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C. I. NYAMWANGE, G. MKOJI, S. MPOKE and H. S. NYANDIEKA

ABSTRACT

***Objectives*: To determine the prevalence of cryptosporidiosis and the associated factors, and characterise the *Cryptosporidium* isolates from children aged five years and less with diarrhoea.**
***Design*: A prospective cross-sectional study.**
***Setting*: This was a health facility and laboratory based study. Screening for *Cryptosporidium* oocysts was done at the Microbiology laboratory, School of Medicine, Moi University, Eldoret and genotyping and sub-genotyping at the Kenya Medical Research Institute, Nairobi, Kenya.**
***Subjects*: Children aged five years and less seen at the outpatient clinic and those admitted in the pediatric wards at MTRH were recruited into the study upon obtaining assent and written consent from the parents or guardians.**
***Results*: The prevalence of cryptosporidiosis was 9.8% (N=317). A duration of diarrhoea of more than two weeks was associated with cryptosporidiosis (OR= 1.8301) compared to those with diarrhoea for less than one week. There were no sex related differences in the cryptosporidiosis prevalence (P= 0.9752). Waste disposal, water sources and treatment, and livestock in homesteads were not associated with cryptosporidiosis. About 82% of the isolates were *C. hominis* and 18% were *C. parvum*. There were 6 subtypes of *C. hominis* and 4 subtypes of *C. parvum* in circulation.**
***Conclusion*: The prevalence of cryptosporidiosis is comparable to other regions of the world with *C. hominis* being the most common followed by *C. parvum*. Human-to-human transmission is the main mode of spread of cryptosporidiosis. All the Cryptosporidium isolates were from children residing in peri-urban and rural areas.**

INTRODUCTION

Cryptosporidium parvum is an apicomplexan protozoan parasite that causes gastrointestinal illness known as cryptosporidiosis in a wide variety of mammals, including humans, cattle, sheep, goats, pigs, and horses. The disease is characterised by watery diarrhoea, and while it can resolve without intervention in immunocompetent individuals, cryptosporidiosis has increasingly become a major public health problem. In humans, clinical disease and shedding of oocysts appears to occur at all ages, but is most common among children and immunocompromised individuals (1). A study in Lima, Peru found that children with cryptosporidiosis experienced growth faltering, both in weight and in height for several months after the onset of infection, suggesting that cryptosporidiosis may have adverse effects on child growth, especially if the infection is acquired during infancy (2). Cryptosporidiosis has also been associated with impaired physical fitness and cognitive function in children (3). Similarly, in developed countries water-borne outbreaks have had a significant economic impact. For example, the Milwaukee outbreak in 1993 in USA cost 96.2 million US dollars in medical costs and productivity losses (4) and it is estimated that the United Kingdom spends 23 million sterling pounds per year to meet legal requirements for removal of *Cryptosporidium* from drinking water (5).

Little is known about the epidemiology of cryptosporidiosis in Kenya and virtually nothing is known about the prevalence and epidemiology of the disease in the North Rift region of Kenya. Simwa *et*

al. (6) observed that 3.8% of the 1420 fecal samples examined from children of five years of age and below with diarrhoea from the Kiambu district were positive for Cryptosporidium oocysts. *C. parvum* is commonly present in Kenya but other species such as *C. felis*, *C. meleagridis*, *C. muris* and C. *canis* have been shown to be present (7-9).

Most of the information available on cryptosporidiosis in Kenya is based on a few field-based or hospital-based studies undertaken in or around Nairobi. The objective of the study was to determine the prevalence and the associated factors of Cryptosporidium infections and characterise Cryptosporidium isolates obtained from children aged five years and below with diarrhoea seen at Moi Teaching and Referral Hospital in Eldoret.

MATERIALS AND METHODS

Collection of human stool samples: Stool samples were collected from children with diarrhoea at the MCH clinic and paediatric wards of MTRH on a daily basis for a period of one year (January through December 2005). The stool samples were collected in poly pots from the children after written informed consent and assent had been obtained from the parent(s) or guardian. The stool samples were screened for *Cryptosporidium* oocysts within eight hours of passage using modified ZN staining and examined by compound microscopy. Positive samples were aliquoted into two portions and preserved in 2.5% potassium dichromate or kept without preservative but frozen at –80^0 C. The samples were then transported in cool boxes to Centre for Microbiology Research in KEMRI, Nairobi, Kenya for DNA isolation and molecular analysis.

