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COMPARISON OF TWO TECHNIQUES FOR DIAGNOSIS OF INTESTINAL HELMINTHIASIS IN DOGS IN ILE-IFE, NIGERIA

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Abstract
This study was conducted to assess for any significant difference in the use of either modified Kato-katz technique or formol-ether concentration technique in diagnosing intestinal helminth infection from dog's faeces. Faecal samples collected randomly from individual-owning dogs from Ile-Ife between January and September, 2005, were transported to the laboratory, processed and then examined for intestinal helminth eggs using both modified Kato-katz and formol-ether concentration techniques. Out of 191 faecal samples examined 51.8% samples were positive for intestinal helminth eggs using modified Kato-katz technique while 49.2% were positive using formol-ether technique. *Toxocara canis* and *Ancylostoma* sp. eggs were the most common helminth eggs identified. The results also showed that there was no significant difference in the efficacy of the two techniques in detecting *T. canis, Ancylostoma* sp., *T. vulpis* and *Toxascaris leonina* eggs. Either of the two techniques could be used in diagnosing intestinal helminth infections in dogs because the overall prevalence of helminth infection using both techniques were similar and comparable to each other.

Key words: Helminth eggs, Kato-katz, formol-ether concentration, *Toxocara canis, Ancylostoma* sp., *Trichuris vulpis.*

Introduction
Parasitic diseases caused by intestinal parasites are a major public health problem in developing countries of Africa (Oduntan, 1974). In the diagnosis of intestinal parasites a lot of techniques can be employed. Several methods have been devised for determining helminth burden through stool examination. The Kato-katz technique is advocated by the World Health Organisation (WHO) for surveillance (WHO, 1991) owing to its simplicity and relative low cost. The formol-ether concentration technique (Ridley and Hawgood, 1956, Allen and Ridley, 1970) is also widely used and it requires the use of ether or ethylacetate as a lipid removing agent and formalin as a fixative. It has the advantages of fixing the parasites, thus rendering the samples non-infections, as well as preserving many types of cysts, which the Kato-katz technique does not (Cheesbrough, 2005). Although any method is less sensitive when used alone than when used in combination with other methods (Brown *et al.*, 2003), for practical reason studies rarely combine methods.

In developed countries, the formo-ether technique is the concentration method of choice. Even for tropical countries, this technique is recommended as the best overall method to concentrate parasite in faeces (Cheesbrough, 1991). The possible reasons why the Kato-katz is less

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recommended are that it is messy, resulting in a high risk of infection for the technician handling fresh stools and that it is not suitable for detection of cyst, larvae, small fluke eggs or thin-shelled eggs such as hookworm (only detectable if the samples are examined immediately) (Kongs *et al.*, 2001).

None of the earlier reports in the literature on coprological studies involving concentration of helminth ova in dog faeces has employed the Kato-katz method. Most of the other studies have employed the formol-ether concentration method (Robinson *et al.*, 1989) which is often more accepted as the standard for efficient concentration of helminth eggs from faeces. Other methods that have also been used include salt floatation method using saturated solutions of magnesium sulphate, zinc sulphate and sodium chloride (Richards and Lewis, 2001).

In this study, two methods of faecal processing for examination of helminth eggs have been used i.e. Kato-katz and formol-ether concentration methods. In this paper, attempt was made to test the efficiency of the two methods, by processing the same quantities of faecal samples using both Kato-katz and formol-ether concentration methods and the numbers of helminth eggs detected were subjected to statistical analysis.

Materials and methods

Areas of study

The study was carried out in Ile-Ife, a peri-urban community located within latitude of 07º 26′N – 07º33′N and longitude 004º 30′E - 044º 35′ E. The climate of the area is typical with a characteristic dry season of about 6 months (April-September) (Akinbuwa and Adeniyi, 1996). The detail description of the area of study is in my earlier report (Sowemimo, 2007).

Faecal collection

Faecal samples were collected randomly from 191 dogs between January and September, 2005 from various homes at Ile-Ife with the assistance of individual owning-dogs after discussion on the purpose of this study. Information was obtained on the approximate age, sex, breed and mode of life of each dog. The faecal samples were placed in clean 30ml bottles and transported to the laboratory for processing and examination for intestinal helminth eggs. In the laboratory, each faecal sample was divided into two consisting of about 10gm of faeces and was preserved separately in 10% aqueous formaldehyde. The preserved faecel samples were later processed using both Kato-katz and formol-ether concentration techniques.

The first set of 191 samples was processed and examined using the modified Kato-katz procedure (Forrester and Scott, 1990). The number of each helminth eggs counted was recorded and multiplied by a factor of 20 to obtain a value of eggs per gram. The second set of another 191 samples were processed and examined using the formol-ether concentration method as outlined by Cheesbrough (2005). The egg sediment produced was transferred to a labeled microscope slide and a drop of saline was added and the slides were examined for helmtinth eggs. Each helminth egg was identified using established structural and morphometric criteria (Bowman, 1999).

Statistical analysis

Statistical tests were performed using SPSS 11.0 (SPSS Inc., Chicago, Illinois, USA). Chi-square tests were used to study the relationship between the parasite prevalence and type of technique. One-way Anova and Mann-Whitney U tests were used to explore the relationship

between the egg intensity and the technique employed.

Results

The eggs of ascarid worm, *Toxocara canis*, hookworm (*Ancylostoma* sp.), *Trichuris vulpis* and *Toxascaris leonina* were observed. Out of a total of 191 faecal samples examined for various helminth eggs using Kato-Katz and formol-ether concentration techniques, 99 (51.8%) samples were positive using Kato-Katz technique while 94 (49.2%) were positive using formol-ether technique. However, there was no significant difference between the overall prevalences of helminth eggs between the two techniques (p>0.05).

