Current status and future trends in *Cryptosporidium* and *Giardia* epidemiology in Malaysia

Y. A. L. Lim, R. A. Ahmad and H. V. Smith

ABSTRACT

Cryptosporidium and *Giardia* are major causes of diarrhoeal diseases of humans worldwide, and are included in the World Health Organisation's 'Neglected Diseases Initiative'. *Cryptosporidium* and *Giardia* occur commonly in Malaysian human and non-human populations, but their impact on disease, morbidity and cost of illness is not known. The commonness of contributions from human (STW effluents, indiscriminate defaecation) and non-human (calving, lambing, muck spreading, slurry spraying, pasturing/grazing of domestic animals, infected wild animals) hosts indicate that many Malaysian environments, particularly water and soil, are sufficiently contaminated to act as potential vehicles for the transmission of disease. To gain insight into the morbidity and mortality caused by human cryptosporidiosis and giardiasis, they should be included into differential diagnoses, and routine laboratory testing should be performed and (as for many infectious diseases) reported to a centralised public health agency. To understand transmission routes and the significance of environmental contamination better will require further multidisciplinary approaches and shared resources, including raising national perceptions of the parasitological quality of drinking water. Here, the detection of *Cryptosporidium* and *Giardia* should be an integral part of the water quality requirement. A multidisciplinary approach among public health professionals in the water industry and other relevant health- and environment-associated agencies is also required in order to determine the significance of *Cryptosporidium* and *Giardia* contamination of Malaysian drinking water. Lastly, adoption of validated methods to determine the species, genotype and subgenotype of *Cryptosporidium* and *Giardia* present in Malaysia will assist in developing effective risk assessment, management and communication models.

Key words | *Cryptosporidium*, genotype, *Giardia*, public health, species, waterborne

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INTRODUCTION

Cryptosporidium and *Giardia* are ubiquitous enteric protozoan pathogens which infect humans, domestic and feral animals, worldwide. They are important diarrhoeal disease-causing organisms in man, especially children, leading to significant morbidity and mortality in both the developing and developed world. *Cryptosporidium* and *Giardia* can be transmitted to humans through any mechanism by which material that is contaminated with faeces containing infectious *Cryptosporidium* oocysts or *Giardia* cysts [(oo)cysts] is swallowed by a susceptible host.

At least seven species (*C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. suis*, and *C. muris*) and two genotypes (monkey and cervine) of *Cryptosporidium*, are associated with human disease. Two of *G. duodenalis* assemblages (A and B) are associated with human disease (Table 1). Person-to-person transmission of *Cryptosporidium*

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Table 1 | Species of *Cryptosporidium* and *Giardia*

Cryptosporidium*		***Giardia*†**	
Species	**Major host**	**Species‡**	**Major host**
C. hominis	Humans, monkeys	*G. duodenalis* (Assemblage A)	Humans and other primates, dogs, cats, livestock, rodents and other wild mammals
C. parvum	Cattle, other ruminants, humans	*G. duodenalis* (Assemblage B - *G. Enterica*)	Humans
C. andersoni	Cattle	*G. duodenalis* (Assemblage C - *G. canis*)	Dogs
C. muris	Rodents	*G. duodenalis* (Assemblage E - *G. bovis*)	Cattle, other hoofed livestock
C. suis	Pigs	*G. duodenalis* (Assemblage F - *G. cati*)	Cats
C. felis	Cats	*G. duodenalis* (Assemblage G - *G. simondi*)	Rats
C. canis	Dogs	*G. agilis*	Amphibians
C. wrairi	Guinea pigs	*G. muris*	Rodents
C. bailey	Poultry	*G. microti*	Muskrats, voles
C. meleagridis	Turkeys, humans	*G. psittaci*	Birds
C. bovis	Cattle, sheep	*G. ardeae*	Birds
C. galli	Finches, chicken		
C. serpentis	Reptiles		
C. saurophilum	Lizard		
C. molnari	Fish		
C. scophthalmi	Fish		

*Details in Xiao *et al*. (2004).
†Details in Thompson & Monis (2004).
‡Other criteria used to distinguish *Giardia* species include trophozoite morphology by electron microscopy, host specificity and molecular taxonomy. Thompson & Monis (2004) propose that the *G. duodenalis* assemblages should be reclassified into six separate species (see Thompson & Monis 2004 for discussion).

and *Giardia* is regarded as the major route, and secondary cases and asymptomatic excretors may be a source of infection for other susceptible persons. Currently, the contributions from other transmission routes remain unclear in Malaysia, but the use of appropriate descriptive epidemiological and molecular epidemiological tools should help us determine the significance of direct (person-to-person, anthropozoonotic, zoonotic, etc.) and indirect (waterborne, foodborne, airborne, zoonotic, fomites, etc.) transmission routes in endemic and non-endemic settings (Cacciò *et al*. 2005). Without the use of appropriate tools for investigating both clinical and environmental settings, the relative significance of different transmission routes cannot be determined (Cacciò *et al*. 2005; Smith *et al*. 2006).

Animals can be infected, experimentally, with (oo)cysts of human origin, and may acquire infection naturally from man. Cryptosporidiosis and giardiasis have been reported in domestic animals, livestock and wildlife, including companion animals which may be reservoirs of human infection. Human cryptosporidiosis can be acquired from non-human hosts, but the role of animals in transmitting *G. duodenalis* to humans and the most likely routes of infection remain unclear. The potential for zoonotic transmission is clear, and the broad host range exemplified by *C. parvum* and *G. duodenalis* Assemblages A and B (Table 1) together with the high output of infective (oo)cysts from numerous mammalian hosts ensure a high level of environmental contamination.

Environmental (indirect) routes of transmission include all vehicles which contain sufficient numbers of infectious (oo)cysts to cause human infection, the most commonly recognized vehicles being water and food. The importance of environmental transmission is augmented by the robustness and disinfection insensitivity of their transmissive

stages [(oo)cysts]. *Cryptosporidium* and *Giardia* are well recognised aetiological agents of waterborne outbreaks of disease, with more than 120 waterborne outbreaks worldwide causing gastrointestinal tract infections in over 600 million people (Smith & Lloyd 1997; Smith *et al*. 1997; Smith & Rose 1998).

