Important zoonotic intestinal protozoan parasites in Asia

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Abstract. Intestinal protozoa are increasingly being studied because of their association with acute and chronic diarrhoea in immunocompromised as well as immunocompetent patients. Various community outbreaks due to contamination of water or food with these protozoa have further highlighted their importance in public health. Among these important pathogens are *Giardia duodenalis*, *Entamoeba histolytica*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli*, and microsporidia. Except for the cyst-forming *G. duodenalis* and *E. histolytica*, the others are intracellular and form spores which are passed out with the faeces. These organisms are also found in various animals and birds and zoonotic transmission is thought to occur. These infections are distributed worldwide, with a higher prevalence in developing compared to developed countries. However, the relative importance of zoonotic infections especially in developing countries has not been studied in detail. The prevalence rates are generally higher in immunodeficient compared to immunocompetent patients. Higher prevalence rates are also seen in rural compared to urban communities. Most studies on prevalence have been carried out in developed countries where the laboratory and other health infrastructure are more accessible than those in developing countries. This relative inadequacy of laboratory diagnosis can affect accurate estimates of the prevalence of these infections in developing countries. However, reports of these infections in travellers and workers returning from developing countries can provide some indication of the extent of these problems.

Most studies on prevalence of amoebiasis in developing countries were based on morphological identification of the parasite in faecal smears. As the pathogenic *E. histolytica* is morphologically indistinguishable from that of non-pathogenic *E. dispar*, estimates of amoebiasis

may not be accurate. The epidemiology of human microsporidia infections is not completely understood. Two species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, are associated with gastrointestinal disease in humans and it is believed that human to human as well as animal to human infections occur. However, the importance of zoonotic infections has not been fully characterised. *G. duodenalis* cysts, microsporidia and *Cryptosporidium* oocysts have been detected in various ground water resources, but their role in community outbreaks and maintenance of the infection has not been fully characterised. The taxonomic classification and pathogenic potential of *B. hominis* are still controversial. While considered by many as yeast, fungi or protozoon, recent sequence analysis of the complete SSUrRNA gene has placed it within an informal group, the stramenopiles. This review covers recent published data on these zoonotic infections and examines their public health importance in Asian countries.

INTRODUCTION

A number of intestinal protozoa are being increasingly subjected to further study because of their association with acute and chronic diarrhoea not only in immunocompromised patients but also in immunocompetent individuals. Community outbreaks of diarrhoea caused by contamination of water or food with these protozoa have further highlighted their importance. Among the important pathogens are *Giardia duodenalis*, *Entamoeba histolytica*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli*, and microsporidia. Except for *G. duodenalis* and *E. histolytica*, the others are intracellular and form spores which are released into the intestinal lumen and passed out in the faeces. Except for *E. histolytica*, *C. cayetanensis* and *I. belli*, these organisms are also found in multiple animals and birds and zoonotic transmission is thought to occur. *G. duodenalis*, *C. parvum*, microsporidia, and *B. hominis* will be considered further in this review. It is generally believed that although these parasitic infections are distributed worldwide, their prevalence is higher in developing compared to developed countries. However, the relative importance of zoonotic infections especially in developing countries has not been studied in

detail. The prevalence rates are generally higher in immunodeficient compared to immunocompetent patients. However, most studies on prevalence have been carried out in developed countries where the laboratory and clinical infrastructure are more easily available than those in developing countries. For example, the prevalence of *C. parvum* (excluding HIV/AIDS patients) in developed countries was estimated to be 4.9% compared to 7.9% in undeveloped countries (Current & Garcia, 1991).

