Etiology and biodistribution of enterobacteria and parasites, and their associated environmental risk factors among children under 5 years old with diarrhea in East-Central Gabon

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Abstract

Background

Better knowledge of endogenous germs and their associated demographic and environmental risk factors in a locality are essential to treat childhood diarrheal diseases. The aim of the study was to identify and characterize bacterial and parasitic pathogens responsible for childhood diarrhea, and to identify environmental risk factors associated with diarrhea in children under 5 years old living in Koula-Moutou, East-Central Gabon.

Methods

A cross-sectional study was performed from May 2016 to February 2018. One-hundred and thirty-two (132) children under 5 years old were enrolled. The detection of pathogens in stool samples was performed using microscopic examination and MIF concentration for parasites, and conventional culture on selective media for enterobacteria.

Results

The prevalence of diarrheal pathogens was 46.1%, including bacterial enteropathogens (25.5%) and parasites (20.6%). A total of 228 pathogenic organisms were isolated, including 199 bacterial strains (87.3%) and 29 parasites (12.7%). The specific richness of the isolated enterobacteria was 24 species with a high prevalence of *E. coli* (39.8%), including 26.7% for the diarrheal cases and 13.1% for the controls. Twelve (12) parasite species were also isolated and the most common types of parasites were rhizopods (44.8%), which accounted for 37.9% of the diarrheal cases and 6.9% of the controls. Univariate analysis showed that the presence of watercourses (OR = 3.37) and domestic animals (OR = 1.65) were significant risk factors for diarrhea.

Conclusion

The findings show a high prevalence of bacterial enteropathogens but a low rate of parasites and bacteria-parasite co-infection in the study area. Risk factors associated with diarrhea among children under 5 years old were the presence of watercourses and domestic animals. These findings highlight the need to strengthen the routine examination of diarrheic stool samples for the diagnosis of pathogenic organisms. Further analyses are required to better understand the etiologies and risk factors associated with the transmission of bacteria and parasites in rural, semi-urban and urban regions of Gabon.

Background

Diarrheal diseases remain the second cause of preventable childhood morbidity and mortality. They are responsible for 9% of global deaths among children under 5 years old (1, 2), despite technological advances and awareness programs issued by the World Health Organization (WHO) and the increased use of oral rehydration therapy in recent decades (3, 4). Today, acute diarrhea still represents a major public health issue worldwide and about 80% of diarrheal deaths in children under two years old occur in South-East Asia and sub-Saharan Africa (5, 6).

Infectious and contagious diarrhea is common in developing countries where the risk of transmission is even greater because of the appalling environmental conditions. They are the result of non-sanitary elimination of human wastes, very limited access to safe water, poor hygiene practices, and the means of food conservation and cooking (7, 8). In addition, the density of urbanization and the increase of populations in cities can also be risk factors contributing to the development and the contraction of diseases in young children (9).

Diarrheal diseases are mostly caused by enteric pathogens, including bacteria, viruses, and protozoa (10). Germs such as rotaviruses, *Escherichia coli* and other pathogenic bacteria, are the most common etiological agents in young children which induce moderate to severe diarrhea (11, 12). In addition, the economic and health impacts due to diarrheal diseases would require monitoring in order to better understand the risk factors leading to their occurrence. Thus, reducing the morbidity of diarrheal diseases requires a comprehensive approach of this pathology that combines the study of therapeutic management, socio-economic, demographic and environmental conditions, the ecology of the pathogens responsible, and knowledge of local communities (5, 13).

In Gabon, like everywhere else in sub-Saharan Africa, the epidemiological plan is largely dominated by communicable diseases which are classified as the first group responsible for morbidity. Ninety percent (90%) of the causes of death for children under five years old in this country are due to malaria (29%), prematurity (15%), acute respiratory infections (11%), HIV (10%), and diarrheal diseases (6%) (14, 15). The prevalence of diarrheal diseases was 50% in 2008 and 15.8% in 2012 in the city of Libreville, and the most vulnerable population was children under 15 years old (15, 16). However, the national prevalence of endemic diarrhea remains only poorly documented and prospections on the etiology of infectious diarrheas are rarely done routinely. Furthermore, it is also necessary to consider the specific etiological characteristics of the different cities because bacterial etiology often leads to systematic and abusive antibiotic therapy (17). Therefore, better knowledge of the possible infectious causes and their associated risk factors will improve diarrhea treatment methods in order to preserve effective antibiotics and fight off antibiotic resistance.

The aims of this study were to **1)** estimate the prevalence of parasitic and bacterial pathogens, and their associated risk factors in children under 5 years old with diarrhea living in Koula-Moutou and **2**) make a map of parasitic and bacterial pathogens responsible for childhood diarrhea in Koula-Moutou, Gabon.

Methods

Study area

This study was conducted at the Paul Moukambi Regional Hospital Center (PMRHC) and different districts in Koula-Moutou, Gabon. Koula-Moutou is the administrative capital of the Ogooué-Lolo Province and a semi-urban area in East-Central Gabon (1° 13' 10" S and 12° 28' 0" E) (**Figure 1**). The city of Koula-Moutou had an estimated population of 23,629 inhabitants in 2017 (18). Among the twenty-eight (28) neighborhoods listed in this study, only twenty-seven (27) are currently listed by the municipal authorities of the province (Figure 1).

Study design and population

A cross-sectional study was carried out in Koula-Moutou from May 2016 to February 2018. The different sampling campaigns were carried out intermittently and during the rainy season which corresponds to the peak of diarrheal disease (19). Samples were collected in the pediatric and emergency wards of the PMRHC and different districts of the city. The definition of "diarrhea case" was based on the recommendations of the World Health Organization (8).

The study population was composed of 132 children aged 0 to 70 months old living in the selected study sites and presenting acute diarrhea or no diarrhea. Children with acute diarrhea, hospitalized patients, children treated on an outpatient or consultant basis and who had not been on antibiotic therapy for more than 24 hours prior to the sampling were included. Children older than 71 months and under treatment for more than 24 hours prior to sampling were excluded from this study. Controls selected during the same period were children who had no history of diarrheal episode over a period of 15 days prior to their recruitment in this study.

Written informed consent was obtained from the parents or guardians of the participating children. For each participant, demographic characteristics (age, sex, origin), clinical characteristics (fever, vomiting, duration of diarrhea before consultation, the type or absence of treatment), and environmental and living conditions (type of drinking water, presence of toilets or a latrine, presence of watercourses, presence of domestic animals, sanitary condition of the plots, proximity of livestock farms) were collected on a standard survey form. Children aged 24 months old or less were considered to be exposed to the disease.

Sample collection

Stool samples were collected in sterile containers from diarrheal pediatric patients. In infants, fecal samples were collected by rectal swab. Each vial was identified by the code number of the participant.

Sample examination

Macroscopic examination was performed according to the Bristol scale on each stool sample to note the appearance, consistency, color and possible presence of blood and mucus. A microscopic examination after specific staining with lugol and merthiolate formalin iodine for the detection of parasites and Gram

staining for the appreciation of the morphology and composition of the bacterial flora was carried out on each stool sample.

