Research Article **AJBB (2017) 1:5**

American Journal of Biotechnology and Bioinformatics
(ISSN:2575-999X)

Surveillance of enteric parasites in an infectious diseases hospital, Kolkata, India

Sumallya Karmakar, Shanta Dutta and Sandipan Ganguly*

ICMR-National Institute of Cholera and Enteric Diseases, Indian Council of Medical Research, P 33 CIT Road, Scheme XM< Beliaghata, Kolkata 700010, West Bengal, India

ABSTRACT

A hospital-based laboratory surveillance was conducted to study the prevalence and intensity of enteric parasites in India, with a special emphasis on West Bengal. The survey was conducted among the patients admitted between January 2010 and December 2015 to the Infectious Diseases (ID) Hospital with diarrhoeal complaints. The present study reports the occurrence and prevalence of 3 most abundant enteric parasites, viz. Giardia lamblia, Entamoeba histolytica and Cryptosporadium sp. in Kolkata and surrounding. Of the 4208 samples screened during the study period from ID Hospital, 297 (7.05%) were positive for Giardia lamblia, 20 (0.475%) were positive for Entamoeba histolytica and 94 (2.23%) were positive for Cryptosporidium sp.

Keywords: Surveillance, enteric parasites, infectious diseases hospital, Kolkata

***Correspondence to Author:**
Sandipan Ganguly
Head, Division of Parasitology, ICMR-National Institute of Cholera and Enteric Diseases, Indian council of Medical Research, Dept. Of Health Research,Govt. of India.
sandipanganguly@hotmail.com

How to cite this article:
Sumallya Karmakar et al.,. Surveillance of enteric parasites in an infectious diseases hospital, Kolkata, India. American Journal of Biotechnology and Bioinformatics, 2017; 1:5 .

eSciencePublisher
eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

Introduction:

Diarrhoea is a major cause of illness and death in the developing countries. It is estimated that globally around 19% of the total child deaths are caused by diarrhoea and a major proportion of these deaths are concentrated in the developing countries (1). Diarrhoea is the 2nd most common cause of death due to infectious diseases in children under 5 years of age. It is interesting to know that adults are also vulnerable to diarrhoea and a major cause of adult deaths in developing countries are attributable to diarrhoea (2). Three clinical syndromes of diarrhoea have been identified that includes acute watery diarrhoea, dysentery and persistent diarrhoea. Each syndrome represents a different pathogenesis and thus requires a different treatment approach (3). Bacteria and viruses are the major causative agents of diarrhoea. Apart from them, parasites also contribute handsomely to this disease in the developing countries (3). Parasitic diarrhoeal infections are sometimes overlooked, leading after a period of time to an uncertain etiology.

A previous epidemiological survey or systematic studies of occurrence and prevalence of parasitic diarrhoea amongst different diarrheogenic cases was conducted by our Division of Parasitology at National Institute of Cholera and Enteric Diseases, Kolkata from November 2007 to October 2008. From that study it was evident that *Giardia* infection was about 13%, *Cryptosporidium*infection was 7.6% and *Entamoeba* infection was about 4.6%. Our present studyon the patients admitted to ID Hospital is a continuation of the previous survey and here we report the occurrence and prevalence of 3 most abundant enteric parasites, viz. *Giardia lamblia, Entamoeba histolytica*and*Cryptosporadium*sp in Kolkata and surrounding areas from January, 2010 to December, 2015.

Method:

For the survey of diarrhogenic parasites, the target population of our study comprised of patients admitted to Infectious Diseases (ID) hospital from different parts of Kolkata between January 2010 to December 2015.A laboratory-based surveillance system was introduced. The I. D. Hospital in Kolkata provides treatment to about 20,000 to 25,000 acute diarrhoea patients annually. Patients admitted to the hospital with diarrhoeal complaints were included in the study using a systematic sampling process: on two randomly selected days each week, every fifth patient with diarrhoea or dysentery but no associated complaints was enrolled. The system remained unbiased for sex and age of the patient at the time of selection. Faecal samples from all the enrolled patients were collected and analysed. A total of 4208samples were collected from patients admitted to the I.D. Hospital during our study period.

