

Comparing concentration methods: parasitrap® versus Kato-Katz for studying the prevalence of Helminths in Bengo province, Angola.

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Abstract

Background: Helminth intestinal parasitoses are responsible for high levels of child mortality and morbidity. Hence, the capacity to diagnose these parasitoses and consequently ensure due treatment represents a factor of great importance.

Objectives: The main objective of this study involves comparing two methods of concentration, parasitrap and Kato-Katz, for the diagnosis of intestinal parasitoses in faecal samples.

Methods: Sample processing made recourse to two different concentration methods: the commercial parasitrap® method and the Kato-Katz method.

Results: We correspondingly collected a total of 610 stool samples from pre-school and school age children. The results demonstrate the incidence of helminth parasites in 32.8% or 32.3% of the sample collected depending on whether the concentration method applied was either the parasitrap method or the Kato-Katz method. We detected a relatively high percentage of samples testing positive for two or more species of helminth parasites. We would highlight that in searching for larvae the Kato-Katz method does not prove as appropriate as the parasitrap method.

Conclusion: Both techniques prove easily applicable even in field working conditions and returning mutually agreeing results. This study concludes in favour of the need for deworming programs and greater public awareness among the rural populations of Angola.

Keywords: Concentration methods, parasitrap® versus Kato-Katz, helminths, Bengo province, Angola.

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Introduction

According to the World Health Organisation (WHO)¹, intestinal parasitoses caused by helminth parasites of the Trematoda, Cestoda and Nematode classes continue to rank among the major public health problems in sub-Saharan Africa, afflicting both children and adults with their level of prevalence depending on the levels of hygiene and sanitation and the climatic conditions in the respective geographic area.

The life cycle of these parasites incorporates man as its host and infected whether by oral or by dermal channels (in the case of infectious larvae)².

These parasitoses prove responsible for the high levels of morbi-mortality and frequently causing organic deficits that compromise the normal development of children and thereby serving to limit their working abilities as adults^{3,4,9}.

As the diagnosis of these parasitoses represents a key step in the treatment and control of intestinal parasites, any regional healthcare unit needs equipping with at least one validated methodology for researching the prevalence of parasite eggs.^{5,6} Only thus are able to provide a quality level of laboratorial response and the appropriate treatment of populations.⁷

For such reason, a detailed à priori evaluation of the sensitivity and specificity the various methods of diagnosis

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available is required within the framework of drafting a tailored strategic plan. These, should be done alongside the corresponding allocation of financial resources to ensure the provision of staff qualified members in scientifically undertaking tests and their respective analysis.

This study derives from the application of two concentration methods for parasitoses diagnosis,^{5,8} the parasitrap^{®10} method and the Kato-Katz¹¹ method with the primary objective of comparing their sensitivity and specificity in the identification of intestinal parasite eggs and/or larvae.

Material and methods

Study area

This study took place in two rural locations in Bengo Province, Angola, within the scope of the Demographic Surveillance System run by CISA (Health Research Center of Angola). The first sample site was the village of Cabungo that belongs to the Caxito commune with an estimated population of 450 inhabitants, distributed across two sectors with agriculture and subsistence fishing representing the main activities.

The second sample site was the school in Porto-Quipiri that belongs to the mabubas commune with its estimated population of 600 inhabitants, with agriculture and trade the main sectors of activity.

Sample

In the period between December 2012 and March 2013, the team collected a total of 610 faecal samples from pre-school and school age children (aged between 2 and 15), 24.1% belong to pre-school age children (aged between 2 and 5) and 75.9% of school age (from 6 to 15 years of age) and with 53.4% male and 46.6% female.

The stool samples were collected in purpose designed flasks and sent to the laboratory for processing.

Concentration methods

Sample processing made recourse to two different concentration methods: the commercial parasitrap[®] method and the Kato-Katz method.

In the parasitrap method, the helminth eggs are concentrated by means of a centrifuge. The test relies on a concentration technique involving formol and ether with

the sediment resulting susceptible to direct visualisation by microscope. This method, beyond its effectiveness in concentrating the eggs, does not cause any morphological alterations to either the helminth eggs or larvae.

The parasitological Kato-Katz stool filter kit method provides a qualitative and quantitative examination. The faecal samples are first clarified by glycine and green malachite and bearing the advantage of enabling the counting of the number of eggs of each parasite species and thereby generating estimates of the intensity of the parasitoses prevailing in any given population. This is the benchmark method for epidemiological studies. Furthermore, the counting of the eggs also provides an indication of the effectiveness of deworming treatments through before and after testing.

The Kato-Katz method was deployed only as a qualitative concentration in this study.

The technical processing was carried out in accordance with the instructions of the manufacturer^{10,11}, with each sample prepared for visualisation on a double slide and observed by microscope by two different researchers.

