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COMPARATIVE DIAGNOSIS OF LARVAE AND OVA OF GASTROINTESTINAL NEMATODES AND TREMATODES IN A CLINICAL LABORATORY

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Abstract

This review examines the comparative diagnosis of larvae and ova of gastrointestinal nematodes and trematodes in a clinical laboratory.

No procedure used for examining specimens is 100% effective- that is, the procedures will not always recover all the species present and, if a particular species is present in only very low numbers, they may fail to demonstrate them when used on a single specimen. Because the techniques are not perfect, we should perform them as carefully as possible for optimum results. Also, we should be sure to use techniques that are appropriate for the material we are examining.

Finding Ascaris lumbricoides (Gastrointestinal nematode) eggs in faeces rarely requires concentration techniques. Direct technique for preparing and examining specimens is used which involves reporting the appearance of the specimen and identifying any parasite and examining the specimen with a microscope for larvae and eggs of the parasite.The need for direct faecal examination technique is essential in direct motile parasites and is usually adequate to detect significant Helminth infections except in Schistosoma species (Gastrointestinal trematode) because only a few eggs are usually produced even in moderate and severe infections.

Therefore faecal concentration technique is used for the detection of schistosomes while direct faecal examination technique is used for the detection and identification of Ascaris lumbricoides.

INTRODUCTION

Gastrointestinal Trematodes and Nematodes are multicellular worms called Helminths.

They do not normally multiply in their human host. Trematodes (Flukes) includes *Schistosoma species, Paragonimus species, Fasciolopsis buski, Fasciola hepatica,* and *Opisthorchis viverrini.* Those of lesser medical importance includes ; *Metagonimus yokagawai, Heterophyes heterophyes,* and *Dicrocoelium dendriticum.*

Flukes are segmented , mostly flat leaf –like worms (*Schistosomes* are an exception) without a body cavity. They vary in size from 1mm (*H. heterophyes)* to 70 mm(*F. buski)* in length. They have an oral and ventral suckers (attachment organs).

The digestive system consists of a mouth and an oesophagus which divides to form two intestinal cerca (branched in some species). There is no anus but an excretory system composed of excretory cells called flame cells, collecting tubules and an excretory pore.

There is a simple nervous system. Parasitic flukes of medical importance belong to the sub-class Digenea.

Two generations are required to complete their life cycle. There is an asexual generation in which multiplication occurs (in sporocyst or redia stage) and sexual generation which produces eggs. In humans, only the adults are found.

With the exception of *Schistosomes,* flukes are haemophrodites (male and female reproduction organs in the same individual) and produce eggs that are operculated (with lids).
To develop, the eggs must reach water. Snails serve as the first or only intermediate host. Most flukes parasitize a wide range of animals. Adult flukes of *Schistosoma species* live in the blood venules around the gut or bladder. Hypersensitivity reactions to the eggs cause tissue damage. *Paragonimus* flukes parasitize the lungs.
F. hepatica, O. sinensis,O.viverrini and *D. dendriticum* live in the biliary tract, and *F. buski, H. heterophyes* and *M. yokagawai* parasitize the small intestines.
Gastrointestinal nematodes are roundworms. Intestinal nematodes includes *Ascaris lumbricoides* (large roundworm), *Enterobius vermicularis,* (Threadworm), *Trichuris trichiura* (Whipworm), *Strongyloides stercoralis, Ancylostoma duodenale* (hookworm) and *Necator americanus* (hookworm).
Filarial and other tissue nematodes includes *Wurchereria bancrofti, Brugia spcies, Loa loa, Onchocerca volvulus, Drancunculus medinensis* (Guinea worm) and *Trichinella species.*
Gastrointestinal nematodes are cylindrical worms. They have a body cavity and a cuticle (skin) which may be smooth, spined, or ridged. The adults of some species are very long, e.g. *D. medinensis* measuring 1 meter or more. The mouth is surrounded by lips, or papillae. In some species, e.g. hookworms, the lip open into a buccal cavity which has cutting or tooth-like plates. The digestive system is a simple tube which ends in an anus. There is an excretory system and a nervous system. Sexes are separate with the male worms being smaller than the females. Females are either viviparous (produce larvae) or oviparous (lay eggs). The discharged eggs may hatch directly into infective larvae or they may require special conditions in which to hatch and up to three developmental stages before becoming infective larvae.
Each stage involves a shedding of the old cuticle (moulting). For most nematodes of medical importance, humans are the only and most significant host.
Most of the medically important intestinal nematodes are geohelminths, ie. soil transmitted (spread by faecal contamination of the soil). A person becomes infected by swallowing infective eggs (*A. lumbricoides, T. trichiura, E. vermicularis)* or by infective larvae penetrating the skin (hookworms, *S. stercoralis).*

Before becoming adults in their human host, the larvae of hookworms, *A. lumbricoides,* and *S. stercoralis* migrate through the heart and lungs for about 10days during which time the larvae grow and develop. The infective larvae of filarial nematodes are transmitted through the bite of an insect vector. *T. spiralis* is transmitted by ingestion of larvae in infected tissue and *D. medinensis* by ingestion of an infected intermediate host (Cyclops).

