First genetic classification of Cryptosporidium and Giardia from HIV/AIDS patients in Malaysia

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Abstract

Given the HIV epidemic in Malaysia, genetic information on opportunistic pathogens, such as Cryptosporidium and Giardia, in HIV/AIDS patients is pivotal to enhance our understanding of epidemiology, patient care, management and disease surveillance. In the present study, 122 faecal samples from HIV/AIDS patients were examined for the presence of Cryptosporidium oocysts and Giardia cysts using a conventional coproscopic approach. Such oocysts and cysts were detected in 22.1% and 5.7% of the 122 faecal samples, respectively. Genomic DNAs from selected samples were tested in a nested-PCR, targeting regions of the small subunit (SSU) of nuclear ribosomal RNA and the 60 kDa glycoprotein (gp60) genes (for Cryptosporidium), and the triose-phosphate isomerase (tpi) gene (for Giardia), followed by direct sequencing. The sequencing of amplicons derived from SSU revealed that Cryptosporidium parvum was the most frequently detected species (64% of 25 samples tested), followed by C. hominis (24%), C. meleagriris (8%) and C. felis (4%). Sequencing of a region of gp60 identified C. parvum subgenotype IIdA15G2R1 and C. hominis subgenotypes IaA14R1, IbA10G2R2, IdA15R2, IeA11G2T3R1 and IIdA11G1R2. Sequencing of amplicons derived from tpi revealed G. duodenalis assemblage A, which is of zoontic importance. This is the first report of C. hominis, C. meleagriris and C. felis from Malaysian HIV/AIDS patients. Future work should focus on an extensive analysis of Cryptosporidium and Giardia in such patients as well as in domestic and wild animals, in order to improve the understanding of transmission patterns and dynamics in Malaysia. It would also be particularly interesting to establish the relationship among clinical manifestation, CD4 cell counts and genotypes/subgenotypes of Cryptosporidium and Giardia in HIV/AIDS patients. Such insights would assist in a better management of clinical disease in immuno-deficient patients as well as improved preventive and control strategies.

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1. Introduction

Cryptosporidium and Giardia are two key genera of protozoan pathogens which are known to significantly affect HIV-infected people (Tzipori and Widmer, 2008; Dib et al., 2008). In immunocompetent hosts, infections by these agents can be acute and self-limiting. However, in immuno-compromised individuals, such as HIV/AIDS patients and people receiving immuno-suppressive therapy, infections with these protozoa may be prolonged and associated with chronic diarrhoea, vomiting, severe colic and/or wasting (Moolasart, 1999; Kosek et al., 2001). Persistent protozoan infections in HIV-infected people are particularly severe, decreasing their quality of life and shortening life expectancy (Miao et al., 2000; Ives et al., 2001). Given that there is no highly effective chemotherapeutic agent against cryptosporidiosis for patients who have low CD4+ cell counts (i.e. <200/ mm³) (Smit et al., 2008) and the occurrence of relapses of giardiasis in AIDS patients (Moolasart, 1999), the prevention and control of protozoal enteritis requires an improved understanding of the transmission patterns of the causative agents. Both Cryptosporidium and Giardia are transmitted directly via the faecal–oral route, with a significant number of cases being linked to drinking water contaminated with the infective stages (i.e. oocysts or cysts) of these parasites (Karanis et al., 2007). The potential risk of immuno-compromised people acquiring such protozoal infections through the accidental ingestion of contaminated water during recreational water activities has been emphasized recently (McOliver et al., 2009).
Although it is acknowledged that the access to highly active anti-retroviral therapy (HAART) in developing countries has significantly reduced the morbidity from cryptosporidiosis and giardiasis in HIV-infected people (Miao et al., 2000; Ives et al., 2001; Zardi et al., 2005), the situation in developing countries is less positive. In the latter countries, infections with Cryptosporidium and Giardia are still major threats to HIV/AIDS patients due to the lack of access to suitable supportive and/or chemotherapy treatment, particularly in poor communities (Smith and Corcoran, 2004; Silva et al., 2005). Cryptosporidium is linked to ~50% of diarrhoeal cases in HIV-infected patients living in developing countries, in contrast to 10–20% in developed countries (Navin et al., 1999; Weber et al., 1999). In Malaysia, where infections with Cryptosporidium and Giardia are widespread (Lim et al., 2008, 2010) and a large proportion of HIV/AIDS patients are still not receiving HAART, it is crucial that efforts are made to improve the understanding of these opportunistic parasites in such patients.