DNA Isolation from Cryptosporidium Oocysts: Total genomic DNA was isolated from *Cryptosporidium* oocysts in stool samples that had been preserved in 2.5% potassium dichromate or kept frozen at -80^{o}C as per the manufacturer's instructions supplied with the QIAamp® DNA Stool Mini Kit (Qiagen Ltd Crawley, West Sussex, United Kingdom). Briefly, a pea-size amount of formed stool or 200 μL of diarrheic stool was aliquoted into a 1.5 ml eppendroff tube, washed four times using 800 μL of distilled water prior to DNA extraction and centrifuged at 13000 rpm for five minutes. The pellet was then suspended in 1.4 ml of ASL extraction buffer supplied in a QIAamp kit. Oocysts were ruptured by subjecting them to a freeze-thaw cycle of -80°C for 30 min and -80°C for 15 minutes followed by thawing at 75 ^{0}C. DNA was then extracted from the suspension using a QIAamp® DNA extraction kit for stool according to the manufacturer's instructions.

PCR-RFLP analysis: The Identification of *Cryptosporidium* Genotypes was done using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the 18s rRNA gene as described by Morgan *et al*. (7) and Xiao *et al*. (10). This technique involves nested PCR of the small subunit of the 18S gene followed by restriction digestion of the nested PCR product using *Ssp*1 and *Vsp*1 DNA restriction enzymes. For the primary PCR a forward primer 5'-TTC TAG AGC TAA TAC ATG CG-3' and reverse primer 5'-CCC TAA TCC TTC GAA ACA GGA-3'were used. The PCR reaction mixture contained 5 μl of Perkin-Elmer 10X PCR buffer (Norwalk, Conn, UK), 3 mM MgCl2, 240 mM (each) deoxynucleoside triphosphate (Promega), 250 nM (each) primer, 1.25 Units of *Taq* polymerase (Boehringer Mannheim, Livingston, United Kingdom), 4µg/µL of non-acetylated BSA (Sigma), and 0.5 μL of DNA template in a total 50 μL reaction mixture. A total of 35 cycles were carried out, each consisting of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for one minute, with an initial hot start at 94°C for three minutes and a final extension at 72°C for seven minutes. The PCR products were stored at 4 ^{0}C and used as template for the secondary PCR. The secondary PCR was performed in a total of 50 μL reaction mixture without BSA using 1 μL of the primary PCR product as template, forward primers 5´-GGA AGG GTT GTA TTT ATT AGA TAA AG-3´ and the reverse primer 5´-AAG GAG TAA GGA ACA ACC TCC A-3´ in a final concentration of 500 nM to amplify an 826 to 864 bp internal fragment. The concentrations of other reaction mix and amplification conditions for secondary PCR were the same as the primary reaction. Amplicons were analysed by electrophoresis in a 2% agarose gel and visualised after ethidium bromide staining.

To differentiate the cryptosporidium genotypes Restriction Fragment Length Polymorphism (RFLP) analysis of the 18S rRNA secondary PCR products was done by digesting 15 μL of each secondary PCR product in a total reaction volume of 40 μL consisting of 2μL (20 units) of *Ssp*1 (Promega) and 4 μL of 1X restriction buffer. The second set of restriction using *Vsp*1 (Promega), was also performed in 40 μL volume except that 1 μL (10-12 units) of the enzyme was used. The restriction reactions were carried out in a water bath at 37°C for a minimum of one hour or overnight. The digestion products were separated on 2% agarose gel and visualised by ethidium bromide staining.

