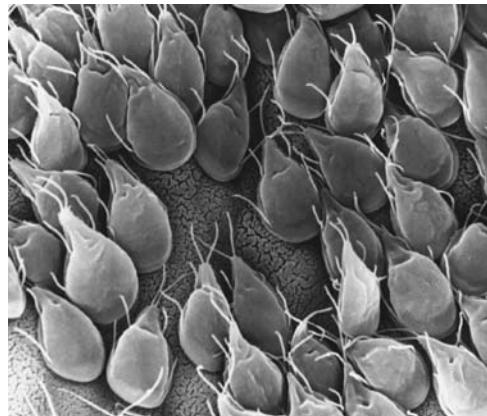


Chapter 5

Protozoa



The protozoan *Giardia* pictured here attached to the lining of the intestine

KEY FACTS

- Protozoa are only of pharmaceutical interest because they cause disease; they are not product contaminants or spoilage organisms in medicines.
- Protozoal infections are much more common in hot countries. Although UK cases of malaria are always caught abroad, there are several endemic pathogenic protozoa, of which *Trichomonas vaginalis*, *Giardia lamblia* and *Cryptosporidium* species are the most important.
- Metronidazole is normally effective for the treatment of *Trichomonas* and *Giardia* infections, but the treatment of some other protozoal infections can be difficult.
- Malaria is caused by five species of *Plasmodium*, of which *P. falciparum* is the most dangerous; *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* usually cause less severe symptoms, and infections by these species are referred to as benign malaria.
- The choice of drug for the treatment or prevention of malaria will be influenced by local resistance patterns and the species of *Plasmodium* responsible.

Protozoa are single-celled eukaryotic organisms that can cause a variety of severe human infections, and they are of pharmaceutical interest for this reason alone. They are extremely unlikely to arise as contaminants of pharmaceutical raw materials, and they are not used in the manufacture of any medicines other than the vaccines that prevent the diseases they cause. As with bacteria, there are very many species of protozoa (some estimates exceed 50 000) but only a relatively small number are capable of causing infections – at least in humans. A few of these pathogenic species are able to survive and reproduce outside the body, for example the pathogenic amoebae,

but most are parasites that can only reproduce inside an animal host; several of these, like the organisms causing malaria, leishmaniasis and trypanosomiasis (sleeping sickness), for example, require animal vectors – insects – to transport them from one human victim to the next.

5.1 Cultivation of protozoa

The pathogenic protozoa differ significantly in terms of their shape and size (Figure 5.1). They can be divided into three groups in terms of the way they are grown:

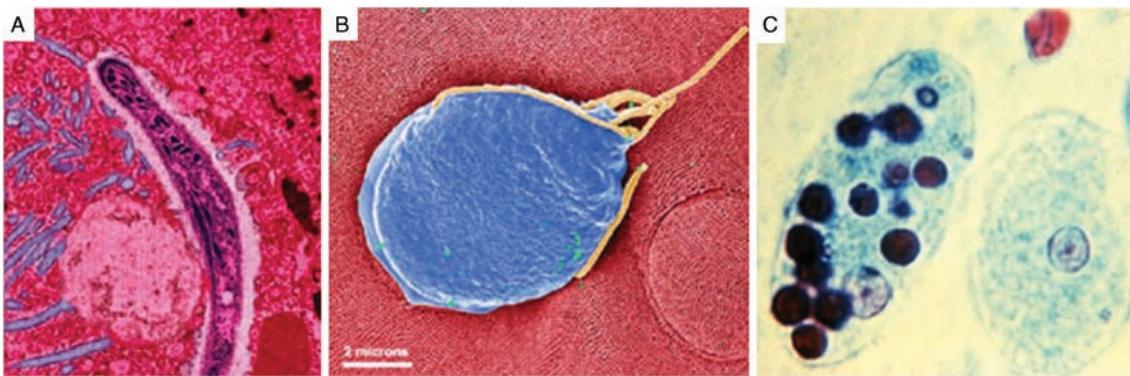


Figure 5.1 Pathogenic protozoa: A. malaria (*Plasmodium*) sporozoite in intestinal epithelium; B. *Giardia* cell attached to an epithelial surface; C. *Entamoeba histolyticum* (the light blue cell) which has ingested host red blood cells (dark circles). A and B are artificially coloured electron micrographs with higher magnification than C. thus, the *Entamoeba* cells are approximately twice the size of the other two. Sources: A. <http://commons.wikimedia.org/wiki/File:Malaria.jpg>. B. PHIL ID #11653; Photo Credit: Dr. Stan Erlandsen, Centers for Disease Control and Prevention. C. CDC DPDx Image Library; http://www.dpd.cdc.gov/dpdx/html/ImageLibrary/Amebiasis_il.htm.

