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Original Research Article

Persistence in Endemicity of NTDs: Case of Intestinal Parasites in the Health District of Lolodorf, South Cameroon

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ABSTRACT

A study conducted on school children aged 3-15 years in the health District of Lolodorf, South region of Cameroon was aimed to be disposed with more epidemiological data on intestinal parasites. Stool samples were collected from 250 school pupils in January 2015 from four different villages and analyzed using two parasitological techniques: the quantitative Kato-Katz and the concentration formalin ether techniques.
A global infection rate of 46.8% with significantly high prevalence of single infections (64.1%) compared to multiple infections (35.9%; P<0.05) was recorded. The parasites identified were the helminthes *Ascaris lumbricoides* (20.5%), *Trichuris trichiura* (31.9%) and hookworms (2.8%), and the protozoan, *Entamoeba coli* (11.6%). The global egg load varied from 24 to 30.000 eggs per gram of stool, 24 to 56400 eggs per gram of stool and 24 to 14520 eggs per gram of stool for *Ascaris lumbricoïdes*, *Trichuris trichiura* and hookworms respectively. Prevalence varied significantly in the different villages and age groups for *A. lumbricoïdes* and *T. trichiura* same as for egg load observed for *T. trichiura* (P<0.05). Significantly high value of ligth infection intensity was obtained for *T. trichiura* (P= 00.001). Infection intensities of parasitic helminthes were significantly high when in presence of another parasite (P=0.0001) for *A. lumbricoides* +*T. trichiura* and vice-versa, *T. trichiura* + hookworms (P<0.05).
These results confirm persistence in the transmission of intestinal parasites with high risk of morbidity in infected children, since morbidity is essentially linked to egg load. Development of more adequate control strategies are thus necessary like biannual mass drug administration irrespective of age and intensive sensitization of the population on sel-preventive measures.

Key words: NTDs, Intestinal parasites, persistence in endemicity, school pupils, Lolodorf health District, Cameroon

INTRODUCTION

Intestinal parasitic infections are endemic worldwide and are found among the most frequent affections, thereby posing in the same line like other parasitic diseases a major public health problem in less developing countries. [1] They are among the most common infections which heavily affect the poorest and most deprived communities where sanitation is inadequate and water supplies are unsafe. [2] Intestinal infections include : infections caused by helminthes with the most common being soil transmitted helminthes (STHs : *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms) which affect up to 2 billions people worldwide (24% of the world's population; and infections caused by protozoans including amoeba species that cause amibiases, affects up to 10% of the world's population and represents the second cause of mortality among human parasitic infections in children between 4 to15 years. [2-4] These two groups of intestinal parasitic infections could be responsible for 155.000 deaths each year. [5] Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa. [2] People at risk of infection are preschool-age children (aged 1-4 years), school-age children (aged 5-14 years), women of reproductive age (including pregnant women in the second and third trimesters and lactating mothers), and adults who are exposed to STH infections (agriculture professionals). [2,6] These diseases, essentially linked to poverty and inadequate hygiene constitute an important cause of morbidity in children, thus the major concern of WHO global target to eliminate morbidity in children by 2020, through regular treatments of at least 75% of the 873 millions children living in endemic areas. [2]

In Cameroon infections caused by helminthes and protozoans are among the most chronic parasitic infections with more than 10 millions (50%) of the people infected by intestinal worms. [7] Infection rates vary from one geographical region to another and even within the same geographical area. According to Villard (2012), these infections are more common in the meridionian part of the country, with the South region of the country being the most infected (52.8%) followed by the East (46.6%), Southwest (46.2%) and the Central region (25.1%). [8-10] equally, 50.0% infection rates were observed in Yaounde, 28.6% in Mfou and 35.5% in Yoro in the Central region; 42.4% in Barombi kotto and 11.9% in Muyuka in the Southwest region; 39.22% in Njombé and 15.2% in Douala in the Littoral region. [7,11-15] A recent study conducted in some villages of Ngovayang locality in the South region showed the area to be hyperendemic for intestinal parasites. [7] This study was however limited to few villages of Lolodorf neighbourhood, posing a limitation on the updated epidemiological data in the entire locality. In order to have a large scale epidemiological status on these infections in the Lolodorf district health area, a study was conducted in additional four neighbouring villages on intestinal parasitic infections. More importantly, the work was aimed to identify the different parasites with their prevalence, evaluate the infection intensity of the different parasites and evaluate the effect of single and multiple infections on parasites intensity. The results of this study could be vital for in strengthening concrete control measures against these infections in some endemic areas.

