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Prevalence of *Salmonella enterica* Subspecies *enterica* Serovar Typhi *(Salmonella typhi)* Infection in Febrile Patients at the Sino-Gabonese Friendship Hospital in Franceville: A Two-Year Retrospective Study in South East Gabon

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ABSTRACT

Introduction: *Salmonella enterica* subspecies *enterica* serovar Typhi *(Salmonella typhi)* causes typhoid fever. This disease is a global public health problem, particularly in developing countries like Gabon. Unfortunately, to our knowledge, little information exists in the epidemiological literature on the prevalence of *Salmonella enterica* infection in patients presenting with febrile illness in Gabon, particularly at the Sino-Gabonese Friendship Hospital in Franceville, in the southeast of the country. **Methods:** A retrospective, cross-sectional study was conducted using the "Widal and Felix" serodiagnostic test results of febrile patients archived in the Medical Analysis Laboratory Records of the Sino-Gabonese Friendship Hospital in Franceville from June 2019 to May 2021. **Results:** The prevalence of *Salmonella typhi* in febrile patients was 58.88% [95% CI: 0.5 - 0.6]. The participants' age ranged from 15 to 49 years (mean 24.79 years). We observed that with 1724 cases and a percentage of 62.15% ($P < 0.001$), women were more infected than men, and the most affected age group was 14 to 49 years, with 1379 cases (49.71%). During the study period, the seasonal distribution of *Salmonella typhi* infection indicated that quarter 6, corresponding to the small rainy season, with 16.58%, i.e., 460 cases, had the highest cases. **Conclusion:** These results highlight the need to strengthen food safety hygiene in community markets and environmental sanitation to break the *Salmonella typhi* transmission in Franceville, Southeast Gabon.

INTRODUCTION

Salmonella belongs to the family Enterobacteriaceae and has more than 2500 serotypes or serovars within two species, *Salmonella bongori* and *Salmonella enterica* [1]. Classified into two major groups, we distinguish the group of the invasive form and the group of the non-invasive form [2]. *Salmonella* is a gram-negative bacterium responsible for typhoid fever, an infection associated with fecal peril [3], and people living in poor socio-economic conditions and with no hygiene are the known natural hosts and reservoirs for *Salmonella typhi* infections [4]. Caused by *Salmonella enterica* serovar Paratyphoid A (SPA), B, C, paratyphoid fever is an infection very similar to typhoid fever but often less severe [4]. Typhoid fever is an acute, febrile, and potentially fatal disease with a real impact. The symptoms of this disease are similar to those of many other febrile infections [5]. In the absence of prompt treatment, typhoid fever is likely to result in death, and without effective treatment, it has a case fatality rate of 10%-30% [6]. Despite the availability of antibiotics and various prevention methods, nearly 80% of cases and deaths still occur in Asia, with the remainder occurring mainly in Africa and Latin America [7]. Nowadays, estimating the real burden of this enteric fever is problematic in many developing countries, especially in sub-Saharan Africa. Limited or no adequate diagnostic resources and surveillance tools result in a poor characterization of the burden of enteric fever [7,8]. The risk of infection is high in low- and middle-income countries where typhoid Salmonella is endemic, sanitation is poor, and access to

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safe food and drinking water is limited [9]. Some studies in South Asia reported the highest rates of enteric fever in children under five years of age [10]. About 1% -5% of patients with acute typhoid infection become chronic carriers of the infection in the gallbladder, depending on age, gender, and treatment regimen. This course should include measures to reduce the spread of infection, including vaccination, hand washing, safer sex, and good food hygiene [11]. In Gabon, as in most African countries, the estimated annual incidence of typhoid fever was 10-100 cases per 100,000, with the highest in children [12]. Despite the existence of a study conducted in Libreville, which showed a worrying trend in the number of typhoid fever cases in Gabon [13], there is a lack of concrete data for this disease in the epidemiological literature due to the presence of other diseases and the absence of a coordinated epidemiological surveillance system in Gabon. As a result, there is no reliable national statistical data on its prevalence. However, to control the spread of typhoid fever, surveillance of *Salmonella typhi* and antimicrobial susceptibility testing are essential [14].

This study set the primary objective to determine the prevalence of S. typhi infection in patients presenting with febrile illness at the Sino-Gabonese Friendship Hospital in Franceville, South-East Gabon.