The largest waterborne *Cryptosporidium* outbreak occurred in Milwaukee, USA in the early spring of 1993 when approximately 1.5 million consumers were exposed to *Cryptosporidium* contamination of the public water supply. Of these consumers, 403 000 became ill and 104 died (MacKenzie *et al*. 1994). The total cost of outbreak-associated illness was $96.2 million, and the average total costs for persons with mild, moderate, and severe illness were $116, $475, and $7,808, respectively (Corso *et al*. 2003).

In Malaysia, studies on *Giardia* were first documented in the scientific literature in 1970 (Bisseru & Aziz 1970) but it was not until 1984 that *Cryptosporidium* was first documented in a young male presenting with diarrhoea (Che Ghani *et al*. 1984). Since this time, published work on *Cryptosporidium* and *Giardia* has increased considerably, peaking in the 1990s.

The possibility of zoonotic transmission increases the number of (oo)cysts in our environment, and as both the robustness and disinfection insensitivity of (oo)cysts also favour environmental transmission, the challenge to unravel the zoonotic potential of these parasites in Malaysia has driven studies of *Cryptosporidium* and *Giardia* in animal populations and environmental samples, especially in water. This review highlights current knowledge on the occurrence of these protozoan parasites in human and non-human hosts and assesses their significance in Malaysia, particularly in water.

Taxonomy of *Cryptosporidium* and *Giardia*

Molecular biology has provided powerful new tools for characterising *Cryptosporidium* and *Giardia*, and the analysis of previously unrecognised genetic differences within these genera has revolutionised both their taxonomy and our understanding of the epidemiology of human disease. Descriptions of *Cryptosporidium* and *Giardia* species by early workers relied largely on host occurrence which resulted in the description of a large number of species and a history of taxonomic confusion and controversy (O'Donoghue 1995; Thompson 2002). The lack of morphological characters to differentiate variants led to much debate over whether phenotypic differences were 'real' and reflected genetic differences, or were the result of environmental- or host-induced changes (Monis & Thompson 2003). Molecular tools have revealed that *Giardia* and *Cryptosporidium* are a phenotypically and genotypically heterogeneous assemblage of species and genotypes which are largely morphologically identical (Thompson 2004; Thompson & Monis 2004; Xiao *et al*. 2004). Molecular approaches enable the direct characterization of (oo)cysts recovered from faecal or environmental samples, thus avoiding any potential bias due to preferential amplification of genotypes by *in vitro* culture or animal models.

We now have a better understanding of *Cryptosporidium* and *Giardia* species, many of which have distinct host specificities and are host adapted (Table 1). In addition, there is growing evidence for differences between species and genotypes within *Giardia* and *Cryptosporidium* with respect to parasite development, growth rates, drug sensitivity and other phenotypic characteristics, in addition to disease presentation.

Currently, the genus *Cryptosporidium* consists of 16 species (Table 1), with over 40 genotypes which show varying genetic diversity, in some cases greater than that observed between named species (Xiao *et al*. 2004). Similarly, *Giardia* is classified into six species, with further genetically distinct assemblages within *G. duodenalis*, which are likely to represent different species (Thompson & Monis 2004) (Table 1).

Although previous use of host occurrence, morphology and morphometry to define species caused confusion, it generated a nomenclature which can be used currently, although as a result of the lack of 'type' (i.e. reference) material for comparison (Thompson & Monis 2004; Xiao *et al*. 2004), a degree of supposition is required to make some of these associations. The taxonomy of both *Cryptosporidium* and *Giardia* remains only partially resolved, and the species status of genetic variants of both parasites (especially in the absence of other data) will be an important and controversial future issue to resolve.

Cryptosporidium, *Giardia* and the World Health Organisation (WHO)

In many regions of the world, *Giardia* and *Cryptosporidium* constitute part of the complex group of parasitic, bacterial and viral diseases which impair the ability to achieve full potential and impair development and socio-economic improvements. All diseases included in the WHO Neglected Diseases Initiative have a common link with poverty and, as the current view is to take a comprehensive approach to all these diseases, both *Giardia* and *Cryptosporidium* were included in the 'Neglected Diseases Initiative' in 2005. Major hopes were that molecular methods would generate significant insight into *Giardia* and *Cryptosporidium* biology and host–parasite interactions (Savioli *et al*. 2006).

Cryptosporidium and *Giardia* in Malaysian humans

Malaysia (Figure 1, population ~27 million) is a multi-ethnic society, the major ethnic groups being Malays and the tribal peoples of Sarawak and Sabah, who have the political and cultural status *Bumiputra* (sons of the soil), Chinese, and Indians, accounting for 61%, 25% and 7% of the population, respectively. Different ethnicities are distributed throughout Malaysia, but in general, Malays predominate in rural areas, while Chinese are concentrated in urban areas. Health statistics identify a total of 533,600 live births and 65,257 cases of digestive system diseases in 2003 (Anon 2005*a*).

The population living in urban and rural settings is 45% and 55%, respectively, with poverty being consistently higher in rural than in urban settings. The reduction in the poverty rate (from 22.8% in 1990 to 5.7% in 2004) is due to increased provision of basic amenities (health services, education and housing), other infrastructure and utilities. The less industrialized states (Kelantan, Terengganu, Perlis, Kedah and Sabah), with their heavy dependence on agriculture and fishing, have a higher incidence of poverty than more industrialized states (Selangor, Pulau Pinang and Negeri Sembilan). While the achievements of poverty eradication programmes are impressive, areas and communities beset with hardship and poverty remain. In Sabah and Sarawak indigenous groups living in the interior are among the worst affected. Among indigenous minorities in peninsular Malaysia, the Orang Asli with their traditional culture, remain desperately poor despite attempts to alleviate poverty. The Orang Asli generally possess a lower economic status and a lack of knowledge and information

