



Intestinal Parasites and *Salmonella typhi* Infection among Food-handlers in Port Harcourt Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors OEO and AEA managed the analyses of the study. Author AEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Intestinal parasites and *Salmonella* infections are of main public health concerns in Nigeria and the world, especially in developing countries where access to safe water and hygienic food handling practices by food handlers is a challenge. Consequently, food-handlers play a major role in the transmission of these types of foodborne diseases.

Objective: This study was to determine the preponderance of intestinal parasites and *Salmonella typhi* among food-handlers in selected areas in Port Harcourt.

Materials and Methods: A total of 480 food-handlers (195 males and 285 females) of various educational levels were sampled. Stool samples were collected and analyses were carried out using direct smear examination and formol-ether concentration technique - adopted for

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investigation of intestinal parasites and deoxycholate citrate agar and Salmonella-Shigella agar for the cultivation of *Salmonella typhi*.

Results: Of 480 food-handlers examined, comprises of 195(40.6%) males and 285(59.4%) females, - majority (85.4%) were young adults aged 20-40 years. 47.9% of them - had education above secondary educational level. The preponderance of intestinal parasites was 48(10.0%) and implicated were *Ascaris lumbricoides* (41.7%), hookworm (33.3%) and *Trichuris trichiura* (25.0%). No intestinal protozoa were identified. Prevalence of 30(6.3%) was recorded for *Salmonella typhi*.

Conclusion: Prevalence of Intestinal Parasites and *S.typhi* infection is relatively low among food handlers in Port Harcourt, but indicates the important role food handlers play in the transmission of the infection. Therefore, awareness should be step-up for the food handlers to avoid escalation of these infections

Keywords: Intestinal parasites; typhoid fever; preponderance; food handlers.

1. BACKGROUND

Food borne diseases are globally important, as they may be responsible for considerable morbidity, mortality, and economic costs [1,2]. Many different diseases, including those due to bacteria, viruses, parasites, chemicals, and prions, may be transmitted to humans by contaminated food [3]. Outbreaks and sporadic cases of foodborne disease are regular occurrences in all countries of the world. In recent decades, globalization of the food supply has also meant that pathogens causing foodborne diseases are rapidly transported across international borders [4]. Foodborne disease outbreaks had led to adverse impacts on trade and food security [5,6]. In response to foodborne diseases, national governments and international bodies have established elaborate systems to control and improve food safety [7].

The centers for disease control and prevention have identified that poor personal hygiene is ranked as the third most frequently reported food preparation practice that contributes to food-borne diseases [8]. Foodborne illnesses from diarrheal and invasive non-typhoidal *S. enterica* resulted in the largest disease burden reflecting the ubiquitous nature of *Salmonella*, the severe nature of illness, and the fact that young children are commonly infected [9]. *Salmonella typhi* is transmitted through food or water contaminated with faeces from infected persons, persistent excretors or from chronic asymptomatic carriers who handle food. Humans are the only host for *S. typhi* and there are no known environmental reservoirs [10,11].

The parasitic infections are considered as one of the major health problems in the world and especially in developing countries [12,13]. According to the World Health Organization

(WHO), nearly two-thirds of the world is infected with one kind of intestinal parasite and *Ascaris* and *Giardia* infections have the highest rate among all kinds [14]. The reasons for high incidence of parasites in some parts of the country are a result of specific climate of the regions, local customs, and the use of human and animal wastes as fertilizers in agriculture and floriculture [15]. Lack of clean and safe water, high population density, lack of proper disposal of waste, noncompliance with health standards (social and individual), lack of adequate washing of vegetables, and lack of well cooked meat lead to high prevalence of intestinal parasites [16,17].

Objects contaminated with faeces can directly or indirectly affect the rate of transmission of intestinal parasites and enteropathogenic bacteria. These include food, water, nails, and fingers, showing the significance of faecal-oral human-to-human transmission [18]. Consequently, food-handlers with low quality of personal hygiene that works in food-serving establishments could be possible sources of infections of many intestinal helminths, protozoa, and enteropathogenic bacteria [19]. Food-handlers that harbour and excrete intestinal parasites and enteropathogenic bacteria may serve as vehicle for contamination of foods from their faeces through their fingers, then to foodstuff processing, and finally to healthy persons [20]. Compared to other parts of the hand, the area beneath fingernails harbours the most microorganisms and is most difficult to clean [21].

