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Asymptomatic Carriage of Salmonella and Intestinal Parasites in Pupils in Yaounde

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Abstract

Background: Feco-oral transmitted diseases (FOTD) remain a public health issue, particularly in developing countries. Data concerning the carriage of *Salmonella* and intestinal parasites in children are available worldwide but are lacking in Cameroon. This study aimed to determine the asymptomatic carriage of *Salmonella* and intestinal parasites in children of two primary schools in Yaoundé. **Methods:** A cross-sectional descriptive study was conducted from October 2017 to May 2018 in two primary schools (from rural and urban areas) in the 7th precinct of Yaoundé. Sociodemographic, clinical and paraclinical (rectal swab, direct examination of fresh stool and bacteriological culture on Hektoen medium) data were collected. **Results:** We included 368 (192 boys) pupils from both schools (184 in each school) with a mean age of 8.99 ± 2.21 years. None of the children was infected by *Salmonella spp*. Intestinal parasite prevalence was 9.80% (6.52% of children from the urban school vs 13.04% from the rural ones). The intestinal parasite prevalence tended to be higher in girls than in boys (11.98% vs. 7.39%). Among intestinal parasites, protozoa were the most widely found. *Entamoeba histolytica* and *Giardia intestinalis* were the most prevalent pathogenic intestinal protozoa (11.11% vs. 25% of all positive stool exams). The helminths, less frequent, were represented by *Ascaris lumbicoides* and *Enterobius vermicularis.* The factors associated with intestinal parasite carriage were mainly rural school location and age between 11 - 13 years. **Conclusion:** Among children in primary school, *Sal-*

monella infection was absent, while intestinal parasites are frequent, represented mostly by protozoa. This parasitism is in our cohort associated with the rural school location and the 11 - 13 years age group. This suggests that there is additional room for the implementation of prevention measures for intestinal parasite infections in our setting.

Keywords

Stool Culture, Intestinal Parasites, Pupils

1. Introduction

Feco-oral transmitted disease (FOTD) refers to the risk of contracting a viral, bacterial or parasitic infection carried by excrements of sick or asymptomatic people or animals [1]. Intestinal parasites and enteropathogenic bacteria are directly or indirectly transmitted through food, water and fingers [2]. Whatever the infectious agent involved, the consequences are digestive disorders, particularly diarrheal episodes which can progress to dehydration or even malnutrition in children [3].

Enteropathogenic bacteria and intestinal parasites remain by far a public health problem in Sub-Saharan Africa (SSA). The incidence of *Salmonella spp* infection in developing countries is 540 cases per 100,000 inhabitants (versus 0.2 cases/100,000 in temperate countries). In the most affected regions, the peak incidence occurs among children and adolescents aged from 2 to 15 years [4]. Among the *Salmonella* infections, *S. Typhi* is responsible for approximately 21 million new infections each year. Annual mortality of this infection has increased by 39% from 1990 to 2010 and is estimated to be more than 190,000 worldwide [5]. *Salmonella* infections are endemic in developing countries and associated with high mortality [6]. On the other hand, intestinal parasitic infection (IPI) is one of the leading causes of childhood morbidity and mortality worldwide [7]. It is estimated that three and a half billion people are infected with 450 million people symptomatic; the majority being children [8]. On the educational level, the impact of IPI is noticeable with the decline in the child's physical and intellectual capacities [9].

The particularity of these infections is that there are healthy carriers for both *Salmonella* and intestinal parasites. In fact, after healing from typhoid fever 2% - 5% of individuals continue to harbor *S. Typhi* which are excreted episodically in the stool and which can, therefore, be the cause of secondary cases [5]. This asymptomatic carriage is common among children of poor neighborhoods as demonstrated by Praharaj *et al.* in India [10]. In Africa, Le Noc *et al.* found an asymptomatic carriage of *Salmonella* in 2.6% of primary school children while the prevalence of 39.7% was found in the city of Buea [11] [12]. Concerning IPI, a prevalence of 11.6% was found in a children's population in Cameroon [13]. Asymptomatic carriers for *Salmonella* and IPI represent a risk for the commu-

nity (as they contribute to the persistence of the endemic state in households) and risk for the carrier (particularly in immunocompromised patients, who are more exposed to invasive salmonellosis and malignant anguillulosis) [14]. However, data on the asymptomatic carriage of *Salmonella* and IPI are rare in our setting. It was, therefore, important to determine their prevalence and identify associated risk factors. This process could reduce the burden of enteropathogens in a limited-resources setting.

