LABORATORY ACTIVITY PARASITOLOGY

Recourse Person : Ratna Dewi I.,dr.,M.Kes.
Subject : Identification of Intestinal Helminths and Protozoa
Department : Parasitology

A	Sequent			
	I	Introduction	:	30 Minutes
	II	Pre-test	:	10 Minutes
	III	Lab. Activities	:	80 Minutes
	IV	Post test	:	-
B	Topic			
		During Lab Activity in parasitology, students are expected to do the following activities: 1. **Microscopic examination** and identification of intestinal helminth (nemotodes, trematodes, cestodes) 2. **Microscopic examination** and identification of intestinal protozoa (trophozoit and cysts) 3. **Gross/macroscopic observation** of adult helminth models 4. **Hands-on exercise** on direct wet mount stool examination and Kato Katz method	: : : :	20 Minutes 20 Minutes 20 Minutes 20 Minute
C	Venue			
	Biomedical Laboratory, Faculty of Medicine, Unisba, Jl. Tamansari No.22 Bandung 40116			
D.	EQUIPMENT			
	Direct Wet Mount Stool Examination	**Materials** - Sample material (stool sample) - Clean glass microscope slide - 22 x 22 mm coverslip - 0.85% NaCl solution - Lugol's iodine solution - Biohazard discard container - Light microscope		
	Kato-katz stool Examination	- Sample material (stool sample) - Clean glass microscope slide - 22 x 22 mm coverslip - Selophan tape which soaked with methylene blue glycerol solution/ malachite green glycerol - Biohazard discard container - Light microscope		
	Diagnostic stage Soil Transmitted Helminth	**Materials** Permanent stained slide of adult: 1. *Trichuris trichiura and* Hook worms (nematode) 2. *Fasciola gigantica (trematode)* 3. Proglotid taenia (cestode) Permanent stained slide of egg : 4. *Ascaris lumbricoides/Trichuris trichiura/hookworm*		

	Diagnostic stage Intestinal protozoa	**Materials** Permanent stained slide of: 1. *Giardia lamblia* (trophozoit and cysts)
	Gross preparat	**Materials** 1. Gross preparat *Ascaris* 2. Gross preparat *Taenia sp.*
E	Lab. Activities	
	1. The Students were divided into six group 2. Each group do lab. activities accompanied by tutor	
	How to Identify Helminths Diagnostic stages of helminths found in feces include ova (eggs), larvae, proglottids, and, occasionally, adult worms. Adult nematodes are rarely found in stool specimens. Exceptions are the pinworm *(Enterobius vermicularis)* and *Ascaris.* Adult trematodes are not found in feces, but proglottids may be found in some cestode infections. Ova are usually seen. Characteristic eggs produced by nematodes, trematodes, and cestodes are most frequently used to make a diagnosis of infection with these parasites. Gravid proglottids of cestodes may rupture, releasing eggs. Eggs produced by a particular species of helminth are usually the same size and shape, and have the same stage of development when passed in feces. Characteristics used most frequently to identify helminth eggs include size, shape, appearance of the eggshell, and stage of development. A calibrated ocular micrometer is necessary to accurately determine measurements of helminth eggs. The size of these eggs ranges from 25 to 90 micrometers in length. The shape of the egg varies from spherical or round to oval. Infertile eggs may have a shape different from fertile eggs. Eggshells may be clear or colored (bile stains appear yellowish or brownish), and may be thick or thin. The eggs of *Ascaris, Enterobius,* and *Trichuris*are thick; those of the hookworms *(Necator*and *Ancylostoma)* are thin. Modifications of the eggshell are characteristic of certain species. Knobs and spines are characteristic of certain species of trematodes. The stage of development of the parasite within the eggs is characteristic for the species. Embryonated and unembryonated eggs may be found in feces. Development of the embryo may occur in the environment. Trematode eggs may contain an embryo known as a **miracidium.** A cestode egg usually contains a six-hooked larva called an **oncosphere.** Unfertilized eggs of *Ascaris lumbricoides* usually contain unorganized masses of globular material. No ova of *Strongyloides stercoralis* are passed in stool specimens; larvae constitute the diagnostic stage found in feces. These are the only nematode larvae usually found in fecal specimens. However, hookworm larvae may occasionally be present if the specimen is allowed to stand at room temperature for more than a day. Distinguishing between these two parasites may be difficult. Infection with filarial nematodes is usually detected by demonstrating the presence of microfilariae in blood or skin. Recognition of characteristic morphologic types may allow more specific identification of the parasite. **BE CAREFUL WITH ARTIFACTS WHICH MAY BE CONFUSED WITH PARASITES** Artifacts may cause confusion by being identified as human parasites. Yeast cells may resemble	

protozoan cysts or helminth eggs, but lack internal structure, and may be observed as budding forms. Pollen grains and vegetable cells may be mistaken for helminth eggs. Striations in the pollen grain wall may be confused with those found in the eggs of *Taenia*species. These structures generally are irregular and vary in size.