1.1 Objective

This study was to determine the preponderance of intestinal parasites and *Salmonella typhi* among food-handlers in selected areas in Port Harcourt metropolis, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in selected area in Port Harcourt Metropolis situated in Rivers State of the South-South region of Nigeria. Coordinates of study area ranges from latitude 4°44'0" - 4°45' 0" N and longitude 6°54'0"-7°8'0" E.

2.2 Sample Collection

This study included selected 480 food-handlers, conducted from April 2018 - September 2018 in Port Harcourt. A well-structured questionnaire aimed at obtaining standard demographic data - was given to each participant to complete and return-. A clean stool container was given to each participant for sample collection.

2.3 Inclusion Criteria

Normal healthy food-handlers (Male and Female).

2.4 Exclusion Criteria

Food-handlers suffering from diarrhoeal illness - or had suffered diarrhoea in last three months and those unwilling to participate in the study.

2.5 Specimen Collection

A stool specimen was collected from each food handler in a clean stool cup and transported into the laboratory. The stool samples were examined microscopically for parasites following direct wet mount preparations in normal saline (0.85% NaCl) and iodine solution (2% lugols iodine) [22].

2.6 Isolation and Identification of Intestinal Parasites

2.6.1 Direct smear examination for stool samples

On a glass microscope slide, about 1-2 mg of stool was emulsified in a drop of normal saline (0.85% NaCl) on the left hand side of the slide, and in Lugol's iodine on the right side of the slide. A cover-slip was then placed on each side, and the slides were scanned under 10× and 40×

objective lenses of a light microscope, as required. Saline direct smear is used mainly for detection of motility of intestinal protozoan trophozoites, which are seen in liquid or semi-liquid specimens. Iodine direct smear shows the characteristic features of the diagnostic stages in more details [23].

2.7 Formol-Ether Sedimentation Concentration Technique Ritchie

Although, this formol ether technique cannot detect trophozoites, it is considered as the best concentration technique used in diagnostic parasitology laboratories for detection of cysts, ova, and larvae [24,25]. Generally, 10% formal saline is used in the Ritchie technique to kill and preserved diagnostic stages. Diethyl ether collects most of debris in a separate layer. All diagnostic stages that are applicable with the Ritchie technique will be concentrated at the bottom of the analysis centrifuge tube. However, safety precaution -was taken, as formalin is carcinogenic, and diethyl ether is flammable and explosive. Quantitatively, one slide from the Ritchie technique is a substitute of about 1000 slides or more from the direct smear technique. Thus the greater the amount of stool used, the greater the chance of recovery of diagnostic stages. The Ritchie sedimentation technique was performed by emulsifying about 2 g of stool in 10-15 ml of 10% formol saline. The suspension was allowed to stand for 30 minutes, and then strained through two layers of gauze into a 15 ml conical centrifuge tube and centrifuged at 2000 rpm for 5 minutes. When needed, the washing step was repeated until supernatant becomes clear. The sediment was resuspended with 10 ml of 10% formal saline and allowed to stand for 5-10 minutes. A total of 3 ml of diethyl ether was added, and then the tube was shaken vigorously for 30 seconds and centrifuged at 2000 rpm for 5 minutes. After centrifugation, the applicable diagnostic stages were sedimented in the bottom of the tube. The fecal debris was separated in a layer between the diethyl ether and the 10% formol-saline layers. A fecal debris layer was loosened by wooden stick and the tube rapidly inverted to discard the top three layers while the sediment remained at the bottom. One to two drops of iodine were added to the sediment and mixed well. Then, part of the sediment was transferred to a microscope slide, covered with a cover glass and scanned microscopically under low and high objective lenses [26].

2.8 Modified Acid Fast Staining Techniques

Smears are prepared after concentration, air dried and then fixed in methanol, stained with Kinyoun carbol-fuchsin for 4-5 minutes, destained with 1% aqueous sulfuric acid for 2-3 minutes, rinsed with distilled water and then counterstained with Loeffler's alkaline methylene blue for 1 minute. Smears are rinsed with distilled water drained and dried [25].

2.9 Isolation and Identification of *Salmonella typhi*

Ten (10) mL of Selenite F broth, an enrichment medium that inhibits the growth of coliforms while allowing the growth of *Salmonella* and *Shigella* species was also inoculated and incubated overnight at 37°C aerobically. The overnight incubated Selenite F broth was subcultured onto DCA and SSA and further incubated for 18-24 hours at 37°C. Non-lactose fermenting (NLF) colonies growing on DCA and SSA plates were identified on the basis of their biochemical properties. Non lactose fermenting colonies 2-3 mm in diameter, moist, circular, converse surface and entire edge (which on DCA plate have black centre) were picked into Kligler iron agar (KIA) slope before streaking on the surface. These were incubated overnight at 37°C.