Detection of parasites

A small amount of the stool sample equivalent to the size of a pea was mixed with a drop of saline (0.9% sodium chloride) and covered with a coverslip. A drop of lugol and merthiolate formalin iodine were also added on a small stool sample and the slide was examined under a light microscope to observe motile parasites. This method is used to detect intestinal protozoa and soil-transmitted helminths (STHs). The sample was considered negative if no intestinal protozoa or helminths were found on the entire slide. The merthiolate-iodine-formaldehyde (thimerosal) concentration (MIFc) method was also used. The MIFc method was carried out as described by M'bondoukwé et al. (20). Samples were considered positive upon the detection of a single parasite.

Isolation and identification of bacterial strains

Fecal samples were streaked on selective agar media for the isolation of Gram negative bacilli: Hektoen, *Salmonella-Shigella*, for the isolation of strains of *Salmonella, Shigella*; Methylene Blue Eosin (MBE) for the isolation of *E. coli* and Drigalski for other enterobacteria. Briefly, the stool samples were streaked on the agar surface. The media were incubated at 37°C for 24 hours. After the isolation of pure colonies, the identification of the strains was carried out using the standard biochemical system Api 20E (Biomérieux, Marcy l'Etoile, France) and the results were interpreted using the Api web^{TM} stand alone V 1.2.1 software (Biomérieux, Marcy l'Etoile, France). However, no confirmation by serotyping was done for *Salmonella, Shigella* and *Yersinia* isolates.

Ethical considerations

The study was approved by the Gabonese National Ethics Committee for Research and the Ministry of Health (PROT N° 0020/2015/SG/CNE). The study protocol was approved and authorized by the Center-East General Direction of Health, the Direction of the Ogooué-Lolo Provincial Academy and the Provincial Direction of Family and Social Welfare. Stool samples were collected from children after obtaining their parents' or guardians' written informed consent.

Statistical analysis

The data collected were typed in an Excel spreadsheet and analyzed using R software version 3.2.2. Heterogeneity between the infectious origins within the two populations and the eco-distribution of enteropathogens were assessed using the chi-square test and Fisher's exact tests. The Shannon diversity index made it possible to assess the bacterial and parasitic diversity observed. Crudes odds ratios (*CORs*) and 95% confidence intervals (*CIs*) were used to assess the association between variables and occurrence of diarrhea. In addition, a factorial correspondence analysis was performed to assess the eco-distribution of enteropathogens. The statistical significance was set at $p<0.05$.

Results

Study population

The children's demographic characteristics are summarized in Table 1.

Table 1
Distribution of diarrhea infection according to demographic data.

Characteristics	Number (%) of children		Total (n = 132)
	Diarrhea cases (n = 102)	Control (n = 30)	
Sex			
Male	54 (52.9)	14 (46.7)	68 (51.1)
Female	48 (47.1)	16 (53.3)	64 (48.5)
Sex-ratio (M/F)	1.13	0.88	1.06
Age groups (months)			
0–24	45 (44.1)	10 (33.4)	55 (41.6)
25–48	29 (28.4)	10 (33.3)	43 (29.5)
49–70	28 (27.5)	10 (33.3)	16 (28.8)

A total of 132 children aged from 0 to 70 months old were included, among whom 102 had a diarrheal syndrome and 30 were controls. Overall, the sex ratio was 1.06 (68 males and 64 females). The sex ratios for the children with diarrhea and the control group were 1.13 (54 males and 48 females) and 0.088 (14 males and 16 females), respectively. The mean age of the children included in the study was 32,6 ± 20,6 months old. Diarrheal syndrome was predominantly found in children aged 0 to 24 months old with a prevalence of 44.1%. Prevalence of diarrheal syndrome was 28.4% and 27.5% in children aged 25 to 48 months old and 49 to 70 months old, respectively.

Clinical examination revealed that the most frequent signs associated with diarrhea were fever (26.5%; n = 27/102), the association of fever and vomiting (16.7%; n = 17/102) and vomiting (5.9%; n = 6/102). These signs were assessed according to the usual clinical criteria: weight-to-height and height-to-age ratio, vomiting and fever. In addition to diarrhea, 11.8% of children had other conditions. Thirty-three (32.4%) of the 102 subjects with diarrhea were on treatment; with a prevalence of 15.7% for antiparasitic treatments, 6.9% for traditional treatment, 5.9% for antibiotic therapy and 3.9% with antibiotic-antiparasitic combination (data not showed).

Eco-distributions of germs and specific richness

The distribution and classification of pathogens responsible for diarrhea in the children study population are summarized in Table 2. Microscopic examination of the 132 stool samples revealed that 71.2% of stool cultures were positive vs. 6.1% negative. Overall, 228 germs were isolated including 199 (87.3%) bacterial strains and 29 parasites (12.7%). Opportunistic pathogens were more common in both diarrheal cases (53.1%, n = 121/228) and controls (21.5%, n = 49/228) than strict enteropathogens (24.1%, n = 55/228; 1.3% n = 3/228) in diarrheal cases and controls, respectively. No strict enteropathogenic bacteria in controls were detected compared to diarrhea cases (16.2%). In parasite infections, 7.9% of strict enteropathogens and 2.6% of opportunistic pathogens were isolated from diarrhea cases. In controls, the prevalence rates were 1.3% and 0.9% for strict enteropathogens and opportunistic pathogens, respectively.

Table 2
Eco-distribution of the total germs isolated in the study according to the type of the population.

	Number of cases (%)	Diarrheal cases n (%)	Control n (%)
Bacteria	**199 (87.3)**	152 (66.7)	47 (20.6)
Strict enteropathogens		37 (16.2)	0 (0.0)
Opportunistic pathogens		115 (50.4)	47 (20.6)
Parasites	**29 (12.7)**	24 (10.5)	5 (2.2)
Strict enteropathogens		18 (7.9)	3 (1.3)
Opportunistic pathogens		6 (2.6)	2 (0.9)

Specific richness of the parasitic strains isolated from the study population

The parasitic strains isolated are summarized in Table 3. In parasitic infections, the highest prevalence was observed with the Rhizopoda class (37.9%), which accounts for the highest prevalence of parasitic infections detected among diarrhea cases. The second most prevalent class was Nematoda (20.7%), followed by Trematodes (Schistosomes; 6.9%) and Flagellata (6.9%). Among Trematodes, *Schistosoma mansonni* (3.4%) and *intercalatum* (3.4%) were the two species detected. In Flagellata, *Giardia intestinalis* was detected (6.9%). *Entamoeba coli* (20.7%) was the most prevalent Rhizopod, *Dientamoeba fragilis* (3.4%), *Entamoeba hartmani* (3.4%), and *Entamoeba hystolitica/dispar* (3.4%) had the same prevalence. In the Nematoda class, the highest prevalence was observed with *Ascaris lumbricoides* (10.3%), followed by *Ancylostoma duodenale* (6.9%) and *Trichuris trichiura* (3.4%). Moreover, some parasitic species were also isolated in the controls among which *Ancylostoma duodenale* (3.4%) and *Ascaris lumbricoides* (3.4%) for the Nematodes, and *Entamoeba coli* (6.9%) for the Rhizopoda class. *Blastocystis hominis* was isolated both in diarrhea cases and in controls with prevalence rates of 10.3% and 3.4%, respectively. The

overall diversity of the parasites was assessed by the Shannon index which was valued at 2.2. The difference was not statistically significant for all parasites between diarrhea cases and controls.

Table 3
Profile of parasitic strains isolated in patients.

