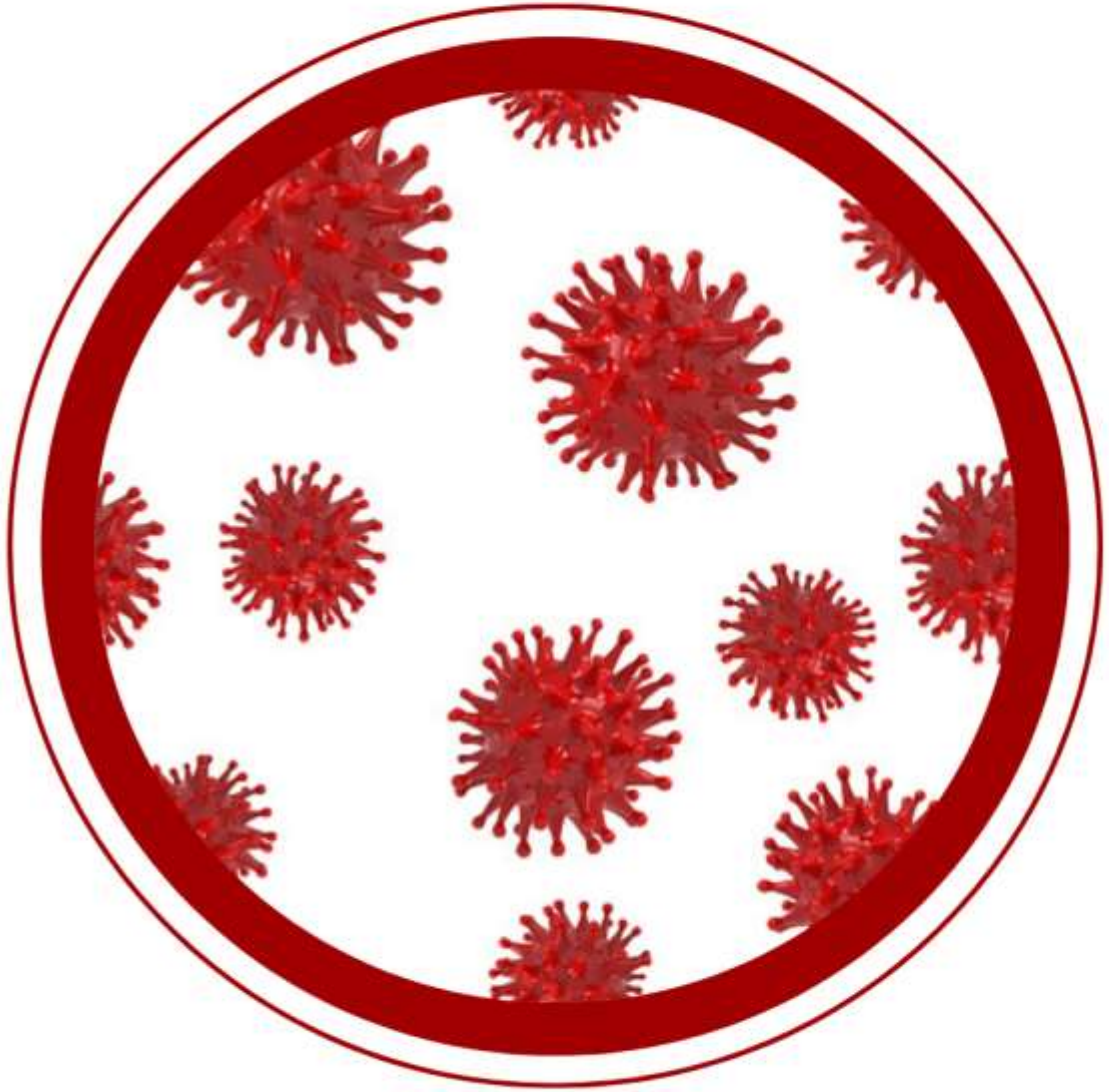
GI

MICROBIOLOGY

#1 LAB



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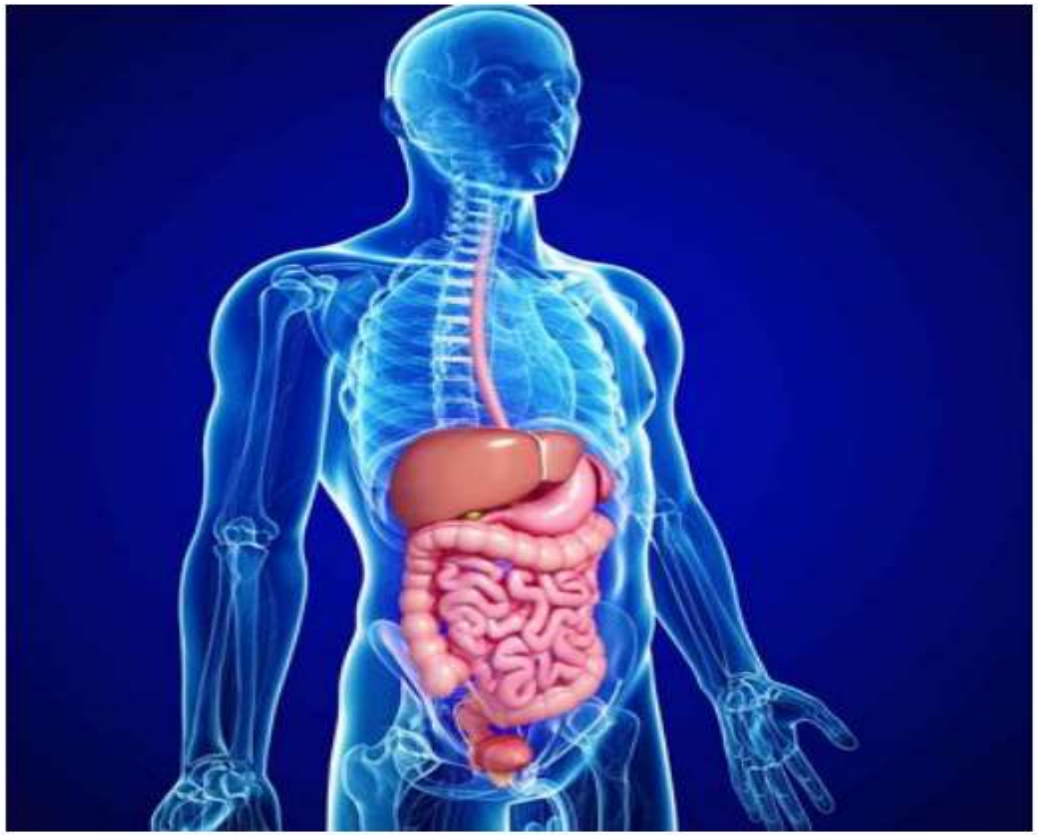
Gastro Intestinal System



-GI pathogens could be viruses, parasites, or bacteria.

-viral infections in GIS are self-limited with electrolytes and water supplements, though:

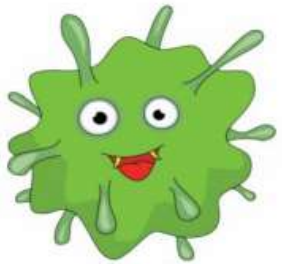
-if common symptoms like gastric pain and diarrhea manifest, we should wait at least 1 day for the diarrhea to resolve alone, but if it continues for more than 3 days, you should seek clinical intervention.

-the doctor will probably ask for a stool sample, for culturing.

-so, why stool, not urine sample??

*urine is sterile, and needs a sterile procedure to deal with, while stool is almost never had been sterile, it is contaminated, which means that it is easier to deal with, collecting the stool must be in clean containers and somehow sterile, but not a must!

Stool Collection & culture



- Stool should be collected in clean wide mouth container not sterile

If you have been showing one of the following symptoms your doctor might ask for a stool sampl

1) **diarrhea that lasts more than a few days .**
2) **Stools that contain blood or mucus .**
3) **Stomach pain or cramping.**
4) **Nausea**