Starch granules may be confused with protozoan cysts, but are very retractile. Plant hairs and undigested food fibers may be mistaken for nematode larvae. Internal structure is usually lacking in these artifacts, which have thick, retractile walls.

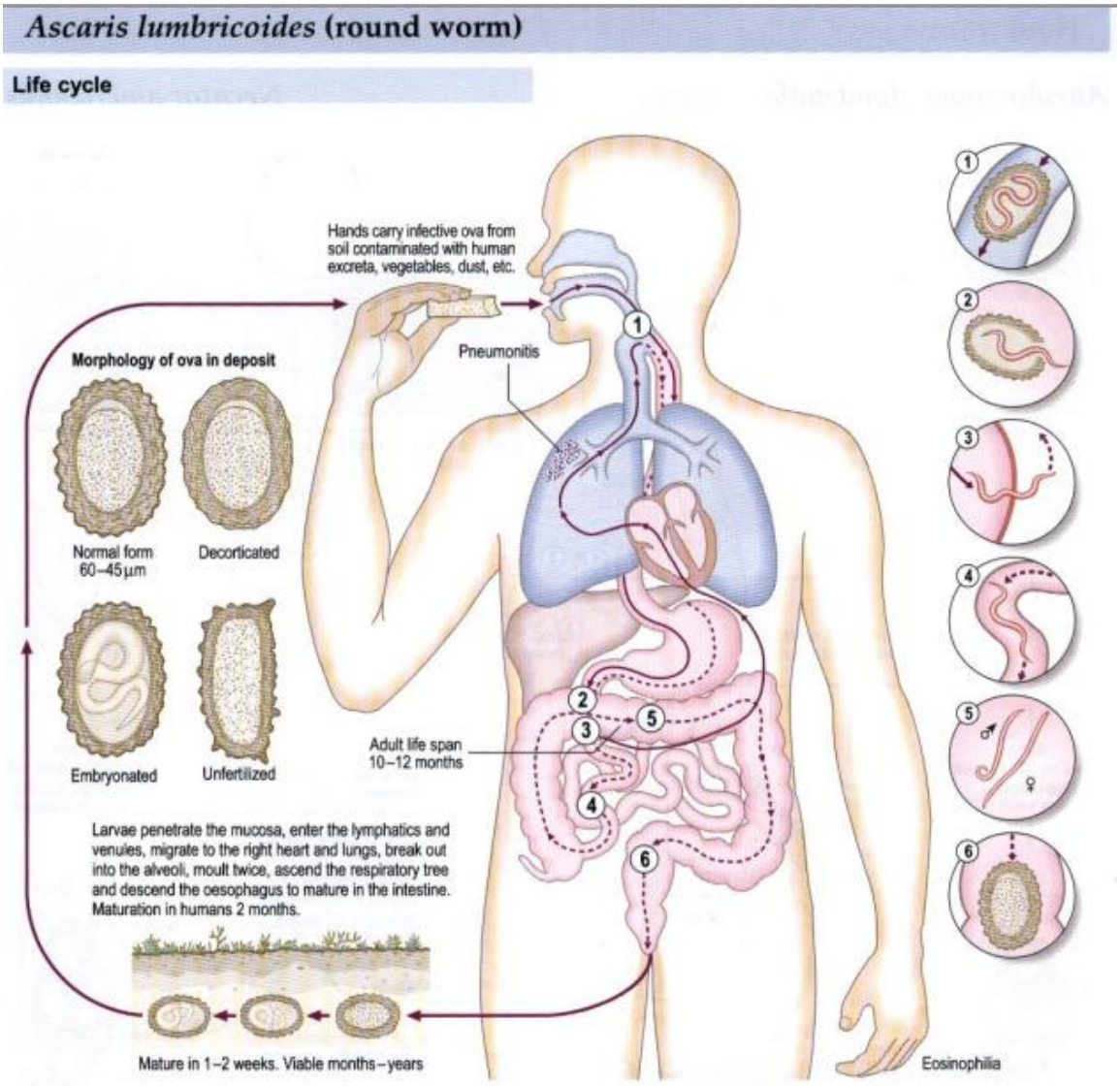
Polymorphonuclear leukocytic neutrophils may resemble amebic cysts, but usually have more irregular, poorly defined borders. The nuclei of these white blood cells are larger in proportion to the cytoplasm than are nuclei in amebic cysts, lack peripheral nuclear chromatin, and may be linked by strands of chromatin.

