Non-pathogenic protozoa are single-celled parasites usually detected in the intestinal tract and with a worldwide distribution. Our aim in this study was to estimate the non-pathogenic intestinal parasites among primary schoolchildren in Sohag Governorate, Egypt and to correlate between these protozoa and the release of symptoms. Stool specimens of 200 child were investigated microscopically by iodine stained smear and formol-ether sedimentation. Non-pathogenic protozoa represented 9% of all studied children (3.5% for Entamoeba coli, 2.5% for Iodamoeba bütschlii and 1.5% for each Entamoeba hartmanni and Chilomastix mesnili). Iodamoeba bütschlii and Entamoeba coli infection showed a statistical significance regarding symptoms. Only Iodamoeba bütschlii infection was affected statistically by the child gender. Infection with these non-pathogenic parasites is a proof of fecal contamination and can also be disordered with the potentially pathogenic parasites. In addition, these parasites are also problematic in that they may be considered the cause of some symptoms after exclusion of other pathogens.

Non-pathogenic protozoa have a worldwide distribution. This high prevalence rate may reflect the state of poor environmental sanitation (Koshak and Zakai 2003). Detection of these non-pathogenic parasites in human would submit ingestion of polluted water or food, and may suggest possible exposure to pathogenic organisms (Kuo et al., 2008).

These non-pathogenic protozoa can be identified based on morphological features, molecular, genetic, immunologic and clinical criteria (Stauffer and Ravdin, 2003).

Although there is still no final evidence of a causal connection between the existence of these parasites and the appearance of symptoms in the host (ISSA, 2014). The available study is to identify the non-pathogenic intestinal protozoa in schoolchildren, utilizing the copro-microscopic techniques and a trial to correlate between the potential occurrence of symptoms with these parasites only.

INTRODUCTION

Non-pathogenic protozoa can be divided into two groups: amoebae and flagellates, commonly range in length between 10 to 52 micrometers, but can expand as vast as 17mm. (Alcamo and Warner, 2010).

The non-pathogenic intestinal protozoa include: Entamoeba coli, Entamoeba dispar, Entamoeba hartmanni, Entamoeba polecki, Endolimax nana, Iodamoeba bütschlii and Chilomastix mesnili (ISSA, 2014).

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Although there is still no final evidence of a causal connection between the existence of these parasites and the appearance of symptoms in the host (ISSA, 2014). The available study is to identify the non-pathogenic intestinal protozoa in schoolchildren, utilizing the copro-microscopic techniques and a trial to correlate between the potential occurrence of symptoms with these parasites only.

SUBJECTS AND METHODS

ETHICAL CONSIDERATION

An acceptance was obtained from the scientific ethics committee of our institute before the onset of the study. Informed consent was obtained from all patients alternate-ly their guardians after clear explanation for the objectives of the study.

POPULATION

The study was conducted from January 2015 to December 2016 in the Parasitology Department, Faculty of Medicine, Sohag University, Sohag, Egypt. A total of 200

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school-aged children between 6 and 12 years, who attend primary schools in our governorate, were randomly enlisted from four elementary schools in our governorate to be enrolled in the study.

**Questionnaire**
Information was collected through a pre-tested questionnaire by children alternately their parents which included socio-demographic information such as age and gender, with a history of symptoms (e.g., diarrhea, nausea, vomiting and abdominal pain).

**Parasitological Methods**
Stool specimens were collected in clean, dry, and labeled containers. Microscopic examination of the iodine stained and formol-ether sedimentation specimens (Garcia, 2016) were used for detection of diagnostic stages of the enteric non-pathogenic protozoa.

**Statistical Analysis**
Data were organized, tabulated, and statistically analyzed using SPSS version (22). Quantitative data were expressed as means ± standard deviation. Qualitative data was expressed as frequencies and percentages. Chi-Square test ($\chi^2$) and Fisher’s Exact test were used when appropriate for comparison between qualitative variables. $P < 0.05$ indicates significant values.

**RESULTS**

**Population Profile**
200 schoolchildren aged between 6 and 12 years (Age Mean ± SD = 8.9±1.9) had participated in this study. Of these children, 103 (51.5%) were boys and 97 (48.5%) were girls, 119 (59.5%) < 10 years and 81 (40.5%) ≥ 10 years.

Non-pathogenic protozoa were recorded in 18 child with a prevalence of 9%. 3.5% (7/200) for *Entamoeba coli*, 2.5% (5/200) for *Iodamoeba bütschlii* and 1.5% (3/200) for both *Chilomastix mesnili* and *Entamoeba hartmani*. (Table 1)

**Table 1: Parasite frequencies and percentages in descending manner.**

<table>
<thead>
<tr>
<th>Non-pathogenic Protozoan</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>7 (3.5)</td>
</tr>
<tr>
<td><em>Iodamoeba bütschlii</em></td>
<td>5 (2.5)</td>
</tr>
<tr>
<td><em>Chilomastix mesnili</em></td>
<td>3 (1.5)</td>
</tr>
<tr>
<td><em>E. hartmanni</em></td>
<td>3 (1.5)</td>
</tr>
</tbody>
</table>

Surprisingly, *Iodamoeba bütschlii* and *E. coli* infection showed a statistical significance regarding symptoms although these are non-pathogenic (P-value was 0.005 and 0.016) respectively. (Table 2)

According to data in Table 3, there was no statistically significant relation between the infecting parasite and the children age groups.