- Amoebae can be cultivated easily in the laboratory in much the same way as bacteria, for instance *Acanthamoeba* species.
- Some can be grown in mixed cultures with companion organisms and, with more difficulty, on their own, for example the pathogenic protozoa possessing flagella, such as *Giardia* and *Trichomonas*.
- Others are extremely difficult to grow in the lab because they have both human and insect stages in their life cycles so they require very complex media supplemented with red blood cells, e.g. the *Plasmodium* species responsible for malaria.

Laboratory cultivation of protozoa is not, therefore, undertaken even in hospital pathology laboratories let alone pharmaceutical quality assurance labs, so this aspect will not be considered further. Diagnosis of protozoal infections does not normally require cultivation of the organism anyway; it is usually based upon symptoms and immunological tests or, in some cases, on microscopic examination of faecal samples to detect the organism's cysts.

5.2 Protozoal infections: the global and UK perspectives

Protozoal infections are far more common in tropical countries than in temperate ones, largely because higher tropical temperatures promote the reproduction of both

the protozoa themselves and their insect vectors. In temperate countries winter frosts tend to kill protozoa, but despite this there are several indigenous protozoal infections in the UK. Only about 10% of the cases of giardiasis, 5% of amoebic dysentery, and 3% of cryptosporidiosis recorded annually by the Heath Protection Agency for England and Wales are associated with foreign travel, so the great majority of these infections are caught within the country. By contrast, all UK cases of malaria and the majority of leishmaniasis infections are caught abroad. This situation may change however, because the geographical ranges of protozoal infections change over the years. Malaria, for example, was relatively common in southern Europe and the southern United States a century ago, and the last recorded indigenous cases in Britain were in the 1950s. The possibility exists that the global ranges of malaria and other tropical protozoal (and some nonprotozoal) infections may expand due to climate change.

Statistics relating to infections may be confusing because of the way they are recorded. Cases (the number of persons with the disease) differ from outbreaks (in which a single source affects many persons), and some databases record the number of new cases per year, which is not the same as the number of sufferers (particularly for diseases of long duration, which may extend over more than one year). Nevertheless, it is clear that the numbers of UK cases recorded in Table 5.1 are minute compared with those globally. Each year, there are approximately 350–500 million cases of malaria worldwide, for example, and more than 66 million people suffer from African trypanosomiasis.

Table 5.1 Incidence of protozoal infections in the UK.

Infection	Protozoan	UK cases per year (approx)
Trichomoniasis	<i>Trichomonas vaginalis</i>	6000
Cryptosporidiosis	<i>Cryptosporidium parvum</i> and <i>Cryptosporidium hominis</i>	4500
Giardiasis	<i>Giardia lamblia</i>	3500
Malaria	<i>Plasmodium falciparum</i> (approx 75%) and other species	2000*
Toxoplasmosis	<i>Toxoplasma gondii</i>	500
Amoebic dysentery	<i>Entamoeba histolyticum</i>	100
Acanthamoebiasis	At least 10 species of <i>Acanthamoeba</i>	50–100
Leishmaniasis	21 different species of <i>Leishmania</i>	60*
Trypanosomiasis	<i>Trypanosoma brucei</i> causes African sleeping sickness <i>Trypanosoma cruzi</i> causes Chagas disease in Central and S America	1 <1

*Almost all caught abroad

5.3 The characteristics and transmission of the major UK protozoal infections

5.3.1 Trichomoniasis

- *Trichomonas vaginalis* causes the greatest number of UK protozoal infections each year and it is the most common protozoal pathogen in industrialized countries, being estimated to affect over 170 million people globally.
- It is a sexually transmitted disease that infects males and females at approximately the same frequency, but infections in both sexes, though particularly in males, are often without symptoms.
- It commonly invades the vagina, particularly in circumstances when the protective acidity of the vaginal secretions is reduced, but it can also infect the urinary tract, fallopian tubes and pelvis in females, and the prostate in males.
- It is not likely to be life threatening and, although it may spontaneously clear, treatment with metronidazole or tinidazole is normally prescribed (Table 5.2) and achieves a high success rate. Treatment is usually necessary for the patient's sexual partners too, even if they are asymptomatic.
- Chronic untreated infection in females may cause premature births during pregnancy and may increase susceptibility to both HIV and cervical cancer.