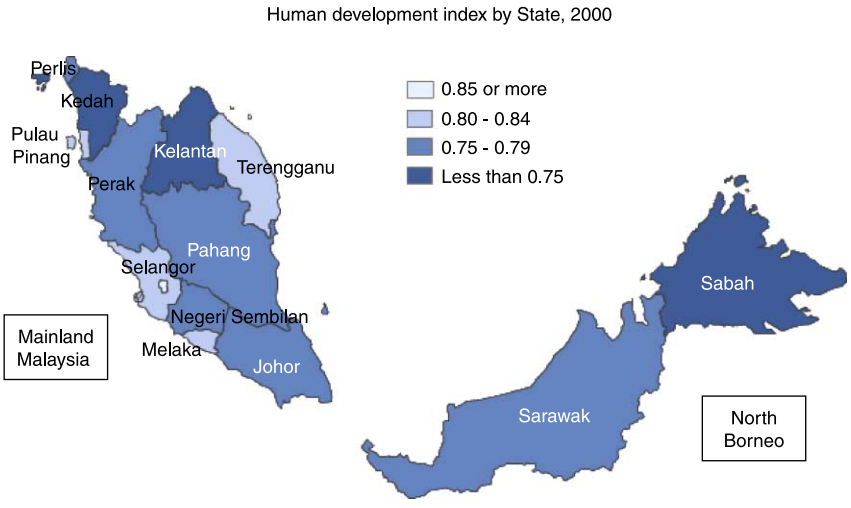

Figure 1 | Map of Malaysia showing Human Development Index.
Key. The human development index is a summary measure of human development. It measures the average achievements in a country in three basic dimensions of human development: a) A long and healthy life, as measured by life expectancy at birth. b) Knowledge, as measured by the adult literacy rate (with two-thirds weight) and the combined primary, secondary and tertiary gross enrolment ratio (with one-third weight). c) A decent standard of living, as measured by GDP per capita (PPP US$).
Source. United Nations Development Programme – Malaysia 2005. Progress and Challenges of Human Development in Malaysia. http://www.undp.org.my/index.php?navi_id = 132.

on how to utilize basic amenities provided for them. Despite state and federal Government efforts to modernize their society, the habits of the Orang Asli, which include poorer personal hygiene and health practices, walking barefooted, indiscriminate defaecation and the use of bare hands to consume food, make them more susceptible to intestinal parasitic diseases (Bisseru & Aziz 1970; Dunn 1972; Dissanaike *et al*. 1977; Kamel *et al*. 1994*a*; Lim *et al*. 1997). Recent rapid urbanisation, and the subsequent mushrooming of slums and shanty towns, populated by lower socioeconomic strata has led to overcrowding and poorer access to drinking water, sanitation and health facilities, which increase the likelihood of infectious diseases transmission. With further rapid urbanization and rural to urban migration, urban poverty might become more critical than rural poverty.

Reported *Cryptosporidium*-predisposing conditions such as HIV/AIDS appear higher in urban than in rural areas, but this may be due to reporting bias and cultural influences. Increased rural to urban migration is probably a component part of the increase in urban cases. In Malaysia, 70% of HIV/AIDS cases are intravenous drug users, the majority being Malay from lower socioeconomic levels (Anon 2004).

Major livestock populations include cattle (Kedah, Perak, Johor, Pahang, Terengganu, Kelantan), buffalo (Perak, Pahang, Terengganu), goats (Kedah, Perak, Johor, Pahang, Terengganu, Kelantan), sheep (Kelantan, Negeri Sembilan) and swine (Pulau Pinang, Perak, Selangor, Melaka, Johor). Cattle and sheep have been implicated in the zoonotic transmission of cryptosporidiosis and giardiasis, previously. Usually livestock farms are located nearby rivers, which act both as water sources and disposal sites for livestock excreta (Anon 2005*a*).

Piped water supplies are widespread in Malaysia, being available to approximately 98% and 86% of the urban and rural populations respectively (Anon 2005*b*). Water treatment includes aeration, chemical mixing, coagulation and flocculation, sedimentation, filtration, disinfection and pH correction. Wells are common drinking water sources in rural Kelantan, and in Malaysian rural and interior areas, untreated river water is a common source. About 60% of the Malaysian population is sewered.

Cryptosporidium

The first reported case of *Cryptosporidium* infection in Malaysia occurred in 1984 in a young man presenting with bloody diarrhoea (Table 2). Cryptosporidiosis was probably an accidental finding in this individual, because cryptosporidiosis usually presents with watery, but not bloody, diarrhoea (Che Ghani *et al*. 1984). Subsequently, studies on cryptosporidiosis have been limited to hospital cases. The incidence of cryptosporidiosis among paediatric patients ranged from 1–11% (Mendez *et al*. 1988; Mat Ludin *et al*. 1991; Lai 1992; Kan & Shekhar 1993; Ng & Shekhar 1993; Menon *et al*. 2001) and among immunosuppressed children, suffering from cancer and receiving chemotherapy, was 2% (Menon *et al*. 1999). In a community study of diarrhoeic young children in Kelantan, 11% had cryptosporidiosis (Lai 1992).

To date, only two cross sectional community studies have been undertaken to determine the prevalence of cryptosporidiosis in the urban Orang Asli (Aborigine) communities. The prevalence of cryptosporidiosis in Orang Asli communities is between 5.5–20.1%, with all cases being asymptomatic (Kamel *et al*. 1994*a*, Lim *et al*. 1997).

Asymptomatic infections appear to be more prevalent in children, young adults and AIDS patients in endemic areas of disease (Gilman *et al*. 1988; Smith & Nichols 2004), and the presentation of *Cryptosporidium* infection in Orang Asli, rural and urban Malaysians may be different, in that rural and urban Malaysians have more overt signs and symptoms than do the Orang Asli. The high prevalences reported suggest that infection with *Cryptosporidium* is underestimated in Malaysian communities particularly as infected, asymptomatic individuals can act as sources of infection for other susceptible individuals.

