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Prevalence of parasitic infections in Motobo and surrounding villages in South-eastern Gabon: a relevant study in rural Central Africa.

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Abstract

Objective: To determine the prevalence of parasitic infections in the locality of Motobo and surrounding villages, located in the department of Mpassa, province of Haut-Ogooué in South-eastern Gabon.

Material and Methods: In order to determine this prevalence, 377 children, regardless of sex, aged between 2 and 17 years were enrolled. Three types of samples, namely stool, urine and blood, collected from the children were examined by microscopic methods using techniques such as Kato-Katz, the MIF two-phase concentration method, the Coproculture technique, the Harada-Mori technique, a method of cultivating larvae on filter paper, a technique of concentrating parasites on a slide from a drop of blood that is thickened with a drop, the membrane filtration technique, a technique for identifying and quantifying S.

haematobium eggs in urine, and leuco-concentration, an enrichment technique based on the destruction of red blood cells by saponification at 2%, often used for the detection of microfilariae in weakly infected subjects.

Results: Of the 377 samples examined, only 231 were positive for parasitic infections, a prevalence of 61.27%. With a sex ratio of 0.90, females were in the majority at 52.52% (n=198) compared to males at 47.48% (n=179). The average age was 8 years. There was a major representation of children in the 2-5 and 6-10 age groups, with 36.6% (n=138) and 35.07% (n=136) respectively. While 121 children (32.1%) were from Motobo I, 256 children (67.9%) were from the surrounding villages (Motobo II and III). Different types of microscopy methods used in this study allowed the identification of 5 groups of parasites in mono, bi, tri or poly infections. The prevalence rates of the study population obtained by microscopy gave respectively: geohelminthiasis 78.35%, urogenital bilharziasis 35.08%, malaria 38.96%, filariasis 14.72%, intestinal protozoa 58.87%. The age group 6 to 10 was more exposed to contracting one or more parasitic infections.

Conclusion: The use of different microscopic methods allowed the identification of several parasite species with high prevalence rates of parasitic infections. Thus, it was found that children living in the locality of Motobo and surrounding villages, located in the Mpassa department, Haut-Ogooué province in south-eastern Gabon, are seriously exposed. These results should drive decision-makers to develop and orientate control strategies to reduce the transmission and consequent morbidity of these parasitic infections in this rural locality.

Key words: Epidemiology, prevalence, microscopy, parasitosis, parasitic infection, Motobo, Franceville, Gabon.

I. INTRODUCTION

Parasitic infections pose a serious threat to the lives of billions of people around the world due to high morbidity and mortality rates. These infections are mainly found in the hot and humid tropics, which provide a favourable environment for the spread of their vectors. Parasitic infections are generally divided into four groups; protozoan, helminthic, micomycete and ectoparasitic infections [1]. In the tropics and subtropics, parasitic diseases such as malaria, schistosomiasis, geohelminthiasis, filariasis and intestinal protozoa are an important public health problem [2]. Today, almost half of the world's population is exposed to malaria, which, according to a WHO report, has recorded 228 million cases and 405,000

deaths due to malaria worldwide [3], with almost 93% of cases and 94% of deaths occurring in Africa [3]. Among the forms of malaria encountered, Plasmodium falciparum malaria remains a considerable burden, as anaemia related to this form is responsible for the mortality of pregnant women and children under 5 years of age [4]. In many endemic regions, as if the heavy burden of malaria alone were not enough, schistosomiasis or bilharzia is also a major problem. These two parasitic infections overlap geographically in Africa [5]. Schistosomiasis is caused by parasitic worms called trematodes of the genus schistosoma that are transmitted in the urine or faeces [6]. They infect about 230 million people in 74 countries worldwide, 90% of which are in sub-Saharan Africa [7]. Of the three species that infect humans, *Schistosoma haematobium* causes urogenital schistosomiasis. It is the most common form in Africa and school children are the most affected [8]. Geohelminthiasis is also a parasitic infection caused by nematode worms of which the three main species are Ascaris lumbricoides, Trichuris trichiura and the hookworms (Necator amricanus and Ancylostoma duodenale). This group of parasitic diseases is transmitted to humans through soil contaminated with faeces. In addition, the WHO in its strategy to control these parasites, reported that more than 1.5 billion of the world's population are infected with geohelminths [9]. These parasites are most prevalent in tropical and subtropical regions and children are the most affected population. In the second edition of the WHO guide for school-age helminth control programmes published in 2011, WHO estimates that 35.4 million school-age children are infected with *Ascaris lumbricoides*, 40.1 million with *Trichuris trichiura* and 41.1 million with hookworms [10]. With regard to *Strongyloides stercoralis*, which has a geographical distribution that coincides with that of the other geohelminths, work on these parasites estimates that it is responsible for more than 600 million cases of infection worldwide [11]. After malaria and bilharziasis, filariasis as a whole represents the third parasitic endemic. Indeed, it has been reported that more than 150 million people are infected with filariasis in inter-tropical areas [12]. *Loa loa* and *Mansonella* perstans filariasis are endemic in several African countries, infecting between 10 and 100 million people. Both filariasis are considered public health problems in Africa [13]. Varying according to age group, their prevalence may be moderate in children, but evolves rapidly with age [14]. In Gabon, as in many other countries in sub-Saharan Africa, parasitic infections are widespread and their prevalence also varies from one region to another. Although a study in the Gabonese capital Libreville and its surroundings showed that only 18.8% of participants were infected with *P. falciparum* [15], a more recent study on the same infection showed a prevalence of 58% among schoolchildren