Macroscopic Examination Of The Specimens

In the identification of *Ascaris species* by the direct technique, the appearance of faeces is reported which includes size, colour, consistency, presence of blood, mucus or pus. On consistency, it is reported whether the faeces is formed, semifomed, uniformed or watery. If blood is present, it is reported whether this is mixed in the faeces. If only in the surface, this indicates rectal or anal bleeding. It is also reported whether the specimen contains woms e.g. *A. lumbricoides (*large roundworms), *E. vermicularis* (threadworm) or tapeworm segments, e.g. *T. solium,* and *T. saginata.*

Blood and mucus may be found in faeces from patients with amoebic dysentery, intestinal schistomiasis, invasive balantidiasis (rare infection) and a severe *T.trichiura* infections. Other non-parasitic conditions in which blood and mucus may be found include bacillary dysentery, *Campylobacter enteritis,* ulcerative colitis, intestinal tumour and haemorroids. The presence of pus can be found when there is an inflammation of the intestinal tract. Many pus cells can be found in faecal specimens from patients with bacillary dysentery. They can also be found in amoebic dysentery but are less numerous. Pale coloured faeces (lacking stercobilinogen) are also excreted by patients with obstrucive jaundice.

Microscopic Examination Of The Specimens

In the microscopic examination of faecal specimens, the faeces should be examined immediately for those specimens containing blood and mucus and those that are unformed because they may contain motile trophozoites of *E. histolytica* or *G. lamblia.* In the examination of dysenteric and unformed specimens, using a wire loop or piece of stick, place a small amount of the specimen, to include blood and mucus on one end of the slide.

Without adding saline, cover with a cover glass and using a tissue, press gently on the cover glass to make a thin preparation. Place a drop of eosin reagent on the other end of the slide. Mix a small amount of the specimen with the eosin and cover with a cover glass. Eosin does not stain living trophozoites but provides a pink background which can make them easier to see.

Examine immediately the preparations microscopically, first using the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the 40x objective to identify motile trophozoites, e.g. *E. histolytica* amoebae or *G. lamblia* flagellates. The eggs of *Schistosoma species* and *T. trichiura,* and the trophozoites of *B. coli* can also result in specimen containing blood and mucus.

In the examination of semi-formed and formed faeces, place a drop of fresh physiological saline on one end of a slide and a drop of iodine on the other end. To avoid contaminating the fingers and stage of the microscope, do not use too large a drop of saline or iodine. Using a wire loop or piece of stick, mix a small amount of specimen, about 2mg (match stick head amount) with the saline and a similar amount with the iodine. Make smooth thin preparations. Cover each preparation with a cover glass. Sample from different areas in and on the specimen or preferably mix the faeces before sampling to distribute evenly any parasites in the specimen.

Do not use too much specimen otherwise the preparations will be too thick, making it difficult to detect and identify parasites.

Examine systematically the entire saline preparation for larvae, ciliates, helminth eggs, cysts and oocysts. Always examine several microscope fields with this objective before reporting " No parasites found". Use the iodine preparation to assist in the identification of cysts.

Report the number of larvae and each specie of egg found in the entire saline preparation as scanty (1-3 per preparation), few (4-10 per preparation), moderate number (11-20 per preparation), many (21-40 per preparation) and very many (over 40 per preparation).

In the identification of larvae in fresh faecal specimen, *S. stercoralis* is the only larva that can be found. It can be easily detected in a saline preparation by its motility and large size. If the specimen is not fresh, *S. stercoralis* will require differentiation from hookworm larvae[1].

Faecal Concentration Diagnostic Techniques

The need for faecal concentration diagnostic techniques have been discussed above. The sedimentation and floatation techniques are commonly used to concentrate faecal parasites in diagnostic laboratories.

In the sedimentation technique, in which parasites are sedimented by gravity or centrifugal force e.g. formol ether concentration method which is the most frequently used technique because it concentrates a wide range of parasites with minimum damage to their morphology [3]. The floatation techniques in which parasites are concentrated by being floated in solutions of high specific gravity, i.e. solutions that are denser than the parasites being concentrated. Examples include the Zinc sulphate method and saturated sodium chloride method.

Unlike the formol ether sedimentation technique, a single floatation technique cannot be used to concentrate a wide range of parasites because of differences in the densities of parasites and the damage that can be caused by floatation fluids to some parasites.

For certain parasites and situations, floatation techniques are recommended and be easily performed in the field with the minimum of equipment, providing adequate health and safety measures are taken. The choice of concentration technique depends on why the technique is being performed, the species of parasite requiring concentration, and how well its morphology is retained by a particular technique; the number of specimens to be examined and time available, the location, e.g; field or laboratory situation and equipment available, experience of staff performing the technique and health and safety considerations.

