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PREVALENCE OF INTESTINAL WORMS AMONG SCHOOL CHILDREN IN CHAD: IMPORTANCE OF HIGHLIGHTING THE CONCENTRATION METHOD IN FECAL EXAMINATION

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SUMMARY
A third party of humanity is infested by intestinal worms. Although not always easy to discover the impact on health of parasitism by intestinal worms is admitted all the same. In Chadian medical analysis laboratory, the analysis limit to a direct coprological examination. This work aims to establish the prevalence rates of the intestinal parasites from three technics of parasitic coprology, to improve the diagnosis in our analysis laboratories and insure better medicinal prescriptions in our hospitals. The present study took place in Sahelian (Abéché, Bokoro and N'Djamena) and Sudanese (Bongor, Pala, Torrok and Lai) zones. Every stool was submitted to a direct macroscopic examination and two technics of concentration. All eight species of Helminthes (*Hymenolepis nana, Taenia saginata, Taenia solium, Ascaris lumbricoides, Ancylostoma spp., Trichuris trichiura, Strongyloides stercoralis and Schistosoma mansoni*) were found during this investigation. The global rates of infection, that is by at least a species of parasite different and equal were in 36.1% for MOD, 51.1% for MFE and 55.2% for MKA. The number of cases of infections mono-, bi- and tri specific revealed in this work was 362 by the MOD, 512 by the MFE and 554 by MKA. Since prevalence's and number of very high parasitic types of associations observed by the methods of concentration in this study, it would be necessary to incite laboratories of medical analyses of hospitals in Chad to use technics of concentration for the improvement of coprological diagnosis.

KEYWORDS: Intestinal helminthiases, children, school environment, Sahelian zone, Sudanese zone, Chad.

INTRODUCTION
One third of the world population is infected by intestinal worms; thus, 1.5 billion people are infected by *Ascaris lumbricoides*, 1.3 billion by ankylostoms (*Ankylostoma* spp.) and 1 billion by *Trichuris trichiura* (Champetier de Ribes *et al*., 2005). We can estimate at least 400 billion; the total number of intestinal worms harbored by man, such a biomass induces the loss of proteins, vitamins and different types of nutrients in patients (Perez, 2000).

Children aged between 5 and 15 years represent the target population that is most affected. Even though it is not easy to put to evidence, the impact on health with respect to intestinal worms is important (Crompton, 1986).

These helminthes are responsible for annual and they are sources of important morbidity, with non-negligible consequences on the nutritional status (anemia) and the psychomotor development of the target population.

Years are lost due to premature death, or incapacity linked to the helminthes on the first stage, scholarised children in developing countries are infected from birth and this continues throughout their life cycle (Montresor, 2001). In Africa today, intestinal parasitesare less studied due to the deviation of available resources towards new priorities such as AIDS, Avian influenza, malaria, tuberculosis or leprosies (Sakti *et al*., 1999). However, Tcheum Tchuenté (2011) estimated 89.9 million the number of children parasitized by one or many species of helminthes and that 44% of these infections are concentrated in 4 countries which are Nigeria, Democratic Republic of Congo, South Africa and Tanzania. OMS (2001) specifies that the intensity of infection by *A. lumbricoides* and *T. trichiura* is maximum in children of school going ages. It reveals

that intestinal helminthiasis facilitates the transmission and the gravity of AIDS, malaria and tuberculosis.

Hamit *et al*. (2008) have noted that the epidemiological studies on intestinal helminthiasis in the human population in Chad are less assessed in the town of N'Djamena, with the rate of prevalence of 9.96% for *A. lumbricoides*, 0.43% for Ankylostoma, 13.42 for *Hemenolepsis* spp. and 0.22% for the bilharzias due to *S.mansoni*.