The prevalences of the various helminth eggs observed using Kato-katz technique were *T. canis* 30%, *Ancylostoma* sp. 35.6%, *T. vulpis* 4.7% and *T. leonina* 3.1% while the prevalences using formol-ether technique were *T. canis* 33.0%, *Ancylostoma* sp. 23.0%, *T. vulpis* 7.3% and *T. leonina* 8.9% (Table 1). It was also observed in Table 1 that the prevalences of *T. canis, T. leonina* and *T. vulpis* eggs were higher with formol-ether technique than Kato-katz technique. However, there was no significant differences in the prevalence of these helminth eggs using the two techniques (P>0.05). Table 1 also showed that the prevalence of *Ancylostoma* sp. was higher with Kato-katz technique than with formol-ether technique, however there was no significant difference (p>0.05).

Comparison of results using the two techniques

Using One-way Anova, Table 2 showed that the number of *T. canis* and *Ancylostoma* sp. eggs detected were higher using Kato-Katz technique than formol-ether concentration technique. However, there was no significant difference between the two techniques (P > 0.05) for detecting both *T. canis* and *Ancylostoma* eggs in the faeces of infected dogs examined. The number of eggs observed in both *T. vulpis* and *T. leonina* were higher using formol-ether concentration technique than with Kato-katz technique (Table 2). There was no significant difference between the two techniques (p > 0.05). On subjecting the types of techniques to further statistical analysis using Mann-Whitney test (non-parametric test), which is more reliable for egg counts, it was observed that there was still no significant difference in the number of each helminth eggs detected using both techniques.

Table 1. Prevalence of intestinal helminth in faecal specimens of 191 dogs using Kato-katz and Formol-ether concentration techniques

Helminth	Kato-katz No. and (%) infected	Formol-ether No. and (%) infected
Toxocara canis	59(30.9)	63(33.0)
Hookworm (*Ancylostoma* sp.)	68 (35.6)	44(23.0)
Toxascaris leonina	6(3.1)	17(8.9%)
Trichuris vulpis	9(4.7)	14 (7.3%)
Totalw	99(51.8)	94 (49.2%)

Table 2. Analysis of variance to test for differences between the number of helminth eggs recovered from dog faeces using modified Kato-katz and formol-ether concentration techniques

Types of Technique	Number of samples	Mean ± S.E.	F
T. canis			
Ether	191	599.91 ± 205.27	0.063**
Kato-katz	191	678.53 ± 237.33	
Ancylostoma sp. (Hookworm)			
Ether	191	73.62 ± 21.94	0.829**
Kato-katz	191	101.88 ± 21.94	
T. vulpis			
Ether	191	10.05 ± 4.70	1.118**
Kato-katz	191	4.61 ± 2.09	
T. leonine			
Ether	191	36.07± 18.09	0.589**
Kato-katz	191	19.79 ± 11.07	

(**): $p > 0.05$

Discussion

In this study, two techniques of analyses were explored to determine whether one was more suitable than the other. From the results, it appears that there was no appreciable difference between the use of Kato-katz and formol-ether concentration techniques in the efficacy of extracting *T. canis*, *Ancylostoma* sp., *T. leonina* and *T. vulpis* eggs from the faeces of infected dogs.

In the detection of *T. leonina* and *T. vulpis* egg the prevalences and intensities of these two helminths were higher using formol-ether concentration technique than Kato-katz techniques. This showed that formol-ether technique was better than Kato-katz technique for the detection of *T. leonina* and *T. vulpis* and both eggs stood out very clearly and remained undistorted for long periods (3-4 hours after preparation).

This study also showed that the prevalence of *T. canis* was higher using formol-ether than Kato-katz, although both methods were effective because the prevalence of *T. canis* using the two techniques were comparatively the same. In contrast, the intensity of *T. canis* was higher using Kato-katz than formol-ether, however there was no significant difference.

The prevalence and intensity of hookworm (*Ancylostoma* sp.) were higher using Kato-katz than formol-ether technique. This is in agreement with the findings of Dacombe *et al.* (2007) who reported that the percentage of hookworm eggs detected was significantly higher using Kato-katz than with formol-ether technique. Dacombe *et al.* (2007) also stated that the differential sensitivity of the methods of different helminths causes problem when considering the effects of helminthes in the host. They further reported that analyses using the word 'any' helminth infection will underestimate prevalence in areas where hookworm is more common than other helminth species,

and where there is a qualitative difference expected in their effects, the effect of hookworm will be less easy to determine than that of other helminth species.

The original reason for using both techniques was the concern as to whether using Kato-katz technique alone would result in low yield of certain helminths, since it has been previously reported that formol-ether concentration technique was more efficient in recovery of *Ascaris* eggs from the faeces of infected humans (Ridley and Hawgood, 1956; Allen and Ridley, 1970). Therefore, this study has shown that any of the two techniques could be used to diagnose intestinal helminth infections in dogs in view of the fact the overall prevalence of intestinal helminth eggs obtained for both techniques were similar and comparable. Meanwhile, the World Health Organisation (WHO) has recommended that Kato-katz should be used when a large scale study is to be conducted because it is cheap, easy to learn and to perform with little special material needed. In addition, no hazardous products have to be used and that the sensitivity of a Kato-katz is nearly as high as the formol-ether technique (Ebrahim *et al*., 1997).

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