Diarrhoea, especially chronic diarrhoea is associated with AIDS but about half the time no specific aetiology can be found for the diarrhoea in Africa (Grant & De Cock, 2001). Among the identified causes the most common are cryptosporidiosis, microsporidiosis, isosporiasis, and bacterial infections. In Brazil, Cimerman *et al.* (1999) found 40% of 200 HIV positive patients to have intestinal parasitic infections, the prevalence rates being 16% for *Giardia lamblia*, 7% for *C. parvum*, 2% for *I. belli* and 0.5% for *B. hominis.*

Gassama *et al.* (2001) studied the relationship between HIV status, diarrhoea and enteropathogens and found that in 121 HIV-ve adults with diarrhoea parasite pathogens were *E. histolytica* (10.7%), and *G. lamblia* (4.9%). In HIV patients *Microsporidium* (9.4%), *Cryptosporidium* (8.2%), *E. histolytica* (5.1%), and *I. belli* (4.4%) were involved. *B. hominis* was 2.5% in HIV patients with diarrhoea and 0.6% in those without diarrhoea.

This review will examine recent published data on these pathogens in Asian countries and analyse their current importance to public health. Data on the prevalence of these infections in domestic and wild animals and evidence on zoonotic sources of transmission will be examined.

IMPORTANT INTESTINAL PROTOZOAN INFECTIONS

Amoebiasis

The World Health Organization (WHO) estimates that there were 48 million new cases and 70,000 deaths due to *E. histolytica* in 1997 (WHO, 1998). In Malaysia Noor Hayati *et al.* (1998) examined aborigines in Perak and found prevalence rates of 11.5% with *E. histolytica*. In an

earlier study among 300 hospitalised patients the prevalence rate was 2.7% in 2001 compared to 3.4% in 198 patients examined in 1981 (Nor Hayati *et al.*, 2003). Noor Hayati *et al.* (1995) reported finding 16 out of 196 (8.2%) duodenal aspirates from immunocompetent patients undergoing endoscopy in Kuala Lumpur to be positive for *E. histolytica* cysts. *E. histolytica* prevalence rates in HIV patients may be lower than those in HIV –ve adults in some studies. Gassama *et al.* (2001) reported more than twice the prevalence rate in Senegalese HIV-ve patients (10.7%) with diarrhoea compared to HIV +ve patients (5.1%) with diarrhoea. All these studies were based on morphology of the parasites and as pathogenic *E. histolytica* is morphologically indistinguishable from that of non-pathogenic *E. dispar*, estimates of amoebiasis may not be accurate. A further complication is that molecular studies on parasites in asymptomatic infections show that *E. histolytica* and *E. dispar* may co-exist in some highly endemic areas as in parts of Mexico (Ramos *et al.*, 2000).

Pai *et al.* (2003) showed that *E. histolytica/ E. dispar* cysts were found on the cuticle and/or digestive tract of 25.4% of 299 American cockroaches (*Periplaneta americana*) and from the digestive tract of 10.3% of 29 German cockroach (*Blattella germanica*) from 11 urban primary schools in Taiwan. This suggests that cockroaches may play a potential role in the mechanical dissemination of amoebiasis.

The natural host range of *E. histolytica* is limited; humans and some non-human primates are the known natural hosts (Stanley, 2003). As there is hardly any zoonotic transmission, amoebiasis will not be discussed further in this review.

Giardiasis

The global incidence of giardiasis is estimated to be 500,000 new cases in 1997 (WHO, 1998). Human infections with *G. duodenalis* belong to two genotypes, A and B based on specific signal sequences in the 5' end of the small subunit (16S) ribosomal RNA gene (van Keulen *et al.* (2002). As they are found in faecal samples from domestic and wild animals and in the

environment, these two genotypes are widespread and possibly zoonotic. It is now shown that the re-emergence of zoonotic human giardiasis corresponds mainly to the genotypes A-I (Ambroise-Thomas, 2000) and to a lesser extent to genotype B (Thompson, 2000). There may also be a correlation between these genotypes and pathogenicity (Ambroise-Thomas, 2000).

The major source of infection is through faecal-oral route (Upcroft & Upcroft, 2001) although large outbreaks have been through contaminated water.

