Genetic Characterization of *Cryptosporidium* spp. in Diarrhoeic Children from Four Provinces in South Africa

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Summary

The diversity of *Cryptosporidium* at species, subtype family and subtype level in diarrhoeic children was investigated in four provinces in South Africa. A total of 442 stool samples from children <5 years of age were collected under a large rotavirus surveillance programme and analysed by Ziehl–Neelsen acid-fast staining. Fifty-four (12.2%) were positive for *Cryptosporidium*, of which 25 were genotyped by polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) and DNA sequence analyses of the 18S rRNA gene. The majority of genotyped specimens were identified as *C. hominis* (76%), and a high genetic diversity was found with five different *C. hominis* subtype families (Ia, Ib, Id, Ie and If). *Cryptosporidium parvum* was found in 20% of the isolates, and three subtype families were identified (IIc, IIe and IIb), with subtype family IIc being the most common. One specimen was identified as *C. meleagridis* of the subtype family IIId. These results are in accordance with findings from other developing countries and report for the first time the presence in South Africa of *C. meleagridis*, various subtypes of *C. parvum* and the subtype family Ie of *C. hominis*. The results suggest that *C. hominis* and anthroponotic *C. parvum* subtypes are the major cause of cryptosporidiosis in South Africa. Further molecular studies are needed to better understand the epidemiology and public health importance of *Cryptosporidium* in humans in South Africa.

Impacts

• *Cryptosporidium* is a widespread parasite in the environment, and the findings of this study indicate a potential public health impact of *Cryptosporidium* in South Africa.

• Cryptosporidiosis represents a 15% of the cases of diarrhoeic children in four different regions of South Africa.

• Genetic analysis reveals that in our study, cryptosporidiosis was mainly anthroponotic and only one species found in this study might be of zoonotic origin.

Introduction

Cryptosporidium is an enteric parasite in a wide range of hosts, including humans, domestic and wild animals. *Cryptosporidium* infection can be associated with profuse, chronic or even life-threatening diarrhoea, particularly in young children and in immunocompromised persons (Xiao and Ryan, 2004). Children younger than 2 years of age are frequently infected in community and hospital settings in developing countries (Bern et al., 2000; Cama et al., 2008). Humans can acquire *Cryptosporidium* infections through direct contact with infected persons (anthroponotic transmission) or animals (zoonotic transmission), or by ingestion of contaminated food (foodborne transmission) or water (waterborne transmission) (Xiao, 2010).

The use of molecular tools to genotype and subtype *Cryptosporidium* parasites has contributed to improved understanding of the transmission of cryptosporidiosis in humans and animals. Humans can be infected by at least 10 *Cryptosporidium* species (*C. hominis*, *C. parvum*, *C. meleagridis*, *C. canis*, *C. felis*, *C. cuniculus*, *C. suis*, *C. muris*, *C. andersoni* and *C. ubiquitum*) and four genotypes (horse, skunk, chipmunk genotype I and pig genotype II), with *C. hominis* and *C. parvum* responsible for most infections (Xiao, 2010).

Molecular characterization of the 60-kDa glycoprotein (gp60) gene has recently been used for *C. hominis* and *C. parvum* subtyping. These analyses have shown at least eight *C. parvum* subtype families: two zoonotic (IIa and IId) and six anthroponotic (IIb, IIc, IIe, IIf, IIh and IIi) ones (Peng et al., 2003; Sulaiman et al., 2005). Within each subtype family, several subtypes have been described based on the variations in the number of trinucleotide repeats (TCA, TCG or TCT) of the gp60 gene (Xiao, 2010).

