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PREVALENCE OF INTESTINAL PARASITES AND BACTERIAL AMONG CHILDREN IN KETU ADIE-OWE COMMUNITY, OGUN STATE, NIGERIA.

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Abstract

Intestinal parasites and bacteria are among the most communal infectious diseases affecting the poorest and most deprived people of the world's population. The study examined the prevalence of intestinal parasites and bacterial load in children in Ketu Adie-Owe Community, Ogun State, Nigeria, and using community based approach. Fresh stool specimens were collected from a total of 120 children using universal specimen bottle. The specimens were processed using direct wet mount method, formalin-ether concentration technique and kato-katz technique for the parasitological analysis while the stool samples were examined for bacteriological analysis by serial dilution and cultured on various selective agars. Structured questionnaires were administered to obtain information on the risk factors associated with the infection. Total prevalence of infection 33.3%. Having, 14.2% of *Entamoebahistolytica*, 12.5% of *Entamoeba coli*, 5% of *Trichuristrichiura*, 1.7% of hookworm. The frequency of bacteria in the stool sample showed 82.5% of *Escherichia spp.*, 60.8% of *Samonella spp*, 75% of *Klebsiella spp* and 21.5% of *Shigella spp*. Prevalence of infection was highest among ages 5years-8years and in females. It was also noted that those that observed more hand wash was least infected. Statistical analysis showed association between age of infection, sex and hand wash for both intestinal parasites and bacteria at $p > 0.05$. This study indicated that personal hygiene affects intestinal parasite and bacterial prevalence. There is need to create awareness in the importance of personal hygiene in the study area.

Key Words: Intestinal parasites, bacteria, Fresh stool sample, Kato-Katz technique and Personal hygiene

1.0 Introduction

Intestinal parasite and bacterial are amongst the most prevalent infection throughout the world. Globally, an estimated 3.5 billion case of intestinal parasite infection occur each year and 450 million people are ill as a result of these infections, the majority being children (WHO, 2019). These communal infectious diseases affect the poorest and most deprived people of the world's population (WHO, 2019). Over 270 million Pre-School-Age children and over 600

million School-Age Children live in regions where the climatic conditions favours the viability of these parasites, which are mainly in the tropical and subtropical region (WHO, 2016). These infections are considered a serious public health problem as they can cause growth retardation, iron deficiency, anaemia, malnutrition, diarrhoea, trauma, poisoning and mental health conditions (Hotez, 2009). The prevalence of these infections is closely associated with poor environmental hygiene, prevailing climatic and environmental condition, poverty and impoverished health services and overpopulation which are common in developing countries (Effiom *et.al.,* 2018). Intestinal parasitic infections and bacteria have been stated to have high prevalence among children in Nigeria because of their exposure (Otubanjo, 2010). In Nigeria, a significant amount of human and animal wastes are discharged into the soil daily leading to soil seepage with pathogenic organisms which include cysts, eggs and larvae of these intestinal parasite and bacterial cells (Suriptiatuti and Widiastuti, 2011). Enteric bacteria are bacteria that live in the intestine of humans and animals. The members of the Enterobacteriaceae family, enteric bacteria are very important because some of them symbiotically aid the digestion of their hosts, while other pathogenic species cause disease or death in their host organism. The pathogenic members of Enterobacteriaceeae include species from the genera Escherichia, Salmonella, Shigella, Klebsiella and Yersinia. All of these pathogens are closely related with faecal contamination of foods and water (Eseigbe *et al*.,2014)). The consumption of contaminated water, eating raw vegetables and fruit, soil eating behaviour and poor sanitation and hygiene are the major contributors to the transmission of this infection. However, lack of access to facilities for safe disposal of human and animal waste, lack of available hygiene resources such as hand washing facilities also contribution to the distribution and transmission of intestinal parasitic infection. In in Ado Odo/Ota Local Government Area, there is paucity of information on the distribution and transmission of intestinal parasite and bacterial in many communities including Ketu Adie-Owe community. To investigate the prevalence of intestinal parasitic infections and bacterial in children from age 1-12 in Ketu Adie- owe community, Ogun State.