**Table 3: Age distribution of non-pathogenic protozoa among studied children.**

<table>
<thead>
<tr>
<th>Age</th>
<th>E. coli</th>
<th>E. hartmanni</th>
<th>I. bütschlii</th>
<th>C. mesnili</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10Y</td>
<td>2 (28.6%)</td>
<td>2 (66.7%)</td>
<td>4 (80%)</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>≥ 10Y</td>
<td>5 (71.4%)</td>
<td>1 (33.3%)</td>
<td>1 (20%)</td>
<td>1 (33.3%)</td>
</tr>
</tbody>
</table>

Girls were slightly higher susceptible to infection with *E. coli* and *Iodamoeba bütschlii* whereas infection with *E. hartmanni*, *Chilomastix mesnili*, were more common among boys. Only *I. bütschlii* was affected by the child gender. All were girls 5 (100%). This was statistically significant, P value was 0.025. (Table 4)

**Table 4: The relation between the type of parasite and child gender.**

<table>
<thead>
<tr>
<th>Gender</th>
<th>E. coli</th>
<th>E. hartmanni</th>
<th>I. bütschlii</th>
<th>C. mesnili</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls</td>
<td>4 (57.1%)</td>
<td>1 (33.3%)</td>
<td>5 (100%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Boys</td>
<td>3 (42.9%)</td>
<td>2 (66.7%)</td>
<td>0 (0.0%)</td>
<td>2 (66.7%)</td>
</tr>
</tbody>
</table>

P-value was calculated by Chi square test and Fisher’s Exact Test

| Statistically significant |

**DISCUSSION**

The recognition of non-pathogenic protozoal parasites is lastly accepted as a useful epidemiological indication of the level of fecal contamination (El Ammari and Nair, 2005) and may indicate probable exposure to pathogenic organisms (Kuo et al., 2008).

In this study *E. coli* was the most predominant non-pathogenic protozoa (3.5%). This is in agreement with Osman et al. (2016) who detected it in (2.4%) but this brings down over with that detected by Matthys et al. (2011) in western Tajikistan (65.7%). Their sample size was more than three times the present work’s sample size.

*Iodamoeba bütschlii* was the second non-pathogenic protozoa in the current study with a prevalence of (2.5%). This is in agreement with Nxasana et al. (2013) who detected it in (3.1%) of Mthatha, Eastern Cape Province, South Africa school children. On the contrary, Al-Delaimy et al. (2014) had recently reported that *Iodamoeba bütschlii* represented...
Table 2: Symptoms of individual parasites

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No symptoms</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>4 (57.1%)</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>Pain</td>
<td>1 (14.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Dysentery</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Perianal itching</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>4 (57.1%)</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>E. hartmanni</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Iodamoeba bütschlii</td>
<td>1 (20%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>C. mesnili</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

P- value *Statistically significant

(6.8%). This may be due to the larger sample size in addition to the pure rural background of the children.

(1.5%) of children in our study were infected with Chilomastix mesnili. This is inconsistent with Nxasana et al. (2013) who reported that its prevalence was (1.2%). Such prevalence was not in agreement with that reported by Al-Delaimy et al. (2014), who found it in (4.8%) in rural Malaysia Schoolchildren around Orang Asli.

Also, E. hartmanni prevalence was (1.5%) which is in line with (1.0%) among primary schoolchildren in Derna District, Libya, which had been reported by Sadaga and Kassem, (2007).

Iodamoeba bütschlii and E. coli infection unexpectedly showed a statistical significance regarding enteric symptoms although these are non-pathogenic. We can elucidate this question firstly by the fact that some non pathogenic protozoa can cause symptoms.

Some previous studies highlight the recovery of the non-pathogenic protozoa (E. coli, Endolimax nana, Chilomastix mesnili, E. hartmanni and Iodamoeba bütschlii) from patients creating intestinal disturbances (El Ammari and Nair, 2015). Mezaid et al. (2014) detected that 18.4% of patients had only E. coli, E. hartmanni and Endolimax nana were associated with intestinal symptoms.

Also recovery of E. dispar and E. moshkovskii from patients with gastrointestinal manifestation (Fotedar et al., 2007; Tanyuksel, 2007).

Although Endolimax nana in humans originally thought to be non-pathogenic. Studies suggested that it singly can cause intermittent or chronic diarrhea (Ash Lawrence, 2007). Also Iodamoeba bütschlii is an indicator of fecal-oral contamination and humans may experience diarrhea (Yamamoto, 2009).

CONCLUSION

There is a definitive proof of a causal link between the presence of non-pathogenic protozoa and the release of symptoms. This study could be considered as a basis for conducting further studies on a larger sample size for the determination and confirmation the relation between the non-pathogenic protozoa and symptoms.

ACKNOWLEDGEMENTS

Our special acknowledgment to Dr. Refaat Mohamed Khalifa, professor of Medical Parasitology, Faculty of Medicine, Assuit University, Egypt, who helped us revising the manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest.

AUTHORS CONTRIBUTION

Idea by Nada AF El-Nadi, Eman F Fadel performed the laboratory works and collected data, Noha S Ahmed helped with the laboratory analysis of samples, collection of papers, data analysis and writing the manuscript and Nada AF El-Nadi, Eman K Omran revised the manuscript.

REFERENCES