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An Irish perspective on *Cryptosporidium*

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Cryptosporidiosis, a protozoal disease which causes significant morbidity in humans, is one of the chief causes of diarrhoea in neonatal ruminants. Although the parasite poses a significant threat to public health and animal health in Ireland, its epidemiology on the island is only poorly understood. Environmental studies have shown the waterborne parasite to be widespread in some untreated waterbodies around Ireland. The island's hydrogeological situation, combined with high stocking rates of livestock and the absence of filtration from regular water treatment, render it vulnerable to large-scale outbreaks. This review discusses the parasite in the Irish context and underlines the need for a reference facility to provide active surveillance on the island.

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Introduction

First described in 1907 (Tyzzer, 1907), *Cryptosporidium* remained obscure for many decades. During the 1960s and 1970s it was increasingly recognised as an important pathogen of neonatal ruminants (Panciera *et al.*, 1971) and humans (Meisel *et al.*, 1976; Nime *et al.*, 1976), but it was not until the emergence of AIDS in the early 1980s that *Cryptosporidium* was identified as a life-threatening parasite in immunocompromised patients. Today, it is known to be one of the most serious causes of waterborne diarrhoea (Tzipori and Ward, 2002); it is a most difficult organism to control and it is one of the most heavily researched protozoan parasites. In contrast, we are only beginning to understand the importance of *Cryptosporidium* on the island of Ireland. This article summarises what we know about the epidemiology of the parasite on the island and how the unique combination of land use, infrastructure and geophysics on the island may affect it.

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Life-cycle and transmission

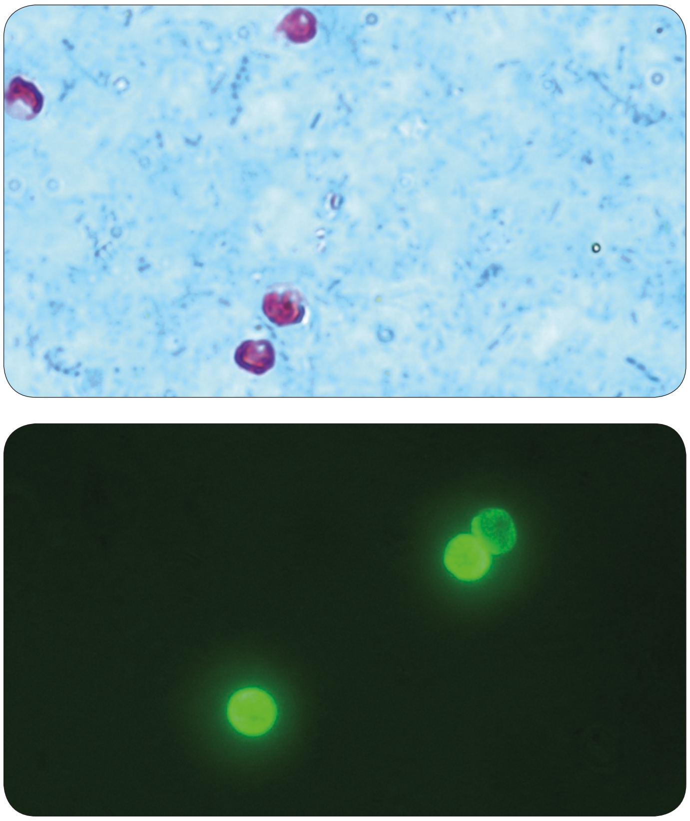

Figure 1: Stained *Cryptosporidium* oocysts; (top) modified Ziehl-Neelsen stain, viewed under bright light, and (bottom) phenol-auramine stain, viewed under fluorescent FITC filter.