5.3.2 Cryptosporidiosis

- The incidence has been rising in recent years so that it is the second most frequent protozoal infection in the UK.
- It causes diarrhoea, abdominal pain and nausea but is usually self-limiting in immunocompetent persons and drug treatment is unnecessary (except for rehydration therapy).
- In developed countries infection without symptoms is rare (approximately 1% of cases), but asymptomatic infection in developing countries is far more common.
- It is a complication in AIDS but the introduction of highly active antiretroviral therapy has reduced the incidence.
- The organism is an obligate intracellular parasite (can only reproduce inside a host cell) but it is capable of producing oocysts that are extremely robust and can survive outside the body for long periods; they even survive water chlorination.
- Transmission is via the faecal-oral route and infection is readily initiated by the low infective dose of 10–1000 oocysts.
- Treatment options in immunocompromised patients are few, and the treatments that have been proposed rarely eradicate the infection without improvement in immune function. Nitoxanide is available in the United States, but only on a named patient basis in the United Kingdom; alternatives mainly comprise the macrolide antibiotics: clarithromycin and azithromycin.

Table 5.2 Drug treatment of protozoal infections.

Infection	Drug therapy
Trichomoniasis	Metronidazole or tinidazole
Cryptosporidiosis	In immunocompetent patients symptomatic treatment (fluid rehydration therapy and anti-diarrhoeal drugs) is normally adequate (nitroxamide is used as an antiprotozoal agent in the United States). No consistently effective antimicrobial agents for immunocompromised patients
Giardiasis	Metronidazole or tinidazole or meprazine
Malaria	See Table 5.3
Toxoplasmosis	Not usually necessary, but if required pyrimethamine and sulfadiazine administered for several weeks under expert supervision
Amoebic dysentery	Metronidazole or tinidazole followed by a 10-day course of diloxanide furoate
Acanthamoebiasis	Propamidine isethionate
Leishmaniasis	Sodium stibogluconate or amphotericin
Trypanosomiasis	Pentamidine and other drugs under expert supervision

5.3.3 Giardiasis

- *Giardia lamblia* is the only other pathogen causing a significant number of indigenous UK infections; it is transmitted via the faecal-oral route following consumption of contaminated food or water.
- Its cysts resist chlorination, survive for long periods outside the body and, following ingestion, cause an infection resulting in acute diarrhoea.
- The infection is quite frequently asymptomatic, and relatively easy to treat with metronidazole or tinidazole which are the drugs of choice (Table 5.2), or, less commonly, meprazine.

5.3.4 Other protozoal infections

The other protozoal infections listed in Table 5.1 are, with the exception of malaria (considered in the next section), relatively uncommon in the United Kingdom, but when infections do arise the drug treatments described in Table 5.2 are normally effective.

- Amoebic dysentery although less common than giardiasis and cryptosporidiosis, may cause a more severe dysentery (defined as diarrhoea with blood or mucus in the faeces), which, if untreated, may lead to a fatal liver abscess. This organism, too, is transmitted by the faecal-oral route and produces cysts, although they are less robust than those of *Giardia* and

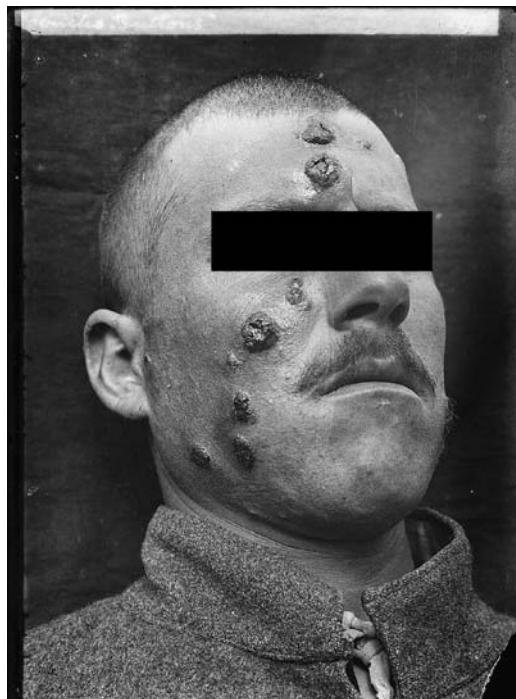


Figure 5.2 Cutaneous leishmaniasis, also known as kala azar, dum-dum fever or 'Jericho buttons'; the last name is relevant here as this photograph, taken in 1917, shows the disease in a soldier serving near the city of Jericho in the Middle East. Source: G. Eric, Edith Matson Photograph Collection, US Library of Congress; <http://commons.wikimedia.org/wiki/File:Jericho-Buttons.jpg>.

Cryptosporidium species and only survive for a few months even in moist conditions. *Acanthamoeba* species are relatively rare problem organisms contaminating contact-lens solutions and have caused ophthalmic keratitis.

