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Giardia lamblia and *Entamoeba histolytica*: Parasitic Causes of Diarrhoea in Children Under - Five Years Old Attending Out – Patient Clinics in South-South Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IAA and OI designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author MIA managed the analyses of the study. Author ANO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to determine the prevalence of some intestinal protozoans among diarrhoeic and apparently healthy children under - five years old in Eket and Ibeno, Akwa Ibom State of Nigeria between October, 2013 and April, 2014. The study also established spatial distribution of *Entamoeba histolytica* and *Giardia lamblia* in the study areas.
Study Design: A total of 150 freshly - voided diarrhoeic samples of children attending Primary Health Care Centre, Eket and General Hospital, Ibeno, and 50 non–diarrhoeic samples were collected which served as controls.

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Methodology: Direct stool microscopy and concentration techniques were utilised to identify parasites. Questionnaires were also administered.
Results: *Giardia lamblia* had the highest prevalence of 11% in Eket while *Entamoeba histolytica* had the highest prevalence of 16% in Ibeno. The prevalence of parasitic pathogens decreased with increase in age of participants. However, there was no statistical significant difference between these parasites and age of the subjects across the study areas (P>0.05). There was no case of the parasites in exclusively breast-fed children whereas a high prevalence was recorded among those who were not breast-fed, Eket (91.1%) and Ibeno (95.0%). The prevalence of pathogens had the highest occurrence in those who used water from the stream in Eket (90.0%). The relationship between the water source and intestinal parasites across the study areas showed a statistical significant difference (P < 0.05).
Conclusion: In this study the incidence of parasitic pathogens in under-five children could be traced to contaminated drinking water sources and feeding patterns of the children. Thus, there is a clarion call for sustained health information, communication and education (IEC) of nursing mothers and caregivers in the proper care and handling of these under-fives to curb this menace of intestinal parasitic pathogens.

Keywords: Parasitic pathogens; intestinal protozoans; Entamoeba histolytica; Giardia lamblia; diarrhoea; under – five children; Nigeria.

1. INTRODUCTION

Gastrointestinal infections are alarming causes of morbidity and mortality in children exceeded only by respiratory infections and mortality. They are usually associated with cases that evolve without proper feeding or rehydration care of persistent diarrhoea that occur especially in children from low-income socio-economic groups [1]. Children, young adults and elderly patients are the most affected, particularly in regions with limited resources and where hygienic measures are not strictly followed. Nevertheless, some pathogens may cause infections in individuals of all ages. In Africa, diarrhoea has been estimated to be responsible for 25–75% of all childhood illnesses. Causes of diarrhoea in endemic areas include a wide variety of bacteria, viruses, and parasites. Intestinal parasites are associated with serious clinical diseases and mortality and are known to cause malnutrition and impairment of physical development in children [2]. It is necessary to have a fairly-accurate picture of the situation to target interventions in an affected area. Diarrheal disease remains one of the top two causes of young children mortality in the developing world. This precarious situation can be diminished by improvements in water/ sanitation infrastructure and hygiene [3].

Epidemiological surveys have shown that parasitic diarrhoea in children is primarily due to *Giardia lamblia* infection, particularly in areas where fresh vegetables and drinking water sources are contaminated with sewage materials, and foodstuffs are purchased from street vendors [4]. It has been estimated that about 200 million people are infected each year in Africa, Asia and Latin America [5]. In the industrialised countries, overall prevalence rate of giardiasis is 2-5%. However, in developing countries, *G. lamblia* infects children early in life thus a prevalence rate of 15-20% in children younger than 10 years is common. Particularly, children who are malnourished are more frequently infected [4].

In Nigeria, available reports indicate that more than 315,000 deaths of pre - school age children are recorded annually as a result of diarrhoeal diseases [6]. Nevertheless, despite the public health implications and the enormous burden imposed on the Primary Health Care Delivery System by infantile diarrhoeal illnesses in the country, there is still negligence on the epidemiology and aetiology of infantile diarrhoea in many communities, including Eket and Ibeno LGAs of Akwa Ibom State. The microbial quality of water in several rural Nigerian communities has been reported to be poor, unsafe and not acceptable for human consumption. Studies have shown that enteric pathogens thrive for long periods in water, despite large number of antagonistic populations. These pathogens are variously incriminated in cases of diarrhoea which in turn accounts for a substantial degree of morbidity and mortality in different age groups worldwide [7].