Cryptosporidium is an obligate enteric parasite of the phylum *Apicomplexa* **(Figure 1)**. The disease is transmitted via long-lived, thick-walled oocysts of 3µm to 8µm diameter, excreted in the faeces of infected humans and animals. The infectious dose is dependent on the species and immune status of the host and on the *Cryptosporidium* species/strain, but generally considered to be very low. Infection experiments in 29 healthy volunteers indicated an infectious dose of 132 oocysts (median value) (DuPont *et al*., 1995), while mathematical modelling suggested it may be as low as one oocyst (Haas and Rose, 1994). Upon ingestion, invasive stages or sporocysts emerge from the oocysts and invade cells in the microvillous border of the intestinal epithelium and, to a lesser extent, extra-intestinal epithelia **(Figure 2)**. Situated intracellularly, just beneath the host cell membrane, but extracytoplasmic and enclosed within parasitophorous vacuoles, the parasites multiply through repeated cycles of asexual reproduction and re-invasion, thereby destroying the functional integrity of the brush border. Ultimately, sexual reproduction occurs resulting in the formation of thick-walled oocysts that are shed with the faeces. The oocysts are already sporulated and capable of immediate infection of a new host. Thin-walled oocysts may occur also; these are believed to be capable of excysting within the same host and causing auto-infection in immunodeficient hosts.

Contamination of the environment with *Cryptosporidium*

Cryptosporidium is a waterborne pathogen and its epidemiology is determined by environmental factors. According to the Geological Survey of Ireland, in many parts of the island there is only a shallow layer of soil and subsoil over karst limestone (Daly, 2003). In other areas, the General Soil Map of Ireland (Gardiner and Radford, 1980)