The parasites were detected firstly by conventional microscopy, which is still considered the gold standard for any epidemiological study, but as this method has comparatively low sensitivity and has limitations in distinguishing among different parasites (e.g. *Entamoeba dispar* from *Emtamoebahistolytica*), we also used molecular detection techniques, ELISA and PCR, to increase the sensitivity and specificity of detection.

For microscopic analysis the samples were concentrated using the method of Ridley (4), mixed with 2.5% potassium dichromate for preservation (5) and stored at 4°C. Three separate aliquots from each sample were stored with no preservative at -80°C for ELISA and PCR studies.

For microscopic screening, the samples were processed immediately on receiving. Three separate techniques were used to identify the parasites in the faecal samples: iodine wet mount staining for trophozoites and parasite cysts; modified Kinyoun's Acid fast staining for *Cryptosporidium sp*. (6); and Trichrome staining for *Giardia* sp. and *Entamoeba* sp. (7)

Molecular screening was performed in two ways, firstly, Antigen-capture Enzyme Linked Immunosorbent Assay (ELISA) was performed on all the samples to detect the above-mentioned parasites using commercially available kits, i.e. GIARDIA II, E. HISTOLYTICA II and CRYPTOSPORIDIUM II (TECHLAB, USA). Secondly, parasite DNA was isolated using a Stool DNA Isolation Kit (QIAGEN) according to the manufacturer's protocol, then PCR was performed on all samples using the parasite-specific primers mentioned in Table 1. For detection of *Giardia lamblia*, a beta-giardin gene fragment-specific primerwith a PCR amplicon size of 218 bp was used (8). Detection of *Entamoeba histolytica* was done by PCR amplification of SSU rRNA(9). The primers used are highly specific for *Entamoeba histolytica* and differentiate it from the non-pathogenic *Entamoeba dispar*.*Cryptosporidium* was detected by the primers described by Xiao *et al*. (1999) with an amplicon size of 1325 bp. Then nested PCR was performed with an amplicon size of 825 bp (10).

Table 1:

Detection of	Primer Name	Forward/Reverse	Sequence (5'-3')
Giardia lamblia	MAH433F	Forward	CATAACGACGCCATCGCGGCTCTCAGGAA
Giardia lamblia	MAH592R	Reverse	TTTGTGAGCGCTTCTGTCGTGGCAGCGCTAA
Entamoeba histolytica	EH1	Forward	GTACAAAATGGCCAATTCATTCAATG
Entamoeba histolytica	EH2	Reverse	ACTACCAACTGATTGATAGATCAG
Cryptosporidium sp. Primary	18 SF	Forward	TTCTAGAGCTAATACATGCG
*Cryptosporidium*s p. Primary	18 SR	Reverse	CCCTAATCCTTCGAAACAGGA
Cryptosporidium sp. Nested		Forward	GAAGGGTTGTATTTATTAGATAAAG
Cryptosporidium sp. Nested		Reverse	AAGGAGTAAGGAACAACCTCCA

Results:

Of the 4208 samples screened from ID Hospital during the study period, 297 (7.05%) were positive for *Giardia lamblia*, 20 (0.475%) were positive for *Entamoeba histolytica* and 94 (2.23%) were positive for *Cryptosporidium* sp. The month wise analysis of the data suggests that the *Giardia* infection in prevalent from the month of April to the month of October (Table 2, Fig. 1). *Cryptosporidium* infection is prevalent from the month of February to June. Infection with *Entamoeba* is highly sporadic and there is no specific pattern of the infection. Yearly survey suggests that *Entamoeba* infection, although low, shows a gradual increase over the study period with only a dip in the year 2013. *Cryptosporidium* infection shows an interesting trend. At the beginning the infection rate rises and then it drops sharply in 2012. The rate of infection again increases in the following years and then dips in 2015. The percentage of infection by *Giardia* decreases in the last two years of our study (Table 3, Fig. 2).