Isolate sub-typing: Nested PCR of the CP47 gene was used to subtype the *Cryptosporidium* speciesas described by Gatei *et al*., (11). The following primers were used for a nested PCR amplification of a 380-500 bp fragment of the gene. For the primary PCR forward primer 5'-GCT TAG ATT CTG ATA TGG ATC TAT-3 and a reverse primer 5'-AGC TTA CTG GTC CTG TAT CAG TT-3' were used, and for secondary PCR forward primer 5'-ACC CCA AGA AGG CGG

ACC AAG GTT-3′ and reverse primer 5′-GTA TCG TGG CGT TCT GAA TTA TCA A-3′ were used. The primary PCR was performed in 50 µL volumes containing 1X PCR buffer, 3 mM $MgCl_2$, 240 µM of each dNTP, 250 nM of each primer, 4 µg/µL of non-acetylated BSA, 1.25 units of *Taq* polymerase and 0.5 µL of DNA template. Then 35 PCR cycles (94^oC for 45 seconds, 43^oC for 45 seconds and 72^oC for 60 seconds) were performed with an initial hot start at 95^oC for three minutes and a final extension step at 72^oC for 10 minutes. The secondary PCR was performed without BSA in 100 µL volumes using 2 µL of the primary PCR product and increasing the final concentration of primers to 500 nM while the rest of the ingredients remained at the same concentration as in the primary PCR. The PCR conditions remained the same except for the annealing temperature which was adjusted to 55°C. The PCR bands were stained with ethidium and visualized under the UV light. The bands were then carefully excised and the PCR products were extracted and purified from the agarose gel using QIAquick® gel extraction kit and protocol as per the manufacturer's instructions. Briefly, the gel slice was weighed and three volumes of buffer QG (Qiagen, Proprietary composition), incubated at 50°C for 10 minutes. One gel volume of isopropanol was added, centrifuged at 16.1g for one minute in a QIAquick spin column, washed using buffer QG (Qiagen, Proprietary composition) and centrifuged at 16.1g for five minutes. The DNA was washed by adding buffer PE (Qiagen, Proprietary composition) and finally eluted from the column using buffer EB ((10 mM Tris-HCl, pH 8.5) and kept at -20°C.

Sequencing of the Purified CP47 Gene PCR Products: The two-directional sequencing of the DNA fragments of the CP47 region was done at the International Livestock Research Institute (ILRI) in Nairobi, Kenya. The PCR products were sequenced in both directions using the CP47 specific forward and reverse primers on an ABI Prism 3730 Genetic Analyser (Applied Biosystems). The nucleotide sequences were read using the Analysis Software (http://www.technelysium.com.au/ChromasPro.html) that uses the four colors to color-code analysed data from all dye/virtual filter set combinations with adenine (A) as green, cytosine (C) as blue, guanine (G) as black, and thymine (T) as red in the electropherogram view.

Data Management and Analysis: The data collected by use of the structured questionnaire, microscopy and molecular biology techniques were stored and maintained in an excel package. Parasitological and epidemiological data were analysed by use of Epi Info computer package for dispersion, central tendency and associations (P-value <0.05 was considered statistically significant). The CP47 sequencing products were blasted using NCBI BLAST (www.ncbi.nlm.nih.gov/BLAST) to check whether they correspond to the CP47 sequences in the database and subsequently aligned by the ClustalW program (www.ebi.ac.uk/Tools/clustalw2/) with manual adjustment (12). A Trinucleotide Analysis was done by assigning the CP47 locus of *C. hominis* as type I and *C. parvum* as type II and for the subtype identification, the trinucleotide TAA was coded as A and the TGA/TAG was coded as G with the following digit showing the number of each trinucleotide repeat to elucidate the subtypes of the isolates.

Ethical approval: This study was approved by both the Institutional ethics and research committee (IREC) of MTRH and Moi University School of medicine, and The KEMRI/National Ethical Review Committee SSCP protocol No. 843.

RESULTS

A total of 317 children aged five years and below were sampled and screened for *Cryptosporidium* oocysts. Their HIV infection status was not established.

Prevalence of cryptosporidiosis in relation to age and sex: The proportion of males was 54.6% (173/317) and females' was 45.4% (144/317). The overall prevalence of cryptosporidiosis in these children was 9.8% (31/317). There was no difference in infection prevalence between males and females. Similarly, there was no significant difference in the risk of *Cryptosporidium* infection between the sexes, (OR= 0.9882, CI= 0.4693 to 2.0810, P= 0.9752). The children were grouped into five age groups and the age-specific prevalence determined as shown in the Table 1.