5) **Throwing up**
6) **fever**

The patient will be given a special wide mouth container with a label of the patient's name , birthdate and the time of sample collection.

Steps to collect the sample:

1) **make sure that the sample does not touch the inside of the toilet**
2) **place the sample into the container using a small disposable spoon or spatula (Make sure to throw them after using them)**
3) **Don't overfill the container and avoid getting urine mixed up with the stool**
4) **Return the sample to the lab as soon as possible (it can be kept at the patient's refrigerator but not for more than 24 hours)**

The sample should be cultured within a duration that doesn't exceed 30 mins, otherwise, it has to be refrigerated, some factorial problems could happen like crowded samples, but it forms only 0.05

Stool should be added to Selenite broth

Why? ?

- ❑ Inhibits the growth of coliforms
- ❑ Enhances the growth of Pathogen

Normal colonizers

❖ Most common pathogens (Bacteria) :

- E.coli
- Salmonella
- Shigella
- Vibrio
- Proteus
- Yersinia , Campylobacter , Clostridium, Bacillus …etc

Rare in Jordan

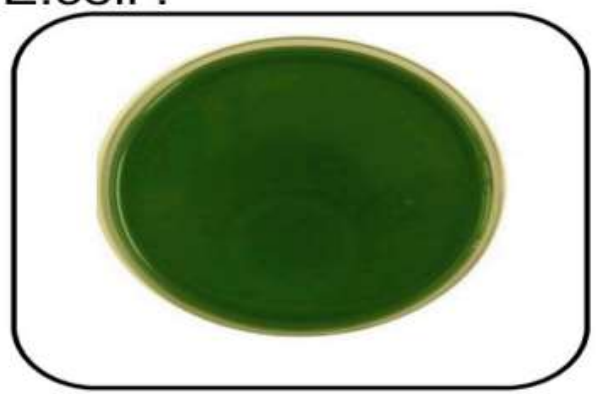
*The following bacteria are not normal inhabitants of the gastrointestinal tract and are known to cause G.I. infections.