Since the first HIV/AIDS case was identified in Malaysia (Goh et al., 1985), the HIV epidemic in this country has expanded rapidly. Until 2008, ~96,000 HIV/AIDS cases, which represent ~0.4% of the Malaysian population, had been reported (Anonymous, 2008). Given that intra-venous drug users (IVDUs) constitute the majority (~70%) of Malaysian HIV-positive cases, a study was conducted to estimate the prevalence of Cryptosporidium and Giardia in 168 asymptomatic HIV-infected IVDUs (Kamel et al., 1994); the results revealed a prevalence of 23% in these people (Kamel et al., 1994). Among hospitalised HIV-infected patients, the prevalence of Cryptosporidium infection was reported to be 3% of 66 people from urban areas (Lim et al., 2005) and 18.6% of 59 humans from semi-urban areas (Zaidah et al., 2008). Although Giardia represents a relatively common group of pathogens (0.2–25%) in the Malaysian population (Lim et al., 2008), epidemiological information on this parasite genus in immuno-compromised patients is scarce. Thus far, giardiasis had only been reported in 6% in 237 of paediatric cancer patients undergoing chemotherapy (Menon et al., 1999). It is expected that the clinical impact of AIDS in Malaysia will be substantially exacerbated by co-infections with Cryptosporidium and Giardia. Therefore, enhanced knowledge and understanding of the epidemiology and population genetics of members of these two parasite genera in this country would assist in the formulation of prevention measures for immuno-deficient individuals. In the present study, we take the first step to genetically characterize Cryptosporidium and Giardia from a range of Malaysian HIV/AIDS patients.

2. Materials and methods

Faecal samples from 122 HIV-infected patients (with or without diarrhoea) from two major teaching hospitals (i.e. Hospital Sungai Buloh and University Malaya Medical Centre [UMMC]) in Malaysia were included in this study. Information on age, sex and stool consistency was also collected. Ethics approval was obtained prior to the commencement of the study from the Ministry of Health Malaysia and the University Malaya Medical Centre. Written consent was obtained from all participants. Faecal samples were examined microscopically for the presence of Cryptosporidium oocysts using the modified Ziehl-Neelsen staining method (Henrikson and Pohlenz, 1981) and for Giardia cysts using an iodine staining method (Garcia and Voge, 1980), prior to molecular testing.

Genomic DNA was extracted from individual faecal samples known to contain oocysts and/or cysts employing the QIAgen DNA Mini Kit (QIAGEN, Germany) and then subjected to nested PCR. Approximately 50–100 ng of each genomic DNA sample were subjected to the primary PCR, and 1 μl of primary amplicon was transferred to the secondary PCR. For Cryptosporidium species, an ~400 bp region of the small subunit (SSU) of the nuclear ribosomal RNA (rRNA) gene was amplified using the primary primer pair N-DIAF2 (forward; 5′-CAATTGGAGGCAAGTCTGGTGCAGC-3′) + N-DIAGR2 (reverse; 5′-CCTCTCATGTGACCGTCTGGTAC-3′) (Nichols et al., 2003) and the secondary primer pair: CPB-DIAFG (forward; 5′-AAGCTCTGATGGATTCTG-3′) + CPB-DIAGR (reverse; 5′-TAAGTGTCTAGAGAAGTAAAG-3′) (Johnson et al., 1995). PCR was conducted in a 50 μl volume containing 0.2 μl of each primer, 200 μl of each dNTP, 400 μg/ml bovine serum albumin, 3.5 mM of MgCl₂, 2.5 U of Taq polymerase (New England Biolabs, Ipswich, USA) in the buffer provided, using the following cycling conditions: 94 °C/5 min (initial denaturation), followed by 35 cycles at 94 °C/30 s (denaturation), 60 °C/1 min (annealing) and 72 °C/30 s (extension), followed by a final extension at 72 °C/10 min. In order to identify Cryptosporidium genotypes/subgenotypes, a 300–450 bp region of the 60-kDa surface glycoprotein (gp60) gene was amplified using the primary primer pair gp15-ATG (forward; 5′-ATGAGATTTGCTCATTATC-3′) + gp15-STOP (reverse; 5′-TCTACAAACGAAATAGGTCG-3′) (Strong et al., 2000) and the secondary primer pair gp15-15A (forward; 5′-CCCATATCACAATTGAACTGGC-3′) + gp15-15E (reverse; 5′-CCCACTATTACAAACGAAATAGGTCG-3′) (Mallon et al., 2003). PCR was conducted in a 50 μl volume containing 25 pmol of each primer, 200 μM of each dNTP, 3 mM of MgCl₂, 1 U of GoTaq® Flexi DNA polymerase (Promega, Madison, WI, USA) in the recommended buffer. The cycling conditions for each PCR were 94 °C/5 min, followed by 40 cycles of 94 °C/30 s (denaturation), 55 °C/45 s (annealing) and 72 °C/1 min (extension), followed by a final extension at 72 °C/10 min.