- *Toxoplasma gondii*, a protozoan transmitted via cat faeces can, particularly in immunocompromised individuals, cause severe, even fatal, brain damage.
- Trypanosomiasis is, like malaria, transmitted via insect bites, and infects the bloodstream as well as muscle and the central nervous system.
- Cutaneous leishmaniasis, also transmitted via insect bites, is one of the few protozoal infections that is visually recognizable from the skin lesions it causes (Figure 5.2), although in its other form, visceral leishmaniasis, it is much more dangerous because internal organs are infected.

5.4 The transmission, prophylaxis and treatment of malaria

There are about 2000 malaria cases in the UK each year and 10–20 deaths, but the number of deaths worldwide is approximately 1.5 million per year, of which 90% occur in Africa. The relatively small number of cases might give the impression that the disease is of only minor importance from a UK pharmaceutical perspective, but the scale of modern international travel means that antimalarial drugs are dispensed far more frequently for disease prevention than for treatment. Data from the National Health Service Prescription Pricing Division shows that 5.28 million prescriptions were written for antimalarial

Table 5.3 Summary of British National Formulary (BNF) recommendations for the treatment and prophylaxis of malaria.

Antimalarial	Falciparum malaria treatment	Benign malaria treatment	Prophylaxis
Artemether with lumefantrine ^a	Yes	Yes	X
Chloroquine ^a	No longer recommended due to resistance	Yes	Yes, in areas with low risk of chloroquine-resistant falciparum malaria
Mefloquine ^a	Rarely used due to resistance	Rarely used	Yes, in areas with high risk of chloroquine-resistant falciparum malaria
Primaquine	X	Yes, for <i>P. vivax</i> and <i>P. ovale</i> ; used with quinine or chloroquine	X
Proguanil	No	No	Yes, usually with chloroquine
Proguanil with atovaquone ^a	Yes	Yes	Yes, particularly in areas with high risk of resistant falciparum malaria
Pyrimethamine ^a	Not used alone for malaria; only for toxoplasmosis		
Pyrimethamine with sulfadoxine ^a	Possibly, together with quinine	X	Not recommended in the United Kingdom
Quinine ^a	Yes, with doxycycline (adults) or clindamycin (children)	X	No
Doxycycline ^a	Yes, together with quinine	X	Yes, in areas of mefloquine or chloroquine resistance

^a= prescription only medicine in the UK; X= no BNF recommendations.

drugs in the period from January 2007 to June 2008 and to this number must be added the private prescriptions and the sales of those products which are not prescription-only medicines (Table 5.3). Advice is also sought in pharmacies regarding the suitability of alternative insect repellents, which, together with appropriate clothing and mosquito nets, are an integral part of a protection strategy designed to avoid being bitten by the female *Anopheles* mosquitoes that transmit the infection.

Swamp drainage, the use of insecticides, and biological control agents like predatory fish that eat insect larvae are further strategies used to control mosquitoes and reduce the incidence of the diseases they transmit.

It is important to recognize the difference between the various forms of malaria caused by different species of the *Plasmodium* parasite. *Plasmodium falciparum* is the most common and the most dangerous species, causing what is sometimes referred to as malignant malaria; it has the highest mortality and complication rates and accounts for more than 90% of malaria infections and deaths worldwide (approximately 75% of cases in the United Kingdom).

Four other species of *Plasmodium* may also cause the disease: *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. They give rise to a less severe infection referred to as benign malaria, which is relatively more common in parts of Asia and South America. The five species exhibit differing susceptibilities to the common antimalarial drugs, so those used to treat or to prevent falciparum malaria are not necessarily recommended for benign malaria and vice versa (Table 5.3). Chloroquine, for

example, was formerly far more widely used than it is now, but resistance has become so widespread that it is currently employed largely for the treatment of benign malaria and for prophylaxis in regions with a low risk of resistance in falciparum malaria. The situation is further complicated by variations in malaria risk during different months of the year since rainfall affects mosquito breeding.

Mosquitoes are also less common at high altitude, so people living in mountainous areas of a malaria-prone country might be at little or no risk. Because of the regional variations in susceptibility then prior to travel it is important to consult the specific recommendations relevant for each country or region, such as the guidelines in the British National Formulary or those of the UK Health Protection Agency (http://www.hpa.org.uk/infections/topics_az/malaria/guidelines.htm).

There have been many attempts to produce a malaria vaccine, but the complexity of the organism's life cycle and its rapid rate of reproduction and mutation have posed major problems. A recent candidate vaccine has been trialled in Mozambique and the Gambia and was reported to be 71% effective in providing short-term protection against falciparum malaria.

Acknowledgement

Chapter title image: PHIL ID #11632, Photo Credit: Dr. Stan Erlandsen, Centers for Disease Control and Prevention.