials have, however, always been rather difficult to use because of their parenteral administration and cumulative toxicity (including local and diffuse pain and discomfort, hypersensitivity, hepatitis, pancreatitis, electrocardiographic alterations and bone marrow depression). Amphotericin B (AmB) was first used in 1963 under a deoxycholate formulation with very good results but the risk of renal and electrolyte disturbances has promoted the development of less toxic lipid formulations (liposomal, colloidal or lipid complex), allowing delivery of similar doses over shorter periods. Cure rates of VL have reached 95% with AmB formulations [140,141], but experience for ML remained limited to small case series [128].

Several other drugs have demonstrated antileishmanial clinical efficacy in different studies, such as pentamidine (for VL and CL in French Guyana, Surinam and Brazil), fluconazole and ketoconazole (for CL in the Old and New World), parenteral paromomycin (for VL and CL/ML of the New World) and topical paromomycin for CL [137,142,143]. Various physical methods (surgery, thermotherapy, infrared light, laser light and radio-frequency waves) have also been investigated for limited CL lesions, mostly in the Old World [138]. Unfortunately, most studies were observational or poorly designed and did not systematically look for causative species; consequently, treatment recommendations have often relied on local experience rather than on strong evidence [136,137,143,144].

Before 2000, combination therapy had been occasionally investigated: the combination of antimonials and paromomycin (for 17 days) compared favorably with antimonials alone (for 30 days) for the treatment of VL in East Africa [137]; in contrast, the studies comparing the combination of antimonials with allopurinol to antimonials alone for CL/ML in the New World provided conflicting results [143].

Developments since 2000

The main therapeutic advances in the last decade have been the introduction of miltefosine as an oral agent against leishmaniasis and the further developments of paromomycin, AmB formulations, physical methods and combination therapies. Molecular techniques have also allowed a better insight into the relationship between specific species and treatment response [145,146]. On the other hand, the HIV pandemics have led to increasing numbers of leishmania–HIV-coinfected patients who require a more complex management [147].

Miltefosine is an oral drug (hexadecylphosphocholine) that interferes with cellular membrane lipid metabolism. It has a long terminal half-life of 31 days and remains detectable beyond 5 months in the plasma of patients treated for 1 month, raising concerns about development of resistance if used as monotherapy in endemic areas [148]. A long-term cure rate of 94% after 28 days of therapy was demonstrated for adult and children VL patients in India [149,150], as well as in HIV-noninfected patients in Northern Ethiopia [151]. Phase IV trials have confirmed these favorable results [152]. Miltefosine (Impavido® [AeternaZentaris Inc.]) has been registered as oral therapy for VL since 2002 in India and in Germany. Few studies have been reported on miltefosine therapy for tegumentary leishmaniasis. One RCT showed a cure rate of 81% at 3 months for *L. major* infection, similar to that of antimonial treatment [153]. A cure rate of 88% was demonstrated with miltefosine in 34 Dutch soldiers with proven *L. major* infection refractory to intralesional antimonials [154]. Miltefosine was also effective in a few cases of Old World CL with no species diagnosis [155]. Data on miltefosine efficacy are too limited for *L. tropica* and *Leishmania infantum* [138]. In Colombia, a cure rate of 81% at 6 months for CL (due to *Leishmania panamensis*) was obtained, while it was only 50% in CL due to *L. braziliensis* in Guatemala (vs 20% in the placebo arm). In Bolivia, however, treatment efficacy of miltefosine was 88% in *L. braziliensis*-related CL [156] and 83% in moderate *L. braziliensis*-related ML [157]. Of note, drug resistance to miltefosine has been induced experimentally [158] and clinical failure has been reported in Nepal [159].

Paromomycin (15 mg/kg intramuscularly for 21 days) was not inferior to AmB (cure rate of 95%) in Indian VL patients [160]. Shorter courses performed less well [161]. This paromomycin dosage did not perform as well, however, in East Africa, where higher dose regimens (20 mg/kg) were necessary for adequate efficacy [162]. For CL in the New World, both parenteral and topical paromomycin were inferior to systemic antimonials [142]. By contrast, topical paromomycin (15%) associated with methylbenzothenium chloride (12%) was superior to placebo and equivalent to intralesional antimonials for CL in the Old World [142]. An expensive ointment is available in Israel (Leshcutan® [TevaPharmaceutical Industries Ltd]).

Various regimens of conventional and liposomal AmB (L-AmB; AmBisome® [Gilead Sciences Inc.]) have been further evaluated and disclosed excellent cure rates for VL [163,164], but less so for CL and ML. Single-dose L-AmB showed similar efficacy (96%) as conventional AmB therapy in India [165,166]. Treatment failures were, however, more frequent in East Africa [167] and in HIV-coinfected patients [168].

Several physical methods have been developed for treating CL, among which radiofrequency thermotherapy and photodynamic therapy appeared the most promising when compared with classic treatments [144]. However, specific devices are often expensive and such therapies are limited to localized cutaneous lesions with no risk of mucosal dissemination.

Today antileishmanial combinations are being intensively explored [169,170]. In *L. braziliensis*-related infections, oral pentoxifylline associated with parenteral antimonials for 30 days has demonstrated a synergistic superiority to antimonials alone, particularly for ML cases [143]. Combining topical paromomycin–benzomethium or topical imiquinod cream (5%) with antimonials remained of unclear benefit for CL [143,144]. For VL, the superiority of the combination of antimonials with paromomycin over antimonials alone has been confirmed in India and East Africa [171,172]. In addition, single-dose L-AmB followed by 14 days of miltefosine showed a high efficacy in Indian VL [173]. Phase III trials with other short-course regimens (single-dose L-AmB with 7 days of miltefosine; single-dose L-AmB with 10 days of paromomycin; 10-day combination of miltefosine–paromomycin) are ongoing in Asia. Similar combinations are being explored by the DNDI in Phase II/III studies in East Africa

Table 3. Recommended treatment of leishmaniasis in travelers (cont.).

	Adults	Children	Pregnant women	Immunosuppressed patients
Old World cutaneous leishmaniasis				
L. major, *L. tropica*, *L. aethiopica*, *L. infantum*, *L. donovani*	• Small lesion with no cosmetic concern: observation (spontaneous healing within 2–6 months for *L. major* and 6–15 months for *L. tropica* and *L. infantum*) • Lesions limited in size and number: – Intralesional injections with either meglumine antimoniate or sodium stibogluconate 1–3 ml, one to three times (maximum ten times) with 2–7-day intervals – Topical paromomycin (15%) plus methylbenzethonium chloride (12%) twice daily for 10 days – Physical methods (thermotherapy or photodynamic therapy) according to local facilities – Fluconazole 200 mg p.o. for 6 weeks (if documented *L. major* infection); itraconazole 200 mg p.o. for 6 weeks (if documented *L. tropica* infection) • Multiple lesions or nonresponse to topical treatment: – Systemic treatment with pentavalent antimonials (meglumine antimoniate or sodium stibogluconate) 20 mg of Sb/kg/day iv. once daily for 10 days • Diffuse cutaneous leishmaniasis (*L. aethiopica*): see 'visceral leishmaniasis' treatment; place of miltefosine still unclear	Same as 'Adults' Antimonials contraindicated in infants Azoles not evaluated in children	Antimonials contraindicated Itraconazole contraindicated L-AmB (or AmB) if systemic treatment is required	Always consider systemic therapy (caution with antimonial toxicity; AmB formulations; possibly a role for miltefosine)
New World cutaneous & mucocutaneous leishmaniasis				
Cutaneous leishmaniasis due to species belonging to the *L. mexicana* complex	Spontaneous cure within 3–9 months for *L. mexicana* Topical paromomycin (15%) plus methylbenzethonium chloride (12%) twice daily for 10 days Intralesional pentavalent antimonials or systemic pentavalent antimonials (meglumine antimoniate or sodium stibogluconate 20 mg/kg/day for 10 days) Ketoconazole 600 mg/day for 28 days Physical methods and miltefosine (limited data)	Same as 'Adults' Antimonials contraindicated in infants Azoles not evaluated in children	Topical treatments L-AmB (or AmB) if systemic treatment is required Antimonials contraindicated Miltefosine contraindicated	Systemic treatment (caution with antimonial toxicity; AmB formulations)

AmB: Amphotericin B; im.: Intramuscular; iv.: Intravenous; L-AmB: Liposomal AmB; p.o.: Per oral; Sb: Sodium stibogluconate.
Data taken from [136–183].

Table 3. Recommended treatment of leishmaniasis in travelers (cont.).

	Adults	Children	Pregnant women	Immunosuppressed patients
New World cutaneous & mucocutaneous leishmaniasis (cont.)				
Mucocutaneous or cutaneous leishmaniasis due to species belonging to the *L.(Viannia)* subgenus, or unknown species, or diffuse cutaneous leishmaniasis	• Spontaneous healing within 6–15 months for cutaneous leishmaniasis due to *L. braziliensis* and *L. panamensis*) • In case of cutaneous leishmaniasis – Due to (confirmed) *L. braziliensis*: - Pentavalent antimonials (meglumine antimoniate or sodium stibogluconate) 20 mg/kg/day iv. once daily over 30–60 min for 20 days - Miltefosine 2.5 mg/kg/day p.o. once daily for 28 days (60–90% efficacy in Colombia and Bolivia; <50% in Guatemala) – Due to (confirmed) *L. panamensis*: - Systemic pentavalent antimonials as for *L. braziliensis* - Ketoconazole 600 mg/day for 28 days - Miltefosine 2.5 mg/kg/day once daily p.o. for 28 days – Due to (confirmed) *L. guyanensis*: - Pentamidine 7 mg/kg iv. slow infusion single dose (if localized lesion), or repeated after 2 days if multiple lesions; or 4 mg/kg iv. slow infusion every other day (three to four courses) - Systemic pentavalent antimonials as second choice in French Guyana, Surinam and Brazil and first choice in Peru • In case of mucocutaneous leishmaniasis (due to any species): – Pentavalent antimonials (dosage as here above) for 28 days plus pentoxifylline 400 mg twice daily p.o. for 28 days – Miltefosine 2.5 mg/kg/day p.o. once daily for 28 days (lower efficacy than in cutaneous leishmaniasis) – AmB 0.75 mg/kg/day for 15 days (quite toxic) or L-AmB 4 mg/kg/day on days 1–5 and on day 10 (6 days)	Same as 'Adults' Antimonials contraindicated in infants Azoles not evaluated in children	L-AmB (or AmB) as first choice Antimonials contraindicated Miltefosine contraindicated	Systemic treatment (caution with antimonial toxicity; AmB formulations)

AmB: Amphotericin B; im.: Intramuscular; iv.: Intravenous; L-AmB: Liposomal AmB; p.o.: Per oral; Sb: Sodium stibogluconate.
Data taken from [136–183].

Table 3. Recommended treatment of leishmaniasis in travelers (cont.).