Initially regarded as an AIDS defining illness, cryptosporidiosis is gaining importance with the increasing numbers of human immunodeficiency virus (HIV) positive cases in Malaysia. Up to June 2005, 77,552 HIV/AIDS cases (approximately 0.3% of the Malaysian population) have been identified with, on average, 17 new cases per day or 1 in every 85 minutes (Ministry of Health Malaysia 2005). Intravenous drug users (IVDUs) constitute the majority of Malaysian HIV positive cases. *Cryptosporidium* oocysts were detected in 23% of asymptomatic, HIV positive IVDUs (Kamel *et al*. 1994*b*). Recently, a prevalence of 3% (2 cases,

Table 2 | Prevalence of cryptosporidiosis in Malaysia

Population	Sample size	Percent infected	Reference
First case in Malaysia			
Young male			Che Ghani *et al*. 1984
Hospital cases – paediatric			
Children	158	4.4	Mendez *et al*. 1988
Children	836	4.3	Mat Ludin *et al*. 1991
Children, <7 years	131	11.4	Lai 1992
Children	192	2.1	Ng & Shekhar 1993
Children with cancer	237	2.0	Menon *et al*. 1999
Children	258	0.9	Menon *et al*. 2001
Community cases			
Children, <7 years	47	10.6	Lai 1992
All ages, Orang Asli (urban)	159	20.1	Kamel *et al*. 1994*a*
All ages, Orang Asli (urban)	127	5.5	Lim *et al*. 1997
HIV patients			
IVDU HIV-infected patients (drug rehabilitation centre)	168	23.0	Kamel *et al*. 1994*b*
HIV-infected patients (hospital)	66	3.0	Lim *et al*. 2005

1 mortality) was determined in 66 hospitalized HIV-infected patients (Lim *et al*. 2005). Given the alarming increase in the number of AIDS cases in Malaysia, *Cryptosporidium* is likely to become increasingly important over the next few years. HIV infected patients on highly active antiretroviral therapy (HAART) have a dramatically lower incidence of cryptosporidiosis, attributable to the effects of intestinal immune reconstitution as well as the effect on the CD4 lymphocyte count. Protease inhibitors used in HAART (e.g. nelfinavir, indinavir, ritonavir) also reduce *C. parvum* sporozoite host cell invasion and parasite development *in vitro* (Hommer *et al*. 2003), and this direct inhibitory effect on *Cryptosporidium* infection not only suggests a further reason for the reduction in the incidence of cryptosporidiosis in HIV-infected patients, but also implies a further possible therapeutic modality for cryptosporidiosis.

There is the requirement for a higher index of suspicion for clinical cryptosporidiosis in Malaysian HIV patients including those with chronic weight loss with or without diarrhoea. Laboratory testing for *Cryptosporidium* in HIV infected cases, with notification to a centralised public health agency should be instigated, in order to provide a better understanding of the epidemiology and management of the disease in Malaysia.

The absence of firm data on *Cryptosporidium* occurrence and prevalence in Malaysia is primarily due to three factors, namely a) laboratory testing for *Cryptosporidium* (by microscopic examination of faecal samples) is not performed routinely, b) guidelines on individuals at greatest risk of contracting cryptosporidiosis: who should be tested?, are not in place, and c) there is no standardised system for laboratory reporting. As a result of this, cryptosporidiosis is underdiagnosed and its occurrence and prevalence are undetermined. This is despite the fact that researchers, since the early 1990s, have regarded *Cryptosporidium* as an important intestinal protozoan in Malaysia and have stressed that *Cryptosporidium* should be included in field studies and routine laboratory diagnosis to ensure that the cases do not go undetected (Mat Ludin *et al*. 1991; Lai 1992).

Giardia

Giardiasis was first documented in Malaysian children in 1970 (Bisseru & Aziz 1970, Table 3). Surprisingly, a higher

prevalence was observed among schoolchildren (7 to 12 years old; range = 0.2 – 15%) compared to children below 7 years old (2.6%) (Bisseru & Aziz 1970; Hamimah *et al*. 1982; Sinniah 1984; Sinniah *et al*. 1988; Rajeswari *et al*. 1994; Shekhar *et al*. 1996; Rahmah *et al*. 1997), although only one study investigated children below 7 years of age. The preponderance of giardiasis in children of 12 years and less is better reflected in community studies (Lai 1992; Che Ghani 1993). Giardiasis among Orang Asli children was high (23%) (Rahmah *et al*. 1997). Among hospitalized children, a high incidence (25%) of giardiasis was observed in Orang Asli children (Bisseru & Aziz 1970). In children with cancer and undergoing chemotherapy, giardiasis was observed in 6% of the patients (Menon *et al*. 1999).

Epidemiological studies of the Orang Asli communities in the 1970s and 1990s revealed that the prevalence of giardiasis ranged between 4.8–25% (Dunn 1972; Dissanaike *et al*. 1977, Kamel *et al*. 1994*a*; Lim *et al*. 1997). The high prevalence rate indicated in these studies is not surprising given that the majority of Orang Asli maintain a traditional lifestyle which promotes unhygienic practices. In other communities (the Malay, Chinese and Indian races, the fishing community in Penang, island dwellers, oil palm estate community), the prevalence of giardiasis is lower,

Table 3 | Prevalence of giardiasis in Malaysia

Population	Sample size	Percent infected	Reference
Community cases			
Children <7 years			
Children (<7 years), Major races	305	2.6	Hamimah *et al*. 1982
Children 6–12 years			
School children, Major races	678	5.6	Bisseru & Aziz 1970
School children, Malays	271	8.5	Sinniah 1984
School children, Pulau Ketam (Island fishing village)	297	9.3	Sinniah *et al*. 1988
School children, Various communities	456	14.7	Rajeswari *et al*. 1994
School children	7,557	0.21	Shekhar *et al*. 1996
Children (all ages), Orang Asli	162	23.1	Rahmah *et al*. 1997
Adults			
Adults, Indonesian immigrants	198	7.1	Sinniah & Rajeswari 1988
All ages			
All ages, Orang Asli	1,273	10.8	Dunn 1972
All ages, Orang Asli	126	4.8	Dissanaike *et al*. 1977
All ages, Oil palm Estate Indians	150	11.3	Sinniah *et al*. 1978
All ages, Island dwellers	83	6.0	Nawalinski & Roundy 1978
All ages, Major races	529	9.5	Che Ghani *et al*. 1987
All ages, Various communities	7,995	8.4	Lai 1992
All ages, Various communities	1,220	15.5	Che Ghani 1993
All ages, Orang Asli (urban)	159	6.9	Kamel *et al*. 1994*a*
All ages, Orang Asli (urban)	127	18.9	Lim *et al*. 1997
All ages, Rural communities	917	19.2	Norhayati *et al*. 1998
Hospital cases Children			
Children, Orang Asli (hospital)	100	25.0	Bisseru & Aziz 1970
Children with cancer (hospital)	237	6.0	Menon *et al*. 1999

ranging from 6 – 19% (Nawalinski & Roundy 1978; Sinniah *et al*. 1978; Che Ghani *et al*. 1987; Lai 1992; Che Ghani 1993; Norhayati *et al*. 1998). Major factors which predispose Malaysians to giardiasis include poor sanitary conditions, low socio-economic level, lack of clean water supplies and lack of sewage disposal facilities (Lai 1992). These reports indicated that, in rural communities, giardiasis was more prevalent than in urban communities (1.4–22.8% compared with 3.1–9.6%, respectively).