Five years after, Hamit *et al*. (2013) put into evidence a disturbing situation in Chad with 33.7% of *Ascaris*, 12.5% of ankylostomes, 12.4% of *Strongyloides*, 2.6% of *Bricocephalus*, 0.7% of teniasis due to *Tenia solium*, 5.7% teniasis due to *T. saginata*, 20% of *Hemenolepsis*, 3.4% of bilharzias due to *S. mansoni*. In another study carried out in the Lake Chad region, Bechir *et al.* (2011) revealed20% of ankylostomiasis, 19% of ascaridiasis and 21% of hymenolepiasis in the Foulbes and 25%, 20% and14% respectively of these infections in Arabs.

Before these studies were carried out, Brooker *et al.* (2002) had established that 32.7% of the rural population of Chad was infected with ankylostomasis. From the results collected from the different big hospitals in Chad from 1998 to 2002, OMS (2003) published the following rate of prevalence of shistosomiasis, 0.3% in Bol, 0.4% in Mongo, 3.77% in Sarh, 3.08% in Moundou, 1.71% in Bongor and 0.34% in N'Djamena.

In the Chadian medical laboratory practices, fecal examination is limited to direct stools assessment. This works is aimed at establishing the prevalence rate of intestinal parasitism using three technics of fecal assessment, in children attending ten schools in seven Chadian towns with the aim of ameliorating the diagnosis in our laboratories and to assure the best medical prescriptions in our hospitals.

MATERIALS AND METHODS

1-Study site

The present study was carried out in the Sahelian zone (Abéché, Bokoro and N'Djamena) and the Soudanian part of the country**.** The Sahelian climate presents two seasons: a rainy season from June to September and a dry season from October to May. However, Abéché that is located in the Ouaddaï mountains, undergoes a short rainy season from August to September (DREM, 2013). The middle belt of Chad benefits from a tropical climate (or soudanien climate) with a presentation of three distinct climates: a dry season from March to July, a rainy season from August to October and a humidity season from November to February of the following year (DREM, 2013). The geo-climatic characteristics of the study sites are presented in table 1 and the geographical location is presented in figure 1.

Table 1: geo-climatic characteristics of the study area

City	Population (inhabitants)	Pluviometry (mm)	Longitude Est (Is)	Latitude North	Altitude (m)
Abéché	138 684	100 à 400	20^0 85'	13^0 85'	545
Bokoro	114 050	300 à 700	17^0 05'	12^0 33'	300
N'Djamena	951 418	500 à 700	15^0 03'	12^0 13'	294
Bongor	69 787	500 à 1300	15^0 40'	10^0 28'	320
Pala	108 374	500 à 1200	14^0 92'	9^0 37'	454
Torrok	49 981	500 à 1200	15^0 02'	9^0 67'	355
Laï	94 695	700 à 1300	16^0 30'	9^0 40'	358

Source: DREM (2013) and INSEED/RGPH2 (2010).

Our data was collected in ten schools that are chosen half-hazardly, thus three in N'Djamena, two in Abéché and one in each of the following towns: Bokoro, Bongor, Laï, Pala and Torrok.

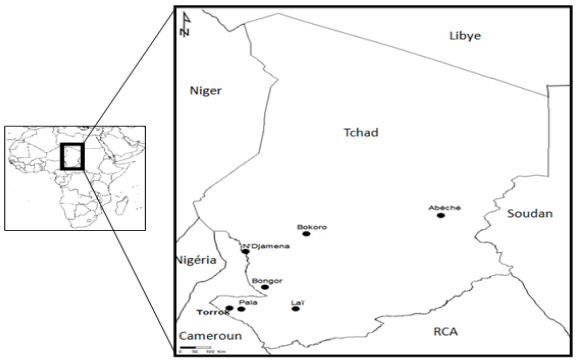

Figure 1: Geographical location of the study sites

2. Target population

This transversal and prospective study that was carried out from March 2010 to February 2011 was carried out on infants whose ages range from 6 to 17 years are all registered in the primary school. The pupils of this age group were retained because, they generally present the highest intensities of specific parasites (Ratard et Greer, 1991; OMS, 2004; PNLSHI, 2005), they are reticent to mass examinations, they are easily transmitted and their situation reflects the parasitic infection of the community. They are highly exposed due to behavioral, hygienic reasons (OMS, 2006).

