

## Laboratory Investigation of Cryptosporidiosis and Giardiasis in Children with Diarrhoea; A Hospital Based Study

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**Abstract**— This study was conducted to determine the proportion of *Cryptosporidium* and *Giardia* infected children less than 12 years of age with diarrhoea and to identify the source of drinking water, level of hygiene in food preparation and hand washing practices of mothers/caretakers of children with diarrhoea. Children who attended to the Lady Ridgeway Hospital, Colombo 08 with diarrhoea during August to October 2012 were enrolled into the study. 145 stool samples were collected and tested them using Modified Ziehl Neelsen stain for cryptosporidium spp and microscopy of saline and iodine wet mount and Formal ether sedimentation technique for giardiasis and other parasitic infections in the laboratory. Interviewer administered questionnaire was used to collect above stated data. *Cryptosporidium* spp were detected in 48 (33%) out of 145 stool samples. There were no positives for giardiasis. Children who were more than 12 months of age were more infected with *Cryptosporidium* compared to children who were less than 12 months in age ( $p < 0.05$ ). The level of hygiene in food preparation was adequate in 74% of the mothers/caretakers. Correct hand washing practices had been adopted by 91% of mothers/caretakers. 73% of families use public tap water as their sole source of water and they use either boiled cooled water (95%) or filtered water (5%) for drinking purpose. Although diarrhoea associated with parasitic infections is less severe compared to bacterial or viral etiological diarrhoea, presence of cryptosporidium spp in diarrhoeic samples indicate the importance of testing stool samples for cryptosporidiosis in routine diagnosis. Our results indicate a higher proportion (33%) of *Cryptosporidium* spp in stool samples collected from children with diarrhoeal illness compared to the other studies conducted locally in recent and decades ago. In the absence of an effective treatment to eradicate cryptosporidiosis from the patient, attention should be paid for preventive measures.

**Keywords**— *Cryptosporidium*, *Giardia*, diarrhoea

### I. INTRODUCTION

Diarrhoea is the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual (WHO, 2012). Diarrhoea can be the presenting symptom of variety of aetiological factors including infections. Infectious causes of diarrhoea are bacteria, viruses and parasites (WHO, 2012). *Giardia intestinalis* and *Cryptosporidium parvum* were the commonest parasitic cause of diarrhoea in developing countries (Snelling et al., 2007).

Acute childhood diarrhoea is one of the leading causes of childhood morbidity and mortality in developing countries (Nkrumah and Nguah, 2011). According to studies done in developing countries such as India, Ghana, Mexico and Uganda parasitic diarrhoea in children is mainly due to *Giardia intestinalis* and *Cryptosporidium parvum* (Nkrumah and Nguah, 2011, Ajjampur et al., 2010, Sanchez-Vega et al., 2006).

*C. parvum* a coccidian parasite, is known to cause childhood diarrhoea and travellers' diarrhoea (Jelinek et al., 1997). In otherwise healthy individuals, it may mostly pass unnoticed or may cause self-limiting water borne diarrhoea whereas immunocompromised patients may suffer from severe life threatening episodes of diarrhoea (Greenwood et al., 2007).

*G. intestinalis* (Syn. *Giardia lamblia*) a flagellate inhabiting the duodenum and upper jejunum, causes giardiasis commonly affecting children (Gendrel et al., 2003). In most of the instances the infection leads to asymptomatic carriage. *G. intestinalis* can cause both acute and chronic diarrhoea. Acute giardiasis presents with diarrhoea subsequently developing nausea, vomiting, bloating, weight loss and steatorrhoea etc. (Ichhpujani and Bhatia, 2002). Intestinal Malabsorption

may occur at times severe enough to cause retardation of growth and development (Ichhpujani and Bhatia, 2002).

