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

Keywords: Odongo-Aginya method, Schistosoma mansoni, intestinal parasites, illustration in stool.

Posted Date: October 31st, 2019

DOI: https://doi.org/10.21203/rs.2.14695/v2

Abstract

Objective World Health Organisation recommends Kato-Katz technique for quantitative diagnosis of Schistosoma mansoni and other soil transmitted intestinal parasites in field research. However, for better visibility especially in hard stool specimens, the prepared slides in Kato-Katz technique are mostly examined after 1-2 hours. This longer clearing time over clear the eggs of parasites with thin cell walls in glycerine and morphology of the eggs are not often preserved in Kato-Katz method. These disadvantages are overcome using Odongo-Aginya method as illustrated by parasite eggs in the results. Result The Odongo-Aginya method uses compound stains of 7.5% nigrosin in 10% formalin and 0.5% eosin yellow in 10% formalin mixed 1:1 ratio. A drop of 50µl of the compound stain is added on 41.7mg of stool and stirred. This method illustrated the morphological appearance of S. mansoni and other soil transmitted intestinal parasites clearly when prepared and six weeks after preparation. The prepared slides maintained the morphological appearance including the eggs of hookworm after being kept in cool dry place for a long time. This method is reliable, reproducible, cost effective, easy to learn, quick and safe because of the base formalin, especially when handling specimens from Human Immunodeficiency virus infected patients.

Introduction

World Health Organisation (WHO) recommends Kato-Katz thick smear techniques for quantification of *S. mansoni* eggs and other soil transmitted intestinal parasites [1, 2]. In Kato-Katz technique prepared thick smear of stool specimen in 1% malachite green covered with cellophane pre-soaked coverslips, often require 1 to 2 hours in most cases under open room conditions to clear for better visibility of the parasite eggs and larva [3]. The major challenge with this method is the distortion of eggs and larva of parasites with thin cell wall which reduces the visibility and hence, makes it difficult to identify and differentiate the parasites from artefacts [4]. This study was carried out to identify and illustrate morphological appearances of *S. mansoni* eggs and other soil transmitted intestinal parasites, protozoa and larvae when malachite green is replaced with the Odongo-Aginya compound stain comprised of 7.5% nigrosin in 10% formalin mixed 1:1 with 5% eosin yellow in 10% formalin [5].

Methods

Study population

This study was conducted among selected primary school children in Lira District Northern Uganda between March to May 2017. The study was approved by the 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 received free treatment of 40mg/kg body weight of praziquantel for *S. mansoni* and single oral dose of 500mg of Albendazole for other soil transmitted intestinal parasites.

Sample preparation

The Kato-Katz technique was substituted by the Odongo-Aginya method where a compound stain consisting of 5% eosin yellow in 10% formalin, mixed v/v with 7.5% Nigrosin in 10% formalin was used. Stool specimens were strained through a stainless-steel sieve mess size 50µl. The strained stool was transferred to fill a template measuring approximately 41.7 mg of faeces and put on the microscope slide. Three slides were prepared for each specimen and a drop of Odongo-Aginya stain (about 10–50µl) was added to 41.7mg stool on the slide and stirred in using an applicator stick [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 stained stool smeared on the slide and to remove excess stain from the slide. The slide was examined immediately [5].

Results

The fresh stool specimens collected from the primary schools were prepared in Lira Regional Referral Hospital (LRRH) and examined immediately at Atek Diagnostic Centre (ADC) Lira. The eggs of *schistosoma mansoni* and other intestinal parasites as illustrated using the Odongo-Aginya method in stool are shown in 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 in stool using the Odongo-Aginya method [5]. In this study, of the advantages using Odongo-Aginya stain to clearly illustrate the eggs of *S. mansoni*, other soil transmitted parasite eggs and larvae have been observed. Odongo-Aginya method has the qualities of the diagnostic methods recommended by WHO. This method is reliable, reproducible, cost effective, quick and is easy to learn in the preparation of slides for microscopy, and the reagents are easily available and at low cost [5]. Furthermore, the slides can be examined immediately after preparation. The morphology of the eggs and larvae are preserved using 10% formalin used as the component of the Odongo-Aginya stain [5]. 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 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 better visualization [8] which causes the eggs of hookworms to become over cleared in the glycerine leading to decolourization and distortion of the morphological appearance of the eggs [6, 9,10]. The prepared slides using Odongo-Aginya stain can be kept in cool dry place for a long time for references without the parasite eggs losing morphology. The bactericidal effect of the malachite green used in the Kato-Katz method is low compared to the usage of 10% formalin used

in fixing faecal pathogens in the Odongo-Aginya method. This 10% formalin has a higher potential to kill bacteria, viruses and other pathogens and is a significant factor to consider especially when working on stool samples from immunocompromised patients such as the Human Immunodeficiency Viruses (HIV) infected persons [6].

Conclusions

This study has shown that slides prepared using Odongo -Aginya method can be examined microscopically immediately or at a later time as the morphology of the eggs and larvae are preserved. Also, the use of 10% formalin in eosin yellow and nigrosin in the preparation of stool thick smear using Odongo-Aginya method makes it safe from faecal pathogens. Therefore, this method is recommended for routine intestinal parasitological surveys where the thick stool smears can be examined immediately and 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 a uniform appearance on the entire slide.