Geographic differences in the distribution of *C. parvum* and *C. hominis* infections have been reported: in European countries, both *C. parvum* and *C. hominis* are responsible for significant numbers of human infections; in the Middle East, *C. parvum* is the dominant species in humans, while in developing countries, the predominant species is *C. hominis* (Xiao, 2010). The anthroponotic IIc subtypes of *C. parvum* are responsible for most human infections in developing countries (Leav et al., 2002; Peng et al., 2003; Akiyoshi et al., 2006; Xiao and Feng, 2008); however in some African countries such as in Malawi and Kenya, unusual anthroponotic *C. parvum* subtype families (IIe and IIb) have been found in addition to the most common IIc subtype family (Peng et al., 2003; Xiao and Ryan, 2004; Cama et al., 2007). Furthermore, in developing countries, a high genetic diversity among *C. hominis* has

been observed, most commonly Ia, Ib, Id, Ie and If (Leav et al., 2002; Peng et al., 2003; Cama et al., 2007; Gatei et al., 2007).

In southern Africa, the number of molecular epidemiological studies of cryptosporidiosis in humans is very limited. Ten years ago, Leav et al. (2002) genetically characterized 20 specimens from hospitalized children with diarrhoea in Durban (KwaZulu-Natal Province) by gp60 sequence analysis; the majority of these samples were identified as *C. hominis* with four different subtype families Ia, Ib, Id and If, and a small number was observed as *C. parvum* with one subtype family (IIc). More recently, a report by Samie et al. (2006), based on PCR–RFLP analysis of the 18S rRNA gene in children and elderly persons from the Vhembe district (Limpopo Province), observed *C. hominis* in most infected individuals (82%; 36/44) and *C. parvum* in a smaller proportion (18%; 8/44). However, there are no published studies from South Africa describing the circulation of *Cryptosporidium* spp. on a wider geographic scale.

In this study, we analysed the diversity of *Cryptosporidium* at the species, subtype family and subtype levels in diarrhoeic children of 0–12 months in age, from four different provinces in South Africa (Cohen et al., 2010). The findings of this study were compared with those conducted in South Africa and other developing countries, particularly those on the African continent.

Material and Methods

Sampling

The study formed part of a large rotavirus surveillance programme carried out at four sites in South Africa (Cohen et al., 2010). The programme aimed to estimate the number of hospitalizations because of severe diarrhoea in children. Patients of <5 years of age were sampled and tested for diarrhoea-causing viruses, bacteria and parasites. Stool samples were collected with parental consent by study nurses over a period of 10 months (June 2010–April 2011) and were tested for rotavirus using an ELISA kit (Oxoid, Basingstoke, UK) and PCR confirmation (Cohen et al., 2010). Any raw faecal material left after rotavirus testing was sent to the Parasitology Reference Unit (PRU) at NICD for the detection of parasitic causes of diarrhoea.

A total of 442 specimens were available for this study. One hundred and 41 samples were from the Chris Hani Baragwanth Hospital (Gauteng Province), 128 from the Agincourt Hospitals (Mpumalanga Province), 147 from the Dr. George Mukhari Hospital (North West Province) and 26 from the Edendale Hospital (KwaZulu-Natal Province) (Fig. 1). Samples were transported to the National Institute for Communicable Diseases for testing and stored at room temperature until analysed. The same samples were tested within 1 week using modified Ziehl–Neelsen staining. Fifty-four samples were *Cryptosporidium* positive, and among those forty (all from children under 12 months of age) were available for molecular analysis.