2. Methods

Study Design and Setting: A cross-sectional descriptive study was carried out in two public primary schools in Yaoundé (Minkoa Meyos II for the rural part and Nkolbisson II-A for the urban part) from October 2017 to May 2018. These schools are located in the 7th precinct of Yaoundé, the capital city of Cameroon, with a catchment of two million inhabitants.

Study Population: The children attending both educational institutions were recruited after the obtention of parents' and administrative authorizations. The precinct, school and educational level of pupils were chosen after a random sample drawn at each level. Detection of IPI was done using a direct examination between slide and cover slide under photonic microscopy while *Salmonella spp* diagnosis was performed using stool culture on Hektoen medium after enrichment on Mueller-Kaufman medium.

Variables and Measurements: All pupils, whose parents/legal guardians had signed an informed consent form, were included. All children whose parents refused their participation or whose consent was withdrawn during the study or were on antibiotics or who presented fever, vomiting or diarrhea were excluded from the study. The screening was conducted by trained medical personnel. All participants (and their parents) were subjected to a face-to-face interview. Data were collected (using a standardized questionnaire) on sociodemographic characteristics (age, gender, vaccination status, risk factors for enteropathogens), social characteristic (availability of water at home or canteens at school). Clinical data were also recorded (general status, weight, abdominal exam findings) as well as bacteriological and parasitological parameters (type of analysis performed, cell morphology).

Laboratory Testing: For each patient, two stool samples were collected, using rectal swabs. The swabs were transported in their packaging to the bacteriology laboratory of the Yaoundé University Teaching Hospital (YUTH) where the analysis was carried out immediately. One rectal swab was used for direct examination under optic microscopy, in order to detect parasites or yeast cells. The other rectal swab was used for performing stool culture. Enrichment was done on Mueller-Kauffmann medium then subculturing was carried out on Hektoen agar with a drop of the solution after a maximum of three to six hours of incubation at 37˚C. The research for *Salmonella spp* on this medium was orientated by the appearance of the colonies. The likely colonies were H_2S-positive and lac-

tose-negative. At least five likely isolated suspicious colonies were identified. We tested for urease on the urea-indole medium. This was carried out on each suspect colony and incubated at 37˚C for 24 hours. Urease-positive colonies (urea-indole medium in two hours) were eliminated. We inoculated a classic mini gallery with urease negative colonies from the indole urea medium. The results were reported on the lab bench and the identification form.

Definitions: IPI was defined by the identification of parasites at the direct stool test (either protozoa or helminths). *Salmonella* infection was defined by the identification of any *Salmonella colony* on the culture medium.

Sample Size and Statistical Analysis: The sample size was calculated using Lorenz's formula (StatCalc of Epi Info software). Using a previous prevalence of 39.7% of *Salmonella* infection in Buea (Cameroon) [12], with an 80% power to detect associations or differences and a 5% accepted margin of error, the minimal sample size estimated was 368 participants. Data were analyzed using EXCEL 2016 and EPI-INFO v.3.5 software. Discrete variables were presented as counts and percentages, and continuous variables as mean (standard deviation). The Chi-square test was used, and also the Student t-test where appropriate.

3. Results

Characteristics of the Study Population

We included 368 (192 boys) pupils from both schools (184 in each) with a mean age of 8.99 ± 2.21 years. The most frequent age group was 8 - 10 years with 154 (41.85%) participants (**Figure 1**). They included pupils who were respectively recruited in the following classes: class 1 (n = 31), class 2 (n = 32), class 3 (n = 30), class 4 (n = 31), class 5 (n = 32) and class 6 (n = 28).