Formol ether concentration method is recommended for the detection and identification of *Strongyloides* larvae, formol ether and saturated sodium chloride method for *A. lumbricoides* and hookworm larvae, formol ether and zinc sulphate recommended for *opisthorchis* and *T. trichiura* eggs and formol ether concentration method for *Schistosoma species.* The formol detergent gravity technique is also suitable for the *Schistosomes.*

DIAGNOSIS OF GASTROINTESTINAL NEMATODES

Finding gastrointestinal nematodes' eggs like *A. lumbricoides* eggs in faeces rarely require concentration techniques. The method of preparing and examining specimens are used and is followed by identifying *A. lumbricoides* worms expelled through the anus or mouth.
Freshly expelled *Ascaris* worms are pinkish in colour with an appearance similar to earthworms. They measure 12-35 cm in length and taper at both ends. The tail of the mail is curved and has small rod-like projections (spicules). There is a small mouth surrounded by three lips. Always use forceps to handle *Ascaris* worms because they can cause asthma and other allergic reactions. If storing *Ascaris* eggs in formol saline e.g. for teaching purposes, first treat the faecal deposit with hot (70-80 ^{o}C) formol saline. This will prevent the eggs from developing to an infective stage.
Usually, fertilized eggs of *A. lumbricoides* are found in faeces but occasionally infertile eggs are produced by unfertilized female worms.
The fertilized eggs are yellow-brown, oval or round, measuring 50-70 uM wide , shell is often covered by an uneven albuminous coat (mammilated) and contains a central granular mass which is the unsegmented fertilized ovum.
Decorticated egg is a term used to describe an egg that has no albuminous coat. A decorticated egg has a smooth shell and appears pale yellow or colourless. Infertile egg is darker in colour and has a thinner wall and more granular albuminous covering.
More elongated than a fertilized egg, measuring about 90x45uM and contains a central mass of large granules.

Trichuris trichiura is also known as whipworm. Its laboratory diagnosis is by finding the eggs in faeces. Concentration techniques are rarely required to detect significant infections. This method of preparing and examining specimens have been described above.
Heavy infections can be diagnosed clinically by examining the rectum for worms using a proctoscope.
The egg of *T. trichiura* is yellow-brown and measures about 50x25 uM . It has a characteristic barrel shape with a colourless protruding mucoid plug at each end. It contains a central granular mass which is the unsegmented ovum.

Laboratory diagnosis of *Capillaria philippinensis* is by finding the eggs. They can be few and therefore if infection is suspected and the eggs are not found in direct preparations, a concentration technique should be used, such as the formol ether concentration method. Features used for the identification of egg of *C. philipinensis* and differentiate it from *T. trichiura* is that they are smaller than *T. trichiura,* measuring about 45x 21 uM. It is yellow-brown but less elliptical in shape than *T. trichiura* and the plugs (one at each end of the egg) are smaller and do not protrude like those of *T. trichiura.*
The laboratory diagnosis of hookworm infection is by finding hookworm eggs in faeces.
Morphologically, the eggs of *Ancylostoma duodenale* and *Necator americanus* cannot be differentiated. The direct examination of faeces is usually adequate to detect the eggs. If required, the eggs can be concentrated by the formol ether concentration technique or the saturated salt floatation technique. Hookworm infection is usually accompanied by a blood eosinophilia.
Egg of hookworm (*N. americanus* or *A. duodenale)* in faecal specimens less than 12 hours old, a hookworm egg is colourless with a thin shell which appears microscopically as a black line around the ovum, oval in shape measuring about 65x40uM and contains an ovum which appears segmented (usually 4-8 cell stage).
If the specimen is more than 12 hours old, a larva may be seen inside the egg. If the faeces is more than 24 hours old, the larva may hatch and must then be differentiated from a *Strongyloides stercoralis* larva.
Eggs that can be mistaken for hookworm eggs are *Trichostrongylus species, Ternidens deminutus, Oesophagostum species* and *Strongyloides fuelleborni.* These nematodes are normally parasitic in animals but can infect humans.
The egg of *Trichostrongylus* compared with a hookworm egg, the egg of *Trichostrongylus* is longer and thinner than a hookworm egg, measuring 85-115 uM in length, more pointed at one or both ends and usually appears more segmented.

The egg of *Ternidens deminutus* has a similar structure to a hookworm egg but is much larger, measuring about 85 uM in length and contains more cells. The egg of *S. fuelleborni* often contains a larva. When at the non-embryonated segmented stage, it closely resembles a hookworm egg except that it is smaller, measuring about

50x35 uM. The egg of Oesophagostum species is about the same size as that of hookworm but is passed in the faeces in an advanced stage of development.
The laboratory diagnosis of *Strongyloides stercoralis (*also known as the dwarf threadworm) infection is by finding motile *S. stercoralis* larvae in fresh faeces. The larvae can also be found in duodenal aspirates, but this method of diagnosis is not usually necessary.
In disseminated infections, larvae can be found in most body fluids.
Because S. stercoralis larva tend to be excreted intermetently and their numbers can be few, the following technique should be used if infection is suspected and the larvae are not detected by direct examination.