The prevalence of giardiasis among different ethnic groups varies greatly. In Malays, the prevalence is greatest, ranging between 0.9–17.0%, while the prevalence in Chinese is 1.5–10.5% and 2.9–9.0% in Indians (Nawalinski & Roundy 1978; Che Ghani *et al*. 1987; Khairul Anuar & Afifi 1990). Infection was more common in children below 12 years of age but was not sex dependent (Che Ghani *et al*. 1987).

The age distribution of cases clearly supports the idea of faecal–oral transmission. Places where young children congregate are important foci of transmission (Boreham *et al*. 1990), and reasons for this age dependent pattern are probably related to specific habits including mouthing of objects, close contact and sharing objects among themselves, which are specific risk factors increasing the incidence of giardiasis in the cohorts studied. The lack of effective immunity and a greater risk of exposure to sources of faecal contamination because of poor personal hygiene practices also augment the risks (Davis & Reynoldson 1994).

Cryptosporidium and *Giardia* in animals

There are limited studies in Malaysia pertaining to the occurrences of giardiasis and cryptosporidiosis in domestic and wild animals (Table 4). The first documented report of *Giardia* in animals was in a lesser mouse–deer, *Tragulus javanicus* which was trapped by the US Army Research Unit, Institute for Medical Research, Kuala Lumpur. While trophozoite morphology was similar to *G. duodenalis*, the parasites were not infectious in a new born goat (Colley & Mullin 1971). Some 20 years later, Rahman (1990) established that the prevalence of *Giardia* in pet dogs was 21.9%, with a greater preponderance in dogs aged 6–12 months. This study did not include the prevalence of giardiasis in dog owners. Given this high percentage of infected pet dogs, it would have been interesting to determine whether their owners were also infected. Most Malaysian housing estates are provided with children's playgrounds which are visited constantly by stray and pet dogs. Preventing dogs from defaecating in children's playgrounds, parks and other public premises could reduce the likelihood of human infection, assuming that the species of *Giardia* present in such dogs was infective to man.

Many studies published in the mid 1990s focused on cryptosporidiosis in farm animals. Studies indicated that the prevalence of cryptosporidiosis in domestic animals was high (>25%). Studies of cryptosporidiosis in goat kids and neonatal lambs indicated that 28% of kids and 36% of lambs were infected (Fatimah *et al*. 1995*a*, 1995*b*). A preliminary study carried out in two cattle farms in Selangor indicated

Table 4 | Occurrence of giardiasis and cryptosporidiosis in domestic and wild animals

Animals	Sample size	Giardiasis (% infected)	Cryptosporidiosis (% infected)	Reference
Lesser mouse–deer	1	Positive *	nt	Colley & Mullin 1971
Dogs (pets)	237	21.9	nt	Rahman 1990
Goat kids	na	nt	28	Fatimah *et al*. 1995*a*
Neonatal lambs	na	nt	36	Fatimah *et al*. 1995*b*
Cattle	na	nt	14.5	See 1997
Wild and domestic rats	49	28.6	4.1	Lim & Ahmad 2001
Cattle	96	14.6	25	Farizawati *et al*. 2005
Zoo birds	100	nt	6	Rohela *et al*. 2005

*Detected in a mouse deer trapped by US Army Research Unit.
na = not available, nt = not tested.

that 14.5% of faecal samples were oocyst positive (See 1997). This high prevalence was confirmed in a larger study of cattle faeces and the immediate 'on farm' environment, from seven cattle farms within Selangor. The study revealed that 14.6% of faecal samples from adult cattle were positive for *Giardia* cysts and 25% were positive for *Cryptosporidium* oocysts (Farizawati *et al*. 2005). (Oo)cyst concentration per gram ranged from 50–3.9 × 10^5 (oo)cysts, and both *Giardia* cysts and *Cryptosporidium* oocysts were detected in 5 samples. (Oo)cysts were also detected in wastewater (range = 4–3.1 × 10^3 (oo)cysts per litre) and river water (from the river situated adjacent to the farm; range = 0.7–240 (oo)cysts per litre). Given the high levels of occurrence and numbers of *Cryptosporidium* and *Giardia* (oo)cysts among the cattle and the positive findings of (oo)cysts in wastewater and river water samples, the risk of the zoonotic spread of human infectious (oo)cysts into rivers appears high (Farizawati *et al*. 2005). Human infectious (oo)cysts present on such farms are also a risk to the owners/tenants and their families, particularly when they are dependent on the river for their source of water for their daily activities, or when farmyard manure, slurry are used as fertilizer or river water for irrigation of crops.

In addition to identifying (oo)cyst contributions from domestic livestock, zoo animals have also been investigated. Of 100 bird faecal samples taken from various locations in the Kuala Lumpur National Zoo, 6 samples from 6 different bird species were *Cryptosporidium* oocyst positive (Rohela *et al*. 2005). The birds included the Wrinkled Hornbill (*Aceros corrugatus*), Great Argus Pheasant (*Argusianus argus*), Black Swan (*Cygnus atratus*), Swan Goose (*Anser cygnoides*), Marabou Stork (*Leptoptilos crumeniferus*) and Moluccan Cockatoo (*Cacatua moluccencis*). These birds were located in the aviary and the lake and the Moluccan Cockatoo was routinely used as a show bird. These results indicate that public animal sanctuaries including zoos and bird parks could be further sources of *Cryptosporidium* infection to susceptible visitors and other animals (Rohela *et al*. 2005).