Stool sample should be cultured on the following media using streak plate method

- MacConkey media is considered as a selective and differential media for enterobacteriaceae looking for E.coli .

S-S agar

Hekton agar



T.C.B.S
(Selective and differential for vibrio spp.)

Selective + differential media for salmonella and shigella spp.

The difference between S-S and Hekton is just commercial, depending on the manufacturing companies, so they differ in color

Selective: only allow salmonella and shigella growth
-differential: differentiate between them

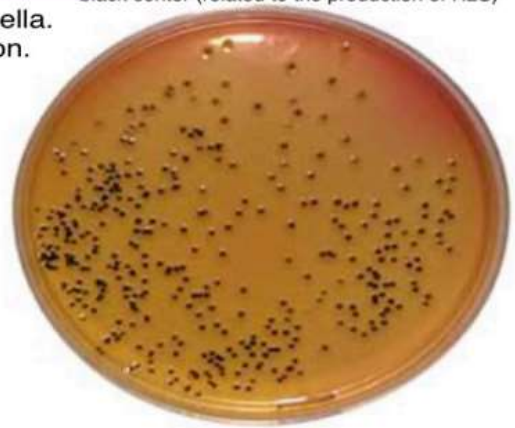
S-S agar

Stands for salmonella -shigella agar : Highly selective and differential for salmonella and shigella. It contains an indicator to detect H2S production.

SS Agar Plate
(Salmonella–Shigella Agar)

Salmonella

Salmonella colonies appear colorless with black center (related to the production of H2S)



Shigella

Shigella colonies appear colorless only

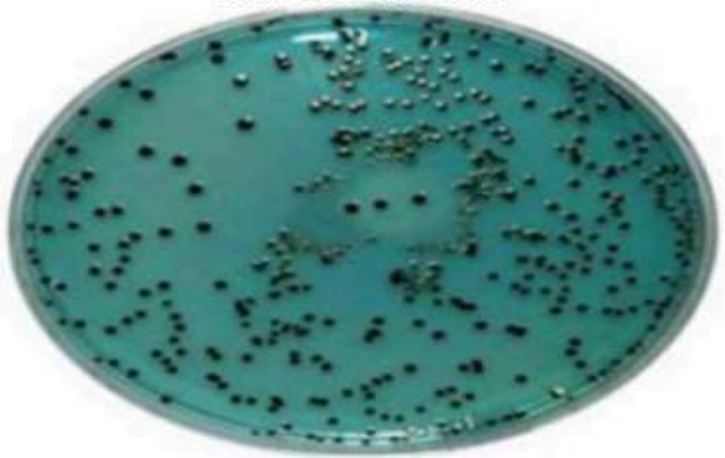
Or yellow but without black dots(no H2S)

Hekton enteric agar

Highly selective and differential for salmonella and shigella. It contains an indicator to detect H2S production.

Salmonella

Green colonies with black center



Shigella

Green colonies only

RECALL from sketchy:
-you can skip this slide☺

Salmonella - the salmon dinner
1. Gram Negative - Non lactose Fermenter, white on
MacConkey's
2. Tail Flopping around - Motile - Indole Negative due to lack
of tryptophanase
3. Plate is black - H2S positive - All motile enteric colonies
stain black on hektoen agar

Shigella: She Gorilla's Circus
-Green Tutu - Green colonies on hektoin agar INDOLE POSITIVE, differentiate salmonella from Shigella: Salmonella will grow black
- Chained to the weight- Immotile, non-lactose, non-H2S

T.C.B.S media

Thiosulfate-citrate-bile salts-sucrose media .