and adolescents [16]. In the south-east of the country, in Franceville, not far from our study area, research has shown that 61% of cases of paediatric diarrhoea were caused by intestinal parasitic infection [17]. *Loa loa* and *Mansonella Perstans* are two blood-borne parasites that are highly endemic in Gabon. The highest prevalences of these two filariasis are recorded more in forest areas and less in the savannahs [18]. In southern Gabon in the department of Tsamba-Magotsi, a study indicated that 50.8% of participants tested were positive for loasis [19]. In Koulamoutou 12% of participants in another study were positive for Mansonellosis [20]. The locality of Motobo and the surrounding villages are all surrounded by a vast equatorial forest, and the rural populations live in climatic and hygienic conditions that are often questionable. Thus, they are exposed to several risk factors for the most common parasitic infections. These include sanitation, environment, level of education, access to drinking water and nutritional status, in short, socio-economic status [15]. It is in this context that this study was undertaken to determine the prevalence of parasitic infections in the locality, located in the department of Mpassa, province of Haut-Ogooué in south-eastern Gabon. Understanding the epidemiology of parasitic infections in this rural area is essential for the design and implementation of control strategies to combat them by raising awareness of environmental sanitation, deworming and individual hygiene in populations that are comparable from one region to another.

II MATERIAL AND METHODS

II.1 Type, Area, and Study Population

This cross-sectional and prospective study was conducted from 25 August 2020 to 18 December 2020 in the Motobo area, a grouping of villages (Motobo I, Motobo II and Motobo III) located on the Franceville-Okondja axis, in the Mpassa department, which is an administrative subdivision of Haut Ogooué, the southern province of Gabon. Franceville serves as the administrative centre of this department and its surrounding rural villages, the population of this department is 1,193 inhabitants in 1993 and 110,568 inhabitants in 2013. [21]. The tropical climate consists of two rainy seasons from September to November and from February to May, which are separated by two dry seasons. The main activities in the department are subsistence farming, game hunting, fishing and some employment in the local administrative sector. The vegetation is equatorial. The locality of Motobo has a primary school and a dispensary. The population of this study consisted of children of both sexes aged

2 to 17 years living in the village of Motobo and the surrounding villages located on the Franceville-Okondja axis.

II.2 Study procedure

II.2.1 Participant inclusion criteria, data collection

Only children who agreed to give all three types of samples; blood, stool and urine, and whose parents or legal guardians had previously signed and freely consented to have their children participate in the study were included. Once informed consent was obtained, a survey form and two identified sterile jars containing coded information from each participant were given to each child. While the collection of blood samples collected by venipuncture into an EDTA tube took place on the same day of registration, stool and urine samples were collected the following day, in sterile jars given to participants the day before. Once the samples were received, they were taken to the medical analysis laboratory of the Amissa Bongo Hospital in Franceville, where they were first recorded in a register or reception folder. The survey form included identification (surname, first names, age and sex) and information on the type of housing and water supply.

II.3 Diagnostic methods

II.3.1 Microscopy

II.3.1.1 Parasitological examination of stools

Stool samples collected from the field were transferred to the sorting room of the Molecular and Cellular Biology Laboratory of the Biology Department of the University of Science and Technology of Masuku where they were sorted, the information on the participants was anonymised by coding and recorded on data collection forms for the microscopic examinations (Kato-Katz, MIF, Harada-Mori, coproculture), and stored at -20°C.

II. 3.1.1. 1 Kato-Katz

Interest

The Kato-Katz concentration technique allows the stools to be lightened by a solution composed of glycerine and malachite green, in order to quickly identify, at low magnification, the eggs of helminths such as Ascaris lumbricoides, hookworms and Trichuris trichiura [22] [23].

Technique

Using a wooden spatula, the faeces were homogenised in a jar, then a small portion was taken and placed on aluminium foil. The stool was then sieved through a metal sieve with 212µm mesh to remove large fragments found in the stool. Using a 1.5 mm thick template with a 6 mm diameter hole, 41.7 mg of faeces were measured and deposited on two slides. These were each covered with a rectangle of cellophane and immersed for at least 24 hours in a 3% glycerine-malachite green solution taken with forceps. The preparation was turned over and crushed with the thumb to obtain a thin and homogeneous spread. The ready preparation was left to stand at room temperature for 10 minutes to brighten, after which it was examined under the microscope with an X10 objective. A second reading was taken after 24 hours to confirm or improve the result of the previous day's reading.

