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Original Research Article

Cryptosporidial diarrhoea in children at a paediatric hospital in Accra, Ghana.

ABSTRACT

Background: Diarrhoeal diseases are common among children in developing countries, and is caused by several aetiological agents including *Cryptosporidium* sp. Several species of this parasite exist which may belong to either anthroponotic or zoonotic forms. With recent application of molecular tools, species involved in human transmission in any locality and sources of infection can now be determined.
Aim: We screened children with acute diarrhoea at a paediatric hospital in Accra, Ghana for enteric parasites to determine frequency of cryptosporidial diarrhoea. *Cryptosporidium* isolates were then characterized by molecular methods to determine the genetic species in transmission.
Methodology: A total of 365 diarrhoeic children of age ≤ 5 years were used in this cross-sectional study. Stool samples were collected and tested for enteric parasites by microscopy and ELISA. *Cryptosporidium* isolates were subsequently genotyped by PCR-RFLP and confirmed by sequencing of the *18S rRNA* gene. Demographic and clinical data were obtained by a structured questionnaire and data analysed for possible association with cryptosporidial diarrhoea.
Results: Enteric parasites detected were *Cryptosporidium* sp. (22.2%), *G. lamblia* (5.8%) and *E. histolytica* (0.8%). Neither gender nor breastfeeding habits, presence of domestic animals, source of children's food, seasons (dry or rainy) appeared to be associated with infection of *Cryptosporidium* sp. However, age of children, source of drinking water, and education level of mother seems to have association with infection of the parasite. Genotyping results show that *C. parvum* is the main species involved in transmission.
Conclusion: *Cryptosporidium parvum* is the commonest enteric parasite causing diarrhoea among children with acute diarrhoea. Children ≤ 3 years and those who drank sachet water were most affected. A carefully planned health education among illiterate mothers and improved sanitary conditions could reduce rate of infections. Further sub-genotyping of *C. parvum* is needed to determine whether source of infection is zoonotic or anthroponotic.

Keywords: Cryptosporidium parvum, cryptosporidial diarrhoea, *18SrRNA* gene, anthroponotic, zoonotic.

1. INTRODUCTION

Diarrhoeal diseases are common among people in developing countries, and they cause considerable amount of morbidity and mortality, especially among children (1, 2). According to a United Nations Children's Fund (UNICEF)/ World Health Organization (WHO) joint report (3), diarrhoea remains the most common cause of death among children under five years globally. The report indicated that each year, about 1.5 million children in this age group die as a result of diarrhoea, and more than 80 per cent of these deaths occur in Africa and South Asia. Cryptosporidiosis caused by *Cryptosporidium* sp. is among the most common gastroenteritis in humans worldwide. Symptomatic infection is characterized by diarrhoea, epigastric pain, nausea, vomiting, and weight loss, though many infections are asymptomatic. The diarrhoea can develop into a persistent life threatening type especially in immune-deficient individuals and malnourished children. *Cryptosporidium* is widespread in the developing world, with 10–30% of individuals being asymptomatic oocyst excretors (4).

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The frequency of cryptosporidiosis worldwide is often dependent on HIV status. Besides humans, cryptosporidiosis is also a common cause of diarrhoeal disease in a number of other mammals (5, 6, 7), which suggests the zoonotic potential of the parasite. The oocyst, which is apparently not host specific, has been found in cattle, sheep, rats, mice, cats, dogs, rabbits and guinea pigs (8, 9).

Cryptosporidium is a protozoan parasite which belongs to the Phylum Apicomplexa, (Class Sporozoasida, Subclass Coccodiasina, Order Eucoccidiorida, Suborder Eimeriorina, Family Cryptosporidiidae). The genus *Cryptosporidium* now comprises 14 species, namely *C. hominis* in humans and monkeys, *C. parvum* in cattle, other mammals, and humans, *C. andersoni* in cattle, *C. muris* in rodents, *C. suis* in pigs, *C. felis* in cats, *C. canis* in dogs, *C. wrairi* in guinea pigs, *C. bailey* in poultry*, C. meleagrides* in turkeys and humans, *C. galli* in finches and chicken, *C. serpentis* in reptiles, *C. saurophilum* in lizard, and *C. molnari* in fish (10). Humans are most frequently infected with *C. hominis* and *C. parvum* (11, 12, 13, 14). Molecular characterization of the 60-kDa glycoprotein (GP60) gene of *C. hominis* and *C. parvum* has enabled further division into subtype families and subtypes (14). Molecular analyses indicate that *C. parvum* which is the major cause of cryptosporidiosis in humans comprises of at least two different genotypes (11). These are genotypes 1 (or human type), and genotype 2 (or calf type). Whilst genotype 1 is restricted to humans, genotype 2 is found in livestock as well as humans. Anthropophilic and zoonotic species such as *C. meleagridis* in turkeys, *C. muris* in mice, and *C. felis* in cats have also all been implicated in human illness (15). Identification of *C. parvum* genotypes has also been achieved through the amplification of the *Cryptosporidium* oocyst wall protein (COWP) (5). Other *Cryptosporidium* gene targets for amplification include the 18SrDNA, Hsp70, Actin, β-Tubulin, GP60, Microsatellites, Minisatellites, and Extrachromosomal double-stranded RNA (16).