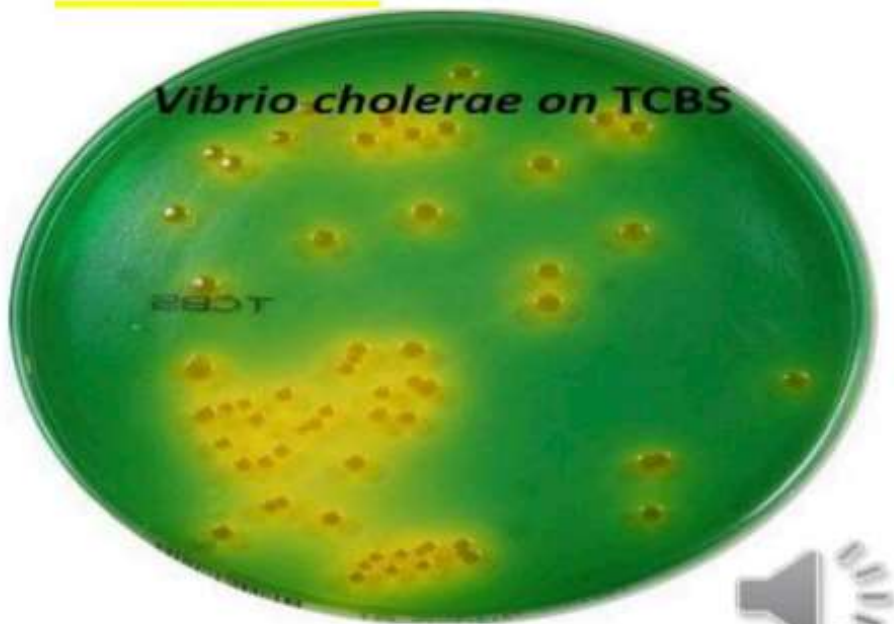
TCBS agar is highly selective for the isolation of V. cholerae and V. parahaemolyticus as well as other Vibrio species.

But not other bacteria

- Selective for Vibrio Spp. (Since its Ph is alkaline (8.5-10))
- Ph (8.5-10)

And differential due to the presence of sucrose and dyes such as Bromthymol blue .
For example sucrose fermentation produces acid which convert the color of Bromthymol blue into yellow colonies in the case of vibrio cholerae .

- **When Vibrio ferment sucrose it turns the media from green to Yellow**



Salmonella

Additional biochemical tests to be 100% sure about the type of the bacteria (for salmonella) :

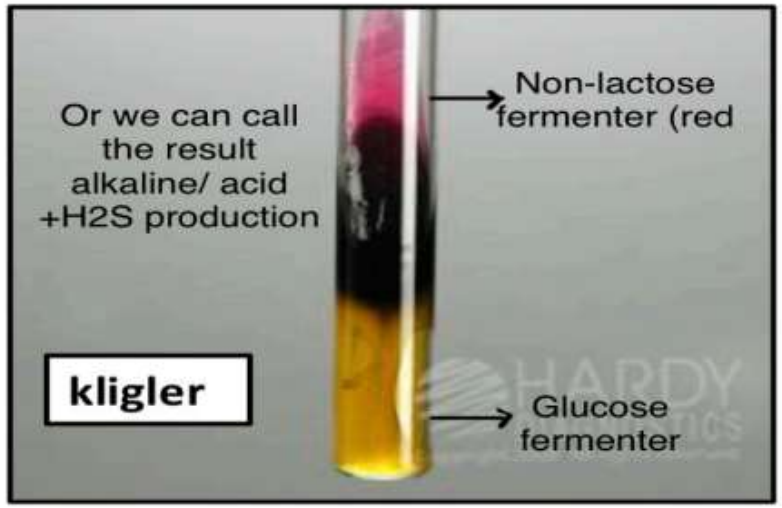
- **Kligler : red/Yellow + H2S**
- **Urease : Negative**
- **Citrate : Positive**

It can produce H2S

- **SIM : Positive / Negative / Positive**

Motile

stands for: Sugar ,Indole and Motility



Yellow -> yellow

Urease test

Green-> blue

citrate test

SIM test

We have to do other tests to ensure that it is salmonella:

Figure 7: Salmonella on TSI agar
A: S. Typhimurium
B: S. Enteritidis
C: Control

- **At the beginning, salmonella is +ve glucose and sucrose fermenter, negative lactose fermenter, oxidase –ve, citrate +ve, indole –ve,H2S producer and motile.**
- -the first test is the KLIGLAR TEST:

It defines 3 features:1- lactose fermentation in the upper part since it is exposed to O2 after a small rotation of the tube

يعني بنميله شوي ليتعرض للاكسجين ليصير مسكوب بالميل

If +ve red becomes yellow

2-H2S production:if +ve black area appears

3-glucose fermentation in the deep part, since it doesn't need O2, if +ve red becomes yellow

-so in the case of salmonella:3 colors appear: red, black, yellow

non-lactose fermenter, H2S producer, glucose fermenter

2nd test is urease test: indicates the ability of hydrolyzing urea to CO2 and ammonia, it is yellow agar

If +ve: yellow becomes red

But salmonella is –ve so yellow remains yellow

3rd test: citrate test:ability to detect the ability of an organism which can utilize citrate as a sole source of carbon for their metabolism with resulting alkalinity. The citrate enzyme hydrolyses the citrate to form oxaloacetic acid and acetic acid

If **+ve green becomes blue, which is salmonella**

4th test: SIM: it tests three features:

