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Research Article

Comparative Prevalence of Enteric Parasitic Infections among Subclinical Breastfeeding/Non-Breastfeeding Mothers and their Infants in Calabar, Nigeria

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ABSTRACT

Enteric parasitic infections (EPIs) affect breastfeeding mothers world-wide and the largely asymptomatic nature makes it under-recognized. This study compared the prevalence of EPIs amongst subclinical breastfeeding/non-breastfeeding mothers and their infants. A health center based cross-sectional study was conducted among 300 participants (75 breastfeeding mothers with 75 of their breastfeeding infants and 75 non-breastfeeding mothers with 75 of their non-breastfeeding infants) in Calabar. Fresh faecal samples were obtained from the subjects and observed under the microscope using direct wet mount and formal-ether faecal concentration technique. Data analysis was done using chi-square test at $p<0.05$ significant level. An aggregate of 12.3% of the participants were infected with at least one enteric pathogen. The most prevalent were *Ascaris lumbricoides* (12.0%), *Entamoeba histolytica/dispar* (7.0%), Hookworm (5.0%) and *Trichuris trichiura* (2.3%). The frequency of enteric parasite was 10.7% among breastfeeding mothers, 0.0% among their infants, 16.0% among non-breastfeeding mothers and 22.7% among their infants. Interestingly, there was no statistically significant difference in the prevalence amongst breastfeeding and non-breastfeeding mothers ($X2 = 0.9231$, $p = 0.3367$). The incidence of EPIs was significant in both breastfeeding mothers and control non-breastfeeding mothers. The outcome from this study suggests that breastmilk provides immunity on infants being breastfed against EPIs and also reveal the need for further investigation on the quality of water sources available to these subjects' trials.

Keywords: *Prevalence, Enteric parasitic infections, Mothers, Infants, Breastfeeding, Subclinical*

INTRODUCTION

Enteric parasitic infection (EPIs) also known as intestinal parasitic infection (IPIs) is a neglected tropical disease (NTD) that thrives and affects mostly the poorest population. EPIs can be essentially classified into two categories, that is, protozoan and helminthic infections.

Globally, more than 2 billion individuals are affected with soil-transmitted helminths (STHs) such as *Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Trichuris trichiura* and *Ascaris lumbricoides* (Brooker *et al.*, 2006). These parasites produce different clinical symptoms, ranging from asymptomatic carriage to diarrhea, general

malaise, weakness and abdominal pain, which could influence learning abilities and physical growth (Ahmed *et al*., 2012; Hotez, 2013; Papier *et al.*, 2014; Hadiza *et al.*, 2019). The mode of transmission of the disease is through the consumption of contaminated water, uncooked animal products and fecal routes (Bhunia 2018; Bharti *et al*., 2018). The ailment is characterized by complications such as intestinal obstruction, malnutrition and growth retardation. These pathogens could influence intestinal bleeding, food intake, fat absorption and compete for nutrients, resulting to malabsorption of nutrients (Robertson *et al.*, 1992, Stephenson *et al.*, 2000). These impacts could result to anaemia, nutrient deficiencies, and protein energy malnutrition (Robertson *et al.*, 1992; Stephenson *et al.*, 2000; Chifunda and Kelly, 2018; Njoku *et al.*, 2018).

Approximately 1.5 billion humans have been infected globally with EPIs, mostly *T. trichiura*, hookworms and *A. lumbricoides* (Farrell *et al.*, 2018; WHO, 2020). EPIs were estimated to have affected approximately 3.5 billion individuals globally, predominantly among the low socio-economic groups and resulted in morbidity in about 450 million people (WHO, 2000, Saki *et al*., 2017, Feleke *et al.*, 2019). The global long term goal was to eliminate EPIs related mortality and morbidity among preschool and school age children by 2020 (WHO, 2016). Unfortunately, the goal is yet to be achieved even in year 2021.

EPIs cause life-threatening conditions with the greater proportion in Sub-Saharan Africa. In most parts of this region, the prevalence of EPIs surpasses 50% (WHO, 2020). The highest-burden for both helminthes and other EPIs is predominant in Sub-Saharan Africa (Dejon-Agob *et al.*, 2018). The most affected are developing countries, with the majority of affected people being school-age children due to their hand to mouth activity, uncontrolled fecal activity and their immature immune systems. Breastfeeding mother could harbor some quantity of enteric pathogens in her gut or organ where the parasite usually lives. It has been documented generally, that breastfeeding reduces infant mortality and defends infants from diarrheal disease (Arifeen *et al.*, 2001)

