Full Length Research Paper

Prevalence of Cryptosporidium and other intestinal parasites using different diagnostic techniques in Enugu Metropolis, South-Eastern Nigeria

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Cryptosporidium is a protozoan parasite and the aetiological agent of a gastrointestinal disease, known as cryptosporidiosis. In this study, a total of 300 faecal samples were collected and analysed for the presence of Cryptosporidium, yeasts and other parasites using direct normal saline, iodine wet preparations, formol-ethylacetate concentration and modified Ziehl Neelsen staining techniques. Using direct normal saline and iodine wet preparation techniques, a total of 25 (8.3%) samples out of 300 were positive for Cryptosporidium and yeasts, but the use of formol-ethylacetate concentration increased the number of positive samples for Cryptosporidium and yeasts to 30 (10%). Further application of modified Ziehl Neelsen staining technique to sediments differentiated the aetiologic agents into Cryptosporidium 6 (20%) and yeast-positive 24 (80%). The prevalence of other parasites encountered in this study include: Giardia lamblia, 1 (0.3%); Blastocystis hominis, 1 (0.3%); Entamoeba histolytica, 13 (4.3%); Clonorchis sinensis, 2 (0.7%); Hookworm, 6 (2%); Ascaris lumbricoides, 3 (1%) and Balatidium coli, 1 (0.3%). Thus, the application of only routine wet preparation was less predictive and resulted in 21.7% misdiagnosis (1.7% undetected plus 20% misdiagnosed as yeast). This study buttresses the need for the confirmation of every suspected yeast infection as may be detected in wet examination of faecal specimens, to avoid misdiagnosis. The study also indicates the importance of formol-ethylacetate sedimentation technique in the detection and diagnosis of yeasts, Cryptosporidium, B. hominis and other parasites from stool samples.

Key words: Cryptosporidium, intestinal parasites, immunocompromised, yeasts, misdiagnosis.

INTRODUCTION

Cryptosporidiosis is an emerging protozoan disease associated with large waterborne outbreaks and contamination of foods like vegetables, through poultry faeces used as sources of manure (Coupe et al., 2005; Upton, 2008; Borchardt and Spencer, 2002). Intestinal parasites such as Cryptosporidium and fungal organisms like yeasts have been associated with gastrointestinal disturbances and other biological disorders including diarrhoea, abdominal pain, vomiting, waist pain and anaemia (Arora and Arora, 2009; Tessema, 2008). Infections by Cryptosporidium and yeasts have also been associated with immune depression (Upton, 2008; Cheesbrough, 2005). The two organisms produce similar signs and symptoms in infected individuals and are not easily distinguished in direct examination of wet preparation of faecal specimens. It is believed by many medical personnel in this part of the

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world that Cryptosporidium and yeasts can only cause gastrointestinal diseases in HIV patients, without considering other conditions that could predispose individuals to such infections. As a consequence of this belief, confirmatory laboratory diagnosis of Cryptosporidium and yeasts are not carried out. Thus, Cryptosporidium species are misdiagnosed as yeasts which have the same appearance in wet preparation of faecal specimen. The non-identification of Cryptosporidium poses a great threat to infected patients’ recovery as it is known to be highly resistant to antimicrobial agents (Upton, 2008; Tessema, 2008). This study was conducted to determine the prevalence of Cryptosporidium in stool samples collected within Enugu metropolis using different diagnostic techniques for their predictive values in diagnosing these infections.

MATERIALS AND METHODS

Sample collection

A total of 300 faecal samples for routine microbiological analysis were collected within Enugu metropolis, south Eastern Nigeria. The collection was made between November, 2011 and March, 2012. Of these, 190 samples were collected from the Microbiology unit of Medical Laboratory Department, University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu; 90 samples were collected from Mascot Diagnostic Laboratory, Abakpa, Enugu and the remaining 20 samples collected from St. Patrick Hospital, Asata, Enugu. The samples were collected with regard to the hospitals’ ethical guidelines. The faecal specimens were immediately preserved in 10% formol-saline, before transportation to the laboratory for analysis.

Sample analysis

The samples collected were analysed by homogenising a pea-sized portion of faecal specimen in 3 ml of 10% formol-saline in a test-tube. The faecal suspensions were filtered through a 2 mm sieve. Four millilitres of ethylacetate was added to the faecal suspension, shaken vigorously for 1 min and then centrifuged at 3000 rpm for 10 min. The ether-faecal debris at the interface was loosened with an applicator stick and the supernatant decanted. The tube was tapped gently to loosen and resuspend the faecal deposit at the bottom. The deposit was put on clean a grease-free glass slide, covered with a cover slip and examined using x10 and x40 objective lens of the microscope. The deposit was allowed to dry and then stained by modified Ziehl Neelsen staining technique.

Modified Ziehl Neelsen staining technique

The faecal smear was fixed in methanol for 2 min. The fixed smear was drained and stained with cold carbol fuschin for 15 min and washed off with water. The stained smear was decolorized with 1% acid-ethanol (that is 1 volume of concentrated HCl into 99 volumes of ethanol) for 15 seconds and washed with water. The decolorized film was counter-stained with 0.25% malachite green for 30 s and washed with water. Then, the stained slides were kept in a draining rack to dry, before examination under the microscope using x100 objective lens.