2.1 Study area

The study was conducted on school children (aged 3 to 15 years) belonging to four primary schools of the health District of Lolodorf (Mangouma, Bibia, Bikoka I and Bikoka II) of the South region of Cameroon. At the moment that this study was carried out, these schools were not included in any MDA program. The area was selected based on previous level of STHs infections in the South region globally (Tchuem Tchuente et al., 2013), and more specifically on the most recent epidemiologial data obtained on intestinal parasites in neigbouring villages. [10,16] The area is a rural locality with a tropical humid climate with four seasons: a long dry season that extend from November to mid March; a long rainy season that extends from mid August to November; a short dry season that extends from mid June to mid August and a short rainy season that extends from mid March to mid June. The annual average temperature varies between 24°C and 28°C. The community members practice agriculture work, fishing, hunting and trading. [17] Access to potable water constitutes a major problem to the community members with the main water sources being wells and rivers. The four primary schools included in the study benefit from annual MDA for intestinal parasitic infections.

Primary school Bibia (3°16.518’N ; 10°41.110’E), situated at about 6km from Bikoka II primary school is characterized by toilets with poor maintenance, absence of potable water sources and no site for garbage disposal. Primary school Bikoka II (3°15.622'N; 10°42.041'E) with no water sources and non functional toilets is situated at about 4km from primary school Bikoka I. Primary schools Bikoka I (3°15.621'N; 10°42.040'E) and Mangouma (3°13.971'N; 10°42.516'E) separated with a distance of about 3km, are characterized by the absence of water sources, no site for garbage disposal. In all these schools, children defecate around the school premises.

2.2 Study Subjects

The study was conducted from December 2015 (administrative contact and sensitization) to January 2016 (samples collection). Of the 314 school children contacted, 250 (144 boys: 57.6% and 106 girls: 42.4%) provided stool samples giving an overall participation rate of 79.6%. The sampled population was between the ages of 3-15 years and was divided into 3 classes with an age interval of 5 years (3-5 years; 6-10 years; 11-15 years).

2.3. Ethical Considerations

This study was ethically approved by the Ethical Committee of the Institute of Medical Research and Medicinal Plant Studies (IMPM) of the Ministry of Scientific Research and Innovation, Cameroon and the Ethics Review Committee of Lolodorf hospital. Permission to conduct the study was obtained from the community leaders and the school administrators who were duly informed on the objectives and benefits of the study. Parents/guardians were informed about the aim and the procedure of the entire clinical trial. All children whose parents or guardians gave informed consent for their participation were included in the study. Participants were recruited on a voluntary basis and their personal information was treated privately and was not divulged to a third party. Treatment with mebendazole 100 mg (2 tablets taking two times per day for 3 days consecutively was administered under the direct supervision of a clinical nurse.

2.4. Samples collection and processing

Registration and samples collection was done during two days. Day one was confined for registration while the second day was mainly for samples collection which was done by two groups of persons (two persons per group). One stool samples was collected from each participant in 50 ml screw-cap vial between 7:30 am and 12 noon. Samples were conserved in a cooler containing ice blocks and were immediately transported to the Parasitology laboratory of the Medical Research Centre of IMPM in Yaounde. On arrival in the laboratory, each sample was immediately divided into two portions and one portion was fixed with 10% formalin, while Kato slides were prepared using the other portion. The quantitative Kato-Katz technique was used for the identification of helminthes eggs following their morphology (*A. lumbricoides, T. trichiura*, and hookworms) and estimation of infection intensity, while the qualitative concentration formalin ether technique was used to identify helminthes eggs and protozoan cysts. [18-19] To avoid complete degeneration of hookworm eggs, all Kato slides were prepared and read within 24 hours following stool samples collection. Eggs were counted under a light microscope at 10X magnification and their number expressed in eggs per gram of stool (epg). Intensity of helminthes infection was evaluated as low (1-4999 epg, 1-999 epg, and 1-1999 epg); moderate (5000-49999 epg, 1000-9999 epg and 2000-3999 epg) and high (≥50000epg, : ≥10000 epg and ≥4000 epg) infection intensity respectively for *A. lumbricoides, T. trichiura* and Hookworms. [20]

2.5 Data Analysis

Parasitological data were analyzed using Statistic logistic PC DOS Version 2.0. The Chi square test was used to compare the prevalence of parasites in relation to sex, age groups and villages, while one - way ANOVA or Kruskal-Wallis tests were used to compare the parasite intensity in relation to sex, age groups, villages and different parasites combinations. The Kruskal-Wallis test was used when the conditions of parametric ANOVA were not fulfilled. The level of statistical significance was at 95% (P <0.05). [21]