Another study examined 49 faecal samples from domestic and wild rats in an Orang Asli community. The results revealed that 14 (28.6%) rats were infected with *Giardia* (range = 10–59 640 cysts per gram) and 2 (4.1%) rats were *Cryptosporidium* oocyst positive (range = 70–320 oocysts per gram) (Lim & Ahmad 2001). The high prevalence of *Giardia* in rats suggested the possibility that rats might be involved in the transmission of giardiasis to susceptible human hosts since in this community and others, where hygiene and sanitation are questionable, rats are ubiquitous, numerous and live in close proximity to man, livestock, food and water supplies, drainage and effluent routes, and contaminate these environments with their droppings. Rodents may represent a significant reservoir of infection for *Giardia* and *Cryptosporidium* with the potential of transmitting infection to humans and livestock due to their cohabitation and contamination of the environment (Chalmers *et al*. 1995, 1997).

Cryptosporidium and *Giardia* in water

Waterborne transmission of cryptosporidiosis and giardiasis can affect large numbers of consumers of contaminated drinking water (MacKenzie *et al*. 1994; Robertson *et al*. 2006), and the possibility of such an event occurring in Malaysia has driven many Malaysian studies on the occurrence of these parasites in the environment, especially in water. Methods used in Malaysia are large volume cartridge filtration, membrane filtration, elution and concentration of the eluate by centrifugation, immunomagnetisable particle separation and detection of (oo)cysts by fluorescence microscopy, and are based upon UK methods (Anon 1999). Many studies report on the occurrence of *Giardia* and *Cryptosporidium* (oo)cysts in rivers and in drinking water in Malaysia (Table 5). By collating published studies, we found that a total of 174 river water samples have been analysed. Of these, 39% were positive for *Giardia* cysts and 11.5% for *Cryptosporidium* oocysts with concentrations ranging from 0.7–12,780 cysts per litre and 0.4–246 oocysts per litre, respectively. These data identify a high level of contamination in rivers, which is worrying because some of these rivers are situated in catchment areas where water is abstracted for drinking water.

The quality of drinking water was also evaluated from 8 drinking water treatment plants. While raw water supplying water treatment plants were highly contaminated with *Giardia* and *Cryptosporidium* (oo)cysts, no (oo)cysts were detected in treated water (Ahmad *et al*. 1997; Tan 1998;

Table 5 | Occurrence of *Giardia* and *Cryptosporidium* (oo)cysts in Malaysian water

		Giardia		*Cryptosporidium*	
Water type	**Numbers examined**	**% positive**	**Range of cysts/L**	**% positive**	**Range of oocysts/L**
Environmental water					
Rivers	174	39%	0.7 – 12,780	11.5%	0.4–246
Well	28	17.9%	0–0.25	7.1%	0–0.75
Drinking water treatment plants					
Dam	28	0	0	0	0
Raw	87	46%	0.03–120.0	6.9%	0.05 – 3.0
Treated	87	0	0	0	0
Backwash water	2	100%	1600–2400	100%	1200–1600
Household water					
Kitchen tap	5	0	0	0	0
Household storage containers	20	10%	0.4 – 2.0	0	0
Sewage treatment works					
Influent	30	100%	18–8480	40%	1–80
Effluent	30	83.3%	1–1462	20%	20–80

Lim & Ahmad 2004*a* and unpublished data). The presence of (oo)cysts in raw water supplying water treatment plants and their absence in treated drinking water led us to postulate that (oo)cysts accumulated in the treatment processes of the treatment plant. This was confirmed when two filter backwash water samples taken from a treatment plant were (oo)cyst positive, at a range between 1200–2400 (oo)cysts (unpublished data). While their removal from drinking water is a public health prerequisite, their accumulation in filters and backwash waters has led to waterborne outbreaks in the UK, when oocysts accumulate in such numbers they tend to overload the filtration capacity of the filter(s) and break through into drinking water. Recycling (oo)cyst containing filter backwash water increases (oo)cyst (and other particulates) load further, increasing the likelihood of (oo)cyst break through into the drinking water (Anon 1990).

Post-treatment contamination of drinking water with *Giardia* and *Cryptosporidium* (oo)cysts has been demonstrated in Malaysia. In a study in an Orang Asli community (Lim & Ahmad 2004*b*), *Giardia* cysts was found at 0.4 to 2 cysts per litre in the household water which was stored in a bucket. The treated water in this house was supplied by the Water Supply Department (JBA). The presence of cysts in drinking water is a public health risk to consumers of that water, because of the low infectious doses for cryptosporidiosis and giardiasis (9–1042 *C. parvum* oocysts and 10–100 *G. duodenalis* cysts) (Rendtorff 1954; Okhuysen *et al*. 1999). The most probable reason for the presence of these cysts is post-treatment contamination by humans or animals. It was noted during sampling visits, that some families kept their water containers on the floor to make it convenient for young children to obtain water from them *ad lib*. Children with contaminated hands could have contaminated the water in the containers. As the prevalence of giardiasis in this community is high (19%) and was preponderant amongst children, the possibility of post-treatment contamination by children is very high (Lim *et al*. 1997).

In a study conducted in the northern state of Malaysia where many households are dependent on well water for their daily chores, 17.9% and 7.1% of the well water samples were contaminated with *Giardia* and *Cryptosporidium* (oo)cysts, respectively (Ahmad & Chan 1994). Upon further investigation it was noted that some wells were located downstream of household toilets. Although most households in Malaysia boil their drinking water, thirsty individuals will drink water straight from the well. The occurrence

of *Giardia* and *Cryptosporidium* (oo)cysts in well water warrants further study especially in communities who remain dependent on wells for their water source, as do interventional and preventive steps, such as building toilets downstream of well locations and increasing hygiene awareness. These issues should also be addressed by members of these communities and local Government officials.

