

BRIEF COMMUNICATION

REFRACTIVE INDEX MATCHING APPLIED TO FECAL SMEAR CLEARING

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SUMMARY

Thick smears of human feces can be made adequate for identification of helminth eggs by means of refractive index matching. Although this effect can be obtained by simply spreading a fleck of feces on a microscope slide, a glycerol solution has been routinely used to this end. Aiming at practicability, a new quantitative technique has been developed. To enhance both sharpness and contrast of the images, a sucrose solution (refractive index = 1.49) is used, which reduces the effect of light-scattering particulates. To each slide a template-measured (38.5 mm³) fecal sample is transferred. Thus, egg counts and sensitivity evaluations are easily made.

KEYWORDS: Turbidity of fecal suspensions; Thick fecal smears; Refractive index matching.

Suspensions of human feces in water tend to be turbid, which is detrimental to the observation of the morphological characters which support the identification of parasites. Light-scattering particulates, including bacteria, degrade the images of microscopic objects. Concentration techniques, based on such properties of the suspended materials as size and density, are designed to selectively remove as much as possible of the light-scattering fraction. The chances of detecting and identifying parasites are thus increased. Another way to tackle this problem is to reduce light-scattering by means of refractive index matching. In that case the optical properties of fecal suspensions are changed without the removal of any fraction of it¹. Cedar wood oil, well known for its high refractive index (n = 1.52), has excellent optical characteristics as a clearing medium, but requires previous dehydration of the fecal smears. The Kato & Miura thick-smear technique, further described by KOMIYA & KOBAYASHI³ in 1960, consists in pressing against a hard, flat surface, a fecal specimen placed between a microscope slide and a "cover glass" of hydrophilic cellophane soaked in a glycerol solution. A version of this technique, by KATZ *et al.*² includes the use of a template to evaluate the volume of the specimen examined. Due to their operational advantages, thick smear techniques promptly gained world-wide acceptance⁵. Further investigation into the possibilities of applying refractive index matching to fecal thick-smear techniques will be essential to their refinement. It has been demonstrated that the addition of glycerol can be dispensed with⁴. Fecal thick-smears obtained by squeezing a fleck of feces between a microscope slide and a dry, impermeable plastic "cover glass", also fulfill the requirements for helminth egg identification. The clearing

effect is probably due to the optical properties of the mucus contained in the fecal mass. A better image quality, in terms of sharpness and contrast, is obtained through the use of an 85.0% (n = 1.49) aqueous solution of sucrose (plus six drops of liquefied phenol and 0.2% of a surfactant agent containing sodium alkyl benzene sulfonate). In addition to low-cost and good optical properties, the sucrose clearing medium does not affect the morphology of the *Schistosoma mansoni* eggs to the same extent as glycerol does.

The approximate volume of the fecal sample to be examined is measured by using a 1.0 mm thick template to be placed on a 26 mm by 17 mm slide. It has a hollow cylinder (7.0 mm in diameter, volume = 38.5 mm³) which should be filled up with feces, care being taken not to include macroscopic detritus. Next, the template should be cautiously removed, the fecal specimen being left on the slide. One drop of sucrose should be added and, with the aid of an applicator, mixed with the fecal specimen. As "cover glass" a 26.0 by 36.0 mm transparent polypropylene sheet is used. The fecal specimen is squeezed as stated above. After a few minutes the smears will be ready for examination. An estimate of the number of eggs per gram of feces is obtained by multiplying the number of eggs per smear by the factor 26.0. Here, 1.0 g/cm³ is accepted as the average density of human feces.

In order to obtain preliminary information concerning the technique described above, we compared the results of the examination, for helminth eggs and larvae, of 110 fecal samples by sucrose-cleared thick smears and gravity sedimentation. A 90% calculated agreement

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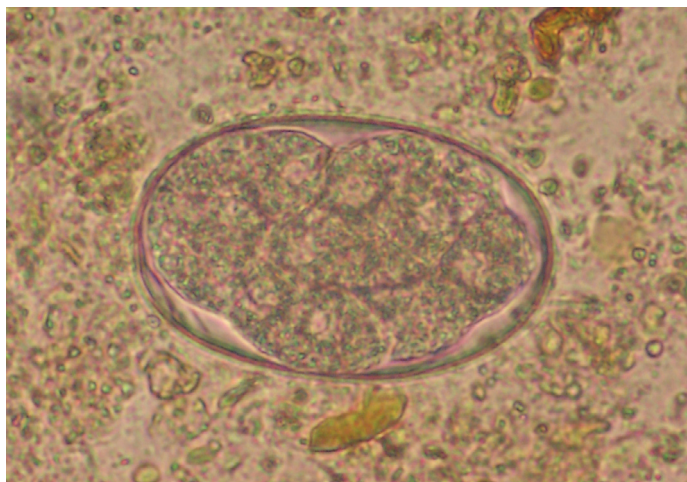


Fig. 1 - *Ancylostoma duodenale*, egg, morula.