According to annual health bulletin in year 2012, intestinal infectious diseases are the 11<sup>th</sup> leading cause of hospitalization in Sri Lanka (Ministry of health, 2012). In that year 35% of cases who were hospitalized due to intestinal infectious diseases were children age of four years old or less (Ministry of health, 2012). Each episode of diarrhoea deprives the child of the nutrition necessary for the growth, which finally leads to malnutrition (Gulati, 2010). Although, diarrhoea associated with parasitic infections is less severe compared to diarrhoea due to bacterial and viral causes, Perera et al (1999) have mentioned that malnutrition is more prevalent in children with diarrhoea due to parasitic causes compared to bacterial and viral causes (Perera et al., 1999).

## II. METHODOLOGY

This study was conducted to determine the proportion of *Cryptosporidium spp* and *Giardia spp* among children less than 12 years old with diarrhoea to identify the importance of laboratory investigation of diarrhoeic stool samples for parasitic infections and to study the source of drinking water, level of hygiene in food preparation and hand washing practices of mothers/caretakers of children with diarrhoea. Children who attended to the Lady Ridgeway Hospital for children with diarrhoea during September to November in year 2012 were enrolled into the study. The Lady Ridgeway hospital for children is the main tertiary hospital for children in Sri Lanka and is located in a highly populated area of people belonging to all level of social categories.

Mothers/caretakers of the children who visited outpatient department of the Lady Ridgeway hospital for oral rehydration treatment were interviewed with a questionnaire to gather information, including the source of drinking water, level of hygiene in food preparation and hand washing practices.

Sub samples were taken from the faecal samples sent to the hospital laboratory for routine investigations and processed using Modified Ziehl Neelsen stain for *Cryptosporidium spp* and direct microscopy of saline and iodine wet mount and Formal ether sedimentation technique for protozoans and helminth in the laboratory at Medical research institute, Colombo-8.

## III. RESULTS

145 stool samples were processed for the detection of parasitic infections in children with diarrhoea. One sample was positive for *Iodamoeba buschlii* as an accidental finding and the same sample was positive for *Cryptosporidium* too. There were 48 (33.1%) faecal specimens which were positive for *Cryptosporidium spp*. Children who were more than 12 months of age were more infected with *Cryptosporidium spp* compared to children who were less than 12 months in age ( $p < 0.05$ ,  $p = 0.000$ ). Male children were more infected compared to female children [ $p < 0.05$ ,  $p = 0.030$ ].

Table 1. Distribution of cryptosporidiosis among children in different age categories

Age categories	Cryptosporidiosis detected by modified Ziehl-Neelsen stain method	
	Positive (%)	Negative (%)
Less than one year old	13 (27.1%)	58 (59.8%)
One year old	15 (31.2%)	17 (17.5%)
Two years old	2 (4.2%)	5 (5.2%)
Three years old	6 (12.5%)	2 (2.1%)
Four years old	5 (10.4%)	1 (1.0%)
Five years old	1 (2.1%)	4 (4.1%)
Six years old	0 (0%)	1 (1.0%)
Seven years old	1 (2.1%)	4 (4.1%)
Eight years old	1 (2.1%)	4 (4.1%)
Nine years old	2 (4.2%)	0 (0%)
Ten years old	2 (4.2%)	0 (0%)
Eleven years old	0 (0%)	1 (1.0%)
<b>Total</b>	<b>48 (100%)</b>	<b>97 (100%)</b>

When the interviewed mothers/caretakers were considered only 3% has had no formal education. Majority (84%) of mothers/care takers had a good educational back ground. Nearly half of the study group

was from families with an income level between Rs. 10,000-20,000.

Table 2 Sources of drinking water

Main water source	Frequency
Public tap/piped water	73
Protected dug well or spring	24
Unprotected well or spring	2
River or stream	1
Total	100

Majority (73%) of interviewed families use public tap water as their sole source of water. All the mothers/care takers said that they use either boiled cooled water (95%) or filtered water (5%) for drinking purpose. The truths of these statements were difficult to assess objectively during the short period of this study.