School and Clinical Characteristics

Schools had no running water or toilets with wet excreta disposal. Latrines were present at the urban school but not in the rural one. There was no canteen

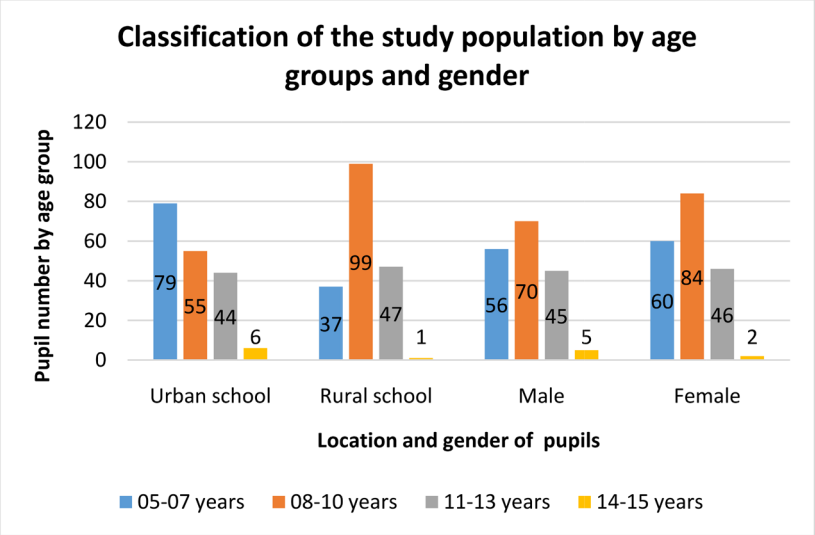

Figure 1. Distribution of the study population by age groups and gender.

in both schools, children have to buy food from itinerant traders. None of the children lived near a farm. All children had been vaccinated as recommended by the national vaccination planning. They all said that they followed basic hygiene rules. The abdominal exam was normal for all children (no tenderness, no mass).

Bacteriological and Parasitological Profile

None of the stool samples was positive for *Salmonella* after stool culture.

The overall prevalence of IPI was 9.80% (6.52% in the urban school and 13.04% in the rural school, p = 0.026). This prevalence tended to be higher in females (11.98%) than males (7.39%) however, no evidence of statistical significance was found (p = 0.095). Monoinfection was found in 33 (91.66% of all infected children) children while coinfection was found in 3 (8.34%).

Among the parasites, we found 8.42% of protozoa (5.43% in the urban school and 11.41% in the rural one) and 1.35% of helminths (1.08% in the urban school and 1.63% in the rural one). This protozoa prevalence was significantly higher in the rural than the urban one (p = 0.028), unlike helminths whose prevalence was almost similar in the two groups. In monovariate logistic regression analysis, the 5 - 7 years (p = 0.008) and 11 - 13 years (p = 0.023) age groups were associated with IPI. However, the 5 - 7 years age group tended to be protective of IPI. Table 1 summarizes the factors associated with IPI.

The pathogenic protozoan species were represented by *Entamoeba histolytica* and *Giardia intestinalis* representing respectively 11.11% and 25% of positive stool exams. However, *Entamoeba coli* was the most common protozoa found in stool exams. The helminths were represented by *Ascaris lumbricoides* and *Enterobius vermicularis* respectively 5.56% and 8.33% of the positive stool exams.

Table 1. IPI associated risk factors.

Variables	Overall	IPI+, n(%)	IPI-, n(%)	OR (95% CI)	P-value
Sex					
Male	176 (100)	13 (7.4)	163 (92.6)	0.61 (0.30 - 1.26)	0.095
Female	192 (100)	22 (11.5)	170 (88.5)	1.61 (0.78 - 3.30)	
Age groups					
5 - 7 years	116 (100)	5 (4.3)	111 (95.7)	0.33 (0.13 - 0.88)	0.008
8 - 10	156 (100)	16 (10.3)	140 (89.7)	1.16 (0.60 - 2.33)	0.338
11 - 13	96 (100)	14 (14.6)	82 (85.4)	**2.14** (1.03 - 4.43)	0.023
School					
Rural	184 (100)	23 (12.5)	161 (87.5)	**2.04** (0.98 - 4.25)	0.026
Urban	184 (100)	12 (6.5)	172 (93.5)	0.48 (0.23 - 1.01)	
Latrine					
Yes	184 (100)	12 (6.6)	172 (93.5)	0.48 (0.23 - 1.01)	0.026
No	184 (100)	23 (12.5)	161 (87.5)	**2.04** (0.98 - 4.25)	

Interestingly, the prevalence of *Blastocytis hominis*, whose pathogenicity is controversial, is similar to that of Entamoeba. The prevalence of all parasites is presented in Figure 2.

