**Original Research Article**

**PREVALENCE OF *SALMONELLA*, *SHIGELLA*, AND INTESTINAL PARASITES AMONG FOOD HANDLERS PREDISPOSE CONSUMERS TO SIGNIFICANT HEALTH RISKS**

**ABSTRACT**

**Background and objectives:** Food borne diseases are a global public health problem and food handlers play a major role for the transmission of food borne diseases.This study was aimed at exploring the prevalence of intestinal parasites, *S. typhi* carrier rate and risk factors of infection with typhoid and /or intestinal parasites among food handlers at Ibb city, Yemen.

 **Subjects and methods:** A cross -sectional survey was conducted among three hundred and fifteen food handlers, at age ranges from 14 to 65 years. All individuals were working in restaurant, cafeteria or schools buffets in Ibb city. A pre-tested structured questionnaire was used for collecting data. Stool samples were investigated for intestinal parasites and *S. typhi* were investigated by stool culture media and blood for detection antibodies per the standard laboratory methods.

**Results:** A total of 315 food workers in Ibb city over a 12-month period were included in this study, ages ranged from 14 to 65 years, with a mean ± SD age of 31.2 ± 11.9 years. The highest prevalence of antibodies against *S. typhi* antigen suspension O was 18.4%, while antibodies against *S. typhi H* antigen suspension were 7.6%. Also, the positive rate for total *S. typhi* antibodies ELISA IgG was 9.5% and the positive rate for *S. typhi* stool cultures was 7.3%. The prevalence of intestinal *protozoa* was 20%, in which the prevalence of *Entamoeba histolytica* was 15.6%, the prevalence of intestinal *Ascaris lumbricoides* 12.1%, *Hymenolepis nana* (4.4%), and *Schistosoma mansoni* (3.2%).

**Conclusion:** Inexperienced and poor personal hygienic food handlers play a role for transmission of food borne infections. Local health authorities should implement food handler’s training on food safety, institute periodic focused medical check-up for food handlers and improve human waste disposal.

**Keywords**: Food handlers, intestinal parasites, *S. typhi*, Ibb city, Yemen

**INTRODUCTION**

Food borne diseases are a public health problem in developed and developing countries. The World health organization (WHO) expected that in developed countries, up to 30% of the population suffer from food borne diseases each year, whereas in developing countries up to 2 million deaths are estimated per year 1. Intestinal parasitic infections are public health problems especially in the developing countries as Yemen. Studies indicated that intestinal parasitic infections result in malnutrition, morbidity, mortality and socioeconomic impact owing treatment cost and hospitalization 2, 3. Intestinal parasites, which have direct life cycle, are transmitted by faecal oral route to human through poor personal hygiene4. *S. typhi* is one of the major causes of food and water borne gastroenteritis in human5 and remains an important health problem in Yemen and worldwide. The World Health Organization estimates 16 million new cases and 600,000 deaths of typhoid fever were estimated each year1. The emergence of antimicrobial resistant *S. typhi* including to chloramphenicol has been an issue4, 6. Studies had demonstrated that food handlers harbor *S. typhi* asymptomatically7. Food handlers with poor personal hygiene and inadequate knowledge on food safety could be the source of food borne pathogens1. The consequence of food contamination varies among countries and regions of the world depending on climate, geography and degree of social and economical development1, 2. In Ibb city, Yemen, eating and drinking in food services establishments, such as hotels, restaurants and snack shop is becoming a common practice. Information on intestinal parasites, *S. typhi* and risk factors among food handlers in the study area is limited. Thus, this study was aimed to determine the prevalence of intestinal parasites, S *.typhi* and exploring risk factors among food handlers working in food service establishments in Ibb city, Yemen.

**SUBJECTS AND METHODS**

**Study population:** This cross sectional study was carried out during a period of one year, starting in 1-2- 2019 and ending in 1-2-2020. Three hundred and fifteen food handlers were included, at age groups ranges from 14 to 65 years. All individuals were working in restaurant, cafeteria or schools buffets in Ibb city.