-S: production of H2S

-I: indole, the ability to convert tryptophan into indole when we add Kovac's reagent, salmonella can not, it is indole -ve

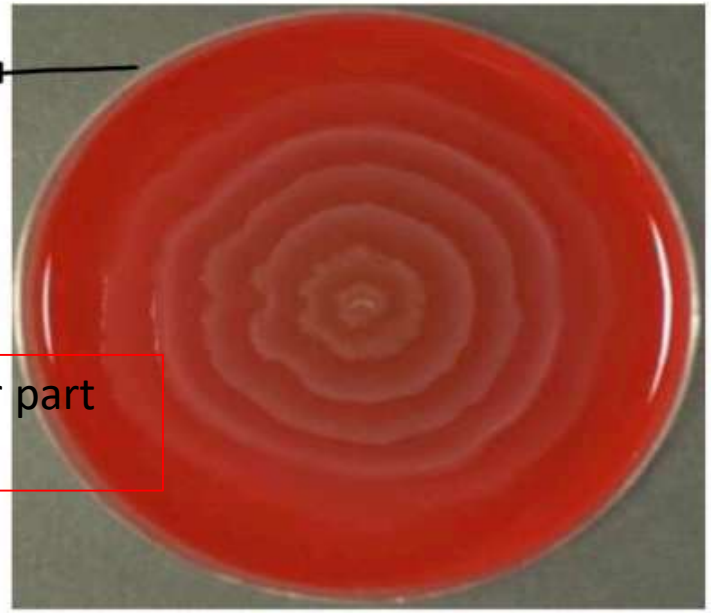
M:motility: we can test it by the line shapes زي شكل الجذور in the black area produced by H2S or by the turbidity of the medium, however in most cases, H2S producer bacteria are motile ones.

(**salmonella is motile, H2S producer, indole –ve**)

Fishy smell

Proteus

Blood agar media (enriched media)
Proteus grow on the Blood Agar plate in successive waves to form a thin filmy layer of concentric circles (swarming) due to the presence of flagella and high motility.



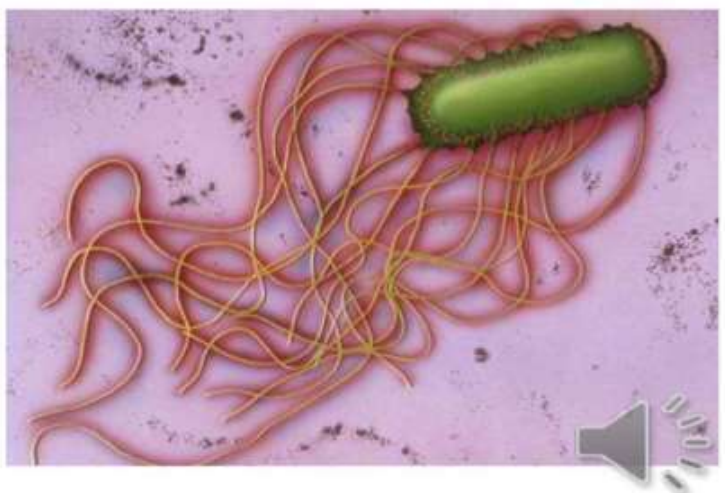
- Gram negative rods , non lactose fermenter

So on kligler agar, the upper part remains red

- Swarming motility (flagellated)