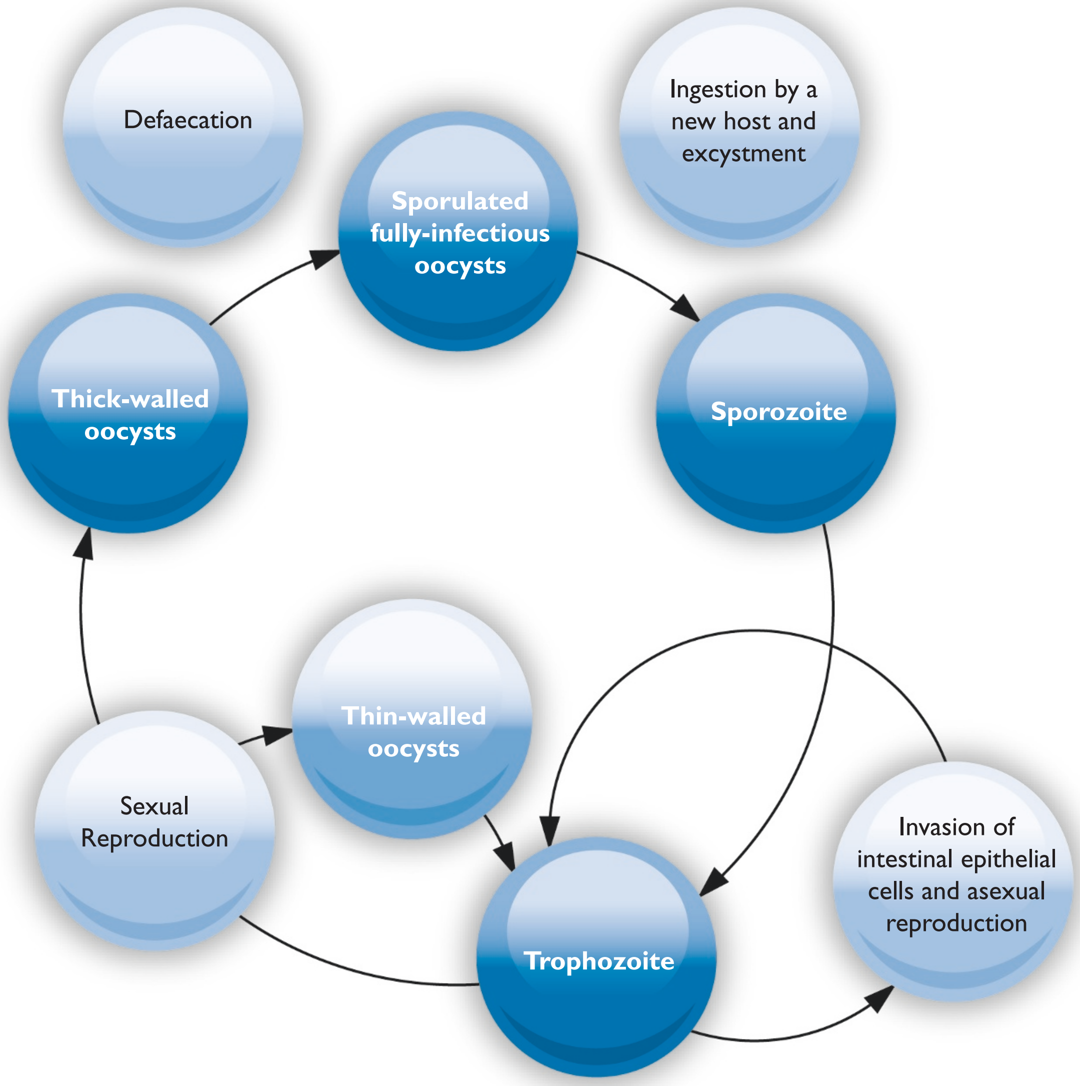

Figure 2: Life-cycle of *Cryptosporidium*

indicates heavy soils that can be prone to either rapid surface runoff after rainfall or channeling of water along large pores, known as preferential flow. In all these situations, instead of being filtered by slow passage through a substantial volume of soil, rainwater that may hold a significant contaminant load rapidly reaches surface water reservoirs or groundwater. A study carried out on Lough Owel, County Westmeath, confirmed this threat. The water level in the lake was shown to rise rapidly after rainfall events, indicating that areas within the catchment have extremely poor permeability, leading to excessive overland flow and facilitating direct ingress of contaminants into the lake (Report, Trinity College Dublin, 1999). Incidentally, three years later the lake water, which is used as a source for drinking water, was linked to one of the largest recorded outbreaks of cryptosporidiosis in the Republic of Ireland.

Cryptosporidium oocysts are extremely resilient and remain viable for long periods in the environment (Kato *et al*., 2004). Long survival periods in the environment, combined with quick passage to surface and ground waters, mean that many of the oocysts are still viable when they reach reservoirs of drinking water. Several studies investigating waterbodies and associated shellfish, on the island of Ireland found that contamination with oocysts was chiefly restricted to more densely populated areas (Belfast, Dublin, Sligo) and the River Shannon, where low levels of oocyst contamination were widespread (Chalmers *et al*., 1997; Finn *et al*., 2003; Graczyk *et al*., 2004; Lowery *et al*., 2001a, b; Skerrett and Holland, 2000). This widespread occurrence of oocysts in Irish waterbodies needs to be considered in view of the fact that the chief disinfection treatment used for drinking water in Ireland is chlorination, which does not kill *Cryptosporidium*. Filtration, the only reliable method to remove the parasite from drinking water, is not included in regular water treatment (Report of Waterborne Cryptosporidiosis Subcommittee of the Scientific Advisory Committee, 2004). This fact, combined with the high stocking rates that are carried on dairy farms in Ireland and the agricultural practices, soils and hydrology found on the island, could mean that Ireland is highly vulnerable to large-scale outbreaks should, for whatever reason, environmental contamination with oocysts increase.

Pathology and prevalence in the human population

The most common clinical signs in humans are diarrhoea, abdominal cramps, fever, nausea and vomiting, which may result in weight loss and dehydration. Sometimes, headache, weakness, fatigue, myalgia and inappetence are also reported. In immunocompetent patients, the primary site of infection is the distal small intestine (Chen *et al.,* 2002). These patients usually recover within one to two weeks; however, shedding of oocysts may continue for several weeks after symptoms cease and clinical relapses may occur up to 14 days after resolution of the initial symptoms (MacKenzie *et al*., 1994). In contrast, immunocompromised persons suffer chronic enteritis, lasting as long as the immune impairment. In these patients, the disease may go through cycles of resolution and recurrence, or it may be persistent and become life-threatening (Fayer *et al*., 1997). In immunocompromised hosts, the parasite can also infect other organs, resulting in hepatitis, pancreatitis, cholecystitis, cholangitis and conjunctivitis (Fayer *et al* .,1997). Respiratory infections are associated with coughing, wheezing, croup, hoarseness and shortness of breath