Recent studies show 19.2% of 917 Malays from villages in Trengganu, Malaysia to be positive for *G. duodenalis* (Norhayati *et al.*, 1998). The infection rates for those 20 years and below were greater that 20%, the rates being 21.8%, 23.4% and 20.8% for the age groups 2-6, 7-12, and 13-20 years respectively. The prevalence rate for those ≥ 21 years was 12.2%. Risk factors identified were age ≤ 12 years old and other family members infected with the parasitic infection. Another study by Noor Hayati *et al.* (1998) among Orang Asli (aborigines) in Perak, Malaysia showed very similar prevalence rate (19.4%). However, the prevalence rates in patients admitted to hospital in 1981 and 2001 were 2.8% and 0.7% respectively (Norhayati *et al.*, 2003). Noor Hayati *et al.* (1995) found 6 out of 196 (3.1%) duodenal aspirates from immunocompetent patients undergoing endoscopy to be positive for cysts or trophozoites of *G. duodenalis*.

Ahmad (1995) studied the occurrence of pathogenic protozoa in Malaysian water resources and detected 15 out of 76 (19.7%) positive for *Giardia* cysts.

Taken as a whole these studies show that giardiasis though more common in rural areas, is also present among other communities and the high proportion (about 20%) of water resources positive for *G. duodenalis* cyst, indicates this to be a possible route of transmission. The source of contamination of these water resources has not been studied. Zoonotic sources of contamination have not been determined. In view of the resistance of the cyst to withstand normal chlorination, the high prevalence of infected water resources is a cause of concern.

Bhatti *et al.* (1999) reporting on the pathogens associated with diarrhoea in Pakistan lamented on the lack of basic laboratory techniques and demonstrated how the introduction of basic microscopy of stools (direct and after concentration) resulted in detection of *G. duodenalis* in 51% (52 out of 98) of stools from cancer patients.

Microsporidiosis

Microsporidia belongs to a huge phylum with about 150 genera and over 1000 species (Franzen & Muller, 2001), infecting almost every kind of insect and animal studied (James, 1997). However, only *Enterocytozoon*, *Encephalitozoon* (including *Septata*), *Pleistophora*, *Trachipleistophora*, *Vittaforma*, *Brachiola* and *Nosema* have been reported in humans (Franzen & Muller, 2001). These are eukaryotic spore forming obligate intracellular protozoan parasites that are common in the environment and have been found in water supplies (Weiss, 2001). No genetic evidence of variability has been demonstrated in the twelve species able to infect humans (Ambroise-Thomas, 2000). There has been a dramatic increase in microsporidiosis in humans in the last 20 years in tandem with the AIDS pandemic (Ambroise-Thomas, 2000). However, the spectrum of clinical presentation ranges from silent carriers in the immunocompetent to severe infections in the immunodepressed patients. The epidemiology of human microsporidia infections is not completely understood but it is believed that human to human as well as animal to human infections occur. However the importance of zoonotic infections has not been fully characterised. Two species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, are associated with gastrointestinal disease in humans.

Dowd *et al.* (1998) confirmed for the first time, the presence of *E. intestinalis* in tertiary sewage effluent, surface water, and groundwater; *E bieneusi* in surface water; and *Vittaforma corneae* in tertiary effluent. The study therefore provided evidence that these are waterborne pathogens.

Although the reservoirs and the modes of transmission of the most frequent microsporidia species in humans, *E. bieneusi*, are still unknown, Dengjel *et al.* (2001) have shown on the basis of molecular phylogenetic analysis of this parasite from humans, cats, pigs, and cattle, the lack of a transmission barrier between *E. bieneusi* from humans and these animals. Buckholt *et al.* (2002) found 32% of pigs positive for *E. bieneusi*, three isolates being identical in their ribosomal internal transcribed spacer sequence to human type D organism. Thus, *E. bieneusi* appears to be a zoonotic pathogen. *E. bieneusi* (genotype 'J') has been reported from chickens and the latter may therefore be another source of human infection (Reetz *et al.*, 2002).