III. RESULTS

3.1.Parasite Prevalence

Out of the 250 children included in the analysis, 117 (46.8%) presented positive result for at least one parasite species by at least one of the analysis techniques used (Table I). The Kato Katz technique revealed 72 (28.8%) positive cases including *Ascaris lumbricoides* (15.2%), *Trichuris trichiura* (21.6%) and hookworms (0.8%). For the formalin ether technique, 117 (46.8%) infected participants were diagnosed for *Ascaris lumbricoides* (20.5%), *Trichuris trichiura* (31.9%), hookworms (2.8%) and *Entamoeba coli* (11.6%). The formalin ether technique revealed statistically high number of infected cases than the Kato Katz technique (P<0.0001). The different parasites diagnosed belonged to the group of helminthes (88.0%: *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms) and Protozoans (24.8%: *Entamoeba coli*). Transmission trend in the different schools varied significantly from 58.9% (Mangouma) to 63.5% (Bikoka II), precisely between Bikoka II compared to the rest of the schools (P= 0.001) and specifically for *A. lumbricoides* (P=0 .0001) and *T. trichiura* (P=0.03) (Table 2).

For all the parasite species, infestation rate was high in males compared to females. Nevertheless, no significant difference was observed (Table 3). Generally, infestation rate varied significantly between the different age groups with children of 6-10 years (P=0.0001) and 11-15 (P=0.004) being more infected compared to those of 3-5 years (Table 2).

Table1. Distribution of the study population with the infection rates given in brackets

Variables	Boys	Girls	Total
Study group	144 (57.6)	106 (42.4)	250 (100)
Infected	73(50.7)	44 (41.5)	117 (46.8)
Uninfected	71 (49.3)	62 (58.5)	133 (53.2)
Single infection	46 (63.0)	29 (65.9)	75 (64.1)
Multiple infection	27 (37.0)	15 (34.1)	42 (35.9)

Table2. Prevalence of the different parasites diagnosed in relation to village, sex and age group (infection rates are given in brackets)

Variables	Number examined	*Ascaris lumbricoides*	*Trichuris trichiura*	Hookworms	*Entamoeba coli*
Village					
Bibia	80	7 (8.8)	24 (30.0)	2 (2.5)	9 (11.3)
Bikoka I	40	7 (17.5)	11 (27.5)	0 (0.0)	6 (15.0)
Bikoka II	85	33 (38.8)	36 (42.4)	4 (4.7)	10 (11.8)
Mangouma	45	4 (8.9)	8 (17.8)	1 (2.2)	4 (8.9)
Sex					
Boys	144	33 (22.9)	47 (32.6)	4 (2.8)	19 (13.2)
Girls	106	18 (17)	32 (30.2)	3 (2.8)	10 (9.4)
Age group					
3-5	28	1 (3.6)	4 (14.3)	0 (0.0)	0 (0.0)
6-10	155	35 (22.6)	52 (33.5)	3 (1.9)	18 (11.6)
11-15	67	15 (22.4)	23 (34.3)	4 (6.0)	11 (16.4)
Total	**250**	**51(20.4)**	**79 (31.6)**	**7 (2.8)**	**29(11.6)**

3.2. Infection Intensities

Egg load varied from 24 to 30.000 eggs per gram of stool, 24 to 56400 eggs per gram of stool and 24 to 14520 eggs per gram of stool for *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms respectively. The mean egg load for *A. lumbricoides*, *T. trichiura*, and hookworms were 19924.1±50572.3 epg of stool 1864.3±8028.8 epg of stool and 7284±10233.2 epg of stool respectively. These values were significantly high in Bibia for *T. trichiura* (P=0.02). For the factors of sex and age group, high egg load values were record though with no significant difference recorded (Table 3). Atleast 50% of light infection intensity was observed for all the three helminthes parasites identified: *A. lumbricoides* (50.0%), *T. trichiura* (50.0%) and hookworms (94.0%), with significant high value obtained for *T. trichiura* (P= 00.001). Heavy infections did not exceed 13.2% and was observed only for *A. lumbricoides* and *T. trichiura.*

Table3. Egg load with standard deviation of different parasites identified during the study according to village, sex and age groups. The number of infected persons are given in brackets