Table 1

Prevalence of cryptosporidiosis by the duration of diarrhoea and the logistic regression output for the in-between group comparisons

Age group (years)	Frequency	*Cryptosporidium* prevalence
>0 ≤ 1	221	7.7% (7/221)
>1 ≤ 2	55	12.7% (7/55)
>2 ≤ 3	28	21.4 (6/28)
>3 ≤ 4	8	0
>4 ≤ 5	2	0

There was a prevalence of 7.7% (7/221) in children aged one year and below, 12.7% (7/55) in those more than one year old but less or two years old, 21.4% (6/28) in those older than two years but less or three-years-old. When comparing the prevalence in between the age groups using the children of one year or less as reference, there was no significant difference in infection between the reference group and children aged between one and two years (OR = 2.00, CI = 0.8154 - 4.9056, P = 0.1300), but the difference was statistically significant between the reference group and those aged between two and three years (OR = 3.2727, CI = 1.1692 - 9.1611, P= 0.0240).

Prevalence of cryptosporidiosis by area of residence: The prevalence of infection by residence was 0% (N= 9) in the urban, 12.2% (N=172) in peri-urban and 7.4% (N= 136) in rural residences. However, the difference in infection prevalence between the peri-urban and rural populations were not significant (OR= 0.5707, CI= 0.2595 - 1.2563, P= 0.1635). The odds of infection were two times higher in children of peri-urban residences compared to those of rural residences.

Prevalence of cryptosporidiosis in relation to water sources: The main sources of water were river/spring 11.5% (N= 317), well/borehole 35.6% and tap water 49.5%. The prevalence of cryptosporidiosis stratified in relation to water sources was 10.6% (5/47), 8.0% (9/113) and 10.8% (17/157) among those using river/spring, well/borehole and tap water, respectively. Using logistic regression analysis, there was no statistically significant association between the prevalence of cryptosporidiosis and water sources (OR= 1.0902, CI= 0.6474 - 1.8360, P= 0.7453).

The major method of treating drinking water was by boiling 44.8% (N= 317), sieving 0.9% (N=317), adding chemical 11.7% (N=317) and none 42.6% (N=317). The prevalence of cryptosporidiosis in relation to method of water treatment was 8.45% (12/124), 8.11% (3/37), 11.85% (16/135) among those who boiled their drinking water, used chemicals and those who did not treat their water at all, respectively. However, there was no significant association between cryptosporidiosis and the methods of water treatment (P= 0.361). Furthermore, there was no significant differences between those who boiled their water or those who used chemical treatment (OR= 0.9559, CI= 0.2553 – 3.5790, P=0.9466) and those who did not treat water (OR=1.4566, CI= 0.6619 – 3.2052, P= 0.3500) when the group boiling water was used as baseline. The odds of infection were 1.46 times more in those who did not boil water as compared to those who boiled their water.

Prevalence of cryptosporidiosis in relation to waste disposal: About 89.9% (285/317) according to the guardians of the children, used pit latrines for human waste disposal, 9.8% (31/317) used flash toilets and 0.3% (1/317) used the bush. The prevalence of cryptosporidiosis stratified for methods of waste disposal was 9.1% (26/285) among pit latrine users and 16.1% (5/31) among flash toilet users. There was no significant association between the occurrence of cryptosporidiosis and methods of waste disposal though the odds of infection were 1.9 times in those using flash toilets as compared to those using pit latrines (OR= 1.9157, CI= 0.6781 - 5.4121, P= 0.2199).

Health symptoms and cryptosporidiosis: The proportion of children who presented with vomiting was 54.9% (174/317). The prevalence of cryptosporidiosis in children presenting with vomiting was 7.5% (13/174) while it was 12.6% (18/143) among the children without vomiting. There was no significant difference in the odds of *Cryptosporidium* infection between those presenting with and without vomiting (OR= 0.5607, CI= 0.2647 - 1.1877, P= 0.1309).

