

Prevalence of Diarrhoea Causing Microorganisms among Children that are Exclusively Breastfed and those on Weaning Foods.

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ABSTRACT

Diarrhoea is an intestinal disorder characterized by abnormal fluidity and frequency of fecal evacuations. Weaning is a gradual process, extending from the time when baby is solely breast fed, until he is feeding entirely on the adult diet. The period of weaning is a major factor to favor the occurrence of diarrhoea. Hydration therapy, severe malnutrition, frequent vomiting, low socioeconomic status, and the presence of associated major infections are recognized risk factors for the development of dehydration in children with diarrhea. To determine the prevalence of diarrhoea causing microorganisms among children that are exclusively breastfed and those on weaning foods. Fresh stool specimens were collected from each patient in sterile wide-mouth plastic universal containers labeled with the name, sex and age of the patient. About 10 ml of stool was obtained from nylon diapers of each child with sterile plastic spoons, placed in sterile plastic universal specimen containers and were taken to the laboratory for microbiological studies, which included culture, isolation of bacteria and microscopy for detection of parasites. This study has shown that infants who are exclusively breastfed from birth to six months of age have a significantly lower prevalence of diarrhoea than those infants who are not exclusively breastfed. The present study has provided insight into the burden of infantile diarrhoea in Nigeria. As a public health measure to reduce the disease burden, an integrated package of immunization services and other childcare programs need to be implemented in addition to well-focused health-education messages to improve treatment-seeking behavior for childhood diarrhoea as well as improved personal and environmental hygiene.

(Keywords: breast-feeding, weaning, diarrhea, stool, infant health)

INTRODUCTION

Diarrhoea is an intestinal disorder characterized by abnormal fluidity and frequency of fecal evacuations. Generally, the result of increased motility in the colon may be an important symptom of such underlying disorders as dysenteric diseases, lactose intolerance and inflammatory bowel disease [1]. Diarrhoea is the passage of watery stools, usually at least three times in a 24 hr. period. However, it is the consistency of the stools rather than the number that is most important. Frequent passing of formed stools is not diarrhoea. Babies fed only breast milk often pass loose, "pasty" stools; this also is not diarrhoea [2].

The World Health Organization defined diarrhoea as the voiding of more than two unformed watery stools in any 24 hour period, or any voiding of watery stools if accompanied by fever, abdominal pain and/or vomiting [3]. Diarrhoeal illness stands, as an important cause of infectious morbidity in children, exceeded only by respiratory tract infections and mortality is currently associated with cases that evolve without proper feeding or rehydration care, invasive diarrhoeas with extra intestinal or systemic involvement, or persistent diarrhoeas that occur especially in infants from low-level socioeconomic groups, who suffer previous deficiencies and develop severe nutritional consequences of enteric infection [2].

Diarrhoeal diseases are among the most common causes of global childhood morbidity and mortality, especially in the developing countries. It is estimated that infectious diseases are responsible for the majority of 5 million deaths annually in children under 5 years of age in Pakistan with diarrhoea and acute respiratory infections standing out as major killers [4].

Lack of breast-feeding, inappropriate rehydration therapy, severe malnutrition, frequent vomiting, low socioeconomic status, and the presence of associated major infections are recognized risk factors for the development of dehydration in children with diarrhoea.

On the other hand, the role of specific microorganisms in the etiology of dehydrating diarrhoea has been systematically assessed in very few studies, all of which have been conducted in cholera-endemic countries of the Indian subcontinent. WHO has accordingly underlined the need for epidemiological surveys of infantile diarrhoea in all geographical area [3].

Many factors, including socio-demographic, environmental and housing characteristics, affect the prevalence of diarrhoea [5]. In Egypt, the widespread use of oral rehydration therapy has successfully lessened the severity of diarrhoeal episodes and sharply reduced the number of subsequent deaths, but the incidence of diarrhoeal diseases has not declined. This indicates that the causative agents of diarrhoea and their environmental sources are still within [6].