Statistical analysis

The prevalence was calculated by simple percentages and significance determined using t-test. Comparison of the prevalence of Cryptosporidium, yeasts, B. hominis and other parasitic infections was made using one-way ANOVA. Chi-square was used to determine the association between age range and occurrence of Cryptosporidium and yeasts in faecal specimens. All statistical significance was set at 5% level.

RESULTS

Prevalence of Cryptosporidium and yeasts among yeasts-like organisms in human faeces

A total of 300 stool samples were analysed for Cryptosporidium, yeasts and other intestinal parasites. Of the 300 samples examined in this study, 25 (8.3%) were observed to have yeast-like organisms in faecal wet examination. However, the use of formol ethyl-acetate concentration technique increased the number of positive samples to 30 (10%) suggesting that 1.7% of the positive samples were undetected by routine faecal wet examinations alone (Figure 2). Of the 30 samples positive for yeast-like organisms, 6 (20%) were identified as Cryptosporidium, while 24 (80%) were yeasts with modified Ziehl Neelsen staining technique (Figure 1). Of the 6 posi-

Figure 1. Prevalence of Cryptosporidium and yeasts among yeast-like organism in faecal specimens.
Figure 2. Effects of methods of analysis in the detection of Cryptosporidium, yeast and B. hominis

Figure 3. Percentage chances of detection of Cryptosporidium and yeast in direct wet preparation of faecal specimens.

tive samples for Cryptosporidium, 4 (13%) samples were initially detected in direct wet examination, while the additional 2 (7%) samples were detected with application of formol-ethylacetate sedimentation technique. Also, of the 24 positive samples for yeasts, 21 (70%) samples were initially detected in direct wet examination, while the additional 3 (10%) samples were detected with the application of sedimentation technique (Figures 2 and 3).

Age-dependent prevalence of Cryptosporidium and yeasts

Of the 6 cases of cryptosporidiosis, 3 were detected among children in the age-group <1 year - 10 years (n = 28) constituting 10.7% prevalence for the group. Also 2 cases were detected among the age group 21 years and above (0.8%; n = 251) while only one of the cases was detected in a female patient within the age group of 11 - 20 years (4.8%; n = 21). Similarly, of the 24 cases of yeast infections, 11 (39.3%) cases were from the age group of <1 year – 10 years, 4 (19%) cases were detected among age group of 11 – 20 years, while age group 21 years and above had 9 (3.6%) cases (Table 1).

Prevalence of other parasites in the faecal specimens

Other parasites encountered during this study included Giardia lamblia, 1 (0.3%); Blastocystis hominis, 1 (0.3%); Entamoeba histolytica, 13 (4.3%); Clonorchis sinensis, 2 (0.7%); Hookworm, 6 (2%); Ascaris lumbricoides, 3 (1%) and Balatidium coli, 1 (0.3%). A total of 57 (19%) samples were infected with parasites (both protozoan and helminthic) (Figure 4). In this study, yeasts were the most prevalent, with 24(8%) positive samples followed by Entamoeba histolytica, with 13 (4.3%) positives and then hookworm and Cryptosporidium, 6 (2%) positives each.

DISCUSSION

The occurrence of yeast-like organisms in faecal specimens is not unusual, since yeasts are normal flora of both human and animal gastrointestinal tracts (Arora and
Table 1. Age-Dependent Prevalences of *Cryptosporidium* and Yeasts.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number examined</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Cryptosporidium</em></td>
</tr>
<tr>
<td>&lt;1yr-10yrs</td>
<td>28</td>
<td>3(10.7%)</td>
</tr>
<tr>
<td>(11-20) yrs</td>
<td>21</td>
<td>1(4.8%)</td>
</tr>
<tr>
<td>21yrs &amp; Above</td>
<td>251</td>
<td>2(0.8%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>300</strong></td>
<td><strong>6(2%)</strong></td>
</tr>
</tbody>
</table>

![Figure 4. Prevalences of parasites and yeasts.](image)

Arora, 2009). The appearance of yeasts in faecal specimens is therefore usually ignored unless they are detected in large numbers, in which case, they are regarded as opportunistic pathogens. Conditions which lead to yeast overgrowth include prolonged intake of antibiotics, immune suppressive chemotheraphy or immune depression due to other diseases (Forbes et al., 2001; Tessema, 2008).