The colonies were also tested for motility and oxidase production using appropriate reagents. They were oxidase negative, motile urease

producing and able to ferment glucose on Kligler iron agar with hydrogen sulphide (H₂S) production. No gas was produced which was typical of *S. typhi* [27].

2.10 Statistical Analysis

Data were analyzed by using Microsoft excel version 2016. Categorical data were analyzed as frequency and percentage. All statistical analyses were performed using SPSS software (version 23, SPSS, Chicago, USA).

3. RESULTS

Out of 480 food-handlers examined for intestinal parasites, 48(10.0%) were positive while 432(90.0%) were negative. Also, 30(6.3%) food-handlers had positive stool cultures for *Salmonella typhi* while 450(93.7%) food-handlers showed negative stool cultures (Table 1). Among the food-handlers, great majority (85.4%) were young adults aged 20-40 years.

Forty-eight (10%) stool specimens were positive for intestinal parasites. The positive stool samples among the food-handlers for intestinal parasites had *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm, their respective prevalence is seen on Fig. 1.

There were 195(40.6%) males and 285(59.4%) females. The prevalence of intestinal parasites and *Salmonella typhi* isolated were low among the male food-handlers when compared to female counterpart (Table 2).

Table 1. Overall Prevalence of Intestinal Parasites and *Salmonella typhi* among Foodhandlers in the study population

Age Group	Number Examined	Prevalence of Foodhandlers with Intestinal Parasites.		Prevalence of Foodhandlers with <i>Salmonella typhi</i>	
		NP	Percentage	NP	Percentage
20-25	110	13	11.8	9	8.2
26-30	102	17	16.7	8	7.8
31-35	106	11	10.4	7	6.6
36-40	92	5	5.4	3	3.3
≥ 40	70	2	2.9	3	4.3
Total	480	48	10.0	30	6.3

Legends: NP- Number Positive

Table 2. Sex-Related Distribution of Food-handlers Examined in Port Harcourt

Sex	Number examined	Number of Positive intestinal parasites	Number of Positive <i>Salmonella typhi</i>
Male	195	13(27.1)	11(36.7)
Female	285	35(72.9)	19(63.3)
Total Prevalence (%)	480 (100)	48(10.0)	30(6.3)

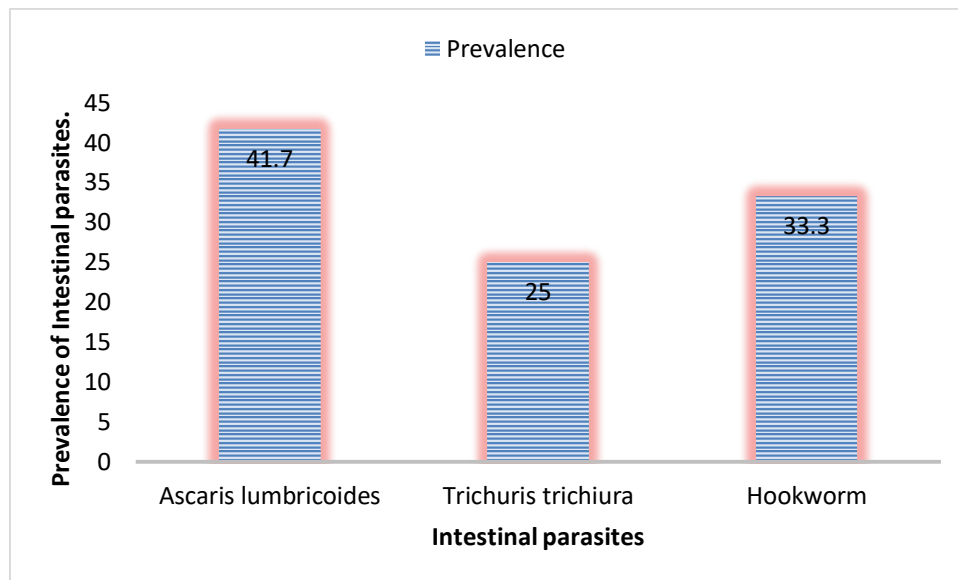


Fig. 1. Prevalence of Intestinal parasites among Food-handlers in Port Harcourt

Table 3. Prevalence of Intestinal Parasites and *Salmonella typhi* along Educational Level in Port Harcourt

Educational level	Prevalence of Food-handlers with intestinal parasites and <i>Salmonella typhi</i> (%).			Total Prevalence Infected (%)
	Number Examined	Intestinal parasites	<i>Salmonella typhi</i>	
Primary	150(31.3%)	26(17.3)	14(9.3)	26.6
Secondary	230(47.9%)	17(7.4)	9(3.9)	8.9
Tertiary	100(20.8%)	5(5.0)	7(7.0)	12.0
Total (%)	480(100)	48(10.0)	30(6.3)	

The study included all the 480 food-handlers, and the response rate was 100%. Only 47.9% of the food-handlers had education above secondary educational level (Table 3).