Table 2

The statistical parameters and association of health complaints with cryptosporidiosis

Health complaints		Prevalence		Odds ratio	95% CI	P-value
Symptom	Status	Symptom	Cryptosporidium			
Fever	Yes	63.3%	9.0%	0.7793	0.3670- 1.6650	0.5164
	No	36.7%	11.2%			
Cough	Yes	57.4%	8.8%	0.7711	0.3670- 1.6202	0.4926
	No	42.6%	11.1%			
Vomiting	Yes	54.9%	7.5%	0.5607	0.2647- 1.1877	0.1309
	No	45.1%	12.6%			

A proportion of the children 63.3% (201/317) had fever. The prevalence of cryptosporidiosis was 9.0% (18/201) among the children with fever, and 11.2% (13/116) among those with no fever. There was no significant association between *Cryptosporidium* infection and fever (OR= 0.7793, CI=0.3670 - 1.6650, P= 0.5164).

About 57.4% (183/319) of the children were reported to have a cough by their parents/guardians. The prevalence of cryptosporidiosis was 8.8% (16/182) among the children having cough and 11.1% (15/135) in those who did not have a cough. The logistic regression analysis did not show significant association of coughing with the occurrence of cryptosporidiosis (OR= 0.7711, CI= 0.3670 - 1.6202, P= 0.4926) as in Table 2.

Cryptosporidiosis and domesticated animals: A proportion of 12.9% (40/317) had cats, 16.7% (53/317) dogs, 67.5% (214/317) chicken, 19.9% (63/317) sheep, 14.8% (47/317) goats and 22.4% (71/317) cattle in their homesteads. The prevalence of cryptosporidiosis among those with and without the various domestic animals together with other statistical findings is shown in Table 3. It is worth noting that there was no statistically significant association of cryptosporidiosis with the domestic animals in homesteads.

About 42.6% (135/317) of the respondents reported not having any animals in their homesteads while 58.0% had at least one type of animal or the other. The prevalence of cryptosporidiosis among those who did not have any animals was 7.4% (10/135) and 11.5% (21/182) in those who had at least one type of animal. The logistic regression output did not show any significant difference in the odds of *Cryptosporidium* infection between those with and without animals within the homesteads, (OR=0.6133, CI= 0.2788 - 1.3492, P= 0.2242).

Table 3
Domestic animals and the statistical values associated with cryptosporidiosis

Domestic animals		Prevalence		Statistical values		
Animal	Status	Animals	*Crypto-sporidium*	Odds ratio	95% CI	P-value
Cats	Yes	12.9%	5.0%	0.4501	0.1032 - 1.9634	0.2881
	No	87.1%	10.5%			
Cattle	Yes	22.4%	7.0%	0.6410	0.2369 - 1.7347	0.3813
	No	77.6%	10.6%			
Chicken	Yes	67.5%	8.7%	0.8225	0.3644 - 1.8565	0.6381
	No	32.5	10.3%			
Dogs	Yes	16.7%	9.4%	0.9535	0.3487 - 2.6072	0.9261
	No	84.3%	9.8%			
goats	Yes	14.8%	6.4%	0.5893	0.1719 - 2.0201	0.4002
	No	85.2%	10.4%			
Sheep	Yes	19.9%	9.5%	0.9642	0.3778 - 2.4608	0.9392
	No	80.1%	9.8%			

Multivariate analysis for the association of domestic animals with cryptosporidiosis showed that none of the animals were predictors of cryptosporidiosis, Table 4.

Table 4
Predictors of cryptosporidiosis among domestic animals

Animal	Odds Ratio	95% C.I.	P-Value
cat	0.4121	0.0878 - 1.9349	0.2612
cattle	0.4880	0.1042 - 2.2867	0.3627
chicken	1.2872	0.4270 - 3.8800	0.6538
dog	0.7456	0.2172 - 2.5586	0.6407
goat	0.5493	0.1237 - 2.4398	0.4309
sheep	1.9489	0.4563 - 8.3240	0.3677

