Identification of *Schistosoma mansoni* eggs and other soil transmitted intestinal parasites in stool using Odongo-Aginya method

John Paul Byagamy (✉ jonepaul2010@gmail.com)

Gulu University <https://orcid.org/0000-0001-6288-0208>

Geoffrey Maxwell Malinga

Gulu University

Harriet Angwech

Gulu University

Richard Echodu

Gulu University

Emmanuel Igwaro Odongo-Aginya

Gulu University Faculty of Science

Research note

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Abstract

Objective World Health Organisation recommends Kato-Katz technique for quantitative diagnosis of *Schistosoma* (*S*) *mansoni* and other soil transmitted intestinal parasites in field research. However, the prepared slides in Kato-Katz technique cannot be examined immediately and after 1 to 2 hours, the eggs of parasites with thin cell walls over clear in glycerine and morphology of the eggs are not preserved. These disadvantages are overcome using Odongo-Aginya method as illustrated by *S. mansoni* eggs and other soil transmitted intestinal parasites in stool in the results. Result The Odongo-Aginya method composed of compound stains of 7.5% nigrosin in 10% formalin and 0.5% eosin yellow in 10% formalin mixed 1:1 ratio can be examined immediately. This method illustrated very clearly morphological appearance of *S. mansoni* and other soil transmitted intestinal parasites. This method showed a clear appearance of intestinal parasites eggs and larvae immediately after preparation and also after six weeks. The prepared slide also maintains the morphological appearance of the eggs even of the hookworm for a long time when the slides are kept in cool dry place. This method is reliable, reproducible, cost effective, easy to learn, quick and safe especially when handling specimens from Human Immunodeficiency virus infected patients.

Background

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 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].

This study was carried out to identify and illustrate morphological appearances and advantages of Odongo-Aginya method using the compound stain 7.5% nigrosin in 10% formalin mixed 1:1 with 5% eosin yellow in 10% formalin over Kato-Katz malachite green technique to identify the eggs of *S. mansoni* and other intestinal parasite eggs and larvae (4,5)

Methods

Study population

This study was conducted among selected primary school children in Lira District Northern Uganda between the month of March to May 2017. 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 40mg/kg body weight of praziquantel while other soil transmitted helminths were treated with single oral dose of 500mg of Albendazole at no cost.

Sample preparation

The Kato-Katz technique was substituted by the Odongo-Aginya method where compound stain consisting of 5% eosin yellow in 10% formalin, mixed v/v with 7.5% Nigrosin in 10% formalin. Stool specimens were strained through a stainless steel sieve mesh size 50µl. 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 the stool on the slide and stirred in using an applicator stick [4,5]. A cellophane cover slip presoaked in 50% glycerin was picked with a pair of forceps and excess glycerin on it was 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, then the slide was examined immediately [4,5].

Results

The fresh stool specimens were prepared in Lira Regional Referral Hospital (LRRH) 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 (Fig.1). The photographs of the eggs and larvae were taken using a digital camera mounted on Olympus binocular microscope CX 21 at ADC - Lira.

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 (4, 5). In this study, advantages of Odongo-Aginya stain over the Kato-Katz technique 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 (4,5). 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 (4,5). The parasite eggs and larvae can be easily identified immediately after preparation of the thick stool smear as compared to Kato-Katz method where the preparation should be allowed to stand for at least 1 to 2 hours for proper visualization [4, 8]. Within this waiting period eggs of hookworms over clear in the glycerine, and thus decolorizes and distorts the morphological appearance of the eggs and other intestinal parasites [6, 9,10]. 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. These characteristics are not possible with Kato-Katz method. 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 as compared to malachite green

which is weakly bactericidal. 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, the morphology of the eggs and larvae are preserved for a longer time compared to Kato-Katz method. Odongo-Aginya method of stool thick smear is safe from faecal pathogens due to fixation with 10% formalin in eosin yellow and nigrosin. Therefore, we recommend this method for routine 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 make the same appearance on the entire slide.

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

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 signing an informed consent form. Children aged 8 years and above were given assent form to sign. All information obtained was kept confidential and codes not participant's name were used. Children found infected were treated with praziquantel for free.

Consent for Publication

Not Applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

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

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Authors' information

¹Department of Biology, Faculty of Science, Gulu University, P.O. Box 166, Gulu, Uganda.

²Department of Microbiology and Immunology, Faculty of Medicine, Gulu University, P.O. Box 166, Gulu, Uganda.

³Health Department, Lira District Local Government, P.O. Box, 49, Lira, Uganda.

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Figures

Parasites identified	Morphological appearances	
<i>Schistosoma mansoni</i> eggs (A) Immediately the slide was prepared (B) Six weeks after the slide was prepared
<i>Trichuris trichura</i> eggs (A) viewed with condenser raised (B) viewed with fully opened aperture of the condenser (C) viewed behind dark area of the slide
Hookworm eggs (A) immediately the slide was made (B) one week after the slide was made (C) six weeks after the slide was made
<i>Ascaris lumbricoides</i> egg (A) immediately the slide was made (B) six weeks after the slide was made
<i>Enterobius vermicularis</i> egg (A) immediately the slide was made (B) six weeks after the slide was made. Note the embryo in both eggs
<i>Strongyloides stercoralis</i> larva
<i>Hymenolepis nana</i> eggs (A) immediately the slide was made. Note a thin membrane pilling out from the egg shell (B) six weeks after the slide was made note the conspicuous hooks in the egg shell

Figure 1

Morphological appearance of intestinal parasites species identified using Odongo-Aginya method at magnification 400. courtesy of professor Odongo-Aginya the author of the new diagnostic method.