	Adults	Children	Pregnant women	Immunosuppressed patients
Visceral leishmaniasis				
L. donovani, *L. infantum/chagasi*	Pentavalent antimonials (meglumine antimoniate or sodium stibogluconate) 20 mg/kg/day iv. once daily over 30–60 min for 28 days (not in case of exposure in India and Nepal) Miltefosine 150 mg/day p.o. once daily for 28 days AmB 0.75 mg/kg/day for 15 days, or L-AmB 4 mg/kg/day on days 1–5 and on day 10 (6 days) Paromomycin 20 mg/kg iv. or im. for 21 days (only licensed in India) Consider combination therapies only for Indian visceral leishmaniasis: single-dose L-AmB followed by miltefosine 150 mg/day for 14 days; also antimonials plus paromomycine for 17 days	Systemic antimonials contraindicated in infants Miltefosine 2.5 mg/kg/day for 28 days	L-AmB (or AmB) as first choice Antimonials contraindicated Miltefosine contraindicated	Longer course of L-AmB recommended (10 doses in total: day 1–5, 10, 17, 24, 31 and 38) Secondary prophylaxis with L-AmB 4 mg/kg every 3 weeks Antimonials very toxic Consider combination therapy (L-AmB plus miltefosine, paromomycin or pentamidine) if relapse

AmB: Amphotericin B; im.: Intramuscular; iv.: Intravenous; L-AmB: Liposomal AmB; p.o.: Per oral; Sb: Sodium stibogluconate.
Data taken from [136–183].

and Brazil [170,174]. If the efficacy of combination therapies is demonstrated in other settings than India, it may become the standard of care for VL.

Treatment recommendations for travelers

Cutaneous & mucocutaneous leishmaniasis

For CL acquired in the Old World, intralesional injections of antimonials, topical paromomycin–methylbenzethonium, or physical methods may be recommended when lesions are limited in number and size (Table 3) [138,175]. For large and multiple CL lesions or CL nonresponsive to local treatment, parenteral antimonials should be offered. Physicians in travel clinics have a wide experience with these drugs and can adequately manage the side effects, although great caution is required in HIV-coinfected patients [147]. Oral therapy with fluconazole is a good option in case of *L. major* infection. Most treatment recommendations, however, rely on rather weak evidence [144,176].

In CL from the New World, subgenus- or species-specific PCR investigation of dermal scrapings is required to identify *L. (Viannia) braziliensis* complex for which a systemic treatment is required (Table 3). Once *L. (Viannia) braziliensis* has been excluded, various recommendations exist for travelers [138,175], but again few of them are based on strong RCT evidence. For example, only oral ketoconazole, oral miltefosine and topical paromomycin–methylbenzethonium were demonstrated to be more effective than placebo for CL due to *L. panamensis* [143]. Many other classic treatment protocols have not been evaluated against placebo or the reference antimonials in adequate RCTs, and rely on local observational studies [177,178].

Visceral leishmaniasis

The treatment of choice for VL in travelers should be L-AmB (4 mg/kg/day on day 1–5 and on day 10) owing to its high efficacy and low rate of side effects. This is also the only safe treatment in pregnant women [179], in HIV-coinfected patients [147] and in transplant recipients [180]. However, antimonials or conventional AmB often remain the first-line therapy in specialized travel clinics because of longer experience and much lower costs (Table 3). Miltefosine appears as a good alternative where available and affordable. However, tolerance is limited by gastrointestinal discomfort (in up to 50% of the patients); serious side effects such as liver enzyme or creatinin elevations are not infrequent [154], leading to treatment discontinuation in approximately 3% of the cases [152]. Miltefosine is abortive and teratogenic in rats [181]. Women of childbearing age should therefore take an efficient contraception up to 5 months after treatment.

In HIV-coinfected patients and in transplant recipients, treatment-limiting toxicity is frequent with systemic antimonials (>30%) [147,180]. In addition, risk of relapse is particularly high in HIV-coinfected individuals, with any type of antileishmanial therapy [147,182]. Longer courses of L-AmB are recommended (up to ten doses within 5 weeks) and various combination treatments are suggested in case of relapse. Although robust clinical data are scarce, maintenance therapy (secondary prophylaxis) with L-AmB seems to reduce the risk of relapse, at least until antiretroviral therapy has restored adequate immunity [147,183].

Other less studied drugs such as paromomycin or pentamidine should be reserved as second-line options in travel medicine. The place of combination therapy is not yet defined in nonendemic settings, but some single-dose L-AmB-based regimens could become very attractive if their positive results are confirmed in other countries than India, in particular for the difficult-to-treat group of immunosuppressed travelers.

Trypanosomiasis

Human African trypanosomiasis (sleeping sickness)

Human African trypanosomiasis (HAT) is caused by two subspecies of *Trypanosoma brucei: Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, transmitted by tsetse flies of the genus *Glossina*. Both infections have distinct epidemiological and clinical characteristics (Table 1) but result in CNS complications invariably fatal if left untreated [184,185]. Diagnosis of *T. b. gambiense* HAT has improved in the field with the combination of serological screening and parasitological confirmatory tests, while *T. b. rhodesiense* detection still relies on classic microscopy [186]. Diagnosis of the second (late) meningoencephalitic stage is based on cerebrospinal fluid (CSF) examination (Table 1) [187]. Staging is, however, not always clear-cut and some borderline cases could benefit from early and specific markers of CNS involvement [188].

The disease affects almost exclusively remote rural areas in 24 countries in sub-Saharan Africa and approximately 95% of the reported cases are due to *T. b. gambiense* [3]. A few cases are diagnosed yearly outside Africa in returning travelers or migrants, and most of these are due to *T. b. rhodesiense*, which is endemic in popular east African game reserves [189,190]. Pathogen differentiation is classically based on the place of exposure, but this may be difficult in case of stay in Uganda, where distribution of both trypanosomes tends to overlap and in case of travel to multiple endemic areas. Molecular techniques for species differentiation are available in only a few reference settings [190,191].

Progress up to 2000

For more than 50 years, selection of therapy has relied on the recognition of the causative trypanosome and on the disease staging [192]. Two drugs have been used for decades to treat the first (early) hematolymphatic stage of the disease: pentamidine and suramine (Table 3). Pentamidine has long been the drug of choice for first-stage infection due to *T. b. gambiense*, because it was rather well tolerated and remained remarkably effective (cure rate of 93%) despite decades of widespread use throughout Africa [193]. Suramin has been indicated mostly for the treatment of first-stage infection due to *T. b. rhodesiense*, which is less sensitive to pentamidine. Suramin is also highly effective against *T. b. gambiense* but risk of severe allergic reactions has limited its use.

For second-stage disease, melarsoprol was the only drug widely used for more than 50 years for both trypanosomes. It has an excellent trypanocidal activity, compensating its rather low CNS penetration. Regimens, initially long and complex, have been progressively simplified to a 10-day course, following the results of the Improved Application of Melarsoprol (IMPAMEL) trials I and II [194,195]. However, toxicity has remained a major issue, in particular with regards to the encephalopathic syndrome occurring in up to 10% of the treated patients and associated with almost 50% mortality [196], and which could only be partly prevented by coadministration of prednisolone [197]. Cardiac rhythmic disorders, probably inflammatory mediated and therefore potentially treatable with corticosteroids, are also frequent [198] as well as other severe adverse events such as peripheral neuropathy, skin reactions, hepatitis or thrombophlebitis at injection sites. In addition, melarsoprol failure was increasingly reported in patients with *T. b. gambiense* HAT in the 1990s, although no parasitic drug resistance has been formally demonstrated to date.

A second drug, the polyamine synthesis inhibitor eflornithine, was developed in the late 1980s. Eflornithine was as effective as melarsoprol against *T. b. gambiense* but less so against *T. b. rhodesiense* [199]. By contrast, its toxicity was much more acceptable, with anemia and leukopenia as the most frequent side effects [200]. Unfortunately, efficacy of oral eflornithine was too limited for second-stage *T. b. gambiense* trypanosomiasis, leaving the expensive, cumbersome, four-times daily 2-week intravenous regimen as a unique alternative to melarsoprol [201]. For this reason, it was kept in rural Africa as a second-choice therapy for late-stage *T. b. gambiense* disease relapsing after melarsoprol. Shorter courses of eflornithine were clearly inferior [202].

Developments since 2000

A trial comparing a 3-day course of pentamidine to the classic 7-day regimen in the treatment of early-stage *T. b. gambiense* HAT has recently been conducted and results are pending [193,302]. For the late stage, the toxicity of melarsoprol and the increasing rate of treatment failure (up to 50% in some settings) renewed international efforts to find alternative effective regimens [203]. Nifurtimox, an anti-*Trypanosoma cruzi* agent, did not demonstrate sufficient activity in monotherapy against second-stage *T. b. gambiense* HAT but, when combined with melarsoprol, was superior to melarsoprol monotherapy [204]. Toxicity of melarsoprol was, however, still unacceptable [205]. Several observational studies meanwhile suggested that eflornithine alone or combined with nifurtimox was safer and more effective than melarsorpol [200,206,207]. In 2009, the Nifurtimox–Eflornithine Combination Therapy (NECT) randomized trial demonstrated that the combination of a 10-day course of oral nifurtimox with a 7-day, twice-daily, intravenous course of eflornithine was not inferior to eflornithine monotherapy (cure rate above 95%) and caused two times less major adverse events (14 vs 29%; p = 0.002) [208]. NECT has been included in the WHO Essential List of Medicines for the treatment of second-stage *T. b. gambiense* trypanosomiasis. A Phase IV study conducted by the DNDI is ongoing [303].

For the treatment of *T. b. rhodesiense*, a recent systematic review did not identify any available RCT to provide guidance [209]. Old drugs with complex administration schedules are still in use. The applicability of an abridged 10-day melarsoprol schedule for second-stage *T. b. rhodesiense* HAT is being explored in Uganda and Tanzania in a nonrandomized trial (IMPAMEL III) owing to the difficulty in recruiting enough patients, and preliminary results suggest similar safety and efficacy as that obtained in historical series [193].

Table 4. Recommended treatment of trypanosomiasis in travelers.

Disease stage	Adults	Children	Pregnant women	Immunosuppressed patients
Trypanosoma brucei gambiense				
First	Pentamidine 4 mg/kg/day im. (or slow iv.) once daily for 7 days Eflornithine 100 mg/kg four times a day (slow infusion >30 min) for 14 days	Pentamidine 4 mg/kg/day im. (or slow iv.) once daily for 7 days Eflornithine 150 mg/kg four times a day for 14 days	Pentamidine 4 mg/kg/day im. (or slow iv.) once daily for 7 days Eflornithine 100 mg/kg four times a day (slow infusion) for 14 days	Same as 'Adults'
Second	NECT: eflornithine 200 mg/kg twice daily (slow infusion) for 7 days combined with nifurtimox p.o. 15 mg/kg/day divided into three doses for 10 days Eflornithine 100 mg/kg four times a day (slow infusion) for 14 days	Eflornithine 150 mg/kg four times a day for 14 days	Eflornithine 100 mg/kg four times a day (slow infusion) for 14 days (nifurtimox contraindicated)	Same as 'Adults'
Trypanosoma brucei rhodesiense				
First	Suramin: test dose of 100 mg iv. on day 1 followed by 1 g on days 1, 3, 6, 14 and 21 (or 1 g once a week for 5 weeks)	Suramin 0.5 mg/kg test dose iv. on day 1 followed by 20 mg/kg on days 1, 3, 6, 14 and 21 (or once a week for 5 weeks)	Same as 'Adults'	Same as 'Adults'
Second	Suramin (as above) plus melarsoprol three series of one injection of 3.6 mg/kg/day for 3 days with an interval of 7 days between each series Consider also the abridged melarsoprol schema: 2.2 mg/kg/day for 10 days (pending the IMPAMEL III results) plus prednisolone	Suramin (as above) plus melarsoprol three series of one injection of 3.6 mg/kg/day for 3 days with an interval of 7 days between each series (consider also the abridged melarsoprol schema: 2.2 mg/kg/day for 10 days) plus prednisolone	Same as 'Adults'	Same as 'Adults'
Trypanosoma cruzi				
Acute or chronic phase	Benznidazole 5 mg/kg/day divided into three doses for 60 days (some national programs recommend 30 days) Nifurtimox 8–10 mg/kg/day divided into three doses for 60 days	Benznidazole 7.5 mg/kg/day divided into three doses for 60 days (some national programs recommend 30 days) Nifurtimox 15 mg/kg/day divided into three doses for 60 days	Both drugs are contraindicated Balance risk–benefit for the pregnant women with acute disease	Same as 'Adults' in case of reactivation Consider secondary prophylaxis with benznidazole 5 mg/kg three times a week

im.: Intramuscular; IMPAMEL: Improved Application of Melarsoprol Trial; iv.: Intravenous; NECT: Nifurtimox–Eflornithine Combination Therapy trial; p.o.: Per oral.
Data taken from [2,3,192–197,208,209,224–247].