2.0 Materials and method

2.1 Study area

The study was conducted in Ketu Adie-owe community in Ketu Odo local government Area of Ogun State, Nigeria, between April 2019 and June 2019.

Ketu Adie-owe community lies on the latitude 6.5102N and the longitude of 3.0927E. It is located at an elevation of 71 meters above sea level and its population amount to 2,7015 people (NPC, 2006).

The climate of ketu Adie-owe community is a true reflection of the climate of the middle belt rainfall start from April and lasts till October. It has a mean annual temperature of 27.0℃ and rainfall of 1,403mm (www.Worldweatheronline). There several source of water (such as well, public tap, borehole, stream, and rainwater) that serve as major sources of water supply to the populace in both rainy and dry season in this community. In addition, there are boreholes

sunk at various locations to compliment water supply especially during dry season. The inhabitants of the area are mostly farmers, herdsmen, business and civil servant. Since farming forms the major occupation of the populace, they depend on rain water, the public taps and wells for their source of drinking water supply and for dry season farming by irrigation.

2.2 Study design

Cluster survey was used for the study. Informed and oral consent was obtained from the parents and the community leaders and participation by children was voluntary. Parents were assured that the information obtained will be treated with full confidentiality. Standard questionnaire were used to obtain information on age, sex, soci-economic and risk factor for every participant. The community health care also helped in the collection of data for correct information of the children.

2.3 Sample collection

Sterile universal sample bottles were given to each participating children. The parents were educated on how to place a few samples of their stool into the bottle. Stool samples were collected and instantly worked on for bacteriological analysis before preservation in 10% formalin. The analysis was carried out at Crawford University Laboratory. Stool samples were divided into two portions for bacteriological analysis and parasitological examination. Stool samples that were not examined that same day were well-preserved. Preservation of the stool sample was done to avoid the stool from decaying by adding 4ml of normal saline to each sample of the first portion (for bacteriological analysis) while 4ml of 10% formalin was added to the second portion (for parasitological examination) and then stored in the refrigerator till the next day.

2.4 Sample analyses

Parasitological Examination of Faecal Samples

The sample were analyzed for cysts, eggs and larvae of parasites using formol-ether concentration technique as described by Alozie, (2018) and kato-katz technique as recommended by WHO (2016). The formol-ether concentration technique was done by weighing 1g of faeces into 4ml of 10% formalin solution with an applicator stick in a screw cap bottle . Another 4ml of the 10% formalin solution was added into the bottle and capped carefully before shaking thoroughly. The emulsified faeces were sieved using cotton gauze and the suspension collected into a centrifuge tube. 3ml of diethyl ether was added to the suspension in the centrifuge tubes, covered with glass stopper and was shaken for 1minute. With a piece of cloth wrapped around the top of the tube, the stopper was loosening (considering the fact that pressure might be built up inside the tube). It was centrifuge immediately at 3000 revolutions per minute. A stick was used to slacken the layer of focal debris from the side of the tube. The tube was inverted to discard the ether, faecal debris and 10% formalin solution leaving behind only the sediment. The tube was returned to its standing position and allowed the fluid from the side of the tube to channel to the bottom. The bottom of the tube was tapped to re-suspend and mix the residue. A few drops of the residue were moved to a slide using a pipette and covered with a cover slip. The preparation was examined microscopically using 10 objectives with the condenser iris closed sufficiently to give good contrast. The