	Cases detected	Diarrhea cases	Control
	N = 29	n (%)	n (%)
Flagellates		**2 (6.9)**	**0 (0.0)**
Giardia intestinalis		2 (6.9)	0 (0.0)
Nematodes		**6 (20.7)**	**2 (6.9)**
Ancylostoma duodenale		2 (6.9)	1(3.4)
Ascaris lumbricoides		3 (10.3)	1 (3.4)
Trichuris trichiura		1 (3.4)	0 (0.0)
Rhizopods		**11 (37.9)**	**2 (6.9)**
Dientamoeba fragilis		1(3.4)	0 (0.0)
Endolimax nanus		2 (6.9)	0 (0.0)
Entamoeba coli		6 (20.7)	2 (6.9)
Entamoeba hartmani		1(3.4)	0 (0.0)
Entamoeba histolytica/dispar		1 (3.4)	0 (0.0)
Schistosoma		**2 (6.9)**	**0 (0.0)**
Schistosoma mansonni		1 (3.4)	0 (0.0)
Schistosoma intercalatum		1 (3.4)	0 (0.0)
NS			
Blastocystis hominis		3 (10.3)	1 (3.4)
Total		**24 (82.8%)**	**5 (17.2%)**

NS: not specified by systematic.

Specific richness of Enterobacteriaceae strains isolated from the study population

Opportunistic pathogens were more common than strict enteropathogens (Table 4). Strict enteropathogens were found in 18.6% (n = 37/199) of the diarrhea samples. The highest prevalence was for *Salmonella spp* (8.6%), followed by *Salmonella enterica* (3.0%; n = 6/199), *Salmonella Typhi* (2.0%; n =

4/199) and *Salmonella Paratyphi A* (1.5%) serovars of this species, whereas the genera *Shigella* and *Yersinia* were the least represented. The global prevalence of opportunistic pathogens was higher among diarrhea samples (57.8%) compared to control samples (23.6%) (see Table 4). Out of the 17 isolated species, the highest prevalence recorded was for *E. coli 1* (25.2% for diarrhea cases and 11.6% for controls), followed by *Raoultella ornithinolytica* (11.6% for diarrhea cases and 3.0% for controls), *Kluyvera spp* (2.5% for diarrhea cases and controls, respectively), and *Serratia fonticola* (2.5% for diarrhea cases).

Table 4
Profile and diversity of enterobacteria in the study.

Cases detected 199	Diarrhea cases n (%)	Control n (%)
Strict enteropathogens	**37 (18.6)**	-
Salmonella enterica	6 (3.0)	-
Salmonella Paratyphi A	3 (1.5)	-
Salmonella **spp**	17 (8.6)	-
Salmonella Typhi	4 (2.0)	-
Shigella sonnei	2 (1.0)	-
Shigella **spp**	3 (1.5)	-
Yersinia pestis	2 (1.0)	-
Opportunistic pathogens	**115 (57.8)**	**47 (23.6)**
Citrobacter braakii	1 (0.5)	-
Citrobacter freundii	1 (0.5)	-
Citrobacter koseri	1 (0.5)	1 (0.5)
Enterobacter aerogenes	4 (2.0)	-
Enterobacter cloacae	2 (1.0)	1 (0.5)
Escherichia coli 1	50 (25.2)	23 (11.6)
Escherichia coli 2	3 (1.5)	3 (1.5)
Escherichia vulneris	2 (1.0)	-
Klebsiella oxytoca	3 (1.5)	-
Klebsiella pneumoniae	4 (2.0)	1 (0.5)
Kluyvera **spp**	5 (2.5)	5 (2.5)
Pantoea **spp**	1 (0.5)	1 (0.5)
Raoultella ornithinolytica	23 (11.6)	6 (3.0)
Raoultella terrigena	4 (2.0)	-
Serratia fonticola	5 (2.5)	2 (1.0)
Serratia liquefaciens	2 (1.0)	-
Serratia odorifera 1	4 (2.0)	4 (2.0)

Regardless of the bacterial species, 7 pathogens were isolated only in diarrhea samples including *Enterobacter aerogenes* (2.0%), *Raoultella terrigina* (2.0%), *Klebsiella oxytoca* (1.5%), *Escherichia vulneris* (1.0%), *Serratia liquefaciens* (1.0%), *Citrobacter braakii* (0.5%), and *freundii* (0.5%). The results of the Shannon Diversity Index showed a great diversity of bacteria within the study population (H = 2.48 and 1.68 for diarrheal and control cases, respectively). The Pielou Fairness Index showed that the observed diversity was due to the high abundance of *E. coli* in both cases and controls (J = 0.7).

Spatial distribution of isolated germs

The spatial distribution of the bacterial and parasite species detected in the study population in Koula-Moutou is presented in Fig. 2. There was no species representative of a district.

Regardless of the bacterial species, bacterial diversity was higher in the Mikoumou district with 11 different species including *Citrobacter freundii and koseri, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca and pneumoniae, Kluyvera spp, Pantoea spp, Raoultella ornithinolytica, Serratia fonticola and Yersinia pestis* belonging to 9 genera (*Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Kluyvera*, *Pantoea*, *Raoultella*, *Serratia* and *Yersinia*). The Bakélé and Bambomo districts harbored 8 different species. *Escherichia coli 1 and 2, Klebsiella pneumoniae, Kluyvera spp, Raoultella ornithinolytica and terrigena, Salmonella Paratyphi A and Shigella sonnei*, belonging to 6 genera (*Escherichia, Klebsiella, Kluyvera, Raoultella, Salmonella* and *Shigella*) were found in the Bambomo district. For the Bakélé district *Enterobacter aerogenes and cloacae, Escherichia coli 1 and vulneris, Salmonella enterica and spp, Shigella spp and Yersinia pestis*, belonging to 5 genera (*Enterobacter*, *Escherichia*, *Salmonella*, *Shigella* and *Yersinia*) were found. Seven species including *Citrobacter braakii, Escherichia coli 1, Klebsiella pneumoniae, Raoultella ornithinolytica, Salmonella spp, Serratia fonticola and Shigella spp*, belonging to 5 different genera (*Citrobacter, Escherichia, Klebsiella, Raoultella*, *Salmonella*, *Serratia*, and *Shigella*) were found in the Mayéla district. The Concorde (*Enterobacter aerogenes, Raoultella ornithinolytica, Salmonella enterica* and *Serratia liquefaciens* and *odorifera 1*), and Jardin-Four-TP (*Escherichia coli 1 and 2, Klebsiella oxytoca, Kluyvera spp* and *Raoultella ornithinolytica*) districts presented intermediate diversities with 5 different species. Four species were also found in Konadembé (*Escherichia coli 1, Salmonella enterica, Serratia liquefaciens* and *Raoultella ornithinolytica* belonging *Escherichia, Salmonella, Serratia* and *Raoultella* genera), in Makadium (*Enterobacter cloacae, Escherichia coli 1, Raoultella ornithinolytica and terrigena* belonging to the *Enterobacter, Escherichia*, and *Raoultella* genera) and in Ménage (*Escherichia coli 1, Raoultella ornithinolytica and terrigena* and *Salmonella spp* belonging to *Escherichia, Salmonella* and *Raoultella)*, respectively. In the rest of the districts, 3 species belonging to various genera were isolated (see Fig. 2). The same profile was observed for Bouvendo (*Escherichia coli 1 and 2* and *Salmonella* spp) and Koungou (*Escherichia coli 1, Salmonella Paratyphi A* and *spp*), Centre-ville and Mandji-Château (*Escherichia coli 1, Raoultella ornithinolytica* and *Salmonella spp*), Lenguébé/Pembébé (*Escherichia coli 1, Salmonella spp* and *Serratia oderifera 1*), Litsébé (*Escherichia coli 1, Salmonella enterica* and *Serratia oderifera 1)*, and Mikalou *(Escherichia coli 1, Salmonella Typhi* and *Serratia fonticola)* districts. The Mandji-Boungouêret district presented a different profile (*Enterobacter aerogenes, Escherichia coli 1* and *Klebsiella pneumoniae*). Babambo (*Escherichia coli 1* and *Raoultella*

ornithinolytica), Dakar (*Escherichia coli 1* and *Shigella spp)* and Moukouagna (*Salmonella spp and Typhi*) presented 2 different genera or the same *Salmonella* genus, respectively.