For Giardia, an ~500 bp region (designated ptp1) of the triose phosphate isomerase (tpi) gene was amplified specifically by nested PCR (Sulaiman et al., 2003) using the primary primer pair AL3543 (forward; 5′-AAATTATGCTGCTGCTGCTG-3′) + AL3546 (reverse; 5′-CAACATTCTCCAGACATAC-3′) and the secondary pair AL3544 (forward; 5′-CCTTCATCTGGTGTAATT-3′) + AL3545 (reverse; 5′-GTCGCCACACCTCCGCCC-3′). Each PCR (50 μl) contained 25 pmol of each primer, 125 μM each of dNTP, 3 mM of MgCl₂, 1.25 U of GoTaq® Flexi DNA polymerase (Promega) in the buffer provided, and was conducted over 35 cycles of 94 °C/45 s, 50 °C/45 s, 72 °C/1 min with an initial hot start of 94 °C/5 min and a final extension of 72 °C/10 min. Known C. parvum or C. duodenalis DNA (reference) samples were included as positive controls in each PCR run, as were samples without DNA template (negative controls).

The quality and intensity of individual amplicons were examined on ethidium bromide-stained, 1.5% agarose gels. Amplicons were purified over minicolumns (Wizard PCR-Preps, Promega), eluted in 30 μl H₂O, and then subjected to bidirectional, automated sequencing (BigDye Chemistry; Applied Biosystems) using the same primers (separately) as employed in the secondary PCR. For each locus amplified, sequences were trimmed and then aligned using the program Clustal X (Hall, 2004). The sequences derived from the SSU amplicons were compared with selected, publicly available sequences representing C. parvum (accession no. AF093493), C. hominis (AF093489), C. meleagridis (AF112574) and C. felis (AF112575) (cf. Xiao et al., 1999).

Phylogenetic analysis of sequence data was conducted by Bayesian inference (BI) using Monte Carlo Markov Chain (MCMC) analysis in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The likelihood parameters set for BI were based on the Akaike Information Criteria (AIC) test in Modeltest v3.7 (Posada and Crandall, 1998). The general time-reversible model of evolution with gamma distribution and a proportion of invariant sites (GTR + I + G) and GTR + Γ were used.
for the analyses of sequence data for partial SSU and gp60, including selected reference and outgroup sequences from previously published studies (Figs. 1 and 2). Estimates of the base frequencies, the substitution rate model matrix and the proportion of invariable sites determined using AIC were fixed for each analysis. Posterior probabilities (pp) were calculated via one or two million generations, utilizing four simultaneous tree-building chains, with every 100th tree being saved. At this point, the standard deviation of split frequencies was <0.01, and the potential scale reduction factor (PSRF) approached one. A 50%-majority rule consensus tree was constructed based on the final 75% of trees generated, which was viewed using the program TreeviewX v.0.5.0 (http://darwin.zoology.gla.ac.uk/_rpage/treeviewx/).

3. Results

Conventional microscopic examination of 122 faecal samples from adult HIV/AIDS patients revealed 27 (22.1%) to be test-positive for Cryptosporidium and seven (5.7%) for Giardia. Both parasite genera were detected in individual samples from three patients. Of these 27 Cryptosporidium-infected patients, some personal and clinical information was available for 24 of them. Most people infected with Cryptosporidium were males (91.7%; 22