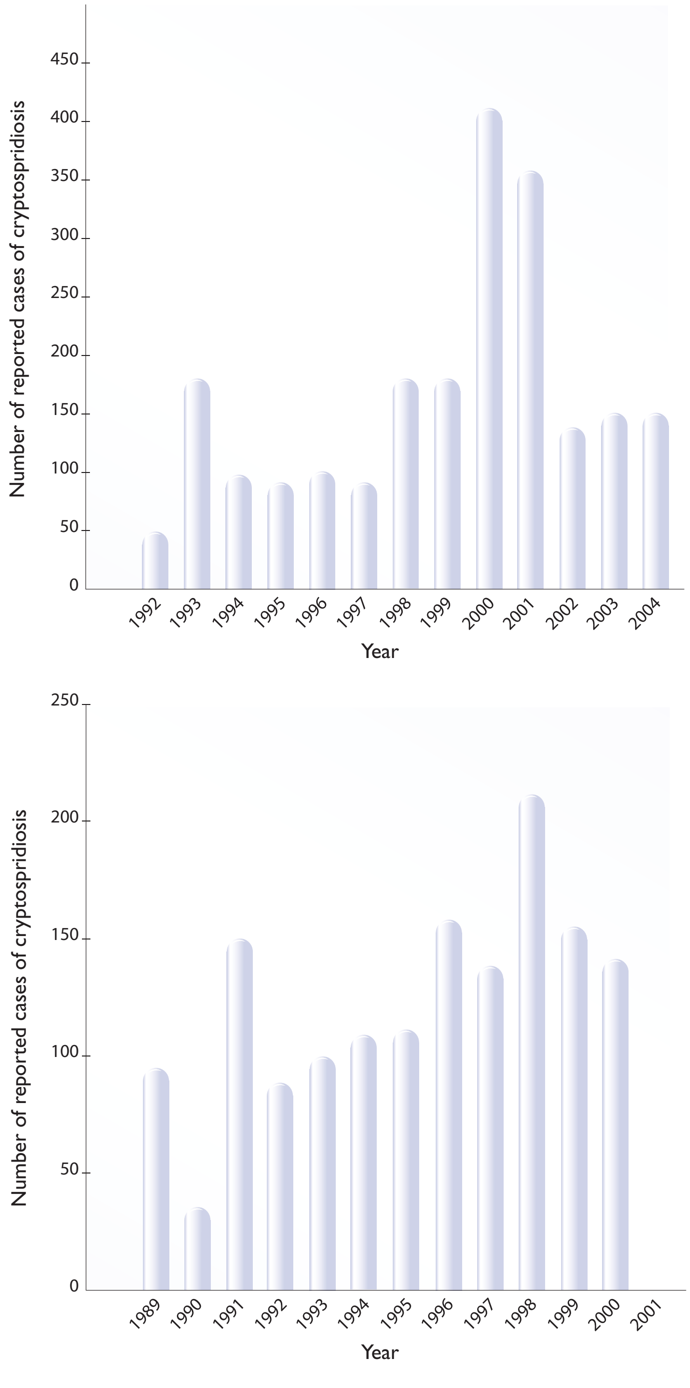

Figure 3: Recent trends of reported cases in (top) Northern Ireland from the Communicable Disease Surveillance Centre, Northern Ireland and (bottom) in the Republic of Ireland from INFOSCAN (Report of Waterborne Cryptosporidiosis Subcommittee of the Scientific Advisory Committee, 2004).

Over the last number of years, there have been several outbreaks of cryptosporidiosis on the island of Ireland. Between April 2000 to April 2001, there were three outbreaks associated with drinking-water in the greater Belfast area and neighboring communities, involving at least 476 cases in total (Smyth, 2001; Communicable Disease Surveillance Centre, 2000; Glaberman *et al*., 2002). Officials blamed Northern Ireland's antiquated system of water pipes (Birchard, 2000), which allowed the ingress of human sewage from a septic tank and of wastewater from a blocked drain (Glaberman *et al*., 2002). In the spring of the following year, an outbreak of cryptosporidiosis in County Westmeath was traced to the drinking water distribution network serviced by Lough Owel. The water from this spring-fed lake is generally considered to be of pristine quality. Following basic gross solid removal and disinfection, it enters the mains unfiltered. Primary land use in the catchment is grassland farming and it is thought that heavy rains in April 2002 may have increased surface water runoff, facilitating the ingress of animal excrement into the lake. During the outbreak, 26 cases were confirmed, a further seven were reported but not confirmed, and four people were admitted to hospital (O'Toole *et al.*, 2002; Bonner *et al.*, 2002; Report of Waterborne Cryptosporidiosis Subcommittee of the Scientific Advisory Committee, 2004). In 2005, there were two outbreaks of *Cryptosporidium* on the island of Ireland. Between March and May, 30 cases of cryptosporidiosis were confirmed in Carlow and residents were advised to consume only boiled or bottled water for a period of six weeks. In June, a cluster of cases was reported from Ennis, County Clare.