Variables	**Parasite species**		
Village	***Ascaris lumbricoides***	***Trichuris trichiura***	**Hookworms**
Bibia	2584±3252.1 (3)	3703.5±14054.5 (16)	14520±0.0 (1)
Bikoka I	19284±27299.9 (6)	1071±2738.9 (8)	0±0.0 (0)
Bikoka II	23994.8±59563.3 (26)	1012.8±1616.5 (25)	48±0.0 (1)
Mangouma	3265.3±3119.2 (3)	72.00±0.0 (5)	0±0.0 (0)
Sex			
Boys	27036.5±62131.5 (24)	814.3±1603.5 (31)	48±0.0 (1)
Girls	7731.4±13997.4 (14)	3314.2±12268.9 (23)	14520±0.0 (1)
Age group			
3-5	0±0.0 (0)	60±50.9(2)	0±0.0 (0)
6-10	19726.6±58995(26)	2566.4±10274.0 (34)	7284±10233.2 (2)
11-15	20352±26313.2 (12)	894.6±1868.0 (18)	0±0.0 (0)
Total	**19924.1±50572.3 (38)**	**1864.3±8028 (54)**	**7284±10233.2 (2)**

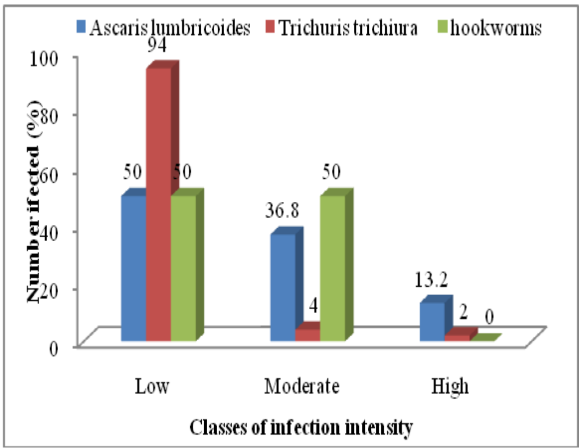

Figure1. Prevalence of infected children in different classes of infection intensity

3.3. Prevalence and infection intensity in co-infected individuals

Out of the 117 infected children, 75 (64.1%) had single infection, while 42 (35.9%) harbored more than one parasite species (Table 1). Single infections were significantly high (P= 0.0001) compared to multiple infections. Overall, *T. trichiura* infections occurred more frequently as single species infection (53.3%; P = 0.0001) compared to other parasite species. The occurrence of multiple species infection was mostly observed for combinations involving *A. lumbricoides and T. trichiura* (64.3%).

Generally, infection intensity of any of the parasite was significantly high when in presence of another parasite (P=0.0001). The mean egg load of *A. lumbricoides* increases significantly when in coexistence with *T. trichiura* and vice-versa, same for *T. trichiura* in the presence of hookworms. Nevertheless, low mean egg load of *A. lumbricoides* and *T. trichiura* were recorded in the presence of *Entamoeba coli* (Table 4).

Table 4 : Variation of mean egg load of parasites in co-infected individuals

Parasite association	**Number of cases**	**Mean egg load**	**Standard deviation**	**P-value**
Al	**18 (25)**	**6798.9**	**13503.2**	0.001
Al+Tt	14 (19.4)	38045.8	78815.2	
Al+Ec	1 (1.4)	48	0	
Al+Tt+Ec	4 (5.6)	24858	27602.8	
Tt	**26 (36.1)**	**357.2**	**725.9**	0.001
Tt+Al	14 (19.4)	663.4	820.6	
Tt+HW	1 (1.4)	56400	0	
Tt+Ec	1 (1.4)	249.6	451.4	
Tt+Al+Ec	4 (5.6)	4248	3949.3	
HW	**1 (1.4)**	**48**	**0**	0.001
Ak+Tt	1 (1,4)	14520	0	