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**Fig. 1.** Phylogenetic analysis of partial gp60 sequence data representing Cryptosporidium hominis (A) and C. parvum (B) from HIV/AIDS patients using Bayesian Inference (BI). Sequences from the present study as well as 51 reference sequences representing C. hominis and C. parvum subgenotypes (from GenBank) were included in the analysis. Sample code (accession number; subgenotype): SB2 (HQ836661; IbA10G2R2), SB23 (HQ631409; IbA10G2R2), SB35 (HQ631410; IbA10G2R2), SB90 (HQ631406; IaA14R1), UM6 (HQ631408; IaA14R1), A17 (HQ836662; IfA11G1R2), A23 (HQ836663; IfA11G2T3R1), A41 (HQ836664; IdA15R2) and UM1 (HQ631423; IdA15G2R1). The accession numbers of publicly available reference or outgroup sequences included are indicated in each tree. Posterior probabilities are indicated at all major nodes.
of 24), >35 years of age (62.5%), asymptomatic (91.7%) and had a CD4 count of <200/mm³ (66.7%). Similarly, for the seven patients shown to be test-positive for *Giardia*, most were male (100%), >35 years of age (57.1%), asymptomatic (85.7%) and had CD4 counts of <200/mm³ (85.7%). Samples from 25 and four patients that were test-positive for Cryptosporidium and *Giardia*, respectively, and for which sufficient faeces were available for DNA extraction, were then tested using molecular methods. Based on the sequences determined from partial SSU and comparisons with selected reference sequences for *C. parvum* (accession no. AF093493), *C. hominis* (AF093489), *C. meleagridis* (AF112574) and *C. felis* (AF112575) (cf. Xiao et al., 1999), *C. parvum* was detected most frequently (64% of 25), followed by *C. hominis* (24%), *C. meleagridis* (8%) and *C. felis* (4%). Based on sequences determined from selected gp60 amplicons (n = 9) representing *C. hominis* and *C. parvum* and following phylogenetic analysis, including 51 reference sequences from the GenBank database for comparison, six distinct genotypes/subgenotypes (i.e. laA14R1, lbA10G2R2, ldA15R2, leA11G2T3R1, lfa11G1R2, and lfdA15G2R1) were identified (pp > 0.95) (see Fig. 1). For *G. duodenalis*, a phylogenetic analysis of the data for the four distinct tpi sequence types, together with 31 reference sequences from GenBank, inferred *G. duodenalis* assemblage A (pp > 0.90) for all four samples (see Fig. 2).

4. Discussion

In the present study, using conventional microscopic techniques, 22.1% and 5.7% of 122 faecal samples from adult HIV/AIDS patients contained *Cryptosporidium* and *Giardia*, respectively, with three having mixed infections of both parasites. The phylogenetic analysis of partial SSU sequence data inferred four parasites of *Cryptosporidium* in these patients. *Cryptosporidium parvum* was detected most frequently (64% of 25), followed by *C. hominis* (24%), *C. meleagridis* (8%) and *C. felis* (4%). Although *C. parvum* has been recorded in HIV-infected human patients (Zaidah et al., 2008) and other animal hosts (Halim et al., 2008; Quah et al., 2011) in Malaysia, the present study is the first report of *C. hominis*, *C. meleagridis* and *C. felis* in such patients in this country. The present data for Malaysia appears to be consistent with those for France, Portugal, Switzerland and the United Kingdom, wherein zoonotic *C. parvum* is responsible for more human infections than *C. hominis* (see McLauchlin et al., 2000; Morgan et al., 2000; Guyot et al., 2001; Alves et al., 2000, 2003). However, elsewhere, such as in Australia, Kenya, South Africa, Thailand, United States and Vietnam, anthropoctic *C. hominis* is reported to be the more common causative agent of cryptosporidiosis in immuno-compromised people (Morgan et al., 2000; Sulaiman et al., 1998; Leav et al., 2002; Tiangtip and Jongwutiwes, 2002; Gatei et al., 2003; Chhin et al., 2006) and has been associated with an increased duration and magnitude of oocyst shedding (McLauchlin et al., 1999; Xiao et al., 2001). However, further study is needed to elucidate the relationship between species/genotypes of *Cryptosporidium* and their virulence and pathogenicity as well as oocyst excretion rates in HIV-infected individuals. Furthermore, the existence of geographical variation in the distribution of *C. parvum* and *C. hominis* in such humans remains inconclusive, as most previous data have been from small-scale studies (e.g., Leav et al., 2002; Alves et al., 2003; Muthusamy et al., 2006; Ajjampur et al., 2007; Meear et al., 2007).