II. 3.1.1.2. Merthiolate Iodine Formol (MIF)

Principle

The MIF two-phase concentration method is commonly used for the diagnosis of geohelminths and is particularly recommended for the detection of cysts of amoebae and other protozoa. It is a physico-chemical method based on the difference in density between the disturbing debris and the different forms of parasites. It is characterised by the presence of two immiscible phases, one aqueous and the other constituted by ether, which is a solvent for lipids. A partition coefficient is created between these two phases and the distribution of each faecal element in each of them will be made according to the hydrophilic or lipophilic power of each element.

Technique

In a sterile plastic jar or glass 10 ml of Formol-Water (10%) was mixed with 1 to 2 g of stool taken from a sample that had been homogenised beforehand. The resulting homogenous solution was filtered through a filter cloth and 7 ml of the filtered solution was collected in a 15 ml Falcon conical tube. Then 3 ml of ether was added to the filtered solution, the tube was closed and shaken by hand for one minute and left to stand for 5 minutes. The resulting ether-liquid solution was centrifuged at a slow speed of about 1600 rpm for 5 minutes. After centrifugation, the constituents of the suspension were divided into 4 layers and the residues of the lipophilic layers were peeled off by tapping the tube lightly with the finger, the tube was turned over the sink. Once the supernatant had been removed two to three drops of lugol were added to allow suspension and staining of the pellet. 2µl of the pellet was removed with

a micropipette and placed on an object slide which was examined under a light microscope at the X10 objective.

II. 3.1.1.3 Coproculture

Principle

This is a method of culturing eggs and cysts to obtain larvae. It allows the detection of helminth larvae, in particular Strongyloides stercolaris larvae and hookworm larvae.

Technique

A slide was wrapped with two layers of absorbent paper and placed in a petri dish. A portion of stool was rubbed onto an absorbent paper and 20 ml of tap water taken with a syringe was added to the petri dish. The petri dish was then closed and placed in an incubator at 25 ± 30°C for 7 days. After 7 days of incubation, the petri dish was removed from the incubator and 10 ml of the culture medium contained in the petri dish was withdrawn with a syringe and filtered through a filtration kit. The millipore membrane was removed from the filter with forceps and placed on an object slide without being turned upside down. Finally the slide was examined under a microscope with an X10 objective.

II. 3.1.1.4 Harada-Mori

Principle

The Harada-Mori technique is a method of culturing larvae on filter paper that uses the water tropism of strongyloid larvae to concentrate them.

Technique

A filter paper was cut into a narrow strip of about five inches with slightly tapered ends. One to two grams of faeces were placed on the end of the filter paper, then 1 ml of tap water was added to a 15 ml Falcon tube. The filter paper containing the faeces was inserted into the tube so that the tapered end of the paper touched the bottom of the tube and the water level was slightly above the faecal material. The tube was then plugged with absorbent paper, held in an upright position and placed in an incubator at 25-28°C for 10 days. A smear was then taken after the 10 day incubation period by removing the water that remained in the tube after homogenisation, 2µl of this solution was placed on a slide covered with a coverslip and examined under the microscope at X10 objective to allow identification and differentiation of hookworm larvae.

II. 3.1.1.5 Thick drop

Principle

This is a technique for concentrating parasites on a slide from a drop of capillary blood, it consists of the lysis of haemoglobins in order to make the trophozoites appear in the field of the slide. The principle is based on a thin circular or rectangular blood drop spread in the centre of a slide.

Technique

Using a micropipette, 10µL of blood was collected and spread on a slide placed on top of a rectangular mould 1.8 cm long and 1 cm wide drawn on a white sheet. The blood was spread with a cone or other slide to cover the rectangular mould and then air-dried for approximately two hours. Then the dried slide was stained with 10% Giemsa for 15 minutes. After being dried again, the slide was examined under the microscope with an X100 objective after applying a drop of immersion oil to the smear. Parasitemia was established by counting the number of trophozoites found in 100 fields and was obtained by the following formula (Number of parasites counted X target number)/Number of fields viewed.

II. 3.1.1.6 Urine filtration

Principle

Membrane filtration is a technique for the identification and quantification of S. haematobium eggs in urine. It is sensitive and the parasite load is estimated as the number of eggs excreted per 10 ml of urine.

Technique

The urine in a plastic jar was first homogenised. Using a syringe adapted to the filtration kit containing a millipore filter, 10 ml were collected and placed on an unturned slide. Two drops of lugol were then placed on the millipore filter and the slide was examined under a microscope with an X10 objective.

II. 3.1.1. 7 Leuco-concentration

Principle

Leuco-concentration is an enrichment technique based on the principle of destroying red blood cells by saponification at 2%. It is often used for the detection of microfilariae in weakly infected subjects [24].