*Cryptosporidium* and yeasts cause the same type of diseases in humans and animals with symptoms including non-bloody, watery diarrhoea (Tessema, 2008; Upton, 2008; Arora and Arora, 2009). Also, both yeasts and *Cryptosporidium* appear round to oval in shape and refractile in microscopy (Cheesbrough, 2005; Upton, 2008), and are therefore presumptively identified as yeasts in wet examinations, in many laboratories. In routine laboratory analysis, effort is rarely made to distinguish these two organisms. Moreover, there is a general belief that *Cryptosporidium* infections occur mostly in HIV patients (Arora and Arora, 2009; Morgan et al., 2000). Based on this, 30 samples yielding yeast-like organisms were initially scored as yeast-positive in this study. However, the use of modified Ziehl Neelsen staining technique was able to differentiate the two infectious agents (*Cryptosporidium* and yeasts). *Cryptosporidium* spp are acid fast, oocyst protozoan parasites that appear reddish in colour after staining (Morgan, 2000; Mahgoub et al., 2004; Cheesbrough, 2005). These features were observed in this study and were used to identify and differentiate the two organisms. The results show that 6 (20%) out of the 30 positive samples were *Cryptosporidium*, while 24 (80%) were yeasts (Figure 1). The prevalence of the two organisms is significant (P< 0.05) among the samples analysed. Also, the appearance of *Cryptosporidium* in low numbers is diagnostic, while that of yeasts is not diagnostic as they are normal flora in the gut (Arora and Arora, 2009).

Concentration of parasites by sedimentation is a technique which uses centrifugal force to concentrate parasites within faecal suspension at the bottom of tube (Weber, et al., 1992). Studies have shown that this method is efficient in the detection of parasites even when they appear in low numbers in a specimen (Weber et al., 1992; Davies et al., 2003; Cheesbrough, 2005). The findings in this study showed higher detection of infectious agents in stool including *Cryptosporidium* in 6 samples, yeasts in 24 samples and *B. hominis* in 1 sample (Figure 2). These findings are consistent with the scientific reports of Davies et al. (2003) and Weber et al. (1992) on this technique as indicated by the extra detection of 7% and 10% of *Cryptosporidium* and yeasts, respectively (Figure 3).

Immune-competence is highly associated with age, with children and the aged having lower immunity (immu-
The excessive use of antibiotics has been associated with increase in intestinal yeast infections, particularly (Mahgoub et al., 2004; Forbes et al., 2001). In this study, the distribution of Cryptosporidium and yeasts infections among age groups showed that children in the age group <1-10 years had the highest prevalence of Cryptosporidium, 3 (10.7%; n=28) and yeasts, 11 (39.3%; n=28), while lower prevalence among age groups of 11 – 20 years and 21 and above were observed (Table 1). The high prevalence of Cryptosporidium, 3 (10.7%) and yeasts, 11 (39.3%) within the age group of <1 year – 10 years is consistent with the report that Cryptosporidium and yeasts cause diseases in the immunocompromised individuals especially children (Mahgoub et al., 2004; Tessema, 2008). Similarly, the lower prevalence among the age groups of 11 – 20 years and 21 years and above agrees with the report of Upton (2008) that these age groups are more immunocompetent, hence infections with these agents are self-limiting. The prevalence of the organisms among children, in this study, may be attributed to factors such as the use antibacterial chemotherapy, without prescription and in unrestricted doses which is widely obtained in Nigeria. The analysis of the prevalence of Cryptosporidium and yeasts among age range using Chi-square shows a significant (P<0.05) association between age range and their rates of infection. This is consistent with the report that age has influence on the immunity of individuals and mostly lowers at infancy and elderly stage (Tessema, 2008).

Of all the parasites detected, Blastocystis hominis 1 (0.3%) is another emerging pathogen in humans in addition to Cryptosporidium and yeasts. Blastocystis hominis is a protozoan parasite that has been recently discovered to be pathogenic in human especially in immune compromised individuals and people with irritable bowel syndrome (Anchalee et al., 2004). This protozoan parasite, B. hominis appears commonly in different forms in human faecal specimens (vacuolar, multivacuolar and cystic forms) and its diagnosis has not given attention (Anchalee et al., 2004). The cystic form, suspected to be the infectious form is difficult to detect in wet examinations, but may be easily detected by formol-ether sedimentation technique (Anchalee et al., 2004; Nascimento and Moitinho, 2005). The B. hominis reported in this study was in cystic form and was detected after sedimentation but not during wet examination which is in agreement with the above reports. The detection and identification of only Blastocystis hominis in one of patient’s faecal sample who had a clinical provisional diagnosis of recurrent diarrhoea is suggestive of its involvement in the diarrhoea. This finding is consistent with the report of Anchalee et al., (2004) where it was implicated as the cause of gastrointestinal disturbances in patients with irritable bowel syndrome. It is therefore important that serious attention should be paid to the diagnosis and identification of B. hominis from human stool specimens. Other parasites encountered during this study include Giardia lamblia, Blastocystis hominis, Entamoeba histolytica, Clonorchis sinensis, Hookworm, Ascaris lumbricoides and Balatidium coli (Figure 4). Cryptosporidium infections occurred at the same rate with hookworm (Figure 4). The prevalence of Cryptosporidium (which is not normally diagnosed) and hookworm (which attracts a great deal of attention) at the same rate is suggestive of the need for serious attention to Cryptosporidium infections.

The findings of this study strongly suggest the need for more attention in diagnostic approaches to infectious agents like Cryptosporidium, yeasts and B. hominis infections, as it will aid in the management and treatment of the ailments (especially cryptosporidiosis which has no adequate drug for its treatment, but is rather managed by immune reconstitution).

REFERENCES


