

RESEARCH ARTICLE

Identification of *Schistosoma Mansoni* Eggs and Other Soil Transmitted Intestinal Parasites in Stool using Odongo-Aginya Method

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Abstract

Background: World Health Organisation had recommended the original Kato technique for quantitative diagnosis of *Schistosoma(S) mansoni* and other soil transmitted intestinal parasites in field research. However, there have been methods modifying the Kato method. Katz introduced the sieve for straining the stool and standard measuring template to deliver constant weight of the stool specimen on the prepared slides in Kato-Katz technique. However, Kato-Katz techniques cannot be examined immediately but after 1 to 2 hours. Using the Odongo-Aginya method, consisting of a compound stains of 7.5% nigrosin in 10% formalin and 0.5% eosin yellow in 10% formalin mixed 1:1 ratio. One of the methods modifying The Kato/Katz method can be used to examine the stool smears prepared in the compound stain immediately. The Malachite green in Kato-Katz technique was substituted with the compound stain. Fresh stool samples sieved through a sieve mesh size 0.315mm (Analysensieb 56557HAAN W. GERMANY) was transferred to the slide using a template measuring 41.7mg. And the Cellophane cover slips cut in the size of 2x4 cm were separately pre-soaked in 50% glycerine, and these were used to cover the stained stool on the slide and can be examine microscopically immediately using x10 and x40 objectives.

The results of using Odongo-Aginya method in this study illustrated mainly *S. mansoni* eggs, other soil transmitted intestinal helminths, and *Strongyloides (S) stercoralis* larva and *Isospora belli* in stool in the results were provided by the Odongo-Aginya to validate the efficacy of the method.

Conclusion: This method illustrated very clearly morphological appearance of *S. mansoni* and other soil transmitted intestinal parasites including larvae of *Strongyloides(S) stercoralis* and *protozoa cysts*. The method showed clear appearances of intestinal parasites eggs and larvae immediately when the slides were prepared and six weeks later. The morphological appearances of the eggs including those of the hookworm in prepared slides are maintained for a long time when the slides are kept in a cool dry place. This method been found to be reliable, reproducible, cost effective, easy to learn, quick and above all because of the formalin the base of the compound stain renders the method safe especially when handling specimens from Human Immunodeficiency virus infected patients.

Keywords: Identification, *Schistosoma Mansoni*, Soil Transmitted Helminths, STH, Intestinal Parasites, Stool, Odongo-Aginya Method

List of abbreviations: ADC Atek Diagnostic Centre; DEO District Education Office; DHO District Health Office; GUREC Gulu University Research Ethics Committee; LRRH Lira Regional Referral Hospital; MOH Ministry of Health; WHO World Health Organization

Introduction

World health organisation (WHO) recommends Kato-Katz thick smear techniques for quantification of *S. mansoni* eggs and other soil transmitted intestinal parasites [1]. In Kato-Katz technique, prepared thick smear of stool specimen in 1% malachite green, requires 1 to 2 hours to examine the slide for clear visibility of the parasite eggs and larvae [2]. The major challenge with this method is that, during the waiting hours for better visibility of the parasite eggs and larvae, parasites eggs with thin cell walls are over cleared and the morphologies are distorted and this makes it difficult to identify and differentiate the parasites from artefacts [3]. There have been methods developed to overcome the major challenges in Kato/Katz [4]

One of these methods is the Odongo-Aginya method; the compound stain comprising of 7.5% nigrosin in 10% formalin mixed 1:1 with 5% eosin yellow in 10% formalin used in this study to identify and illustrate morphological appearances of the eggs of *S. Mansoni* and other intestinal parasite eggs, cysts and larvae. In Odongo-Aginya method the slides are read immediately they are prepared and the eggs are clearly distinguished from artefacts and these are maintained for a long time as illustrated in the results of comparative studies [4].

Materials and Methods

Study population

This study was conducted among selected primary school children in Lira District Northern Uganda between the months of March to May 2017. It was a cross sectional study and purely evaluating the prevalence of *Schistosoma mansoni* in the post national control program.

Ethical Approval

The study was approved by Gulu university research ethics committee (GUREC no. 04/03/2017). Further approval from the District Health office, District Education office and head teachers of the selected primary schools were sought. Written consent was obtained from participants or their guardians. All infected children with *S. Mansoni* and other soil transmitted intestinal parasites were treated with a single oral dose of praziquantel 40mg/kg body weight while other soil transmitted helminths were treated with single oral dose of 500mg of Albendazole at no cost.

