International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

SOI: http://s-o-i.org/ 1.15/ijarbs-2-11-17

Prevalence of human intestinal protozoa parasites and enteric Bacteria among school children in shagari Federal housing estate, Akure South local Government, Ondo state, Nigeria

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Abstract

Study was carried out from February to April 2015 to determine the prevalence of human intestinal protozoan parasites associated with enteric bacteria and to identify the risk factors among the school children of Shagari area in Akure South local government of Ondo state. A total of two hundred and fifty (250) stool samples were collected randomly from both males and females. Parasitological and bacteriological examinations of the faecal samples were carried out using standard techniques. The overall prevalence of intestinal protozoan parasites and enteric bacteria was found to be 31.6 % and 38.0% respectively. *Entamoeba histolytica* was found to be 19.2% followed by *Giardia lamblia* 12.4%. The prevalence of protozoa parasites and enteric bacteria in male children were 13.6% and 20.4% respectively and the prevalence of protozoa parasites and enteric bacteria in female children were 18.0% and 17.6% respectively. The highest prevalence of protozoa infection was recorded among the age group of 5 – 9 years (13.2%) in both sexes, and the highest prevalence of enteric bacteria was recorded among the age group of 10 – 14 years (16.4%) in both sexes. Prevalence of protozoan parasite infection was the highest among children using tap water (19.6%), compared with those using wells (12.0%). Prevalence was high (15.2%) among students defecating in bush than those using pit (5.6%). The prevalence of bacterial isolates from faecal samples are; *Escherichia coli* (21.2%), *Enterobacter* spp (1.6%), *Salmonella* spp (1.6%), *Shigella* spp (2.0%), *Staphylococcus aureus* (4.0%), *Streptococcus faecalis* (2.8%), *Proteus* spp (1.2%), *Klebsiella pneumoniae* (1.6%) *and Pseudomonas* spp (2.0%).

Keywords: Prevalence, Human, Intestinal, Parasites, Enteric, Bacteria

Introduction

Human intestinal protozoa parasitic infections result in gastrointestinal morbidity and mortality worldwide, particularly among young children in developing countries (Speich *et al* 2013*).* It has been estimated that *Entamoeba histolytica*, the causative agent of amoebiasis, kills between 40,000 and 100,000 people each year; hence, it is one of the deadliest parasitic infections worldwide (Sah *et al*., 2013 and Stanley 2003). The prevalence of *Giardia lamblia* has been estimated at 2-3% in the industrialized world and 20-40% of the global burden of *G. lamblia* is reported in developing countries, especially in children (Escobedo, 2009). The intestines of man are colonized by a large number of microbes. Most of these are harmless, or even beneficial. Others are harmless in normal individuals, but can produce disease in the very young, those with weakened immune systems, or in a new host that has no prior experience with the microbe. Some of the enteric bacteria are known to cause diseases in human worldwide, with the majority of these diseases occurring among children of the developing countries (Jawetz, 2007 and Farthing *et al*., 2012). An estimated 1.8 million children die each year from largely preventable enteric illnesses, with the large majority of these mortalities occurring in developing countries (Kevin *et al*., 2014). Nearly all

types or strains of *Escherichia coli* cause disease in children in the developing world (Farthing *et al*., 2012); around 600, 000 deaths are reported worldwide yearly due to *Shigella* infection with the majority of these deaths in developing countries (Scharf, 2010). *Shigella* which is one of the most communicable and severe forms of the bacteria induced diarrhoea (Heymann, 2008) is acquired by small children at the highest rate (Replogle, 2000 and Rooney, 2004). Successful colonization of intestinal protozoan parasites depends on factors such as inoculum size, intestinal motility, transit time, the presence or absence of specific intestinal flora, the host's diet and the ability to adhere to the colonic mucosa epithelia cells. As soon as the parasites bind to the mucosa cells, they attack and kill the cells and this lead to the development of infection (Jawetz, 2007). The essential pathologic process of enteric bacteria is invasion of the mucosa epithelial cells by induced phagocytosis, multiplication and spread within the epithelial cell cytoplasm. After the bacteria have been ingested, they disrupt the phagosome membrane and are released into the cytoplasm where they reproduce. The bacteria then invade the mucosa cells where they produce endotoxins that destroy the cells and initiate an inflammatory reaction in the mucosa (Prescott, 2008). Generally, situations involving poor sanitation and unhygienic conditions promote transmission of human intestinal protozoa parasites and enteric bacteria. Intestinal protozoa are transmitted by the faecal-oral route; faecal-oral transmission involves the ingestion of food or water contaminated with cysts. Human infection with enteric bacteria is generally associated with consumption of faecal contaminated food or drinking of contaminated water. Inter-personal transmission also occurs where level of hygiene may be particularly poor, e.g. mental healthcare units and schools (Hellard *et al*., 2000 and Prescott, 2008). Food handlers may also play an important role in the transmission even though they rarely survive more than ten minutes on the hands except under the finger nails. Faulty plumbing and water systems may cause water borne transmission (Washington *et al*., 2006). The intestinal protozoa organisms may be common worldwide, however, the prevalence is higher in developing countries. Their frequency may be related to inadequate sanitation, water supply, healthcare, education and poverty (Ibrahim *et al*., 2014).

