First lecture. by Dr. Hiro M. Obaid

Classification of Protozoa

Protozoan parasites of medical importance have been classified into kingdom Protista, subkingdom Protozoa which is further divided into the following four phyla

1-Sarcomastigophora.

2-Apicomplexa

3-Microspora

4-Ciliophora

Phylum Sarcomastigophora

Phylum Sarcomastigophora has been subdivided into 2 subphyla based on their modes of locomotion.

 Sarcodina (Sarcos meaning flesh or body): It includes those parasites, which have no permanent locomotory organs,but move about with the aid of temporary prolongations of the body called pseudopodia (e.g. Amoebae).

 Mastigophora (Mastix, meaning whip or flagellum) It includes those protozoa which possess whip-like flagella (e.g. Trypanosoma and Trichomonas).

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Phylum Apicomplexa

Phylum Apicomplexa was formerly known as **sporozoa**. Members of this group possess, at some stage in their life cycle, a structure called the **apical complex** serving as the organ of attachment to host cells.

- They are tissue parasites.
- They have a complex life cycle with alternating sexual and asexual generations.
- To this group, belongs the malarial parasites (suborder: Haemosporina, Family: Plasmodiidae), *Toxoplasma*, *Sarcocystis*, *Isospora,* and *Cryptosporidium* (under the Suborder: Eimeriina), *Babesia* (under the subclass: Piroplasma), and the unclassified *Pneumocystis jirovecii.*

Phylum Ciliophora

These protozoa are motile by means of cilia, which cover their entire body surface. The only human parasite in this group is *Balantidium coli,* which rarely causes dysentery.

Phylum Microspora

Phylum Microspora contains many minute intracellular protozoan parasites, which frequently cause disease in immunodeficient subjects. They may also cause illness in the immunocompetent, rarely.

Phylum	Subphylum	Superclass	Class	Subclass	Order	Suborder	Genus
Sarcomasti-gophora	**Mastigophora (having one or more flagella)**		Zoomastigopho-rea		Kinetoplastida	Trypanosomatina	*Trypanosoma Leishmania*
					Retortamonadida		*Retortamonas Chilomastix*
					Diplomonadida	Enteromonadina	Enteromonas
						Diplomonadina	*Giardia*
					Trichomonadida		*Trichomonas Dientamoeba*
	Sarcodina (pseudopodia present)	Rhizopoda	Lobosea	Gymnamoe-bia	Amoebida	Tubulina	*Entamoeba Endolimax Iodamoeba*
						Acanthopodina	*Acanthamoeba*
					Schizopyrenida		*Naegleria*
Apicomplexa			Sporozoea	Coccidia	Eucoccidia	Eimeriina	*Crypto-sporidium Isospora Sarcocystis Toxoplasma*
						Haemosporina	*Plasmodium*
				Piroplasmia	Piroplasmida		*Babesia*
Ciliophora			Kinetofragmino-phorea	Vestibuliferia	Trichostomastida	Trichostomatina	*Balantidium*
Microspora			Microsporea		Microsporida	Apansporoblas-tina	*Enterocytozoon Encephalito-zoon Microsporum*

Terms used in parasitology:

•An obligatory parasite that is completely dependent on its host and can't survive without it e.g. hookworms.

•A facultative parasite that can change its life style between free-living in the environment and parasitic according to the surrounding conditions. e.g. Strongyloides stercoralis.

•An accidental parasite that affects an unusual host e.g. Toxocara canis (a dog parasite) in man.

•A temporary parasite that visits the host only for feeding and then leaves it. e.g. Bed bug visiting man for a blood meal.

•A permanent parasite that lives in or on its host without leaving it e.g. Lice.

•An opportunistic parasite that is capable of producing disease in an immunedeficient host (like AIDS and cancer patients). In the immuno-competent host, it is either found in a latent form or causes a self limiting disease e.g. Toxoplasma gondii.

•A zoonotic parasite that primarily infects animals and is transmittable to humans. e.g. Fasciola species.

-2Types of Hosts:

Hosts are classified according to their role in the life cycle of the parasite into:

•Definitive host (DH) that harbors the adult or sexually mature stages of the parasite (or in whom sexual reproduction occurs) e.g. man is DH for Schistosoma haematobium, while female Anopheles mosquito is DH for Plasmodium species (malaria parasites.(

•Intermediate host (IH) that harbors larval or sexually immature stages of the parasite (or in whom asexual reproduction occurs) e.g. man is IH of malaria parasites. Two intermediate hosts termed 1st and 2nd IH may be needed for completion of a parasite's life cycle, e.g. Pirenella conica snail is the 1st IH, while Tilapia (Bolty) fish is the 2nd IH for Heterophyes heterophyes.

•Reservoir host (RH) harbors the same species and same stages of the parasite as man. It maintains the life cycle of the parasite in nature and is therefore, a reservoir source of infection for man. e.g. sheep are RH for Fasciola hepatica.

•Paratenic or transport host in whom the parasite does not undergo any development but remains alive and infective to another host. Paratenic hosts bridge gap between the intermediate and definitive hosts. For example, dogs and pigs may carry hookworm eggs from one place to another, but the eggs do not hatch or pass through any development in these animals.