According to the scoring system, 74% showed excellent practices in food preparation and handling while only 1% showed poor practices. In case of hand washing 91% showed excellent and 2% showed poor practices.

#### IV. DISCUSSION

The prevalence of cryptosporidium reported from the same study setting in 1999, was 2.7% among children with diarrhoea and 0.4% in the control group (Perera et al., 1999). Giardiasis had been present only in 0.9% of children with diarrhoea and none in the control group (Perera et al., 1999). Sirisena et al., reported 5.7% of *cryptosporidium* infection among children with diarrhoea and all the positive cases were less than 3 years old (Sirisena et al., 2014). Presence of *Cryptosporidium* oocysts and *Giardia* cysts had been reported from all sources of drinking water in Southern area of Sri Lanka (Shortt et al., 2006). The prevalence of intestinal parasites had been 4.54% in diarrhoeal stools but with no cases of Cryptosporidiosis in a hospital based study conducted among children in the Northern part of Sri Lanka (Suhail and Morel, 2011).

In our study, we did not get any positives for *G. lamblia*. This may be due to either actual prevalence of *G.lamblia*

is less compared to previous studies, but the most likely explanation is that we would have missed the positive cases as we tested only one stool sample per child.

The difference observed in the studies of Perera et al, 1999 and Sirisena et al, 2014 and the our study may be due to Population congestion in Colombo can bring the hygienic standards to low levels and in such an environment, access to safe water can be a problem faced by the residents favouring the increase of *C.parvum* infection. However, the responses given by the mothers/caretakers on enquiry did not favour this concept. There is a possible discrepancy between the answer given to a researcher and the real situation at home. A seasonal variation of the prevalence of *C.parvum* infection may also occur with rain fall (Manson, 1891).Our study was conducted during a time when Sri Lanka was experiencing heavy rains and floods and therefore it may have had some effect on the higher number of cases.

During microscopy of the faecal wet smears we did not observe any eggs of intestinal helminth among these children with diarrhoea, except one case of *Enterobius vermicularis*. This could be a result of the liberal use of anthelmintic drugs due to the low cost as an over the counter medicine, availability from government hospitals free of charge, and awareness of health hazards caused by helminth infections. On the other hand 48% of the children in our were less than 12 months age category and chances of getting intestinal parasitic infections in this group are less compared to children >12 months of age as they were under breast feeding until they are six months old and the chances of consumption of contaminated food are less likely to occur.

Although, direct fluorescent antibody testing (DFA) method is used as the bench mark test for the diagnosis of cryptosporidiosis, commercially available antigen detection immunoassay tests can be used instead of DFA since these methods are more diagnostically sensitive compared to microscopic examination (Painter et al., 2015). A more sensitive testing method for the diagnosis process of *Cryptosporidium* spp, other than microscopy was not used and correlates of clinical data with the presence of cryptosporidiosis were not made due to Lack of available data, resources and time since this study was done in undergraduate level. Above mentioned reasons can be mentioned as limitations of this study.

Medical professionals should pay more attention to *Cryptosporidium spp* especially bearing in mind the non-availability of an effective treatment for it and the rising number of immunocompromised individuals who are at risk of developing severe life threatening episodes of diarrhoea in *Cryptosporidium spp* infection. This should also awaken the researchers and experts in the field to do large scale survey with a confirmatory diagnostic tool to find the prevalence among different age groups and individuals with normal and compromised immunity in Sri Lanka.

#### V. CONCLUSION

Proportion of *Cryptosporidium spp* infection among children with diarrhoea is high in this population. Therefore we conclude that the proportion of *Cryptosporidium spp* infection has increased over the years irrespective of better life styles of present day Sri Lankans.

The determination of hygienic practices in food preparation and handling of mother/ caretaker of the child was done using the questionnaire. But the reliability of the responses is highly doubtful and an objective assessment of the situation in the form of home visits would have been more appropriate.