4. Discussion

We conducted this cross-sectional descriptive study for assessing the prevalence and associated factors of asymptomatic carriage of *Salmonella* and IPI in a pupil population from two primary schools in a sub-Saharan African setting. This study revealed no asymptomatic carriage of *Salmonella*, a prevalence of IPI of 9.80% (with protozoa as the main intestinal parasites) and an association with 8 - 10 years age group and rural school.

We did not find any asymptomatic carriage of *Salmonella* in our study. This result differs from that of Devi *et al.* and Le Noc *et al.*, who respectively found a *Salmonella* carriage in 1% of pupils in India, and 2.6% of pupils in Cameroon and Ivory Coast [11] [15]. This difference could be explained either by the intermittent excretion of *Salmonella* in the stool [5]. Similarly, this result is lower than the 39.7% of carriage found in Buea (Cameroon) [12]. The difference in age group could also explain this discrepancy, as the study in Buea was carried out on the global population and knowing that the risk for chronic carriage of salmonella increases with age [5].

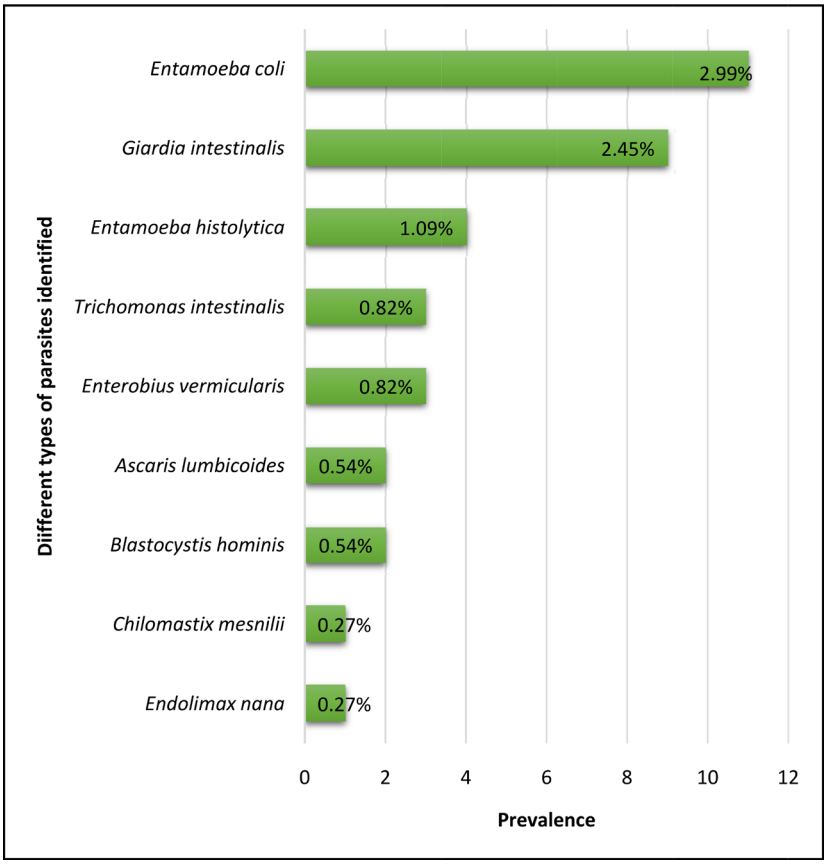

Figure 2. Prevalence of identified species of intestinal parasites.

We found an IPI prevalence of 9.80% in this pupil population (these subjects harbor one or more intestinal parasites). This prevalence rate is lower than that of Ahmed Salem *et al.* (33.4%), in 2012, among school children of the rural area of Mauritania and that of Adou-Bryn *et al.* (38.9%) among school children in Ivory Coast in 1997 [16] [17]. Our prevalence is comparable to that found by Saotoing *et al.* (10.74%) in schoolchildren of the Far-North region, in Cameroon [18]. This difference could be explained by the systematic deworming program initiated in schools' settings in Cameroon since 2004 and the laboratory methods (they used concentration and enrichment techniques for improving their results).