CHARACTERISTIC OF HELMINTHS

Differences between Cestodes, Trematodes & Nematodes			
	CESTODES	TREMATODES	NEMATODES
Shape	Tape like segmented	Leaf like unsegmented	Elongated, cylindrical, unsegmented
Sexes	Sexes not separate Monoecious Hermaphrodite	Sexes not separate Monoecious except Schistosoma	Sexes are separate Diecious
Head end	Suckers, often with hooks	Suckers, no hooks	No suckers, no hooks, well developed
Alimentary canal	Absent	Present, incomplete, no anus	Present, complete, anus present
Body cavity	Absent	Absent	Present

8/31/2017 4

Life cycle of *Ascaris lumbricoides*



CHARACTERISTICS OF INTESTINAL AMEBAE

AMOEBA	TROPHOZOITE	CYST
Entamoeba histolytica	10-60 µm Finely granular cytoplasm Single nucleus has peripheral nuclear chromatin with a small, central, compact karyosome Inclusions: ingestion of RBC is diagnostic Progressive motility	10-20 µm Mature cyst contains 4 nuclei Elongated chromatoidal bars with blunt, smooth ends Nucleus with small, central, compact karyosome

	Entamoeba hartmanni	5-12 μm Finely granular cytoplasm Evenly distributed peripheral nuclear chromatin Central, compact karyosome Inclusion: bacteria, no RBC Non-progressive motility	5-10 μm Mature cyst contains 4 nuclei Elongated chromatoidal bars with blunt, smooth ends Central, compact karyosome
	Entamoeba coli	15-50 μm Coarsely granular cytoplasm Unevenly distributed peripheral nuclear chromatin Large, discrete, eccentric karyosome Inclusion: bacteria, yeast, debris Sluggish, non-progressive motility	10-35μm Mature cyst contain 8 nuclei Splinter shaped chromatoidal bars with sharp, ponted ends Large, eccentric karyosome
	Balantidium coli	30-100 μm by 30-80 μm Very large ovoid shape Large been-shaped macronucleus Small spherical micronucleus Numerous cilia Cytostome/cytopyge	45-65 μm Oval or spherical shape Large macronucleus
	Giardia lamblia	9-20 μm by 5-15 μm Pear-shaped or teardrop shaped with 2 nuclei, linear axonemes, curved median bodies, and 8 flagella; sucking disc Falling leaf motility	8-18 μm by 7-10 μm Round to oval with 4 nuclei, axonemes and median bodies

Life cycle of *Giardia lamblia (Giardia duodenalis)*

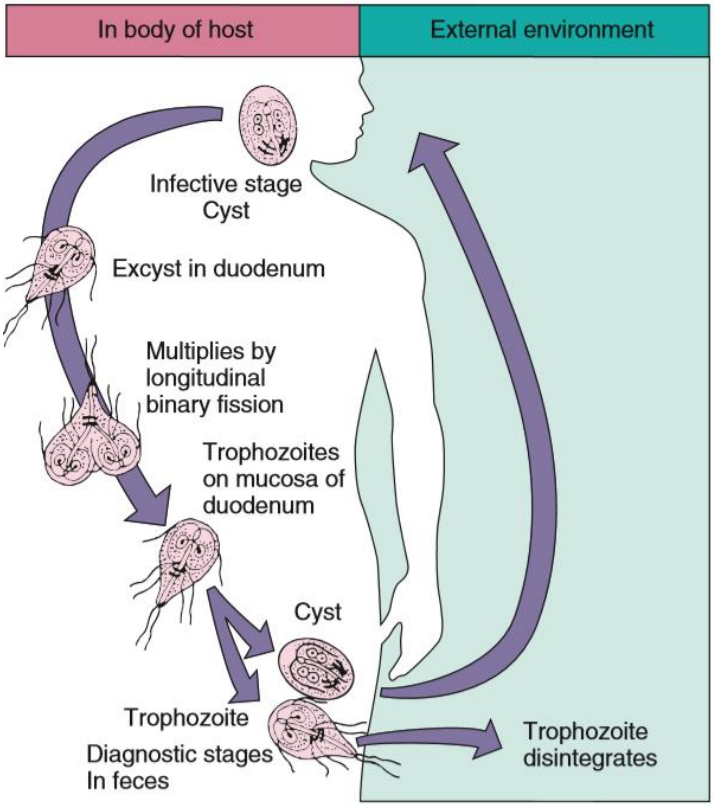


FIGURE 73-4 Life cycle of *Giardia duodenalis*.

A. DIRECT WET MOUNT

Principle

The direct wet mount is performed to identify parasite worm eggs and detect motile protozoan trophozoites and to determine cellular morphology.