Sample Preparation

Stool specimens were strained through a stainless steel sieve mesh size 0.315mm (Analysensieb 56557HAAN W. GERMANY). The strained stool was transferred to fill a template measuring approximately 41.7 mg of faeces, put on the microscopic slide. Three slides were prepared for each specimen and a drop of Odongo-Aginya stain (about 10–50µl) was added to each of the measured stool on the slides and stirred in using an applicator stick [4]. A cellophane cover slip cut in 2x4cm, presoaked in 50% glycerin was picked with a pair of forceps and excess glycerin on it blotted out on an absorbent paper. This cellophane cover slip was then

placed on the stained stool on the slide. The slide was inverted upside down and pressed down gently on tissue paper to spread out the stool smeared on the slide and to remove excess stain from the slide. The slide could have been examined immediately at field research but the prepared slides were stored in cool dry place to be examined later [4].

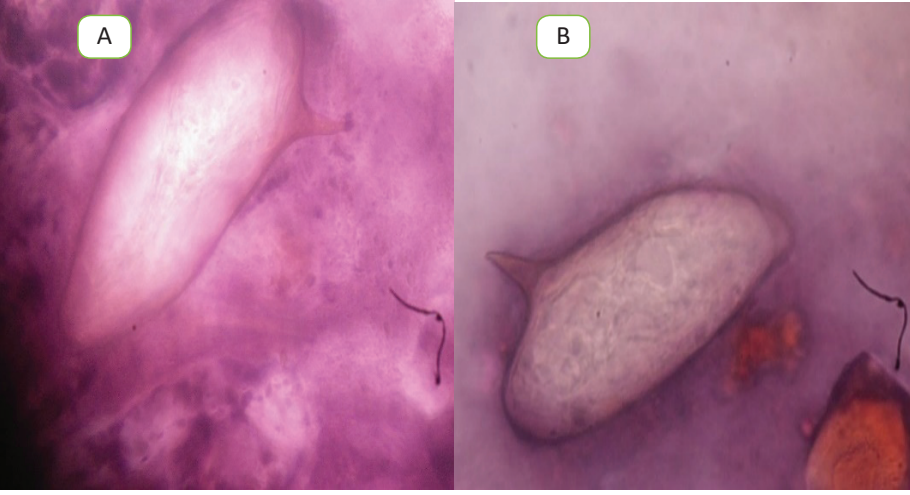
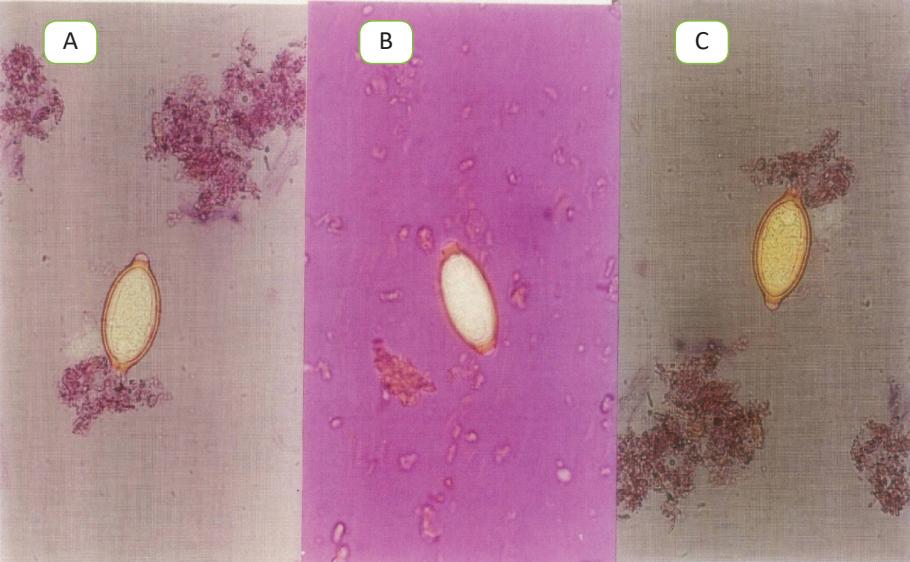
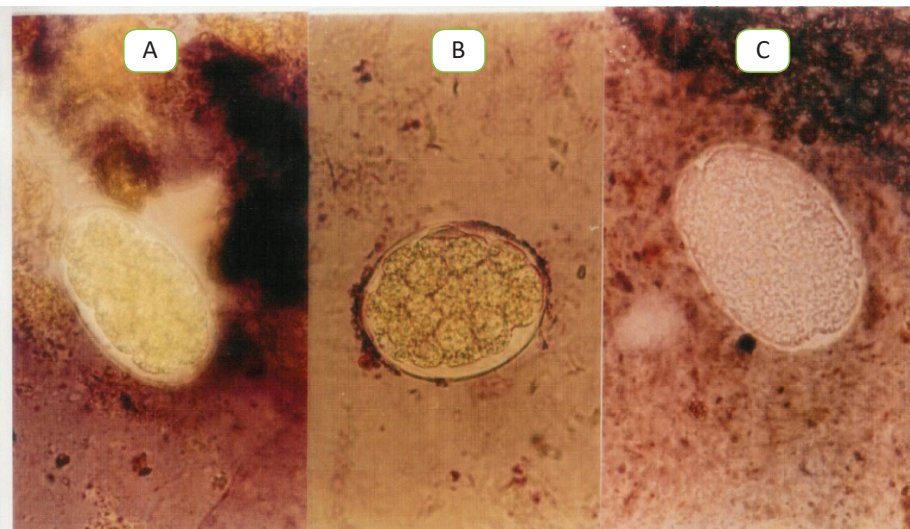