3. Sampling method

The sampling technic (Hamit *et al*. 2013; Fleiss, 1981; Spiegel *et al*., 1989) enabled us to select 1002 participants in the 7 towns enrolled in the study. Their

number was established by the formula $n = \frac{P(1-P)Z^2}{i^2}$, where n = sample size, P= prevalence of the last study in the south of Chad, thus 37.52% (Brooker *et al*. 2002), Z= 1.96 in the table of normal distribution at 95% of Security and i= precision value of 0.03.

4. Inquiry on children

The permission required for carrying out the inquiry was obtained from the ministry of public health, then the ministry of national education, after the ethical comity had given us a clearance of our project on the basis ofits usefulness to the society. At the eve of the inquiry in a given school, a visit was carried out to inspect the health infrastructures, sensitize the pupils and the teachers on the nature, the importance and the necessity of the study and to obtain their adhesion and that of the representatives of the parent's teachers association. Socio-economic and sanitary questionnaire elaborated on the individual question sheet led to the information on the identity each pupil, mode of life and to bring out the general hygienic and food problems of each participant involved in the study.

5. Stool sampling

On the day of sampling, after the roll call, each participant received an identification number and a plastic recipient to put the stools and a Petri dish to insert part of the fecal sample for onward identification. In order to avoid the contamination of excreta by soil, the participant also receives a stick to sort out the faces, a process that was carefully controlled in child of the junior primary school. Individual Petri dishes containing part of the fecal material, that were labeled and contained 3 to 5 drops of formol were collected and transported to the laboratory of the host school for parasitological examination.

6. Laboratory examination of stool samples

Each stool was submitted to a macroscopic examination, in order to note the consistence, the color, the aspect of the stool and the eventual presence of segments of cestodes, larva and adult worms of nematodes (Anonyme, 2008). Then direct microscopically analysis was carried out according to two technics, the formol /ether method (Marti et Escher, 1990; Utzinger *et al*., 2009) and that of Kato (OMS, 1983 and OMS, 1994).

For the direct identification (MOD), a drop of physiological saline was put on the slide. The eggs of helminthes which measure a minimum of 40 µm, were first observed under objective X10 and then the internal components are further observed with a precision at objective 40 (Gentillini, 1993; Anonyme, 2008).

For the Kato method (MKA), a filter is pressed on the stool sample, with the help of a spatula; the upper part of the shifter is screened to collect the sample to be assessed. A quantity is then placed on the microscope slide (Odongo-Aginya *et al*., 2007). The sample is covered with cellophane that was first inserted into a Kato solution for 24 hours. The preparation is realized evenly with a test tube so that the thickness can enable a reading through the characters of a journal, the sample is allowed to rest for some fifteen minutes so that the water evaporates while glycerol makes the organisms to be clearer. It could be necessary to clean the excess quantity of Kato solution, with a piece of toilet tissue, or to compress it in order to insure that the cellophane is well positioned and the observation are carried out as described before the number of eggs obtained are multiplied by 24 as the model used has 6 pits of 6 mm of diameter on 1.5 mm of thickness and the stool analyzed weighs about 41.5mg (Katz *et al*., 1972; OMS, 1983; Golvan, 1984; OMS 1993).

For the formol/ether concentration method, or MFE **(**Cheesbrough, 2009; Golvan, 1984; Marti et Escher, 1990; Utzinger *et al*., 2009), with the help of stool stick, stool is introduced into a test tube containing 10ml of formol at 10%. The whole preparation is mixed in a suspension and poured unto 2 layers of surgical gaze, directly into the tube to be centrifuged.

This suspension is added to a volume of 10ml, with some slides of 10% formol. After an addition of 0.3ml of ether, the suspension is homogenized for 10 seconds and then centrifuged for 2 to 3 minutes at 400–500g. Four layers are obtained, an upper layer of ether, a fat layer that adheres to the walls of the tube, a layer of formol and the concentrated sample. The cover of the tube is carefully opened and the three layers are discarded at the same time, with the help of a stool stick, the sample is allowed to rest, for seconds. The concentrate at the bottom of the tube is mixed, with drops of physiological Saline and then transferred to a slide for observation as was done with the previous sediments.