Technique

Blood contained in EDTA tubes was homogenised beforehand. Then 1 ml was collected and introduced into a 15 ml Falcon tube, 2 ml of 0.9% NaCl physiological water and 200 µl of 2% saponin were added. The mixture was left to stand at room temperature for 5 minutes to allow the saponin to lyse the red blood cells, after which the solution was centrifuged at 2000 Rpm for 10 minutes. After centrifugation, 10 to 20 µl of the pellet was removed and placed on a slide covered by a coverslip. The resulting smear was observed under a microscope with an X10 objective to look for microfilariae (Loa loa and Mansonella perstans).

II.4 Ethical considerations

. The parents or guardians of the children included in the study had signed an informed consent form before any sample was taken.

II.5 Data processing

The results of the parasitological examinations were recorded on pre-established forms stored in binders. These were supplemented by molecular analysis results sheets. All these results were then saved on a common database in an Excel file which was in turn saved in a document on Dropbox version 123.4.4832.

II.6 Statistical analyses

The statistical analyses were carried out using the R software version 4.0.5 and the Rstudio environment with the package "tidyverse". The database was imported into R from an Excel spreadsheet on which all parasitological and molecular results had been recorded. The report was produced using R markdown and the tinytex package. Confidence intervals and p-values were determined at the 95% confidence level and the comparison of proportions test, respectively, using the "binom.approx" and "prob.test" functions of the "epitools" package.

III.RESULTS

III.1. Descriptive analysis of the study population

III.1.1 Socio-demographic characteristics of the study population

During the present study, a total of 377 children between the ages of 2 and 17 years were registered. The average age of this population was 8 years. With a sex ratio (M/F) of 0.90, females were in the majority at 52.52% (n=198) compared to males at 47.48% (n=179). The 2-5 and 6-10 age groups were in the majority, with 36.6% (n=138) and 35.07% (n=136) of children respectively. Also in this study, 121 children or 32.1% were from Motobo I, and 256 children or 67.9% were from the surrounding villages, namely Motobo II and Motobo III. Table 1.

Table 1: Socio-demographic characteristics of the study population

Characteristics		**Number of participants**	**Percentage(%)**
Age			
	2-5	138	36.6
	6-10	136	35.07
	11-17	103	28.33
Sex			
	Fermale	198	52.52
	Male	179	47.48
	Sex Ratio [M/F]		0.90
Location			
	Motobo I	121	32.1
	Motobo II et III	256	67.9

III.1.2 Distribution of parasitic infection rates by locality

Of the 377 samples examined, only 231 were found to have at least one or more parasite infections, giving an overall prevalence of 61.27%. The distribution of parasitic infections by village showed that children in the two surrounding villages (Motobo II and Motobo III) were more parasitic than those living in Motobo I, which is less rural. A 95% confidence interval indicated statistical significance of these results. Table 2.

Table 2: Distribution of parasite infection rates by locality

Villages	**Examined**	**Positive**	Percentages%	IC95%	p-value
Motobo I	121	73	6.33	[0.510 – 0..691]	0.028*
Motobo II et Motobo III	256	158	61.71	[0.554 - 0.677]	< 0. 0 1*

Total	377	231	61.27	[0.561- 0.662]	< 0. 0 1*

* Signifincant Test

III.2. Presentation of different parasitic infections identified in the study population

With a 95% confidence interval, the different microscopic diagnostic methods used in this study and summarised in Table 3 allowed the identification of 14 species of parasites with mono, bi, or poly infections, divided into 5 types of parasitic infections. The prevalences of these identified in the study area were as follows: Geohelminthiases (Soil-transmitted Helminthiases (STH)) caused by *Ascaris lumbricoides*, Hookworms, *Trichuris trichiura*, *Strongyloides stercoralis*, were detected in 181 children or 78.35%. Malaria, of which Plasmodium falciparum and Plasmodium malareas are responsible, was identified in 90 children, i.e. 38.96%. Urogenital bilharzia, for which Schistosoma haematobium is responsible, was diagnosed in 61 children, i.e. 26.41%. Filariasis with loasis and mansonnelosis were diagnosed in 34 children, i.e. 14.72%. Finally, intestinal protozoa with *Giardia duodenale*, *Entamoeba coli*, *Entamoeba histolytica*, *Blatocystis hominis*, Chilomastix were detected in 136 children, i.e. 58.87%.

Table 3: Different parasitic infections identified in the study population.