40 objective was used to examine cysts. Lugol's iodine solution was added under the cover slip to succour in the identification of the cysts. The identification of the parasite was done using the morphology of cysts, eggs and larvae using Atlas of medical Helminthology and Protozoolgy (Effiom *et.al*., 2018). The kato-katz technique was used for the qualitative and semi-quantitative analysis of intestinal helminthic infections caused mainly by *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm. In this technique the faeces were passed through a mess screen to remove any large particles. A portion of the sieved sample is then transferred to the hole of a template on the slide using a spatula. After filling the hole, the template was removed and the remaining sample was covered with a piece of cellophane that had been soaked in glycerol. This was pressed evenly downward to spread the faeces in a circle. The slide was then placed with the cellophane upward. What the glycerol does is to clear the faecal material from around the eggs. The slide was placed under the microscope and the whole area was examined in a systematic zigzag pattern. The egg was then counted and the number calculated per gram of faeces. The intensity of the infection was assessed by the standard measurement in the inlet-information of the Kato-set (WHO, 2019).

2.5 Bacteriological analysis of faecal samples

One gram of each faecal sample was weigh using a microbiological weigh balance and homogenized into 9ml sterile distilled water. 1ml from the stock culture was inoculated into the second test tube which also contain 9ml of sterile distilled water following the principles of serial dilution, until the tenth dilution. The tenth dilution is then inoculated into a sterile petri dish using an auto pipette. 15ml of nutrient agar was then poured and carefully homogenized. This was done for the total viable count of the faecal samples. The tenth dilutions of the samples were also cultured on differential and selective media for bacteria cultivation in order to isolate bacteria entero-pathogens. The cultured plate was incubated overnight within the temperature of 37℃ (Hyeng-II, 2010). MacConkey agar, Eosin methylene blue agar (EMBA) and salmonella-shigella agar were used for the isolation of bacteria pathogens.

2.6 Gram Staining

The cosonies of microorganisms were picked from the growth of the cultured media plate and smeared on a clean grease free slide. This was heat fixed, by passing it through the light Bunsen burner (with the bacteria mounted on it) for 5 times. Too much flaming which could cause rapture of the bacterial should be avoided. The entire slide was flooded with crystal violet and allowed to stand for about 60 seconds. When the time has elapsed, the slide was washed for 5seconds with the water bottle. The specimen appears blue-violet when observed with the naked eye.

After this, the slide was flooded with iodine solution and is allowed to stand for about a minute. When time expired, the slide was rinsed with water for 5 seconds and immediately proceeds to step three. At this point, the specimen was still blue-violet. The Decolourizer was dropped on the stained slide until the blue-violet colour is no longer emitted from the specimen, the slide was rinsed with water for 5 seconds. The slide was flooded with saffranin and allowed to stand for about a minute to allow the bacteria

to incorporate the saffranin. Gram positive bacteria stained blue-violet in appearance and the Gram negative bacteria however, stained pink colour and are easily distinguished from Gram positives. After the staining, the slide was dried and oil immersion was dropped on the stained region and examined under X 100 objective of the microscope.

3.0 RESULT

Prevalence of intestinal parasite in the children in Ketu Adie-Owe

Table 1 showed that a total of 120 children were examined of which 40(33.3%) were positive for intestinal parasites. Out of the 40 infected children 13(32.5%) children had scanty helminthic load, 21(52.5%) had moderate helminthic load and 6(15%) were highly infected with intestinal parasite.

3.1 Species-specific prevalence

Figure 1 showed that four species of parasites were identified in this study *Entamoeba histolytica* was recorded in 17 (14.2%) children followed by *Entamoeba coli* 15(12.5%), *Trichuris trichuira* 6(5%) and hookworm 2(1.7%).

3.2 Prevalence of intestinal parasites in relation to age of children

Table 2 showed that infection occurred in all the age-groups. The age-group 5-8 years 18 (42.9%) had the highest prevalence, the age-group 9-12 years had a prevalence of 14 (29.8%) and the age-group 1-4 years had the least prevalence of 8(25.8%). The difference in prevalence across age groups was highly significant ($p<0.05$). The odd ratio value of 2.135 for the age-group of 5-8years showed associations between the age group and the infection (Table 2).