Results

This study made simultaneous recourse of two different methods for concentrating faecal samples, the parasitrap method and the Kato-Katz method, for the diagnosis of helminth parasites in a population of pre-school and school age children in Bengo Province, Angola. The results returned indicate the presence of helminth parasites in 32.8% or 32.3% of the samples collected (either 200 or 197 children infected out of the total of 610 samples subject to analysis), depending on whether the concentration method applied was the parasitrap method or the Kato-Katz method (Table 1) respectively.

Despite the vast majority of samples testing positive for helminth parasite containing only one species (27.9% and 170 samples according to the parasitrap method or 27.2% and 166 samples by the Kato-Katz method), a relatively high percentage of the positive samples contained two or more species of helminth parasites, corresponding to 4.9% and 30 samples when testing by the parasitrap method or 5.1% and 31 samples by the Kato-Katz method (Table 1)

Table 1 – Sample detection frequency for one or more different parasites through recourse to the two methodologies

	Frequency – parasitrap	%	Frequency – Kato-Katz	%
Negative – 0	410	67.2	413	67.7
1 Parasite	170	27.9	166	27.2
2 or more parasites	30	4.9	31	5.1
Total of (1+2)	200	32.8	197	32.3

We identified the presence of eggs from six different species of helminth parasites (Table 2), with *Trichuris trichiura* attaining the greatest incidence followed by *Ascaris lumbricoides* and along with larvae from two species: Ancylostomidae and *Strongyloides stercoralis*. In pre-school age children, the most commonly observed

parasite was *Ascaris lumbricoides* (11.3%), followed by *Hymenolepis nana* (6.3%) while school age children were most susceptible to the *Trichuris trichiura* (16.9%) parasite followed by *Ascaris lumbricoides* (11.3%). Moreover, we have not found differences in prevalence between boys and girls.

Table 2- Incidence of the different parasites observed by each applied method.

PARASITES	Positive parasitrap	%	POSITIVE Kato-Katz	%
<i>Trichuris trichiura</i> eggs	86	14.10%	93	15.20%
<i>Ascaris lumbricoides</i> eggs	71	11.60%	72	11.80%
<i>Hymenolepis nana</i> eggs	40	6.60%	40	6.60%
Ancylostomidae eggs	24	3.90%	20	3.30%
<i>Schistosoma mansoni</i> eggs	1	0.20%	2	0.30%
<i>Enterobius vermicularis</i> eggs	1	0.20%	1	0.20%
Ancylostomidae larvae	4	0.70%	0	0.00%
<i>Strongyloides stercoralis</i> larvae	7	1.10%	1	0.20%

Despite the incidence of the positive results proving similar irrespective of the method of concentration applied, there were certainly differences between the respective results as set out in Table 2. *Trichuris trichiura* was the parasite found with greatest frequency with the incidence of positive samples identified by the Kato-Katz method (93 samples, 15.2%) slightly higher than the results from the parasitrap method (86 samples, 14.1%). Equally, the num-

ber of positive samples for the *Ascaris lumbricoides* parasite rose when applying the Kato-Katz method rather than the parasitrap method, despite the difference between the two methods being only one positive result (72 samples, 11.8%, and 71 samples, 11.6%, respectively). The third most prevalent parasite, *Hymenolepis nana*, was identified to an equal incidence by both methods (40 samples, 6.6%) with only *Ancylostomatidae* eggs gaining a higher level of

numerical identification by the parasitrap concentration method than by the Kato-Katz method (24 samples, 3.9% and 20 samples, 3.3%, respectively). The frequencies of *Schistosoma mansoni* (1 sample, 0.2%; 2 samples, 0.3%) and *Enterobius vermicularis* (1 sample, 0.2%; 1 sample, 0.2%) eggs proved similar whatever the method and with only comparatively residual levels of the remaining parasites. In what concerns larvae identification, there were great differences between the two concentration methods, being the Kato-Katz not suitable for larvae detection. We have detected 4 samples with *Ancylostomidae* larvae, not detected by Kato-katz, and also 7 samples with *Strongyloides stercoralis* larvae detected by parasitrap, but just one sample detected by Kato-Katz.

In order to determine the detection capacity and relative sensitivity of the Kato-Katz method, we took the parasitrap method as our reference method and compared the number of samples with a positive diagnosis for helminth parasites through recourse to both methods of concen-

tration. Despite the results displaying some variation in accordance with the helminth parasite species present in the sample (Table 3), some species, such as *Schistosoma mansoni* and *Enterobius vermicularis*, are easily identifiable by both methods. For these parasites, every positive sample was susceptible to mutually concordant diagnosis, irrespective of the concentration method being the parasitrap method or the Kato-Katz method.

The diagnosis of other helminth parasites, such as those belonging to the *Ancylostomatidae* family, are not equally facilitated by utilisation of the Kato-Katz method as by utilisation of the parasitrap method. Only 83.3% (n=20) of the samples positively identified by the parasitrap method as containing parasites from this family were also detected when applying the Kato-Katz method. This thus reflected a different diagnosis for four separate samples that were reported as negative for *Ancylostomatidae* family parasites when making recourse to the Kato-Katz method despite parasites from this same family having been detected when applying the parasitrap method.