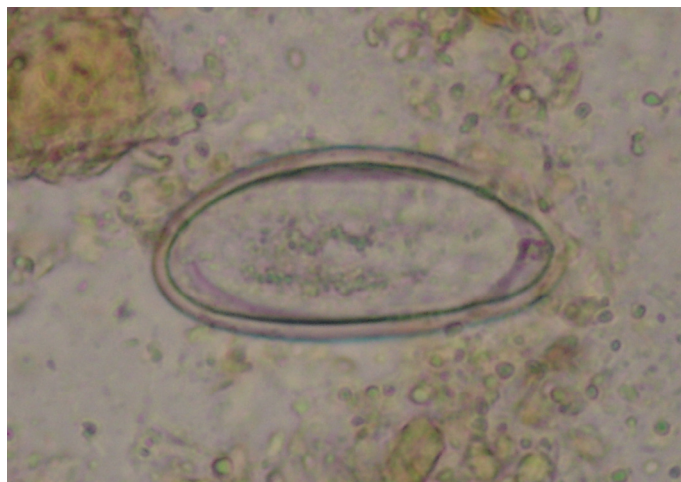


Fig. 4 - *Enterobius vermicularis*, egg with larva.



Fig. 2 - *Ancylostoma duodenale*, egg with larva.

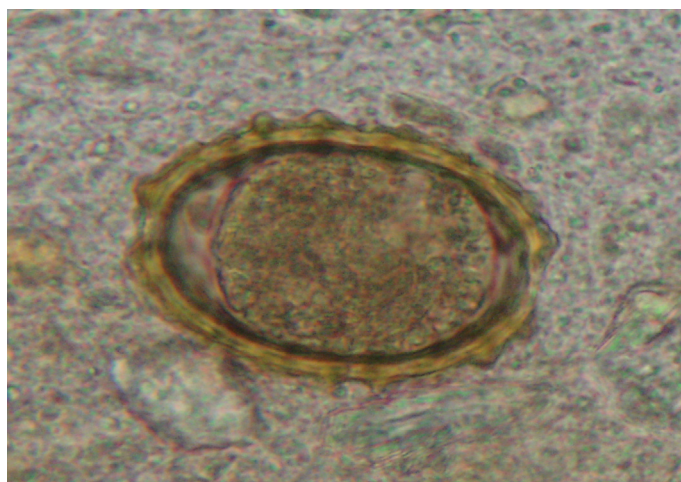


Fig. 5 - *Ascaris lumbricoides*, fertile egg.

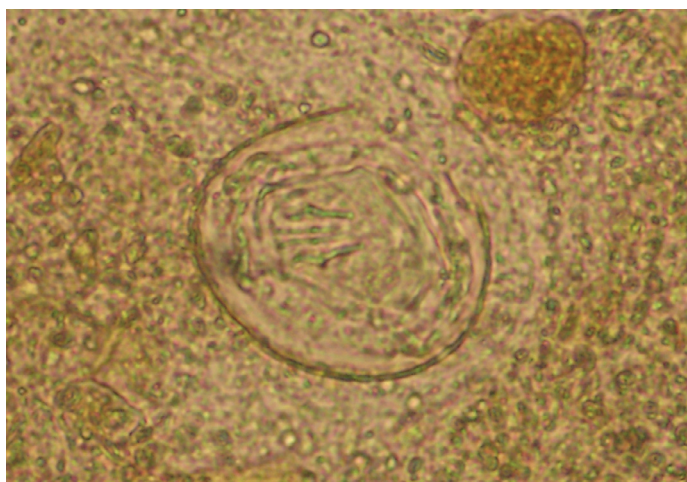


Fig. 3 - *Hymenolepis nana*, egg with embryo.

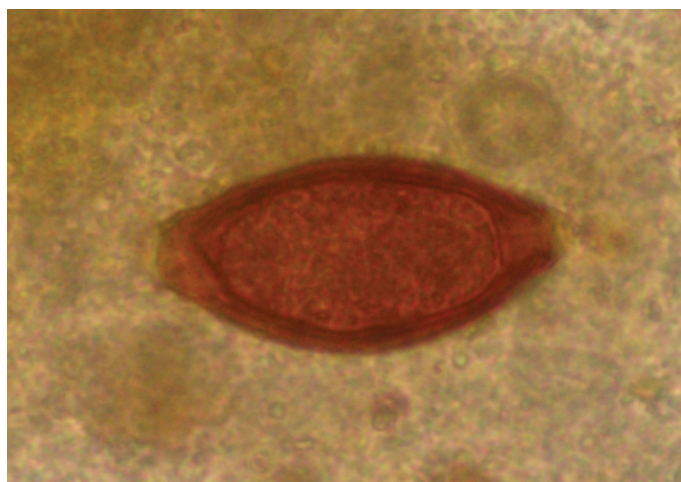


Fig. 6 - *Trichuris trichiura*, egg.