Sewage treatment works (STWs) can also contribute (oo)cysts into receiving waters used for the abstraction of drinking water (Smith *et al.* 1995; Robertson *et al.* 1999, 2000), and research on Malaysian STWs is of concern because of the high percentage of effluents which discharged *Giardia* (72.2%) and *Cryptosporidium* (22.2%) (oo)cysts (Lim 1996; unpublished data). (Oo)cyst concentrations ranged from 1–1462 per litre for *Giardia* cysts and 20–80 per litre for *Cryptosporidium* oocysts. (Oo)cyst contaminated STW effluents can contaminate downstream water sources used by rural communities using river water as their main drinking water supply and services such as drinking water treatment works, livestock farms and food producers.

Cryptosporidium and *Giardia* in soil

(Oo)cysts have also been found in soil in Malaysia. In a study where 138 soil samples from house compounds were collected, *Giardia* cysts and *Cryptosporidium* oocysts were present in 0.7% of soil samples, and the concentration of *Giardia* cysts and *Cryptosporidium* oocysts in the soil were 2.9 cysts per gram and 2.0 oocysts per gram respectively (Lim 2000) (Table 6). The soils were predominantly sandy in nature. In this study, the likely source of contamination was human excreta because of high prevalence of these parasitic infections in the community studied, and the habit of defaecating indiscriminately. The low concentrations of (oo)cysts detected might be due to a) low levels of defaecation in house compounds, b) rapid transportation of (oo)cysts across the surface of soils by rain, and/or c) percolation of (oo)cysts into the sandy soil. Sandy soil does not entrap (oo)cysts as efficiently as clay soil. It is more porous and does not retain water well. The occurrence of (oo)cysts in soil within the house compound, especially in the vicinity of children who regularly play with soil, should be of concern to public health officials.

Table 6 | Occurrence of *Giardia* cysts and *Cryptosporidium* oocysts in soil samples

	No. of samples	% positive	Mean of (oo)cysts per gram ± SD	Range of (oo)cysts per gram
Giardia	138	0.7%	0.02 ± 0.25	0–2.9
Cryptosporidium	138	0.7%	0.01 ± 0.17	0 – 2

Viability of *Cryptosporidium* in the Malaysian environment

The viability of *Cryptosporidium* oocysts in Malaysian water and soil was assessed in two studies using fluorogenic vital dyes, 4′ 6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) (Campbell *et al.* 1992). The results from each study indicate that it takes 1–2 months for *Cryptosporidium* oocysts to be rendered non-viable (Lim *et al.* 1999; Farizawati *et al.* 2005). *Cryptosporidium* oocysts survive longer in soil than in river water. The reasons why oocyst survival is reduced in river water include their high temperatures (range = 25–26°C), the adverse effect of sunlight-derived UV radiation and the presence of chemical residues. *Cryptosporidium* oocysts survive better in cool, moist dark microclimates, as afforded by the soil in this study, which had a high moisture content (range = 20.69–25.27%). Given a survival time of 1–2 months, river water and soil contaminated with *Cryptosporidium* oocysts can be important mechanical, abiotic agents of transmission, especially in communities who are dependent on river water for drinking and washing unheated foods and in risk groups such as children who do not wash their hands after playing with contaminated sand or soil.

CONCLUSIONS AND FUTURE PERSPECTIVES

The pathogens *Cryptosporidium* and *Giardia* occur commonly in Malaysian human and animal populations. The commonness of contributions from human (sewage treatment works effluents, indiscriminate defaecation) and non-human (calving, lambing, muck spreading, slurry spraying, pasturing/grazing of domestic animals, infected wild animals) hosts indicate that many Malaysian environments, particularly water and soil, can act as potential vehicles for the transmission of disease.

Without insight into the morbidity and mortality caused by human cryptosporidiosis and giardiasis in Malaysia, it becomes very difficult to determine their impact. This issue is particularly significant now, since WHO included both these diseases in their 'Neglected Diseases Initiative' in 2005, identifying that the risks associated with these diseases should be determined. We have identified various risk factors using current technology, but advances in molecular techniques, especially those associated with species identification and epidemiology, now provide us with the armoury required to determine the level of risk. Differences in geography, climate and lifestyle will influence the designation of risk.

Insight into the impact of infectious diseases is based on reliable baseline data backed by an informative, national surveillance system. With knowledge of the impact of such diseases, effective control measures can be implemented. Symptoms caused by intestinal pathogens such as *Cryptosporidium* and *Giardia* are often viewed lightly and regarded as a 'fact of life', especially amongst the poorer communities. Acute gastroenteritis, which is a clinical sign of giardiasis and cryptosporidiosis, is not notifiable in Malaysia. The scarcity of laboratory-based data about *Cryptosporidium* in Malaysia is due to the fact that microscopic examination of faecal samples for *Cryptosporidium* is not performed routinely and rarely is cryptosporidiosis included in the differential diagnosis. As a result, cryptosporidiosis remains underdiagnosed and its prevalence cannot be determined. However, the contribution from infected human beings in urbanized areas can be assessed readily by monitoring sewage influent and effluent discharges. Dependent on disease patterns, the identifiable risks may be seasonal.

The contribution from livestock and wildlife is less easy to assess, but, for water, monitoring of catchments and abstraction points can produce useful background information, especially when treatment plants may be categorised as high risk. During periods of high risk, changes in treatment plant operation should be avoided where possible. Dependent upon the perceived risk, and based upon data from monitoring surveys, water treatment plants may vary in their risk category at different times of the year, with the differences being reflected in the parasite sampling strategy.

Many outbreaks of *Cryptosporidium* and *Giardia* have been due to drinking contaminated water. However, *Cryptosporidium* and *Giardia* (oo)cysts are inactivated by bringing drinking water to the boil (Fayer 1994), and most Malaysians boil their drinking water prior to its consumption. This culture of boiling drinking water provides some insight into the perceived low numbers of clinical cases of giardiasis and cryptosporidiosis. However, not all Malaysians can afford sufficient fuel to boil water, as some communities are so poor. In other communities (e.g. Orang Asli), lifestyles dictated through numerous generations identify that boiling water destroys the cooling effect of water. In these communities, unboiled water is the preferred choice. Lifestyle changes also affect more affluent communities who, by choice, prefer to drink bottled water or use water filters which advertise the complete removal of waterborne pathogens. This identifies the lack of confidence in the quality of drinking water supplied, but does not indicate that these lifestyle changes automatically reduce the likelihood of contracting waterborne disease, as there is no regulatory body to monitor the presence of *Cryptosporidium* and *Giardia* in bottled water in Malaysia. The use of validated methods, the auditing of laboratory performance for determining the occurrence of (oo)cysts in drinking water, and the reporting of results to Government Regulators must increase confidence in the quality of drinking water supplied. Similarly, an informal and voluntary agreement between bottled water producers and a laboratory with validated and accredited methods will address any issues raised regarding bottled water. Here, the availability of accurate and timely data is of paramount importance.