It limits the ability to see the growth of other bacteria in the culture

- Prevent swarming by culturing it on CLED or MacConkey media



Parasites that are pathogenic to GI system

You have to memorize the shape for each one

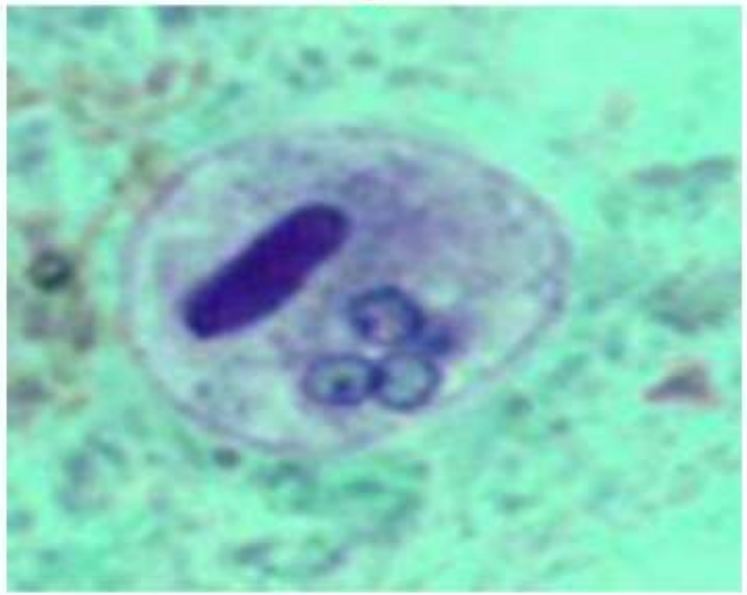
Entamoeba histolytica

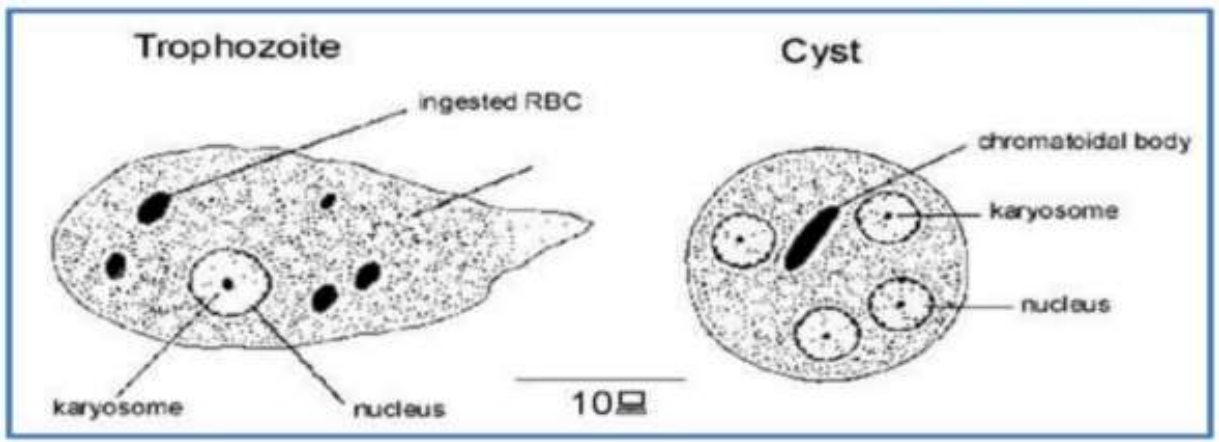
Trophozoite

- trophozoites
  - 15-20 μm
  - extended pseudopodia
  - progressive movement

Cyst indicates past infection, while trophozoites indicates recent infection
-if the sample is frozen, you will see cysts