**Sample size:** The sample size was calculated in Epi Info 6 version 6.04 taking into consideration the following: The size of population from which the sample selected was 5000. The expected frequency of the factor was 5%. If 5% is the true rate in the population and the worst acceptable resent is 1%, with confidence level of 99%, the sample size would be not less than 302 selected individuals. The number increased to 315 to have more precise results.

**Data collection:** A full history of risk factors of contracting infections among food handlers and their demographic data were taken from each studied individual; and the findings were recorded in a predesigned questionnaire. The data collected included name, age at the time of the study, sex, residence, occupational status, and personal hygiene practices, history of typhoid, intestinal protozoa infections and intestinal parasitic infections etc. Also laboratory results of stool investigations, stool culture and ELISA IgG for typhoid were included in this questionnaire.

**Laboratory methods**

**Collection and transferring stool samples:** Stool specimens were collected from food handlers in Ibb city. Specimens were collected in sterile screw caped containers. Then prepared for microscopic examination and bacteriological culturing.

**Microscopically:** Each fresh sample were examined microscopically for cysts and Trophozoites of *Entamoeba histolytica*, and *Giardia lamblia* by using a saline and trachoma stain and examined specimens by concentration method for cysts of *Entamoeba histolytica*, and *Giardia lamblia* and intestinal helminthes8.

**Isolation of Pathogenic Bacteria:** All specimens were cultured in various selective media such as; xylose lysine deoxycholate agar (XLD), MacConkey sorbitol agar, and selenite broth. Plates were incubated for 18 hours at 37°C aerobically, the selenite broth then subculture onto *Salmonella-Shigella* agar (S.S agar)8.

**Identification of Isolated Bacteria:** Colonies had been identified based on morphologic characteristics and other standard Biochemical reaction, Motility Indol Urea (MIU), Kligler Iron Agar (KIA) and Oxidase tests are recommended to identify or differentiae Species of bacteria8.

**Detection of *Salmonella* Spp (pathogenic strains):** Isolated *Salmonella* Spp were examined by *Salmonella* Vi Antisera.

**Widal test:** Widal test is an agglutination test which detects the presence of serum agglutinins (H and O) in serum with typhoid and paratyphoid fever. The food handler's serum was tested for O and H antibodies (agglutinins) against the following antigen suspensions (stained suspensions): (antibodies titer higher than 1/80): S***. typhi* 0 antigen suspension** 9, 12, S***. typhi* H antigen suspension, d,** S***. Paratyphoid A O* antigen suspension, 1, 2, 12;** S***. Paratyphoid A H* antigen suspension, a**

**ELISA IgG for typhoid:** Total anti-bodies IgG agonist *typhoid* and paratyphoid quantitative were determined by an Enzyme-linked immunosobent assay (ELISA) using a commercially available kit provided by Biokit, Spain.

**Ethical consideration:** Consents were taken from all the participants and the participants were informed that participation is voluntary and that they can refuse without giving any reason.