Methodology

Study area

This study was carried out in Shagari village, Federal Housing Estate (7°26′N and 5°19′E) in Akure South local government area of Ondo state, Nigeria. The inhabitants are mainly civil servants, traders and artisans. Wells and public boreholes are the major sources of water that the residents depend upon for domestic activities.

Study design

Informed consent was obtained from the head teachers and participation by the students was voluntary after obtaining consent. Information on age, sex and socio-economic factors was collected from every participant in the study using standard questionnaires. Participants were assured that information obtained will be treated with utmost confidentiality. The school teachers also helped in the collection of data for correct information from the students.

Sample collection

Sterile universal bottles were given to each participating student. They were instructed on how to place a few samples of their stool into the bottle. Stool samples were collected and preserved in 10% saline before taken to The Federal University of Technology, Akure (FUTA) Microbiology laboratory for analysis. Stool samples were divided into two portions for parasitological examination bacteriological analysis. Stool samples that were not examined the same day were preserved. Preservation of stool samples was done to avoid the stool from decaying by adding 4 ml of 10% formalin to each sample of the first portion (for parasitological analysis) while 4 ml of normal saline was added to the second portion (for bacteriological analysis) and then stored in the refrigerator till the next day.

Parasitological examination of faecal samples

The Formol-ether concentration method (Cheesbrough, 2006) was used to carry out the analysis in the laboratory. About 2 g of faeces was emulsified in 7 ml of 10% formalin in a centrifuge tube, using a swab stick and was mixed thoroughly. The mixture was passed through a sieve (0.75 mm mesh size) into an evaporating basin. It was then placed in the centrifuge tube. Three millilitres (3ml) of ether was added and shaken vigorously for at least thirty seconds. The tube was then centrifuged at 1500 rpm for 5 minutes. After the spinning of the stool sample, the debris and supernatant were separated by decanting. Then, a Pasteur pipette was used to take 0.05 ml of the sediment and mounted on a clean grease-free microscope slide, covered with a cover slip

and viewed under ×10 and ×40 objective lens of microscope for intestinal protozoa parasites.

Bacteriological analysis of faecal samples

The faecal samples were cultured on differential and selective media for bacteria cultivation in order to isolate bacteria entero-pathogens and incubated overnight at 37℃ (Hyeng-II *et al*., 2010). MacConkey and Eosin methylene blue agar were used for the isolation of bacteria pathogens. The bacteria isolates were identified by standard biochemical tests according to Cheesbrough (Cheesbrough, 2006).

All data obtained in the study were subjected to SPSS. Chi-square, cross tabulation and general descriptive statistics were used to summarise the data obtained from the study at 95% confidence interval, $P < 0.05$ were considered to be significant.

Results

The intestinal protozoa parasites encountered from parasitological investigation are *Entamoeba histolytica* and *Giardia lamblia*. Out of 250 stool samples examined, 79 were infected with intestinal protozoa parasites, which represent a prevalence of 31.6%. The mixed infection with bacteria among protozoan infected cases was investigated (Table 1). A total of 32 children infected with protozoan parasites were mixed-infected with bacteria.

Table 1: Overall Prevalence of human intestinal protozoan parasite among the children

Protozoans parasites encountered	Number infected: Protozoa only	Mixed infection (protozoa and bacteria)	Total number infected	Prevalence (%)
Entamoeba histolytica	33	15	48	19.2
Giardia lamblia	14	17	31	12.4
Total	47	32	79	31.6

N (Total number of children examined) = 250

Table 2 shows the occurrence of bacteria isolates from faecal samples with the prevalence of each bacterium. *Escherichia coli* (21.2%), *Enterobacter* spp (1.6%), *Salmonella* spp (1.6%), *Shigella* spp (2.0%), *Staphylococcus aureus* (4.0%), *Streptococcus faecalis* (2.8%), *Proteus* spp (1.2%), *Klebsiella pneumoniae* (1.6%) *and Pseudomonas* spp (2.0%).

Table 2: Prevalence of bacteria organisms isolated from faecal sample

Bacteria Isolates	Number infected	Prevalence (%)
E.coli	53	21.2
Salmonella spp	4	1.6
Shigella spp	5	2.0
Staphylococcus aureus	10	4.0
Streptococcus faecalis	7	2.8
Proteus spp	3	1.2
Klebsiella pneumonia	4	1.6
Enterobacter spp	4	1.6
Pseudomonas spp	5	2.0
Total	95	38.0

N (Total number of children examined) = 250

Table 3 shows the prevalence of intestinal protozoan parasites and enteric bacteria by sex; prevalence of protozoa parasite in females was high (18%) compare to males (13.6%) but prevalence of enteric bacteria in males was high (20.4%) compare to females (17.6%) with no significant difference (P- value > 0.05).