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

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 during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable

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. Manuscript writing and revision: BJP, MGM and EIOA. All the authors' read and approved the final version of manuscript.

Acknowledgements

We thank the management of LRRH and ADC, and the head teachers of selected schools for allowing the study to be conducted in their premises. We thank the research assistants, Phiona, Rita and Lydia for assistance with data collection. We are grateful to the staff of LRRH and ADC, especially Solomon, Bernard and Coletta for their assistance in sample collection, preparation and examination. Finally, we thank all the pupils of the selected schools who volunteered to participate in the study.

Authors' information

[1]Department of Biology, Faculty of science, Gulu University, P.O. Box 166, Gulu, Uganda.

[2]Department of Microbiology, Faculty of Medicine, Gulu University, P.O. Box 166, Gulu, Uganda.

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

Corresponding author: jonepaul2010@gmail.com

References

1. World Health Organization. Basic laboratory methods in medical parasitology. Geneva: World Health Organization; 1991.
2. Utzinger J, Becker SL, Knopp S, Blum J, Neumayr AL, Keiser J & Hatzb CF. Neglected tropical diseases: diagnosis, clinical management, treatment and control. Swiss Med. Wkly. 2012;142:w13727.
3. Martin LK, Beaver PC. Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. J. Trop. Med. Hyg. 1968; 17 (3):382-391.
4. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev. Inst. Med. Trop. Sao Paulo. 1972;14(6):397-400.
5. Odongo-Aginya EI, Narcis K, Ludwig S, Wabinga H, Fenwick A, Montresor A. Substitution of Malachite Green with Nigrosin-Eosin Yellow Stain in the Kato-Katz method: microscopical appearance of the helminth eggs. Afr Health Sci. 2007;7(1).
6. Odongo-Aginya EI, Taylor MG, Sturrock RF, Ackers JP & Doehring, E. Field evaluation of an improved Kato-Katz thick smear technique for quantitative determination of helminth eggs in faeces. Med. Parasit. 1995; 46 (4): 275-277.
7. Martin LK, Beaver PC. Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. Am J. Trop. Med. Hyg. 1968;17(3):382-91.
8. Ramsan M, Montresor A, Foum A, Ameri H, Di Matteo L, Albonico M, Savioli L. Independent evaluation of the Nigrosin-Eosin modification of the Kato-Katz technique. Trop. Med. Int. Health. 1999;4(1):46-9.
9. Sleigh AC, Hoff R, Mota EA, Sherlock I, Mott KE, Barreto ML, Maguire JH, Weller TH. Three-year prospective study of the evolution of Manson's schistosomiasis in north-east Brazil. The Lancet. 1985; 326 (8446):63-66.
10. Sleigh A, Hoff R, Mott K, Barreto M, de Paiva TM, de Souza Pedrosa J, Sherlock I. Comparison of filtration staining (Bell) and thick smear (Kato) for the detection and quantitation of Schistosoma mansoni eggs in faeces. *R Soc. Trop. Med. Hyg*. 1982; 76(3): 403-406.

Figures

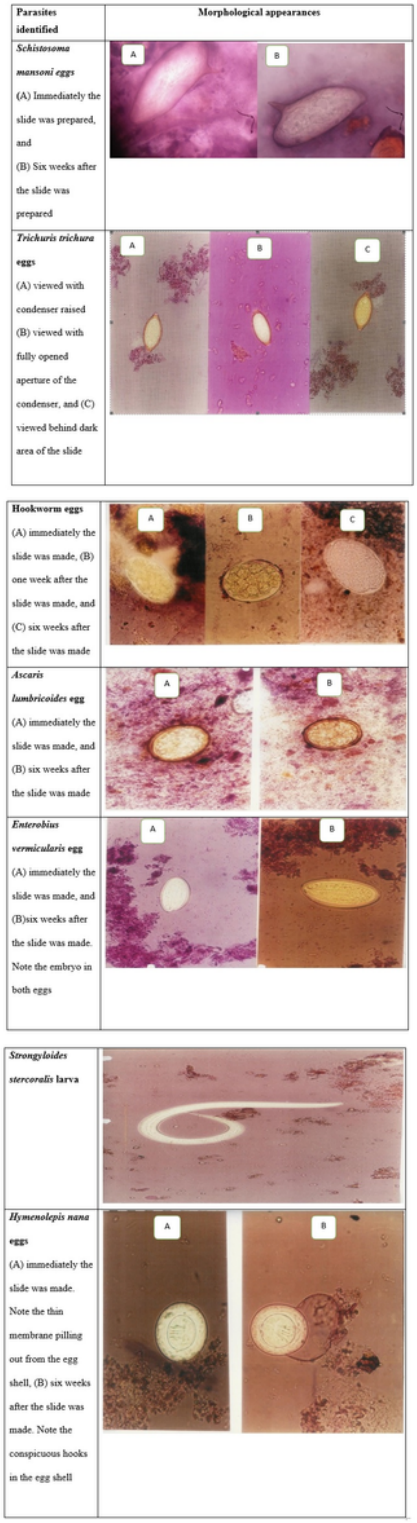

Figure 1

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