Of note, it has recently been demonstrated that the post-treatment follow-up could be simplified to two lumbar punctures maximum and shortened to 1 year maximum, instead of 2 years and four to five lumbar punctures previously. Useful algorithms based on CSF examination have been proposed to identify definitive cure or treatment failures early [210–212]. In addition, novel biological predictors for treatment failure outcome have been developed [210].

Treatment recommendations for travelers

Recommendations in travelers are similar to those in endemic settings (Table 4). Side effects of pentamidine mainly consist of pain and swelling at the injection site, abdominal discomfort, glucose instability and diabetes. For suramine, a test dose of 100 mg should be given under monitoring, the first day before full administration of the total dose. Adverse reactions are frequent but reversible and include, besides hypersensitivity, nephropathy, peripheral neuropathy and bone marrow toxicity. For second stage *T. b. gambiense* HAT, NECT should now be the standard of care in nonendemic settings as well. Children require a higher dosage of eflornithine because of increased renal clearance. In pregnant women, eflornithine should be administered alone since nifurtimox is mutagenic. Treatment in immunosuppressed patients has never been specifically studied. Although highly toxic, melarsoprol (in combination with steroids) remains the first choice therapy for second stage *T. b. rhodesiense* infection.

American trypanosomiasis (Chagas disease)

American trypanosomiasis, or Chagas disease, is caused by the protozoan *T. cruzi*, transmitted by triatomine bugs. The disease is characterized by an acute stage, predominantly asymptomatic, and a chronic stage where cardiac and digestive complications may progressively develop. Chagas disease was originally confined to poor and rural areas of Central and South America, where multisector efforts have dramatically impacted on prevalence and incidence for 30 years. However, rural exodus and migration have brought many Chagas patients to Latin American cities and to nonendemic countries, where the infection is increasingly being diagnosed [2,213]. Transmission of Chagas disease in nonendemic countries has emerged since the beginning of 2000, mainly in North America [214] and Europe [215,216], with congenital transmission [217] and blood or organ donation [218,219] as the main routes of transmission. The large majority of *T. cruzi* infections in nonendemic countries, however, go unrecognized [220,221]. Chagas disease has been rarely reported in returning short-term travelers [222,223]. Diagnosis has long relied upon blood microscopy for acute stages, and on xenodiagnosis for chronic infection (Table 1). Serology is now widely used for diagnosing chronic Chagas. Serological diagnostic tests currently in use have recently been thoroughly reviewed [2] and reported to be appropriate for use in nonendemic settings [216].

Progress up to 2000

Antiparasitic treatment, also termed etiological treatment, of Chagas disease is limited to two drugs discovered approximately 40 years ago, with similar trypanocidal activity: benznidazole, a nitroimidazole, and nifurtimox, a 5-nitrofuran derivative (Table 3). Rapid eradication of parasites could be demonstrated when the treatment was administered in the acute phase (60–85% cure rate), in the congenital forms (90% cure rate) and during immune depression-related disease reactivation (case series); treatment has long been indicated for such cases [224]. Treatment of children (18 years of age and younger) with early chronic infection has also been widely recommended, since two RCTs have shown rates of negative *T. cruzi* seroconversion reaching 60% (vs less than 5% in the placebo group) [225,226] One clinical study demonstrated less cardiac deterioration in such children given benznidazole than in untreated controls [227]. A meta-analysis further confirmed that benznidazole treatment was effective in improving parasitic-related outcomes, but also highlighted that no definitive conclusions could be drawn for relevant clinical outcome [228]. For older patients with chronic Chagas infection, etiological treatment remained controversial because all studies had serious limitations (nonrandomized design, nonblinded, inadequate power and short follow-up) [229]. The major problem faced by researchers has always been the absence of early markers of a parasitological cure in chronic stages and the need to rely on serology with a very long delay before negative seroconversion [2,213].

Developments since 2000

The management of Chagas disease has evolved in the last decade mainly thanks to advances in the laboratory. Ease-of-use serological screening tests have been developed [230]. Molecular techniques have been standardized [231], and have significantly improved the early diagnosis of congenital Chagas infection [232,233]. Progress in molecular techniques has allowed confirmation of persistent *T. cruzi* infection in the tissues of patients chronically infected and have underlined the need for parasite elimination at any moment of disease evolution [234,235]. New surrogate biomarkers of parasitic presence (e.g., IFN-γ-secreting T cells specific for *T. cruzi*) have suggested that benznidazole also has some efficacy in chronic stages [236], even if inferior than in acute forms [237]. Finally, a large but nonrandomized and nonblinded clinical study in 2006 has shown a significant reduction of progression to cardiac disease in adult patients treated with benznidazole (4 vs 14% for untreated controls) [238]. There is a consensus to etiologically treat all children and young adults diagnosed with Chagas to prevent cardiac complications. Although not yet fully conclusive, the aforementioned indirect arguments support a more liberal use of benznidazole treatment for chronic infections in older adults as well, taking into account that its side effects are manageable (see later: treatment recommendations in travelers) [239]. There is therefore a growing acceptance that etiological treatment should be offered to any (treatment-naive) patient fulfilling serological criteria for *T. cruzi* infection [2,213,224,234,235,240]. The question should be definitively answered in 2012 with the results of the Benznidazole Evaluation for Interrupting Trypanosomiasis study, a RCT evaluating (since 2004) the preventive effect of benznidazole on the clinical progression of early Chagas cardiopathy

in more than 3000 patients aged 18–75 years [241]. Efficacy of alternative therapeutic drugs such as allopurinol, itraconazole or fluconazole has been inconsistent and nonreproducible to date [228,242,243]. Combination of nifurtimox and benznidazole has not yet been studied [244].

Treatment recommendations in travelers

Therapeutic guidelines for congenital, acute, chronic and reactivated Chagas disease in nonendemic countries are similar to those in endemic countries (Table 4) [245]. Both nifurtimox and benznidazole are administered for 2 months and are poorly tolerated. Digestive and CNS disturbances were reported in up to 50% and led to treatment interruption in approximately 30% of patients treated with nifurtimox in endemic settings [234,243]. In a recent observational study in Switzerland, almost all 73 patients treated with a 60-day course of nifurtimox had some adverse events, mostly gastrointestinal or neurological [246]. Most side effects occurred during the first month and some were life-threatening. Approximately 40% of patients did not complete their treatment. Benznidazole has a better tolerance profile, with a low rate of severe adverse events compared with nifurtimox. In large series, however, up to one third of the patients present with side effects (mainly hypersensitivity, digestive intolerance, peripheral polyneuritis and bone marrow suppression), and approximately 15% of the patients had to discontinue their therapy [224,239]. A 30-day course of benznidazole is recommended by some experts and national programs, for example, in Argentina; in observational studies using a 30-day regimen, efficacy appeared similar (disappearance of parasites assessed by serial xenodiagnosis), but with less neuropathic and bone marrow toxicity compared with cohorts on a longer course [227,238,239]. However, no RCTs comparing 30-day and 60-day treatments have been conducted to date. Tolerability is less of a problem in children. Presently, no pediatric formulation is available, but dispersible pediatric tablets are being investigated by the DNDI and should be delivered in Brazil in 2011.

Both antiparasitic drugs are mutagenic [224] and are contraindicated in pregnancy, at least to treat asymptomatic infection. Immunosuppressed patients with Chagas reactivation should be treated as acute cases and secondary prophylaxis with benznidazole has been suggested [2,247]. The WHO can be contacted for drug delivery in Europe (and CDC in the USA).

Expert commentary & five-year view

Global Epidemiology of vector-borne protozoan infections will continue to depend on the multiple interactions between pathogens in endemic countries and mobile populations. In nonendemic settings, the past 10 years has witnessed substantial improvements in clinical awareness, diagnostic facilities and treatment optimization.

For malaria, clinical practice in travel medicine has been revolutionized by two potent oral FDCs (AP and AL) and by intravenous artesunate. This should not lead to complacency, however: efficacy of AP may be jeopardized by single point mutations, while partial resistance to artemisinin has emerged. All Phase II and III studies of antimalarial treatment are for innovative artemisinin-based therapies, but there is no new drug to be expected in the coming decade [37]. Development of the peroxide compound arterolane has been discontinued before Phase III trials because of instability in blood and more stable molecules like trioxaquine have not yet entered Phase I trials. The only new promising class of drug, spiroindolones, is at its earliest stage of development [248].

For leishmaniasis, therapy has benefited from the development of miltefosine and new AmB formulations, and the renewed interest for an old drug such as paromomycin. Optimized short course combinations of existing drugs are the most near-term promising advances for severe leishmaniasis. Sitamiquine, a promising oral 8-aminoquinoline, has not reached further development. A few other drugs are only in preclinical development (buparvaquone, alternative AmB formulations and 8-aminoquinolines). There is also a desperate need to improve evidence-based strategies for ML and CL by well-designed multicenter trials [142,143,249].

For HAT, the combination eflornithine–nifurtimox has been the only therapeutic breakthrough for second-stage *T. b. gambiense* infection. Other drugs have seen their development stopped such as pafuramidine (DB289) owing to unexpected nephrotoxicity [193]. Only a nitroimidazole, fexinidazole, has entered into a 2009 Phase I trial conducted by the DNDI [193,250,304].

For American trypanosomiasis, no new treatment has been developed. However, the third-generation triazole derivatives have displayed potent curative activity against *T. cruzi*. A Phase II trial with posaconazole is planned in Spain in 2011; the less expensive prodrug of ravuconazole (E12-34) has entered a Phase II trial in Bolivia. Among other explored molecules, a drug targeting the major cysteine protease (K777) will soon enter a Phase I safety trial [245,251].

In the near future, combination therapy for protozoan diseases should be further explored in order to obtain synergistic efficacy, reduced (dose-dependant) toxicity and less parasite resistance. Further molecular development should allow improved monitoring of parasitological outcome and accelerate efficacy measures in clinical trials. Finally, international networks of travel physicians may contribute greatly to the global control of these poverty-related conditions, through monitoring epidemiological changes, standardizing evidence-based disease management and exploring adequately unresolved diagnostic and therapeutic issues.