Among the sub-Saharan Africa countries, Nigeria has the utmost number of individuals infected with soil-transmitted helminths (STHs) (Hotez and Kamath, 2009; Hotez *et al*., 2012; Federal Ministry of Health, 2013). Studies conducted in several parts of Nigeria on the prevalence of EPIs among children employed microscopy-based techniques. These studies revealed high prevalence of EPIs. For instance, Ajayi *et al*. (2017) reported 78.1% and 17.1% among children in public and private schools. Hadiza *et al*. (2019) recorded a prevalence of 9.2%, Salawu *et al*. (2015) found a prevalence of 59.2%, while Odinaka *et al*. (2015) recorded 30.3% prevalence. Despite control measures against parasitic infections, they still pose a challenge among women and children worldwide (Kliegman *et al*., 2011). Hence, this research was aimed at determining and comparing the prevalence of enteric parasitic infection among mothers and their infants in relation to breastfeeding. This study is expected to add to existing knowledge and make useful suggestions towards better management and control of this problem.

MATERIALS AND METHODS

Study design and location

A health Centre based cross-sectional study was carried out among breastfeeding mothers with their infants attending routine postnatal care at the Primary Health Centers in Calabar, Cross River State, Nigeria. Calabar is the capital of Cross River State, Nigeria. The city of Calabar is divided into Calabar Municipal and Calabar South Local Government Areas. It has an area of 406 square kilometers and population of 371,022 people (Ottong *et al*., 2010) and the main occupation of the residents is agriculture.

The study location was stratified into four zones, Ekpo Abasi health center, Esiereobom health center, Primary health center Ewa Henshaw and Primary health center Atu. All the centres are within Calabar, Cross River State, Nigeria.

Ethical consideration

The study was approved by the Ethical Review Committee of Cross River State Ministry of Health, Calabar, Nigeria in accordance with the guidelines provided by the World Medical Association declaration of Helsinki on ethical principles for medical research involving humans. Verbal assent and informed consent were obtained from all participants. In addition, a verbal enlightenment about the study was given to the participants before performing the study-related procedures. Confidentiality of study subjects was assured and kept by desisting from mentioning participant's names, initial or hospital numbers.

Sample size determination

A single population proportion formula was used to calculate the sample size with the prevalence of enteric parasitic infection (27.66%) obtained from a previous study in River State (Abah and Arene, 2015), at 95% confidence level and 5% precision. This brought a total sample size of 300 participants used in this study.

Sampling techniques

The subjects (breastfeeding mothers plus infants and non-breastfeeding mothers plus infants) were carefully chosen using systematic sampling techniques with a sampling

interval of 5. The first subject was carefully chosen by a simple random sampling method. Successive subjects were carefully chosen at a regular interval of the 5th subjects until the required sample size was achieved. Seventy-five subjects were selected from each of the four health centres. All subjects were given a unique code to be recognized during study period.

Eligibility criteria and data collection

Consented participants in this study were verbally asked about their breastfeeding status which was used to determine the breastfeeding mothers. Participants were verbally asked if there had taken any anthelminthic drugs and those that responded that they had any form of anthelminthic therapy within the past twenty-one days were excluded from the research.

Sample collection and preservation

Pre-labeled clean screw capped plastic stool containers with wide neck and unique identification numbers were provided to the participants to put the fecal samples. About 5g to 10g of freshly passed single stool samples were obtained using a sterile wide-mouthed container with tightly fitted lid. All samples were immediately taken to the laboratory and were preserved in 10% normal saline until use.

Samples analysis

Physical assessment: The visual assessment of collected stool samples was carried out for mucus, color, smell, presence of blood, pus and consistency (watery, soft and formed)

Direct wet mount technique: Approximately half of the 2 g of stool samples of each participant was emulsified in 0.5 ml of normal saline. A drop was placed on each of two glass slides. A drop of Lugol's iodine solution was added to the wet preparation on one slide. Coverslips were applied and the slides examined microscopically at 10X and 40X objective lenses, respectively for parasites identification.

Formalin ether sedimentation method: approximately 0.5g of fecal sample was transferred to 10 ml of formalin in a test tube. For adequate fixation, the sample in the tube was mixed and allowed for 30 minutes, sieved via two layers of gauze in a funnel and into centrifuge tube. Then 3ml to 4mL of diethyl ether was added to the tube, closed with a glass stopper and shaken for 15 minutes. The tube was centrifuged at 5000 rpm for 5 minutes. The supernatant was poured out, leaving the sediment at the bottom of the tube. The sediment was mixed and one transferred onto a clean grease-free slide, covered with coverslip and examined for larva, cysts, eggs and Oocysts of parasites using 10X and 40X objectives.