Cyst



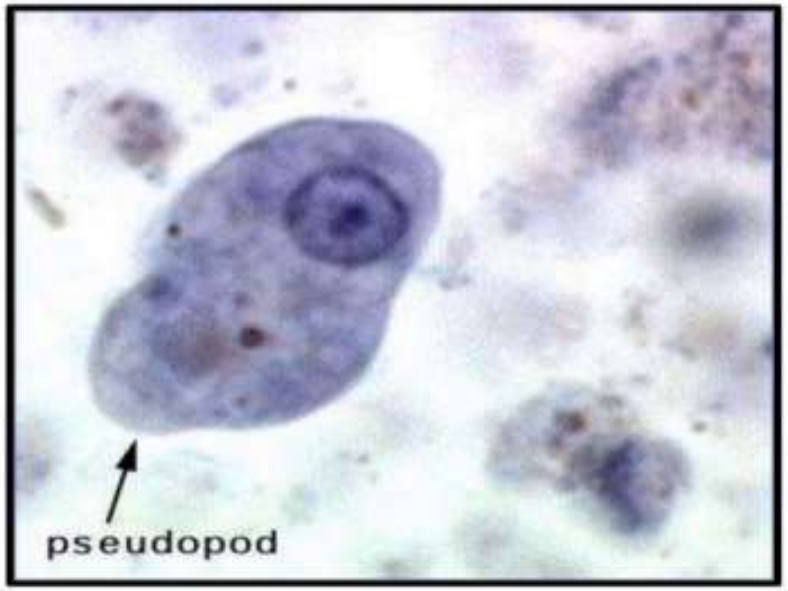


- cysts
  - 12-15 μm
  - 4 nuclei (mature)
  - blunt chromatoid bodies

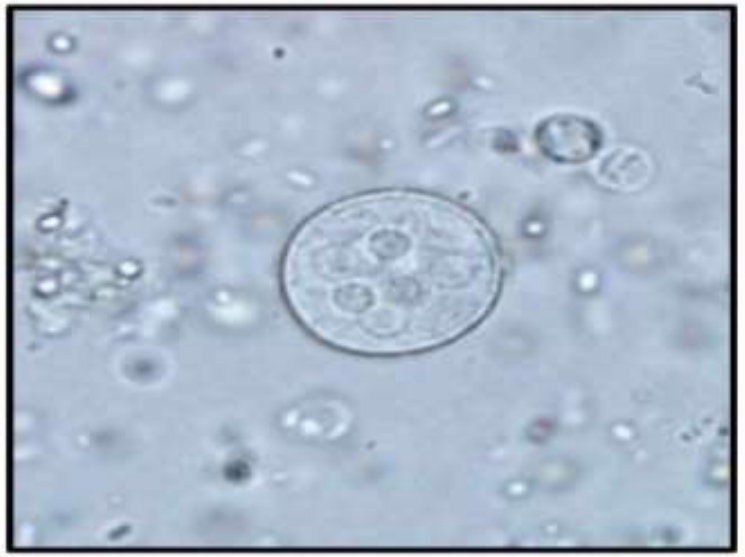
Entamoeba Coli

Trophozoite

- trophozoites
  - 20-25 μm
  - broad blunt pseudopodia



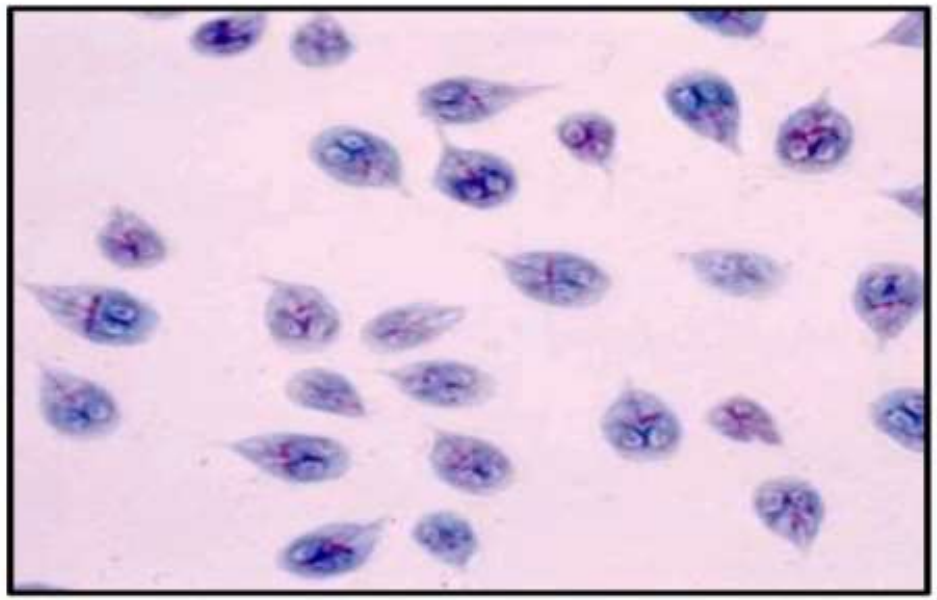
Cyst



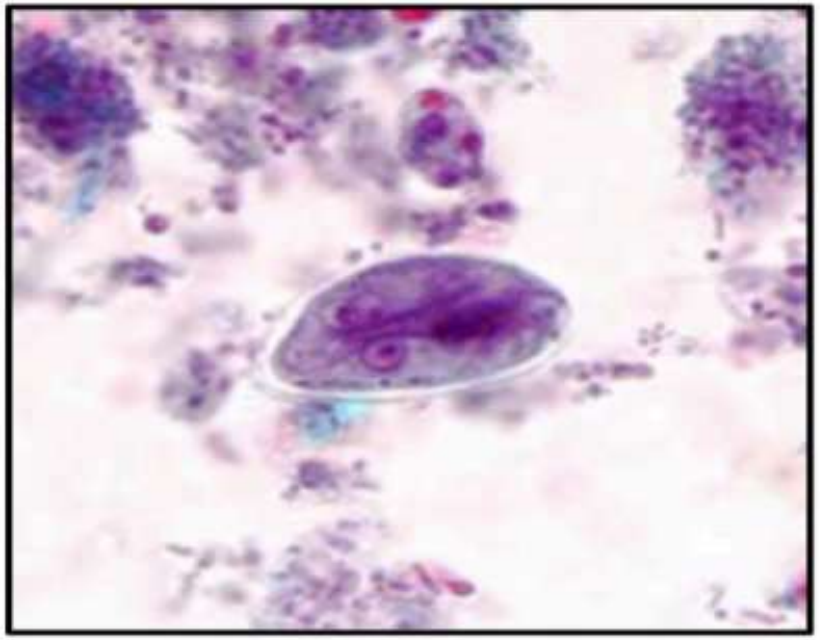
- cysts
  - 15-25 μm
  - 8 nuclei (mature)
  - pointed chromatoid bodies (less prominent