Table 3 – Specificity and sensitivity of the Kato-Katzo method in parasite identification in relation to the parasitrap method.

Parasites	Sensitivity	Specificity
<i>Schistosoma mansoni</i>	100%	99.8%
<i>Enterobius vermicularis</i>	100%	100%
<i>Ascaris lumbricoides</i>	98.6%	99.6%
<i>Trichuris trichiura</i>	97.7%	98.3%
<i>Hymenolepis nana</i>	92.5%	99.5%
Ancylostomatidae	83.3%	100%

Furthermore, we also evaluated the specificity of helminth parasite detection through the utilisation of both concentration methods and counting the number of negative samples returned by the parasitrap reference method that were reported as positive when applying the Kato-Katz method (Table 3). The results indicate how both the sensitivity and the specific detection characteristics vary in accordance with the species of helminth parasite present in the sample. In any case, the specific differences between

the two respective methods hold little overall significance for the majority of parasites with concordance in over 99% of samples testing negative irrespective of the actual concentration method applied. The lowest specificity value of the Kato-Katz method in relation to that of the parasitrap method was recorded for the diagnosis of the incidence of *Trichuris trichiura* parasites for which seven more samples were returned as positive by the Kato-Katz method but attaining a negative result in accordance with parasitrap method.

Discussion

The pathologies caused by parasites are, in the majority of cases, a consequence of environmental, cultural and socioeconomic conditions.¹ Hence, we may consider that research into the incidence of parasites in these endemic contexts should be deemed a priority given the importance of their effective control to overall public health.

The application of different methods in intestinal parasite diagnostic is essential, taking into account the morphological and biological variability presented by parasites.² The parasitrap and Kato-Katz methods both enable the concentration of a large number of faecal parasite eggs from either fresh or preserved samples, being these methods the most sensitive to the detection of parasites present in low levels of concentration.^{12,13}

The study results obtained demonstrate the presence of helminth parasites in approximately one third of children analysed. We verified that the highest levels of incidence were Nematode class parasites, and especially *Trichuris trichiura* and *Ascaris lumbricoides*. Also present from this class are eggs from the Ancylostomidae family, *Enterobius vermicularis* eggs and *Ancylostomidae* and *Strongyloides stercoralis* larvae. From the Cestoda class, we found a very representative frequency of *Hymenolepis nana* eggs. From the Trematoda class, we detected *Schistosoma mansoni*, however with a very low level of frequency due to the fact that this parasite is not endemic in the study area. The two samples identified were from children who do not regularly inhabit the study area and having fallen into the scope of the study due to their spending a period of time staying with family members living in the study area.

We would furthermore note the high number of children (approximately 5%) testing positive for the presence of two or more different species of parasites (co-infections). These findings provide due evidence of the need to undertake deworming campaigns as suggested by the WHO.¹

The majority of intestinal parasitoses result from the drinking of water and consumption of foodstuffs contaminated by parasite eggs such as *Ascaris lumbricoides* and *Trichuris trichiura* with this possibly being the main cause of parasitoses found in this area of study.

In the pre-school age group (aged between two and five), there is a very representative incidence of infection by *Hymenolepis nana* (6.6%), which results primarily from the

consumption of contaminated foodstuffs. Furthermore, this incidence is fostered by the poor hygiene habits of children with this parasitic infection commonly known as the dirty hands disease.² Health awareness campaigns, including the practicing of hygiene habits, the utilisation of latrines and consuming treated water clearly remain a need in this area.

In determining the relative sensitivity and specificity of the Kato-Katz method, taking the parasitrap as our reference method, we report that there are no clear differences between these two methodologies as regards the diagnosis of helminth parasites. The small differences encountered may result from inter-observer variability as well as inter-sample variability. Hence, in samples with low levels of infection, we may detect the presence of parasites in one aliquot and not in another. Moreover, due to observer failure (inter-observer variability), a parasite may get detected in one sample and not in another sample when making recourse to two distinctive methods.

We would nevertheless highlight that the Kato-Katz method is not appropriate for researching larvae due to the morphologic destruction of the parasite during processing and thereby preventing its determination as demonstrated in Table 1. Hence, the Kato-Katz method returns a lower level of detection (83.3%) for parasites in the *Ancylostomidae* family when compared with the parasitrap method. This fact proves extremely important when choosing the intended methodology for field studies.

This study sets out a comparison of two diagnostic methods for intestinal parasites in stool samples. Both techniques are easy to apply even in field working conditions and return concordant results. This study once again demonstrates the need for deworming and awareness campaigns among rural populations in Angola in order to reduce the still high incidence of intestinal parasitoses in children.

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