The specific problems associated with diarrhoea that prompted the study are enormous, confronting both the individuals concerned in particular and society in general. Diarrhoea has caused many problems ranging from morbidity,

mortality and antibiotic resistance in some cases. Nevertheless, despite the public health implications and enormous burden imposed on the Primary Health Care Delivery System by infantile diarrhoeal illness in Nigeria, there is still paucity of information on the aetiology and epidemiology of infantile diarrhoea in many communities including the South - South region of Nigeria. Studies have shown that the pathogens responsible for diarrhoea infections employ ingenious mechanisms to establish the disease therefore regional variation in the microbiological profile may exist even in the same country. Some remote communities of the study area depend on surface water for their domestic use. Therefore, there is tendency of infiltration of faecal waste matter into the water bodies which serve as source of drinking water to the community dwellers. Therefore there is need for proper scrutiny of the prevalence of intestinal pathogens in the community which are commonly incriminated in diarrhoeal cases, since little or no information has been documented so far in the study areas.

2. METHODOLOGY

This work was carried out on fresh diarrhoeic faecal samples of 150 children and non-diarrhoeic faecal samples of 50 children which served as control in Eket and Ibeno. The age range of the children recruited into this study was between 5 months and 26 months. The male to female ratio of the sample population was 2:3. Eket is an urban area while Ibeno is more of a rural area. The study design was a "cross sectional" study.

2.1 Sample Collection

The faecal samples not contaminated with urine were collected into clean, dry, transparent, leak proof, wide-necked universal bottles. Sterile spoons were used to scoop a teaspoon amount of specimen, transferred into the universal bottles and labeled with the participant's name, age and sex. Mothers of subjects selected for the study were given questionnaires to obtain information about their demographic data, all written in English and interpreted in their local dialect for those who are not educated.

2.2 Laboratory Analysis

All samples were transported in improvised iced pack within 3 hours of collection to Public Health Laboratory, University of Uyo, Uyo - Nigeria. The samples were observed macroscopically for colour, consistency and atypical components such as presence of blood, mucus or pus cells and presence of worms.

2.3 Identification of the Parasitic Pathogens

2.3.1 Microscopic examination

2.3.1.1 Wet or Direct Preparation

A drop of normal saline and iodine was placed respectively at each end of a clean grease free slide. Using an applicator, a small amount (that is, the size of matches head) of sample was picked and emulsified first in normal saline and in iodine. A smooth and thin preparation was made by removing all coarse particles. Each preparation was covered with coverslip respectively. The saline preparation was examined under the microscope, starting with x10 and then x40 for larvae, ciliate, eggs of helminthes, cysts, etc. while the iodine preparation was examined for cysts using x40 objective.

2.3.1.2 Concentration Method (Formol Acetone Method) [8]

Into a tube was added 1g of sample containing 10ml of 10% formol solution and shaken vigorously. The homogenized sample was filtered into a centrifuge tube and 5ml of acetone was added to the supernatant, covered and mixed vigorously for 1 min. The sample centrifuged immediately at approximately 2000rpm for 5 minutes. Then the faecal debris was removed and the tube inverted to discard acetone, faecal debris and formol solution.

3. RESULTS

The prevalence of intestinal parasites among children in Eket and Ibeno shows that out of 100 children examined in Eket, 74 (74%) were infected; of 50 children examined in Ibeno, 36 (72%) were infected, and in the apparently healthy subjects (Control), 15 (30%) were infected with these pathogens out of 50 examined (Table 1). There was a statistical significant difference amongst the subjects in the study areas (P>0.001). The relationship between intestinal parasitic pathogens and age of subjects is shown on Table 2. The findings showed that children in the age group 7–12 months in both

Eket and Ibeno had the highest prevalence of 90%, respectively. The prevalence of these pathogens decreased with increase in age of participants, except in older children (≥ 2 years). Among the apparently healthy subjects, similar age-related pattern of infection was observed. The relationship between age of subjects and intestinal parasites across the study areas showed no statistical significant difference (P > 0.05).