E. intestinalis has been shown to occur in donkey, pig cow and goat, suggesting the possibility that this may be zoonotically transmitted (Bornay-llinares *et al.*, 1998).

Infection of an AIDS patient with *Encephalitozoon cuniculi* III which is identical to that isolated from domestic dogs (Didier *et al.*, 1996) also provides evidence of the zoonotic potential of the organism.

Microsporidia infections are commonly seen in immunosuppressed patients like AIDS and Gumbo *et al.* (1999) reported on four patients who received solid organ transplants and developed microsporidia infections. The microsporidian protozoon *E. bieneusi*, first recognised as a cause of chronic diarrhoea in AIDS patients in 1985, was described in the small bowel of a Haitian patient with AIDS (Desportes-Livage, 1996). Extraintestinal localization is uncommon, but *E. bieneusi* can spread to the biliary ducts and nasopharyngeal epithelium, thereby causing cholangitis and rhinosinusitis (Kotler & Orenstein, 1998; Pol *et al.*, 1993). Respiratory tract microsporidiosis has rarely been reported and is mainly due to *Encephalitozoon* sp. (Scaglia *et al.*, 1998). Pulmonary involvement by *E. bieneusi* has been documented for patients with intestinal microsporidiosis (Botterel *et al.*, 2002; de Aquila *et al.*, 1997; Weber *et al.*, 1992).

In Singapore microsporidial keratoconjunctivities has been reported and the authors are of the view that it may be more common than expected in healthy non-immunocompromised individuals (Chan *et al.*, 2003).

It appears that exposure to microsporidia is common as a high seroprevalence against *E. intestinalis* was found in Dutch blood donors (8% of 300) and pregnant French women (5% of 276) who were all presumably immunocompetent (van Gool *et al.*, 1997).

The availability of sensitive and specific methods to detect microsporidia is crucial in estimating the realistic prevalence of microsporidiosis. An excellent review of the laboratory methods in the detection of microsporidia is available (Garcia, 2002). While the microscopical examination of stained faecal specimens is usually used, a PCR-based assay for the detection of microsporidia in sodium hypochlorite-treated stool specimens has been developed. Fedorko *et al.* (1995) used a single primer pair complementary to conserved sequences of the small-subunit rRNA to amplify DNA from the four major microsporidian pathogens of humans: *E. cuniculi*, *Encephalitozoon hellem*, *E. bieneusi*, and *Septata intestinalis*. Differentiation of the microsporidian gastrointestinal pathogens *E. bieneusi* and *S. intestinalis* could be accomplished by restriction endonuclease digestion of PCR products using *Pst*I and *Hae*III.

Ignatius *et al.* (1997) has developed a modified acid-fast Trichrome staining method to demonstrate both acid-fast oocysts of *C. parvum* and other coccidia, as well as microsporidia spores. This acid-fast Trichrome stain yields results comparable to those obtained by the Kinyoun and modified Trichrome methods and considerably reduces the time necessary for microscopic examination.

In a study of 95 Thai HIV+ve and 87 HIV-ve children aged 13.4 ± 13.2 and 13.7 ± 12.9 years respectively, admitted for diarrhoea, 24(25.3%) and 13 (14.9%) had intestinal microsporidiosis (Wanachiwanawin *et al.*, 2002). The difference in infection rates was not significant ($p = 0.122$). Five of the isolates were identified as *E. bieneusi*. In an earlier study among hospitalised HIV+ve children aged < 15 years in Bangkok, Leelayoova *et al.* (2001) found 9 of 83 (10.8%) and 1 of 58 (1.72%) of those with and without diarrhoea to be positive for microsporidia, the difference being statistically significant ($p<0.05$). The authors used calcofluor fluorescent stained samples for screening followed by gram-chromotrope stained slides for

confirmation. TEM confirmation was used as the gold standard and infections were found to be due to *E. bieneusi.* In another study among 66 AIDS patients with chronic diarrhoea 22 (33.3%) were positive for microsporidia spores (Wanachiwanawin *et al.*, 1998). TEM confirmed *E. bieneusi* in 18 of 22 positive specimens examined.