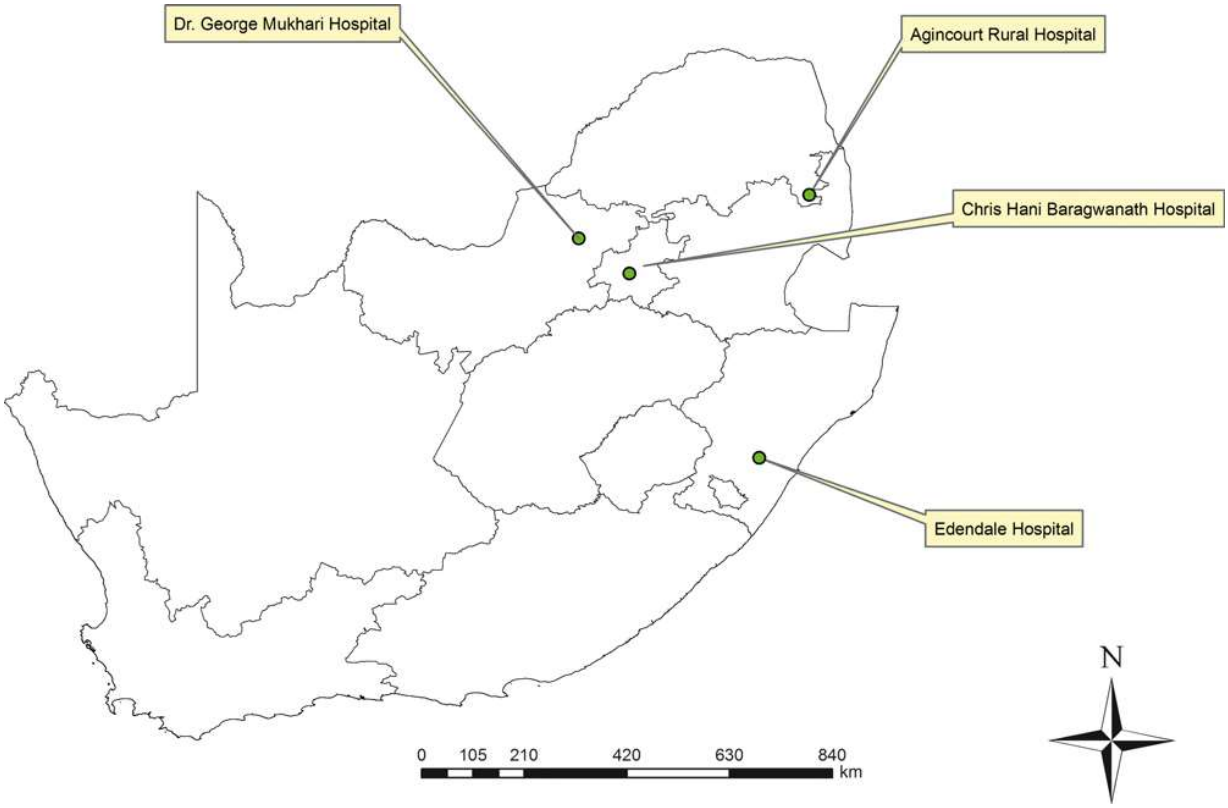

Figure 1. Location of clinics where sampling took place.

DNA extraction and *Cryptosporidium* genotyping and subtyping

Molecular analysis was carried out at the Centers for Disease Control and Prevention in Atlanta, Georgia, within fourteen months of receipt. DNA was extracted using the FastDNA SPIN kit for soil (BIO 101, Carlsbad, CA, USA). Briefly, approximately 0.5 g of faecal sample was transferred into a 2-ml tube containing lysing matrix E. After adding 100 µl sodium phosphate buffer and 122 µl MT buffer, the tube was vortexed in a FastPrep instrument (BIO 101). The sample was processed further following the manufacturer's suggested protocol. Initially, all specimens were genotyped by a PCR–restriction fragment length polymorphism (RFLP) technique in which an approximately 830-bp fragment of the 18S rRNA gene was amplified by a nested PCR. Primers and amplification conditions used in this study have been described previously (Xiao et al., 2001). Genotype identification was made by restriction digestion of the secondary PCR product with the enzymes SspI and VspI (Xiao et al., 2001).