**RESULTS**

The tested food handlers ages were ranged from 14 to 65 years old, most of individuals were in age groups of 20 -29 years (40 %), followed by age group 30-39 years (30.2 %). The mean age ±SD for our tested food handlers was 31.2 years ± 11.9 years (Table1). Most of individuals had primary school level (46.3 %), followed by illiterate level (30.2 %), but secondary level and higher were only 13% and 10.5% respectively (Table 2). The prevalence of *S. typhi* positive stool culture was 7.3% and the prevalence of *Shigella* species positive stool culture was 0.95% (Table 3). The highest prevalence of antibodies against S**. *Typhi O* antigen suspension** 9, 12 was 18.4%, while antibodies against S***. Typhi H* antigen suspension, d** were only 7.6%. Also the prevalence of antibodies against S**. *Paratyphoid A O* antigen suspension, 1, 2, 12** and *S****. Paratyphoid A H* antigen suspension were 7.6% and 6.3% respectively.**  The positive rate of ELISA IgG total *S. typhi* antibodies was 9.5% and the positive rate of stool culture *S. typhi* was 7.3% (Table 4). The total prevalence of intestinal protozoa was 20%; *Entamoeba histolytica* was 15.6%, in and *Giardia lamblia* was 4.4% (*Trophozoites* was 3.5%, *Cysts* was 4.4%) (Table 5). The total prevalence of intestinal parasites was 19.7%; *Ascaris lumbricoides* was 12.1%, *Hymenolepis nana* (4.4%), and *Schistosoma mansoni* (3.2%) (Table 6). In hand washing practices, 210 (66.7%) food handlers had a habit of hand washing by water only after toilet. However only 189 (60%) food handlers had a habit of hand washing by water and soap after toilet. However, a less number (49.8%) of food handlers had a habit of hand washing after touching dirty materials and different body parts (hair, nose and ear) between handling of food items. Only 31(9.8%) of the participants had had medical checkup including stool examination previously. Two hundred and ten (66.7%) food handlers were certified for training in food handling and preparation (Table 7). When we considered sources of water use in the restraints 80% of sites were used tape water, while 20% of the sites were using tank water. 60 % of food handlers were wearing special food clothes and 40% not practices that 79% of participants using reuse plastic tools. There were only 6 (1.9%) food handlers had past history of *typhoid* (Table 8).

**DISCUSSION**

 The current study established the Salmonellae carriage among a population of food handlers in Ibb city, Yemen was 7.1%. This high rate contrasts with the rate of 0.13% quoted for the developed world9,10, and is interestingly higher than the rate of 1.7% of Abera *et al.*11 in Ethiopia and 3.2% of Mensah *et al*. in Accra12. Yet others; Gelosa *et al.* in Italy13, and Yamada *et al.* in Tokyo14 have reported only 1.68% and 0.7% respectively. High prevalence of carriage intestinal *S. typhi* in our study is attributed by poor personal hygienic practices, poor environmental sanitation and absent of policy regulates food safety in Yemen. The current result also confirmed the finding of Tsen *et al.*15, and Turki *et al.*16 in which they found that *S. typhi* is one of the major causes of food and water borne gastroenteritis in human and remains an important health problem worldwide. Also the World Health Organization estimates 16 million new cases and 600,000 deaths of typhoid fever were estimated each year17. Studies had demonstrated that food handlers harbor *S. typhi* asymptomatically9. The high rate of carriage of *S. typhi* might lead to outbreak of typhoid in Ibb city, a report from Spain18 where one chronic carrier, a casual food handler, was shown to have infected 70 others, still highlights the continued importance of chronic *Salmonellae* carriers especially food handlers in the spread of the disease a fact that has been long established12, 14, 19. An attempt was also made through this study to establish the *Salmonella* carriage among a group of food handlers in the city of Ibb, Yemen by Widal test; 18.4% has been identified. Regardless of the constraint, the obtained value is thus a partial reflection of the expected total20. In the publication, Frimpong *et al.*20 proposed to be adopted for the diagnosis of enteric fever, Widal titer 1/160 and 1/320 for anti-O and anti-H, respectively. Remarkably, food handlers with positive stool cultures of non- *typhoidal Salmonellae* had a low titer. A rate of 9.5% (Table 4) was determined by ELISA for typhoid. The previous method of using an antibody detection assay was recently introduced in favor of using stool cultures and an ELISA assay, although only with limited differentiation of carriers and prior infection21. So the method used is appropriate. Regardless of the constraint, the obtained value is thus a partial reflection of the expected total21. In our finding, all food handlers who had a culture positive for the proposed ELISA IgG antibody suggested adoption for the diagnosis of enteric fever.