7. Analysis of data

The data collected enabled a calculation of the prevalence rate P, which is in relation with the number n of samples infected by each species of the given parasite, multiplied by 100, divided by the total number of samples examined: P = (n x 100)/ N (Margolis *et al*., 1982; Bush *et al*., 1997; Feingold, 1998). A subject was considered as infested and thus positif, for given species, if he or she eliminated an adult worm, or when each of the three coprological methods reveals the presence of a larva or an egg of the worm. Infra community is the set of parasites of each species infesting an individual host (Combes, 1995).

8. Statistical treatment of the data

The CSPRO and SPSS statistical programs enabled a comparison of the difference in frequencies by the Khi-twos (χ^2) test.

RESULTS AND DISCUSSION

The results of the infestation rates, the prevalence of the parasites and the various associations are presented in tables 2, 3 and 4 respectively. There are three species of Cestodes (*H. nana*, *T.saginata* and *T. solium*), four species of Nematodes (*A. lumbricoides*, *Ancylostoma* spp, *T. trichiura* and *S. stercoralis*) and a Trematode (*S. mansoni*) were put into evidence during this study. This parasitological diversity has been discussed by Hamit *et al*. (2013). The rate of global infection by at least one specie were different ($P < 0.001$) and equal to 36.1% for the MOD, 51.1 for MFE and 55.2% for MKA (table 2) however, a comparison of the two methods of concentration did not reveal any difference ($\chi^2 = 3.4$, ddl= 1; $P> 0.05$).

The analysis of the test of prevalence of each species of helminthes identified in the stool and according to the methods used, has revealed differences (table 3) that are generally very significant, ($P < 0.001$), except for *T. solium* for which these three sampling methods have provided statistically equal values ($P > 0.05$).

The larva of *S. stercoralis* were put into evidence only with the direct method. These results explain why the species appears only in bispecific associations revealed by MOD. However, it is known that the best procedure to identify the larva is to practice the Baermann culture method (ANOFEL, 2010). The prevalence of this parasite seems under estimated in this study. These results also testify to the fact that in the absence of the appropriate Baermann method, the MOD even though presents low parasite counts, can give indications on the cause by this parasitic species.

For Ancylostoma spp. And *T. trichiura* ($\chi^2 = 00$) on one hand and *T. saginata* ($\chi^2 = 0.2$) on the other. The two technics of concentration were equivalents and more sensitive than that of the direct method MOD. In the case of *S. mansoni*, the rates of prevalence that were determined, with the MOD and MEF were equal ($\chi^2 = 1.2$; ddl = 1) but lower than that of the MKA.

A. lumbricoides and *H. nana* were the helminthes species that were more isolated (Hamit *et al*., 2013) because they were assessed by the three analytical methods as presented in (table 3). *A.lumbricoides*, followed by *H. nana*, was associated with other parasitic forms (table4).

A part from *A. lumbricoides*, the bi specific or tri specific associations with *Ancylostoma* sp with one or other intestinal helminths were only put into evidence, with concentration methods as a result of the low sensitivity of the direct method.

The cestodes *T. Saginata* and *T. solium* were in association with *A. lumbricoides*; the three methods indifferently detected an association of the first species, while that with T *solium* and were revealed equivocally, with MFE and MKA. In addition *S. stercoralis*, *T. saginata* and *T. solium*, were not identified in the tri specific group. These results can be partially expressed by the fact that tenias are solitary worms **(**ANOFEL, 2010; Golvan, 1984). The five types of tri specific associations obtained in which four integrate *T. trichiura* and one *H. nana*, were revealed due to the diagnosis methodology employed. The low sensitivity of MOD for *T. trichiura*, with respect to *H nana* seems un explained in this failure.