Although *C. parvum* and *C. hominis* have been reported as the most common species causing infections in HIV-infected people in developed countries (e.g., France, Switzerland, Spain and the USA) (Sulaiman et al., 1998; Morgan et al., 2000; Guyot et al., 2001; Llorente et al., 2007) and developing countries (e.g., Jamaica, Kenya, Malaysia, Peru, Portugal, South Africa, south India, Thailand...
and Vietnam) (Leav et al., 2002; Tiangtip and Jongwutiwes, 2002; Alves et al., 2003; Muthusamy et al., 2006; Cama et al., 2007; Llorente et al., 2007; Gatei et al., 2008; Zaidah et al., 2008). C. felis and C. meleagridis have also been reported (Morgan et al., 2000; Guyot et al., 2001; Alves et al., 2003; Matos et al., 2004). Interestingly, C. muris, C. canis and C. suis appear to be relatively common in HIV-infected people in developing countries, such as Jamaica (Gatei et al., 2008), Peru (Cama et al., 2007), south India (Muthusamy et al., 2006) and Thailand (Gatei et al., 2002; Tiangtip and Jongwutiwes, 2002), although information is still limited to date.

Given that C. parvum and C. hominis constituted 88% of the Cryptosporidium infections in the HIV-infected patients studied here, a further evaluation of the genetic variation within each of these two species was carried out using partial gp60. Six subgenotypes (i.e. C. hominis LaA14R1, lbA10G2R2, ldA15R2, leA11G2T3R1 and IfA11G1R2 and C. parvum lIdA15G2R1) were identified. C. hominis lbA10G2R2 has been regarded as the most prevalent and widely distributed subgenotype in infected humans in Australia (Chalmers et al., 2005; Jex et al., 2007, 2008), France (Cohen et al., 2006), Ireland (Zintl et al., 2009), Kuwait (Sulaiman et al., 2005), the Netherlands (Wielinga et al., 2008), Portugal (Alves et al., 2003), South Africa (Leav et al., 2002), USA (Zhou et al., 2003), Wales and northeast England (Chalmers et al., 2008). In addition, C. hominis lbA10G2R2 has been implicated as a dominant subgenotype in some waterborne and foodborne outbreaks of human cryptosporidiosis (cf. Sulaiman et al., 2001; Cohen et al., 2006). Although C. hominis lbA10G2R2 is mainly found in humans, this species has also been reported in a three-day old suckler calf and a six-year old Aberdeen Angus-cross cow (Smith et al., 2005).

Given the potential of C. hominis lbA10G2R2 to infect non-human hosts, Jex and Gasser (2010) hypothesized, based on a comprehensive review of the literature and sequence data, that C. hominis lbA10G2R2 could, at least in some circumstances, be transmitted from animals to humans.

Although less frequently reported than C. hominis lb, subgenotype C. hominis la is more genetically diverse at the subgenotypic level (Jex and Gasser, 2010). Within C. hominis la, laA12G1R1 is the commonest subgenotype, with reports from countries including Japan (Abe et al., 2006), Nepal (Wu et al., 2003), Pakistan (Chalmers et al., 2008) and Peru (Cama et al., 2008). C. hominis laA14R1 was identified herein; although not common, this subgenotype has also been documented for children with diarrhoea in Peru (Cama et al., 2008; Jex and Gasser, 2010). As for C. parvum, the most frequently identified C. parvum subgenotypes are within Iic and iid (Jex and Gasser, 2010). In this present study, C. parvum lIdA15G2R1 was identified, which had previously been associated with humans in the Netherlands (Wielinga et al., 2008) and dairy calves in Spain (Quilez et al., 2008). However, C. hominis IdA15R2, C. hominis leA11G2T3R1 and C. hominis IfA11G1R2, also detected in the present study, have not been reported previously.

An analysis of the partial tpi sequence data herein revealed all four positive isolates from HIV-infected patients as G. duodenalis assemblage A. Prior to this study, assemblage B had been identified as the predominant assemblage among the Semai indigenous people of peninsular Malaysia (Mohammed Mahdy et al., 2009a). Although both major G. duodenalis assemblages A and B have been found in humans throughout the world, their propensity to cause disease might vary. Assemblage A appears to be more widespread, whereas assemblage B is more restricted to localised endemic loci (Thompson and Meloni, 1993; Meloni et al., 1995). It remains to be established whether there is geographical variation in the prevalences of different G. duodenalis genotypes, as assemblage A has been found to be more prevalent in Canada (van Keulen et al., 2002), Ethiopia (Gelanew et al., 2007), Italy (Lalle et al., 2005), Mexico (Eligio-Garcia et al., 2005) and the USA (van Keulen et al., 2002). However, in Albania (Berrilli et al., 2006), Australia (Read et al., 2004; Yang et al., 2010), India (Sulaiman et al., 2004), Nepal (Singh et al., 2009), Peru (Sulaiman et al., 2004), the Philippines (Yason and Rivera, 2007), Turkey (Aydin et al., 2004) and the UK (Cacciò et al., 2005), assemblage B has been reported to predominate.