Currently in many African countries, through application of genotyping and sub-genotyping techniques, the knowledge and understanding of the transmission dynamics of this parasite has improved. For instance, in Tunisia, *C hominis*, *C. parvum*, and *C. meleagridis* were all identified, with *C. hominis* and *C. parvum* being higher in diarrhoeal stools than in formed ones (17) Also, in that study, *C. hominis* was more prevalent in children from urban areas than those from rural areas. Among children at the Mulago hospital, Kampala, Uganda (18), it was revealed through application of the PCR-RFLP tool that the main species involved in transmission in the area was *C. parvum*. Similarly, the tool has been used to determine the extent of genetic diversity and transmission pathways of the parasite in the Kaduna state of Nigeria (19). According to that study *C. hominis* (Ia), *C. parvum* (IIe) were the commonest in the population. Importantly, the authors recognized that mode of transmission in this population was likely to be anthroponotic, as revealed by their genotyping results. In an earlier study at Osun State, Nigeria, similar techniques were employed to determine the species, genotypes, and subgenotypes of *Cryptosporidium* sp. in the area (20). The authors reported a high diversity of the parasite in the area, which include three species, namely *C. hominis*, *C. parvum*, and *C. meleagridis,* as well as *Cryptosporidium* rabbit genotype, the cervine genotype and *C. canis*. Observations from this study showed that source of infection could be anthroponotic, zoonotic and/ or environmental.

A recent study of paediatric diarrhoea in southern Ghana (21) revealed that *Cryptosporidium* was one of the aetiological agents. Although a prevalence rate of 8.7% was reported by the authors, like other previous studies in the country (22) the particular species involved in transmission was not determined. In many Ghanaian communities companion animals including cats and dogs, usually kept as pets as well as farm animals reared in close proximity to human habitations is a common practice. Our observations of frequent contamination of homes by faecal material of these animals and poor sanitary conditions

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prompted us of need to investigate possible association of environmental risk factors with cryptosporidiosis. In our study, we determined rate of cryptosporidial diarrhoea in children hospitalized for acute diarrhoea and the species involved in transmission by polymerase chain reaction (PCR) and nucleotide sequence analysis of the (18S) subunit of rRNA gene. Our results show the clinical significance of the parasite in paediatric diarrhoea in Accra, Ghana and provide preliminary information on the extent of genetic diversity. We also report on aspects of socioeconomic, behavioural and environmental risk factors needed to be considered in the planning and future implementation of intervention methods against transmission of this parasite in Ghanaian children.

2. MATERIAL AND METHODS

Study site and study participants

The study which was hospital-based, prospective and cross-sectional was conducted between the periods of March, 2010 and June, 2011 at the Princess Marie Louise Children's hospital (PML) at Accra, Ghana. The hospital is the main paediatric health facility located within Accra and accessed by people from all parts of the city. These include people from different socio-cultural, economic, political or educational background. The hospital has both out-patients and in-patients departments which are fully and well patronized by patients. According to the hospital's recent report, the daily average attendance of patients to the hospital is 143. This comprises of an average of 85 new attendants, and 58 old attendants. It is estimated that about 90 percent of these patients are children below 5 years, of which about 11 percent report with diarrhoea and other related problems (PML records).

The study participants were children (of age ≤ 5 years) who had been hospitalized at PML primarily due to acute diarrhoea and dehydration. Diarrhoea was defined as passage of loose or watery stools in the previous 24 hours and still present when the faecal specimen was collected at the hospital. Patients who developed diarrhoea after admission at the hospital, as well as patients with diarrhoea resulting from food intolerance were all excluded from our study. In all, 485 patients were referred to the study by paediatricians and nurses at the ward, 365 with diarrhoea and 120 without diarrhoea (control).