Fig. 7 - *Schistosoma mansoni*, fertilized egg.

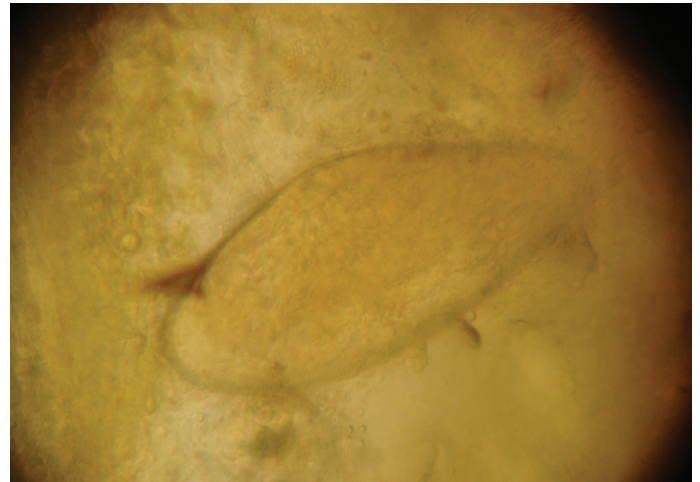


Fig. 8 - *Schistosoma mansoni*, egg.

was found (12 positive and 87 negative results). A disagreement was observed in 11 cases, as shown below:

Frequency	Sedimentation technique	CSF thick smear technique
4	<i>S. stercoralis</i>	Negative*
1	<i>S. stercoralis</i>	<i>A. lumbricoides</i> **
1	<i>S. stercoralis</i>	<i>S. mansoni</i> ***
1	<i>S. stercoralis</i>	Ancylostomatidae, <i>T. trichiura</i> **
1	<i>H. nana</i>	<i>H. nana</i> , <i>T. trichiura</i> , <i>S. mansoni</i>
1	Negative	<i>S. mansoni</i>
1	<i>Taenia</i> sp., <i>S. mansoni</i>	<i>Taenia</i> sp.
1	<i>E. vermicularis</i>	Negative

*Fecal sample collected respectively 8, 7, 6 and 10 days before examination;
Fecal sample collected 7 days before examination; * Fecal sample collected 5 days before examination.

The following comments are suggested:

- a) *Strongyloides stercoralis* larvae may not be identifiable after such time intervals.
- b) Ancylostomatidae larvae are sometimes difficult to identify as such.
- c) *Schistosoma mansoni* are easily identified in sugar-cleared thick smears.
- d) *Enterobius vermicularis* eggs are not, in this case, good indicators of sensitivity.
- e) According to such results, the technique under investigation is satisfactorily sensitive.

In actual laboratory work, the practicability of this technique has already become evident. The templates were designed for comfortable handling and the height/diameter ratio of the hollow cylinder produces a fecal specimen which is easily detached from it. As a result of a good

refractive index matching, sharp images of microscopic objects are expected. The process of scanning the smears and identifying the parasites is correspondingly easier than otherwise. This technique is recommended for the identification of helminth eggs or larvae in either clinical or epidemiological investigation. Liquid or mushy feces, as well as those contaminated by materials which could interfere with the transparency of the slides or alter the morphology of parasites are not adequate for thick smear examination. The photomicrographs annexed show the images of some worm eggs in sugar-cleared thick fecal smears.

RESUMO

Homogeneidade de índices de refração aplicada ao clareamento de esfregaços de fezes

Os esfregaços espessos de fezes humanas podem tornar-se adequados para a identificação de ovos e larvas de helmintos por meio da busca de homogeneidade de índices de refração. Embora seja possível obter esse efeito por meio de simples espalhamento de um fragmento de fezes sobre uma lâmina de microscopia, uma solução de glicerol tem sido usada rotineiramente para este fim. Visando à praticabilidade, elaborou-se uma técnica quantitativa em que é usada uma solução de sacarose (Índice de refração = 1,49) para reduzir o efeito da difusão da luz produzido por material particulado. O volume da amostra fecal a examinar em uma lâmina corresponde ao da cavidade cilíndrica da placa medidora (38,5 mm²). Avaliações de sensibilidade e contagens de ovos tornam-se, portanto, de fácil execução.

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