The distribution of bacterial and parasitic enteropathogens by district is presented in Fig. 3.

The enteropathogens were mainly distributed in the city center of Koula-Moutou (73.9%, n = 17/23). In 7 of the 17 districts, two different bacterial and parasitic species were isolated including *Salmonella spp* and *Ancylostoma duodenale* in Balomba, *Salmonella spp* and *Ascaris lumbricoides* in Bouvendo, *Salmonella Typhi* and *Ancylostoma duodenale* in the city center, *Salmonella enterica* and *Schistosoma intercalum* in Concorde, *Salmonella spp* and *Giardia intestinalis* in Mandji-Château, *Salmonella Typhi* and *Ascaris lumbricoides* in Mikalou and *Yersinia pestis* and *Ancylostoma duodenale* in Mikoumou. However, for the 2 other districts, 3 species were isolated including 2 bacterial species and 1 parasitic species. For the Mayéla district, the pathogens isolated were *Salmonella spp*, *Shigella spp* and *Endolinax nanus* and *Salmonella Paratyphi A*, *Salmonella spp* and *Ascaris lumbricoides* for the Koungou district. In the 8 other districts, only bacterial enteropathogens were found. Mapping showed that some pathogens were specific to a few districts. In fact, *Shigella spp* was isolated in patients from Dakar, *Salmonella Paratyphi A* from Mibaka, *Salmonella enterica* from Litsébé, and *Salmonella spp* from diarrheal patients from the Ménage district. However, a diversity of species was recorded in the Bakélé (*Salmonella enterica, Salmonella spp* and *Shigella spp*), Konadembé (*Salmonella enterica* and *Shigella sonnei*) and Moukouagna (*Salmonella spp* and *Typhi*) districts.

Enteric organisms associated with diarrhea

Bacterial etiology is the most observed with a prevalence of 25.5% (26/102) in diarrheal cases compared to 0.0% (0/30) for controls. However, the prevalence of parasitic etiology for diarrhea is 20.6% (21/102). In controls, this prevalence is 10.0% (3/30) (no statistically significant difference).

Salmonella spp (29.4%) was the most prevalent enteric pathogen in diarrheal cases, followed by *Entamoeba* spp (7.8%), *Shigella* spp (4.9%), *Ascaris lumbricoides* and *Blastocystis hominis* (3.0%). The most common enteric pathogens among controls were *Entamoeba* spp with 6.7%, *Ancylostoma duodenale*, *Ascaris lumbricoides*, and *Blastocystis hominis* which account for respectively 3.3% (see Table 5). *Entamoeba* spp was significantly associated with diarrhea (OR 1.8, 95% CI 0.21–12.13). Other organisms were not significantly associated with diarrhea in this study (Table 5).

Table 5
Prevalence of microorganisms in cases and controls and their association with diarrhea.

	Diarrhea cases N = 102	**Witnesses N = 30**	**COR**
	n (%)	n (%)	**95% CI**
STH			
Ancylostoma duodenale	2 (2.0)	1(3.3)	0.58 (0.02–35.38)
Ascaris lumbricoides	3 (3.0)	1 (3.3)	0.87 (0.06–45.7)
Trichuris trichiura	1 (1.0)	0 (0.0)	-
Schistosoma spp	2 (2.0)	0 (0.0)	-
Intestinal Protozoa			
Blastocystis hominis	3 (3.0)	1 (3.3)	0.87 (0.06–45.7)
Dientamoeba fragilis	1(1.0)	0 (0.0)	-
Endolimax nanus	2 (2.0)	0 (0.0)	-
Entamoeba spp	8 (7.8)	2 (6.7)	1.18 (0.21–12.13) *
Giardia intestinalis	2 (2.0)	0 (0.0)	-
Bacteria			
Salmonella spp	30 (29.4)	(0.0)	-
Shigella spp	5 (4.9)	(0.0)	-
Yersinia spp	2 (2.0)	0 (0.0)	-
CI : Confidence interval; **STH** : Soil-transmitted helminth; **COR** : Crude odds ratios.			
*COR and CI values above 1.0 that indicate association with diarrhea.			

Infections and co-infections among cases and controls

Microscopic examination revealed at least one pathogen in 50 positives stools samples with a 37.9% global prevalence. Among positive stool samples, 46.1% of diarrheal cases were infected, vs. 10.0% (3/30) in controls. This stool examination did not lead to the detection of strict pathogens among the 132 participants examined. However, opportunistic pathogens were detected in 82 children (62.1%) among which 53.9% (55/102) in diarrheal cases and 90.0% (27/30) in controls ($p < 0.001$). The frequency of single and multiple infections was analyzed in all populations. Multiple infections predominated (34.8% versus 25.8% for single infections) (see Table 5). Multiple infections carrying two to four different pathogens were detected in children, including 33.3% (34/102) in diarrheal cases and 10.0% in controls

(p = 0.011). Thirty of the children with multiple infections and diarrhea harbored two pathogens (29.4%). The most representative multiple infections are parasite-opportunistic bacteria (13.7%) and *Salmonella spp* - opportunistic bacteria (10.8%) for co-infections. The least representative are *Salmonella - Entamoeba coli* (2.0%) and *Salmonella spp-Shigella spp* (1.0%), *Salmonella spp-Giardia intestinalis* (1.0%), *Shigella spp- opportunistic bacteria* (1.0%). Three of them harbored three and four pathogens (2.9% and 1.0%, respectively). *Salmonella spp - Shigella spp - Yersinia spp*, *Salmonella* – opportunistic bacteria – *Dientamoeba fragilis and Shigella spp* - opportunistic bacteria – *Entamoeba coli* were found with the same prevalence (0.9%), and *Salmonella spp* – opportunistic bacteria – *Endolimax nanus* – *Entamoeba hystolitica/dispar* were found with a prevalence of 1.0%. Three (10.0%) children with multiple infections in the control group harbored two pathogens (10.0%) among which *Ancylostoma duodenale* – opportunistic bacteria (3.3%), *Ascaris lumbricoides* – opportunistic bacteria (3.3%) and *Blastocystis hominis* – opportunistic bacteria (3.3%) were found.