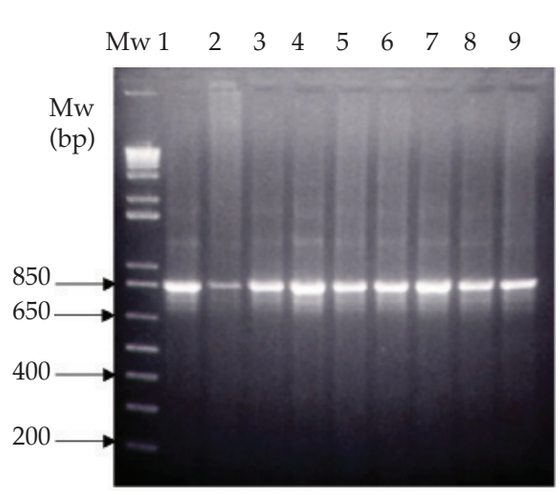
Prevalence of cryptosporidiosis in relation to residence: The overall mean number of rooms was 2.0. The mean number of rooms in the urban residences was 2.1 with a mean occupancy of four persons, peri-urban 1.7 with four persons and 2.2 in the rural residences with a mean occupancy of five persons. On stratifying for cryptosporidiosis the mean number of rooms ($P \leq 0.001$) and mean occupancy ($P = 0.003$) were found to be significantly different in the cryptosporidiosis negative households. The mean number of rooms were not significantly different in the cryptosporidiosis positive households ($P = 0.7951$) but the mean occupancy was significantly different ($P = 0.003$) between the areas of residence. On logistic analysis though, there was no significant association between the number of rooms (OR= 1.1864, CI= 0.8342 - 1.6872, P= 0.3415) and the mean occupancy (OR= 1.0271, CI= 0.8563 - 1.2319, P= 0.7731) with the occurrence of cryptosporidiosis.

MOLECULAR ANALYSIS

The Circulating Cryptosporidium Genotypes: Of the 31 isolates 28 were PCR amplified and the secondary PCR band products (826-864 bp) that are diagnostic of the *Cryptosporidium* species are shown in Figure 1.

Figure 1

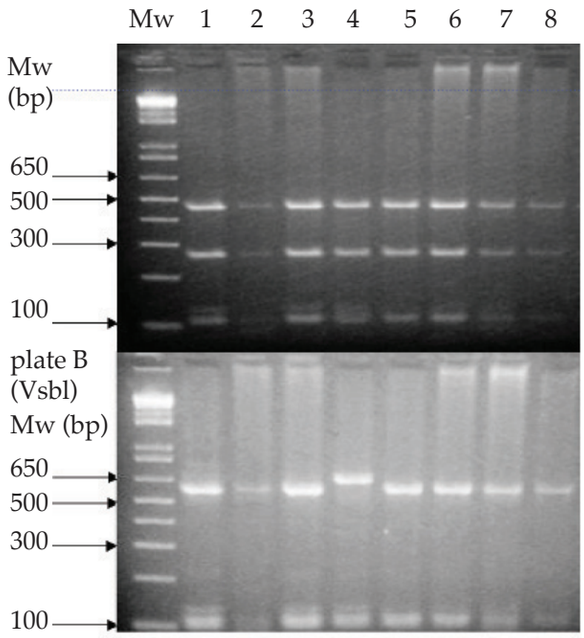
Agarose gel electrophoresis showing the nested PCR products of the of 18S rRNA gene (826 - 864 bp), diagnostic of Cryptosporidium species separated on 1.5% agarose gel stained with ethidium bromide. Lane 1:Sample A243, lane 2: P125, lane 3:A566, lane4: C504, lane5: P253, lane 6: P252, lane7: A594, lane 8: C537 and lane 9: A659



The RFLP analysis using *Ssp*1 enzyme, and *Vsp*1 restriction enzymes was used to identify the genotypes whose sample gel photograph is shown in Figure 2.

Figure 2

Genotyping of the Cryptosporidium species by a nested PCR-RFLP procedure based on the 18s rRNA gene sequences. Plate A: Ssp1 digestion products and Plate B Vsp1 digestion products. The fragments were separated on 2.0% agarose gel stained with ethidium bromide. Lane1 Molecular weight marker, Lanes 2,3,4,6,7 and 8, C. hominis and lane 5 C. parvum