Table 1. Prevalence of Intestinal Parasites among Breastfeeding, Non-breastfeeding Mothers and Their Corresponding Infants

Subject	No. Examined	No. (%) with Infections
Breastfeeding Mothers	75	8(10.7)
Breastfed infants	75	0(0.0)
Non-breastfeeding Mothers	75	12(16.0)
Non-breastfed infants	75	17(22.7)
Total	**300**	**37(12.3)**

Statistical analysis

All statistical analyses were done using simple descriptive analysis and SPSS software program (IBM SPSS v.25 Inc., Chicago Il, USA). Compiled data was analyzed using Chi square. Results were presented in frequency and percentage. P-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Results

The prevalence of EPIs among breastfeeding and non-breastfeeding mothers and their corresponding infants (Table 1) is 37 out of 300 (12.3%). The prevalence of enteric parasitic infection among non-breastfeeding infants (22.7%) was greater than those of breastfeeding infants (0.0%).

Moreover, the prevalence of intestinal parasitic infection among breastfeeding mothers (10.7%) was lower than that of non-breastfeeding mothers (16.0%). The prevalence of EPIs amongst non-breastfeeding mothers and their infants was 12 (16.0%) and 17(22.7%) respectively ($x2 = 1.0687$, $p = 0.3012$).

There was statistically significant higher parasites prevalence in non-breastfed infants 17 (22.7%) than in breastfed infants 0(0.0%) ($X2 = 19.1729$, $p = 0.000$). That implies an association between infant's parasite status and breastfeeding. The most prevalent (Table 2) parasite recovered from the stool samples in order of their prevalence are *A. lumbricoides* (12.0%), *E. histolytica/dispar* (7.0%), Hookworm (5.0%) and *T. trichuria* (2.3%).

Discussion

Subclinical parasitic infections are widespread in adults but many pathogens are not transmitted through breastmilk to the breastfeeding infant. Findings on the geospatial distribution of *T. trichiura, E. histolytica/dispar, Ascaris* and Hookworm infections are uncommon, but emerging in Nigeria (Oluwale *et al*., 2015; Oluwale *et al.,* 2018; Yaro *et al.,* 2018). The findings in this study add to the existing epidemiological data on the prevalence of EPIs in Cross River State, Nigeria. The prevalence of EPIs among breast-

feeding mothers was higher compared to their infants and was lower among non-breastfeeding mothers compared to their infants. The non-breastfeeding mothers had a higher prevalence of enteric parasites than breastfeeding mothers. However, breastfed infants had no case of enteric parasitic infection while the non-breastfed infants had the highest case of the infection. This may be linked to the report of Katona and Katona-Apte (2008), which reveals that immune capability of breastmilk might confer significant defense and that malnutrition that results due to non-breastfeeding encourages parasitic infections to occur. The result of this study consented with the finding of Leda and Luis (2000), where breast-feeding mothers had substantially higher parasite prevalence of 37.3% against 0.0% of their breast-fed infants. However, these results were lower compared with that of Haileeyesus *et al.* (2006) who reported 44.4% among breastfed infants as against 22.7% among non-breastfed infants. This could be attributed to the differences in living conditions. The prevalence of enteric parasites among non-breastfeeding children was high with no prevalence among breastfeeding children in this study. Exclusive breastfeeding plays a substantial role in protecting against common infectious agents during infancy (Quigley *et al*., 2007). The study revealed a prevalence of EPIs with 12.3% of all respondents being infected with at least one species of the parasites. The result recorded in this study area is in line with the finding of Kumurya *et al.* (2020) in Nigeria who reported 14.3% of intestinal parasitic infection. The most frequently detected pathogenic parasites were *T. trichiura, E. histolytica/dispar, A. lumbricoides* and Hookworm with the frequency of detection comparable to that recorded by Kumurya *et al.* (2020). Also, the result of this study is in line with the findings of 17.2%, 13.8% and greater than 20% prevalence reported by Mogaji *et al*. (2020), Oluwole *et al.* (2015) and Yaro *et al*. (2018), respectively. The result observed in this study is lower than the 48.7% (Yoseph and Beyene 2020), 25.4% (Staudacher *et al*., 2014) and 24.1% (Tchuem-Tchuente *et al*., 2012) reported in Southern Ethiopia, Rwanda and Cameroon, respectively. The two leading pathogens found in this study are *A. lumbricoides*

Table 2. Distribution of Intestinal Parasites among Breastfeeding/Non-breastfeeding Mothers and their Corresponding Infants