As shown on Table 3, the prevalence of these pathogens, (90.0%) was highest in those whose source of water was stream in Eket, whereas in Ibeno (where no one among the examined subjects used stream water), borehole water had the highest prevalence of parasites (82.5%). The relationship between water source and parasitic pathogens across the study areas showed a statistical significant difference (P<0.001)**.** There was no positive cases recorded among the exclusively breast–fed babies whereas a high prevalence was recorded among children who were not fed with breast milk (91.1%) and (95.0%) in Eket and Ibeno, respectively (Table 4). The findings showed a statistical significant difference between the feeding patterns and prevalence of intestinal parasitic pathogens in children across the study areas (P > 0.05).

The prevalence of *Giardia lamblia* was 11.0%, while *E. histolytica* was 9.0% in Eket. In the control, mixed infection had the highest prevalence of 8.0%, with *G. lamblia* being the most frequently encountered parasite. *Entamoeba histolytica* had the highest prevalence of parasites (16.0%), followed by *G. lamblia* (12.0%), while mixed infection had the least (2.0%). In the control, *G. lamblia* and mixed infection had the prevalence of 8.0% and 4.0%, respectively (Table 5). *Entamoeba histolytica* had the highest prevalence of parasites (16.0%), followed by *G. lamblia* (12.0%), while mixed infection had the least (2.0%). In the control, *G. lamblia* and mixed infection had the prevalence of 8.0% and 4.0%, respectively (Table 6).

4. DISCUSSION

In this study, the prevalence of intestinal parasitic pathogens in children attending health facilities in Eket and Ibeno was observed to be considerably higher than the frequency of pathogens in the control subjects. This finding was comparable to the finding made in Abakaliki [9] and Lagos [10], where intestinal pathogens were respectively detected in 81.3% and 73.7% of children with acute diarrhoea. Data obtained from the control samples has revealed that a child who may

Table 1. Prevalence of intestinal parasites among children in the study areas

Study area	No. examined	No. (%) positive	Chi square	P-value
Eket	Diarrhoeic participants (n = 100)	74(74.0)	30.01	> 0.001
	Apparently healthy subjects (Control) (n = 25)	9 (36.0)		
Ibeno	Diarrhoeic participants (n = 50)	36 (72.0)		
	Apparently healthy subjects (Control) (n=25)	6 (24.0)		
Total	200	125 (62.5)		

Table 2. Relationship between intestinal parasitic pathogens and age of participants

Age (Month)	Eket		Ibeno		Control	
	No. examined	No. +ve (%)	No. examined	No. +ve (%)	No. examined	No. +ve (%)
≤ 6	20	13(65.0)	10	6(60.0)	10	2(20.0)
7-12	20	18(90.0)	10	9(90.0)	10	4(40.0)
13-18	20	15(75.0)	10	8(80.0)	10	3(30.0)
19-24	20	11(55.0)	10	4(40.0)	10	1(10.0)
≥ 24	20	17(85.0)	10	9(90.0)	10	5(50.0)
Total	100	74(74.0)	50	36(72.0)	50	15(30.0)

Key: +ve – Positive; No. - Number

Table 3. Relationship between intestinal parasitic pathogens in children and source of drinking water

Source of water	Eket		Ibeno		Control	
	No. examined	No. +ve (%)	No. examined	No. +ve (%)	No. examined	No. +ve (%)
Pipe borne	10	4(40.0)	0	0	15	2(13.3)
Bore-hole	70	59(84.2)	40	33(82.5)	15	5(33.3)
Stream	10	9(90.0)	NA	NA	15	8(53.3)
Treated	10	2(20.0)	10	3(30.0)	5	0(0.0)
Total	100	74(74.0)	50	36(72.0)	50	15(30.0)

Key: 0 - 'Absence of infection'

Table 4. Relationship between intestinal parasitic pathogens and feeding patterns of children

Feeding pattern	Eket		Ibeno		Control	
	No. examined	No. +ve (%)	No. examined	No. +ve (%)	No. examined	No. +ve (%)
Exclusive	10	0	10	0	10	0
Mixed (Breast milk and other foods)	45	33(73.3)	20	17(85.0)	20	4(20.0)
Other foods without breast milk	45	41(91.1)	20	19(95.0)	20	11(55.0)
Total	100	74(74.0)	50	36(72.0)	50	15(30.0)

Table 5. Relationship between stool consistency and distribution of parasites in Eket