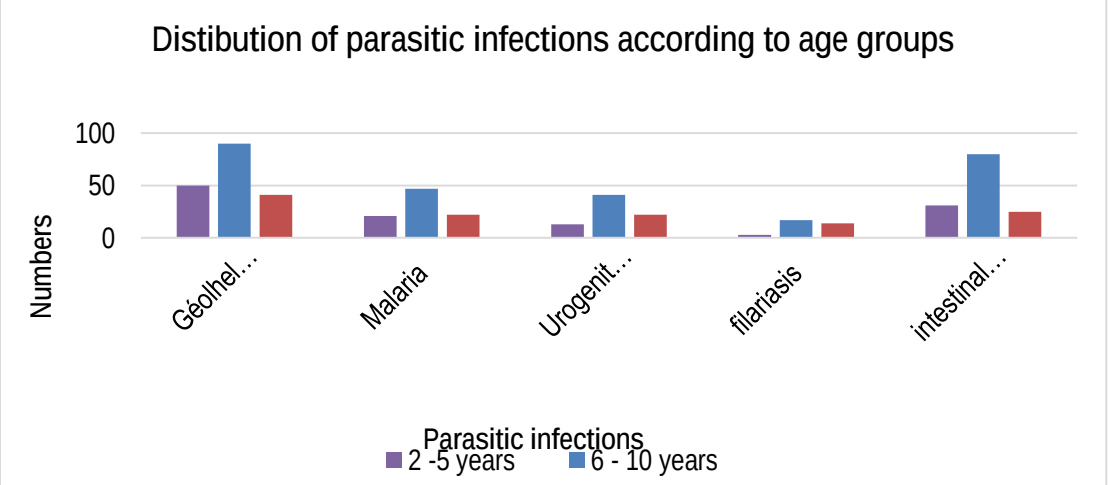
Parasitic infections	Number of children with parasites	**Percentage %**	Confidence interval **IC95%**
Geohelminthiases (HTS) *Ascaris lumbricoides* *Trichuris trichiura* *Hookworms* *Strongyloides stercoralis*	**181** 35 98 27 21	**78,35** 15,15 42,42 11,69 9,09	[0.72- 0.83]
Malaria *Plasmodium falciparum* *Plasmodium malarea*	**90** 87 3	**38,96** 37,66 1,3	[0.326 - 0.455]
Bilharzia-urogenital *Schistosoma haematobium*	**81**	**35.08**	[0.289 - 0.415]
Filariasis *Loa loa* *Mansollena p*	**34** 27 7	**14,72** 11,68 3,04	[0.104 - 0.199]]

Intestinal protozoa	**136**	**58,87**	[0.522 - 0.652]
Giardia duodenale	17	7,36	
Entamoeba histolytica	21	9,09	
Entamoeba coli	84	36,36	
Blatocystis hominis	7	3,03	
Chilomastix	7	3,03	

III.3. Distribution of parasitic infections among to age group

Figure 1 shows that among the 231 children diagnosed as positive for the various parasitic infections, the age group 6 to 10 was the most affected by all the parasitic infections identified in this locality. Indeed, 90 or 49.73% of the children in this age group were infected with geohelminthiasis (STH), compared to 50 or 27.62% and 41 or 22.65% respectively for the 2 to 5 and 11 to 17 age groups. Forty seven (47) children (52.22%) in the 6-10 age group were infected with malaria, 17 children (50%) with filariasis, 41 children (20%) with urogenital bilharzia, and finally 80 (58.82%) with intestinal protozoa. The lowest prevalence rates for all parasitic infections were recorded in the 2-5 year age group except for intestinal protozoa where the prevalence rate (22.79%) was higher than in the 11-17 year age group where 18.38% were infected.

Figure 1: Distribution of parasitic infections among toage group

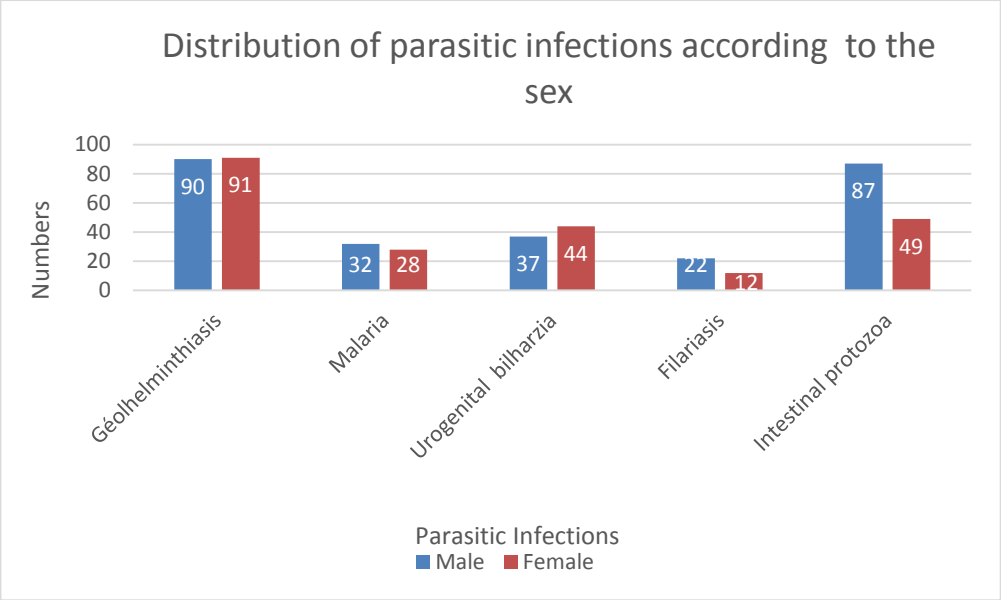


III.4 Distribution of parasitic infections according to sex.