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Key issues

- Vector-borne protozoan infections cause major mortality and morbidity in tropical and subtropical areas, and are increasingly diagnosed in returning travelers and migrants in nonendemic settings.
- Malaria causes, by far, the highest burden of protozoan disease in travel medicine.
- Introduction of fixed-dose combination therapies such as atovaquone–proguanil and artemether–lumefantrine has substantially improved the management of uncomplicated drug-resistant *Plasmodium falciparum* malaria.
- Intravenous quinine is being supplanted by artesunate as first-choice therapy for severe malaria in nonimmune travelers of any age.
- Miltefosine is the first oral therapy of visceral leishmaniasis. Its use in cutaneous and mucocutaneous leishmaniais remains to be defined.
- Several short course combination regimens based on amphotericin B formulations, paromomycin or miltefosine are being evaluated for visceral leishmaniasis.
- Large well-conducted studies are urgently needed to adequately evaluate the current treatment strategies for cutaneous and mucocutaneous leishmaniasis.
- The combination of eflornithine and nifurtimox has become the treatment of choice for second-stage *Trypanosoma brucei gambiense* infection.
- Benznidazole should be offered to any patient found seropositive for *Trypanosoma cruzi*, while waiting for definitive proof of clinical benefit in patients with chronic Chagas infection.
- Optimized combination therapies will most likely be the only achievable improvements for treatment of vector-borne protozoan diseases in the near future.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Hay SI, Guerra CA, Gething PW *et al.* A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Med.* 6(3), e1000048 (2009).

2 Lescure FX, Le Loup G, Freilij H *et al.* Chagas disease: changes in knowledge and management. *Lancet Infect. Dis.* 10(8), 556–570 (2010).

• **Comprehensive and detailed review of all recent advances in knowledge and management of Chagas disease.**

3 Brun R, Blum J, Chappuis F, Burri C. Human African trypanosomiasis. *Lancet* 375(9709), 148–159 (2010).

• **Excellent update on epidemiological, clinical, diagnostic and therapeutic aspects of human African trypanosomiasis.**

4 Bottieau E, Clerinx J, Schrooten W *et al.* Etiology and outcome of fever after a stay in the tropics. *Arch. Intern. Med.* 166(15), 1642–1648 (2006).

5 Freedman DO, Weld LH, Kozarsky PE *et al.* Spectrum of disease and relation to place of exposure among ill returned travelers. *N. Engl. J. Med.* 354(2), 119–130 (2006).

•• **Global large-scale study investigating the burden and spectrum of illnesses in patients presenting in travel clinics.**

6 Hotez PJ, Molyneux DH, Fenwick A *et al.* Control of neglected tropical diseases. *N. Engl. J. Med.* 357(10), 1018–1027 (2007).

7 Moran M. A breakthrough in R&D for neglected diseases: new ways to get the drugs we need. *PLoS Med.* 2(9), e302 (2005).

8 Muhlberger N, Jelinek T, Behrens RH *et al.* Age as a risk factor for severe manifestations and fatal outcome of falciparum malaria in European patients: observations from TropNetEurop and SIMPID Surveillance Data. *Clin. Infect. Dis.* 36(8), 990–995 (2003).

9 Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. *Lancet* 365(9469), 1487–1498 (2005).

10 Singh B, Kim SL, Matusop A *et al.* A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet* 363(9414), 1017–1024 (2004).

11 World Health Organization. Guidelines for the Treatment of Malaria, Second Edition. World Health Organization, Geneva, Switzerland (2010).

• **The current international reference in malaria treatment.**

12 Genton B, D'Acremont V, Rare L *et al.* *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. *PLoS Med.* 5(6), e127 (2008).

13 Price RN, Tjitra E, Guerra CA *et al.* Vivax malaria: neglected and not benign. *Am. J. Trop. Med. Hyg.* 77(Suppl. 6), 79–87 (2007).

14 Cox-Singh J, Davis TM, Lee KS *et al.* *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clin. Infect. Dis.* 46(2), 165–171 (2008).

15 Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 434(7030), 214–217 (2005).

16 Mali S, Steele S, Slutsker L, Arguin PM. Malaria surveillance – United States, 2008. *MMWR Surveill. Summ.* 59(7), 1–15 (2010).

17 Jelinek T, Schulte C, Behrens R *et al.* Imported falciparum malaria in Europe: sentinel surveillance data from the European network on surveillance of imported infectious diseases. *Clin. Infect. Dis.* 34(5), 572–576 (2002).

18 Wilson ME, Weld LH, Boggild A *et al.* Fever in returned travelers: results from the GeoSentinel Surveillance Network. *Clin. Infect. Dis.* 44(12), 1560–1568 (2007).

19 Bruneel F, Tubach F, Corne P *et al.* Severe imported falciparum malaria: a cohort study in 400 critically ill adults. *PLoS ONE* 5(10), e13236 (2010).

20 Hawkes M, Kain KC. Advances in malaria diagnosis. *Expert Rev. Anti Infect. Ther.* 5(3), 485–495 (2007).

21 Marx A, Pewsner D, Egger M *et al.* Meta-analysis: accuracy of rapid tests for malaria in travelers returning from endemic areas. *Ann. Intern. Med.* 142(10), 836–846 (2005).

22 Van der Palen M, Gillet P, Bottieau E *et al.* Test characteristics of two rapid antigen detection tests (SD FK50 and SD FK60) for the diagnosis of malaria in returned travellers. *Malar. J.* 8, 90 (2009).

▶23 Baird JK. Effectiveness of antimalarial drugs. *N. Engl. J. Med.* 352(15), 1565–1577 (2005).

- **Detailed review on all therapeutic aspects of malaria.**

▶24 White NJ. The treatment of malaria. *N. Engl. J. Med.* 335(11), 800–806 (1996).

▶25 White NJ, Olliaro PL. Strategies for the prevention of antimalarial drug resistance: rationale for combination chemotherapy for malaria. *Parasitol. Today* 12(10), 399–401 (1996).

▶26 Brockman A, Price RN, van Vught M *et al. Plasmodium falciparum* antimalarial drug susceptibility on the north-western border of Thailand during five years of extensive use of artesunate–mefloquine. *Trans. R. Soc. Trop. Med. Hyg.* 94(5), 537–544 (2000).

▶27 Patel SN, Kain KC. Atovaquone/proguanil for the prophylaxis and treatment of malaria. *Expert Rev. Anti Infect. Ther.* 3(6), 849–861 (2005).

▶28 Bouchaud O, Monlun E, Muanza K *et al.* Atovaquone plus proguanil versus halofantrine for the treatment of imported acute uncomplicated *Plasmodium falciparum* malaria in non-immune adults: a randomized comparative trial. *Am. J. Trop. Med. Hyg.* 63(5–6), 274–279 (2000).

▶29 Bottieau E, Clerinx J, Colebunders R *et al.* Selective ambulatory management of imported falciparum malaria: a 5-year prospective study. *Eur. J. Clin. Microbiol. Infect. Dis.* 26(3), 181–188 (2007).

- **Observational study demonstrating that ambulatory treatment of uncomplicated falciparum malaria may be safe.**

▶30 Thybo S, Gjorup I, Ronn AM, Meyrowitsch D, Bygberg IC. Atovaquone–proguanil (malarone): an effective treatment for uncomplicated *Plasmodium falciparum* malaria in travelers from Denmark. *J. Travel. Med.* 11(4), 220–223 (2004).

▶31 Chiodini PL, Conlon CP, Hutchinson DB *et al.* Evaluation of atovaquone in the treatment of patients with uncomplicated *Plasmodium falciparum* malaria. *J. Antimicrob. Chemother.* 36(6), 1073–1078 (1995).

▶32 Fivelman QL, Butcher GA, Adagu IS, Warhurst DC, Pasvol G. Malarone treatment failure and *in vitro* confirmation of resistance of *Plasmodium falciparum* isolate from Lagos, Nigeria. *Malar. J.* 1, 1 (2002).

▶33 David KP, Alifrangis M, Salanti A, Vestergaard LS, Ronn A, Bygbjerg IB. Atovaquone/proguanil resistance in Africa: a case report. *Scand. J. Infect.Dis.* 35(11–12), 897–898 (2003).

▶34 Farnert A, Lindberg J, Gil P *et al.* Evidence of *Plasmodium falciparum* malaria resistant to atovaquone and proguanil hydrochloride: case reports. *Br. Med. J.* 326(7390), 628–629 (2003).

▶35 Kuhn S, Gill MJ, Kain KC. Emergence of atovaquone–proguanil resistance during treatment of *Plasmodium falciparum* malaria acquired by a non-immune north American traveller to west Africa. *Am. J. Trop. Med. Hyg.* 72(4), 407–409 (2005).

▶36 Schwobel B, Alifrangis M, Salanti A, Jelinek T. Different mutation patterns of atovaquone resistance to *Plasmodium falciparum in vitro* and *in vivo*: rapid detection of codon 268 polymorphisms in the cytochrome b as potential *in vivo* resistance marker. *Malar. J.* 2, 5 (2003).

▶37 Wichmann O, Muehlen M, Gruss H, Mockenhaupt FP, Suttorp N, Jelinek T. Malarone treatment failure not associated with previously described mutations in the cytochrome b gene. *Malar. J.* 3, 14 (2004).

▶38 Musset L, Bouchaud O, Matheron S, Massias L, Le Bras J. Clinical atovaquone–proguanil resistance of *Plasmodium falciparum* associated with cytochrome b codon 268 mutations. *Microbes Infect.* 8, 2599–2604 (2006).

▶39 Sutherland CJ, Laundy M, Price N *et al.* Mutations in the *Plasmodium falciparum* cytochrome b gene are associated with delayed parasite recrudescence in malaria patients treated with atovaquone–proguanil. *Malar. J.* 7, 240 (2008).

▶40 Schwartz E, Bujanover S, Kain KC. Genetic confirmation of atovaquone–proguanil-resistant *Plasmodium falciparum* malaria acquired by a nonimmune traveler to East Africa. *Clin. Infect. Dis.* 37(3), 450–451 (2003).

▶41 Rose GW, Suh KN, Kain KC, Le Saux N, McCarthy AE. Atovaquone–proguanil resistance in imported falciparum malaria in a young child. *Pediatr. Infect. Dis. J.* 27(6), 567–569 (2008).

▶42 Savini H, Bogreau H, Bertaux L *et al.* First case of emergence of atovaquone–proguanil resistance in *Plasmodium falciparum* during treatment in a traveler in Comoros. *Antimicrob. Agents Chemother.* 52(6), 2283–2284 (2008).

▶43 Legrand E, Demar M, Volney B *et al.* A first case of *Plasmodium falciparum* atovaquone–proguanil treatment failure in French Guiana. *Antimicrob. Agents Chemother.* 51(6), 2280–2281 (2007).

▶44 Perry TL, Pandey P, Grant JM, Kain KC. Severe atovaquone-resistant *Plasmodium falciparum* malaria in a Canadian traveller returned from the Indian subcontinent. *Open Med.* 3(1), e10–e16 (2009).

▶45 Korsinczky M, Chen N, Kotecka B, Saul A, Rieckmann K, Cheng Q. Mutations in *Plasmodium falciparum* cytochrome b that are associated with atovaquone resistance are located at a putative drug-binding site. *Antimicrob. Agents Chemother.* 44(8), 2100–2108 (2000).

▶46 Wichmann O, Muehlberger N, Jelinek T *et al.* Screening for mutations related to atovaquone/proguanil resistance in treatment failures and other imported isolates of *Plasmodium falciparum* in Europe. *J. Infect. Dis.* 190(9), 1541–1546 (2004).

▶47 Happi CT, Gbotosho GO, Folarin OA *et al.* Confirmation of emergence of mutations associated with atovaquone–proguanil resistance in unexposed *Plasmodium falciparum* isolates from Africa. *Malar. J.* 5, 82 (2006).

▶48 Musset L, Pradines B, Parzy D, Durand R, Bigot P, Le Bras J. Apparent absence of atovaquone/proguanil resistance in 477 *Plasmodium falciparum* isolates from untreated French travellers. *J. Antimicrob. Chemother.* 57 (1), 110–115 (2006).

▶49 Ekala MT, Khim N, Legrand E *et al.* Sequence analysis of *Plasmodium falciparum* cytochrome b in multiple geographic sites. *Malar. J.* 6, 164 (2007).