Stool consistency	No. examined	*G. lamblia*	*E. histolytica*	Mixed infection
		No. +ve (%)	No. +ve (%)	No. +ve (%)
Infected				
Loosed	41	4(9.8)	2(4.9)	1(2.4)
Loosed/bloody	24	0	5(20.8)	2(8.3)
Loosed/mucoid	28	7(25.0)	1(3.6)	1(3.6)
Loosed, bloody/mucoid	7	0	1(14.3)	0
Total	100	11(11.0)	9(9.0)	4(4.0)
Control				
Formed	13	0	0	0
Semi formed	12	0	0	2(16.7)
Total	25	0	0	2(8.0)

Table 6. Relationship between stool consistency and distribution of parasites in Ibeno

Stool consistency	No. examined	*G. lamblia*	*E. histolytica*	Mixed infection
		No. +ve (%)	No. +ve (%)	No. +ve (%)
Infected				
Loosed	11	1(9.1)	2(18.2)	0
Loosed/bloody	9	0	3(33.3)	0
Loosed/mucoid	23	5(21.7)	1(4.3)	1
Loosed, bloody/mucoid	7	0	2(28.6)	0
Total	50	6(12.0)	8(16.0)	1(2.0)
Control				
Formed	12	0	0	0
Semi formed	13	2	0	1
Total	25	2(8.0)		1(4.0)

appear apparently healthy may be an asymptomatic carrier harbouring a wide range of pathogenic microorganisms. Therefore, there should be a growing concern by the public for

regular medical check–ups of children who may be apparently healthy.

The prevalence of enteric parasitic pathogens such as *E. histolytica* and *Giardia lamblia* among the children found in the study is of concern and deserves careful consideration in the development of health policies in the region. This is even more important because of the lasting detrimental effects of enteric infections that occur during early childhood on later physical and cognitive development [11]. Other factors, such as sources of drinking water, could play decisive role in the occurrence of these parasites. The sources of drinking water used by participants were investigated in this study. The study showed that prevalence of intestinal pathogens decreased among participants who used pipe-borne water. Pipe-borne water usually undergoes chemical treatment to remove a number of infectious agents before being distributed to users, even though *Giardia* cysts are resistant to normal chloride concentrations used in water treatment. These precautions provide relatively good water quality and its consumption contributes to the reduction of infection by protozoa [3].

In the study areas, as in the majority of developing countries, sanitary/hygiene conditions are poor and could support propagation of *G. lamblia* through contamination of stream water by human faeces. In addition, animals such as rats bathe or drink in streams and then leave many *Giardia* cysts, making the water bodies highly contaminated, especially in the rainy seasons [6]. During the rainy season, water bodies are highly polluted with rain runoff, charged with both human and animal wastes from homes, hospitals, markets and industries. These waste matters contain organic substances charged with parasite cysts from animals and human droppings. Consumption of these polluted water bodies, in an area with high rainfall like Eket, would be the basis for the spread of parasitic infection in the population [9].

The highest prevalence of intestinal parasitic pathogens was found in children between 7–12 months in Eket, Ibeno and control with the prevalence rate of 90%, 90% and 40%, respectively. These findings are similar to reports made in Abakaliki [12], where the highest prevalence of gastroenteritis in children was found within the age range of 7-12 months, when weaning practices begin in many parts of the world, including Nigeria. During the weaning period, mothers supplement breast milk with milk formulas and other foods, exposing their children to infections which may be caused by eating meals that are not prepared in an hygienic environment. Also, during this period a child learns how to crawl and toddle, which exposes them to pick up and bite things from the dirty floor. These may be some of the reasons why this infection is most prevalent among children in this age group. The prevalence rate decreases with increase in age, but increases above 2 years which may be attributed to the period when infants have frequent contact with the natural environment [13].

The prevalence of intestinal parasitic pathogens in Eket (90%) and in control (53.3%) was highest in those whose source of drinking water was stream. A similar report was observed by Okeke [9] in Ebonyi where 84% of pathogens were isolated among children whose mothers depended on stream water for domestic use. Ibeno is a community surrounded by creeks, tributaries and an ocean, a coastal region which is not convenient for the existence of stream. Therefore, borehole water had the highest prevalence of 82.5%. The study also revealed that pipe-borne water in Ibeno had no pathogens, because its potability is absolute as it is being treated and provided by Exxon Mobil Unlimited.