Cryptosporidium parvum and *C. hominis* were further subtyped by a gp60 gene locus that amplifies an 850-bp fragment of the gp60 gene by nested PCR (Sulaiman et al., 2005). All PCR positive products were sequenced, using the forward and reverse primer used in secondary PCR and an intermediary sequencing primer (5′-GAGATATATCTTGTTGCG-3′), and the ABI BigDye Terminator Version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequences were read on an ABI1300 Genetic Analyzer (Applied Biosystems).The nucleotide sequences obtained in this study were assembled using the ChromasPro software (http://www.technelysium.com.au/ChromasPro.html) and aligned with

Cryptosporidium reference sequences using ClustalX software (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/). Subtypes were named by an established terminology (Sulaiman et al., 2005; Xiao, 2010).

Results

Of the 442 specimens tested by ZN staining and microscopy, 54 (12.2%) were positive for *Cryptosporidium* spp. The highest prevalence was found at the Chris Hani Baragwanth Hospital (17.7%; 25/141), followed by Edendale Hospital (15.4%; 4/26), Dr. George Mukhari Hospital (9.5%; 14/147) and Agincourt Hospital (8.6%; 11/128) (Table 1). Owing to the insufficient amount of faecal material, of these 54 samples, only 40 specimens were available for molecular analysis. Thirty samples were positive using 18S PCR and gp60 PCR, and 25 samples were successfully subtyped at the gp60 locus. Sequence analysis of the gp60 locus identified *C. hominis* (76%; 19/25) most frequently, followed by *C. parvum* (20%; 5/25) and

Table 1. *Cryptosporidium* species identified in diarrhoeic children in hospitals in South Africa

	C. parvum	***C. hominis***	***C. meleagridis***	**TOTAL**
Chris Hani Baragwanth (GP)	1	8	1	10
Agincourt (MP)	3	3	0	6
Dr. George Mukhari (NW)	1	7	0	8
Edendale (KZN)	0	1	0	1

GP, Gauteng Province; MP, Mpumalanga Province; NW, North West Province; KZN, KwaZulu-Natal Province.

Table 2. *Cryptosporidium* gp60 subtype families and subtypes identified in diarrhoeic children in hospitals in South Africa

Cryptosporidium species	gp60 subtype family	gp60 subtype	No. of isolates
C. hominis (5 subtype families)	Ia	Ia A20R3	1
		Ia A25G1R3	1
		Ia A17R3	1
	Ib	Ib A9G3	1
		Ib A10G1	1
	Id	Id A20	1
		Id A25	1
		Id A26	2
		Id A24	1
	Ie	Ie A11G3T3	4
	If	If A14G1	3
		If A12G1	2
Subtotal	5	13	19
C. parvum (3 subtype families)	IIb	IIb A11	1
	IIc	IIc A5G3b	3
	IIe	IIe A12G1	1
Subtotal	3	3	5
C. meleagridis	IIId	IIId A4	1
Total	9	17	25

C. meleagridis (4%; 1/25). Five subtype families were identified within *C. hominis*: Ia, Ib, Id, Ie and If. The most common *C. hominis* subtype families observed were If and Id (5/19 each), followed by Ie (4/19), Ia (3/19), and Ib and Id (1/19 each). The highest diversity of *C. hominis* subtypes was found within subtype family Id with four subtypes, followed by Ia with three subtypes, Ib and If, each with two subtypes and Ie with one subtype (Table 2).

Infections with *C. parvum* belonged to three subtype families: IIc, IIe and IIb, and the subtype family IIc was the most commonly found. In this study, only one isolate was identified as *C. meleagridis*, of the subtype family IIId and the subtype IIIdA4 (Table 2).

Discussion

The present study produced data on the importance of *Cryptosporidium* in children with diarrhoea and the predominant *Cryptosporidium* species and subtypes circulating in this patient population in several provinces of South Africa. This is the third study on genetic characterization of *Cryptosporidium* spp. in children in South Africa and the first one providing information on a wide geographic scale.