Table 2:

Months	Infection by *Giardia*	Infection by *Etamoeba*	Infection by *Cryptosporidium*
Jan	15	0	3
Feb	12	0	13
Mar	16	4	15
Apr	28	2	10
May	33	2	11
June	22	0	14
July	27	3	6
Aug	39	0	7
Sep	40	8	3
Oct	27	1	3
Nov	18	0	5
Dec	20	0	2

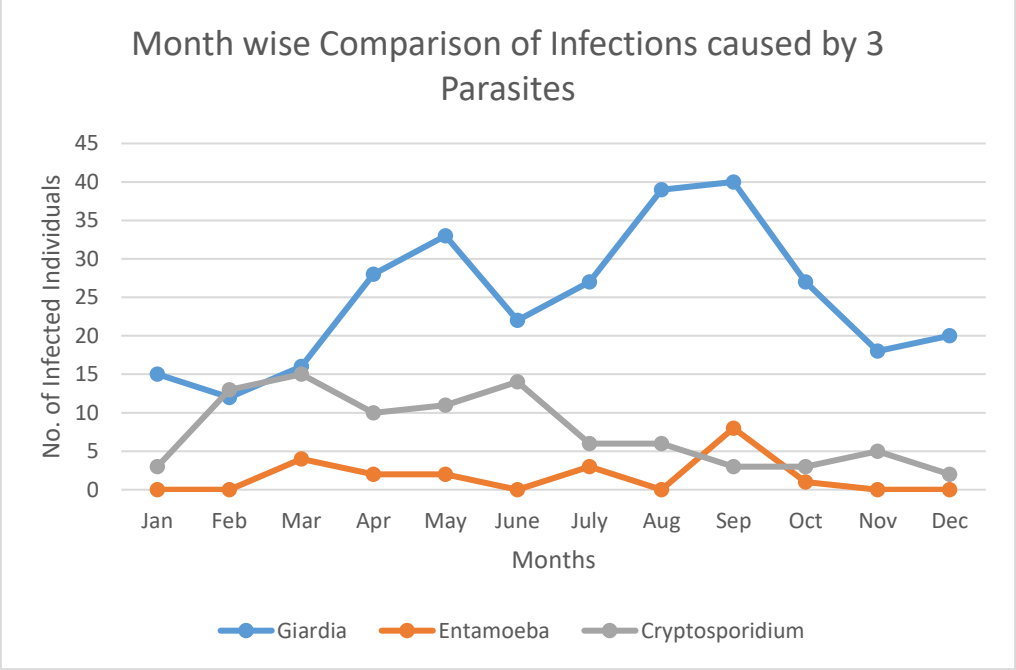

Figure 1: Figure showing the month wise rate of infection caused by the 3 parasites, viz. Giardia lamblia, Entamoeba histolytica and Cryptosporidiumsp in and around Kol-kata.

Table 3:

Year	2010	2011	2012	2013	2014	2015
Total	601	468	691	826	774	848
Giardia lamblia	56 (9.31%)	37 (8.1%)	56 (8.1%)	74 (8.95%)	37 (4.7%)	37 (4.36%)
Entamoeba histolytica	2 (0.33%)	2 (0.42%)	5 (0.72%)	1 (0.12%)	4 (0.516%)	6 (0.70%)
Cryptosporidium sp.	18 (2.99%)	20 (4.27%)	11 (1.59%)	19 (2.3%)	16 (2.06%)	10 (1.17%)