In this research study, children who were exclusively breast-fed were not infected. Those with mixed feeding had the prevalence of 73.3%, 85.0% and 20.0% for Eket, Ibeno and Control, respectively, while those who were fed with other foods without breast feeding had a prevalence of 91.1%, 95.0% and 55.0%, respectively. The absence of pathogens in exclusively breast-fed children corroborates findings from previous studies regarding the protective role of colostrum contained in breast milk which contains a high level of Immunoglobulin A against bacterial gastroenteritis [14]. Faulty weaning practices, interruption of breast milk with milk formulas, cereals, etc. and poor hygiene during food preparation may contribute to increase gastroenteritis in mixed fed and no breast milk groups.

In Eket, *G. lamblia* had the highest prevalence of 11.0% and *E. histolytica* and mixed infection had the least, each having 9.0% and 4.0%, respectively. The data obtained for *G. lamblia* is comparable to previous data (12.8%) obtained in Ethiopia [10]. In Ibeno, the result showed that *E. histolytica* had the highest frequency (16.0%) while *G. lamblia* was least encountered (12.0%).

The finding of this study is comparable to a previous study done by Reuben et al. [15] in Lafia, with prevalence of *G. lamblia (*26.7%) and *E. histolytica* (18.8%), respectively. The result has shown that Ibeno had the highest prevalence of *E. histolytica*, compared to Eket with a low prevalence. This may be attributable to the swampy nature of soil in Ibeno which encourages easy contact of children with infective stage of the parasites. However, people who generally reside in rural or under-developed areas are more prone to the ingestion of infective parasites as compared to those who live in urban/suburban or well developed areas where sanitation is presumably better, hence possess a lower chance of infection. The water supply in developed areas is obviously safer, which reduces the chance of contamination. Also, in rural areas, the nature of everyday activities brings people, especially children, into close contact with natural sources of soil and water, therefore increasing their risk of ingestion [16].

The lower prevalence of parasites in Eket may be attributable to the fact that people who live in sub-urban areas where sanitation is presumably better possess a lower chance of infection. Whereas in Ibeno, where subsistence farming, fishing and animal husbandry are the major occupations of residents, most households have domestic animals such as dogs, sheep, goats, etc. which are often allowed to roam outdoors either unsupervised or in the company of children. Due to the lack of potable water on their farms, the farmers and their children drink from tributaries and creeks which are sometimes also used by these animals. These factors might have contributed to the high prevalence rate of parasitic infection in children within the study area.

The result of this study reveals negligence of hygienic measures by the mothers, family members and the infected children. Some subjects in this study area were school-aged children and thus they had very active playing habits in and out of school. These children normally played in the soil which harbours these parasites and are less mindful of some very important personal hygiene practices such as washing of hands with soap and water before eating, after playing in the soil and after visiting the toilets. They also buy food from street vendors, some of whom do not practice proper personal hygiene and may also be carriers of some of these infective pathogens [16].

All together, these pathogens can be transmitted through contaminated water or food, or through poor hygienic measures. Factors that might be associated with the transmission of these pathogens may include low socio-economic status, low level of education, use of untreated water from well or river, and a low level of personal hygiene. These factors reflect the living conditions, life style, and environmental conditions of the local population. The level of gastrointestinal disease associated with the faecal-oral route of transmission could be decreased significantly by implementing relatively simple strategies, such as better wastewater treatment and hygiene education. Thus, there is a great need to upgrade water-treatment procedures and sanitation standards which do not seem to be well-handled in the study area. Such strategies could alleviate a great deal of unnecessary sufferings and loss of productivity, reduce the number of lives lost due to these diseases, and this could result in significant savings in healthcare costs.

5. CONCLUSION

It is noted in this study that the incidence of parasitic pathogens in under-five children could be traced primarily to contaminated drinking water sources and feeding patterns of the children. This brings to focus the need for a systematic effort to inculcate in nursing mothers and caregivers the attitude and practice of good personal hygiene as the best approaches to the reduction of the scourge of intestinal pathogens.

CONSENT

Informed consent was obtained from the caregivers of the children and ethical clearance was obtained from the State Ministry of Health, Akwa Ibom State, Nigeria.

ETHICAL CONSIDERATION

Ethical approval for this study was received from the PHC, Eket and General Hospital, Ibeno Management, and the parents'/caregivers of the subjects selected for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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