MATERIAL AND METHODS

Study setting. This work was performed in the laboratory of the Sino-Gabonese Friendship Hospital in Franceville. Located in the 2nd district, this hospital has an adequate technical platform that allows bacteriological examinations under good conditions.

Franceville is the provincial capital of Haut Ogooué, in the southeast of Gabon. The seasonal average maximum temperature is 31°C, and the minimum is 23°C, with an average of 27°C. As in all other cities in the interior of Gabon, the efforts of the political authorities do not prevent a not very good supply of drinking water in this city, which has urban slums and under-integrated neighborhoods as neighbors. Using contaminated water from the Mpassa River to wash clothes, dishes, or even drink makes the population very vulnerable to food and waterborne diseases [15].

Type, period, and study population. This retrospective, cross-sectional study was conducted between June 2019 and May 2021. It consisted of the 'Widal and Felix' serodiagnostic results of all febrile persons who had visited the Sino-Gabonese Friendship Hospital in Franceville for treatment during the study period. Therefore, the study was to know the number of typhoid fever cases recorded during the study period.

Sampling method. Purposive sampling was used to target and focus only on typhoid fever diagnostic results by 'Widal and Felix' serodiagnosis during the study period. To ensure the representativeness of the study, the sample size depended on the number of cases registered in the laboratory database of the Sino-Gabonese Friendship Hospital in Franceville. This study included variables such as gender, age, and serological diagnosis results from Widal and Felix.

In developing countries such as Gabon, bacterial culture remains expensive, and patients are usually seen at an advanced stage of the disease [16]. Therefore, the Widal-Felix serodiagnosis remains one of the most accessible means of diagnosis for most typhoid fever cases in our study site.

Serodiagnosis of typhoid fever. According to the manufacturer's protocol, the principle of typhoid fever serodiagnosis at the medical analysis laboratory of the Sino-Gabonese Friendship Hospital consists of performing the semi-quantitative Widal agglutination test on serum samples from each patient. This test consists of a series of tube dilutions of Salmonella typhi and paratyphi A, B, and C serotyping kits manufactured by Bio-Rad Laboratories France. It uses an antibody titer ≥1:80 for O antigen and ≥1:160 for H antigen as cutoff values for positive titers. A negative saline control is included for each batch of Widal tests [17].

Procedure for obtaining the data. The data used for the study were results from the medical analysis laboratory of the Sino-Gabonese Friendship Hospital in Franceville. Access to the data was granted by the hospital manager (letter No. 417/MS/SG/DRSSE/ HASG) authorizing the performance of this study. The extracted data were made available to us in digital form. All the results of typhoid fever examinations from June 2019 to May 2021 were extracted and used for the study.

Inclusion and exclusion criteria. Only the results of the "Widal et Félix" serodiagnosis, obtained from blood samples of all persons who came to the Sino-Gabonese Friendship Hospital in Franceville, were retained in this study. Indeed, the patient was considered positive when both agglutinins were positive for O, and H. Results from persons suspected of having typhoid but without laboratory confirmation and doubtful or incomplete results were excluded from the study.

Ethical considerations. The data received did not include the identity or personal information of the patients.

Statistical analysis of the data. Entered in a Microsoft Excel 2016 format, the data were then analyzed with R software version 3.6.1, including the measurement of rates and associations. An exact binomial test was used to determine correlations between typhoid fever prevalence and particular values. A 95% confidence interval was estimated, and a $P \leq 0.05$ value was considered statistically significant.

RESULTS

Demographic characteristics of patients. A total of 4711 patients consulted the medical analysis laboratory of the Sino-Gabonese Friendship Hospital during the study

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period. The distribution of these patients according to gender gave a sex ratio (F/H) of 1.58. The female gender was in the majority with 2884 cases (61.22%) against 1827 (38.78%) for men. With an average age of 24.79, the most significant number of patients was in the females compared to the males in all age groups except for the 0-4 years age group, in which 396 participants were recorded compared to 404 participants in the male gender (Table 1).

Table 1. Demographic characteristics of patients seen at the medical analysis laboratory of the Sino-Gabonese Friendship Hospital.

Age groups	Male	Female	Total (%)
0 – 4 years	404	396	800 (16.98)
5 – 14 years	525	571	1096 (23.27)
15- 49 years s	698	1421	2119 (44.98)
> 50 years	200	496	696 (14.77)
Total	1827	2884	4711(100%)

Patients distribution by gender. Figure 1 shows an overall prevalence of typhoid fever of 58.88% (2774/4711). With 1724 patients and a percentage of 62.15% ($P < 0.001$), females were more infected than males (37.85%, n=1050).