Parasites	Number of participants with parasite species infection			
	Breastfeeding Mothers (%)	Breastfed Infants (%)	Non-breastfeeding Mothers (%)	Non-breastfed Infants (%)
Ascaris lumbricoides	4(50.0)	0(0.0)	6(50.0)	10(58.8)
Entamoeba histolytica/dispar	2(25.0)	0(0.0)	3(25.0)	5(29.4)
Hookworm	1(12.5)	0(0.0)	2(16.7)	2(11.8)
Trichuris trichiura	1(12.5)	0(0.0)	1(8.3)	0(0.0)
Total	8((100)	0(0.0)	12(100)	17(100)

(12.0%) and *Entamoeba histolytica/dispar* (7.0%). The result was below the value of 52.4–65.8% recorded from previous studies in sub-Saharan African countries (Center for Infectious Diseases Control Rwanda 2008, Tadege and Shimelis, 2017). The disparities could be attributable to difference in hygiene and environmental contamination (Lee *et al*., 2010) as well as changes in rainfall, temperature, soil moisture and humidity (Steinmann *et al*., 2007; Cundill *et al.,* 2011). The EPIs reported in this study have a comparable prevalence values with the results of Oluwole *et al*. (2018), Mogaji *et al.* (2020), and Gemechu *et al.* (2020). This could be as a result of similar factors such as pH, soil moisture or temperature which favors the transmission of EPIs. The result recorded in this study is in contrast with the prevalence of more than 50% recorded by Oluwole *et al.* (2018). However, finding was found to be within the range of 9.4%–28.6% documented among adults in Nigeria (Egwunyenga *et al*., 2001; Baasey and Asor, 2009; Wokem and Onosakpondme 2014). In sub-Saharan African countries including Nigeria, reports of EPIs in adults indicate that the burden of the disease is not limited to children (Cham *et al.,* 2015; Siza *et al.,* 2015; Masaku *et al*., 2017).

This present study further buttresses the need for Nigerians to adhere strictly to the WHO recommended policy for the control of STH infections at community levels. The policy involves prevalence-based targeted distribution of mebendazole and albendazole in both school and preschool-aged children and women of childbearing age (WHO, 2001). The species of parasites reported in this study are comparable to those recorded in other sub-Saharan African countries (Tchuem-Tchuente *et al*., 2003), Ethiopia (Tadege and Shimelis, 2017) and Kenya (Kepha *et al.*, 2015).

This result reveals that EPIs is still endemic in the study area signifying that efforts are necessary to achieve the WHO's goal of elimination in sub-Saharan Africa. The high prevalence of *A. lumbricoides* reported could be attributed to high environmental contamination (Brooker *et al.,* 2006), viability of *Ascaris* eggs under changing environmental conditions (O'Lorcain and Holland, 2000) and the humid nature of the shell of *Ascaris* egg which helps its attachment on human hands, fruits and vegetables (Quilès *et al*., 2006).

In a study conducted in Addis Ababa, Ethiopia, it was revealed that there was a tremendously high prevalence (71.8%) of enteric parasitic infection with major infection cause by *A. lumbricoides* (34.9%), *T. trichiuria* (22.8%) and *G. lamblia* (9.6%) (Mekonnen *et al.*, 2014). The 12.0% prevalence reported for *Ascaris lumbricoides* in this study is higher than 4.9% and 0.8% recorded by Al-lahham *et al.* (1990), and Majed *et al*. (2007), respectively. The low prevalence of *Trichuris trichiura* reported in this study is similar to 1.1% by Al-lahham *et al*. (1990) and 1.6% recorded by Kumurya *et al*. (2020).

CONCLUSION

The overall result of this study provides epidemiological data on the prevalence of *A. lumbricoides, E. histolytica/dispar*, Hookworm and *T. trichiura* infections which will enhance the baseline data in providing suitable control strategies. The result shows that enteric parasites are more prevalent among non-breastfeeding mothers and their infants. But whether lactation and/or breastfeeding reduce risk of maternal parasitic infections is uncertain and needs further investigation. Proper sanitary practice and exclusive breastfeeding as already being advocated should therefore be encouraged.

Limitation and strength

The limitation in this study is that a single stool sample by individual was analyzed, though the sensitivity of microscopy technique is better enhanced by analyzing several samples from a single or multiple faecal samples for parasite detection. In spite of this limitation, valuable results are provided in this study which could enhance the baseline data in providing suitable control strategies for future investigations.

AUTHORS' CONTRIBUTIONS

Author IBO got the concept of the study. Authors BEI, IBO, SAB and SIRO participated in the study design and interpretation of the results obtained. Authors ELO and SAB conducted the Laboratory work. Authors IBO, BEI, SAB and ELO drafted the manuscript and participated in data analysis. Authors BEI, SIRO, ELO and IBO critically reviewed and revised the manuscript for important intellectual content. All authors read through the final version of the manuscript and gave consent for its publication.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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