Figure 2 shows that among the 181 children diagnosed as positive for geohelminthiasis (HTS), 91 or 50.28% were female and 90 or 49.72% were male. With 32 children, i.e. a prevalence of 53.33%, males were more infected with Plasmodium than females who had a prevalence of 46.67%. Similarly for filariasis and intestinal protozoa, with respectively 22 and

67 infected subjects, male children had prevalences of 64.70% and 63.97% compared to female children who had prevalences of 35.3% and 36.03% for filariasis and intestinal protozoa respectively. The results of the urinary filtration for Schistosoma haematobium in the case of urogenital bilharzia showed a majority of infections in female children with 44 (54.32%) of children, compared to male children who had a prevalence rate of 45.68%. Basically, it was the male children who were more likely to be at increased risk of parasitic infection. These results were statistically significant when p-value ≤ 0.05.

Figure 2: Distribution of parasitic infections according to sex



III.5 Distribution of parasitic infections according to locality.

The analysis of table 4 shows that among the 181 children positive for geohelmintia (HTS), 76 (41.98%) were from Motobo I, while 105 (58.02%) were from the surrounding villages (Motobo II and Motobo III). 51 children (56.66%) with malaria resided in the surrounding villages (Motobo II and Motobo III) compared to 39 children (43.34%) in Motobo I. Bilharzia was detected in 66 children (81.48%) of Motobo I compared to only 15 children (18.52%) in the surrounding villages (Motobo II and Motobo III). 23 children (67.64%) positive for filariasis came from the surrounding villages (Motobo II and Motobo III), against 11 children (32.36%) . Finally, the surrounding villages (Motobo II and Motobo III) had 111 children (81.61%) positive for intestinal protozoa compared to only 25 children (18.39%) for Motobo I. Considering a 95% confidence interval and the value $p \leq 0.005$, an exact binomial test was used to analyse the level of significance of the observed differences in the percentages of the distribution of parasitic infections, according to the localities of Motobo I compared to the

surrounding villages (Motobo II and Motobo III). The test was considered significant when p-value ≤ 0.05.

Table 4: Distribution of parasitic infections according to locality

	Localities			**Binomial test**		
Parasitic infections	**Motobo I**	**Motobo II et III**	**Total**	**P (M I)**	**IC 95%**	**p-value**
Geohelminthiasis	76	105	181	0.42	[0.37 – 0,49]	0.03*
Malaria	39	51	90	0.43	[0.32 – 0.54]	0.2
Uro-genital bilharzia	66	15	81	0.81	[0.71 – 0.89]	< 0.01*
Filariasis	11	23	34	0.32	[0.17 – 0.50]	0.05*
Intestinal Protozoa	25	111	136	0,81	[0.74 – 0.87]	< 0.01*
Total	217	305	522	0.58	[0.54 – 0.62]	< 0.01*

*Significant test

IV. DISCUSSION

This prospective cross-sectional study conducted in the rural area of Motobo and surrounding villages all located on the Franceville - Okondja axis, aimed to determine the prevalence of parasitic infections of interest using microscopic methods. The results of all the examinations indicated the presence of numerous parasitic infections. In contrast to a study carried out in another province of Gabon in which male participants were more representative than female participants [25], our study showed a slight female predominance. Indeed, out of the 377 participants recorded, 198 (52.52%) were female compared to 179 (47.48%) male. The sex ratio was 0.90 in favour of girls and the average age of the children included was 8 years. The difference in participation with our study can be explained on the one hand by the fact that in contemporary societies, women are more aware of well-being and health than men. On the other hand, the number of participants and the periods in which the two studies were conducted are different. In addition, children in the 2-5 and 6-10 age groups were in the majority, with 36.6% (n=138) and 35.07% (n=136) of children respectively, compared to

28.33 (n=103) in the 11-17 age group, which is normal as these are the two most vulnerable age groups, exposed to several risk factors for parasitic infections. From this study, it appears that 14 species of parasites were identified from faecal, urine and blood samples. This number of species is almost similar to that found elsewhere [27]. [27]. Of these parasites, 78.35% (n= 181) of the children who underwent a parasitological examination were found to harbour at least one species of geohelminth, i.e. more than one child in two. This prevalence rate is much higher than those reported by other studies carried out in another province of Gabon, which recorded a prevalence rate of 48% [25]. Similarly, Franceville, the capital of Haut-Ogooué province in south-eastern Gabon, close to our study area, reported a prevalence of 33.3% [17]. In our study, the high prevalence of geohelminthiasis is not surprising as it has been reported in one study that geohelminths are very common parasites in rural areas such as our study area [25]. The difference between our results and those of other studies can be explained by the variability in geographical, socio-economic and hygienic data of the populations considered. Among the 78.35% prevalence rates of geohelminths identified in our study, we noted a predominance of Trichuris trichiura with a prevalence rate of 42.42%, followed by Ascaris lumbricoides 15.15%, hookworms 11.69% and the lowest rate was Strongyloides stercoralis 9.09%. These prevalences are lower than those reported elsewhere [15] [26]. However, the prevalences of the different geohelminth species recorded in our study are higher than those reported in a study conducted in Rwanda in 2019 in which only 5% of the study population was infected with hookworms [27]. Our results are almost similar to those obtained in some previous work [28]. Our study noted that with a prevalence rate of 35.08% (n=81), Schistosoma haematobium, responsible for urogenital bilharzia is one of the most endemic parasites in Motobo and surrounding villages. The results of our study are similar to those of a study carried out in another province of Gabon where a prevalence of 30% was reported [25]. The similarity of the results of these two studies can be explained on the one hand by the use of the same diagnostic methods and on the other hand by possible migratory flows of people through the different provinces of Gabon, in which the same subtropical climate and the same dubious socio-economic conditions are found. Other results even closer to ours, obtained in southern Benin, show a prevalence of 32.78% [29]. The blood samples provided by the participants showed a prevalence of 38.96% assigned to malaria and Plamodium falciparum was the most detected parasite with 32.66% presence in children. This high prevalence of malaria in the locality of our study is not surprising because, despite the progress made in the fight against malaria, it remains a worrying public health problem as