It follows from this inquiry that the direct examination of fecal samples is less efficient with the kato test or the formol ether test (table 2). The consideration of specific infestations, which are higher than the MFE and MKA compared to the MDO (table 3 and 4) attest to this also.

The number of cases of mono, bi and tri specific infections reveal in this research was 362 for MOD, 512 for MFE and 554 for MKA. By taking into consideration the value of 554 as a reference, MOD and MFE respectably covered 65.3% and 92.4% of the efficiency of MKA. Many cases of infections are unknown due to the application of MOD in particular. This study reveals that, in fact the concentration studies are recommended for massive inquiries (Tchuem Tchuenté *et al*., 2012; Knopp *et al.*, 2008; Savioli *et al.*, 2002 and OMS, 1983) and therefore suggest their systematic application in hospitals in Chad. These methods are simple, fast, low costing and adapted for low-income countries (Tchuem Tchuenté *et al*., 2012; Knopp *et al.*, 2008; Savioli *et al.*, 2002 and OMS, 1983).

According to OMS (2004) and Montresor *et al*. (2002), we particularly recommend the use of MKA due to its viability and ease of usage, it also equally enables a mastery of the specific parasitic ovular charge and therefore an adaptation of the treatment strategies recommended for helminthiasis. For some helminthes that do not lay their eggs in the gastro intestinal tract, such as *Enterobius vermicularis* (Adou-Brin et *al*., 2001; ANOFEL, 2010) or in which the eggs hatch quickly to free mobile larva such as *S. stercoralis* (Garcia, 2001; Koga *et al.*, 1991), the putting into evidence of the right technique is imperative.

Whether by the MOD or the concentration methods many cases of poly parasitism were observed. This is mostly the case with helminthiasis (Oyewole *et al*., 2002; Tchuem Tchuemté *et al.*, 2003; Hamit *et al*., 2008 and 2013; El Guamri *et al*., 2009). Keusch and Migasena (1982) also precising that in endemic areas children are rarely parasite by a single species. Like in Cameroon (Tchuem Tchuemté *et al.*, 2012), the presence of parasitic associations in this study indicates a very low level of health and food sanitation, a risk of peril fecal matter in the schools enrolled in the study and a deplorable living conditions in the homes of children who took part in the study. *A. lumbricoides* remains the species that is most implicated in the infra community

and could be considered as the species that is responsible for the malnutrition of school pupils in Chad (Hamit *et al*., 2013).

Table 2: Global infestation rate as a function of the analytical technology

Method used	N	n'	%	χ^2	P
Direct observation of fresh samples(MOD)	1002	362	36.1	81.5	< 0.001
formol/éther concentration method(MFE)		512	51.1		
Kato technique(MKA)		554	55.2		

Legend: N: total number of stool samples examined per method applied; **n'**: number of positive sample with at least one helminth specie; **%** = global infection rate

Table 3: Prevalence rate of each helminth specie, identified, according to the analytical method applied

Helminths species	Methods applied			Value of χ^2	Value of P
	MOD	MFE	MKA		
A. lumbricoides	12.9 (129)	24.6 (246)	32.9 (330)	113.3	< 0.001
H. nana	11.5 (115)	19.7 (197)	14.6 (146)	26.5	< 0.001
Ankylostomes	1.3 (13)	10.6 (106)	10.5 (105)	82.5	< 0.001
T. trichiura	0.4 (4)	2.3 (23)	2.4 (24)	15.2	< 0.001
S. stercoralis	12.4 (124)	0 (0)	0 (0)	-	-
T. saginata	3.2 (32)	5,1 (51)	5.6 (56)	7.2	< 0.05
T. solium	0.5 (5)	0,8 (8)	0.7 (7)	0.7	>0.05
S. mansoni	1.3 (13)	0,8 (8)	3.1 (31)	17.3	<0.001
Value of χ^2	447.1(ddl= 7)	668.7(ddl = 6)	847.1(ddl = 6)	-	-
Value of P	<0.001	<0.001	<0.001	-	-

Legend: N= 1002**; NB: each value in parenthesis represents the number of pupils infested by at least one helminth specie; MOD** =direct observation; **MFE**= formol/ether concentration method; **MKA** = Kato technique.