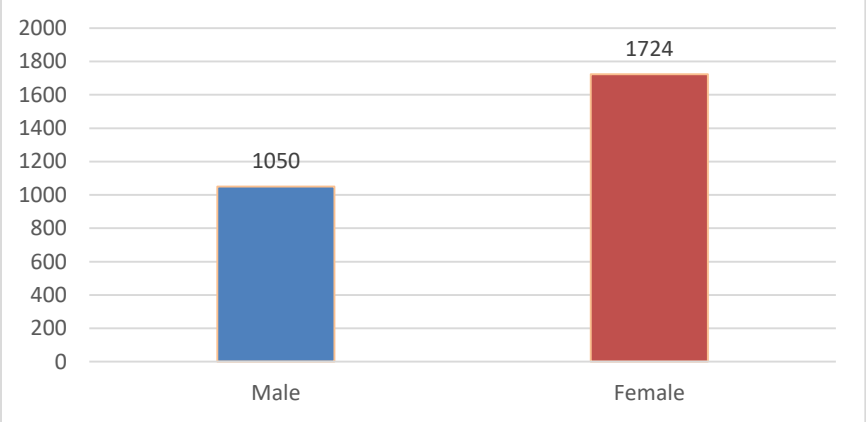

Fig. 1. Distribution of typhoid fever cases by gender

Patients distribution by age group. Among all age groups, the highest number of typhoid fever cases was among women compared to men. The age group most affected by typhoid fever was 15-49 years old, with 1378 cases (49.68%), followed by 5-14 years old, with 613 cases (22.1%). The age group least affected by typhoid fever was 0-4 years old, with 325 positive patients (11.72%), followed by 50 years old and above with 458 cases (16.51%). While the lowest percentage of typhoid fever cases in men was in the 50 and over age group, with 149 patients (14.19%), the most affected age group in men was the 15-49 years age group, with 435 patients (41.43%). The age group with fewer positive cases of typhoid fever in females was 0-4 years, with 164 patients (9.51%). As for men, the age group with the highest number of cases among women was 15-49 years, with 943 patients (54.70) Figure 2.

Correlation between typhoid fever, gender, and age groups. An exact binomial test with a 95% confidence interval was performed to analyze the significance level of differences observed in the percentages of typhoid fever cases in males versus females according to age groups. The test was considered significant when the $P \leq 0.05$.

The statistical analysis showed that men could be significantly affected by typhoid fever in a 50/50 ratio to women. However, during the study period, more typhoid fever cases were recorded in women than men, especially in the age group of 15-49 years (Table 2).

Patients distribution by quarter. The prevalence of typhoid fever showed an uneven distribution across the eight quarters that made up our study period (Fig. 3). The highest prevalence of typhoid fever cases was in quarter 6 (460 cases (16.58%), followed by quarter 3 with 402 cases (14.49%), quarter 7 with 373 cases (13.45%), Quarter 5 with 368 cases (13.27%), quarter 2 with 353 cases (12.73%), quarter 4 with 349 cases (12.58%), quarter 8 with 329 cases (11.86%) and finally quarter 1 with 140 cases (5.04%), was the one with the least number of cases.

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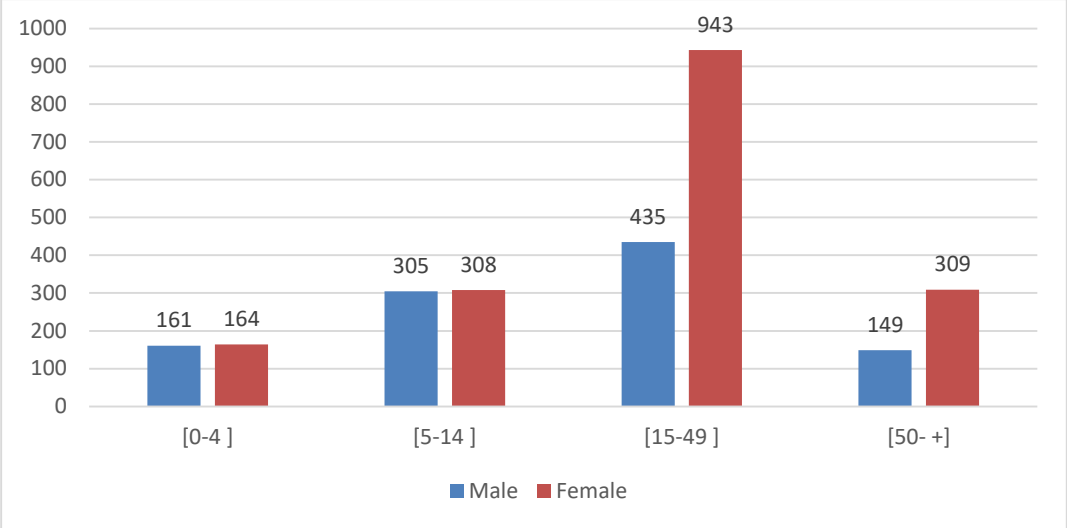