Only 25 of the 40 specimens analysed were positive in the 18S rRNA-based PCR. The number of false positive specimens in our study appears to be considerably higher than reported in literature; Morgan et al. (1989) have demonstrated that PCRs improved the sensitivity to detect *Cryptosporidium* compared with ZN acid-fast staining and microscopy showed 83.7% sensitivity and 98.9% specificity compared with 100% sensitivity and specificity for PCR. This discrepancy with our study may have been due to the fact that a small amount of faecal material was available for testing (as the samples were used for rotavirus testing and acid-fast staining beforehand), and therefore, a lack of rRNA amplification might have contributed to a high number of false positives.

Of the 25 isolates that were subtyped, 19 (76%) belonged to *C. hominis*, 5 (20%) were *C. parvum*, and 1 (4%) was *C. meleagridis*. These findings are consistent with results found in previous studies in Africa and other developing countries, where 79–90% of infections are caused by *C. hominis* (Xiao and Feng, 2008). Likewise in South Africa, Leav et al. (2002) detected mainly *C. hominis* and a small proportion of *C. parvum* in their isolates at the gp60 locus, and Samie et al. (2006) identified 82%*C. hominis* and 18%*C. parvum* in their study. The predominance of *C. hominis* has also been observed in other paediatric populations in Africa, such as in Malawi (Peng et al., 2003), Kenya (Gatei et al., 2006) and Uganda (Tumwine et al., 2005).

Four common *C. hominis* subtype families (Ia, Ib, Id and Ie) are usually observed in humans in developing countries (Xiao, 2010). In a previous study of HIV-positive children in South Africa, a high diversity of *C. hominis* was observed by Leav et al. (2002). However, a subtype family that is rare in other countries, If, was commonly detected, whereas the subtype family Ie was not detected. The results of the present study confirm and expand these findings: the most common *C. hominis* subtype family observed was If, followed by Ie, Id and Ia, and Ib (Table 2).The *C. hominis* Ie subtype identified, IeA11G3T3, has been reported in human infections from other developing countries (Xiao, 2010), such as Nigeria (Akinbo et al., 2010).

The *C. hominis* subtype family Id has previously been widely reported in other countries in Africa (Kenya, Malawi and South Africa), India and Latin America (Peru) (Strong et al., 2000; Leav et al., 2002; Peng et al., 2003; Xiao, 2010). Among the four Id subtypes identified in this study, subtype IdA24 was previously reported in Kenyan children (Gatei et al., 2006). Both *C. hominis* Ib subtypes identified in this study, IbA9G3 and IbA10G2, are commonly found in humans in developing countries (Peng et al., 2003; Gatei et al., 2007).

In this study as well as in previous studies conducted in South Africa (Leav et al., 2002) and in other developing countries (Xiao, 2010), the anthroponotic IIc subtype was the dominant *C. parvum* parasite. However, some other unusual anthroponotic *C. parvum* subtype families, such as IIe and IIb, have occasionally been described in India, Malawi, Kenya and Uganda (Xiao, 2010). This is consistent with the findings in our study, where three different *C. parvum* subtype families were identified: IIc, IIb and IIe, with IIc being the most common subtype family (Table 2). These results suggest that *C. parvum* in children with diarrhoea in South Africa is likely to be of anthroponotic nature.