▶50 Parola P, Pradines B, Simon F *et al.* Antimalarial drug susceptibility and point mutations associated with drug resistance in 248 *Plasmodium falciparum* isolates imported from Comoros to Marseille, France in 2004–2006. *Am. J. Trop. Med. Hyg.* 77(3), 431–437 (2007).

▶51 Legrand E, Volney B, Meynard JB, Mercereau-Puijalon O, Esterre P. *In vitro* Monitoring of *Plasmodium falciparum* rug esistance in French Guiana: a synopsis of continuous assessment from 1994 to 2005. *Antimicrob. Agents Chemother.* 52(1), 288–298 (2008).

▶52 Taylor WR, White NJ. Antimalarial drug toxicity: a review. *Drug Saf.* 27(1), 25–61 (2004).

▶53 White NJ. Cardiotoxicity of antimalarial drugs. *Lancet Infect. Dis.* 7(8), 549–558 (2007).

54 White NJ. Qinghaosu (artemisinin): the price of success. *Science* 320(5874), 330–334 (2008).

•• **Excellent and clear review of the antimalarial activity of artemisinin derivatives and challenges ahead.**

55 White NJ. Antimalarial drug resistance. *J. Clin. Invest.* 113(8), 1084–1092 (2004).

56 Nosten F, White NJ. Artemisinin-based combination treatment of falciparum malaria. *Am. J. Trop. Med. Hyg.* 77(Suppl. 6), 181–192 (2007).

57 D'Alessandro U. Existing antimalarial agents and malaria-treatment strategies. *Expert Opin. Pharmacother.* 10(8), 1291–1306 (2009).

• **Excellent review on antimalarial agents and strategies, in particular artemisinin-based combinations.**

58 Wernsdorfer WH. Coartemether (artemether and lumefantrine): an oral antimalarial drug. *Expert Rev. Anti Infect. Ther.* 2(2), 181–196 (2004).

59 Sinclair D, Zani B, Donegan S, Olliaro P, Garner P. Artemisinin-based combination therapy for treating uncomplicated malaria. *Cochrane Database Syst. Rev.* 8(3), CD007483 (2009).

60 Ashley EA, McGready R, Hutagalung R *et al.* A randomized, controlled study of a simple, once-daily regimen of dihydroartemisinin–piperaquine for the treatment of uncomplicated, multidrug-resistant falciparum malaria. *Clin. Infect. Dis.* 41(4), 425–432 (2005).

61 Valecha N, Phyo AP, Mayxay M *et al.* An open-label, randomised study of dihydroartemisinin–piperaquine versus artesunate–mefloquine for falciparum malaria in Asia. *PLoS ONE* 5(7), e11880 (2010).

62 Zwang J, Ashley EA, Karema C *et al.* Safety and efficacy of dihydroartemisinin–piperaquine in falciparum malaria: a prospective multi-centre individual patient data analysis. *PLoS ONE* 4(7), e6358 (2009).

63 Ndiaye JL, Randrianarivelojosia M, Sagara I *et al.* Randomized, multicentre assessment of the efficacy and safety of ASAQ – a fixed-dose artesunate–amodiaquine combination therapy in the treatment of uncomplicated *Plasmodium falciparum* malaria. *Malar. J.* 8, 125 (2009).

64 Tshefu AK, Gaye O, Kayentao K *et al.* Efficacy and safety of a fixed-dose oral combination of pyronaridine–artesunate compared with artemether–lumefantrine in children and adults with uncomplicated *Plasmodium falciparum* malaria: a randomised non-inferiority trial. *Lancet* 375(9724), 1457–1467 (2010).

65 Hombhanje FW, Linge D, Saweri A *et al.* Artemisinin–naphthoquine combination (ARCO) therapy for uncomplicated falciparum malaria in adults of Papua New Guinea: a preliminary report on safety and efficacy. *Malar. J.* 8, 196 (2009).

66 Laufer MK, Thesing PC, Eddington ND *et al.* Return of chloroquine antimalarial efficacy in Malawi. *N. Engl. J. Med.* 355(19), 1959–1966 (2006).

67 World Health Organization. Global report on antimalarial drug efficacy and drug resistance: 2000–2010. World Health Organization, Geneva, Switzerland (2010).

•• **Recent and comprehensive report on the current status of *P. falciparum* and *P. vivax* resistance worldwide.**

68 Dondorp AM, Nosten F, Yi P *et al.* Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* 361(5), 455–467 (2009).

69 Noedl H, Se Y, Sriwichai S *et al.* Artemisinin resistance in Cambodia: a clinical trial designed to address an emerging problem in southeast Asia. *Clin. Infect. Dis.* 51(11), e82–e89 (2010).

70 Rogers WO, Sem R, Tero T *et al.* Failure of artesunate–mefloquine combination therapy for uncomplicated *Plasmodium falciparum* malaria in southern Cambodia. *Malar. J.* 8, 10 (2009).

71 Dondorp AM, Yeung S, White L *et al.* Artemisinin resistance: current status and scenarios for containment. *Nat. Rev. Microbiol.* 8(4), 272–280 (2010).

• **Very recent update of issues and challenges related to artemisinin resistance.**

72 Newton PN, Fernandez FM, Plancon A *et al.* A collaborative epidemiological investigation into the criminal fake artesunate trade in South East Asia. *PLoS Med.* 5(2), e32 (2008).

73 Wongsrichanalai C, Varma JK, Juliano JJ, Kimerling ME, MacArthur JR. Extensive drug resistance in malaria and tuberculosis. *Emerg. Infect. Dis.* 16(7), 1063–1067 (2010).

74 Shahinas D, Lau R, Khaimar K, Hancock D, Pillai DR. Artesunate misuse and *Plasmodium falciparum* malaria in a traveler returning from Africa. *Emerg. Infect. Dis.* 16(10), 1608–1610 (2010).

75 Dondorp AM, Day NP. The treatment of severe malaria. *Trans. R. Soc. Trop. Med. Hyg.* 101(7), 633–634 (2007).

76 Dondorp AM, Nosten F, Stepniewska K, Day N, White N. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* 366(9487), 717–725 (2005).

•• **Pivotal study demonstrating the superiority of artesunate over quinine for severe malaria in adults in low endemic settings.**

77 Dondorp AM, Fanello CI, Hendriksen ICE *et al.* Artesunate versus quinine in the treatment of severe falciparum malária in African children (AQUAMAT): an open-label, randomised trial. *Lancet* 376(9753), 1647–1657 (2010).

•• **Pivotal study demonstrating the superiority of artesunate over quinine for severe malaria in African children.**

78 Gomes MF, Faiz MA, Gyapong JO *et al.* Pre-referral rectal artesunate to prevent death and disability in severe malaria: a placebo-controlled trial. *Lancet* 373(9663), 557–566 (2009).

•• **Study demonstrating the lifesaving contribution of pre-referral rectal artesunate for severe malaria in rural endemic areas.**

79 Baird JK. Resistance to therapies for infection by *Plasmodium vivax*. *Clin. Microbiol. Rev.* 22(3), 508–534 (2009).

80 Lacy MD, Maguire JD, Barcus MJ *et al.* Atovaquone/proguanil therapy for *Plasmodium falciparum* and *Plasmodium vivax* malaria in Indonesians who lack clinical immunity. *Clin. Infect. Dis.* 35(9), e92–e95 (2002).

81 Looareesuwan S, Wilairatana P, Glanarongran R *et al.* Atovaquone and proguanil hydrochloride followed by primaquine for treatment of *Plasmodium vivax* malaria in Thailand. *Trans. R. Soc. Trop. Med. Hyg.* 93(6), 637–640 (1999).

82 Douglas NM, Anstey NM, Angus BJ, Nosten F, Price RN. Artemisinin combination therapy for vivax malaria. *Lancet Infect. Dis.* 10(6), 405–416 (2010).

83 Ratcliff A, Siswantoro H, Kenangalem E *et al.* Two fixed-dose artemisinin combinations for drug-resistant falciparum and vivax malaria in Papua, Indonesia: an open-label randomised comparison. *Lancet* 369(9563), 757–765 (2007).

84 Bassat Q, Mulenga M, Tinto H *et al.* Dihydroartemisinin–piperaquine and artemether–lumefantrine for treating uncomplicated malaria in African children: a randomised, non-inferiority trial. *PLoS ONE* 4(11), e7871 (2009).

85 Radloff PD, Philipps J, Hutchinson D, Kremsner PG. Atovaquone plus proguanil is an effective treatment for *Plasmodium ovale* and *P. malariae* malaria. *Trans. R. Soc. Trop. Med. Hyg.* 90(6), 682 (1996).

86 Borrmann S, Szlezak N, Binder RK *et al.* Evidence for the efficacy of artesunate in asymptomatic *Plasmodium malariae* infections. *J. Antimicrob. Chemother.* 50(5), 751–754 (2002).

87 Walsh DS, Wilairatana P, Tang DB *et al.* Randomized trial of 3-dose regimens of tafenoquine (WR238605) versus low-dose primaquine for preventing *Plasmodium vivax* malaria relapse. *Clin. Infect. Dis.* 39(8), 1095–1103 (2004).

88 Daneshvar C, Davis TM, Cox-Singh J *et al.* Clinical and laboratory features of human *Plasmodium knowlesi* infection. *Clin. Infect. Dis.* 49(6), 852–860 (2009).

89 Jiang N, Chang Q, Sun X *et al.* Co-infections with *Plasmodium knowlesi* and other malaria parasites, Myanmar. *Emerg. Infect. Dis.* 16(9), 1476–1478 (2010).

90 Van den Eede P, Van HN, Van Overmeir C *et al.* Human *Plasmodium knowlesi* infections in young children in central Vietnam. *Malar. J.* 8, 249 (2009).

91 Centers for Disease Control. Simian malaria in a U.S. traveler – New York, 2008. *MMWR Morb. Mortal. Wkly Rep.* 58(9), 229–232 (2009).

92 Bronner U, Divis PC, Farnert A, Singh B. Swedish traveller with *Plasmodium knowlesi* malaria after visiting Malaysian Borneo. *Malar. J.* 8, 15 (2009).

93 Kantele A, Marti H, Felger I, Muller D, Jokiranta TS. Monkey malaria in a European traveler returning from Malaysia. *Emerg. Infect. Dis.* 14(9), 1434–1436 (2008).

94 Ta TT, Salas A, li-Tammam M *et al.* First case of detection of *Plasmodium knowlesi* in Spain by real time PCR in a traveller from Southeast Asia. *Malar. J.* 9, 219 (2010).

95 van Hellemond JJ, Rutten M, Koelewijn R *et al.* Human *Plasmodium knowlesi* infection detected by rapid diagnostic tests for malaria. *Emerg. Infect. Dis.* 15(9), 1478–1480 (2009).

96 Franco-Paredes C, Santos-Preciado JI. Problem pathogens: prevention of malaria in travellers. *Lancet Infect. Dis.* 6(3), 139–149 (2006).

97 Griffith KS, Lewis LS, Mali S, Parise ME. Treatment of malaria in the United States: a systematic review. *JAMA* 297(20), 2264–2277 (2007).

98 Hatz C, Soto J, Nothdurft HD *et al.* Treatment of acute uncomplicated falciparum malaria with artemether–lumefantrine in nonimmune populations: a safety, efficacy, and pharmacokinetic study. *Am. J. Trop. Med. Hyg.* 78(2), 241–247 (2008).