3.3 Prevalence of intestinal parasites in relation to sex

The prevalence of the parasites in the 120 stool samples from children age 1-12 was higher in females compared to the males. From the total of 54 male and 66 female children enrolled for the screening 16 (29.6%) males and 24(36.4%) females were positive for parasites. There was statistically significant association between the sexes and the prevalence rate. Odd ratio of 1,187 shows an association between sex and the infection (Table 3).

3.4 Responses of the children to questionnaire

The responses of the children to the questionnaire and the results of the statistical analysis showed some association between the various epidemiological variables and the infection Prevalence of intestinal parasitic infections according to water sources showed that the prevalence was higher in children who used public taps and rain water as their source of drinking water and the least in those that used borehole water as source of drinking water.

Out of a total of 26 children who indicated that they use public tap water as their source of drinking, 15(57.7%) tested positive for intestinal parasites, 34 respondents that use rain water, 18(52.9%) positive cases and out of 60 who indicated that they use borehole water, 7(11.7%) children were infected. The result did not show significant difference ($P<0.05$). An odd ratio of 13.665 obtained from the respondents that uses public tap waters showed association between the water source and the disease (Table 4).

Prevalence of intestinal parasite infection according to how they cook their vegetables

showed that individuals that parboil or steam their vegetable have higher prevalence than the people who boil their vegetable.

Out of a total of 26 individuals who indicated that they parboil their vegetables 15(57.7%) tested positive for intestinal parasites, 46 respondents that steam their vegetable 16(34.8%) positive cases, and individuals who boil their vegetable 48 of which 9(18.8%) cases where positive. The result did not show significant difference ($P<0.05$). An odd ratio of 6.131 obtained from the respondents that parboil their vegetable showed association between how they cook their vegetable and the disease (Table 5). Prevalence of intestinal parasitic infections according to how frequent they de-worm showed that individuals that had never de-worm had higher prevalence, followed by those who de-worm once in a year while the respondent that indicated that they de-worm every 3month and 6months had the least prevalence.

Out of a total of 26 children who indicated that they de-worm every 3months 4(15.4%) were positive, 58 respondents indicated that they deworm at 6months interval of which 10(17.2%) were positive, 23 children indicated that they de-worm aunally, 15(65.2%) were infected and children who indicated that they had never de-worm 13 of which 11(84.6%) were infected. The result did not show significant difference ($P<0.05$). An odd ratio of 36.676 obtained from the respondents that had never de-worm showed association between the de-worming interval and the disease (Table 6).

Prevalence of intestinal parasitic infections according to how often they experience symptoms of intestinal parasites showed that respondents that indicated that they never experience the symptoms had the least prevalence, followed by the respondents that indicated that they experience the symptoms less often while those that indicated that they experience the symptoms often had the highest prevalence.

Out of a total of 29 respondents that specified that they never experience the symptoms of intetinal parasite 4(13.8%) were infected, 68 respondent indicated that they experience the symptoms of intestinal parasite less often 24(35.3%) were positive and 23 respondents signified that they experience symptoms of intestinal parasite often 12(52.2%) were positive. The result did not show significant difference ($P<0.05$) (Table 7).

3.5 Species-specific distribution of bacteria

Four organisms were identified in this study are *Escherichia spp., Samonella spp,. Shigella spp., Klebsiella spp.* Out of the 120 children, 99 (82.5%) of *E. coil* was recorded, 73 (60.8%) of Klebsiella, 90 (75%) of Samonella and shigella had the least frequency of 26 (21.2%) (Figure 2).

3.6 Bacterial load in relation to age of children

The children between the age-group of 5-8 years had the highest frequency of bacteria while the children between the age group of 1-4 had the least bacterial load. The children between the age group of 5-8 had a bacteria load range from $8x10^7$cfu/g - $6x10^7$cfu/g, the children between the ages of 9-12 had a bacterial load between $7.7x\ 10^7$cfu/g - $4x10^7$cfu/g and the children between the age group of 1-4 had a bacteria load range between $4x10^7$cfu/g - $1x10^7$cfu/g (Table 8).