Materials

- Sample material
- Clean glass microscope slide
- 22 x 22 mm coverslip
- 0.85% NaCl solution
- Lugol's iodine solution
- Biohazard discard container

Method

A. *Saline Mount*

1. Place 1 drop of 0.85% NaCl on a clean, dry, 2 x 3 inch microscope slide.
2. With an applicator stick, transfer a small amount (~2 mg) of fecal sample and emulsify in the saline drop.
3. Place a 22 x 22 mm coverslip over the suspension.
4. Using the low power objective (10X), systematically scan the entire surface area of the coverslip

for parasites.
5. The high dry objective (40X) should be used for investigation of suspicious objects.

B. *Iodine* Mount

1. An iodine stained mount can be prepared in the above manner, substituting one drop of Lugol's iodine for the saline.
2. A drop of iodine can also be added to the edge of the coverslip of a previously examined saline mount. The iodine will diffuse into the stool-saline mixture, kill the organisms, and stain the cellular elements.

A. KATO-KATZ TECHNIQUE

In areas with moderate to high transmission rates of STH (i.e. where the proportion of infected individuals is >20– >50%) or intestinal schistosomiasis (>10–50%), WHO recommends community diagnosis using the Kato-Katz technique.

Where the prevalence of STH is <20%, the specificity of this technique makes it less appropriate, and more sensitive tools should be used.

The principle behind the Kato-Katz technique is straightforward: people infected with STH or intestinal schistosomes pass the eggs of the worms through their faeces. By examining a stool sample under a microscope it is possible to count the number and the type of eggs that are present

Kato-Katz kits contain a roll of cellophane that is cut into small pieces and soaked in methylene blue glycerol solution (not included in the kit) the night before the field work. The cellophane is then placed directly on the faeces sample, making the eggs more easily visible and allowing long-term storage of the slides

Procedure:

1. Place the template with hole in the centre of a microscope slide
2. Place a small amount of the faecal sample on a newspaper and press a piece of nylon screen on top. Using a spatula, scrape and press a piece of nylon screen on top so that only the debris remains.
3. Scrape up some of the sieved faeces to fill the hole in the template, avoiding air bubbles and levelling the faeces off to remove any excess.
4. Place one piece of the cellophane, which has been soaked overnight in methylene blue glycerol solution, over the faecal sample.
5. Place a clean slide over the top and press it evenly downwards to spread the faeces in a circle. If done well, it should be possible to read newspaper print through the stool smear
6. If hookworm is present in the area the slide should be read within 30–60 minutes, irrespective of the technique used. After that time, the hookworm eggs disappear.
7. Place the slide under a microscope and examine the whole area in a systematic zigzag pattern. Record the number and the type of each egg on a recording form alongside the sample number. Finally, multiply the number of eggs by 24 to give the number of eggs per gram (epg) – the standard measurement to assess the intensity of infection.

	Student task: Draw various the diagnostic stage of *Ascaris lumbricoides (the eggs)*
	Student task Draw the diagnostic stage and infective stage of *Trichuris trichiura (the egg)*
	Students task: Draw the diagnostic stage of Hookworm (the egg)
	Student task: Draw the diagnostic stage of *Giardia lamblia (trophozoit and Cyst)*
	Student task : Draw the adult stage of Trichuris trichiura (male and female)
	Student task : Draw the adult stage of hookworm (male and female)

	Student task : Draw the adult stage of Fasciola hepatica
	Student task : Draw the proglotid of Taenia
F.	**HOMEWORK**
	1. Explain life cycle of *Ascaris lumbricoides* **2. Explain life cycle of *Fasciola hepatica*** **3. Explain life cycle of *Taenia saginata*** **4. Explain life cycle of *Giardia lamblia*** **5. Explain the characteristic of Adult nematodes, adult trematodes and adult cestodes** **6. Explain the characteristic of trophozoit and cyst** **7. Mention procedure of Saline Wet Mount** **8. Mention the procedure of Kato Katz method**
G.	**REFFERENCES**
	1. Essentials of Human Parasitology. Judith S. Heelan, Frances W. Ingersoll, Delmar Thomson Learning, 2002. 2. Parasitology: An Integrated Approach. Alan Gunn, Sarah Jane Pitt. Wiley Blakwell, 2012. 3. Diagnostic Medical Parasitology. Garcia L.S., Bruckner D.A., ASM Press Washington D.C., Fifth Edition, 2007. 4. Human Parasitology, 4th ed. Burton J. Bogitsh, Clint E.Carter, Thomas N.Oeltman. Elsevier Academic Press. 5. Atlas of Medical Helminthology and Protozoology, 4th ed, P.L. Chiodini, A.H. Moody, D.W. Manser, Churchill Livingstone, 2001.