Table 3: Prevalence of human intestinal protozoa parasite and enteric bacteria among children based on sex

Gender	No examined	No infected		Prevalence (%)	
		Protozoan parasite	Enteric bacteria	Protozoan parasite	Enteric bacteria
Male	119	34	51	13.6	20.4
Female	131	45	44	18.0	17.6
Total	250	79	95	31.6	38.0

P-value > 0.05 (0.314)

Table 4 shows the prevalence of intestinal protozoan parasites and enteric bacteria by age; prevalence of protozoa parasite in children below 10 years was marginally high (13.2%) compare to children in age group below 14 years and 19 years (10.8 and 7.6% respectively) but prevalence of enteric bacteria in children of age group below 9, 14 and 19 years was 13.6%, 16.4% and 8% respectively (P-value < 0.05).

Table 4: Prevalence of human intestinal protozoa parasite and enteric bacteria among children based on age

Age (yrs)	No examined	No infected and Prevalence	
		Protozoan parasite	Enteric bacteria
5-9	69	33(13.2)	34(13.6)
10-14	113	27(10.8)	41(16.4)
15-19	68	19(7.6)	20(8.0)
Total	250	79(31.6)	95(38.0)

P-value < 0.05 (0.001)

Table 5 shows the prevalence of infection based on the source of water used. The highest prevalence was recorded in those children that use tap water (19.6%), followed by the least infection rate on those that made use of wells (12%).

Table 5: Prevalence of human intestinal protozoan parasite among the children based on source of water

Source of water	No examined	No infected	Prevalence (%)
Well	97	30	12.0
Tap	153	49	19.6
Total	250	79	31.6

P-value < 0.05 (0.033)

Table 6 shows the prevalence of infection based on toilet facilities used by the children. The prevalence was significantly high in children who made use of bush (15.2%), followed by those students who made use of water closet (10.8%) and the least prevalence with pit latrine users (5.6%).

Table 6: Prevalence of human intestinal protozoan parasite among the children based on toilet facilities

Toilet facilities	No examined	No infected	Prevalence (%)
Bush	84	38	15.2
Pit latrine	47	14	5.6
Water closet	119	27	10.8
Total	250	79	31.6

P-value < 0.05 (0.003)

Discussion

This study found that the overall prevalence of human intestinal parasite infection was highest between the ages of 5 and 14 years which concur with Mukhiya *et al*., (2012) and Oguoma *et al.,* (2008) who respectively stated that this can be associated with their unhygienic habit and age. Higher prevalence rate in females compared with males agrees with Rai *et al.,* (2007) and Ngui *et al*., (2011) both opined that the association of gender with parasitic infection differs from one community to another and might be attributed to socio-behavioural activities and generally the increased mobility of the male increases the risk of infection among them, while female have more soil contact during growing vegetables and eat raw vegetable with prepared food more often than males.

The relatively higher prevalence of infection recorded among students that use water closet and bush compare to those that use pit latrines may be attributed to the poor quality hygiene of the toilet and unacceptably higher numbers of persons per toilet (overcrowding). This observation is in line with that of Marieke *et al.,* (2014).

Children whose source of drinking water is tap had higher prevalence of protozoan infection than the ones using well water, this infection might be due to poor handling of tap and tap knobs with faecal-contaminated hands which could be responsible for the higher prevalence of intestinal protozoan infections. This study is in contrast to the study conducted by Sah *et al*., (2013).

In this study, result shows that nine bacteria species (*Escherichia coli*, *Salmonella species*, *Shigella species*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterobacter spicies*, *Pseudomonas species* and *Proteus* species) were isolated from the children, and *Escherichia coli* was more prevalent than other bacteria. This agrees with the study conducted by Ifeanyi *et al.,* (2010).

Conclusion

Intestinal parasitic infections are intimately associated with poverty, poor personal hygiene, poor environmental sanitation and lack of clean water supply; intestinal protozoa infections in people will remain a public health threat for as long as these factors persists in the developing countries. Morbidity and mortality attributable to intestinal protozoa infections are on the increase with emergence and worldwide spread of the occurrence among the people, especially in children. Health education regarding hygienic practices in the school at primary levels can have substantial effect in prevention of intestinal protozoan parasites among the children. Proper monitoring from public health authorities could also be emphasized. Inhabitants of various areas should be regularly educated on the importance of reducing the spread of infections. The provision of basic amenities such as pipe borne water by the government would assist in reducing the occurrence of human intestinal parasites, and eradicating the diseases they cause. Practice of personal hygiene and good sanitation on daily basis as means of controlling or containing intestinal protozoa infections should be emphasized. With all these in place, these children will have a greater opportunity for a better future in terms of health and educational achievement.

Acknowledgments

The authors would like to thank the technologists in the Department of Microbiology of The Federal University of Technology Akure for their assistance during this study. Our gratitude and sincere appreciation goes to all staff and the student participants from the Schools in Shagari area who gave their stools for this study. We indeed appreciate all the parents who have allowed their children to participate. Thank you all for your co-operation.

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