Fig. 2. Distribution of typhoid fever cases by age group

Table 2. Correlation between typhoid fever cases, gender, and age groups

Age groups	Male	Female	Total	Binomial test		
				p(H)	95%CI	*P*-value
0 – 4 years	161	164	325	0.49	[0.43 – 0.55]	0.9117
5 – 14 years	305	308	613	0.49	[0.45 – 0.53]	0.9356
15- 49 years	435	943	1378	0.31	[0.29 – 0.34]	< 0.001*
> 50 years	149	309	458	0.32	[0.28 – 0.37]	< 0.001*
Total	1050	1724	2774	0.37	[0.36 – 0.39]	< 0.001*

* significant result

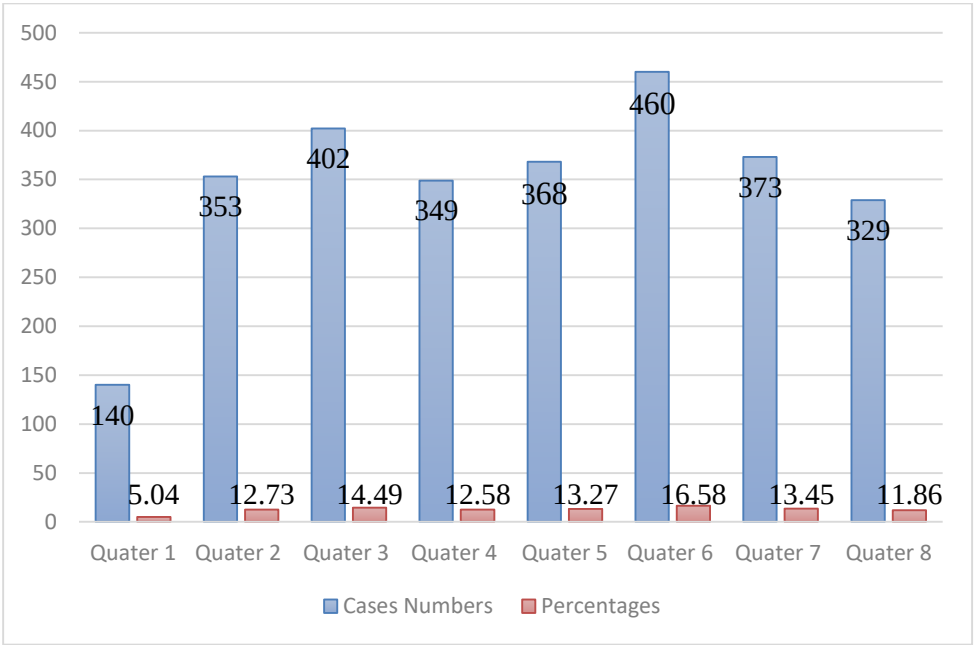

Fig. 3. Distribution of the rate of patients diagnosed positive for typhoid fever by quarter

Seasonal distribution of patients. The prevalence of typhoid fever showed an uneven distribution over the different quarters (eight quarters) which constituted our study period (Fig. 4 and Table 3). The highest prevalence of typhoid fever cases was in quarter 6 (460 cases), which includes the months of September, October, and

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November 2020, compared to quarter 2 (353 cases), which includes the same months in 2019 at the beginning of the study, because these quarters 6 and 2 correspond to the short rainy season (September to December). Similarly, quarter 3 (402 cases) and quarter 7 (373 cases), which respectively contain the months of December, January, and February 2020 and 2021, corresponding to the short dry season (December to January) and the beginning of the long rainy season (February to May). Quarter 1 (140 cases) and Quarter 5 (368 cases), which respectively contain the months of June, July, and August 2019 and 2020, corresponding to the long dry season (March to September), have respectively recorded 141 cases (5.04%) and 368 cases (13.27%). Quarter 4 and Quarter 8, corresponding to March, April, and May 2020 and 2021, represent the long dry season and recorded 349 cases (12.58%) and 329 cases (11.86%), respectively. The number of typhoid fever cases was lower in quarter one, corresponding to June, July, and August 2019 (long dry season, running from March to September). The distribution of typhoid fever cases among seasonal patients was significantly different between quarters ($P < 0.001$, CI 95% [0.04 0.05]).