Table 4: Distribution model, in percentage (and in absolute frequency) of the helminthes species (infra populations or infra commual method nitires) in infested school children as a function of the analytical method

Types of parasitism	Helminth species	Technique applied			Value of χ^2	Value of P
		MOD	MFE	MKA		
Mono specific	AL	7.3 (74)	15.5 (136)	20.7 (208)	74.98	< 0.05
	AN	0.4 (4)	6.5 (66)	6 (60)	56.41	< 0.05
	TT	0 (0)	0.5 (5)	0.5 (5)	0.1	> 0.05
	SS	10.2 (103)	0 (0)	0 (0)		
	HN	8 (80)	14.1 (142)	8.6 (87)	24.95	< 0.05
	TA	1.8 (18)	3.2 (32)	3.3 (33)	5.23	> 0.05
	TO	0.5 (5)	0.7 (7)	0.6 (6)	0.34	> 0.05
	SM	0.5 (5)	0.2 (2)	1.5 (15)	12.73	< 0.05
Subtotal	**7 species**	**28.8 (289)**	**39 (390)**	**41.3 (414)**	**37.96**	**< 0.05**
Bi-specific	AL-HN	2.7(27)	4.4(44)	4.7 (47)	6.1	< 0.05
	AL-AN	0.1(1)	2.7 (27)	2.7 (27)	25	< 0.05
	AL-SM	0.6(6)	0.4 (4)	0.9 (9)	2	> 0.05
	AL–TA	1.4(14)	1.9 (19)	2.3 (23)	2.2	> 0.05
	AL-TT	0.2(2)	1.1 (11)	1 (10)	6.4	< 0.05
	AL-SS	0.5 (5)	-	-		
	AL-TO	-	0.1 (1)	0.1 (1)	-	-
	AN-HN	-	0.7 (7)	0.9 (9)	0.2	> 0.05
	AN-SM	-	-	0.4 (4)	-	-
	AN-SS	0.8(8)	-	-	-	-
	AN-TT	-	0.2 (2)	0.4 (4)	0.6	> 0.05
	HN-SS	0.6(6)	-	-	-	-
	HN-SM	0.1 (1)	0.1 (1)	-	-	-
	HN-TT	0.1 (1)	0.1 (1)	0.1 (1)	-	-
	SM-SS	0.1(1)	-	-	-	-
	SS-TT	0.1(1)	-	-	-	-

Subtotal	**16 associations**	**7.2 (73)**	**11.6 (117)**	**13.4 (135)**	**22.2**	**< 0.05**
Tri-specific	AL-AN-HN	0	0.1(1)	0.1 (1)	-	-
	AL-AN- TT	0	1.2 (2)	0	-	-
	AL-HN-TT	0	0	0.1 (1)	-	
	AL-SM- TT	0	0.1(1)	0.3 (3)	1	< 0.05
	AN–HN-TT	0	0.1 (1)	0	-	-
Subtotal	**5 associations**	**0**	**0.5 (5)**	**0.5 (5)**	**-**	**-**

Legend: N = 1002; **NB: each value in parenthesis represents the number of pupils infested by at least one species of helminth AL** = *A. lumbricoides*, **HN** =*H. nana*, **AN** = *Ancylostoma SP.*, **SM** = *S. mansoni*, **TA** = *T. saginata*, **TO** = *T. solium*, **TT** =*T. trichiura*, **SS** = *S. stercoralis.*

CONCLUSION

In this study, we can suggest the technics of concentration methods in the coprological analysis in Chadians Hospitals. The presence of *S. mansoni*, among the parasitic species identified in this investigation, suggests the presence of the intermediary hosts in some Chadians biotopes, their identification is of utmost importance due to the consequences of helminthiasis in Chad as presented in tables 2-4. A good representative cartography of the distribution of this parasitosis is necessary in order to improve on the medical attention to patients but also to create an accessible database for the competence of authorities that are in charge of public health.

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