Distribution between enteropathogens and age groups

The distribution of the different species of enteropathogens according to the different age groups was determined using the correspondence factor analysis (CFA) (Fig. 4). The results of the distribution of enteropathogens according to age groups showed that parasitic enteropathogens, including *Entamoeba hystolitica, Dientamoeba fragilis, Schistosoma intercalatum and Schistosoma mansonni* were mainly associated with children aged 0 to 24 months old. This same association is found for *Salmonella enterica*. Enteropathogens of the *Shigella* genus including *shigella spp*, *shigella sonnei*, as well as *Yersinia spp* and *Endolimax nanus* were associated with children aged 25 to 48 months old. *Salmonella thyphi* and *Salmonella paratyphi A* were associated with children aged 49 to 70 months old. No association between age groups and pathogens was found for *Giardia intestinalis, Ancylostoma duodenale* and *Salmonella spp*.

Risk factors among environmental determinants for infections due to enteropathogens

Potential risk factors (drinking water, type of toilet, watercourses, domestic animals, and sanitation of the plot) associated with diarrheal diseases are summarized in Table 6.

Table 6
Determinants of diarrhea in children of Koula-Moutou.

Characteristics	Diarrhea cases (n = 102)	Control (n = 30)	COR (CI 95%)	*p*-Value
Housing conditions				
Drinking water				
Tap	70 (68.6)	18 (60.0)	1.5 (0.45–5.43)*	0.59
Public pump	22 (21.5)	11 (36.7)	0.83 (0.15–4.64)	0.8
River	2 (2.0)	1(3.3)	/	/
Well	1 (1.0)	0 (0.0)	/	/
Source	1(1.0)	0 (0.0)	/	/
Rain	2 (2.0)	0 (0.0)	/	/
Water treatment	4 (3.9)	0 (0.0)	/	/
Type of sanitary facilities				
Modern latrines	41 (40.2)	9 (30.0)	1.56 (0.2-10.91)*	0.7
Traditional latrines	61 (59.8)	21 (70.0)	1.37 (0.45–4.41)	0.6
Watercourses				
Rivers	22 (21.6)	5 (16.7)	4 (0.30-214.2)*	0.2
Puddles	20 (19.6)	6(20.0)	/	0.06
Wastewater	6 (5.9)	3 (10.0)	1 (0.01–34.08)	/
Absence	54 (52.4)	16 (53.3)	0.76 (0.2–2.65)	0.6
Domestic Animals				
Hens and roosters	21 (20.6)	6 (20.0)	2.66 (0.29–34.43)*	0.3
Sheep	1 (1.0)	0 (0.0)	/	/
Dogs/cats	20 (19.6)	6 (20.0)	2.69 (0.21-142.12)*	0.62

Mixed : herbs + garbage bins/farms ; **CI at 95%** : confidence intervals at 95%, **COR** : crude odds ratios. The percentages are will reference to totals given at the top of each column. Odd ratios obtained by logistic regression. *COR and CI values above 1.0 that indicate association with diarrhea

Characteristics	Diarrhea cases (n = 102)	Control (n = 30)	COR (CI 95%)	*p*-Value
Mixed	28 (27.5)	8 (26.7)	1 (0.15–6.55)	/
Absence of domestic animals	32 (31.4)	10 (33.3)	1.02 (0.19–5.97)	/
Sanitation of the parcel				
Herbs	87 (85.3)	26 (86.7)	1.6 (0.59–4.55)*	0.3
Garbage bins	7 (6.9)	1 (3.3)	/	/
Farms	2 (2.0)	0 (0.0)	/	/
Mixed	6 (5.9)	3 (10.0)	1 (0.02–88.07)	/

Mixed : herbs + garbage bins/farms ; **CI at 95%** : confidence intervals at 95%, **COR** : crude odds ratios. The percentages are will reference to totals given at the top of each column. Odd ratios obtained by logistic regression. *COR and CI values above 1.0 that indicate association with diarrhea

The results showed that more than half of the population consumed water from taps (68.6% for diarrheal cases vs 60.0% for controls). Regarding amenities, the majority of children used traditional latrines (59.8% in diarrheal cases and 70.0% in controls, respectively). An unsanitary condition found for plots was the presence of grass (85.3% and 86.7% for diarrheal cases and controls, respectively). Although no significant association between the different potential risk factors and the occurrence of diarrhea was observed, the data showed that the presence of a watercourse in the immediate environment increased the risk of diarrhea in children which was multiplied by 4, as well as the presence of domestic animals such as chickens and dogs / cats which multiplied the risk by 2.66 and 2.69, respectively.

Discussion

This study on diarrheal disease in Gabonese children is the first to assess the etiology of diarrheal syndromes in Koula-Moutou, Gabon. While data on adults and children already exist, children under 5 years old with diarrheal syndromes living in the semi-urban environment of the city of Koula-Moutou have not been previously studied.

In this study, the prevalence of children aged up to 5 years with diarrhea was 77.3%, it is similar to that reported by a previous study in another city in Gabon (21). The results of our study also showed that 41.6% of the children with diarrhea were aged 0 to 24 months old and were the most affected age group with a prevalence of 44%. Our results corroborate those of several studies led in Africa, with a higher prevalence found in children aged 0 to 12 months (8, 16, 22, 23). This predominance could be explained by the fact that generally, between 6 to 23 months of age, children are more vulnerable compared to those aged over 36 months old (24). Indeed, pathologies such as diarrhea are recurrent in this age group due to

the gradual decline in maternal antibodies as well as the introduction of new dietary practices sometimes correlated with poor hygienic practices (24, 25).

A range of different pathogenic organisms can cause pediatric diarrhea in the world, especially in tropical and developing countries, including rotaviruses and adenoviruses (26), intestinal parasites such as *Giardia intestinalis, Entamoeba histolytica* (27), and bacteria such as *Escherichia coli, Shigella spp, Salmonella, Campylobacter spp* (8). This study shows a strong heterogeneity of parasites and bacteria in diarrheal cases in which most of the species are not isolated in control cases. The most common parasitic species isolated in diarrheal cases were *Entamoeba spp, Ascaris lumbricoides, Blastocystis hominis, Ancylostoma duodenale, Giardia intestinalis, Endolimax nanus*, *Schistosoma spp* and *Trichuris trichiura.* This profile is similar to that reported in previous studies (28, 29). In addition, *Ascaris lumbricoides, Blastocystis hominis, Giardia intestinalis, Entamoeba spp* and *Trichuris trichiura* appear to be the intestinal parasites most frequently involved in parasitic infections in Gabon (20). These species have a worldwide distribution and are characteristic of some countries (30). Regarding the bacterial group, 11 genera belonging to the Enterobacteriacea family were recorded, a similar profile was found in the work of Mbuthia et al. in Kenya. The diversity was specific to the Enterobacteriaceae, *Pseudomonaceae* and *Vibrionaceae* families (2). This bacterial diversity was marked by a clear predominance of *Escherichia coli* strains with an isolation rate of 26.6%, which is similar to other studies with variable frequencies (25, 31, 32). Likewise, this study isolated a high rate of bacteria less commonly incriminated for causes of diarrhea and belonging to the *Raoultella, Serratia, Klebsiella, Enterobacter, Citrobacter* and *Pantoea* genera (31.1%). Previous studies reveal that some of these strains, such as the *Citrobacter* spp and *Klebsiella* spp species, have acquired specific virulence genes, which can induce diarrhea and / or hemorrhagic colitis (2, 33).