Kumar *et al.* (2002) could only find 1 out of 59 (1.7%) HIV+ve patients with chronic diarrhoea positive for microsporidia. None of 50 normal persons was infected.

The above studies show that *E. bieneusi* appears to be the predominant microsporidium responsible for diarrhoea in Asia.

Blastocystosis

The taxonomic classification as well as the pathogenicity of *Blastocystis hominis* is still controversial. While considered by many as a yeast, fungi or protozoon, data from sequence analysis of the complete SSUrRNA gene has placed it within an informal group, the stramenopiles (Siberman *et al.*, 1996). This is a heterogeneous group of unicellular and multicellular protists, including brown algae, diatoms, water moulds, etc. A recent view of its life cycle has been proposed by workers in Singapore (Singh *et al.*, 1995).

The organism is considered by many as capable of causing diarrhoea, but a recent case-control study of 99 individuals stool-positive for *B. hominis* and 193 matched controls, could not demonstrate pathogenicity based on the association with the development of gastrointestinal symptoms or pathologic findings on endoscopic examination (Chen *et al.*, 2003). However, it is associated frequently with diarrhoea in immunosuppressed patients like AIDS and organ transplant patients; it has been reported in renal transplant patients in India (Rao *et al.*, 2003). In Thailand, 2 of 58 (3.4%) of HIV+ve children hospitalised for diarrhoea were positive for the parasite (Leelayoova *et al.*, 2001).

A number of studies on blastocystosis have been carried out in Malaysia. A prevalence rate of 0.3% was found in 300 hospitalised patients in 2001 (Nor Hayati *et al.*, 2003). Noor Hayati *et al.* (1995) found 2 out of 196 (1.0%) duodenal aspirates from immunocompetent patients undergoing endoscopy in Kuala Lumpur to be positive for *B. hominis* cysts.

Suresh *et al.* (1996) studied laboratory animals and found all 5 each of Wistar, SHR, and Sprague Dawly rats to be positive for *B. hominis* infection based on *in vitro* culture of faeces in Jones medium. Two out of 5 (40%) each of PVG and Dark Agoti rats were positive. In contrast all 10 each of laboratory mice (ICR, CBA, Balb/c and C3HCJ) examined were negative. Of 5 each of sheep, Guinea pig and hamster examined, only 1 (20%) sheep was positive. All 10 *Macaca fasicularis*, 2 of 10 (20%) rabbits, and none of the 2 dogs and 2 cats were positive.

In Japan, Abe *et al.* (2002) examined various livestock, pets and zoo animals in Osaka, for *B. hominis* and found variable infection rates. Farm animals had high prevalence rates, these being 95% (58/61) in pigs and 71% (39/55) in cattle. Primates, pheasants, and ducks had infection rates of 85% (29/34), 80% (8/10), and 56% (9/16) respectively. None of 58 various carnivores and herbivores was positive.

The above studies show that there is still controversy as to whether *B. hominis* is a pathogen in animals and humans. Based on prevalence surveys asymptomatic human infections can occur, and most domestic and wild animals examined carried organisms very similar in morphology to those recovered from humans with or without diarrhoea.

Cryptosporidiosis

Cryptosporidium parvum is a cause of diarrhoea in both immunocompromised and immunocompetent persons. The infective stage, the oocyst, is 5 μm in diameter and contains four sporozoites each measuring 5 x 1 μm. It is highly infectious and as low as 30 oocysts can cause infection in healthy volunteers (DuPont *et al.*, 1995).