Weaning, which is often referred to as "mixed feeding", proceeds in stages from liquids to solids, and from one method of feeding to another. Weaning is a gradual process, extending from the time when baby is solely breast fed, until he is feeding entirely on the adult diet. The period of weaning is a major factor to favor the occurrence of diarrhoea, while many other risk factors should also be considered, such as low socioeconomic level, poor hygiene habits, unsavory dwelling, elevated environmental exposure to enteropathogens and poor nutritional status [7]. The World Health Organization recommends exclusive breastfeeding until the baby is six months old. After six months, breastmilk alone does not provide the baby with enough nutrients, in particular iron, so other foods are needed.

Breastfeeding and weaning of an infant are not only crucial for optimal growth and development but also are important determinants of future physical and mental wellbeing because of the rapid growth spurt and development of organs and tissues during the first year of life. The importance of breastfeeding has been emphasized in various studies, so also the imperative role of exclusive breastfeeding to an

infant and the immunological and nutritional values of breast milk [8]. These practices play an important role in reducing child mortality and morbidity; however it may vary among different regions and communities.

METHODOLOGY

Study Area

The research work was conducted in Abeokuta metropolis, Ogun-State, Nigeria. Abeokuta is a city in Ogun State in southwest Nigeria and is situated along the Ogun River; 64 miles north of Lagos by railway, or 81 miles by water. As of 2010, the Abeokuta proper had an estimated population of about 230,000, while the figure for the city and outlying environs was approximately 500,000 individuals. The hospitals selected for the study were Sacred Heart Hospital, Lantoro; Federal Medical Centre, Idiaba; Ogun State Hospital, Ijaye. Traditional herbal centers in Abeokuta were also used as points of sample collection.

Sample Size and Selection

The sample size was determined using the sample size expression:

$$n = \frac{NZ^2 P (1 - P)}{d^2 (N - 1) + Z^2 P (1 - P)}$$

Where N= Total population.

Z= The standard normal deviate, usually set at 1.96 which corresponds to the 95% Confidence Level.

d= absolute precision, which was set at 0.05.

P= expected proportion in the population.

The total population of people in Abeokuta, Ogun state was estimated to be 593,140 (Dimeji 2010) and 95% confidence interval with an absolute precision of 0.05 was used. Therefore, the calculated sample for the study period was 250.

Ethical Approval

Approval for the study was obtained from the Research Ethics Committees of Federal Medical Centre, Idi-Aba; Sacred Heart Hospital, Lantoro and Ogun State Hospital, Ijaye, Abeokuta. The approval was on the agreement that patients' anonymity must be maintained, good laboratory

practice/quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. Informed consent form was given and obtained from parents and/or care givers of the children.

Sample Collection and Analysis

Fresh stool specimens were collected from each patient in sterile wide-mouth plastic universal containers labeled with the name, sex and age of the patient. About 10 ml of stool was obtained from nylon diapers of each child with sterile plastic spoons, placed in sterile plastic universal specimen containers and were taken to the laboratory for microbiological studies, which included culture, isolation of bacteria and microscopy for detection of parasites.

Laboratory Analysis

The fecal samples were examined macroscopically for consistency, color, presence of worm or segments of tapeworm and for typical components such as mucus and blood.

The fecal samples were also examined microscopically for the presence of leucocytes, red blood cells, parasitic ova and cysts using saline preparation and formalin-ether method of concentration. Each stool specimen was cultured on MacConkey Agar (MCA), Salmonella-Shigella Agar (SS) and Selenite F broth. Cultures on MacConkey Agar and Salmonella-Shigella Agar plates was obtained by using a sterile platinum wire loop to pick a loopful of the stool in each sterile plastic universal specimen containers and streaked on the appropriate media plate.

Plates were incubated at 37°C for 24 hours. Cultured plates were examined after 24hrs for lactose-fermenting colonies on MacConkey agar and non-lactose fermenting colonies on SS agar. On MacConkey agar, deep red colonies were produced if the organism is lactose-positive, and fermentation of this sugar caused the pH of the medium to drop, leading to darkening of the medium. On Salmonella-Shigella Agar, both *Salmonella* and *Shigella* species produced colorless colonies because they are unable to ferment lactose.

