Intestinal coccidian parasitic infections in rural community in and around Loni, Maharashtra

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Abstract Fecal samples examination of 310 individuals with variable gastro-intestinal (GI) disturbances but negative for intestinal parasites or bacterial pathogens, revealed 97 persons positive for infection with coccidian parasites employing modified Ziehl–Neelsen (ZN) staining and Sheather’s sucrose floatation technique. *Cryptosporidium* oocysts alone were detected in 64 (64/97; 66%) cases whereas *Cyclospora* oocysts along with *Cryptosporidium* were present in other 30 cases (31%); remaining three cases yielded only *Cyclospora* oocysts (3%) in their feces. None of the 97 cases positive for the two coccidian parasites was reactive for antibodies to human immunodeficiency virus (HIV) in their blood nor did any individual have history of taking immuno-suppressive drugs or antibiotics in the recent past. A breakdown of these 97 cases according to age and sex revealed not much difference in infection with two coccidian parasites. Further, comparison between two techniques revealed modified ZN staining was far superior to Sheather’s floatation. As the coccidian parasites, particularly *Cryptosporidium* is gaining much importance; therefore modified ZN staining should be included preferably along with other techniques in routine fecal examination which may lead to an overall improvement in the health-care facilities.

Keywords Intestinal coccidian parasites · *Cryptosporidium parvum* · *Cyclospora cayetanensis* · Infection in humans

Numerous GI protozoan parasites causing intra-cellular infections predominantly in intestinal epithelial cells have emerged in recent years, including two important coccidian parasites—*Cryptosporidium parvum* and *Cyclospora cayetanensis*. Infection occurs through ingestion of contaminated food and water containing parasites’ sporulated oocysts and in humans, the infection with *Cry. parvum* was first documented in 1976 (Miesel et al. 1976; Nime et al. 1976), whereas with *Cyc. cayetanensis* in 1977 (Brown and Rotschafer 1999; Satheeshkumar et al. 2004). Both the parasites have been recognized as a cause of GI illness in immuno-competent as well as in immuno-deficient persons that results in diarrhea of varying frequencies (Current et al. 1983). The infection in immuno-competent individuals is self-limited though intermittent intestinal discomfort may be experienced, whereas it can be unrelenting and fatal in immuno-compromised individuals unless treated properly (Current and Gracia 1991; Navin and Juranek 1984). Massive out-breaks caused by the two coccidian parasites have been reported in general population, particularly in persons from the United States (Brown and Rotschafer 1999; MacKenzie et al. 1994). As both the parasites are gaining importance in India also (Barua et al. 2008; Iyer 2006; Shrikhande et al. 2009), the present study therefore was undertaken to find out infections with the two coccidian parasites as a possible cause of GI disturbances in rural community in and around Loni, Maharashtra.

Fecal samples from 1,292 persons aged about 1–65 years, attending out-patient department of Pravara Rural Hospital, PIMS, Loni were received from Sep., 2007 to Aug., 2009 and were routinely examined for parasites as approved by the Institutional Ethical and Scientific Committee at PIMS, Loni. Freshly-passed fecal samples were collected in clean, dried bijou bottles (5 ml of capacity) devoid of any disinfectant/antiseptics and were grossly

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examined for any expelled worms along with mucus and blood. Wet saline and Lugol's iodine mount preparations of the samples were prepared and these were examined microscopically for the parasites. As about 40% (517) of the individuals were referred cases for routine check-up only and none had any GI disturbances, therefore in remaining 60% (775 out of 1,292) of the individuals three samples were collected, one each on three consecutive days and subsequently examined for parasites as given above. Also concentration of the samples was carried out for the parasites using zinc sulphate floatation and formal-ether sedimentation methods as per standard protocol. Any samples suspected of *Salmonella* and/or *Shigella* were further cultured on MacConkey's agar, Salmonella–Shigella agar, Wilson and Blair bismuth sulphite agar and were sub-cultured on MacConkey's agar also after enrichment in Selenite F medium at 37°C. The samples of children aged 1 year were additionally cultured on blood agar.