• Vector is an arthropod that transmits parasites from one host to another, e.g. female sand fly transmits Leishmania parasites .

The parasite is a living organism that lives in (endoparasite) or on (ectoparasite) another organism, termed its host. It obtains nourishment and protection while offering no benefit in return. Consequently, the host suffers from various diseases, infections, and discomforts. However, in some cases, the host may show no signs at all of infection by the parasite.

Fecal specimens

Faecal specimens are examined for the presence of protozoa and helminthes larvae or egg.

The stage of protozoa found in stools are Trophozoites and cysts .The stages of helminthes usually found in stools are eggs and larvae. Though whole adult worms or segments of worms may also be seen. Adult worms and segments of tapeworms are usually visible to the naked eye , but eggs , larvae, Trophozoites and cysts can be seen only with the microscope . In order to see these structures the fecal material must be properly prepared and examined.

Collection of fecal specimens

Because of the fragile nature of intestinal parasites , and need to maintain their morphology for accurate identification reliable microscopic diagnosis can not be made unless the stool is collected properly.

1-Give the patient the following:

-A plastic cup or box with a tight –fitting , lid.

-2 applicator sticks.

*If plastic cups are not available , tin boxes or glass jars can be used.

In control programmers , it is often sufficient to examine a single specimen, but for patients, three specimens are usually required, at 3-day intervals, to detect all parasitic infections .A variety of substances may interfere with the examination of stool specimens for parasites(e.g. laxatives , antacids , ingested contrast media , certain antibiotic)

2-Tell the patient to pass the stool specimens directly into the container, or to pass the stool on to a piece of paper and use the applicator sticks to transfer it to the container

. However the stool must be transferred immediately to the specimen container . It should not remain on the paper or be brought to the laboratory on the paper.

3-Some organisms especially amoebic Trophozoites will begin to disintegrate or change within a short time after passage and became unrecognizable . Warm temperatures will hasten these change . Therefore specimens must reach the laboratory very soon (i.e. within half an hour) after passage . If this is not possible the specimens must be treated with preservative .

4-The container with specimen should be labeled clearly with the following information:

-Patients name or number.

-Date of collection.

-Time the patient passed the stool (ask the patient when he\she passed the stool)

5-The stool specimen must be large enough for satisfactory examination. The smallest quantity that should be accepted is about the size of a pigeons egg. Urine and dirt should be excluded . Urine will destroy any amoebic Trophozoites and dirt will interfere with the examination.

If the specimen is too small or if it is mixed with urine or dirt it should not be accepted . Ask the patient to pass another specimen.

6-Keep the carton containing the specimen in a refrigerator or if this is not possible in the coolest ,shadiest area in the laboratory. Do not keep the specimen artificially warm and do not leave it in the sun.

Examination of stool specimens for parasites:

Stools may be send to a specialized laboratory for the identification of rare parasites that are difficult to recognize .In such cases a preservative should be added to the specimens before they are dispatched for examination . The following preservatives are used:

-Formaldehyde 10% solution .

-MIF (merthiolate iodine formaldehyde) .

-Polyvinyl alcohol (PVA) fixative.

-SAF (sodium acetate acetic acid formaldehyde).

Note: Never leave stool containing mucus or blood first as they may contain motile amoeba (which die quickly).

Visual or macroscopic examination:

Faecal samples are best described by their color consistency and presence or absence of macroscopic blood or exudates . Make sure that any adult worms or segments passed are included.

Color

Normally adult feces are light to dark brown due to the presence of bile pigments .Children 2-4years yellowish . Infant 1 day – 2 years yellow-greenish. The color of the stool may effected by food intake.

The color can be described as:

-black (occult blood) black or tarry black stools indicate bleeding into the upper gastric-intestinal tract , the blood having been altered by the intestinal juices

(melaena). Black stools may also result from iron administration in the treatment of anemia.

-Pale yellow (fat).

-White (obstructive jaundice, cholera) .

Consistency

The consistency can be described as:

-Formed

-Soft

-Loose

-Liquid (watery)

Consistency and form: Normally feces when passed tens to be well formed .

-If feces appear **hard** they might indicate constipation .

-**Flattened and ribbon** like stool indicate some obstruction in the lumen of the bowel.

-**Semi-sold:** In mild diarrhea digestive upsets or after taking laxatives.

-**Watery stool:** Purgatives digestive upsets bacterial infections occasionally some poison like arsenic.

-Rice water stools are typical of cholera the stools being copious thin ,watery , colorless and containing numerous white flakes of desquamated epithelium.

-Clay colored stool indicates an obstruction to the flow of bile into the intestinal canal and is due to the increased fat. After barium salts intake stool will appear white.

-Blood and mucus: Found blood in large amount is suggestive of bleeding piles or contamination with menstrual blood . Occasionally it may be due to the destruction of a blood vessel by an ulcerative process . Blood may be present in certain medical conditions like colitis, schistosomiasis . More rarely this may be the result of uremia or cancer.