3.7 Bacterial load in relation to sex

The male children had higher bacterial load than the female children. The male children had a bacteria load of $8x10^7$cfu/g - $3x10^7$cfu/g and the female children had a range of $7.1x10^7$cfu/g - $1x10^7$cfu/g (Table 9).

Table 1. Prevalence of intestinal parasite in the children in Ketu Adie-Owe.

Helminthic load	Positive (%)	Prevalence(%)
Scanty helminthic load	13(32.5)	10.8
Moderate helminthic load	21(52.5)	17.5
High helminthic load	6(15)	5
Total	**40(100)**	**33.3**

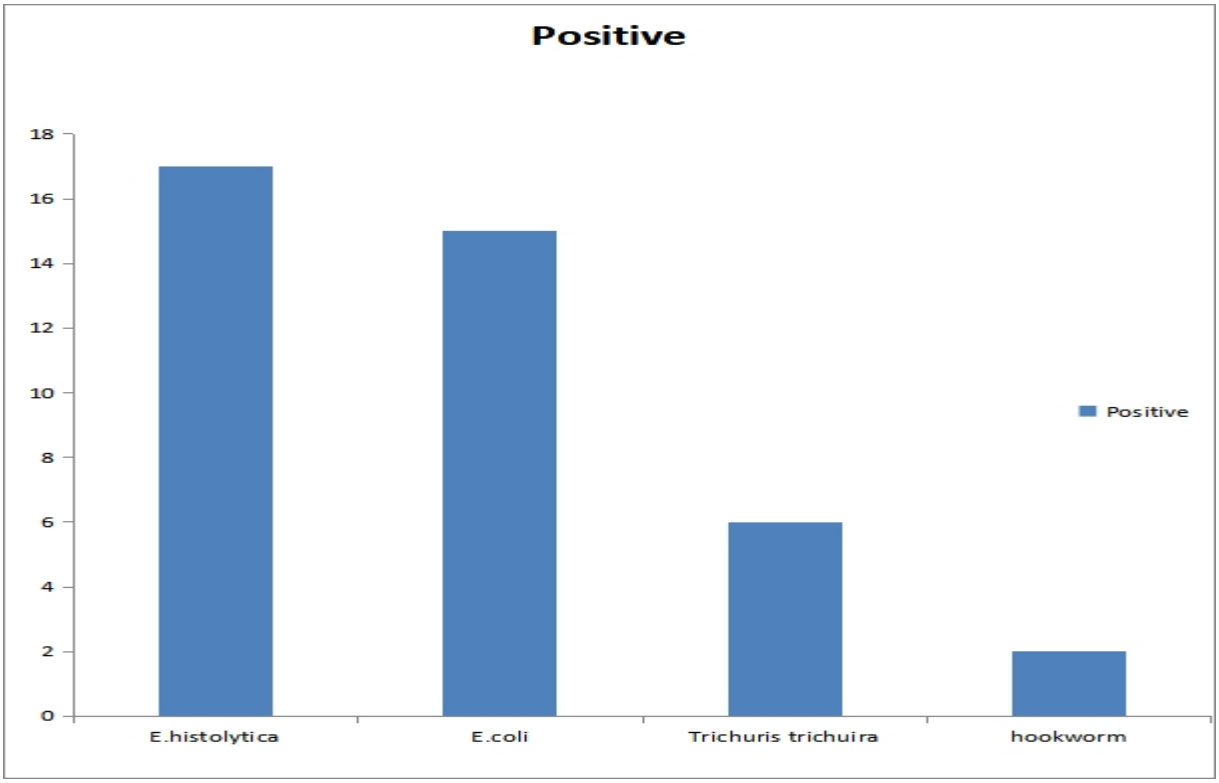

Figure 1. Species-specific prevalence.