To our knowledge, this is the first time that *C. meleagridis* has been detected and subtyped in South Africa. *Cryptosporidium meleagridis,* originally a possible mammalian *Cryptosporidium* species (Xiao et al., 2002), has been widely reported in avian hosts (Qi et al., 2011). This may explain the ability of *C. meleagridis* to infect a wide range of mammals, including humans, rodents and calves (Xiao et al., 2002). In several developing countries, a high prevalence of *C. meleagridis* has been reported in both immunosuppressed and immunocompetent humans (Xiao, 2010). Therefore, it has been recognized as an important human pathogen in Africa (Kenya), Latin America (Peru) and Asia (Thailand) (Xiao et al., 2001; Gatei et al., 2006; Hung et al., 2007). Whether *C. meleagridis* is of zoonotic origin is not yet clear (Xiao, 2010). However, its common occurrence in pet birds and humans has potentially important public health implications. This study highlights the presence of *Cryptosporidium* in our sample of diarrhoeic children; however, diarrhoea in our study population could also have been caused by other concomitant pathogens, such as *Escherichia coli* or rotavirus. Owing to the absence of asymptomatic control subjects, it is difficult to document the real importance of the presence of *Cryptosporidium* as a diarrhoea-causing agent in children in South Africa. This study also extends our knowledge of the genetic diversity of *Cryptosporidium* species among diarrhoeic children in several regions of the country and reports for the first time the presence of *C. meleagridis*, various subtypes of *C. parvum* and the subtype family Ie of *C. hominis*. The presence of anthroponotic *C. parvum* subtype families and the predominance of *C. hominis* found in this study suggest that cryptosporidiosis in humans in South Africa is predominantly of anthroponotic nature. Nevertheless, studies in developing countries specifically targeting rural areas, where conditions of poverty and contacts with animals are more common, may reveal a higher importance of zoonotic transmission in human cryptosporidiosis. Further molecular epidemiologic studies targeting populations over wider geographic areas are needed to better understand the distribution, epidemiology and public health impact of *Cryptosporidium* species and subtypes in southern Africa. In addition, the associated environmental risk factors, such as water sources and contact with domestic and wild animals, should be investigated further.

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References

Akinbo, F. O., C. E. Okaka, R. Omoregie, T. Dearen, E. T. Leon, and L. Xiao, 2010: Molecular characterization of Cryptosporidium spp. in HIV-infected persons in Benin City, Edo State, Nigeria. *Fooyin J. Health. Sci*.2, 85–89.

Akiyoshi, D. E., J. K. Tumwine, S. Bakeera-Kitaka, and S. Tzipori, 2006: Subtype analysis of Cryptosporidium isolates from children in Uganda. *J. Parasitol.*92, 1097–1100.

Bern, C., B. Hernandez, M. B. Lopez, M. J. Arrowood, A. M. De Merida, and R. E. Klein, 2000: The contrasting epidemiology of Cyclospora and Cryptosporidium among outpatients in Guatemala. *Am. J. Trop. Med. Hyg*.63, 231–235.

Cama, V. A., J. M. Ross, S. Crawford, V. Kawai, R. Chavez-Valdez, D. Vargas, A. Vivar, E. Ticona, J. Williamson, Y. Ortega, R. H. Gilman, C. Bern, and L. Xiao, 2007: Differences in clinical manifestations among Cryptosporidium species and subtypes in HIV-infected persons. *J. Infect. Dis*.196, 684–691.

Cama, V., C. Bern, J. Roberts, L. Cabrera, C. Sterling, Y. Ortega, R. Gilman, and L. Xiao, 2008: Cryptosporidium species and subtypes and clinical manifestations in children, Peru. *Emerg. Infect. Dis*.14, 7.

Cohen, C., D. Du Plessis, J. Frean, K. Keddy, K. Kistiah, T. Kruger, B. Mogoye, J. Moyes, V. Msimang, Z. Makhari, S. Nadan, N. Page, B. Poonsamy, A. Smith, and S. Walaza, 2010. Rotavirus Surveillance in South Africa. Communicable Diseases Surveillance Bulletin (Johannesburgh, South Africa).

Gatei, W., C. Wamae, C. Mbae, A. Waruru, E. Mulinge, T. Waithera, S. Gatika, S. Kamwati, G. Revathi, and C. Hart, 2006: Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. *Am. J. Trop. Med. Hyg*.75, 78–82.

Gatei, W., P. Das, P. Dutta, A. Sen, V. Cama, A. A. Lal, and L. Xiao, 2007: Multilocus sequence typing and genetic structure of *Cryptosporidium hominis* from children in Kolkata, India. *Infect. Genet. Evol*.7, 197–205.