 In this study, the total prevalence of intestinal *protozoa* was 20%. The prevalence of intestinal *Entamoeba histolytica* was 15.6%, in which Trophozoites was seen in 1.3% only and cyst was seen in 15.2% (Table 5). However, a low prevalence of *Giardia lamblia* present among food handlers in which it was 4.4%. The overall prevalence of intestinal *protozoa* among food handlers in our study was similar compared to previous study done at Gondar town (20.1%) in North West Ethiopia22, but lower than that reported by Abera *et al.* in Bahir Dar Town, Ethiopia in which 6.5% food handlers had intestinal *protozoa* 11. High prevalence of intestinal *protozoa* is attributed by poor personal hygienic practices and poor environmental sanitation. Active *Trophozoites* forms of *E. histolytica*, *and G. lamblia* were associated with diarrheic food handlers. *G. lamblia* infected food handlers can directly transmit to consumers if ingested *via* contaminated food and water because *G. lamblia* cysts does not need environmental maturation8. Moreover, Mintz *et al.* found that food handlers infected with *G. lamblia* were a vehicle for *Giardia* outbreak in commercial food establishment 23. Thus, food handlers should be in a good health and those suffering from diarrhea must be excluded from work until they have been completely free of symptoms after treatment. In this study, most food handlers working in the kitchens were very young adults in age groups of 20 -29 years (40 %), (table 1) and the majority had inexperienced with low educational levels, in which most of individuals had only primary school level (46.3 %), or illiterate (30.2 %) (Table 2), which agrees with previous studies in developing countries11, 16, 24, 25.

 In our study only 31(9.8%) of the participants had had medical checkup including stool examination previously. Two hundred and ten (66.7%) food handlers were certified for training in food handling and preparation. However, in developing countries in Africa and Asia from 22.7% to 46% of the food handlers had medical checkup including stool examination in the past11, 24, 25. Assessment of hand washing practices revealed varied results in our study 210 (66.7%) food handlers had a habit of hand washing by water only after toilet. However only 189 (60%) food handlers had a habit of hand washing by water and soap after toilet. Our results were in parallel with the previous reports in Ethiopia and India[11, 22, 25. However, less had practices of hand washing after touching dirty materials and different body parts between handling of food items. These reflected that food handlers lack awareness about food contamination with poor hygienic practices. Health education intervention on food safety and hygiene must be strengthening to ensure food safety during processing, preparation and storage in food services establishments.

**LIMITATION OF THE STUDY**

Despite the many recent studies that discussed food borne diseases, gastrointestinal infections, bacterial infections and protozoa in the digestive system in Yemen26-39, the current study did not include other bacterial and viral infections that may be transmitted from workers in restaurants to customers from community members, and therefore these shortcomings must be taken into account in this study. We recommend that different techniques be evaluated more systematically to study this health problem and include infectious pathogens that have been performed among other populations previously in Yemen and performed among food handlers.

**CONCLUSIONS**

In conclusion, *salmonella typhi* intestinal carriage rate, the intestinal protozoa infections and intestinal parasitic infection rates of food handlers of Ibb city were relatively high. The findings emphasize that food handlers with different pathogenic microorganisms may predispose consumers to significant health risks. Therefore, constant epidemiological surveillance through biannual routine parasitological tests and treatment of the infected cases along with the improvement of environmental sanitation are recommended to control *salmonella typhi*, the intestinal *protozoa* infections and intestinal parasitic infection in food handlers in Ibb city. Inexperienced and poor personal hygienic food handlers play a role for transmission of food borne infections. Local health authorities should implement food handler’s training on food safety, institute periodic focused medical check-up for food handlers and improve human waste disposal.

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**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

**AUTHOR CONTRIBUTIONS**

This research is part of a master's degree in the Department of Medical Microbiology, first author GMA, who conducted field work, and who did laboratory work and other authors contributed to data analysis, drafting and review of the paper, and gave final approval to the research.

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Table 1: Age distribution of the food handlers whom tested for intestinal S.typhi and parasitic positivity among 315 food handlers in Ibb city - Yemen.

|  |  |
| --- | --- |
| Age groups | Total (n =315 ) |
| No. | % |
| < 20 years | 31 | 9.8 |
| 20-29 years | 126 | 40 |
| 30 -39 years  | 95 | 30.2 |
| ≥ 40 years | 63 | 20 |
| Mean Age | 31.2 years |
| SD | 11.9 years |
| Min | 14 years |
| Max | 65 years |

Table 2: Educational distribution of the food handlers whom tested for intestinal S.typhi and parasitic positivity among 315 food handlers in Ibb city - Yemen.