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#### REFERENCES

Ajjampur, S. S. R., Liakath, F. B., Kannan, A., Rajendran, P., Sarkar, R., Moses, P. D., Simon, A., Agarwal, I., Mathew, A. & O'connor, R. 2010. Multisite Study Of Cryptosporidiosis In Children With Diarrhea In India. *Journal Of Clinical Microbiology*, 48, 2075-2081.

Gendrel, D., Treluyer, J. & Richard-Lenoble, D. 2003. Parasitic Diarrhea In Normal And Malnourished Children. *Fundamental & Clinical Pharmacology*, 17, 189-197.

Greenwood, D., Slack, R., Peutherer, T. & Barer, M. 2007. *Medical Microbiology*, Churchill Livingstone, Elsevier.

Gulati, J. K. 2010. Child Malnutrition: Trends And Issues. *Anthropologist*, 12, 131-140.

Ichhpujani, R. & Bhatia, R. 2002. *Medical Parasitology*, India, Jaypee Brothers Medical Publishers.

Jelinek, T., Lotze, M., Eichenlaub, S., Löscher, T. & Nothdurft, H. 1997. Prevalence Of Infection With *Cryptosporidium Parvum* and *Cyclospora Cayetanensis* Among International Travellers. *Gut*, 41, 801-804.

Manson, P. 1891. *Manson's Tropical Diseases*, Elsevier.

Ministry Of Health 2012. Annual Health Bulletin, Sri Lanka In: Unit, M. S. (Ed.).

Nkrumah, B. & Nguah, S. B. 2011. *Giardia Lamblia*: A Major Parasitic Cause Of Childhood Diarrhoea In Patients Attending A District Hospital In Ghana. *Parasit Vectors*, 4, 163.

Perera, J., Jayawardene, I., Mendis, L. & Abeyratne, K. 1999. Intestinal Parasites And Diarrhoea In A Children's Hospital In Sri Lanka. *The Ceylon Journal Of Medical Science*, 42, 7-12.

Sanchez-Vega, J. T., Tay-Zavala, J., Aguilar-Chiu, A., Ruiz-Sanchez, D., Malagon, F., Rodriguez-Covarrubias, J. A., Ordonez-Martinez, J. & Calderon-Romero, L. 2006. Cryptosporidiosis And Other Intestinal Protozoan Infections In Children Less Than One Year Of Age In Mexico City. *The American Journal Of Tropical Medicine And Hygiene*, 75, 1095-1098.

Shortt, R. L., Boelee, E., Matsuno, Y., Madramootoo, C., Van Der Hoek, W. & Faubert, G. 2006. *Cryptosporidium* And *Giardia* As Determinants For Selection Of An Appropriate Source Of Drinking-Water In Southern Sri Lanka. *J Health Popul Nutr*, 24, 64-70.

Sirisena, U., Iddawela, W., Noorden, F. & Wickramasinghe, S. 2014. Prevalence And Identification Of *Cryptosporidium* Species In Paediatric Patients With Diarrhoea. *Ceylon Medical Journal*, 59, 75-78.

Snelling, W. J., Xiao, L., Ortega-Pierres, G., Lowery, C. J., Moore, J. E., Rao, J. R., Smyth, S., Millar, B. C., Rooney, P. J. & Matsuda, M. 2007. Cryptosporidiosis In Developing Countries. *The Journal Of Infection In Developing Countries*, 1, 242-256.

Suhail, M. A. A. M. & Morel, R. P. 2011. Prevalance Of Diarrhoea And Intestinal Parasites And Possible Factors Affecting Transmission In Children Admitted To Kattankudy Base Hospital. Proceedings Of The Peradeniya University Research Sessions, 2011 Sri Lanka. University Of Peradeniya, Sri Lanka, 73.

Who. 2012. *Health Topics: Diarrhoea* [Online]. Available: [Http://www.who.int/topics/diarrhoea/en](http://www.who.int/topics/diarrhoea/en) [Accessed 05/04 2012].



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