Bacterial Transformation:

1. Competent cells (200ul) were pipetted into each of 3 ice cold Eppendorf tubes. The tubes was labeled as Control, 1 ng, and 10 ng (1 ng is 10^{-3} ug, or 10^{-9} milligrams). The unknown plasmid was at a concentration of 1 ng/ul. One ng and the unknown plasmid were added to one tube and 10 ng to the other. The tubes were placed on ice for 30 min.
2. The tubes were put at 42°C for exactly 90 seconds and the cells were return to ice for 1-2 minutes thereafter.
3. The transformation mixture was pipette onto labeled plates containing ampicillin and spread around using a sterilized, bent glass rod spreader.
4. It was placed upside down in the 37°C incubator overnight.
5. After 20 hours, the number of colonies on the plate with well-isolated colonies was counted. Parafilm was put around the edge of a plate and put it in a refrigerator for later use. The control plate was checked to see that no colonies grew on it. The plate and the control plate were disposed in the biohazard bag.

RESULTS AND DISCUSSIONS

Table 1 shows socio-demographic characteristics of the children and their mothers. Out of the 250 children studied, 146 (58.4%) were male while 104 (41.6%) were female with a significance difference of 0.0002. Majority (118; 47.2 %) of the children are within the age group 1-12 months, this was followed by 13-24 months (75, 30%), 25-36months (36;14.4%) and neonates age (21;8.4%) with a significant difference of 0.0002. Results presented in Table 1 also show 235 (94%) of the patients were Yoruba while other tribes were 15 (6%). Of the sampled population, 182(72.8%) of the nursing mothers were traders and artisans, 45 (18%) were civil servants and 23(9.2%) were housewives. Fifty- nine (23.6%) mothers practice exclusive breast feeding while 127 (50.8%) practice partial breastfeeding and 64 (25.6%) were not breastfeeding and has a significant difference of $p < 0.05$.

Table 1: Socio-Demographic Characteristics of Children and Nursing Mothers.

Category	Frequency	Percentage (%)
Gender of children		
Male	146	58.4
Female	106	41.6
Total	250	100
P=0.000172		
Age months		
< a month	21	8.4
1-12 months	118	47.2
13-24 months	75	30.0
25-36	36	14.4
Total	250	100
P=0.0001		
Ethnic group of mothers		
Yoruba	235	94.0
Other tribes	15	6.0
Total	250	100
P=0.0000		
Occupation of mothers		
Traders/Artisan	182	72.8
Civil servants	45	18.0
Unemployed/housewife	23	9.2
Total	250	100
Weaning practices		
Exclusive breastfeeding	59	23.6
Partial breastfeeding	127	50.8
No breastfeeding	64	25.6
Total	250	100
P=0.0000		
Type of weaning foods		
Solid	136	71.2
liquid	43	22.5
solid+ liquid	12	6.3
Total	191	100
P=0.0001		

Results of the type of weaning food given to the babies showed that 43 (22.5%) were on liquid foods such as tea, milk, Pap, etc, 136 (71.2%) were on both solid and liquid foods while 12 (6.3%) were on solid foods alone.

Table 2 shows the sex distribution of children in relation to their age. Within the neonate (<1 month) age group, which has a total of 21(8.4%) of the population, 10(47.6%) were male while 11(52.4%) were female. Out of the 21 children within the neonate age group 10 (47.6%) were male while 11(52.4%) were female. Age group

13-24 months had 75 (30%) of the population in which 43 (57.3%) were male and 32 (42.7%) were female. The 25-36 months age group had 36 (14.4%) of the entire population. Within this age group, 21(58.3%) were male while 15 (41.7%) were female. There is no significant difference in the sex distribution as $P>0.05$.

Table 3 shows the household characteristics of the sampled children. Within the Studied population, 175 (70%) were given portable water (borehole, sachet, bottled and tap water) while 75 (30%) were given well water.

Table 2: Sex-Age Distribution of Children.