Table 2. Prevalence of intestinal parasite in relation to age

Class (Age)	Number Examined	Status			Confidence Interval	
		Positive	Negative	Odds ratio	Lower	Upper
1-4	**31**	**8(25.8)**	**23(74.2%)**	**2.135**	**0.776**	**5.877**
5-8	**42**	**18(42.9)**	**24(57.1)**	**1.233**	**0.444**	**3.424**
9-12	**47**	**14(29.8)**	**33(70.2)**	**1.731**	**0.720**	**4.164**
Total	**120**	**40(33.3)**	**80(66.7)**			

Table 3. Prevalence of intestinal parasite in relation to sex.

Gender	Number Examined	Status		Odds ratio	Confidence Interval	
		Positive	Negative		Lower	Upper
Male	54	16 (29.6)	38 (70.4)	1.187	0.762	1.851
Female	66	24 (36.4)	42 (63.6)	0.875	0.630	1.214
Total	**120**	**40 (33.3)**	**80 (66.7)**			

Table 4. Prevalence of intestinal parasite in relation to their water sources.

Source of drinking wat	Number Examined	Status		Odds ratio	Confidence Interval	
		Positive	Negative		Lower	Upper
Tap	**26**	**15**	**11**	**13.665**	**4.190**	**44.570**
Rain	**34**	**18**	**16**	**0.838**	**0.290**	**2.420**
Borehole	**60**	**7**	**53**	**0.073**	**0.22**	**0.239**
Total	**120**	**40**	**80**			

Table 5. Prevalence of intestinal parasite according to how they cook vegetables.

How do you cook your vegetables?	Number Examined	Status		Odds ratio	Confidence Interval	
		Positive	Negative		Lower	Upper
Parboil	**26**	**15**	**11**	**6.131**	**2.093**	**17.964**
Steam	**46**	**16**	**30**	**0.407**	**0.150**	**1.104**
Boil	**48**	**9**	**39**	**0.163**	**0.056**	**0.478**
Total	**120**	**40**	**80**			

Table 6. Prevalence of intestinal parasite in relation to deworming.

Month interval for deworming	Number Examined	Status		Odds ratio	Confidence Interval	
		Positive	Negative		Lower	Upper
3 Months	26	4 (10)	22 (27.5)	0.027	0.004	0.184
6 Months	58	10 (25)	48 (60)	0.033	0.006	0.180
1 year	23	15 (37.5)	8 (10)	0.339	0.059	1.985
Never	13	11 (27.5)	2 (2.5)	36.676	5.428	247.799
Total	**120**	**40**	**80**			

Table 7. Prevalence of intestinal parasite to symptoms.

SYMPTOMS	Number Examined	Status		Odds ratio	Confidence Interval	
		Positive	Negative		Lower	Upper
Never	**29**	**4 (10)**	**25 (31.2)**	**0.136**	**0.035**	**0.035**
Less Often	**68**	**24 (60)**	**44 (55)**	**0.499**	**0.190**	**1.312**
Often	**23**	**12 (30)**	**11 (13.8)**	**7.347**	**1.885**	**28.639**
Total	**120**	**40**	**80**			