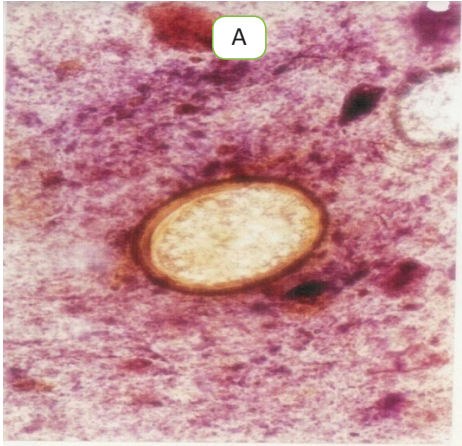
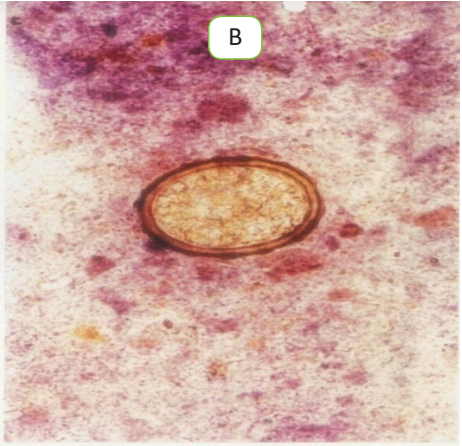
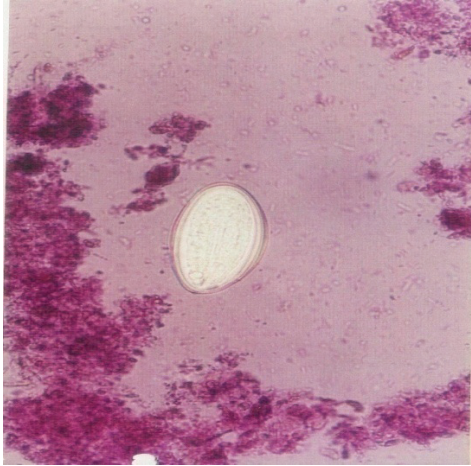
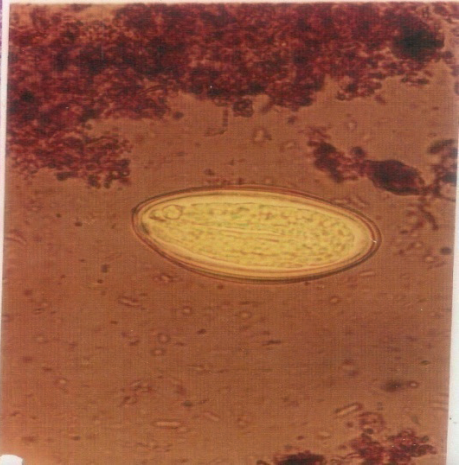
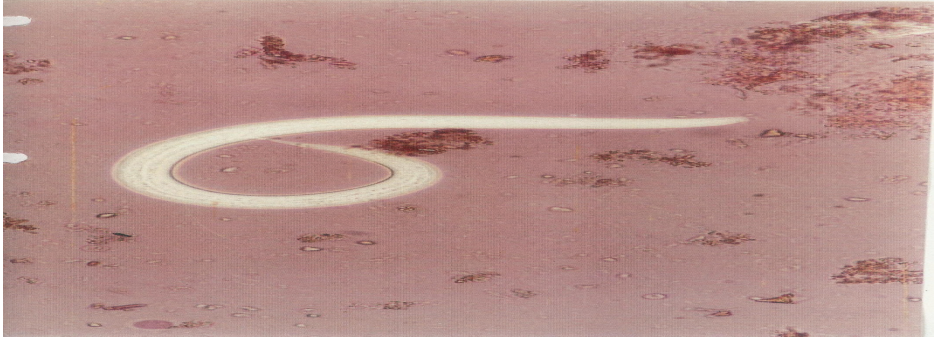
Figure 1: (A) Researcher preparing thick smear of stool using Odongo-Aginya method