Socio-demographic and clinical data

Socio-demographic and clinical data were obtained by a study nurse at the hospital ward by use of a structured questionnaire. These included sex, age, parents educational background, source of drinking water, breastfeeding habits and source of food for children, and presence of domestic animals at home. Clinical data included vomiting, malaise, fever, abdominal pain, frequency of passage and consistency of stools, drugs used for treatment or management, as well as duration, if admitted, in the hospital.

Sample collection

A single stool sample was collected from each patient by parents or guardians and placed into a clean disposable plastic tube with tight fittings. The consistency of the stool was directly observed, classified and recorded by the study nurse as loose, semi-formed, formed, mucoid, slimy, or watery. Each sample was then divided into two portions (one portion was preserved in 10% formalin whilst the other was unpreserved with any reagent) and stored frozen at a temperature of – 20°C.

Stool Microscopy and Enzyme immunoassay test

Each stool sample preserved in 10% formalin was processed by direct smear after formal-ether concentration procedures as previously described (23). Slides were examined under microscopy for enteric parasites including *Giardia* and *Entamoeba* cysts. The modified Ziehl-

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Neelsen method (23) was used to detect *Cryptosporoidium* sp oocysts. The enzyme immunoassay test was performed on the -20°C stored faecal samples using the Wampole™ *Giardia/Cryptosporidium/E. histolytica* Check® ELISA kit (TechLab Inc. Blacksburg VA) following the manufacturer's instructions. Results were read both visually by assessing the colour formed in each well of a microtitre plate, and quantified by measuring the absorbance at 450nm on a microplate ELISA reader (Labsystems Multiskan MS, Finland, serial RS-232C). The recommended values (cut-off points) were <0.150 OD_{450}, for the negative control, and ≥0.500 for the positive control. In the test wells, any value greater than, or equal to 0.150 (≥ 0.150) was considered positive, and recorded.

DNA extraction, RFLP- PCR and Sequencing of samples

Cryptosporidium DNA was directly extracted from the stool samples by using the MO BIO UltraClean®Fecal DNA Isolation kit. The procedure was performed by following manufacturer's instructions and the purified DNA was stored frozen at -40°C until further use. *Cryptosporidium 18S rRNA* gene was amplified by a semi-nested PCR following previously described methods (24). Amplifications were carried out in an Applied Biosystem thermocycler (USA), model 2720. Restriction digests were carried out directly on the second-round PCR products in a final volume of 20µL containing 10µL of PCR product, 10U Vsp1 or SSp1 (Thermoscientific, EU, Lithuania), 1X reaction buffer and 0.2µg/µl BSA. Reaction was incubated at 37℃ for 30 minutes. All reactions were controlled with negative and positive samples. PCR (Fig. 1) and RFLP products were run, alongside 100bp ladder, on 2% agarose gel stained with ethidium bromide. Purification and sequencing of PCR products were performed by Macrogen Inc, Amsterdam, Netherlands. BLAST searches and multi sequence alignments were performed in MEGA V5 (25).

Statistical analysis

All data were entered into Microsoft Excel 2010 and analyzed using SPSS V17. The results were expressed as means for the continuous variables and as numbers and percentages for the categorical data. Univariate analysis was performed using independent-sample t-test, Pearson X^2test or Fisher's exact tests, as appropriate. A P-value of < 0.05 was considered statistically significant.

3. RESULTS

Prevalence of *Cryptosporidium parvum* and other intestinal parasites

The parasitological results of stool from both diarrhoeal and non-diarrhoeal cases are represented in Tables 1 and 2. Among children with diarrhoea, 81/365 (22.2%) were positive *Cryptosporidium* sp. cases, 21/365 (5.8%) *G. lamblia*, and 3/365 (0.6%) were *E. histolytica* cases. None of the non diarrhoeal cases had *E. histolytica* infection but had both *Cryptosporidium* sp and *G. lamblia* present in significantly lower rates compared with the diarrhoeal samples (P < 0.0001). We also identified 3/365 (0.8%) diarrhoeal cases as dual infections (*Cryptosporidium* sp. and *G. lamblia*).

The risk factors suspected to be associated with *Cryptosporidium* sp infection were investigated among children with diarrhoea only. These factors included sex and age of child, educational background of mother, source of drinking water for the child, breastfeeding habits of mother, presence of animals at home, source of food for the child, and seasonality (Table 3).