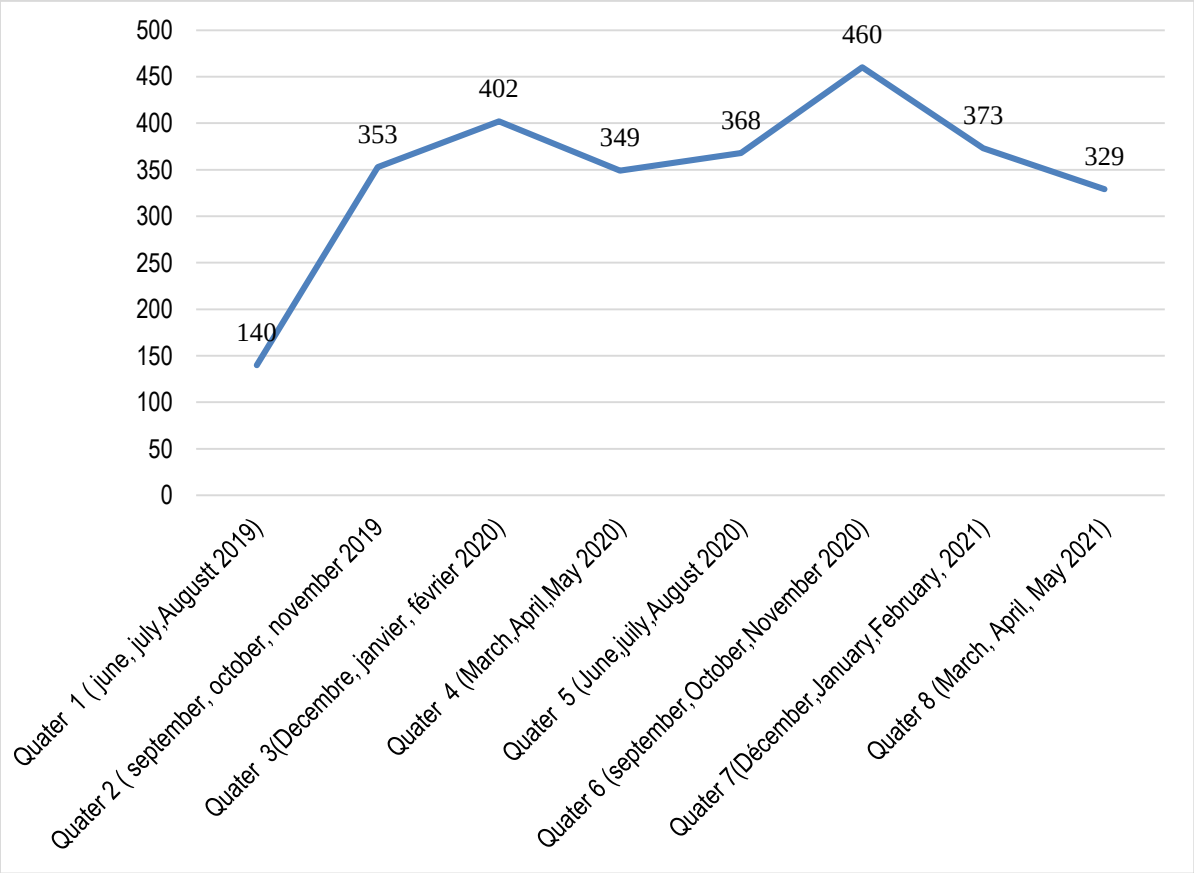

Fig. 4. Seasonal distribution of typhoid fever cases in patients.

Table 3. Seasonal correspondences of the different quarters.