Children from the 11 - 13 years age group seem to be more infested than the other age groups. This finding is comparable to that of Adou-Bryn *et al.*, who described that the 11 - 13 years age group was most affected by IPI [17]. Ahmed Salem *et al.*, however, found that children who are aged 10 years or less were the most infested [16]. These results could be explained by the fact that between 11 - 13 years, it is the beginning of adolescence and this age group has less awareness of disease transmission methods and less adherence to hygiene measures.

IPI prevalence was statistically higher in rural than in urban areas ($p = 0.026$). This observation was similar to that made by Lehman *et al.* who found a prevalence of 14.67% in Douala and 39.22% in Njombé ($p < 0.001$) [19]. This result could be explained in our study by the absence of latrines in rural areas, the absence of water and the presence of itinerant traders.

IPIs among children in Yaoundé were mainly protozoan (protozoan prevalence 8.4%). This finding is similar to that of Saotoing *et al.* (8.52%) and Adou-Bryn *et al.* (69.8% of the parasites encountered) [17] [18]. This high rate of intestinal protozoan diseases indicates the high level of contamination of water and food with feces and the lack of hygiene and sanitation measures in these regions [20].

The pathogenic protozoan species were represented by *Entamoeba histolytica* and *Giardia intestinalis*. A similar observation was made by Ahmed Salem *et al.* who found a prevalence of 3.75% for *Entamoeba histolytica* and 9% for *Giardia intestinalis* [16]. The same protozoa in different proportions (30.7% for *Entamoeba histolytica* and 5.5% for *Giardia intestinalis*) were found by Bamba *et al.* [21]. The prevalence of helminths in our study was 1.35% represented by *Ascaris lumbicoides* and *Enterobius vermicularis* (pinworm). This result is different from that of Adou-Bryn *et al.* (10.4% for helminths represented by *Trichuris trichiura*, *Hymenolepis nana*) and of that from Kyambikwa *et al.* (73.8% for helminths, represented by *Strongyloids stercoralis, Ascaris lumbricoides*, *Trichuris trichiura*, and *Taenia saginata*) [17] [22]. It is surprising to have cases of *E. vermicularis*. This should be because we used the swabs method which is close to the scotch test, the recommended technique for the search of *E. vermicularis.*

This study should be interpreted in light of some limitations. The stools were collected by swabbing which did not allow us to have a sufficient quantity of stool to carry out the stool concentration methods. These limitations could lead

to an underestimation of the prevalence of intestinal parasites.

5. Conclusion

This study suggests that among pupils in Yaoundé, *Salmonella spp* infection is zero prevalent, while intestinal parasites are frequent. This parasitism is essentially protozoan and associated with the rural area and the 11 - 13 years age group. Regular screening followed by adequate treatment associated with water supply, toilet facilities and environmental hygiene will ensure the prevention of these parasitic infestations.

Availability of Data and Materials

The dataset analyzed during this study is available from the corresponding author on a reasonable request.

Authors' Contribution

Conception and design: RNM, MT, HG. Data collection: RNM. Administrative support: MT, HG. Data analysis and interpretation: RNM, SRSN, NCNA. Drafting of the manuscript: SRSN, RNM, NCNA. Reviewing manuscript: MT, ELM, HG. All the authors read and approved the final draft for publication.

Ethical Consideration

This work was approved by the institutional review board of the Faculty of Medicine and Biomedical Sciences, Yaoundé, Cameroon (registration number 0333/UY1/FMSB/VDRC/CSD). Administrative authorization from the Yaounde University Teaching Hospital, the Ministry of Basic Education, the Regional delegate for basic education and the Inspector of Basic Education of the Yaoundé 7th precinct and parents' consent were obtained before collection. This work was carried out in accordance with the declarations of Helsinki [23]. Patients were free to attend the study without any outside constraint. Consent forms were obtained, informed and signed by each participant's parent. This work is reported in compliance with the STROBE checklist.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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List of Abbreviations

FOTD: Faeco-oro transmitted disease

IPI: Intestinal parasite infection

SSA: Sub-Saharan Africa

SPSS: Statistical Package for Social Sciences