Age group	Male (%)	Female (%)	Total (%)
< a month	10(47.6)	11(52.4)	21(8.4)
1-12 months	72(61.0)	46(39.0)	118(47.2)
13-24 months	43(57.3)	32(42.7)	75(30)
25-36months	21(58.3)	15(41.7)	36(14.4)
P>0.05(P=0.712)	146(58.4)	104 (41.6)	250(100)

Table 3: Household Characteristics of Respondent Mothers.

Characteristics	Frequency	Percentage
Water source		
Portable water	175	70
Well	75	30
Total	250	100
P=0.0000		
Refuse disposal method		
Public refuse dump	169	67.6
Bush	81	32.4
Total	250	100
P=0.0000		
Toilet Type		
Pit	105	42.0
Water system	139	55.6
Bush/River	6	2.4
Total	250	100
P=0.0000		

In disposing refuse, 169 (67.6%) of the population uses public refuse dump, 81 (32.4%) uses bushes or disposes anywhere on the street. Water system type of toilet is used by 139 (55.6%) of the population, 105 (42%) uses pit latrine and 6 (2.4%) uses bushes or river for their toilet systems.

Table 4 shows the age and sex distribution of children in relation to their weaning practices. This shows that within the neonate (< a month) age group, 12 (57.1%), were breastfed exclusively, 6 (28.6%) had partial breastfeeding while 3(14.3%) do not breastfeed at all. Within the 1-12 months age group, 36 (30.5%) practices exclusive breastfeeding, 67 (56.8%) practices partial breastfeeding and 15 (12.7%) do not

breastfeed at all. Within the 13-24 months age group, none was going through exclusive breastfeeding, 38 (50.7%) practices partial breastfeeding while 37 (49.3%) was not breastfeeding. Within the 25-36 month age group, none was practicing exclusive breastfeeding, 6 (16.7%) practices partial breastfeeding while 30 (83.3%) was not breastfeeding. There is no significant difference within the age group in correlation to their weaning practices at P>0.05. (P=0.573). The proportion of male children that practices exclusive, partial and none-breastfeeding were 36 (24.7%), 73 (50.0%), 37 (25.3%), respectively, while the proportion of female children that practices exclusive, partial and none-breastfeeding were 23 (22.1%), 54 (51.9%), 27 (26.0), respectively.

Table 4: Age and Sex Distribution of Children in Relation to their Weaning Practices.

Age group	Breastfeeding Practices			
	Exclusive Breast-Feeding (%)	Partial Breast-Feeding (%)	Non Breast-Feeding (%)	Total (%)
< a month	12 (57.1)	6(28.6)	3(14.3)	21(100)
1-12 months	36(30.5)	67(56.8)	15(12.5)	118(100)
13-24 months	0(0)	38(50.7)	47(49.3)	75(100)
25-36months	0(0)	6(16.7)	30(83.3)	36(100)
Total	59(23.6)	127(50.8)	64(25.6)	250(100)
Sex	Exclusive Breast-Feeding (% within sex)	Partial Breast-Feeding (% within sex)	Non Breast-Feeding (% within sex)	Total (%)
Male	36(24.7)	73(50.0)	37(25.3)	146(100)
Female	23(22.1)	54(51.9)	27(26.0)	104(100)
Total	59(23.6)	127(50.8)	64(25.6)	250(100)

P> 0.05(P=0.896 for sex, P=0.573 for age).

Table 5: Age-Related Prevalence of Enteropathogens among Children with Diarrhoea in Abeokuta