Although both assemblages A and B can cause disease, there have been a few attempts to correlate the severity of diarrhoea with genotypes detected. Homan and Mank (2001) discovered in their study of 18 Dutch patients infected with G. duodenalis, that assemblage A isolates were solely detected in patients with intermittent diarrhoeal complaints, while assemblage B isolates were present in patients with acute or persistent diarrhoeal complaints, suggesting that genetic characteristics of G. duodenalis may be a major determinant in the severity of giardiasis in humans. In contrast, a study of children of <5 years of age attending 10 day care centres in Western Australia indicated that assemblage B was more prevalent in asymptomatic children, whereas assemblage A was 26-times more likely to cause diarrhea in children (Read et al., 2002). In a recent study of Giardia in Malaysia, assemblage B infection was shown to be significantly correlated with clinical signs of giardiasis (e.g., gastroenteritis) (Mohammed Mahdy et al., 2009b). In the present study, the relationship between clinical manifestation and genotypes was not possible because of the small number of test-positive samples. Therefore, a large-scale molecular epidemiological investigation is warranted to determine the geographical distribution and prevalence of human-infective genotypes in Malaysia and the significance of ‘strain’-related differences in virulence.

Giardiasis is endemic in Malaysia, with prevalences ranging from 0.2% to 25% (Lim et al., 2008). Giardia has also been isolated from animals (i.e. rats, dogs and cattle) and environmental samples (Lim et al., 2008). In order to assist the Malaysian public health authorities to better understand the epidemiology of Giardia, recent studies have utilized molecular tools to elucidate the genetic composition of Giardia isolates found. Thus far, G. duodenalis assemblages A and B have been identified in an indigenous population and environmental water samples using PCR-based techniques, targeting the SSU gene (Mohammed Mahdy et al., 2009a; Lim et al., 2009a,b). Currently, G. duodenalis (which is a group of species) includes at least eight genetic assemblages (designated A–H) (Monis et al., 1999; Lasek-Nesselquist et al., 2010), with assemblages A and B having wide host ranges (including humans and a variety of other animals), while assemblages C–H are affiliated with animals other than humans (Olson et al., 2004; Thompson and Monis, 2004). Common hosts that assemblages A and B have been reported to occur in include livestock (e.g., cattle, sheep), horses, cats, dogs, beavers, muskrats, voles and slow loris (Smith et al., 2007; Cacciò and Ryan, 2008; Monis et al., 2009). On the other hand, assemblages C and D have been reported in dogs, E in livestock, F in cats, G in rats and H in marine vertebrates (e.g., Monis et al., 2003; Trout et al., 2005; Lasek-Nesselquist et al., 2010). To date, there is limited information regarding Giardia assemblages in HIV-infected individuals. This area warrants detailed investigation.

The results from the present study represent the first report of C. hominis, C. meleagridis and C. felis in HIV patients in Malaysia. This study also reports, for the first time, on genotypes of Giardia (assemblage A) in HIV-infected individuals in Malaysia. The significant prevalence of species of C. parvum and G. duodenalis inferred to be zoonotic begs further comprehensive epidemiological studies to conclude whether zoonotic transmission predominates in relation to these immuno-deficient people. In order to fully understand the public health significance of the different Cryptosporidium and Giardia species and genotypes in immuno-
compromised/-deficient patients, it will be imperative to establish the extent of genetic diversity within Cryptosporidium and Giardia affecting humans or animals through comprehensive studies of the molecular epidemiology of cryptosporidiosis and giardiasis in Malaysia. Considering the known resilience of Cryptosporidium and Giardia against conventional water treatments (MacKenzie et al., 1994, 1995) and of Cryptosporidium against most therapeutic agents (Kosek et al., 2001; Rigs, 2002), valuable insights gained from molecular epidemiological studies will enhanced efforts to prevent clinical disease linked to these protists in HIv-infected humans.

Acknowledgements

We thank Mohammad Mahdy (University of Malaya), Rosely Nichols, Lisa Connelly (Scottish Parasite Diagnostic Laboratory) and Ross Hall (Gasser Lab) for their assistance. Current research in the Gasser Lab is funded mainly by the Australian Research Council (ARC), the National Health & Medical Research Council (NHMRC) and Melbourne Water Corporation.

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