Pathogen infection was significantly more prevalent in patients with diarrhea, with a prevalence of 46.1% compared to controls (10.0%). These results are similar to those reported by other studies (34, 35). On the other hand, this overall prevalence of pathogens recorded in diarrheal cases in this study was lower than those obtained by Okon et al. in Nigeria and Knee et al. in Mozambique who found prevalence rates of 61.8% and 86%, respectively (23, 32). This contrast could probably be related to the lack of characterization of diarrheagenic *Escherichia coli* (DEC) in this study. Furthermore, this study also shown the presence of parasitic pathogens in this population, with a frequency of 20.6%. This prevalence was, however, lower than that recorded in Nigeria (37.1%) (32), Cameroon (59.2%) (7) and Gabon (61.0%) (21). The high prevalence of infections due to bacterial pathogens in these conditions is similar to that found in other studies (8, 23).

One of the particularities of this study was the lack of bacterial pathogens isolated from controls. The frequency of bacterial infections (25.5%) is higher than that reported in a previous study by Koko et al. carried out in Libreville (17) who obtained a prevalence of 12.9%. This variability in epidemiological characteristics could undoubtedly be linked to the level of urbanization of the two zones, the environmental conditions and hygiene of the respective populations, which are all factors that influence the etiology of diarrheal diseases (9, 36). *Salmonella* spp strains were the most predominant in the

bacterial profile of this study and accounted for 29.4%. This prevalence is in contrast with the one obtained in previous studies concerning developing countries, and particularly countries in the African region where the prevalence was markedly lower (37, 38). However, these data corroborate the work of Koko et al. in Gabon (17) and Rathaur et al. in India (39). Indeed, these studies identified *Salmonella* spp as a major etiological agent responsible for diarrhea. In addition, its presence could be correlated with domestic animals like poultry, which could constitute reservoirs of infection and a potential source of *Salmonella* diarrhea (7). The prevalence rates of the *Shigella* and *Yersinia* strains observed in this study, 4.9% and 2.0% respectively, can be explained by the epidemiological heterogeneity of the geographical areas (32, 37, 40, 41).

In this research, multiple infections of enteric pathogens were more found in diarrheal cases than in controls. This has also been reported in other studies particularly in patients with low or average income (35, 42). These results would suggest that more than one pathogen was responsible for the diarrheal disease in children living in Koula-Moutou. The presence of mixed infections complicates the diagnosis of a specific pathogen responsible for the disease and may result in an additive effect, which may lead to new clinical profiles (42). A reliable basic diagnosis must be established for better patient care and treatment.

Factorial correspondence analysis (CFA) reveals a predominance of parasites, *Salmonella* and *Shigella* in children over 12 months old. The parasitic data from this study are comparable to the literature which highlights an increase in parasitic infections in subjects over 12 months of age, for which the most likely explanation would be frequent contact with soil (7, 43). Likewise, this distribution of bacterial strains has already been reported by other authors (17, 23, 39).

The transmission and spread of diarrheal diseases are closely linked to environmental factors but also to living conditions, personal hygiene, behavior and domestic environment (44). In this study, univariate analysis of the data showed that the presence of rivers and domestic animals were the risk factors significantly associated with the occurrence of diarrhea in the town of Koula-Moutou. These results are consistent with the data reported by Bouba Djourdebbé et al. (24). Watercourses create a habitat conducive to the proliferation of microorganisms. Combined with other parameters, such as the proximity of animals which are potential pathogen reservoirs and the lack of knowledge on good hygiene practices, the presence of rivers influences the risk of contamination (24, 45). Given that diarrheal diseases can be linked to the presence of feces in water, the population of Koula-Moutou can be highly exposed to this health risk. Indeed, this city has a very dense water network which unfortunately serves for domestic use but in which wastewater containing the feces of some animals or even humans can be thrown away. These practices can obviously contribute to the fairly high prevalence of diarrheal diseases in this area.

This study has some limitations. It used only microbiological analyzes, including stool bacterial cultures, as they are the most common diagnostic routine methods used in developing countries. Stool culture may have a lower sensitivity of detection of bacterial pathogens than PCR, which allows the discrimination of different pathotypes and a better appreciation of the involvement of multiple infections

with enteric pathogens associated with diarrhea (42). Moreover, the low proportion of controls in this study may not be representative of the correlation between enteric pathogens and acute diarrhea.

Conclusion

This preliminary study in the city of Koula-Moutou on children with diarrhea reveals a great diversity of enterobacteria and parasites. Although the profile of pathogenic enterobacteriaceae strains is very classic, this study highlights a high prevalence of unusual bacteria which may be involved in childhood diarrhea. The map of the distribution of pathogens shows no particular characteristic in any area of the city. In addition, the occurrence of diarrhea in this city is linked to environmental determinants such as the presence of rivers and animals.

Faced with this diversity of microorganisms and risk factors, further data are needed to develop a better mapping of the widespread strains and improve etiological knowledge. These studies would help to target and broaden preventive and treatment strategies for diarrheal disease.

Abbreviations

CFA: Correspondence factor analysis; PMRHC: Paul Moukambi Regional Hospital Center; CI: Confidence interval; ACC: Ambulatory care center; COR: Crud odd ratio; HIV: Human immunodeficiency virus; STH: Soil-transmitted helminth; WHO: World Health Organization.

Declarations

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Authors' contributions

RMM, JFY and SLOL coordinated the study, performed the statistical analysis, conducted data analysis and wrote the manuscript. RMM was the principal investigator of the study, collected all data in the field, collected biological samples and carried out the microbiological analyses. RMM and MGM coordinated the field study at PMRHC and AMD was the physician of the study. FM carried out statistical analyses and the interpretation of data. SED contributed to the writing of the manuscript. JFY, AS and JBLD

conceived, designed and coordinated the study, and conducted data analysis. All authors have read and approved the final manuscript.

Availability of data and materials

The datasets that were used for the analysis and the preparation of this manuscript are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This is detailed in the “Methods” section.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