Water Emergence Diagnostic Technique

The water emergence technique can be used to detect *Strongyloides* larvae in faeces. A fresh (not more than two hours old) formed or semi-formed faecal specimen is required. The method involves using a piece of stick, make a central deep depression in the specimen. Fill the depression with warm water (not over 37 o C). Incubate the specimen in a 35- 37 o C incubator or on a warm part of the bench for one and half hours to three hours during which time the larvae will migrate out of the faeces into the warm water. Using a plastic bulb pipette or pasteur pipette, transfer some of the water to a slide and cover with a cover glass.
Alternatively, transfer all the water to a conical tube, centrifuge, and transfer the sediment to a slide.
Examine the preparation microscopically for motile larvae using the 10x objective with the condenser iris closed sufficiently to give good contrast. *S. stercoralis* larvae are also well concentrated by the formol ether technique. Rhabditiform larvae of *S. stercoralis* is actively mobile. Following formol ether concentration, the larvae are immobilized. The larvae is large measuring 200 - 250 uM x 16 uM , and unsheathed. It shows a typical rhabditiform large bulbed oesophagus and it can be distinquished from a hookworm larvae (sometimes seen in faeces more than 24 hour old) by its shorter buccal cavity (mouth cavity). The buccal cavity can be more easily seen by running a drop of Dobell's iodine under the cover glass to immobilize the larva and using the 40x objective to examine the depth of the buccal cavity.

The egg of *Strongyloides fuelleborni* are often mistakenly reported as those of hookworm. It is colourless, oval in shape and measures about 50x35 uM , ie. smaller than hookworm eggs which they can resemble. They may contain a partially developed larva. If there is delay in examining the faeces, the larva may hatch.

DIAGNOSIS OF GASTROINTESTINAL TREMATODES

Eggs of the following intestinal *Schistosoma species* can be found in faeces. Widespread species like *Schistosoma mansoni* and *Schistosoma japonicum* and less widespread species like *Schistosoma intercalatum, Schistosoma makongi* and related species like *S. malayensis.*
Animal schistosomes may occasionally infect humans. *S. mattheei* is the most significant species. *S. haematobium* causes urinary *schistomiasis.*
The laboratory diagnosis of intestinal *schistomiasis* is by finding schistosome eggs in faeces by direct examination or more commonly by using a concentration technique. The specimen will often contain blood and mucus.
Examining a rectal biopsy for eggs when they cannot be found in faeces, especially after a patient has been partially treated.
The preparation and examination of faeces by direct technique is not adequate for *Schistosoma species* because only a few eggs are usually produced even in moderate and severe infections, therefore a concentration technique should be performed when intestinal *schistomiasis* is suspected and no eggs are found by direct examination .
The formol ether technique is suitable for *Shistosoma species.*This technique is also recommended for use in district laboratories because it is rapid and can be used to concentrate a wide range of faecal parasites from fresh and preserved faeces[2].

The Formol Ether Concentration Diagnostic Technique

In this technique, a rod or stick is used to emulsify an estimated 1g (pea size) of faeces in about 4ml of 10 % formol water contained in a screw- cap bottle or tube. Faeces from the surface and several places in the specimen are

included in the sample. Then 3- 4ml of 10% volume to volume formol ether is further added and the bottle is capped and mixed well by shaking. The emulsified faeces is sieved and the sieved suspension is collected in a beaker. The suspension is then transfered to a conical (centrifuge) tube made of strong glass, copolymer, or polypropylene. Then 3-4 ml of diethyl ether or ethyl acetate.

It should be noted that ether is highly flammable and ethyl acetate is flammable, therefore , use well away from an open flame, e.g. flame from the burner of a gas refrigerator, bunsen burner or spirit lamp. Ether vapour is anaesthetic, therefore make sure that the laboratory is well ventilated. Stopper the tube and mix for one minute. If using a vortex mixer, leave the tube unstoppered and mix for about 15 seconds (It is best to use a boiling tube). Do not use a rubber bung or a cap with a rubber liner because ether attacks rubber. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube). Centrifuge immediately at 750- 1000g (approx. 3000rpm) for 1 minute. After centrifuging, the parasites will have sedimented to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formol water.

Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover glass.

Examine the preparation microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the 40x objective lens to examine small eggs. The number of eggs of each species are counted and this will give the approximate number per gramme of faeces.

Depending on geographical area, eggs of the following *Schistosoma species* can be found in faeces ; *S. mansoni(*Africa, S. America, Carribean, Middle east); *S. intercalatum* (West and Central Africa); *S. japonicum* (China, Philippines, Indonesia); S. mekongi (Lao PDR, Cambodia, Thailand).

Egg of *S. mansoni* is pale yellow- brown, and oval measuring about 150 x 60uM and has a characteristic side (lateral) spine. Sometimes the spine may appear terminal like that of an *S. haematobium* egg but if the egg is rolled over by pressing gently on the cover glass, the spine will be seen to be lateral. It contains fully developed miracidium.

The egg of *S. japonicum* is colourless or pale yellow-brown, large and round to oval, measuring about 90 x 65 uM. A very small hook-like spine (rudimentary spine) can sometimes be seen projecting from the egg wall but often it is hidden by faecal debris and red cells. It contains a fully developed miracidium.