ENTEROPATHOGENS	AGE IN MONTHS				
	<1(21)	1-12(118)	13-24(72)	25-36(36)	TOTAL(250)
	NUMBER (%) OF ENTEROPATHOGEN				
<i>Escherichia coli</i>	2 (4.6)	22(50)	13 (29.6)	7 (15.9)	42
<i>Salmonella species</i>	1 (16.7)	4 (66.7)	0 (0)	1 (16.7)	6
<i>Klebsiella species</i>	0 (0)	1 (50)	0 (0)	1 (50.0)	2
<i>Enterobacter species</i>	0 (0)	3 (37.5)	2 (25.0)	3 (37.5)	8
<i>Citrobacter species</i>	0 (0)	2 (50)	0 (0)	2 (50.0)	4
<i>Giardia lamblia</i>	0 (0)	0 (0)	1 (100)	0 (0)	1
<i>Ascaris lumbricoides</i>	0 (0)	1 (33.3)	1 (33.3)	2 (33.3)	4
Hook worm	0 (0)	0 (0)	1 (100)	0 (0)	1
<i>Entamoeba histolytica</i>	0 (0)	0 (0)	1 (100)	0 (0)	1
<i>Trichuris tricurua</i>	0 (0)	0 (0)	1 (100)	0 (0)	1

This gives a total of 59 (23.6%) children that practices exclusive breastfeeding, 127 (50.8%) children that practices partial breastfeeding and 64 (20.6%) children that were not breastfeeding. There is no significance difference within the sex in correlation to their weaning practices as P>0.05. (P=0.896).

Table 5 shows the Age-related prevalence of enteropathogens among children with diarrhoea in Abeokuta, Nigeria. *E. coli* was the most prevalence enteropathogen isolated. Most (50%)

of the *E. coli* isolated was from children in the age group 1-12 months, followed by age group 13-24months (29.6%). *Salmonella spp* isolated was also more prevalent in age group 1-12 months (4;66.7%) and the *Salmonella spp* (2 isolates) isolate was found in the age group 13-24months and 25 -26 months. The two *Klebsiella spp* isolate was found in age group 1-12 months and 25 -26 months. Other microorganisms such as, *Citrobacter spp*, *Enterobacter spp*, were also observed. *Entamoeba histolytica*, an enteric parasite was detected in 5 (3.3%) of the cases.

Table 6: Frequency of Children Weaned and Isolated Organisms in Relation to the Months of Initiation of Weaning Food.

Month of Initiation of Weaning Foods	Frequency of Children (%)	Frequency of Microorganisms
1	22(8.8)	7
2	35 (14.0)	11
3	32 (12.8)	7
4	45(18.8)	9
5	48(19.2)	10
6	52 (20.8)	2
7 and above	16 (6.4)	2
Total P=0.056	250 (100)	52

Other enteric parasites namely, *Giardia lamblia*, *Trichuris trichiura*, *Ascaris lumbricoides* and *Ancylostoma duodenale* were also observed.

Table 6 shows the frequency of children weaned and isolated organisms in relation to the months of initiation of weaning foods. A majority 52 (20.8%) of the mothers initiated weaning foods at the age of six months, followed by 5 months (19.2%), 4 months, (18.8%), 2 months (14.0%), 3 months (12.8%), 1 month (8.8%), and 7 months (6.4%). The highest number of microorganisms was isolated from children weaned at age of 2 months. This shows a significance difference of 0.056.

CONCLUSION

This study has shown that infants who are exclusively breastfed from birth to six months of age have a significantly lower prevalence of diarrhoea than those infants who are not exclusively breastfed. The present study has provided insight into the burden of infantile diarrhoea in Nigeria. As a public health measure to reduce the disease burden, an integrated package of immunization services and other childcare programs need to be implemented in addition to well-focused health-education messages to improve treatment-seeking behavior for childhood diarrhoea as well as improved personal and environmental hygiene.

APPENDIX



Figure 1: Administration of Auestionnaires in the Out-Patients' Department of Sacred Heart Hospital, Lantoro Abeokuta, Ogun State Nigeria.



Figure 2: Microscopic Detection of Parasites in the Laboratory by the Author.

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SUGGESTED CITATION

Egbontan, A.E., O.A. Ojo, and S.J. Jacob. 2013. "Prevalence of Diarrhoea Causing Microorganisms among Children that are Exclusively Breastfed and those on Weaning Foods". *Pacific Journal of Science and Technology*. 14(1):334-341.

 [Pacific Journal of Science and Technology](http://www.akamaiuniversity.us/PJST.htm)