99 Lalloo DG, Shingadia D, Pasvol G *et al.* UK malaria treatment guidelines. *J. Infect.* 54(2), 111–121 (2007).

100 Committee to Advise on Tropical Medicine and Travel (CATMAT). Canadian recommendations for the prevention and treatment of malaria among international travellers – 2009. *Can. Commun. Dis. Rep.* 35(Suppl. 1), 1–82 (2009).

101 CDC. Malaria Guidelines for Health Professionals in the Northern Territory, 5th Edition. CDC, Atlanta, GA, USA (2007).

102 Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R. Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. *Lancet Infect. Dis.* 7(2), 136–144 (2007).

103 Nosten F, McGready R, Mutabingwa T. Case management of malaria in pregnancy. *Lancet Infect. Dis.* 7(2), 118–125 (2007).

104 Bethell D, Se Y, Lon C *et al.* Dose-dependant risk of neutropenia after 7-day courses of artesunate monotherapy in Cambodian patients with acute *Plasmodium falciparum* malaria. *Clin. Infect. Dis.* 51(12), e105–e114 (2010).

105 Adjei GO, Goka BQ, Binka F, Kurtzhals JA. Artemether–lumefantrine: an oral antimalarial for uncomplicated malaria in children. *Expert Rev. Anti Infect. Ther.* 7(6), 669–681 (2009).

106 McGready R, Cho T, Keo NK *et al.* Artemisinin antimalarials in pregnancy: a prospective treatment study of 539 episodes of multidrug-resistant *Plasmodium falciparum*. *Clin. Infect. Dis.* 33(12), 2009–2016 (2001).

107 McGready R, Tan SO, Ashley EA *et al.* A randomised controlled trial of artemether–lumefantrine versus artesunate for uncomplicated *Plasmodium falciparum* treatment in pregnancy. *PLoS Med.* 5(12), e253 (2008).

108 Wongsrichanalai C, Pickard AL, Wernsdorfer WH, Meshnick SR. Epidemiology of drug-resistant malaria. *Lancet Infect. Dis.* 2(4), 209–218 (2002).

109 Pradines B, Pistone T, Ezzedine K *et al.* Quinine-resistant malaria in traveler returning from Senegal, 2007. *Emerg. Infect. Dis.* 16(3), 546–548 (2010).

110 Miller RS, Wongsrichanalai C, Buathong N *et al.* Effective treatment of uncomplicated *Plasmodium falciparum* malaria with azithromycin-quinine combinations: a randomized, dose-ranging study. *Am. J. Trop. Med. Hyg.* 74(3), 401–406 (2006).

111 Adegnika AA, Breitling LP, Agnandji ST *et al.* Effectiveness of quinine monotherapy for the treatment of *Plasmodium falciparum* infection in pregnant women in Lambarene, Gabon. *Am. J. Trop. Med. Hyg.* 73(2), 263–266 (2005).

112 D'Acremont V, Landry P, Darioli R *et al.* Treatment of imported malaria in an ambulatory setting: prospective study. *Br. Med. J.* 324(7342), 875–877 (2002).

113 Melzer M, Lacey S, Rait G. The case for outpatient treatment of *Plasmodium falciparum* malaria in a selected UK immigrant population. *J. Infect.* 59(4), 259–263 (2009).

114 Jelinek T. Intravenous artesunate recommended for patients with severe malaria: position statement from TropNetEurop. *Euro Surveill.* 10(11), E051124 (2005).

115 Shanks GD. For severe malaria, arstesunate is the answer. *Lancet* 376, 1621–1622 (2010).

116 Rosenthal PJ. Artesunate for the treatment of severe falciparum malaria. *N. Engl. J. Med.* 358(17), 1829–1836 (2008).

117 Morch K, Strand O, Dunlop O *et al.* Severe malaria and artesunate treatment, Norway. *Emerg. Infect. Dis.* 14(11), 1816–1818 (2008).

118 Bartoloni A, Tomasoni L, Bartalesi F *et al.* Combined intravenous treatment with artesunate and quinine for severe malaria in Italy. *Am. J. Trop. Med. Hyg.* 83(2), 274–276 (2010).

119 Newton PN, Chierakul W, Ruangveerayuth R *et al.* A comparison of artesunate alone with combined artesunate and quinine in the parenteral treatment of acute falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.* 95(5), 519–523 (2001).

120 John CC, Kutamba E, Mugarura K, Opoka RO. Adjunctive therapy for cerebral malaria and other severe forms of *Plasmodium falciparum* malaria. *Expert Rev. Anti Infect. Ther.* 8(9), 997–1008 (2010).

121 Bottieau E, Clerinx J, Van den Enden E *et al.* Imported non-*Plasmodium falciparum* malaria: a five-year prospective study in a European referral center. *Am. J. Trop. Med. Hyg.* 75(1), 133–138 (2006).

122 White NJ. *Plasmodium knowlesi*: the fifth human malaria parasite. *Clin. Infect. Dis.* 46(2), 172–173 (2008).

123 Daneshvar C, Davis TM, Cox-Singh J *et al.* Clinical and parasitological response to oral chloroquine and primaquine in uncomplicated human *Plasmodium knowlesi* infections. *Malar. J.* 9(1), 238 (2010).

124 Murray HW, Berman JD, Davies CR, Saravia NG. Advances in leishmaniasis. *Lancet* 366(9496), 1561–1577 (2005).

125 Desjeux P. Leishmaniasis. *Nat. Rev. Microbiol.* 2(9), 692 (2004).

126 Caumes E, Carriere J, Guermonprez G *et al.* Dermatoses associated with travel to tropical countries: a prospective study of the diagnosis and management of 269 patients presenting to a tropical disease unit. *Clin. Infect. Dis.* 20(3), 542–548 (1995).

127 Blum J, Desjeux P, Schwartz E, Beck B, Hatz C. Treatment of cutaneous leishmaniasis among travellers. *J. Antimicrob. Chemother.* 53(2), 158–166 (2004).

128 Schwartz E, Hatz C, Blum J. New world cutaneous leishmaniasis in travellers. *Lancet Infect. Dis.* 6(6), 342–349 (2006).

129 Scope A, Trau H, Bakon M *et al.* Imported mucosal leishmaniasis in a traveler. *Clin. Infect. Dis.* 37(6), e83–e87 (2003).

130 Malik ANJ, John L, Bryceson ADM, Lockwood DNJ. Changing pattern of visceral leishmaniasis, United Kingdom, 1985–2004. *Emerg. Infect. Dis.* 12(8), 1257–1259 (2006).

131 Perez-Ayala A, Norman F, Perez-Molina JA *et al.* Imported leishmaniasis: a heterogeneous group of diseases. *J. Travel. Med.* 16(6), 395–401 (2009).

132 Rijal S, Boelaert M, Regmi S *et al.* Evaluation of a urinary antigen-based latex agglutination test in the diagnosis of kala-azar in eastern Nepal. *Trop. Med. Int. Health* 9(6), 724–729 (2004).

133 Boelaert M, Rijal S, Regmi S *et al.* A comparative study of the effectiveness of diagnostic tests for visceral leishmaniasis. *Am. J. Trop. Med. Hyg.* 70(1), 72–77 (2004).

134 Chappuis F, Rijal S, Singh R *et al.* Prospective evaluation and comparison of the direct agglutination test and an rK39-antigen-based dipstick test for the diagnosis of suspected kala-azar in Nepal. *Trop. Med. Int. Health* 8(3), 277–285 (2003).

135 Reithinger R, Dujardin JC. Molecular diagnosis of leishmaniasis: current status and future applications. *J. Clin. Microbiol.* 45(1), 21–25 (2007).

136 Goto H, Lindoso JA. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. *Expert Rev. Anti Infect. Ther.* 8(4), 419–433 (2010).

• **Comprehensive recent review of tegumentary leishmaniasis.**

137 Mondal S, Bhattacharya P, Ali N. Current diagnosis and treatment of visceral leishmaniasis. *Expert Rev. Anti Infect. Ther.* 8(8), 919–944 (2010).

• **Comprehensive recent review of visceral leishmaniasis.**

138 Reithinger R, Dujardin JC, Louzir H *et al.* Cutaneous leishmaniasis. *Lancet Infect. Dis.* 7(9), 581–596 (2007).

•• **Excellent review on the complex topic of cutaneous leishmaniasis, with a clear and exhaustive list of cure rates obtained with all available treatments.**

139 WHO Technical Report Series 949. Control of the Leishmaniases. Presented at: *WHO Expert Committee on the Control of Leishmaniases*. Geneva, Switzerland, 22–26 March 2010.

140 Sundar S, Agrawal G, Rai M, Makharia MK, Murray HW. Treatment of Indian visceral leishmaniasis with single or daily infusions of low dose liposomal amphotericin B: randomised trial. *Br. Med. J.* 323(7310), 419–422 (2001).

141 den Boer M, Davidson RN. Treatment options for visceral leishmaniasis. *Expert Rev. Anti Infect. Ther.* 4(2), 187–197 (2006).

142 Kim DH, Chung HJ, Bleys J, Ghohestani RF. Is paromomycin an effective and safe treatment against cutaneous leishmaniasis? A meta-analysis of 14 randomized controlled trials. *PLoS Negl. Trop. Dis.* 3(2), e381 (2009).

143 Gonzalez U, Pinart M, Rengifo-Pardo M *et al.* Interventions for American cutaneous and mucocutaneous leishmaniasis. *Cochrane Database Syst. Rev.* 15(2), CD004834 (2009).

•• **Meta-analysis providing the current (limited) evidence for treatment of American cutaneous and mucocutaneous leishmaniasis.**

144 Gonzalez U, Pinart M, Reveiz L, Alvar J. Interventions for Old World cutaneous leishmaniasis. *Cochrane Database Syst. Rev.* 8(4), CD005067 (2008).

•• **Meta-analysis providing the current (limited) evidence for treatment of Old World cutaneous leishmaniasis.**

145 Arevalo J, Ramirez L, Adaui V *et al.* Influence of *Leishmania* (*Viannia*) species on the response to antimonial treatment in patients with American tegumentary leishmaniasis. *J. Infect. Dis.* 195(12), 1846–1851 (2007).

146 Llanos-Cuentas A, Tulliano G, raujo-Castillo R *et al.* Clinical and parasite species risk factors for pentavalent antimonial treatment failure in cutaneous leishmaniasis in Peru. *Clin. Infect. Dis.* 46(2), 223–231 (2008).

147 Alvar J, Aparicio P, Aseffa A *et al.* The relationship between leishmaniasis and AIDS: the second 10 years. *Clin. Microbiol. Rev.* 21(2), 334–359 (2008).

148 Dorlo TP, van Thiel PP, Huitema AD *et al.* Pharmacokinetics of miltefosine in Old World cutaneous leishmaniasis patients. *Antimicrob. Agents Chemother.* 52(8), 2855–2860 (2008).

149 Sundar S, Jha TK, Sindermann H *et al.* Oral miltefosine treatment in children with mild to moderate Indian visceral leishmaniasis. *Pediatr. Infect. Dis. J.* 22(5), 434–438 (2003).

• **Study which demonstrated the efficacy of the first oral therapy miltefosine in Indian visceral leishmaniasis.**

150 Sundar S, Jha TK, Thakur CP *et al.* Oral miltefosine for Indian visceral leishmaniasis. *N. Engl. J. Med.* 347(22), 1739–1746 (2002).

151 Ritmeijer K, Dejenie A, Assefa Y *et al.* A comparison of miltefosine and sodium stibogluconate for treatment of visceral leishmaniasis in an Ethiopian population with high prevalence of HIV infection. *Clin. Infect. Dis.* 43(3), 357–364 (2006).