In addition to these larger outbreaks, there have been numerous smaller outbreaks, frequently indistinguishable from stochastic cases. According to the Communicable Disease Surveillance Centre, Northern Ireland, rates of cryptosporidiosis in Northern Ireland have risen since 1997 and in 2000, it was the highest in the UK with 417 reported cases **(Figure 3)**. Since then, it has decreased somewhat, with between 125 and 140 reported cases per annum in the last three years (Communicable Disease Surveillance Centre, N. Ireland website: www.cdscni.org.uk/surveillance/Gastro/Cryptosporidium_sp.htm).

In the Republic of Ireland, cryptosporidiosis in humans became a notifiable disease on January 1, 2004, under new infectious disease legislation. Two hospital studies carried out before that date identified cryptosporidiosis as the second most common cause of childhood gastroenteritis after *E. coli,* with *Cryptosporidium* being detected in 4.0% to 4.3% of stools of children under 12 years old suffering from gastroenteritis (Carson, 1989; Corbett-Feeney, 1987) **(Figure 3)**. According to estimates made by the Health Protection Surveillance Centre for the period before 2004, *Cryptosporidium* accounted for approximately 8% of laboratory-confirmed cases of gastroenteritis in children under two years old. The number of cases reported in the Republic of Ireland since January 2004 can be viewed on the HPSC website (www.ndsc.ie/NotifiableDiseases).

In both jurisdictions, the majority of cases involve children under five years of age. In common with the UK, there is a significant urban-rural divide with a higher incidence amongst the rural population, indicating farmers and animal health workers as occupational risk groups. In addition, private water schemes, more common in remote areas, are known to be at higher risk from contamination than publicly managed water supplies in urban areas (Environmental Protection Agency, 2003). There is also a marked seasonal pattern with a peak in spring/early summer and often a further smaller rise in the autumn (Carson, 1989; Corbett-Feeney, 1987; Moore *et al*., 2002; Communicable Disease Surveillance Centre, 2000; Garvey and McKeoan, 2004; Lowery *et al.*, 2001a). Cryptosporidiosis in animals is not a notifiable disease in either jurisdiction.

In all, there are currently 15 recognised species of *Cryptosporidium* (Xiao *et al*., 2004; Ryan *et al*., 2004; Fayer *et al*., 2005). The main species infecting humans are *C. hominis* and *C. parvum*. Reports of natural infections with *C. hominis* have been restricted to humans so far, with the exception of two cases identified last year in Scottish cattle (Smith *et al.*, 2005). Experimental infections with *C. hominis* have been carried out in neonatal pigs and lambs (Morgan-Ryan *et al.*, 2002). In contrast, *C. parvum* infects most, if not all, mammals including humans and is a major pathogen of calves. Recent studies suggest that a number of other zoonotic species, particularly the avian pathogen, *C. meleagridis*, also cause diarrhoea in humans. Morphologically indistinguishable, the various species are most easily discriminated genetically, mostly by polymerase chain reaction (PCR) combined with restriction fragment