4. DISCUSSION

In this study, 10.0% and 6.3% of the 480 subjects were positive for intestinal parasites and *Salmonella typhi* respectively. This study had demonstrated that food-handlers harbour *Salmonella typhi* asymptotically [28]. The transmission of parasitic and bacterial diseases by food-handlers as danger has been stressed by several authors all over the world [28].

On the prevalence of intestinal parasites, lower findings were also reported in the country and elsewhere 14.5% [29], 24.3% [30], 29.1% [22] and 30.5% [31]. High prevalence of intestinal parasites 41% was reported in Ethiopia [32], 49.4% [33] in Southeastern Anatolia (52.2%) [34] and Abeokuta, Nigeria (97%) [35] as related with

the present study. A wide difference in magnitude of IPs across surveys could be due to difference among personal hygiene practices, environmental sanitation and ignorance of health-promotion practices.

Ascaris lumbricoides was most prevalent parasite identified among others from food-handlers in the current study. Similar findings have been reported in previous studies in Ethiopia [22,36,37]. Soil transmitted Helminthes, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm reported in this study may indicate low personal hygiene in food-handlers and the habit of open field defecation of the community. Other studies reported *Giardia* as the most prevalent in India [38] and Iran [39]. *Entamoeba histolytica/dispar* was the most predominant parasite in study conducted by Eshetu et al. [40] in Ethiopia.

The prevalence of *Salmonella typhi* is low though higher than some studies elsewhere with the observations by Olalekan et al. [41] with 4.2%,

5.5% in Abeokuta [35], 1.6% in Bahir-Dar town, North West Ethiopia [36], and 1% in Mekele [33], 3.1% in Gondar [42], 5% in Haromaya [43] 3.5% in Addis Ababa [44]. On the contrary, higher findings of 31.5% was recorded in Abuja [45] and 42.3% by David and Oluyeye [46], 92.50% and 67.8% in Ogun State and Niger state by Okonko et al. [47] and Adogo et al. [48], Eze et al. [49] reported prevalence of 92% in Enugu, 62.70% in Imo by Anumudu et al. [50] and 13.56% were also reported in Ethiopia [51].

The stool culture for *Salmonella typhi* is statistically significant ($P < 0.05$). Considering that typhoid fever is endemic and the causative agent, *S. typhi*, is chiefly spread by faecal-oral route following acute infection, it is likely that the contamination of food materials or drinking water as a result of wash off from the faecal contaminated environment during early raining season must have been one of the sources of typhoid fever in the studied areas. This indicates a low prevalence of typhoid fever in the sampled population. However, some of the food-handlers may not be having the active disease.

There were 195(40.6%) males and 285(59.4%) females. It was observed that there were differences in the prevalence from male food-handlers when compared with female food-handlers with 63.3% and 72.9% in both intestinal parasites and *Salmonella typhi* infection. This is similar in reports by Abioye et al. [52] in Karu, Nasarawa State, Ezeigbo et al. [53] in Aba, Abia State and in Abeokuta, Nigeria [54]. A contrary report was recorded in Biu, Borno State by Isa et al. [55]. However, this variation had no statistical significance ($P > 0.05$).

The food-handlers infected with both intestinal parasites and *Salmonella typhi* had different educational levels with different prevalence. Those who had access to primary school education were 26.6%, secondary education 8.9% and only 12.0% had tertiary education. However, there was no statistical significance ($P > 0.05$) between infection and education of the respondents.

5. CONCLUSION

In conclusion, 10% intestinal parasites and 6.3% *Salmonella typhi* carrier rate of food-handlers suggests that the prevalence rate is low in this locality and it could be due to awareness of public health measures and personal hygiene. However, health education about personal

hygiene, especially regarding hand-washing after toilet use and before food preparation; provision of a safe water supply, proper sanitation systems and excluding disease carriers from food handling should be encouraged.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

The data were collected after written informed consent was obtained from the research ethical committee of the University of Port Harcourt and all study participants. The results were kept confidential, and food-handlers who were found positive for *Salmonella typhi* or parasitic infection were given appropriate advice.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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