Results

A total of 532 stool samples were examined. The fresh stool specimens were prepared in respective primary schools studied and examined at Atek Diagnostic Centre (ADC) Lira. The eggs of *schistosoma mansoni* and other intestinal parasites were illustrated using the Odongo-Aginya method in stool (Figure 2). In summary, the prevalence of *Schistosoma mansoni* was (190) 35.7% indicating a moderate infection. There were also low grade rates of infection 13.7% with other intestinal parasites like *Ascaris lumbricoides* 16.4%, *Hookworm* 12.3%, *Enterobius vermicularis* 8.22%, *Strongyloides stercoralis* larva 4.1%, *Trichuris trichuria* 2.7% and *Hymenolepis nana* 1.4% respectively. The photographs of the eggs and larvae were taken using a digital camera mounted on Olympus binocular microscope CX 21 at ADC - Lira.

Courtesy of professor Odongo-Aginya the author of the new diagnostic method.

Parasites Identified	Morphological Appearances
<p><i>Schistosoma Mansoni</i> Eggs</p> <p>(A) Immediately the slide was prepared</p> <p>(B) Six weeks after the slide was prepared</p>	
<p><i>Trichuris Trichura</i> Eggs</p> <p>(A) viewed with condenser raised</p> <p>(B) viewed with fully opened aperture of the condenser</p> <p>(C) viewed behind dark area of the slide</p>	
<p>Hookworm Eggs</p> <p>(A) immediately the slide was made</p> <p>(B) one week after the slide was made</p> <p>(C) six weeks after the slide was made</p>	

<p><i>Ascaris Lumbricoides</i> Egg</p> <p>(A) Immediately the slide was made</p> <p>(B) Six weeks after the slide was made</p>	 
<p><i>Enterobius Vermicularis</i> Egg</p> <p>(A) Immediately the slide was made</p> <p>(B) six weeks after the slide was made. Note the embryo in both eggs</p>	 
<p><i>Strongyloides Stercoralis</i> Larva</p>	