The egg of *S. mekongi* is similar to but smaller and rounder than the egg of *S. japonicum* , measuring about 56x 66 u M and has a small knob- like spine similar to the egg of *S. japonicum* .

The egg of *S. intercalatum* is pale yellow-brown, large, and elongate, measuring about 180x60 uM. They have a characteristic long spine at one end (terminal spine) which may appear bent. Because of the terminal spine, the egg of *S. intercalatum* can resemble that of *S. haematobium* but unlike *S. haematobium*, the egg of *S. intercalatum* is usually found in faeces, not in urine and it is also larger. *S. intercalatum* contains a fully developed miracidium.

Unlike other terminally -spined schistosome eggs, the egg of *S. intercalatum* is usually acid- fast (Ziel- Neelsen staining).

When *Schistosome* eggs cannot be found in faeces, they can sometimes be found in a rectal biopsy. The eggs are often non- viable and calcified. A biopsy is examined immediately after removal by placing the tissue in physiological saline and soaking for 30- 60 minutes. Then transfer the tissue to a slide and cover with a cover glass. With care, press on the cover glass to spread out the tissue and make a sufficiently thin preparation.

Examine the entire preparation microscopically for eggs using the 10x objective with the condenser iris closed sufficiently to give good contrast. Constant focusing is necessary to detect the eggs. If the preparation is too thick to examine, add a drop of lactophenol solution and wait for a few minutes for the tissue to clear sufficiently. Identify the eggs and estimate the number of uncalcified eggs in the biopsy and the proportion that are calcified (black).

Uncalcified and calcified (black) eggs of *S. mansoni* are found in the tissue. A rectal biopsy depending on geographical area, may contain the eggs of *S. mansoni*, *S. intercalatum*, *S. japonicum*, *S. mekongi* and occasionally the eggs of *S. haematobium*.

Kato- katz Diagnostic Technique

In recent years, several modified kato- katz techniques have been developed for the semi- concentration and semi-quantitative estimation of *Schistosome* eggs in faeces. Such techniques have been shown to be of value in *Schistosomiasis* epidemiology and control work.
In the kato- katz technique, faeces are pressed through a mesh screen to remove large particles. A portion of the sieved sample is then transfered to the hole of a template on a slide. After filling the hole, the template is removed and the remaining sample (approx. 10mg, 20mg, or 50mg depending on the site of the template) is covered with a piece of cellophane soaked in glycerol (glycerine). The glycerol 'clears' the faecal material from around the eggs. The eggs are then counted and the number calculated per gram (g) of faeces. Further information on the kato- katz technique can be found in the WHO publication manual of basic techniques for a health laboratory, 2nd edition, WHO, Geneva.
Compared with other field techniques for detecting and quantifying *schistosome* eggs in faeces, the kato- katz technique is less sensitive, is unsuitable for fluid and hard specimens, can alter the morphological appearances of eggs, and the technique is less safe and hygienic. Alternative field techniques have been suggested such as the sodium hydroxide digest technique, details of which can be found in the paper of Marshal et al[3]. and the Stoll helminth egg counting technique described in the following text.

The formol detergent field technique

This technique is for the concentration and quantification of *schistosome* eggs. It is more sensitive than the kato-katz technique because more faeces are used. With simple modification, the technique can also be used to detect other faecal parasites. Formol detergent solution, universal container with a conical base and measuring spoon (sterilin type) and sieve (strainer) with small holes (preferably 400- 450 uM in size). The small nylon tea strainer available in most countries is suitable and are required for this test. In the test, we dispense about 10ml of the formol detergent solution into a universal container. Using the spoon attached to the cap of the container, transfer a level spoonful of faeces to the container (approx. 300mg when using a sterilin spoon), and mix well in the solution to break up the faeces. Tighten the cap and shake for about 30 seconds.
Sieve the emulsified faeces, collecting the sieved suspension to the conical based universal container. Stand the container upright in a rack for one hour (do not centrifuge). Using a plastic bulb pipette or a pasteur pipette, remove and discard the supernatant fluid, taking care not to disturb the sediment (containing *schistosome* eggs) which has formed in the base of the container. Add about 10ml of 10% formol detergent solution, and mix well for a minimum of 30 seconds. Leave to sediment for a further 1 hour. Further 'clearing' of the faecal debris will take place.
The *schistosome* eggs are fixed and will not overclear or become distorted.
Using a plastic bulb pipette or pasteur pipette, remove and discard the supernatant fluid, taking care not to remove the fine sediment that has collected in the conical base of the container. Transfer the entire sediment to a slide and cover with a 22x40mm cover glass or if unavailable with two smaller square cover glasses. Systematically examine the entire sediment microscopically for *schistosome* eggs using the 10x objective with the condenser iris closed sufficiently to give good contrast. Count the number of eggs and multiply the number counted by 3 to give the approximate number per gram (g) of faeces.
The definition of a heavy infection is area- specific and may vary from 100 to 800 eggs per gram of faeces are used in the interpretation of *schistosome* egg counts.
In the serological diagnosis of *schistomiasis*, serum antibody tests have a limited application because they do not differentiate between active and previous infection or reinfection and they give no indication of intensity of infection. Active infection can be diagnosed by detecting circulating *schistosome* antigen.
Enzyme immune assays (EIA) to diagnose urinary and intestinal *schistosomiasis* by detecting circulating *schistosome* antigens, ie. CAA (Circulating anodic antigen) and CCA (Circulating cathodic antigen) in serum and urine have been developed and assessed by Van Lieshout et al (6). Antigen tests , however, are not yet available commercially.