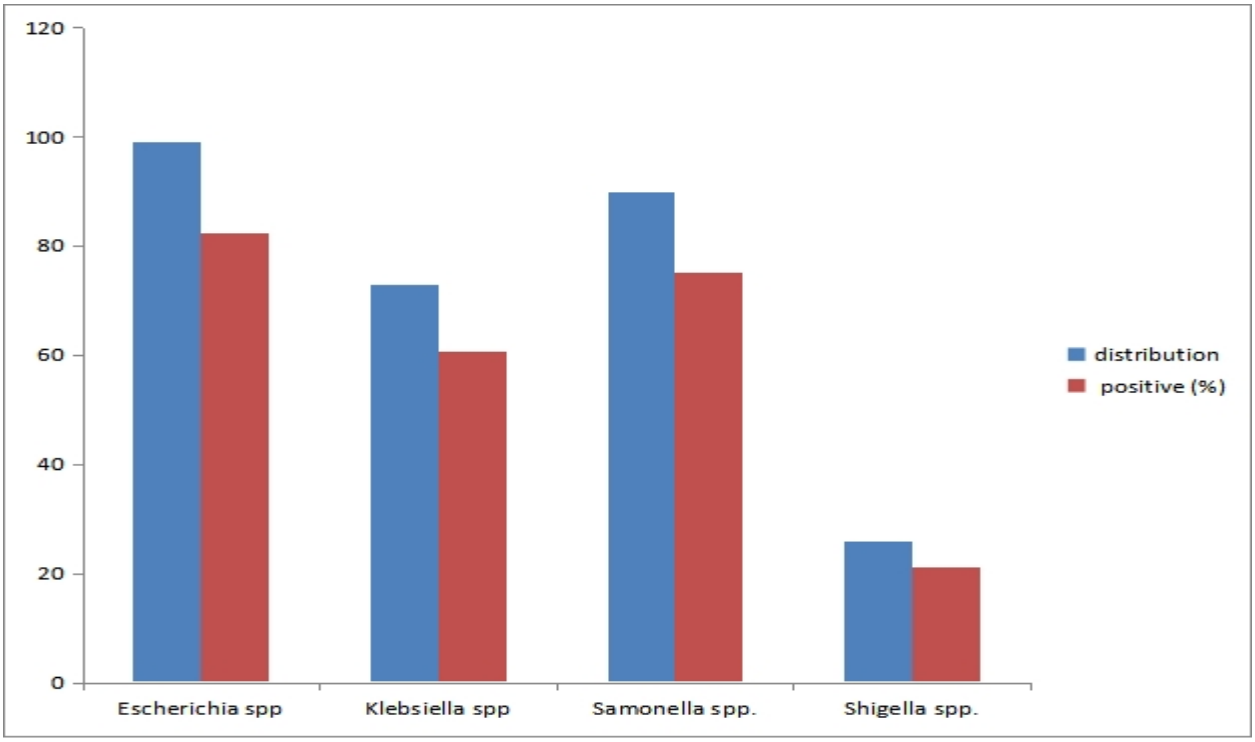

Figure 2. Species-specific distribution of bacteria

Table 8. Bacteria load in relation to age of the children

Age-group	Bacteria load
4	$4x10^7$ cfu/g – $1x10^7$ cfu/g
5- 8	$8x10^7$ cfu/g – $6x10^7$ cfu/g
9- 12	$7.7x10^7$ cfu/g - $4x10^7$ cfu/g

Table 9. Bacteria load in relation to sex.

Gender	Bacteria load
Male	**$8x10^7$ cfu/g – $3x10^7$ cfu/g**
Female	**$7.1x10^7$ cfu/g – $1x10^7$ cfu/g**

4.0 DISCUSSION

The result of this study has indicated low prevalence of intestinal parasite when compared to other areas in Ogun State. This is in line with reports from previous studies. Ogbolu *et.al*. (2009) reported 46.9% prevalence of intestinal parasite at Ota, Ogun State and Sam-Wobo, et al. (2008) reported 58% prevalence of intestinal parasite in Abeokuta, Ogun State. The low prevalence in ketu Adie-Owe community can be attributed to the as semi-urban environment, standard of living, their moderate personal and environmental hygiene, available health facility and Health personess. The nearness to a University who from time to time engage in Advocacy to sensitize and create awarereness on health related issues had been a great advantage to the community. The prevalence and distribution of this parasites are favoured by poverty, poor hygiene and poor sewage disposal. The low prevalence can also be due to the sandy soil type in Ketu Adie-Owe that does not favours the development of intestinal parasites. This finding is similar to Damen (2010), who recorded a low prevalent of 30.2% in Doi village Plateau State as a result of soil type.