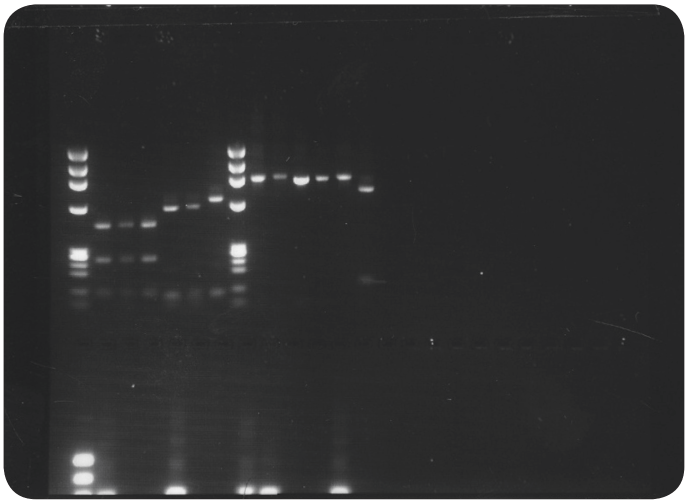

Figure 4: Identification of *C. hominis* (lanes 2, 3 and 5, 6) and *C. parvum* (lanes 4 and 7) by PCR-RFLP. Digestion with the endonuclease SspI results in products of approx 500 and 250bp in both spp. Digestion with the endonuclease VspI renders products of approx 590 (*C. hominis*) and 610 bp (*C. parvum*). Lanes 1 and 8 are molecular weight markers.

length polymorphism (RFLP) analysis (Leng *et al.*, 1996) **(Figure 4)**.

Most work to date on the biology and pathogenicity of *Cryptosporidium* in relation to human infection has been done using *C. parvum*. Therefore, compared to *C. parvum*, little is known of the biology of invasion of the human-restricted *C. hominis*. Recent studies carried out in the Children's Research Centre, Our Lady's Hospital for Sick Children, Crumlin, Dublin, indicate that there is diversity in the mechanisms used by *C. parvum* and *C. hominis* to infect cells of different origin (Hashim *et al.*, 2004; Hashim *et al.*, 2006). More specifically, it was shown that host cell invasion by the two species was mediated by different receptor-ligand interactions. For instance, while a galactose-N-acetylgalactosamine (Gal/GalNAc)-specific sporozoite epitope is crucial for host cell invasion by *C. parvum*, (Chen *et al.*, 2000), Hashim *et al.*(2004) found that *in vitro*, *C. hominis* interacted with potential host cells via a Gal/GalNAc-independent mechanism. These data have important implications for understanding the pathogenesis of cryptosporidiosis and for improved chemotherapy.

The distribution of *C. hominis*, *C. parvum* and *C. maleagridis* in the human population is dependent on the geographical region. For example, in North and South America *C. hominis* appears to be more prevalent (Widmer, *et al.*, 1998; Peng, *et al.*, 1997; Xiao, *et al.*, 2001; Harp, 2003), while in Europe zoonotic infections with *C. parvum* are more common (McLauchlin *et al.*, 1999). In Latin America, the prevalence of *C. meleagridis* may exceed that of *C. parvum* (Xiao *et al.*, 2001; Cama *et al.*, 2003). On the other hand, in the UK this species accounted for only 0.3% of clinical cases (McLauchlin *et al.*, 2000). Generally, it is thought that the prevalence of zoonotic species reflects the importance and type of farming and agriculture in a region. Farming practices, such as pasture grazing, the storage and spreading of slurry/manure, or events such as lambing and calving, all contribute to environmental contamination with oocysts which may enter surface waters either directly or through surface runoff. In fact, it has been suggested that *C. parvum* is more common in areas where a greater proportion of surface water is used in the public water supply (Lowery *et al.*, 2001a). The spring peak of cryptosporidiosis cases is usually attributed to *C. parvum*, as it coincides with the calving and lambing seasons. In contrast, *C. hominis*, a strictly human parasite, is significantly more common in patients infected during the late-summer/autumn peak and in those with a history of foreign travel (McLauchlin *et al.*, 2000). Infections with this parasite are due to contamination with human faeces originating from leaking waste water pipes or septic tanks. Other common routes of transmission are recreational facilities, particularly swimming pools (Report of Waterborne Cryptosporidiosis Subcommittee of the Scientific Advisory Committee, 2004).

Considering the important role of the agricultural sector on this island and the pronounced spring peak, one would assume that *C. parvum* is the most prevalent species in the human population here. Unfortunately, species has been determined in only a very small number of cases. A study of 39 positive samples collected in the greater Belfast area during 1998 reported that *C. parvum* accounted for the greatest number of sporadic cases: 87.2% (Lowery *et al.*, 2001a). However, two of the three outbreaks in the same geographical area between April 2000 and 2001 were ascribed to *C. hominis* and only one to *C. parvum* (Glaberman *et al.*, 2002). To date, there has been no report of *C. meleagridis* infection in Ireland. Clearly, in order to improve our understanding of the epidemiology of the parasite, there is an urgent need for a reference facility to provide active surveillance, including routine typing.

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