Sex and age of child

We observed that 21.8% (N=211) males had *Cryptosporidium* sp. infection whilst 22.2% (N=154) females were infected with the parasite. The difference in infection rates was statistically insignificant (P >0.05) (Table 3), suggesting that the infection is not associated

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with sex (OR=0.948). Although no infection was recorded among children of age 3 years and above, our results show that there was relatively high risk of *Cryptosporidium* infection associated with ages less than 3 years. Children of age 25-36 months had the highest risk (OR=3.432), followed by 6-12 months old (3.111), and then less than 6 months (OR= 2.856).

Maternal education, source of drinking water, and breastfeeding habits

The highest *Cryptosporidium* infection (33.3%) occurred among children whose mothers had no formal education. Their odds ratio (OR= 3.629) also suggests that they could be associated with risk of infection more than any other group (Table 4). Although children belonging to mothers of all levels of education were infected at significantly different levels (P= 0.011), our study shows that children whose mothers have tertiary education could be protected (OR=0.926).

The children depended on two main sources of water, namely Sachet (bagged) water and Pipe borne water. We recorded 23.1% infections for children who drank sachet water (N=337) and 11.5% for those who drank pipe-borne water (N= 26). Although there was no significant difference (P =0.293) between the two sources, pipe-borne water appears to be protective (OR= 0.725), whilst drinking sachet water could be associated with some risk (OR= 1.935).

In Ghana, mothers are encouraged to practice 6-months exclusive breastfeeding, and we observed from our study that 21.6% of children who were exclusively breastfed (N= 218) had infections. Infection among children who were not exclusively breastfed was 23.7% (N= 131). We did not find any significant difference (P= 0.850) between the two groups but rather observed that there could be a higher risk associated with exclusive breastfeeding (OR= 3.118).

Presence of domestic animals and seasonal variations

From this study, 25.6% of children who had domestic animals at home (N=156) had *Cryptosporidium* infection whist 19.6% of children who did not have any domestic animal at home (N= 209) had infection of the parasite. The difference however was not significant (P= 0.171).

Cryptosporidium infections in the dry season (December – March) was 37/189 (19.6%), and 44/176 (25%) during the rainy season (April- November). The observations made suggest

Table 1. *Cryptosporidum sp., G. lamblia* and *E. histolytica* infections among 365 diarrhoeic and 120 non-diarrhoeic children at the PML hospital, Accra, Ghana

Parasite	Number and Percentage (%) infected			P- value
	Symptomatic **n** (%)	Asymptomatic **n** (%)	Total, **n** (%)	
G. lamblia	21 (5.8)	6 (5.0)	27 (5.6)	P< 0.0001
Cryptosporidium sp.	81 (22.2)	4 (3.3)	85 (17.5)	P<0.0001
E. histolytica	3 (0.8)	0 (0.0)	3 (0.6)	P=0.0143

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Table 2: Detection of *Cryptosporidium sp.* infection in diarrhoeic and non-diarrhoeic stool by Microscopy and Enzyme immunoassay

Diarrhoea stool					
	No. tested	No. Positive	%	No. Negative	%
Microscopy	365	32	8.77	333	91.23
ELISA	365	81	22.19	344	94.25
Non-diarrhoea stool					
	No. tested	No. Positive	%	No. Negative	%
Microscopy	120	2	1.67	118	98.33
ELISA	120	4	3.33	116	96.67

Table 3. Clinical characteristics of *Cryptosporidium sp.* infections in 81 positive cases

		Number (N)	Percentage (%)
Consistency of stool	Mucoid	1	1.2
	Slimy	5	6.2
	Loose	75	92.6
Symptoms	Diarrhoea alone	0	0
	Diarrhoea & vomiting	69	85.2
	Diarrhoea, vomiting & fever	12	14.8
Days of hospitalization	1 day	3	3.7
	3 days	32	39.5
	4 days	46	56.8

Table 4.Odds ratios and 95% confidence intervals for *Cryptosporidium* infection in children and associated risk factors (N = 365)