152 Bhattacharya SK, Sinha PK, Sundar S *et al.* Phase 4 trial of miltefosine for the treatment of Indian visceral leishmaniasis. *J. Infect. Dis.* 196(4), 591–598 (2007).

153 Mohebali M, Fotouhi A, Hooshmand B *et al.* Comparison of miltefosine and meglumine antimoniate for the treatment of zoonotic cutaneous leishmaniasis (ZCL) by a randomized clinical trial in Iran. *Acta Trop.* 103(1), 33–40 (2007).

154 van Thiel PP, Leenstra T, Kager PA *et al.* Miltefosine treatment of *Leishmania major* infection: an observational study involving Dutch military personnel returning from northern Afghanistan. *Clin. Infect. Dis.* 50(1), 80–83 (2010).

155 Keynan Y, Larios OE, Wiseman MC *et al.* Use of oral miltefosine for cutaneous leishmaniasis in Canadian soldiers returning from Afghanistan. *Can. J. Infect. Dis. Med. Microbiol.* 19(6), 394–396 (2008).

156 Soto J, Rea J, Balderrama M *et al.* Efficacy of miltefosine for Bolivian cutaneous leishmaniasis. *Am. J. Trop. Med. Hyg.* 78(2), 210–211 (2008).

157 Soto J, Toledo J, Valda L *et al.* Treatment of Bolivian mucosal leishmaniasis with miltefosine. *Clin. Infect. Dis.* 44(3), 350–356 (2007).

158 Seifert K, Matu S, Javier Perez-Victoria F *et al.* Characterisation of *Leishmania donovani* promastigotes resistant to hexadecylphosphocholine (miltefosine). *Int. J. Antimicrob. Agents* 22(4), 380–387 (2003).

159 Pandey BD, Pandey K, Kaneko O, Yanagi T, Hirayama K. Relapse of visceral leishmaniasis after miltefosine treatment in a Nepalese patient. *Am. J. Trop. Med. Hyg.* 80(4), 580–582 (2009).

160 Sundar S, Jha TK, Thakur CP, Sinha PK, Bhattacharya SK. Injectable paromomycin for Visceral leishmaniasis in India. *N. Engl. J. Med.* 356(25), 2571–2581 (2007).

161 Sundar S, Agrawal N, Arora R *et al.* Short-course paromomycin treatment of visceral leishmaniasis in India: 14-day vs 21-day treatment. *Clin. Infect. Dis.* 49(6), 914–918 (2009).

162 Musa AM, Younis B, Fadlalla A *et al.* Paromomycin for the treatment of visceral leishmaniasis in Sudan: a randomized, open-label, dose-finding study. *PLoS Negl. Trop. Dis.* 4(10), e855 (2010).

163 Bern C, Adler-Moore J, Berenguer J *et al.* Liposomal amphotericin B for the treatment of visceral leishmaniasis. *Clin. Infect. Dis.* 43(7), 917–924 (2006).

164 Sundar S, Chakravarty J, Rai VK *et al.* Amphotericin B treatment for Indian visceral leishmaniasis: response to 15 daily versus alternate-day infusions. *Clin. Infect. Dis.* 45(5), 556–561 (2007).

165 Sundar S, Jha TK, Thakur CP *et al.* Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. *Clin. Infect. Dis.* 37(6), 800–804 (2003).

166 Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. *N. Engl. J. Med.* 362(6), 504–512 (2010).

167 Mueller M, Ritmeijer K, Balasegaram M *et al.* Unresponsiveness to AmBisome in some Sudanese patients with kala-azar. *Trans. R. Soc. Trop. Med. Hyg.* 101(1), 19–24 (2007).

168 Laguna F, Videla S, Jimenez-Mejias ME *et al.* Amphotericin B lipid complex versus meglumine antimoniate in the treatment of visceral leishmaniasis in patients infected with HIV: a randomized pilot study. *J. Antimicrob. Chemother.* 52(3), 464–468 (2003).

169 Chappuis F, Sundar S, Hailu A *et al.* Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nat. Rev. Microbiol.* 5(11), 873–882 (2007).

170 Olliaro PL. Drug combinations for visceral leishmaniasis. *Curr. Opin. Infect. Dis.* 23(6), 595–602 (2010).

171 Thakur CP, Kanyok TP, Pandey AK *et al.* A prospective randomized, comparative, open-label trial of the safety and efficacy of paromomycin (aminosidine) plus sodium stibogluconate versus sodium stibogluconate alone for the treatment of visceral leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 94(4), 429–431 (2000).

172 Melaku Y, Collin SM, Keus K *et al.* Treatment of kala-azar in southern Sudan using a 17-day regimen of sodium stibogluconate combined with paromomycin: a retrospective comparison with 30-day sodium stibogluconate monotherapy. *Am. J. Trop. Med. Hyg.* 77(1), 89–94 (2007).

173 Sundar S, Rai M, Chakravarty J *et al.* New treatment approach in Indian visceral leishmaniasis: single-dose liposomal amphotericin B followed by short-course oral miltefosine. *Clin. Infect. Dis.* 47(8), 1000–1006 (2008).

- **Study evaluating a promising combination therapy for visceral leishmaniasis.**

174 van Griensven J, Balasegaram M, Meheus F *et al.* Combination therapy for visceral leishmaniasis. *Lancet Infect. Dis.* 10(3), 184–194 (2010).

- **Overview of the combination therapies explored for viseral leishmaniasis.**

175 Blum JA, Hatz CF. Treatment of cutaneous leishmaniasis in travelers 2009. *J. Travel. Med.* 16(2), 123–131 (2009).

- **Updated therapeutic guidelines for cutaneous leishmaniasis in travelers.**

176 Khatami A, Firooz A, Gorouhi F, Dowlati Y. Treatment of acute Old World cutaneous leishmaniasis: a systematic review of the randomized controlled trials. *J. Am. Acad. Dermatol.* 57(2), 335–329 (2007).

177 Roussel M, Nacher M, Fremont G *et al.* Comparison between one and two injections of pentamidine isethionate, at 7 mg/kg in each injection, in the treatment of cutaneous leishmaniasis in French Guiana. *Ann. Trop. Med. Parasitol.* 100(4), 307–314 (2006).

178 Nacher M, Carme B, Sainte MD *et al.* Influence of clinical presentation on the efficacy of a short course of pentamidine in the treatment of cutaneous leishmaniasis in French Guiana. *Ann. Trop. Med. Parasitol.* 95(4), 331–336 (2001).

179 Moudgal VV, Sobel JD. Antifungal drugs in pregnancy: a review. *Expert Opin. Drug Saf.* 2(5), 475–483 (2003).

180 Antinori S, Cascio A, Parravicini C, Bianchi R, Corbellino M. Leishmaniasis among organ transplant recipients. *Lancet Infect. Dis.* 8, 191–199 (2008).

181 Sindermann H, Engel J. Development of miltefosine as an oral treatment for leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* 100(Suppl. 1) S17–S20 (2006).

182 Sindermann H, Engel KR, Fischer C, Bommer W. Oral miltefosine for leishmaniasis in immunocompromised patients: compassionate use in 39 patients with HIV infection. *Clin. Infect. Dis.* 39(10), 1520–1523 (2004).

183 Lopez-Velez R, Videal S, Marquez M *et al.* Amphotericin B lipid complex versus no treatment in the secondary prophylaxis of visceral leishmaniasis in HIV-infected patients. *J. Antimicrob. Chemother.* 53(3), 540–543 (2004).

184 Pepin J, Meda HA. The Epidemiology and control of human African trypanosomiasis. *Adv. Parasitol.* 49, 71–132 (2001).

185 Blum J, Schmid C, Burri C. Clinical aspects of 2541 patients with second stage human African trypanosomiasis. *Acta Trop.* 97(1), 55–64 (2006).

186 Chappuis F, Loutan L, Simarro P, Lejon V, Buscher P. Options for field diagnosis of human african trypanosomiasis. *Clin. Microbiol. Rev.* 18(1), 133–146 (2005).

187 Lejon V, Buscher P. Review Article: cerebrospinal fluid in human African trypanosomiasis: a key to diagnosis, therapeutic decision and post-treatment follow-up. *Trop. Med. Int. Health* 10(5), 395–403 (2005).

188 Lejon V, Legros D, Savignoni A *et al.* Neuro-inflammatory risk factors for treatment failure in "early second stage" sleeping sickness patients treated with pentamidine. *J. Neuroimmunol.* 144(1–2), 132–138 (2003).

189 Gautret P, Clerinx J, Caumes E *et al.* Imported human African trypanosomiasis in Europe, 2005–2009. *Euro Surveill.* 14(36), pii: 19327 (2009).

190 Lejon V, Boelaert M, Jannin J, Moore A, Buscher P. The challenge of *Trypanosoma*

brucei gambiense sleeping sickness diagnosis outside Africa. *Lancet Infect. Dis.* 3(12), 804–808 (2003).

191 Deborggraeve S, Buscher P. Molecular diagnostics for sleeping sickness: what is the benefit for the patient? *Lancet Infect. Dis.* 10(6), 433–439 (2010).

192 Legros D, Ollivier G, Gastellu-Etchegorry M *et al.* Treatment of human African trypanosomiasis – present situation and needs for research and development. *Lancet Infect. Dis.* 2(7), 437–440 (2002).

193 Burri C. Chemotherapy against human African trypanosomiasis: is there a road to success? *Parasitology* 137, 1987–1994 (2010).

194 Burri C, Nkunku S, Merolle A *et al.* Efficacy of new, concise schedule for melarsoprol in treatment of sleeping sickness caused by *Trypanosoma brucei gambiense*: a randomised trial. *Lancet* 355(9213), 1419–1425 (2000).

195 Schmid C, Richer M, Bilenge CM *et al.* Effectiveness of a 10-day melarsoprol schedule for the treatment of late-stage human African trypanosomiasis: confirmation from a multinational study (IMPAMEL II). *J. Infect. Dis.* 191, 1922–1931 (2005).

196 Blum J, Nkunku S, Burri C. Clinical description of encephalopathic syndromes and risk factors for their occurrence and outcome during melarsoprol treatment of human African trypanosomiasis. *Trop. Med. Int. Health* 6(5), 390–400 (2001).

197 Pepin J, Milord F, Guern C *et al.* Trial of prednisolone for prevention of melarsoprol-induced encephalopathy in gambiense sleeping sickness. *Lancet* 1(8649), 1246–1250 (1989).

198 Blum JA, Zellweger MJ, Burri C, Hatz C. Cardiac involvement in African and American trypanosomiasis. *Lancet Infect. Dis.* 8, 631–641 (2008).

199 Clerinx J, Taelman H, Bogaerts J, Vervoort T. Treatment of late stage rhodesiense trypanosomiasis using suramin and eflornithine: report of six cases. *Trans. R. Soc. Trop. Med. Hyg.* 92(4), 449–450 (1998).

200 Chappuis F, Udayraj N, Stietenroth K, Meussen A, Bovier PA. Eflornithine is safer than melarsoprol for the treatment of second-stage *Trypanosoma brucei gambiense* human African trypanosomiasis. *Clin. Infect. Dis.* 41(5), 748–751 (2005).

201 Milord F, Pepin J, Loko L, Ethier L, Mpia B. Efficacy and toxicity of eflornithine for treatment of *Trypanosoma brucei gambiense* sleeping sickness. *Lancet* 340(8820), 652–655 (1992).

202 Pepin J, Khonde N, Maiso F *et al.* Short-course eflornithine in Gambian trypanosomiasis: a multicentre randomized controlled trial. *Bull. World Health Organ.* 78(11), 1284–1295 (2000).