indicated by its morbidity, which varies between 31% and 71% depending on the region of Gabon [30]. Our prevalence of malaria is close to that indicated in the study by Bouyou-Akotet et al. who reported a rate of 42.1%. [15] Furthermore, a prospective cross-sectional study conducted in Lambaréné, a town in the Middle Ogooué province in central Gabon, and surrounding villages reported that 72% of school children (6-12 years), 55% of young children (2-5 years), and 68% of adolescents (13-18 years) in this region were positive for Plasmodium [16]. This could be explained by the fact that malaria is endemic in Gabon. These differences in results may be explained by the size of the study population and the inclusion criteria may also be at the origin of these differences.

For filariasis and intestinal protozoa, the prevalence rates were 14.72% (Loa loa and Mansonella perstans) and 58.87% (*Giardia duodenale*, *Entamoeba histolytica*, *Entamoeba c*oli, *Blatocystis hominis*, Chilomastix) respectively. These results are consistent with the results of the M'bondoukwé study [15], but are higher than the results of a study conducted in four regions of Gabon, which reported an overall prevalence rate of 6.7% of microfilariae carriage [19]. This lower prevalence rate found in our study justifies the fact that even if the locality of Motobo and the surrounding villages could constitute an endemic zone, our study population was mostly made up of children under 11 years of age. This means that they were not engaged in field work, hunting or fishing, which are risk factors for contracting filariasis. In contrast to the results reported in a study conducted in Butajira, Ethiopia, in which 43.5% of participants were positive for intestinal protozoa[31], our results are rather close to those of studies conducted elsewhere, which found overall prevalences of 53.5% in Cocodi in Côte d'Ivoire [32], 63.8% in Djohong in Adamaoua in Cameroon [33], and a study in Gabon in which 66.7% of participants (children under 5 years) were positive for intestinal protozoa [18]. This high rate of intestinal protozoa found in our study could be explained by the relevance of the examinations carried out for the identification of protozoa, but also by the coprological technique used. Indeed, the technique we used (MIF) allowed the identification of eggs and cysts of intestinal protozoa, which are difficult to destroy in nature, allowing the parasite to resist to different harsh environmental conditions such as heat. Thus, the parasite can be easily identified in the stool. Our study indicated that the 6-10 year age group was the most infected with all parasite species with rates of 49.72% for geohelminths, 52.22% for malaria infection, 50.62% of the participants in this age group had bilharzia, they were also the most affected by intestinal protozoa with a rate of 58.82% and for filariasis 50%. This can be explained by the fact that in this age group, children are very active in the peri-domestic

space, carefree, in the yard of the house, playing with pets and are therefore vulnerable and susceptible to many infections. Primary immunodeficiencies in this age group are innate causes of infections that affect the functioning of the immune system (deficiencies in antibodies, phagocytic cells, etc.) [34] Also included in this age group are the poor hygiene conditions of children who, in rural areas, consume or wash in dirty rivers or streams, walk barefoot outside the home, and defecate in the open. Most of the identified parasites are transmitted orally and through faeces. Results from a study in Ghana and Ethiopia also revealed a higher prevalence of parasitic infections in this age group [35]. Children aged 2-5 years were the least affected by all parasitic infections. 23.33% were infected with malaria, 8.82% with filariasis and 17.28% with urogenital bilharzia, except for geohelminthiasis (27.62%) and intestinal protozoa (22.79%), for which the prevalence rates were higher than those of the 11-17 age group, which were infected at 22.65% and 18.38%. This may be due to the fact that in rural areas, at this age, the child's diet is diverse. And when this is not done properly, the child is very exposed to intestinal parasitic infections by introducing into their mouths everything they pick up from the ground. On the other hand, various risk factors such as geographical and environmental factors, the low socio-economic status of parents, poor sanitation and hygiene conditions, the lack of drinking water and the low level of literacy of parents, all play a role in this susceptibility to contracting intestinal parasitic infections. This has also been observed in Côte d'Ivoire [36]. The monitoring of trends in parasitic infections by sex showed that contrary to the work carried out in Kinshasa, which indicated that girls were more infected [38], our study indicates that among the 181 children diagnosed as positive for geohelminthiasis (HTS), 91 or 50.28% were female and 90 or 49.72% were male. Although it has been reported in some studies in Kenya that females were more exposed [39], our study shows that with 32 children or 53.33% prevalence, male children were more infected with Plasmodium compared to females who had a prevalence rate of 46.67%, which is in agreement with the fact that our study was conducted in an area of high malaria transmission For all children infected with filariasis and intestinal protozoa, male children had a clinical prevalence of 64.70% and 63.97% respectively compared to female children who had a prevalence of 35.3% for filariasis and 36.03% for intestinal protozoa. The results of urine filtration for Schistosoma haematobium in urogenital bilharzia contradict those found in Maroua, Cameroon, which indicated that male children were most at risk of urogenital bilharzia [39]. They indicate a majority of infections in female children with 44 (54.32%)of children, compared to male children who had a prevalence rate of 45.68%. In a study in