In all, a total of 310 persons were selected who revealed negative for the intestinal parasites or bacterial pathogens, were checked further for infection with coccidian parasites employing modified ZN staining and Sheather's sucrose floatation technique (Gracia et al. 1983). All the individuals were having varying degree of abdominal disturbances like intermittent cramping, nausea, anorexia and occasionally low-grade fever also. None of the persons included in the study were on immuno-suppressive drugs or having taken antibiotics in the recent past (within 3–6 months time). As coccidian parasites is one of the commonest causes of intestinal infections in HIV cases, therefore individuals' blood samples except from small children, were collected and screened for antibodies to HIV by TRI-DOT (Biomed Industries, H P, India) and none were found reactive to HIV. All these individuals had opted for voluntary testing and thus informed consent was obtained as per standard guidelines (ICMR 2004; NACO 2003, 2006).

The results revealed a total of 97 out of 310 individuals (about 31%) positive for oocysts of *Cryptosporidium* and/or *Cyclospora* by modified ZN staining and Sheather's floatation technique (Fig. 1). Majority of the infections were either due to *Cryptosporidium* alone (64/97; 66%) or in association with *Cyclospora* (30/97; 31%); however, rare infections with *Cyclospora* (3/97; 3%) alone was also observed. A break-up of the 310 persons (158 males and 152 females) revealed no differences as both the sexes of all age groups were equally affected with the two coccidian parasites (Table 1). Initial indications of repeated fecal examination in some of the cases on follow up after 2 and 4 weeks of initiating antibiotic therapy (spiramycin or trimethoprim-sulfamethoxazole) further revealed no infection with either of the two coccidian parasites and the persons were much relieved of the GI disturbances/discomfort-

Fig. 1 Oocysts of *Cryptosporidium parvum* (a, b) and *Cyclospora* (c, d) by Sheather's floatation method (a, c) and in modified ZN staining (b, d)

ness. Also a correlation for mucus and/or pus cells with the presence of two coccidian parasites in their feces was recorded. However, some of the cases having mucus and pus cells in their feces even did not reveal any of the two coccidian parasites. Therefore, it is likely that some of these cases might be infected with bacterial pathogens including those with enteric viruses and/or protozoan parasites viz. *Microsporidium* species. Although infections with *Salmonella* and *Shigella* were ruled out in the present study, the presence of enterotoxigenic *Escherichia coli*, *Campylobacter* and *Yersinia* species as well as Rotaviruses along with other enteric viruses and *Microsporidia* remains to be elucidated.

Comparison of the two techniques used in the present study revealed modified ZN staining was far superior to Sheather's floatation as 39 cases additionally were detected with the former method (95 out of 97 cases). In contrast, only 2 cases more were detected by Sheather's floatation (58 out of 97 cases) which were negative by modified ZN for infection with the two coccidian parasites. As the oocysts of these two coccidian parasites are visible as refractile round bodies in wet mount preparation, therefore sole reliability on Sheather's floatation technique may lead to errors in their identification, particularly by the inexperienced personnel (Eberhard et al. 1997). Although sporulation assays, immunological and molecular methods are more sensitive and reliable but are costly, tedious and not available except in some sophisticated laboratories. Some of the reports indicate that microscopic examination employing modified ZN is quite comparable with ELISA which would suffice the purpose as a routine procedure in screening fecal samples particularly, for cryptosporidiosis

Table 1 Age and sex-wise distribution of the two coccidian parasites amongst the 310 persons

Age of the individuals	Males Number of positives/ number of samples tested	Females Number of positives/ number of samples tested
Age up to 1 year	4/23	6/35
>1–5 years	10/27	8/22
>5–10 years	11/15	9/11
>10–15 years	2/10	3/9
>15–50 years	20/68	15/53
>50–65 years	4/15	5/22
Positives/total number	51/158 (32.3% approx.)	46/152 (30.3% approx.)

(Barua et al. 2008; Kehl et al. 1995). Since both the parasitic infections are amenable to antibiotic therapy, therefore modified ZN staining along with other techniques such as Sheather's floatation or some better methods, if possible, should preferably be included in feces examination. This may obviate unnecessary treatment particularly, in the children who are more prone to side-effects of the anti-parasitic drugs whereas erroneous treatment with the antibiotics might augment the antibiotic resistance amongst the bacterial population as well as change resulting in normal flora that is usually present in the human GI tract. Since the antibiotics and other drugs are easily available in the market to general public, therefore the overall effect of self-medication on the outcome of intestinal infections remains to be determined. Further, the coccidian parasites (*Cryptosporidium*, *Cyclospora*, and *Isospora*) along with *Microsporidia* appear to be accounting for persistent diarrhea in more than 50% of HIV-infected persons as reported from different parts of the world especially in many of the developed countries (Hunter and Nichols 2002; Kulkarni et al. 2009; Nagamani et al. 2007; Sitara Swarna Rao et al. 2008; Tumwine et al. 2005). The work therefore is to be undertaken to find out the correlation of parasites' load thereon along with clinical findings/symptoms and CD4 cell counts in HIV cases in rural population in and around Loni.