Various modes of transmission have been identified among which are consuming contaminated water and food, through recreational water activities, close person-to-person contact, and through zoonotic sources. Hospital cross infections have been reported (Baxy *et al.*, 1983; Koch *et al.*, 1985). Large outbreaks due to contamination of water supply have been documented in recent years and contamination of the water-treatment plant in Milwaukee was estimated to result in 403,000 people with diarrhoea (Mac Kenzie *et al.*, 1994). However, Goldstein *et al.* (1996) in a case-control study showed that a cryptosporidiosis outbreak was associated with municipal drinking water, despite state-of-the-art water treatment and water quality better than that required by current federal standards in the USA, thus highlighting the importance of surveillance for cryptosporidiosis and the need for guidelines for the prevention of waterborne-*Cryptosporidium* infection among HIV-infected persons. Of the 78 laboratory-confirmed cases in the first quarter of 1994, 61 (78.2%) were in HIV-infected adults. In the case-control study, persons who drank any unboiled tap water were four times more likely than persons who drank only bottled water to have cryptosporidiosis (odds ratio = 4.22; 95% CI = 1.22 to 14.65; P = 0.02). For persons with $CD4^+$ cell counts less than 100 cells/mm^3, the association between tap water and cryptosporidiosis was even stronger (odds ratio = 13.52; CI = 1.78 to 102.92; P = 0.01). Additionaldata indicate that this outbreak also affected persons who were not infected with HIV.

In Malaysia a study among 300 hospitalised patients showed an infection rate of 0.3% with *Cryptosporidium* sp. (Nor Hayati *et al.*, 2003). Leelayoova *et al.* (2001) found the infection in 8 of 83 (9.6%) and 2 of 58 (3.4%) of HIV-infected children with and without diarrhoea respectively in Bangkok, Thailand to be positive. The first report of *C. parvum* infecting animals in Malaysia was the report by Lee *et al.* (1989) who found 4 out of 11 calves in Johore, with diarrhoea to have the organism.

Ahmad (1995) detected 8 out of 76 (10.5%) samples of Malaysian water resources to be positive for *Cryptosporidium* sp. oocysts. In another study (Lim *et al.*, 1999) conducted viability

assays based on morphology and the exclusion and inclusion of fluorogenic vital dyes, and showed that *C. parvum* oocysts do not survive long in tropical climates; almost all oocysts will die after about 30 days of exposure in the river environment and in 56 days in the soil environment. Most oocysts in the soil or water were killed within 1-2 months of exposure.

Using direct and concentration techniques Bhatti *et al.* (1999) reported that 3.2% (1 out of 31) stool samples from a cancer hospital in Pakistan to be positive for *Cryptosporidium* sp.

Kumar found 14 out of 102 (13.7%) HIV+ve with acute or chronic diarrhoea with *Cryptosporidium* compared to 4 out of 50 (8.0%) HIV +ve without diarrhoea. None of the 50 normals was infected.

In a study of acute diarrhoea in 160 children 5 years and below in Nepal, *Cryptosporidium* was detected in 9 (5.6%) (Shariff *et al.* 2002). All 50 control children were negative. In another study in Nepal in children aged 1 month to 15 years there was no difference in prevalence rates between the pre-monsoon (April and May) and monsoon (July and August) periods, the rates being 3.1% (6 out of 195) and 5.6% (11 out of 195) respectively (Oda *et al.*, 1998).

Jelinek *et al.* (1997) examined 469 travellers returning to Germany with diarrhoea and detected 13 (2.8%) infected with *Cryptosporidium.* Of these 13, 4 (30.8%) had visited India and 3 (23.1%) had visited Malaysia and Thailand.

***Cyclospora cayetanensis* infection**

Cyclospora cayetanensis is an obligate intracellular parasite, infecting the cells of the upper portion of the small intestine. The spherical oocyst measuring 8-10 µm in diameter has two sporocysts each with two sporozoites. Infection is through contaminated food and water, causing severe recurrent diarrhoea especially in immunocompromised patients.