The laboratory diagnosis of *Opisthorchis* (*clonorchis*) *sinensis* infection is by finding the eggs of *O. sinensis* in faeces and detecting eggs in aspirates of duodenal fluid. An eosinophil leukocytosis is common. Anti-P1 antibodies may be found in the sera of persons infected with *Opisthorchis* flukes who lack antigen P1 on their red cells, i.e. P2 positive. The flukes are known to contain P1 substances that can stimulate the production of Anti-P1 antibodies.

In the examination of faeces for *O. sinensis* eggs, the eggs can be few and because they small, they can be easily missed. A concentration technique should therefore be used if infection is suspected and no eggs are found by direct examination.
The method of preparing and examining specimens can be done by the formol ether concentration technique. Substitution of formol water with a citric acid- Tween solution in the formol ether technique is recommended for the best recovery of *Opisthorchis* eggs. The 40x objective is required to identify *O. sinensis* eggs and the preparation must not be too thick otherwise the small eggs will be overlooked.
The eggs of *O. sinensis* are yellow-brown and small measuring 27-32x 15-18uM. It is shaped like an electric light bulb and contains a ciliated miracidium but this is difficult to see through the surface of the egg. It has a clarly seen operculum (lid). It is often described as having 'shoulders' (rim on which the operculum rests). A small projection can sometimes be seen at the other end of the egg. When examined with the high power objective , an indistinct outer covering of the shell can often be seen. The eggs of *O. sinensis* closely resemble those of *O. viverrini* and *O. felineus*. In the examination of duodenal fluid for *O.* sinensis eggs, a filtration technique can be used to recover the eggs of *O. sinensis* from aspirates of duodenal fluid. A membrane filter of 8uM pore size is required for filtering the eggs of *O. sinensis* . Staining the membrane with 1% weight to volume trypan blue in saline helps to show the eggs.
If unable to perform a filtration technique or the specimen is not suitable for filtration, transfer a drop of the aspirate (especially a piece of mucus) to a slide, cover with a cover glass and examine microscopically.
Make sure that the preparation is sufficiently thin otherwise the eggs will be missed. Use the 40x objective to identify the small eggs. If the aspirate is watery, centrifuge it first and examine a drop of the sediment.
In the serological diagnosis of *O. sinensis*, details of antibody tests and tests to detect circulating antigens have been described by many authors[2].
In the diagnosis of *Opisthorchis viverrini* infection, the method is the same as that described for *O. sinensis*. The eggs can be found in faeces and aspirates of the duodenal fluid. The egg of *O. viverrini* resembles that of *O. sinensis* except that it is slightly smaller, measuring 19-29 x12-17uM.
Opisthorchis felineus egg closely resembles that of *O. sinensis* except that it is slightly narrower, measuring 26-32 x 11-15uM. Most of the eggs are asymmetrical, being slightly less convex on one side.
In the laboratory diagnosis of *Fasciola species,* eggs are found in faeces in chronic conditions. *Fasciola* eggs can also be found in duodenal aspirates and in bile. Eggs will not be found in faeces in acute *fascioliasis* when the immatre flukes are migrating through the liver and causing serious symptoms but not yet producing eggs.
Diagnosis is best made serologically. Serological diagnosis by testing serum for antibodies is particularly valuable in the early stages of infection when eggs are not present in faeces. Cross- reactivity with other trematodes such as *Schistosomes* can occur.
In endemic areas where *Fasciola* infections are prevalent, laboratories should find a suitable antibody test or faecal antigen test. In the examination of faeces for *Fasciola* eggs, a concentration technique such as the formol ether method is recommended because the eggs are usually few. Several specimens may also need to be examined to detect the eggs. If eggs are found in human faeces, it must be confirmed that they are present due to a *Fasciola* infection and not from eating animal liver containing *Fasciola* eggs. Repeated finding of the eggs in faeces establishes parasitic infection. The egg of *Fasciola hepatica* or *F. gigantica* is yellow- brown, large and oval. *F. hepatica* eggs measure 130-145x 70-90 uM. *F. gigantica* eggs are larger, measuring 156-197x90-104uM and has an indistinct operculum (lid). It contains an unsegmented ovum surrounded by many yolk cells. Morphologically , *Faasciola* eggs resemle those of *Fasciolopsis buski* and *Echinostoma species*.