The commonest cause of blood and mucous in stool is dysentery which may be 2 types

A-Protozoal or amoebic

B-Bacillary

Occult blood in stools

This test is used for screening of bleeding in the intestine caused by polyps , tumors or inflammation.

PH determination

Adult PH is 6-6.5 m children 5-5.5 .

Acidic PH may indicate amoebic dysentery , alkaline PH indicate bacillary dysentery .

Microscopic examination

Direct microscopic examination of feces in saline or iodine suspension.

Clinical significance of the test:

Many pathogenic parasites are excreted in stool. Often when a person is infected with intestinal parasites, other symptom such as anemia, eosinophilia , diarrhea and malabsorption are also present. However diagnosis by physical examination is not sufficient to identify intestinal parasitic infection. Stool examination is essential to identify parasites that cause the disease.

It is useful for the following reasons:

-to detect motile Trophozoites .

-to detect ova and cyst present in moderate numbers.

-to detect erythrocytes, cellular debris or excess fat.

Select unformed or liquid feces when using direct microscopy for detection of Trophozoites .Formed stools rarely contain motile Trophozoites. Also perform a direct examination of any external blood or mucus.

Equipment requirements :

-Glass slides

-Cover slip (20 ×20mm).

-Wooden applicator.

-Grease pencil .

-Microscope.

Reagents and stain Requirements :

-Normal saline (0.9 % sodium chloride solution)

-Lugol's iodine

How to prepare 0.9 % sodium chloride solution –Normal saline

Sodium chloride NaCl ….9 g

Distilled water …….. 1000 ml

How to prepare Lugol's iodine :

Potassium iodide …..2 g

Iodine …… 1 g

Distilled water …. Up to 1000 ml

Mix the potassium iodide with about 200 ml of water until it is completely dissolved. Add the iodine to the potassium iodide solution, mix until dissolved. Add water until the 1000 ml mark. Store in dark brown bottle. Use a small amount in a brown dropper bottle. Prepare a new solution if Colour fades.

Procedure:

1- Label a glass slide with the patient name and or lab number.

2-Put one drop of normal saline in the middle of the left of the slide.

3-Put one drop of Lugol's iodine in the middle of the right half of the slide .

Note\ You can examine the slide using normal saline only but you would not been able to see cysts well. Therefore it is advisable to examine each stool sample with Lugol's iodine and normal saline . iodine solution the chromatin granules and karyosome of nuclei stain brown . The glycogen vacuole stains brown and the chromatid bars remain unstained.

4-Take a small piece of stool with the wooden applicator .

Note\ If the stool is formed take the piece from inside and the surface of the sample. If the stool is liquid take drop. Any part is o.k. If the specimen is very liquid place one or two drops of stool directly onto the slide and cover it with the cover glass , do not add the saline as this would further dilute the specimen.

5-Mix the sample first with the drop of warmed to 37℃ normal saline on the left half of the slide.

Note\ If the stool contains mucus or blood stained parts, prepare a second slide with a drop of normal saline and take the piece from the mucus or blood-stained part.

Amoeba Trophozoites are often more easily found in mucus . Careful , do not mix the mucus with the Lugol's iodine .

The iodine preparation is useful to identify cysts but kills all living organisms in the specimen . 0.5% eosin solution helps in the search for Trophozoites of protozoa.

6-Take a small piece again and mix it with the drop of Lugol's iodine on the right half of the slide.

The iodine preparation is useful to identify protozoa cysts.

7-Place a cover slip over each drop.

Put the cover slip slowly letting it move down from the side to avoid air bubbles.

Parasitic amoeba , flagellates, ciliates, eggs, larvae and cyst are found at thick part of cover.

8-Examine the entire coverslip systematically.

For the saline preparation use the 10X objective and 40X objective .

For the Lugol's iodine preparations use the 40X objective , as there you might find cysts.

When searching for Cryptosporidium and Cyclospora cayetnensis it is better to prepare a modified ziehl neelsen stained smear.

Usually reported as the number seen in the entire preparation as follows.

Scanty (rare)n1-3, 3-10(+) 10 to 20 (++) , 20 to 40(+++), and more than 40(++++).

Cells are usually reported as the number seen per high power field(HPF).

Buffered methylene blue (BMB) mount (to be prepared if amoebic Trophozoites are seen in the saline mount)

Place a large drop of BMB on the slide instead of saline or iodine . wait 5-10 minutes before examining , to allow the stain to penetrate the Trophozoites.

BMB will over stain the Trophozoites in about 30 minutes .Therefore the slide must be examined within 30 minutes after preparation .

Its prepared from:

Methylene blue……..0.1 g

Isotonic saline ……100 ml

For use filter small amount in to a dropper bottle.

Eosin Y stain solution (1% aqueous):

1. eosin Y stain (1.0 g)

2. glacial acetic acid (0.2 ml)

3. deionized water (100 ml)

Report for general stool examination. (G. S .E)

1-Macroscopic examination .

Color : brown, yellow, green, black, ….

Consistency : formed , loose, soft, watery, …

Blood or mucus

Occult blood

PH:

Others

Adult worm

Larvae

Segments

2-Microscopic examination.