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References

- Barua P, Hazarika NK, Rasul E, Laskar N (2008) Microscopy for cryptosporidiosis screening in remote areas. *Indian J Med Microbiol* 26:203–204
- Brown GH, Rotschafer JC (1999) *Cyclospora*: review of an emerging parasite. *Pharmacotherapy* 19:70–75
- Current WL, Gracia LS (1991) *Cryptosporidiosis*. *Clin Microbiol Rev* 4:325–358
- Current WL, Reese NC, Ernst JV, Bailey WS, Heyman MB, Weinstein WM (1983) Human cryptosporidiosis in immunocompetent and immunodeficient persons: studies of an outbreak and experimental transmission. *N Engl J Med* 308:1252–1257
- Eberhard ML, Pieniazek NJ, Arrowood MJ (1997) Laboratory diagnosis of *Cyclospora* infections (review). *Arch Pathol Lab Med* 121:792–797
- Gracia LS, Bruckner DA, Brewen TC, Shimizu RY (1983) Techniques for the recovery and identification of *Cryptosporidium* oocysts from stool specimen. *J Clin Microbiol* 18:185
- Hunter PR, Nichols G (2002) Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. *Clin Microbiol Rev* 15:145–154
- ICMR (2004) Ethical guidelines for biomedical research. <http://www.icmr.nic.in>. Accessed Jan 2004
- Iyer RN (2006) *Cyclosporiasis* in an infant. *Indian J Med Microbiol* 24:144–145
- Kehl KS, Cicirello H, Havens PL (1995) Comparison of four different methods for the detection of *Cryptosporidium* species. *J Clin Microbiol* 33:416–418
- Kulkarni SV, Kairon R, Sane SS, Padmawar PS, Kale VA, Thakar MR et al (2009) Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhea by the level of immunosuppression. *Indian J Med Res* 130:63–66
- MacKenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE et al (1994) A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N Engl J Med* 331:161–167
- Miesel JL, Perera DR, Meligro C, Rubin CE (1976) Overwhelming watery diarrhoea associated with *Cryptosporidium* in an immunosuppressed patient. *Gastroenterology* 70:1156–1160
- NACO (2003) Manual of quality assurance practices in HIV testing laboratories. Published by NICD, Directorate General of Health Services, and NACO, Ministry of Health and Family Welfare, Govt. of India, New Delhi, p 14
- NACO Fact sheets (2006) <http://www.nacoline.org>. Accessed 3 Jun 2008
- Nagamani K, Pavuluri PR, Gyaneshwari M, Prasanthi K, Rao MI, Saxena NK (2007) Molecular characterisation of *Cryptosporidium*: an emerging parasite. *Indian J Med Microbiol* 25:133–136
- Navin TR, Juranek DD (1984) *Cryptosporidiosis*: clinical, epidemiologic and parasitologic review. *Rev Infect Dis* 6:313–327
- Nime FA, Burek JD, Page DL, Holscher MA, Yardley JH (1976) Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. *Gastroenterology* 70:592–598
- Satheeshkumar S, Avanthan S, Lakshmi P (2004) Intestinal infection in HIV infected patients with diarrhoea in Chennai. *Indian J Med Microbiol* 20:88–90
- Shrikhande SN, Chande CA, Shegokar VR, Powar RM (2009) Pulmonary cryptosporidiosis in HIV negative, immunocompromised host. *Indian J Pathol Microbiol* 52:267–268
- Sitara Swarna Rao A, Premi S, Gagandeep K (2008) *Cryptosporidium* species in HIV-infected individuals in India: an overview. *Natl Med J India* 21:178–184
- Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S, Ndeezzi G, Downing R, Feng X et al (2005) Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. *Am J Trop Med Hyg* 73:921–925