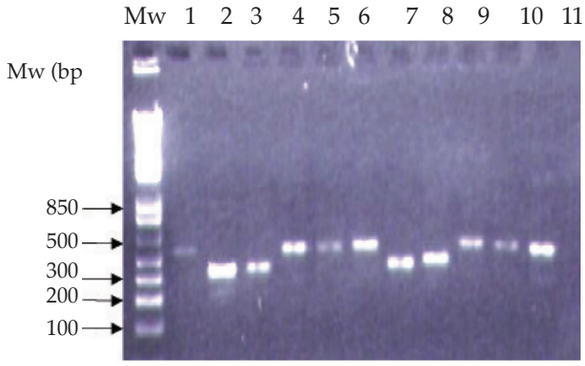


Distribution of Cryptosporidium Species: The genotypes isolated were *C. hominis*, which constituted 82.1% (23/28) and *Cryptosporidium parvum* at 17.9% of the isolates. The *C. hominis* isolates were from patients from peri-urban areas 60.9% (14/23) and 39.1% (9/23) from patients from rural areas. Of the 5 *Cryptosporidium parvum* isolates four were from patients staying in the peri-urban areas and one from the rural residence. The youngest patient with *C. hominis* infection was six months old and the oldest was twenty eight months old.

Sub-typing of Cryptosporidium Species: The nested PCR of the CP47 locus gave bands of 300-500 bp as shown in Figure 3.

Figure 3

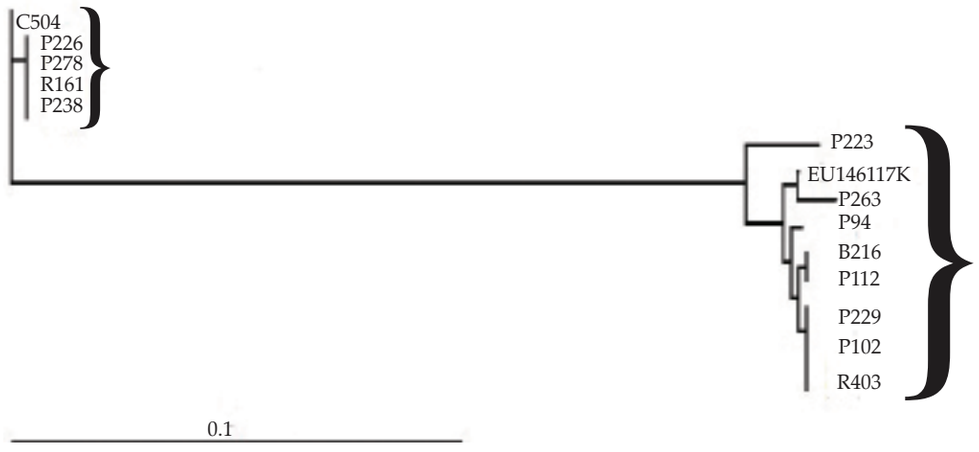
Agarose gel electrophoresis picture of the purified CP47 PCR fragments (300 – 500 bp) separated on a 2% agarose gel stained with ethidium bromide. Lane 1: Sample P94, lane 2: P263, lane 3: P353, Lane 4: P233, lane 5:A242, lane 6:P229, lane 8: P238, Lane 9: P102, Lane 10:P112 and lane 11:R161



Sequencing of the CP47 PCR Products: Of the 16 samples that were sequenced, 3 failed to amplify during sequencing. The 13 samples were aligned using ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The aligned sequences were used to draw the phylogenetic trees shown in Figure 4.

Figure 4

Phylogenetic tree of C. hominis and C. parvum isolates from the children seen at MTRH. The distance is at 10%. Two samples EU14611K and EU146118K were Kenyan sequences deposited at the EMBL database from the study by Gatei et al. (9)



Trinucleotide Analysis: This was done by assigning the CP47 locus of *C. hominis* as type I and *C. parvum* as type II and for the subtype identification, the trinucleotide TAA was coded as A and the TGA / TAG was coded as G with the following digit showing the number of each trinucleotide repeat. Six subtypes within the *C. hominis* genotype were identified IA52G29, IA52G30, all at (22.2%), IA53G29 IA56G33, IA54G30 and IA46G22 all at (11.1%) and four subtypes were identified in the *C. parvum* genotype as IIA37G15 (40%) , IIA36G14, IIA38G15 and IIA34G14 all at 20%.