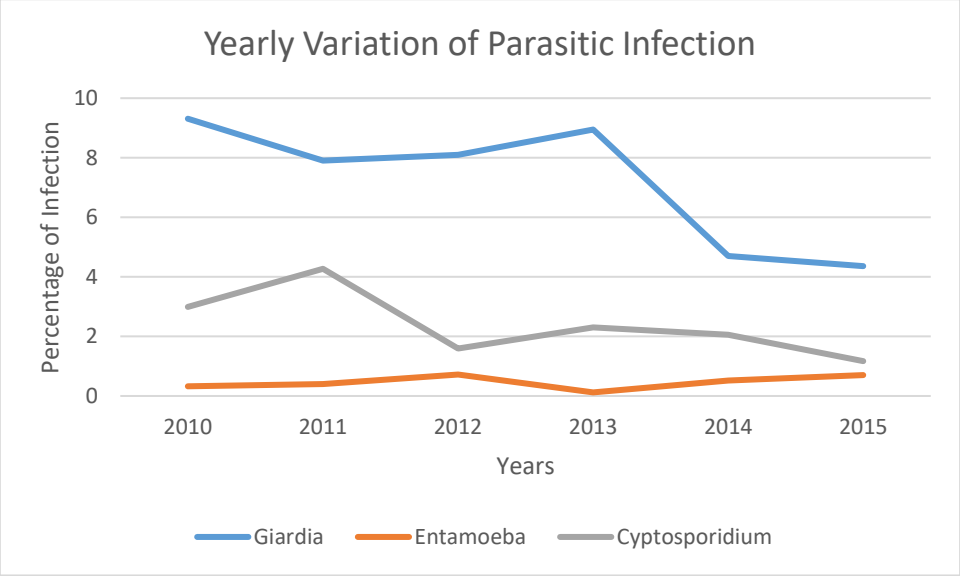

Figure 2: Figure showing the year wise variation in the rate of infection of the 3 enteric parasites in and around Kolkata.

Discussion:

The total number of samples screened during the survey was 4208 out of which 7.05% were positive for *Giardia*, 0.475% were *Entamoeba histolytica* positive and 2.23% infections were positive for *Cryptosporidium*sp.*Giardia* infection increases during the pre-monsoon period (April-May) and subsides in the post monsoon period (October-November). The maximum rate of infection is observed in the month of August-September. *Cryptosporidium* infection increases in the post winter season and is also maximum during that time of the year (February-March). The infection decreases with the advent of the monsoon season. It is interesting to note that with the increase in *Giardia* infection, there is a decrease in the infection by *Cryptosporidium*. The infection by *Entamoeba histolytica* is very low and seasonality of this infection is hard to predict.

References:

1. Boschi-Pinto Cynthia, Velebit Lana, Shibuya Kenji. Estimating child mortality due to diarrhoea in developing countries. Bulletin of the World Health Organization. 2008;86
2. A simple solution. Times Europe; 2006. pp. 42–47.
3. Mukherjee AK, Chowdhury P, Bhattacharya MK, Ghosh M, Rajendran K, Ganguly S. Hospital-based surveillance of enteric parasites in Kolkata. *BMC Research Notes*. 2009;2:110. doi:10.1186/1756-0500-2-110.
4. Allen AV, Ridley DS. Further observations on the formol-ether concentration technique for faecal parasites. J ClinPathol. 1970;23:545–6. doi: 10.1136/jcp.23.6.545.
5. Garcia LS, Bruckner DA, Brewer TC, Shimizu RY. Techniques for the recovery and identification of *Cryptosporidium* oocysts from stool specimens. J ClinMicrobiol. 1983;34:185–190.
6. Fayer R. Cryptosporidium and Cryptosporidiosis. CRC Press; 1997.
7. Diagnostic procedures for stool specimens http://www.dpd.cdc.gov/dpdx/HTML/DiagnosticProcedures.htm
8. Rochelle PaulA, De Leon Ricardo, Stewart MicH, Wolfe RoyL. Comparison of primers and optimization of PCR conditions for detection of *Cryptosporidium parvum*and *Giardia lamblia* in water. App Environ Microbiol. 1997;63:106–114.
9. GoninPatric, Trudel Louise. Detection and differentiation of *Entamoeba histolytica*and *Entamoeba dispar*isolates in clinical samples by PCR and Enzyme- Linked Immunosorbent Assay. J ClinMicrobiol. 2003;41:237–241. doi: 10.1128/JCM.41.1.237-241.2003.
10. Xiao Lihua, Morgan UnaM, limor Josef, Escalante Ananias, Arrowood Michael, Shulaw William, Thompson RCA, Ronald Fayer, Lal AltafA. Genetic Diversity within *Cryptosporidium parvum*and Related *Cryptosporidium* Species. App Environ Microbiol.