203 Priotto G, Kasparian S, Ngouama D *et al.* Nifurtimox–eflornithine combination therapy for second-stage *Trypanosoma brucei gambiense* sleeping sickness: a randomized clinical trial in Congo. *Clin. Infect. Dis.* 45(11), 1435–1442 (2007).

204 Bisser S, N'siesi FX, Lejon V *et al.* Equivalence trial of melarsoprol and nifurtimox monotherapy and combination therapy for the treatment of second-stage *Trypanosoma brucei gambiense* sleeping sickness. *J. Infect. Dis.* 195(3), 322–329 (2007).

205 Chappuis F. Melarsoprol-free drug combinations for second-stage Gambian sleeping sickness: the way to go. *Clin. Infect. Dis.* 45(11), 1443–1445 (2007).

206 Balasegaram M, Harris S, Checchi F *et al.* Melarsoprol versus eflornithine for treating late-stage Gambian trypanosomiasis in the Republic of the Congo. *Bull. World Health Organ.* 84(10), 783–791 (2006).

207 Balasegaram M, Young H, Chappuis F *et al.* Effectiveness of melarsoprol and eflornithine as first-line regimens for gambiense sleeping sickness in nine Medecins Sans Frontieres programmes. *Trans. R. Soc. Trop. Med. Hyg.* 103(3), 280–290 (2009).

208 Priotto G, Kasparian S, Mutombo W *et al.* Nifurtimox–eflornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a multicentre, randomised, Phase III, non-inferiority trial. *Lancet* 374(9683), 56–64 (2009).

•• **Pivotal study demonstrating the noninferiority of the combination eflornithine–nifurtimox compared to eflornithine alone for second-stage Gambiense trypanosomiasis.**

209 Lutje V, Seixas J, Kennedy A. Chemotherapy for second-stage human African trypanosomiasis. *Cochrane Database Syst. Rev.* 8, CD006201 (2010).

210 Lejon V, Roger I, Mumba ND *et al.* Novel markers for treatment outcome in late-stage *Trypanosoma brucei gambiense* trypanosomiasis. *Clin. Infect. Dis.* 47(1), 15–22 (2008).

211 Mumba ND, Lejon V, N'siesi FX, Boelaert M, Buscher P. Comparison of operational criteria for treatment outcome in gambiense human African trypanosomiasis. *Trop. Med. Int. Health* 14(4), 438–444 (2009).

212 Mumba ND, Lejon V, Pyana P *et al.* How to shorten patient follow-up after treatment for *Trypanosoma brucei gambiense* sleeping sickness. *J. Infect. Dis.* 201(3), 453–463 (2010).

213 Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. *Lancet* 375(9723), 1388–1402 (2010).

214 Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. *Clin. Infect. Dis.* 49(5), e52–e54 (2009).

215 Gascon J, Bern C, Pinazo MJ. Chagas disease in Spain, the United States and other non-endemic countries. *Acta Trop.* 115(1–2), 22–27 (2010).

216 Jackson Y, Getaz L, Wolff H *et al.* Prevalence, clinical staging and risk for blood-borne transmission of Chagas disease among Latin American migrants in Geneva, Switzerland. *PLoS Negl. Trop. Dis.* 4(2), e592 (2010).

217 Munoz J, Coll O, Juncosa T *et al.* Prevalence and vertical transmission of *Trypanosoma cruzi* infection among pregnant Latin American women attending 2 maternity clinics in Barcelona, Spain. *Clin. Infect. Dis.* 48(12), 1736–1740 (2009).

218 Piron M, Verges M, Munoz J *et al.* Seroprevalence of *Trypanosoma cruzi* infection in at-risk blood donors in Catalonia (Spain). *Transfusion* 48(9), 1862–1868 (2008).

219 Bern C, Montgomery SP, Katz L, Caglioti S, Stramer SL. Chagas disease and the US blood supply. *Curr. Opin. Infect. Dis.* 21(5), 476–482 (2008).

220 Bowling J, Walter EA. Recognizing and meeting the challenge of Chagas disease in the USA. *Expert Rev. Anti Infect. Ther.* 7(10), 1223–1234 (2009).

221 World Health Organization. Control and Prevention of Chagas Disease in Europe. Report of a WHO Informal Consultation, Geneva, Switzerland, 17–18 December 2009 (2010).

222 Lescure FX, Canestri A, Melliez H *et al.* Chagas disease, France. *Emerg. Infect. Dis.* 14(4), 644–646 (2008).

223 Brisseau JM, Cebron JP, Petit T *et al.* Chagas myocarditis imported into France. *Lancet* 1(8593), 1046 (1988).

224 Bern C, Montgomery SP, Herwaldt BL *et al.* Evaluation and treatment of chagas disease in the United States: a systematic review. *JAMA* 298(18), 2171–2181 (2007).

225 de Andrade AL, Zicker F, de Oliveira RM *et al.* Randomised trial of efficacy of

benznidazole in treatment of early *Trypanosoma cruzi* infection. *Lancet* 348(9039), 1407–1413 (1996).

226 Sosa ES, Segura EL, Ruiz AM *et al.* Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas disease. *Am. J. Trop. Med. Hyg.* 59(4), 526–529 (1998).

227 Viotti R, Vigliano C, Armenti H, Segura E. Treatment of chronic Chagas disease with benznidazole: clinical and serologic evolution of patients with long-term follow-up. *Am. Heart J.* 127(1), 151–162 (1994).

228 Villar JC, Marin-Neto JA, Ebrahim S, Yusuf S. Trypanocidal drugs for chronic asymptomatic *Trypanosoma cruzi* infection. *Cochrane Database Syst. Rev.*(1), CD003463 (2002).

229 Reyes PA, Vallejo M. Trypanocidal drugs for late stage, symptomatic Chagas disease (*Trypanosoma cruzi* infection). *Cochrane Database Syst. Rev.* 19(4), CD004102 (2005).

230 Chappuis F, Mauris A, Holst M *et al.* Validation of a rapid immunochromatographic assay for diagnosis of *Trypanosoma cruzi* infection among Latin-American migrants in Geneva, Switzerland. *J. Clin. Microbiol.* 48(8), 2948–2952 (2010).

231 Deborggraeve S, Coronado X, Solari A *et al. T. cruzi* OligoC-TesT: a simplified and standardized polymerase chain reaction format for diagnosis of Chagas disease. *PLoS Negl. Trop. Dis.* 3(6), e450 (2009).

232 Bern C, Verastegui M, Gilman RH *et al.* Congenital *Trypanosoma cruzi* transmission in Santa Cruz, Bolivia. *Clin. Infect. Dis.* 49(11), 1667–1674 (2009).

233 Fitzwater S, Calderon M, Lafuente C *et al.* Polymerase chain reaction for chronic *Trypanosoma cruzi* infection yields higher sensitivity in blood clot than buffy coat or whole blood specimens. *Am. J. Trop. Med. Hyg.* 79(5), 768–770 (2008).

234 Viotti R, Vigliano C. Etiological treatment of chronic Chagas disease: neglected 'evidence' by evidence-based medicine. *Expert Rev. Anti Infect. Ther.* 5(4), 717–726 (2007).

235 Moncayo A, Ortiz Yanine MI. An update on Chagas disease (human American trypanosomiasis). *Ann. Trop. Med. Parasitol.* 100(8), 663–677 (2006).

236 Laucella SA, Mazliah DP, Bertocchi G *et al.* Changes in *Trypanosoma cruzi*-specific immune responses after treatment: surrogate markers of treatment efficacy. *Clin. Infect. Dis.* 49(11), 1675–1684 (2009).

237 Urbina JA. Specific chemotherapy of Chagas disease: relevance, current limitations and new approaches. *Acta Trop.* 115(1–2), 55–68 (2010).

- **Detailed overview of the current and promising therapeutic approaches for Chagas disease.**

238 Viotti R, Vigliano C, Lococo B *et al.* Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a nonrandomized trial. *Ann. Intern. Med.* 144(10), 724–734 (2006).

239 Viotti R, Vigliano C, Lococo B *et al.* Side effects of benznidazole as treatment in chronic Chagas disease: fears and realities. *Expert Rev. Anti Infect. Ther.* 7(2), 157–163 (2009).

240 Sosa-Estani S, Segura EL. Etiological treatment in patients infected by *Trypanosoma cruzi*: experiences in Argentina. *Curr. Opin. Infect. Dis.* 19(6), 583–587 (2006).

241 Marin-Neto JA, Rassi A Jr, Morillo CA *et al.* Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: the BENznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT). *Am. Heart J.* 156(1), 37–43 (2008).

242 Rassi A, Luquetti AO, Rassi A Jr *et al.* Specific treatment for *Trypanosoma cruzi*: lack of efficacy of allopurinol in the human chronic phase of Chagas disease. *Am. J. Trop. Med. Hyg.* 76(1), 58–61 (2007).

243 Apt W, Arribada A, Zulantay I *et al.* Itraconazole or allopurinol in the treatment of chronic American trypanosomiasis: the results of clinical and parasitological examinations 11 years post-treatment. *Ann. Trop. Med. Parasitol.* 99(8), 733–741 (2005).

244 Coura JR. Present situation and new strategies for Chagas disease chemotherapy: a proposal. *Mem. Inst. Oswaldo Cruz* 104(4), 549–554 (2009).

245 Urbina JA, Docampo R. Specific chemotherapy of Chagas disease: controversies and advances. *Trends Parasitol.* 19(11), 495–501 (2003).

246 Jackson Y, Alirol E, Getaz L *et al.* Tolerance and safety of nifurtimox in patients with chronic Chagas disease. *Clin. Infect. Dis.* 51(10), e69–e75 (2010).

- **Recent observational study quantifying the side effects of nifurtimox for Chagas disease in a nonendemic setting.**

247 Prata A. Clinical and epidemiological aspects of Chagas disease. *Lancet Infect. Dis.* 1(2), 92–100 (2001).

248 Rottmann M, McNamara C, Yeung BK *et al.* Spiroindolones, a potent compound class for the treatment of malaria. *Science* 329(5996), 1175–1180 (2010).

249 Gonzalez U, Pinart M, Reveiz L *et al.* Designing and reporting clinical trials on treatments for cutaneous leishmaniasis. *Clin. Infect. Dis.* 51(4), 409–419 (2010).

250 Yun O, Priotto G, Tong J, Flevaud L, Chappuis F. NECT is next: implementing the new drug combination therapy for *Trypanosoma brucei gambiense* sleeping sickness. *PLoS Negl. Trop. Dis.* 4(5), e720 (2010).

251 Ribeiro I, Sevcsik AM, Alves F *et al.* New, improved treatments for Chagas disease: from the R&D pipeline to the patients. *PLoS Negl. Trop. Dis.* 3(7), e484 (2009).

Websites

301 International Travel and Health www.who.int/ith/en/index.html

302 Assessing Three Day Pentamidine for First Stage Human African Trypanosomiasis in Uganda www.controlled-trials.com/ISRCTN55042030

303 Clinical Study to Assess the Tolerability, Feasibility and Effectiveness of Nifurtimox and Eflornithine (NECT) for the Treatment of *Trypanosoma Brucei* Gambiense Human African Trypanosomiasis (HAT) in the Meningoencephalitic Phase (NECT-FIELD) http://clinicaltrials.gov/ct2/show/NCT00906880

304 Human African Trypanosomiasis: First in Man Clinical Trial of a New Medicinal Product, the Fexinidazole http://clinicaltrials.gov/ct2/show/NCT00982904