Character/Risk factor		**No**	**No infected**	**% infected**	**X^2**	**P-value**	**OR**	**95% CI**
1. Gender	Male	211	46	21.8	0.044	0.883	0.948	0.576-1.561
	Female	154	35	22.7				
2. Age (months)	< 6	74	13	17.6	13.043	0.011	2.856	1.672-3.042
	6 - 12	165	49	29.7			3.111	1.973-3.201
	13 - 24	107	16	15			2.050	0.432-2.854
	25 - 36	9	3	33.3			3.432	0.325-3.654
	37 - 48	10	0	0			-	-
3.Education level of mother	Tertiary	28	13	17.6	13.043	0.011	0.926	0.654-1.453
	Secondary	250	49	29.7			2.437	1.768-2.763
	Primary	40	16	15			1.232	1.108-2.319
	None	47	3	33.3			3.629	2.456-3.896
4.Source of drinking water	Sachet	337	78	23.1	2.457	0.293	1.935	0.763-2.176
	Pipe-borne	26	3	11.5			0.725	0.564-1.342
	Both	2	0	0			1.000	0.895-1.241
5.Breast-feeding habits	Exclusive	218	47	21.6	0.325	0.850	3.118	0.567-3.344
	Not exclusive	131	31	23.7			2.827	0.679-3.129

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	N/R	16	3	18.8			1.724	0.768-1.881
6.Presence of domestic animals at home	Yes	156	40	25.6	1.877	0.171	1.433	0.861-2.320
	No	209	41	19.6				
7.**Seasonal variations**	Dry	189	37	19.6	1.553	0.213	0.730	0.443-1.199
	Rainy	176	44	25.0				

that the parasite has no specific preference for any of the seasons, as difference between seasonal occurrence was not significant (P=0.213, OR=0.73, 95%CI=0.443-1.199).

Microscopy and enzyme immunoassay tests

Of the 365 diarrhoea samples tested, 81 and 32 were positive for ELISA and microscopy, respectively (Table 2). Also, of the 120 non-diarrhoea samples tested, 2 and 4 samples were positive by microscopy and ELISA, respectively. From a total of 85 positive samples by ELISA 72 tested positive by PCR, which amplified a 450bp fragment of the 18S rRNA gene.

Clinical data

The observed clinical characteristics associated with *Cryptosporidium* sp. infection have been reported in Table 3. Majority (92.6%) of samples tested were loose diarrhoeic samples. Diarrhoea and vomiting constituted the predominant clinical symptom (85.2%). Days of hospitalization varied between 1-4 days, and most patients spent 3-4 days on admission at the hospital.

PCR-RFLP analysis and sequencing of *Cryptosporidium* sp.

PCR amplification was successful for 72 ELISA-positive samples (Fig. 1). The RFLP with *SspI* and *VspI* enzymes gave single patterns of fragments for all PCR products which when compared with previously reported work (24) (Table 5) were indicative of *C. parvum.* Sequencing was carried out for 6 representative PCR products, and all the samples, EMO312, EMO303, EMO289, EMO311, EMO325 and EMO346, had good sequence reads.

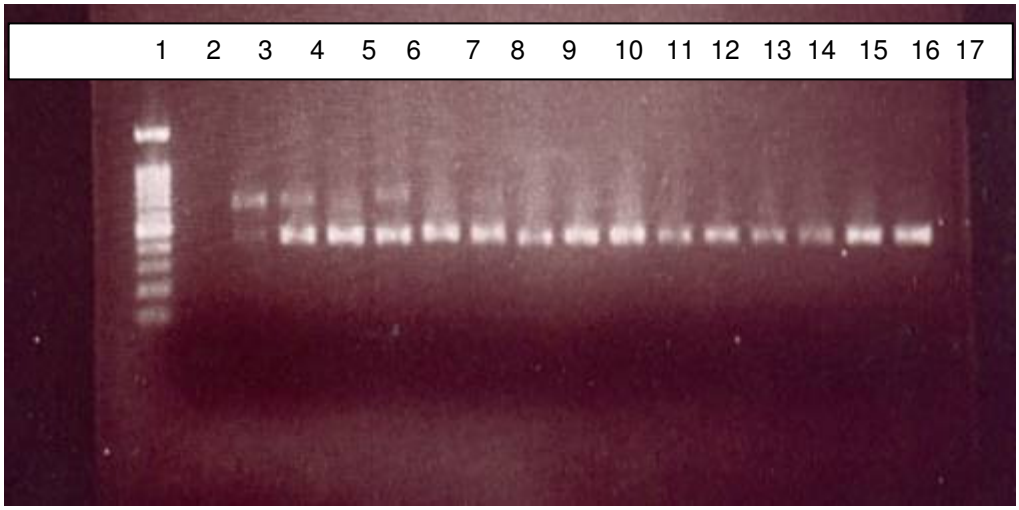

Fig 1 A Nested PCR at the 18S ribosomal RNA gene locus for identification of *Cryptosporidium* sp. (Lane 1 is 100bp molecular ladder, Lanes 3 to 16 are patient samples. Lanes 17 and 2 are positive & negative controls respectively).