Hung, C.-C., J. C. Tsaihong, Y.-T. Lee, H.-Y. Deng, W.-H. Hsiao, S.-Y. Chang, S.-C. Chang, and K.-E. Su, 2007: Prevalence of intestinal infection due to Cryptosporidium species among Taiwanese patients with human immunodeficiency virus infection. *J. Formos. Med. Assoc*.106, 31–35.

Leav, B. A., M. R. Mackay, A. Anyanwu, R. M. Connor, A. M. Cevallos, G. Kindra, N. C. Rollins, M. L. Bennish, R. G. Nelson, and H. D. Ward, 2002: Analysis of sequence diversity at the highly polymorphic Cpgp40/15 locus among Cryptosporidium isolates from human immunodeficiency virus-infected children in South Africa. *Infect. Immun*.70, 3881–3890.

Morgan, U. M., L. Pallant, B. W. Dwyer, D. A. Forbes, G. Rich, and R. C. A. Thompson, 1998: Comparison of PCR and Microscopy for detection of Cryptosporidium parvum in human fecal specimens: Clinical Trial. *J. Clin. Microbiol*.36, 995–998.

Peng, M. M., S. R. Meshnick, N. A. Cunliffe, B. D. M. Thindwa, C. A. Hart, R. L. Broadhead, and L. Xiao, 2003: Molecular epidemiology of Cryptosporidiosis in children in Malawi. J. Eukaryot. *Microbiol*.50, 557–559.

Qi, M., R. Wang, C. Ning, X. Li, L. Zhang, F. Jian, Y. Sun, and L. Xiao, 2011: Cryptosporidium spp. in pet birds: genetic diversity and potential public health significance. *Exp. Parasitol*.128, 336–340.

Samie, A., P. O. Bessong, C. L. Obi, J. E. A. D. Sevilleja, S. Stroup, E. Houpt, and R. L. Guerrant, 2006: Cryptosporidium species: Preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa. *Exp. Parasitol*.114, 314–322.

Strong, W. B., J. Gut, and R. G. Nelson, 2000: Cloning and sequence analysis of a highly polymorphic *Cryptosporidium parvum* gene encoding a 60-Kilodalton glycoprotein and characterization of its 15- and 45-kilodalton zoite surface antigen products. *Infect. Immun*.68, 4117–4134.

Sulaiman, I. M., P. R. Hira, L. Zhou, F. M. Al-Ali, F. A. Al-Shelahi, H. M. Shweiki, J. Iqbal, N. Khalid, and L. Xiao, 2005: Unique endemicity of Cryptosporidiosis in children in Kuwait. *J. Clin. Microbiol*.43, 2805–2809.

Tumwine, J., A. Kekitiinwa, S. Bakeera-Kitaka, G. Ndeezi, R. Downing, X. Feng, D. Akiyoshi, and S. Tzipori, 2005: Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. *Am. J. Trop. Med. Hyg*.73, 921–925.

Xiao, L., 2010: Molecular epidemiology of cryptosporidiosis: an update. *Exp. Parasitol*.124, 80–89.

Xiao, L., and Y. Feng, 2008: Zoonotic cryptosporidiosis. *FEMS Immunol. Med. Microbio*l.52, 309–323.

Xiao, L., and U. M. Ryan, 2004: Cryptosporidiosis: an update in molecular epidemiology. *Curr. Opin. Infect. Dis*.17, 483–490.

Xiao, L., C. Bern, J. Limor, I. Sulaiman, J. Roberts, W. Checkley, L. Cabrera, R. H. Gilman, and A. A. Lal, 2001: Identification of 5 types of Cryptosporidium parasites in children in Lima, Peru. *J. Infect. Dis*.183, 492–497.

Xiao, L., I. M. Sulaiman, U. M. Ryan, L. Zhou, E. R. Atwill, M. L. Tischler, X. Zhang, R. Fayer, and A. A. Lal, 2002: Host adaptation and host-parasite co-evolution in Cryptosporidium: implications for taxonomy and public health. *Int. J. Parasitol*.32, 1773–1785.