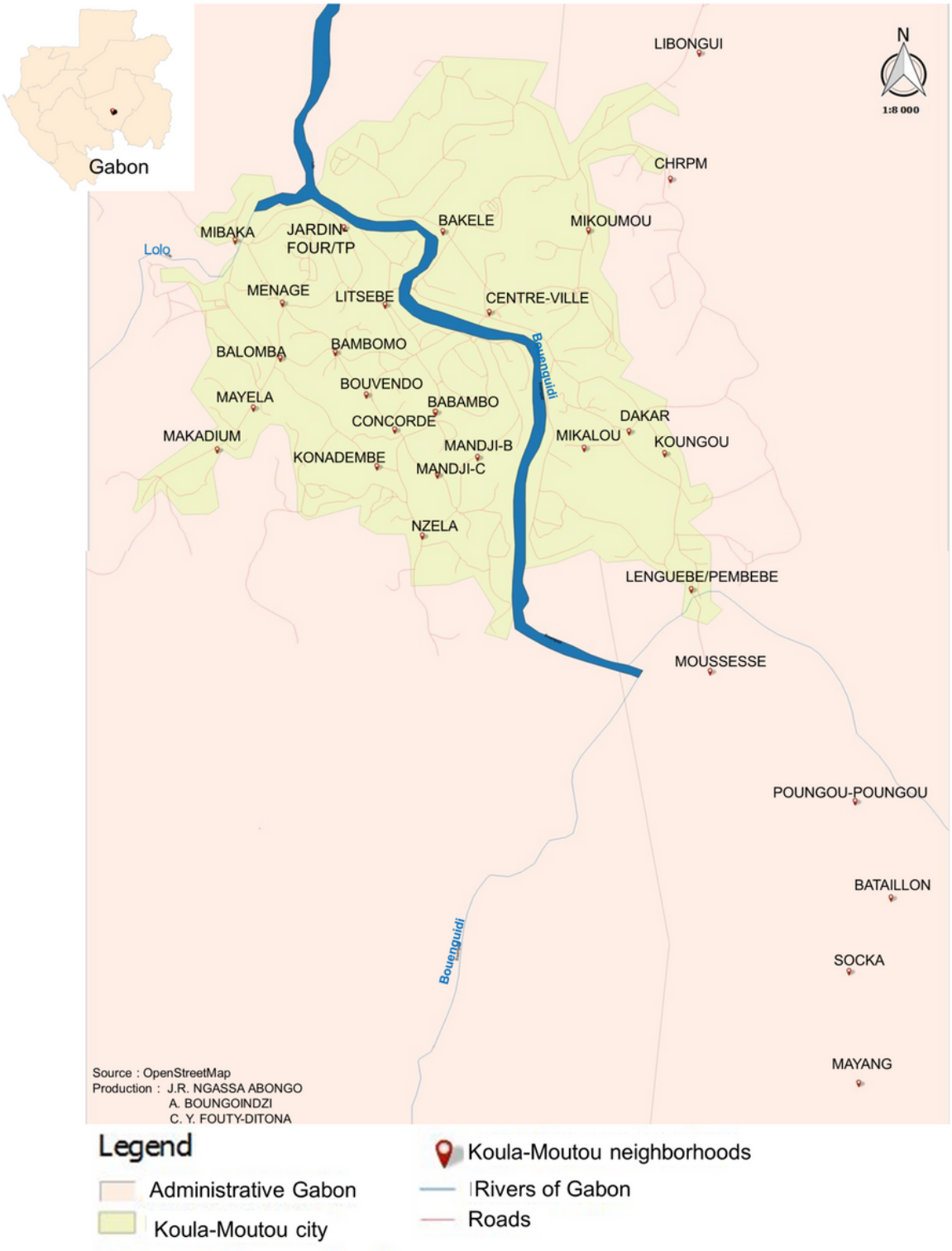

Figure 1

Map of the city of Koula-Moutou Legend: The background map in pink represents administrative Gabon and in yellow the city of Koula-Moutou. The lines in blue represent rivers and in red the roads, and the red location markers represent the neighborhoods.

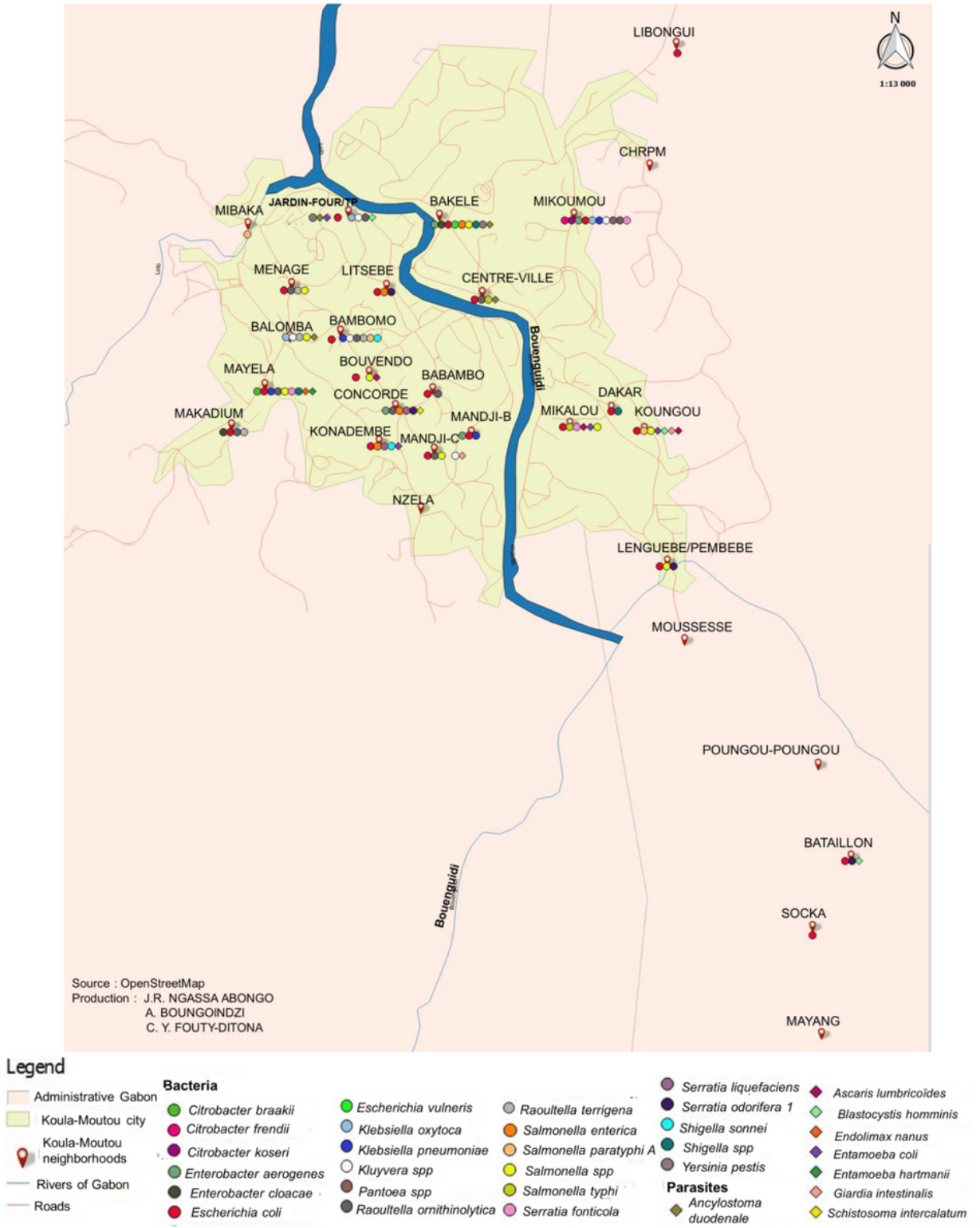

Figure 2

Map of the diversity of microorganisms isolated in the case-control study. Mandji-C: Mandji-Castle; Mandji-B: Mandji-Bougoueret. Legend: The background map in pink represents administrative Gabon and in yellow the city of Koula-Moutou. The lines in blue represent rivers and in red the roads, and the red location markers represent the neighborhoods. The circles in different colors represent the different bacterial species and the triangles represent the various parasitic species