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Table 5. *Cryptosporidium* sp. and genotypes determined by RFLP of the amplicon defined by CPB-DIAGR/F primers after digestion with the enzymes VspI, DraI and DdeI (Nichols et al., 2003)

Cryptosporidium spp. and genotypes(total amplicon length in bp)	Amplicon length(s) (bp) as defined with			GenBank accession number
	VspI	DraI	DdeI	
C. *parvum* 1 (438)	222,104,112	None	204,68,166	L16997
C. *parvum* 2 (435)	219,104,112	None	201,68,166	L16996,AF161856
C. *muris* (432)	320,112	None	42,224,166	AF093498,AF093498,AF093497
C. *andersoni* (431)	319,112	None	265,166	AF093496,49069
C. *felis* (455)	239,104,112	50,405	221,68,166	AF087577
C. *baileyi* (428)	212,104,112	84,344	262,166	L19068,AF093495
C. *maleagridis* (434)	47,171,104,112	None	200,68,166	AF112574
C. *serpentis* (430)	312,112	None	264,166	AF093502
C. *wrairi* (435)	219,104,112	None	201,68,166	AF115378

BLAST searches of the NCBI database however confirmed only one of the sequences, EMO346, with >95% sequence identity, as *C. parvum*. In a separate phylogenetic analysis (Fig. 2), sample EMO346 formed a clade with *C. parvum* KKU and *C. parvum* M31, together with other 10 strains, also previously reported as *C. parvum*. Samples EMO312, EMO303, EMO289, EMO311, and EMO325 formed a separate cluster with unspecific identity (Fig. 2).

DISCUSSION

In many developing countries including Ghana, diarrhoeal diseases remain a major cause of morbidity and mortality among children. *Cryptosporidium* sp. is an important enteric parasite associated with diarrhoea particularly in communities without proper sanitation and potable water.

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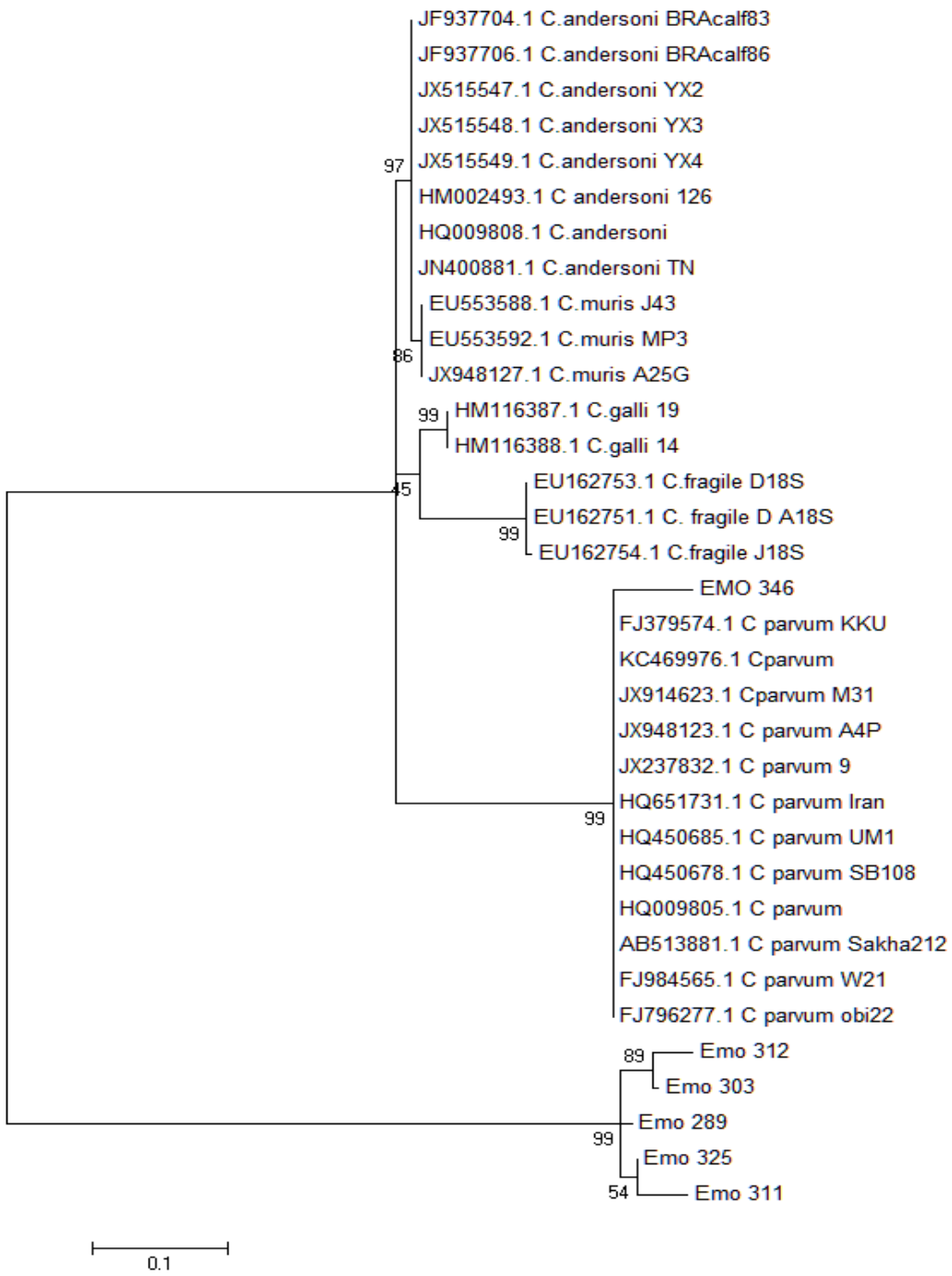