In order to raise national perceptions of the quality of drinking water in Malaysia, the detection of *Cryptosporidium* and *Giardia* should be an integral part of the water quality requirement. Currently, there is no regulatory structure which makes it mandatory to monitor for these parasites, therefore water purveyors are not required to submit water samples for testing, even though the research identified above indicates a degree of risk. The extra cost of testing is also a contributory factor.

The presence of these parasites in water at the concentrations recorded is significant, considering that *Giardia* and *Cryptosporidium* have low infectious doses [i.e. 9–1042 (oo)cysts] (Cacciò *et al.* 2005) and that numerous waterborne outbreaks have occurred where low numbers of parasites were detected in drinking water (Smith & Rose 1998). A multidisciplinary approach among public health

professionals in the water industry (collaborative efforts of the Water Supply Department and the Sewerage Services Department of the Ministry of Energy, Water and Communications, as well as the support of state water authorities and private water companies) and other relevant health- and environment-associated agencies is required in order to determine the significance of *Cryptosporidium* and *Giardia* contamination of Malaysian drinking water.

Studies on the occurrence of these parasites in Malaysian water have relied on small ($\leq$20 litres as a grab sample) volume sampling strategies, which does not necessarily provide the most representative picture. Currently, the use of large ($\geq$1,000 litres over 22–24 hours) volume sampling is hampered by technical difficulties associated with water quality. Some physico-chemical characteristics, particularly turbidity, of Malaysian water samples reduce (oo)cyst recovery. Malaysia has plentiful rainfall and the rivers generally flow in abundance however, rainfall increases the turbidity of water which further reduces (oo)cyst recoveries and leads to an underestimation of (oo)cyst contamination. The development of more sensitive methods, with particular application to Malaysian water concentrates, is of paramount importance.

Molecular biology has provided powerful new tools for characterizing *Cryptosporidium* and *Giardia*, and the analysis of previously unrecognized genetic differences within these genera has revolutionized both their taxonomy and our understanding of the epidemiology of human disease. Molecular tools have enabled not only the identification of species and genotypes in the faeces of infected hosts but also their recognition in environmental samples, including water (Cacciò *et al*. 2005). Validated methods to determine the species, genotype and subgenotype which are present in heterologous mixtures should be applied to environmental samples to enable the monitoring and characterization of infection sources, disease tracking and the establishment of causative links to both waterborne and foodborne outbreaks (Smith *et al*. 2006). The adoption of these criteria in Malaysia will assist in developing effective risk assessment, management and communication models. Once the species, genotypes, subtypes of *Cryptosporidium* and *Giardia* present in Malaysia have been identified, source, contamination and disease tracking tools can be used.

To date, studies on *Cryptosporidium* and *Giardia* in Malaysia have focused on creating baseline data on the prevalence and occurrence of these parasites in human, non-human and environmental samples by employing microscopic techniques. These are fundamental and valuable assets. Molecular tools must be incorporated into current methodologies to provide better insights into *Cryptosporidium* and *Giardia* taxonomy and the epidemiology of disease in Malaysia.

Clearly, with the adoption of these recommendations, an in depth insight into the impact of disease and morbidity and cost of illness of these protozoan parasites in Malaysia is now possible, particularly with the adoption of molecular methods into our current armoury of detection technologies. To understand transmission routes and the significance of environmental contamination better will require further multidisciplinary approaches and shared resources between the players, which include public and veterinary health professionals, water and food industry personnel, state water authorities, private water companies, state and federal regulators and other relevant government and non-government organisation health- and environment-related agencies. These approaches are necessary to generate stable and powerful partnerships in our efforts to understand and address the significance of cryptosporidiosis and giardiasis in Malaysia and South East Asia.

In order to advance our understanding of *Cryptosporidium* and *Giardia* in Malaysia, we recommend the timely investigation of the issues identified below.

- Formulation of a national database on the occurrence of *Cryptosporidium* and *Giardia* (oo)cysts in human and non-human hosts and in the Malaysian environment.
- Instigation of Malaysian (oo)cyst and DNA banks for storage and exchange of samples (within Malaysia and among the South East Asian countries where similarities in geography, climate and lifestyle will provide effective models for comparative studies).
- Use of validated and recognised conventional and molecular methods for isolating, purifying and identifying *Cryptosporidium* and *Giardia* species, genotypes and subtypes.
- Determine the significance of transmission routes in Malaysia.

- Determine the zoonotic potential in Malaysian communities.
- Determine associations between the presence of *Cryptosporidium* and *Giardia* in water and their health significance.
- Develop methods and determine the survival of (oo)cysts in the environment and the likelihood of viable organisms a) entering source waters used for the abstraction of potable water and treated water in distribution and b) contaminating foodstuffs which are consumed unheated.
- Develop disease reduction strategies through the combined analysis of clinical and environmental typing data and descriptive epidemiological data so that 'in depth' hypotheses regarding the significance of different transmission routes in endemic and non-endemic settings can be determined. Develop measures that reduce direct and indirect transmission (e.g. hygienic, sanitary, water catchment and food hygiene policies).
- Develop disease reduction/prevention strategies through effective health education with ample inter-institutional and inter-sectoral collaboration, by targeting well characterized populations, using simple, pertinent and focused educational materials which highlight hygienic and sanitary practices known to reduce infection and transmission, and the benefits of targeted drug intervention (where available). Information dissemination should cover several infectious diseases with similar epidemiological profiles. A sustained change in attitudes and behaviour of the targeted populations should be coupled to the positive impact of health messages identifying health, socio-economic and educational benefits at local, state and country levels.

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