In the laboratory diagnosis of *F. buski* infection, examination of the faeces is by direct technique which is used to find the eggs. Concentration technique is rarely needed. The preparation and direct examination of faecal specimens have been described earlier in this text. The egg of *F. buski* is yellow - brown, large and oval, measuring 130-154x 78-98uM. It has a small operculum (lid) which is usually difficult to see and contains an unsegmented ovum surrounded by yolk cells. Morphologically, the eggs of *F. buski* resemble those of *F. hepatica*, *F. gigantica* (similar but smaller) and *Echinostoma species*.
The laboratory diagnosis of *Heterophyes heterophyes* is carried out by finding its small eggs. The small egg of H. heterophyes morphologically resembles the eggs of *Opisthorchis* and *M. yokagawai* . It is light brown, small and oval, measuring 23-27x14-16uM. It has a less distinct operculum and lacks the 'shoulders' of *opisthorchis* eggs .
A small knob can sometimes be seen at the base of the egg. It has no outer indistinct coat as seen with *opisthorchis* eggs.
The laboratory diagnosis of *M. yokagawai* is by finding its small eggs in faeces. A concentration technique such as

the formol ether method may be necessary to detect and identify the eggs. The egg of *M. yokagawai* morphologically resembles the eggs of *opisthorchis* and *H .heterophyes*. It is yellow-brown, small. oval, measuring 28-32 x14-18uM. It has an operculum but lacks the "shoulders" of *opisthorchis* eggs.
The laboratory diagnosis of *Gastrodiscoides hominis* is by finding the eggs of *G. hominis* in faeces. The eggs of *G. hominis* is spindle shaped and large, measuring about 152x50uM. It has a small operculum and contains an immature ovum and yolk cells.

CONCLUSION

The cellophane faecal thick-smear examination technique has proved to be an efficient means of diagnosis of gastrointestinal *schistosomiasis* and other gastrointestinal *helminth* infections. Also known as the kato-katz technique, the cellophane faecal thick-smear slides can be prepared in the field, stored in microscopic slide boxes, and shipped great distances, for examination at a central laboratory, if required. The technique is not suitable for examining larvae, cysts, or eggs from certain intestinal parasites.
Materials and reagents required for this technique include wooden applicator sticks, screen, stainless steel, nylon or plastic-60-105 mesh, template, plastic or card board, microscope slides, cellophane, 40 – 30um thick, strips, 25 x 30 or 25 x 35mm flat bottomed jar, forceps, toilet paper absorbent tissues, newspaper, glycerol – malachite green (or methylene blue) solution.

In this technique, care must be taken during collection of stool specimens. Always wear gloves to avoid contamination of the fingers. Different materials are used, this includes plastic spatula, plastic template and nylon screen in a commercial available kato-katz kit.

The first two items and the microscope slides may be reused. The nylon screen is disposable. A few kits may be ordered to provide standard reusable templates. The nylon screen and the cellophane required for the thick smear technique may be purchased in bulk; from the roll, cellophane is cut into 25 – 30mm sections and placed in a wide – mouth, flat bottom jar containing a 50% (or greater) glycerol solution with malachite – green or methylene blue stain (100ml water, 100ml glycerol, 1ml 3% aqueous malachite green or methylene blue). The procedure for this technique is the same no matter which material is used. The faecal specimen is forced through the screen by a spatula to separate faecal material from the large debris. The screened faecal material is transferred to the template which is laid flat centrally on a microscope slide. The template hole is completely filled with screened faecal material and leveled to the surface of the template. The kato-katz template delivers 41.7mg of faeces. The number of eggs observed is multiplied by 24 to obtain the number of eggs per gram of faeces. The cellophane square soaked in glycerol for at least 24 hours is placed over the faecal specimen. The slide is inverted against a piece of glass or another glass slide and the faecal specimen spread evenly under the cellophane. After the slide is prepared, an additional drop of glycerol may be placed on the cellophane and the edges of the cellophane pressed smooth to ensure conservation of the slide. If air bubbles form under the cellophane during storage a couple of the glycerol on the cellophane allowed to stand overnight will eliminate the stand bubbles. Cellophane thick-smear slides can be prepared in the field, stored in microscope slide boxes and shipped great distances, which permits examination at a central laboratory if required within days or weeks after preparation. *Ascaris* and *Trichuris* eggs are visible at any time. Hookworm eggs are visible for up to 30 minutes after preparation. The ideal time for observing *S. masoni*, *S. intercalatum*, or *S. Japonicum* eggs is 24 hours after preparation.

In bright sunlight, the slides clear rapidly and a 24 hours delay may not be necessary.

Ascaris species in faeces can be detected with direct saline and wet mount. The eggs of *Ascaris* species can also be detected using the Kato-katz technique and the eggs are visible at any time.

The eggs of *Ascaris* species may develop to multi cellular stages. In addition, larvae may degenerate in old stools making it impossible to identify the species.

The *schitosoma* species are seldom in faeces. They are usually in urine or bladder biopsy. The eggs are detected with the concentration technique. Gastrointestinal *schistomiasis* can be detected using the cellophane faecal thick-smear (Kato-katz) technique. The ideal time for detecting *schistosome* is 34 hours after the preparation of the specimen.

The syringe filtration method can be used to detect *schistosoma* infection by obtaining quantitative data from urine examinations. Results can be reported according to egg count categories[4].