DISCUSSION

A total of 317 fecal samples were examined for *Cryptosporidium* oocysts and a prevalence as 9.8% was recorded. A previous study found cryptosporidium prevalence of 4% and suggested that it was a major cause of diarrhoea in children (9). Higher prevalence values of between 10% and 32% in Africa have been reported in Uganda (13), Tanzania (14) and Egypt (15). In Switzerland, a prevalence of 5.5% was reported in children (16). In as much as there are variations observed in the prevalence levels between regions, these reports and the results of the current study reinforce the fact that *Cryptosporidium* infections are associated with diarrhoeal morbidity among children not only in the North Rift region but in other parts of the world.

PCR- RFLP analysis of *Cryptosporidium* isolates from children identified 28 out of the 31 isolates that were positive with stool microscopy giving a molecular prevalence of 8.8% (28/317). A proportion (78.6%) of the isolates were identified as *C. hominis*, 21.4% as *C. parvum* and there were no *C. meleagridis* isolates. The foregoing suggests that there is more of an anthroponotic and or environmental transmission, than a zoonotic transmission in children in the North Rift region of Kenya. These findings are consistent with those of Tumwine *et al.* (13) among children in Mulago hospital in Kampala in which 73.7% of the isolates were *C hominis* and 19.2% were *C. parvum.* Reccurt *et al.* (17) has also reported that 72% of the children regardless of their HIV status in a study conducted in Haiti were infected with *C.*

hominis. These studies underpin the role played by person-to-person and environmental transmission of cryptosporidiosis. Therefore, there is need for concerted efforts towards proper sanitation, personal hygiene and environmental management in a bid to reduce and control the spread of this disease.

Cryptosporidiosis prevalence was higher in children from peri-urban and rural areas of residence compared to those residing in urban areas. Even then, living in the peri-urban areas presented a greater risk of infection with *Cryptosporidium* (OR=1.75) than living in the rural areas. This trend compares well with the findings of Gatei *et al.* (9) elsewhere in Kenya, which showed that most children with cryptosporidiosis lived in peri-urban areas followed by those in rural areas and then urban areas. The peri-urban residential areas present a greater risk because they are densely populated mostly with families of low socioeconomic status, have limited or poor sanitary infrastructure and water supply system, a situation that is made worse by having pit latrines located a few meters from water points within small plots. Elsewhere on the Texas-Mexico border, Leach *et al*. (18) have too demonstrated that areas that are densely populated and with poor sanitary facilities portend greater risk for *Cryptosporidium* infection among children. The rural and peri-urban areas of the North Rift region of Kenya need a comprehensive field based study to investigate fully the risk factors associated with cryptosporidiosis.

None of the health complaints was significantly associated with cryptosporidiosis. These results are in agreement with those of Adjei *et al.* (19) in diarrheic children in Korle-Bu Teaching Hospital, Accra, Ghana in which the clinical symptoms of abdominal pain, nausea, vomiting, fever and blood in stool were not significantly associated with cryptosporidiosis. However, this is converse to the findings of a study conducted in Nairobi, Kenya by Gatei *et al*. (9) in which vomiting was significantly associated with cryptosporidiosis in children and by Al-Hindi *et al*. (20) in Al-Nasser paediatric hospital in which abdominal pain was significantly associated with cryptosporidiosis in children aged five years and below.

In conclusion, the prevalence of cryptosporidiosis among diarrheic children seen at MTRH is 9.8%. All the isolates of *C. hominis* and *C. parvum* were from the peri-urban and rural areas. Overall, the frequency of *C. hominis* was higher in the peri-urban population as compared to the rural population, whereas *C. parvum* frequency was higher in the peri-urban residences. Most of the homes of cryptosporidiosis positive subjects did not have domesticated animals thereby suggesting that transmission of the disease is mainly through anthroponotic and / or environmental transmission though zoonotic transmission cannot be ruled out.

That stool samples be examined for *Cryptosporidium* oocysts as a routine procedure among diarrheic children in our health facilities so as to institute appropriate management of diarrhoea if this organism is involved and that a comprehensive field based study be undertaken to establish the risk factors associated with the transmission of cryptosporidiosis among children in Kenya. Health education emphasizing on the improvement of sanitation and proper handling of human and animal waste disposal be conducted regularly among mothers.

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