Fig 2. Phylogram (maximum likelihood) of all the *Cryptosporidium* 18S ribosomal RNA gene partial sequences and selected members of the *Cryptosporidium* sp. species inferred from 18S rDNA sequence comparisons. Bootstrap values (within branches at relevant nodes) are reported when equal or greater than 40%. Emo 346, Emo 312, Emo 303, Emo 289, Emo 325, Emo 311 are isolates from the present study at PML, Accra, Ghana.

at Mulago hospital in Uganda (18), Cuban children (31, 12) and Ethiopian children (28). In many places worldwide, children of both sexes in same location are likely to be equally

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In our hospital based study among children hospitalized for acute diarrhoea at PML, Accra, we recorded a 22.2% prevalence of *Cryptosporidium* sp., and observed cryptosporidial diarrhoea to be more common than diarrhoea caused by other enteric parasites. The prevalence appears to be reasonably high and an increase over previous observations of 8.7% in the year 2010 at the same hospital (21). Our findings suggest that the parasite has increasingly become more important in paediatric diarrhoea and now needs urgent attention by health authorities and researchers in the country than ever. The association of *Cryptosporidium* with acute to very severe forms of diarrhoea in children leading to hospitalization and deaths has been reported worldwide (19, 26, 27, 28).

Knowing the importance of accurate reporting, we employed a combination of two separate laboratory methods for diagnosis of parasites in the stool samples. Although microscopy has a high specificity, it could be less sensitive in its detection of parasites. Immunoassays however have high sensitivity and can be used as effective tool for epidemiological investigations (29, 30). We are convinced to a large extent that an application of the two methods for each sample has enabled us to report the magnitude of the disease correctly and accurately. Our findings underscore a critical look at the laboratory methods currently used for diagnosis of *Cryptosporidum* sp. in our hospitals. The use of microscopy alone, as being done now in many hospitals may not be enough to diagnose the infection.

An effective prevention strategy requires a good understanding of factors associated with occurrence of the disease. We investigated risk factors that could be associated with cryptosporidial diarrhoea among the children and found no association with gender. Our observation was similar to those made among Ugandan children hospitalized for diarrhoea

exposed to any environmental contamination. Based on this assumption, observations made in the present study were expected.

Cryptosporidium infection was relatively common among age group 3 (13-24 months) and below, with no infection among children above 3 years. In Ghana, a previous study reported a peak of infection in children 12-24 months, and a decrease with age thereafter (22). Reports from investigators worldwide indicate that most infections occur among younger children of ages 2 years and below (32, 33, 22). The observations from the present study therefore agree with many others worldwide. An exception however was in Lagos, Nigeria, where the parasite was found to be most common among the age group 4- 5 years (34), in contrast to findings in our study.