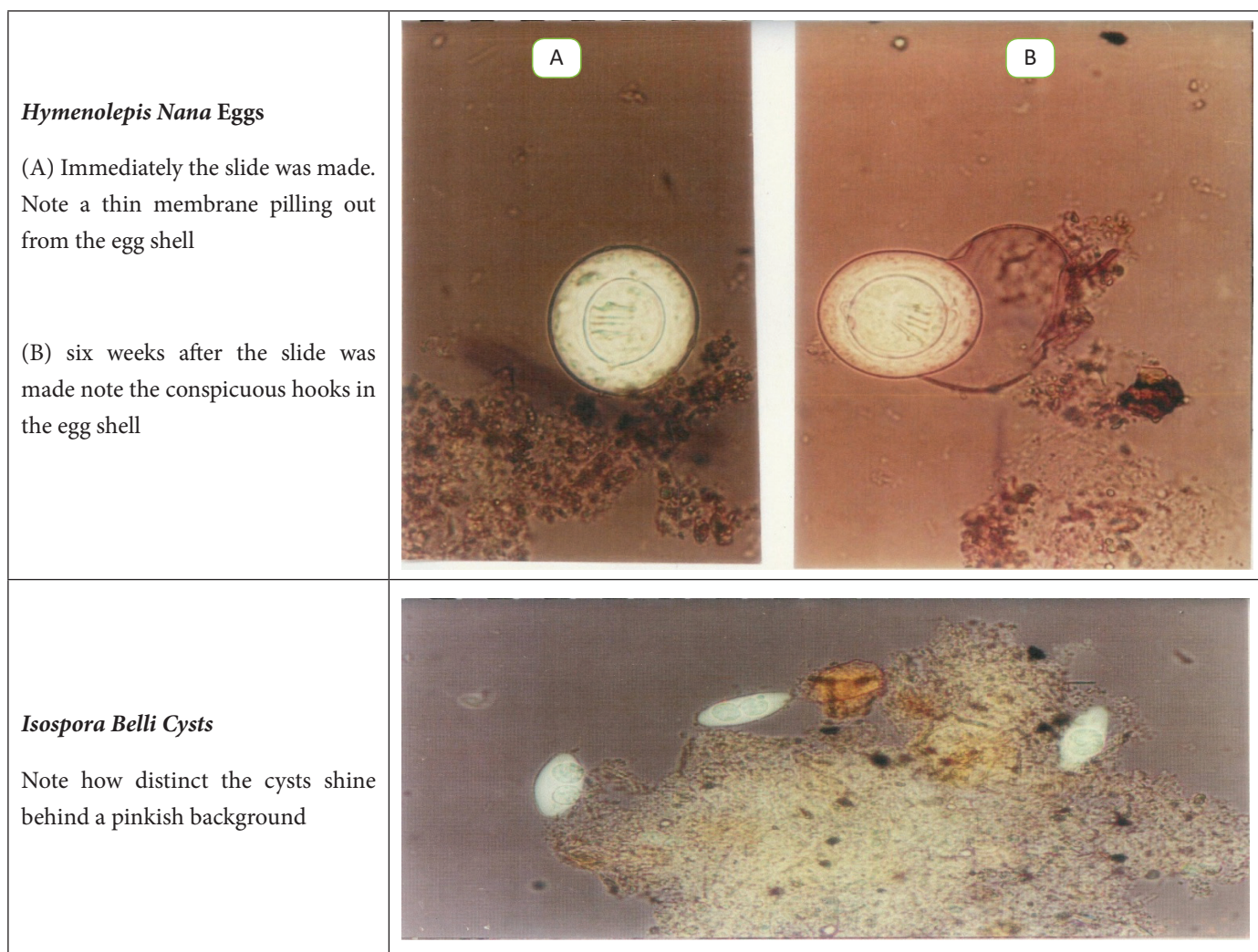


Figure 2: Morphological appearance of intestinal parasites species identified using Odongo-Aginya method at magnification 400.

Discussion

The aim of this study was to identify and illustrate the morphological appearance of *Schistosoma mansoni* and other intestinal parasites using the Odongo-Aginya method in stool [5]. In this study, the rapidity and reproducibility of Odongo-Aginya staining method to illustrate the eggs, larvae and cysts of parasites in stool have been observed.

Odongo-Aginya method is easy to learn and also to prepare the slides for microscopy and the reagents to prepare the stain are easily available and at low cost [5]. Further the prepared slides can be examined immediately the slide is prepared. The morphology of the eggs and larvae are preserved using 10% formalin used as the component of the Odongo-Aginya stain [5]. The parasite eggs, larvae and cysts can be easily identified immediately and after preparation of the stool smears. This may not be possible in the Kato-Katz method. The slides prepared using Odongo-Aginya stain can be kept in cool dry place for a long time for references without the parasites eggs losing morphology. Additionally, Odongo-Aginya method is safe because the faecal pathogens are fixed using 10% formalin which has a higher potential to kill bacteria, viruses and other pathogens. This is significant especially if working on stool samples from Human Immunodeficiency Viruses (HIV) infected patients [6].

Conclusions

This study therefore concludes that, the slides prepared using the Odongo -Aginya method can be examined microscopically immediately and after sometime, without losing the morphology of the eggs, cysts and larvae. Odongo-Aginya method is safe because

faecal pathogens are fixed with 10% formalin used as base in preparing eosin yellow and nigrosin. Therefore, we recommend this method for intestinal parasitological surveys where the thick stool smears can be examined immediately and even later without distortion of the parasite morphology.

Limitations

Odongo- Aginya method is reliable and reproducible. Nevertheless, the volume of the stain to be added on the stool smear on the slide need to be standardised to give consistencies in appearance of the slides prepared.

Declarations

Ethical Approval and Consent to Participate

The study was approved by the Gulu University Research Ethics Committee (Reference no. **GUREC 04/03/2017**). Further approval from DHO, DEO and authorities of selected primary schools in Lira district. Informed consent was sought from parents and legal guardians by for children aged 8 years and below. Older children were explained the purpose and the risks of the study and were requested to assent by signing the consent forms. All information obtained was kept confidential and codes not participant's name were used. Children found infected were treated with praziquantel for free.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Conflicts of Interest

The authors declare that they have no conflicts of interest

Funding

Not applicable

Author's Contributions

Conception and Design

BJP and EIOA. Acquisition of data: BJP and EIOA. Laboratory analyses: BJP and EIOA. Data curation and management: BJP and EIOA. Analysis and interpretation: BJP and EIOA. Manuscript writing: BJP and EIOA. Manuscript revision: BJP and EIOA. All authors' read and approved the final manuscript.

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