Only 1 out of 50 HIV+ve patient with chronic diarrhoea was found to have *C. cayetanensis* infection (Kumar *et al.*, 2002).

Of 469 German patients with travellers' diarrhoea 5 (1.1%) were infected with *C. cayetanensis*. Of these 5, one (20%) was probably infected in Thailand (Jelinek *et al*., 1997).

Humans are the only known hosts for *C. cayetanensis* with the exception of some monkeys and baboons (Eberhard *et al*., 1999a). Thus it will not be considered further in this review.

Isosporidiosis

Human infection with *Isospora belli* can result in severe diarrhoea especially in the immunocompromised. Like cryptosporidiosis and cyclosporiasis, this parasite is an obligate intracellular parasite of the epithelial cells of the small intestine. The infective stage is the ellipsoidal oocyst measuring 20-30 µm. The mature cyst contains two sporocyst each with four sporozoites.

Mukhopadhya *et al.* (1999) studied 111 consecutive HIV +ve southern Indians with and without diarrhoea, using stool microscopy, and culture methods. If diarrhoea persisted with negative stool examination, jejunal biopsy and fluid examination were performed. They found *I. belli* infection in 11 out of 61 (18.0%) patients with diarrhoea and 2 out of 50 (4.0%) in those without, the difference being statistically significant.

Using direct and concentration techniques Bhatti *et al.* (1999) reported 2.8% (2 out of 98) stool samples in cancer patients in Pakistan to be positive for *I. belli.*

Kumar *et al.* (2002) found coccidian parasites to be important agents of diarrhoea, especially chronic diarrhoea in HIV positive patients. *I. belli* was the most frequent coccidian parasite, being found in 14 of 102 (13.7%) patients with acute and chronic diarrhoea. *Cryptosporidium* was next being seen in 7 patients with acute and chronic diarrhoea, and in 4 patients without diarrhoea. *C. cayetanensis* and *E. bieneusi* was each seen in an HIV patient with chronic diarrhoea.

As it is believed that *I. belli* infection is mainly a human infection and transmission is through faecal contamination of water and food from a human source, the infection will not be considered further here.

Garcia *et al.* (2003) evaluated the ImmunoCard STAT! *Cryptosporidium*/*Giardia* rapid assay (Meridian Bioscience, Inc.) which is a solid-phase qualitative immunochromatographic assay that detects and distinguishes between *G. lamblia* and *C. parvum* in aqueous extracts of human faecal specimens (fresh, frozen, unfixed, or fixed in 5 or 10% formalin or sodium acetate-acetic acid-formalin). On the basis of the results of the reference methods, the sensitivities, specificities, and positive and negative predictive values were as follows: for *G. lamblia*, 93.5, 100, 100, and 95.5%, respectively; for *C. parvum*, 98.8, 100, 100, and 99.7%, respectively. False-negative results for *G. lamblia* were obtained with specimens with low parasite numbers or specimens containing trophozoites only; one specimen with a false-negative result contained numerous cysts. The one specimen false negative for *C. parvum* was confirmed to be positive by immunofluorescence. No cross-reactivity was seen with 10 different protozoa (152 challenges), nine different helminths (35 challenges), or human cells (4 challenges) found in faecal specimens. This rapid test system may be very beneficial in the absence of trained microscopists; however, for patients who remain symptomatic after a negative result, the ova and parasite examination and special stains for other coccidia and the microsporidia should always remain options.

Conventional microscopy will continue to be used in most developing countries and may the most practical in diagnostic laboratories. The recent technique of prestaining the faecal specimen with Loeffler's methylene blue followed by trichrome for the staining and screening of microsporidia (Sianongo *et al.*, 2001) is one such example.

Molecular techniques will play a greater role in epidemiology of giardiasis (Thompson, 2000) and other diarrhoea pathogens to trace and confirm the source of infection, as well as to understand the dynamics of transmission.

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