This study revealed that the prevalence of intestinal parasite was high among the children between the age-group of 5-8years. This may be due to their level of exposure to the risk factors. This study indicated an association between the age and the disease. The children within the age group of 5-8years are usually more engaged in soil activities during recreational activities. The same group were associated with maintaining poor personal hygiene and this plays a major role in the prevalence of intestinal parasitic infections. This is having also been reported in previous study (Chioma *et.al*., 2015). However, the children between the age-group of 1-4 had the least prevalence. This may be because of the hygiene level that is maintained by the parent along with special attention and pampering that is given to children between this age-group. This is similar to the observation made by Kalu, (2013) in Mbaitoli in Imo State.

This study further revealed that prevalence was higher in the female children than the male children. The reason may be due to the fact that females are more engaged in the sand play activities and increased chance of exposure of females to contaminated water as they engaged in fetching water for use at home which is the case in most family set up in Ketu Adie-Owe community. This is in agreement with a study conducted in Ethiopia by Adamu, and Endeshaw, (2016) also similar observation where made by Yahaya, and Dogara (2018), in Dutse, Jigawa State, Nigeria.

This study also shows that prevalence was higher in children that indicated that they had never dewormed and those who deworm yearly. This observation emphasises the importance and relevance of periodic anthelminthic therapy as people who deworm every 3 months and 6 months had the least prevalence as (Danladi, 2015). had similar observation. However, positive cases were recorded in those that were de-wormed consistently. This may be due to re-infection after treatment. Chemotherapy is the best way of reducing the worm burden though there is still a debate on the long-term effect on nevertheless improved living standards, environmental sanitation, agricultural and industrial hygiene can contribute to the

success of the use of chemotherapy (Absar *et.al*, 2010).

This experimental research work showed an association between the infection and the symptoms indicated by the respondent. This means that intestinal parasites such as *Entamoeba histolytica*, Hookworm, *Entamoeba coli* and *Trichuris trichiura* may be responsible for the observed symptoms. Frederick *et.al*, 2011 also reported a similar result. This could be controlled by regular deworming, personal hygiene and environment sanitation.

This research work showed that children between the age-group of 5-8 years had the highest bacteria load, this can be because of their poor personal hygiene, improper washing of hands especially before eating and after the use of the toilet, sand play attitude and bumpy attitude. Eseigbe, *et.al*, (2014) had similar findings and suggested that this can be controlled by proper hand wash (with soap and sanitizers) and proper hygiene.

This study indicates the presence of *Escherichia spp., Samonella spp,. Shigella spp., Klebsiella spp.* in the stool samples. Okike-Osisiogu and Ike-Amadi (2018) made such observation in Aba, Nigeria. The presence of *Escherichia spp., Samonella spp,. Shigella spp., Klebsiella spp.* have been notably associated in diarrhoea and other gastrointestinal infection (WHO 2019). This can also be controlled by safe drinking-water, use of improved sanitation and hand washing with soap (WHO 2019).

This study further revealed that the male children had greater bacteria load than the female children. This observation can be as a result of the kind of recreational activities they are involved in (such football, westling etc). In addition, this outcome may be due to the fact that most of them don't clean up or wash hands after play especially when they are hungry. This observation had also been reported by Okike-Osisiogu and Ike-Amadi (2018) in Aba, Nigeria.

Despite the low prevalence of intestinal parasites found in Ketu Adie-Owe community, there is still need to improve personal and environmental hygiene, engage in periodic de-worming of children and provide social amenities which will make available potable water supply. As the above are predisposing risk factors that enhance the occurrence of the infection. However, the high bacteria load could be controlled by regular hand wash and better still personal hygiene and environment sanitation. The Health Service Unit of the community should embark on creating more awareness on health relation issues and improved hygiene.

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