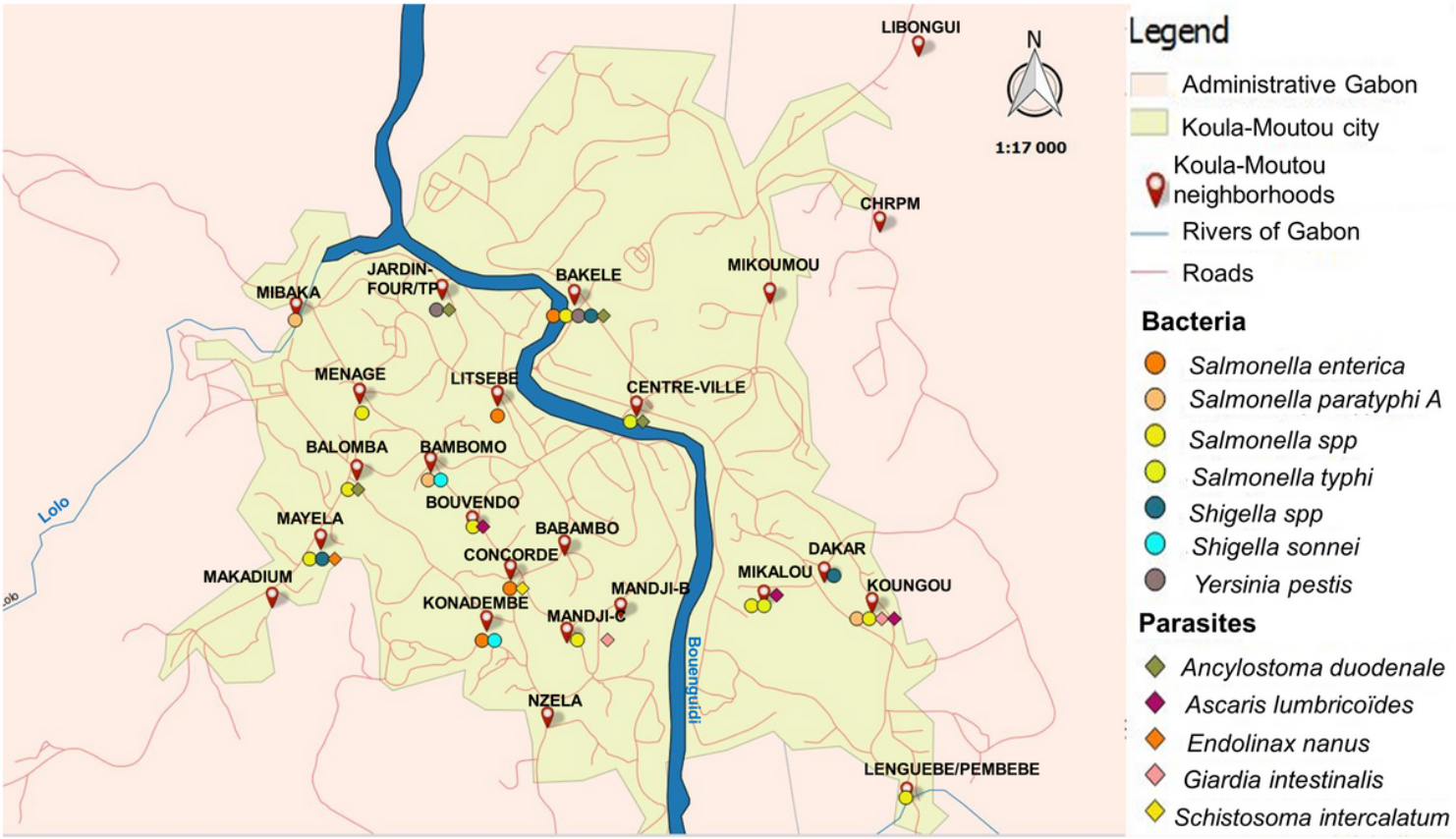

Figure 3

Spatial distribution of enteropathogens in the city of Koula-Moutou. Legend: The background map in pink represents administrative Gabon and in yellow the city of Koula-Moutou. The lines in blue represent rivers and in red the roads, and the red location markers represent the neighborhoods. The circles in different colors represent the different pathogenic bacterial species and the triangles represent the various pathogenic parasitic species.

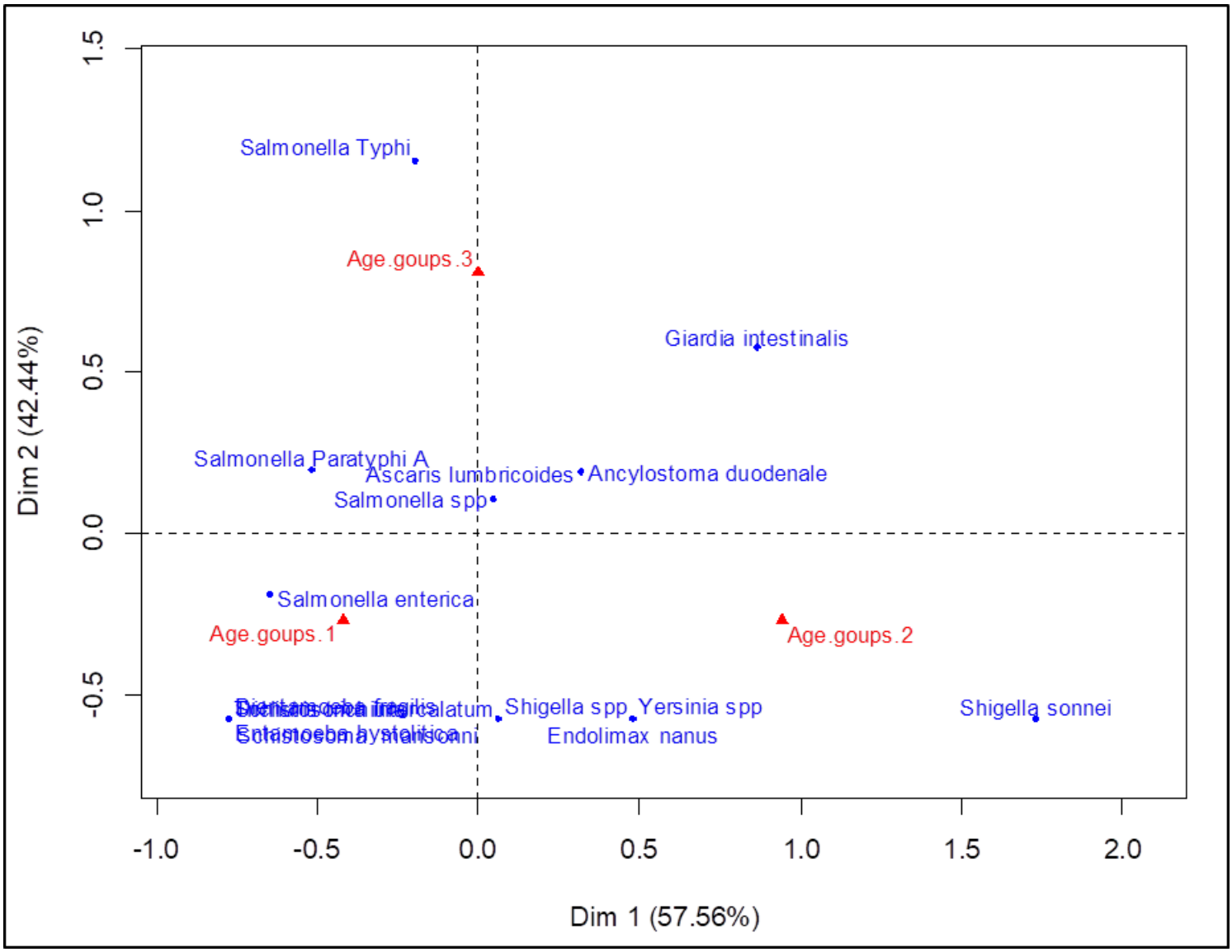

Figure 4

Distribution of enteropathogens by age group. Legend: The triangles on the map represent the different age groups and the dots represent enteric pathogens (Age group 1: 0 – 24 months; Age group 2: 25 – 48 months; Age group 3: 49 – 70 months).