As successful chemotherapy programmes reduce intensities of infection and transmission, it is becoming more difficult to show unequivocally whether a person is infected. The parasitological demonstration of eggs or larvae in specimens continues to be the most reliable and widely used method for the initial diagnosis and has proved to be a dependable and non-invasive aid to clinical examination in the field. Yet, serological techniques, which are urgently needed for low-level infections, continue to suffer from poor sensitivity and standardization.

Nevertheless, several promising antigen-capture tests have been reported[5]. Such a non-invasive technique would obviously be more acceptable to patients than one that requires the drawing of blood for serological testing. The use of recombinant DNA techniques in the search for candidate vaccine antigens has led to promising new applications for specific diagnosis of human infections which will also help to assess its field applicability in clinical trials and promote more research on these species. A techniques using acetone instead of the formol ether concentration technique has been described by Parija and workers, 2003[6]. Commercially available devices like paracep and evergreen faecal concentration kit eliminate the tube and filter process thereby reducing the risk of aerosol contamination.
Immunodiagnostic assays are already used in many developed countries for selective chemotherapy and for the large-scale screening of target populations. Over the past 10-20 years, the most promising alternative diagnostic methods developed against *schistosomiasis* are the intradermal test (ID)[7], the circumoval precipitin test (COPT) [8], an indirect hemagglutination assay (IHA) [9], and enzyme-linked immunosorbent assay (ELISA)[10] . These methods have high sensitivity and specificity and have been widely used in many developed countries. Nonetheless, these methods have some shortcomings, such as being time- consuming and requiring special equipment and reagents, which make them unfit for field and bed application. Thus, better and more convenient screening methods are urgently needed for the detection of schistosomiasis.
Immunochromatography assay is a simple, rapid and convenient method. As a type of new diagnostic technology, it was developed after the immunofluorescence assay, ELISA and radioimmunology assay. An immunochromatographic assay was first used during the early 1990s to test for human chorionic gonadotropin (HCG) [11] and then subsequently for the detection of hepatitis B virus surface antigen (HBsAg)[12] . It is widely used in various fields of medicine.

In some studies , latex microspheres can be used as a color probe to establish a novel, simple, rapid and convenient immunoassay with high sensitivity and specificity for the screening of *Schistosoma* infected persons on a large scale in endemic areas and this can be used assess its usefulness for clinical diagnosis. On the basis of the immunochromatographic assay, labelled mouse anti-human IgG with latex microspheres, and developed rapid anti-*Schistosoma* antibody detection dipstick, which can be called the dipstick with latex immunochromatography assay (DLIA) can be used in field trials. Its application can be used to assess the diagnostic value of the DLIA in the laboratory and field for detection of anti- *Schistosoma* antibodies, and the result can also be compared to the results of the ELISA and Kato-Katz method.

REFERENCES

1.Crompton et al; (2009) .WHO report on prevention and control of schistosomiasis and soil transmitted helminthiasis. www.who.int/health-topic/schisto.
2.Klinkert, MQ et al (1987): Molecular and biochemical parasitology; 25:247-255.
3.Marshal. I, et al (1989); comparison of potassium hydroxide digestion and a modified Kato-katz technique for the semi-quantitative estimation of schistosome masoni eggs in faeces. Annals of Tropical Medicine and parasitology :. 83 (1) : 31 – 35.
4.WHO 1984: Basic laboratory methods in medical parasitology.
5.Monica Cheesbrough: (2008): District laboratory practice for developing countries Part 1 : 25 - 30.
6.Parijah et al (2003) Tropical Doctor 33 : 163 – 163.

7.Li Y. (1991): Advances in the study of immunodiagnosis of schistosomiasis. Nanjing, China: Jiangsu Science Technology.
8.Zhu YC. (2005): Immunodiagnosis and its role in schistosomiasis control in China: a review. Acta Trop. ;96:130–136. doi: 10.1016/j.actatropica.2005.07.007. [PubMed] [Cross Ref]
9.Hamilton JV, Klinkert M, Doenhoff MJ.(1998): Diagnosis of schistosomiasis: antibody detection, with notes on parasitological and antigen detection methods. Parasitology;117(Suppl):S41–57. [PubMed]
10.Doenhoff MJ, Chiodini PL, Hamilton JV.(2004): Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies? Trends Parasitol. ;20:35–39. doi: 10.1016/j.pt.2003.10.019. [PubMed] [Cross Ref]
11.Beggs M, Novotny M, Sampedro S. A (1990): Self-performing chromatographic immunoassay for the qualitative determination of human chorionic gonadotrophin(HCG) in urine and serum. Clin Chem. ;36:1084–1085.
12.Sumathy S, Thyagarajan SP, Latif R, Madanagopalan N, Raguram K, Rajasambandam P, Gowans E.(1992): A dipstick immunobinding enzyme-linked immunosorbent assay for serodiagnosis of hepatitis B and delta virus infections. J Virol Methods. ;38:145–152. doi: 10.1016/0166-0934(92)90177-F. [PubMed] [Cross Ref]