High prevalence of the parasite among children whose mothers had no formal education appears to conform to the general notion that illiterate mothers practice less personal hygiene. In a semi-urban slum in India, children whose mothers had no formal education suffered multiple symptomatic *Cryptosporidium* infections significantly (35). We presume that in Ghana majority of illiterate mothers lack knowledge of transmission of such infections which could have resulted in the high prevalence of cryptosporidiosis observed in this study. It will be a laudable idea to expand health education among prospecting mothers to cover knowledge on common infectious diseases at antenatal clinics.

Breast milk has been highly recommended for use in feeding infants worldwide because of its immunological and nutritional protection value. In Ghana, there have been so much campaign by the Ghana Health Service (GHS), an outfit of the Ministry of Health (MOH) for exclusive breastfeeding by mothers as it is more protective (12, 36). In Israel (37), children who were exclusively breastfed received at least, some protection from *Cryptosporidium* sp. infections within the first 3 months after birth. According to our study, majority of mothers practiced exclusive breastfeeding but unfortunately it appears not to have provided the

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expected protection against *Cryptosporidium* parasites. We are of the view that the subject of exclusive breastfeeding in relation to enteric parasitic infections in Accra needs further investigation.

Consumption of untreated water is one of the major routes by which the parasite is transmitted into humans. In our study, most infections occurred in children whose drinking water was from sachet (bagged) water. It is not surprising, as the purity and safety of sachet water in Accra has been previously challenged by a study (38). According to that study 77% of the samples screened contained parasitic agents including oocysts of *Cryptosporidium* sp. To ensure public health safety of the consumption of sachet water in Accra, regular screening and monitoring of brands of sachet water sold in the city of Accra is largely recommended.

Cryptosporidiosis being a zoonotic infection, we suspected that there could be some association between infection of the parasite and pet ownership at home. The keeping of pets and rearing of domestic animals at home is a common practice in the Ghanaian society. We observed that a majority of children had animals at home, and these were either dogs or cats, or both. Most of these animals contaminate the environment which also serves as playing grounds for children by their faeces. We found that having pets or domestic animals at home was not associated with *Cryptosporidium* infection. Perhaps good hygiene and sanitation in homes with such animals played a role to prevent infection.

It was noticed in our study that the parasite was present all year round and seasonal variations were not significant. The pattern seems to be in line with erratic supply of potable water in Accra for domestic use which occurs throughout the year. Large population of the city depends on untreated water which is also likely to increase the risk of infection all year round. Elsewhere, *Cryptosporidium* infections vary significantly from one season to the other (39, 40, 41).

Our genotyping results indicate that *Cryptosporidium parvum* is a major cause of paediatric cryptosporidial diarrhoea at the hospital. *C. parvum* comprises of both anthroponotic and zoonotic species (subtypes 1 and 2). Subtype 1 of *C. parvum* is anthroponotic whist subtype 2 is zoonotic. Being unable to perform sub-genotyping analysis to determine specific subtypes, our study is limited in reporting whether all infections were human to human, or animals involved in transmission. That notwithstanding, we consider our study to be significant for being able to determine the particular species of *Cryptosporidium* in paediatric diarrhoea at the hospital for the first time.

Further investigations are needed to confirm the identity of other genotypes with unclear identity found in our study. They could be new genetically modified species being introduced into the population of Accra.

4. CONCLUSION

Our study suggests that *Cryptosporidium parvum* is the commonest enteric parasite which causes diarrhoea among children with acute diarrhoea at the Princess Marie Louise children's hospital at Accra, Ghana. Infection was highest among children 3 years and below, and children who drank sachet water had infections most. The findings of our study shows that laboratory methods presently employed in routine diagnosis of the parasite in many hospitals need a critical consideration. A carefully planned health education among illiterate mothers and improved personal hygiene could reduce rate of infections among children. Further studies on sub-genotyping of *C. parvum* isolates are needed to determine whether source of infection is zoonotic or anthroponotic.

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CONSENT

Selection of participants (patients) in the study was strictly voluntary and based on informed consent (verbal) of their guardians or parents, after being informed of the objectives and goals of the study.

ETHICAL APPROVAL

An ethical approval of the study (SAHS-Et/SAHS/PSM-ML/1/26A/2010-2011) was obtained from the Research and Ethical Review Committee of the University of Ghana School of Allied Health Sciences, College of Health Sciences, Korle Bu, Accra.

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