Al= *A. lumbricoides*; Tt= *T. trichiura* ; HW =*Hookorms* ; Ec= *E. coli*

IV. DISCUSSION

The present study is among some of the first previous work conducted in the Lolodorf neighbourhood which earlier characterized it as an area of high risk of infection for intestinal parasites. Though the global prevalence obtained during this study (46.8%) is lower than that obtained (75.9%) in previous work in the area, the transmission risks of intestinal parasites is still not negligible, which can be characterized as mesoendemic after two years followed up. [17,20] This persistence of infections is observable despite the annual National Control program which is effective in the area. This could on one hand be linked to the fact that 17% of children between 6-11 years do not go to schools in the South region (the targeted age group by the control program), and could serve as a potential source in the maintenance of parasites transmission (PNUD, 2010), and on the other hand due to other members of the community who are not often included in the drug administration program. [22] Also, absenteeism is a regular phenomenon in other neighbouring school of the Lolodorf health District, which makes children not to benefit from drug administered by the control program; consequently these children serve as an additional source in the maintenance of parasites transmission. [16] This study was conducted in a rural area with poor sanitation conditions. The results obtained is similar to those obtained in a rural area of the Southwest region (42.4 %), but higher than those obtained in Mfou (29.6%) and Yoro (35.5%) in the Central region, in Douala (15.2%) and in Njombé (39.22%) in the Littoral region and in Muyuka (11.9%) in the Southwest region. [7,11-15] This shows that parasitic transmission is not homogeneous and varies from one geographical area to another, be it in the urban or rural settings. [23] This variation may be linked to climatic factors that could favour the persistence of contaminated eggs or cysts in the environment in one way and in another way on variations in sensitivity of the diagnostic techniques used during the different studies. It can be noted during this study that, the formalin ether concentration technique revealed significantly high number of infected persons (46.8%) compared to the quatitative Kato Katz technique (28.8%) same as a difference in the number of each parasite species. This observation is in line with the proposal made in previous work showing the need of associating the formalin ether technique in the diagnosis of intestinal parasites in order to have a clear image of the epidemiological situation of the different parasites. [24] The parasites diagnosed during our study belonged to the group of helminthes (88.0%) and Protozoans (24.8%).This could reflect the climatic conditions of this geographical area that might favour the development and maintenance of the biological cycles of these parasites. Similar studies have equally put into evidence the presence of these two groups of parasites in other areas of Cameroon. [12,14,15] However, other studies revealed the unique presence of Helminthes and Protozoans. [25,26]

Infection caused by *Trichuris trichiura* predominated during our study (31.9%). This can reflect in addition to the already known resistance of this parasite to anthelmintic drugs, the high reproductive output of female worms (up to 30000 eggs per day) together with the high resistance of these eggs in the environment. [13,27]

Infection rate of hookworms was lower (2.8%) than that obtained previously in the same geographical zone (12.5%), but similar to results obtained in Yoro in the Central region of Cameroon (2.3%). [12,16] This shows that hookworm transmission is not only linked to farming activities and the habit of walking barefooted, but could vary within the same geographical area and in different geographical areas. [16,28] Still in the same line, the low prevalence cannot be attributed to the time put to read the slides, since part of each stool sample was immediately fixed with 10% formalin on return to the laboratory to prevent the decomposition of parasite eggs. The infection rate of *Entamoeba coli* (11.6%) was comparable to that obtained in some localities of Cameroon but high compared to the results obtained in Douala (1.72%). [7,12] Even though the low infections rates of this amoeba species have been recorded, some studies have shown a reduction of heamoglobin concentration in infected individuals. [29,30] In our study area, exposure to infection did not vary with sex, what has equally been observed in previous works. [31,32] Nevertheless, some studied have shown high level of parasitic infections in females than in males. [33] Equally, children of all age groups were infected in the same way, what could reflect the fact that they frequent the same sites, leading to exposure to equal infection risks same as their exposure to the same sanitation condition within and out of the school premises.

The egg load obtained during this study was high, what could mainly be attributed to repeated infections and not really link to long lasting deworming, since the annual drug administration program is equally effective in the study area. This can also reflect the high capacity of egg laid by the female worms of the different helminth species concerned (200.000 eggs /day for *A. lumbricoides* and 30000 eggs per day for *T. trichiura*. [34] High egg loads were equally observed in the case of multiple infections. Previous studies have demonstrated the coexistence of two or more parasites in the same individual to lead to increase egg laid by the parasites concerned and has mainly been attributed to a depression of the host immunity, thus favouring the development of these parasites. [35]

The analysis methods used during this work were more general than specific which permitted to diagnose only the eggs of three helminthes and the cysts of one protozoan. This could constitute a major limitation and by consequent an underestimation of the real epidemiological status of intestinal parasitic infections in the study sites.

CONCLUSION

The results obtained in this work show the persistence of intestinal parasites in the Lolodorf area situated in the South region of Cameroon. Despite the annual Mass drug administration given by MINSANTE, transmission is still intensive. Infected children had single and multiple infections with high egg load for the helminthes parasites identified (*A. lumbricoides*, *T. trichiura*, and hookworms) with a non negligible infection rate of the amoeba species, *E. coli*. These infections might be linked to many endemicity factors like poor sanitation practices, the use of non potable water for consumption and domestic purposes and the absence of good drainage systems. Even though it is difficult to develop concrete strategies which could lead to total elimination of morbidities caused by these infections, continuous attention should be focused on control strategies like: evaluation of the endemicity level coupled to KAP studies, sensitization of community members on their knowledge on KAP studies and intensive mass treatment at the level of entire community followed by re-evaluation of the different intervention strategies. All these associated to the provision of good drainage systems and portable water could significantly limit the extension of these infections within this study area and in other endemic areas.

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