Madagascar, the same finding was made by [40]. This result could be explained by the fact that girls are most often involved in household work, which multiplies their contact with water favourable to the dissemination of the infecting form of schistosomes [39. As a binomial test revealed a highly significant difference for all parasitic infections detected in our study, we concluded that male children were more susceptible to parasitic infections in Motobo locality and surrounding parasitic villages than girls. The distribution of parasitic infections according to locality showed that among the 181 geohelmintic-positive children (GHP), 76 (41.98%) were from Motobo I, while 105 (58.02%) resided in the surrounding villages (Motobo II and Motobo III). 51 children (56.66%) with malaria resided in the surrounding villages (Motobo II and Motobo III) compared to 39 children (43.34%) in Motobo I. Bilharzia was detected in 66 children (81.48%) of Motobo I compared to only 15 children (8.52%) in the surrounding villages (Motobo II and Motobo III). 23 children (67.64%) positive for filariasis came from the surrounding villages (Motobo II and Motobo III), against 11 children (32.36%). Finally, the surrounding villages (Motobo II and Motobo III) had 111 children (81.61%) positive for intestinal protozoa compared to only 25 children (18.39%) for Motobo I. Considering a 95% confidence interval and the value $p \leq 0.005$, an exact binomial test was used to analyse the significance level of the differences observed in the percentages of the distribution of parasitic infections, depending on the locality. This study shows that parasitic infections are epidemic among children in Motobo I, but especially in the surrounding villages (Motobo II and Motobo III), where the largest number of participants in the study were recorded. The overall results are statically significant. The rural habitat thus seems to have an influence on the carriage of parasitic infections, and this is in line with the observation made by Aurélien FRANCKEL in his doctoral thesis entitled "Health care seeking behaviour in rural Senegal" [41].

Limitations of the study

Knowing that the identification of parasitic infections, the determination of their prevalences and the evaluation of their infection intensities necessarily depend on the diagnostic method used [19]. Our study was carried out using only microscopic examinations. The latter could have been combined with a molecular diagnosis, because when one wants to determine the prevalence or surveillance of a disease, the use of more sensitive methods is necessary. This is the example in recent years of molecular methods such as conventional and real-time PCR

that have been developed for the diagnosis of parasitic infections. These methods are based on the identification of nucleic acids and are widely used in epidemiological studies [42,43,]. Other studies have also shown that diagnosis by molecular methods allows more accurate quantification and better detection of parasites and increases the detection threshold for parasitic infections such as HTS, bilharzia and intestinal protozoa [19,42].

Conclusion

The main objective of this study was to determine the prevalence of parasitic infections of interest in the rural area of Motobo and surrounding villages. Using several methods such as Kato-Katz, Merthiolate Iodine Formol MIF, Harada-Mori examination and coproculture on all stool samples, urine filtration on all urine samples, thick drop and leuco-concentration on all blood samples, we were able to identify the different parasites in our study area. The main findings of this study are that Motobo is a rural area with a high prevalence of parasitic infections such as urogenital schistosomiasis (25.08%), malaria (38.96%) and geohelminthiasis (78.35%). The age group of children aged 6 to 10 years is considerably more exposed to one or more of the above infections. The results of this work have provided knowledge of the different parasitic infections encountered in the target communities and may help to develop control strategies to reduce the transmission and consequent morbidity of these infections. To this end, several measures could be taken to carry out molecular diagnosis of stool, blood and urine samples from school-age children in the area, in order to gain a more accurate picture of the prevalence of geohelminths and protozoa. It would also be necessary to study possible polyparasitism in this same study area to better understand the different impacts of certain parasitic infections on others. Similarly, a study on the risk factors associated with urogenital bilharzia in Motobo would be welcome.

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Interests

The authors declare that they have no interests.

Authors' contribution

The data reported in the study were available to the authors who contributed equally to the preparation, writing and proofreading of this manuscript

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