|  |  |
| --- | --- |
| Educational level  | Total (n =315 ) |
| No. | % |
| Illiterate | 95 | 30.2 |
| Primary School | 146 | 46.3 |
| Secondary  | 41 | 13 |
| Higher | 33 | 10.5 |

Table 3: The prevalence of intestinal Salmonella typhi and Shigella species positive culture among 315 food handlers in Ibb city, Yemen

|  |  |
| --- | --- |
| Bacteria | frequency |
| Number | percentage |
| Salmonella typhi positive culture | 23 | 7.3 |
| Shigella species positive culture | 3 | 0.95 |

Table 4: The prevalence of Salmonella typhoid antibodies by Widal test, IgG anti-salmonella antibody by ELISA and stool culture among 315 food handlers in Ibb city, Yemen

|  |  |
| --- | --- |
| Markers | frequency |
| Number | percentage |
| Widal test ( antibodies titer higher than 1/80) |
| S**. *Typhi O* antigen suspension** 9,12 | 58/315 | 18.4 |
| S**. *Typhi H* antigen suspension, d**  | 24/315 | 7.6 |
| S**. *Paratyphoid A O* antigen suspension, 1, 2, 12** | 24/315 | 7.6 |
| S**. *Paratyphoid A H* antigen suspension, a**  | 20/315 | 6.3 |
| ELISA IgG total *S.typhi* antibodies | 30/315 | 9.5 |
| Positive stool culture for *S. typhi* | 23/315 | 7.3 |

Table .5: The prevalence of intestinal protozoa among 315 food handlers in Ibb city, Yemen

|  |  |
| --- | --- |
| Protozoa | frequency |
| Number | percentage |
| *Entamoeba histolytica* *Trophozoites**Cysts* | 49/3154/31548/315 | 15.61.315.2 |
| *Giardia lamblia**Trophozoites**Cysts* | 14/31511/31514/315 | 4.43.54.4 |
| Total | 63/315 | 20 |

Table 6: The prevalence of intestinal parasites (helminthes) among 315 food handlers in Ibb city, Yemen

|  |  |
| --- | --- |
| Parasites | frequency |
| Number | percentage |
| *Ascaris lumbricoides*  | 38/315 | 12.1 |
| [*Hymenolepis nana*](https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjYkY3DyvTSAhVHhiwKHcNrB7EQFggZMAA&url=https%3A%2F%2Fen.wikipedia.org%2Fwiki%2FHymenolepis_nana&usg=AFQjCNFHmjgr9hIpSS8UGrq5fJZze3epPQ&bvm=bv.150729734,d.bGg) | 14/315 | 4.4 |
| *Schistosoma mansoni* | 10/315 | 3.2 |
| Total | 62 | 19.7 |

Table 7: The frequency of hygienic practices of food handlers in Ibb city

|  |  |
| --- | --- |
| **Variables** | **Frequency** |
| **No.** | **%** |
| Certified in food trainingYes  | 210 | 66.7 |
| No  | 105 | 33.3 |
| Hand washing after toilet by water onlyYes | 189 | 60 |
| No  | 126 | 40 |
| Hand washing after toilet by soapYes  | 60 | 20 |
| No  | 252 | 80 |
| Hand washing after touching dirty materialsYes  | 157 | 49.8 |
| No  | 158 | 50.2 |
| Touching body parts during food handlingYes  | 221 | 70.2 |
| No | 94 | 29.8 |
| Medical check upYes  | 31 | 9.8 |
| No  | 284 | 90.2 |

Table 8: The frequency of factors that affect spread of bacterial and protozoa as source of water etc. among food handlers in Ibb city

|  |  |
| --- | --- |
| **Variables** | **Frequency** |
| **No.** | **%** |
| Source of waterTape water | 252 | 80 |
| Tank water | 63 | 20 |
| Wearing food clothesYes  | 189 | 60 |
| No =126 | 126 | 40 |
| Reuse plastic toolsYes  | 249 | 79 |
| No | 66 | 21 |
| Past History of *typhoid*Yes | 6 | 1.9 |