Quarters	Seasons
1	Large dry season
2	Small rainy season
3	Small dry season and the beginning of the big rainy season
4	Large dry season
5	Large dry season
6	Small rainy season
7	Small dry season and the beginning of the big rainy season
8	Large dry season

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DISCUSSION

With the overall objective of determining the prevalence of *Salmonella typhi* infection in patients with febrile illness, this study recorded a total of 4711 patients who consulted the medical analysis laboratory of the Sino-Gabonese Friendship Hospital in Franceville, southeastern Gabon, over two years. The distribution of participants according to sex indicated a sex ratio (F/H) of 1.58, showing that women were in the majority, with 2884 cases (61.22%) against 1827 (38.78%) for men. This result may be related to the large female population in Gabon or our study area. The study reported that 2774 patients out of 4711 were positive for typhoid, i.e., an overall prevalence of 58.88%. Our results show a higher prevalence than in Rwanda, with 0.4% [18], and a similar prevalence in Nigeria, with 63.9% [19]. The geographical locations and the disparity between the study populations and the study periods can justify this variability in results. In addition, the difference in investigative techniques of each laboratory may also affect the result [18]. With 1724 patients and a percentage of 62.15% ($p < 0.001$), women were more infected than men, with a percentage of 37.85%.

In contrast to the studies conducted in Ghana and Gabon, which indicated that most cases of typhoid fever were in men [20 21], our result is similar to that obtained in a previous study in the same hospital [2]. This difference may be related to the fact that in our traditional societies, the nature of women's occupations and outdoor activities differs from that of men. Also, the differences in infection prevalence between men and women could be because, in Gabon, the prevalence of HIV is more than twice as high in women (5.8%) than in men (2.2%) [22]. HIV could contribute to the higher prevalence of typhoid fever in women, as this disease compromises immunity [23].

Regarding the distribution of typhoid fever cases by age group, our study indicated that the age group most affected by typhoid fever was 15-49 years. Although all social strata are affected by this disease, economic insecurity plays a significant role in the occurrence of typhoid fever. In contrast to our result, some studies conducted in Nigeria, Uganda, and Malaysia have reported a high prevalence of typhoid infection among those under 15 years of age [24 25]. Our result is similar to that reported elsewhere, indicating that most typhoid fever cases in the 15-49 age group [2]. Although it is sometimes difficult to confirm which age groups are more susceptible to typhoid fever, the variability of results obtained in various studies may be justified because many studies often report different age ranges and class intervals regarding susceptibility to typhoid fever.

Nevertheless, the high prevalence of typhoid fever among women in the 15-49 age group in our study may be related to the fact that in rural areas, women are involved in household chores such as cleaning garbage cans. In contrast, we find an increasingly young population in urban centers, which is no longer adept at family meals. These young adults, who are increasingly active, prefer "street food," thus exposing themselves to the consumption of sometimes contaminated food [2]. These results corroborate those obtained in previous studies [26, 27, 28, 29]. Furthermore, it can also be assumed that the water used and consumed by this age group could be contaminated with *Salmonella typhi*. In addition, the prevalence of the disease could be attributed to the fact that when tap water is cut off, the population resorts to well water or buys water of unsafe origin, sold on the sly by street vendors. In addition to the exposure of food on the ground in community markets and the lack of public toilets, most households do not have latrines and defecate in the open. There are also many links between the occurrence of the disease and places with poor hygiene and sanitation [20]. The seasonal distribution of the number of positive typhoid fever cases during this study indicated that the prevalence of typhoid fever was unevenly distributed over eight quarters. Quarter 6, corresponding to September, October, and November 2020, was observed to have the highest prevalence of typhoid fever cases. Comparing quarter 2, which comprises the same months in 2019, it was noted that these two quarters correspond to the rainy seasons, covering the months of September through December. As shown by Mefane et al. in 1986 [30] and reinforced by M. Okome-Nkoumou in 2000 [20], our study showed that high typhoid fever cases occur during the rainy seasons in Franceville. The association of high typhoid fever cases in different years with rainy seasons was also observed by others [31].

The present study indicates that, despite its low frequency, typhoid fever remains a crucial febrile disease for our populations. In Gabon, particularly in Franceville, typhoid fever is a real public health problem. Therefore, our results are essential for public health practitioners and policymakers to plan and implement targeted and effective preventive measures to curb this infection in semi-urban areas like Franceville.

One limitation of this study was that the Widal and Felix test was the only method to diagnose typhoid. It would have been interesting to deploy a culture test to detect false-negative results, which occur when previous antimicrobial treatment inhibits the antibody response [24].

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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