Giardia lamblia

Trophozoite



Cyst



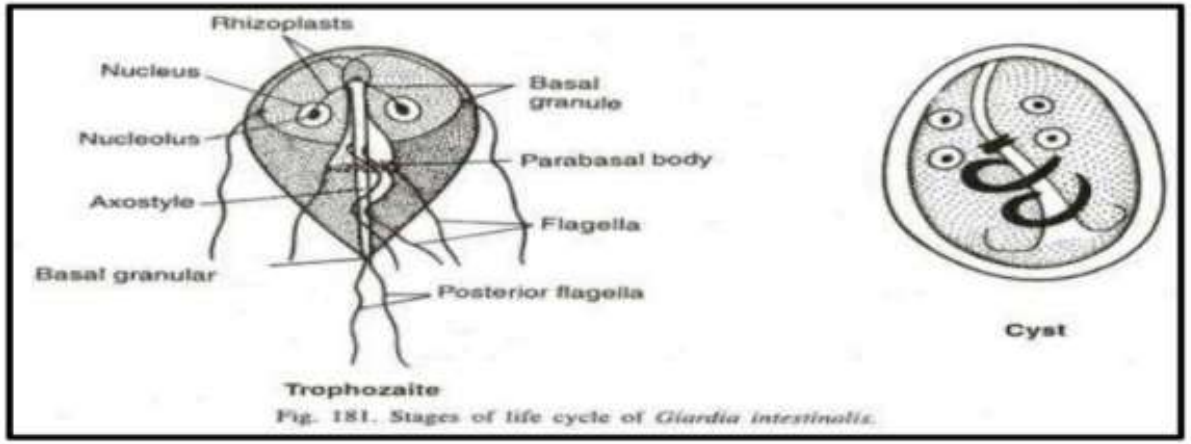
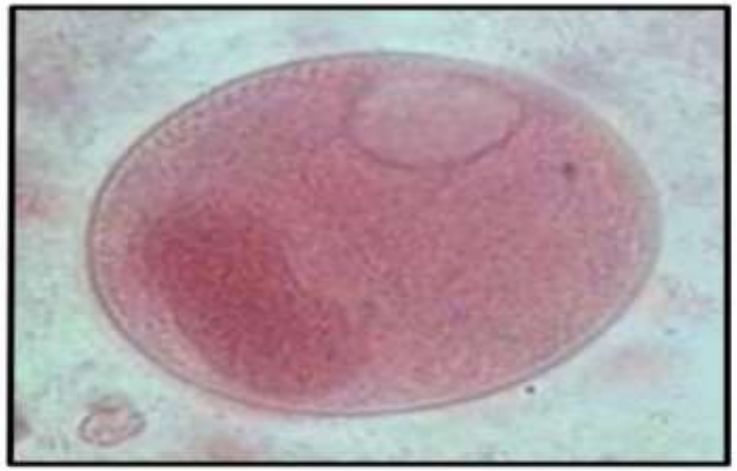


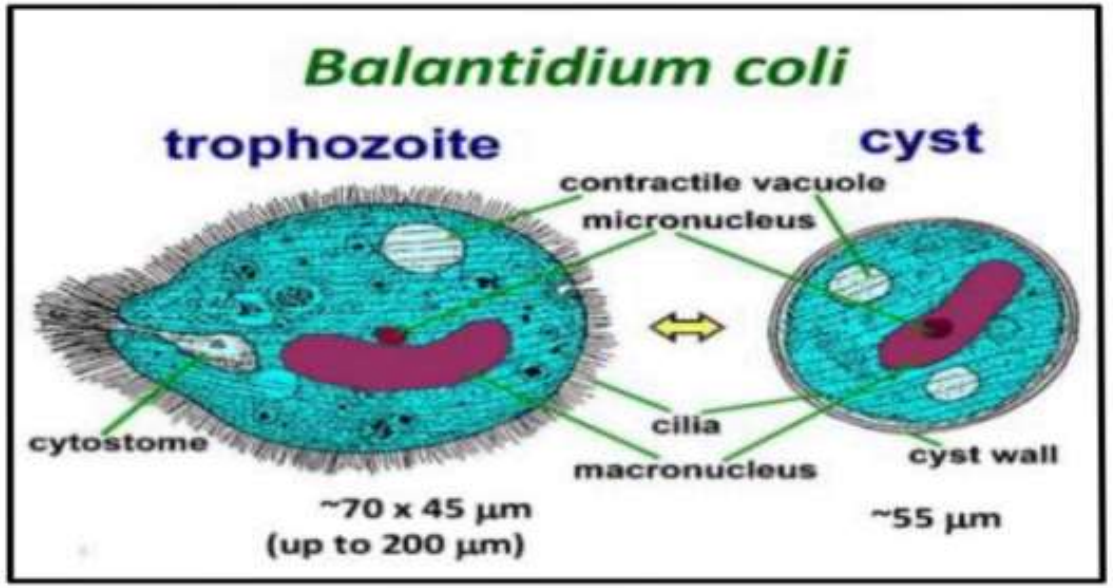
Fig. 181. Stages of life cycle of *Giardia intestinalis*.

Balantidium coli

Trophozoite

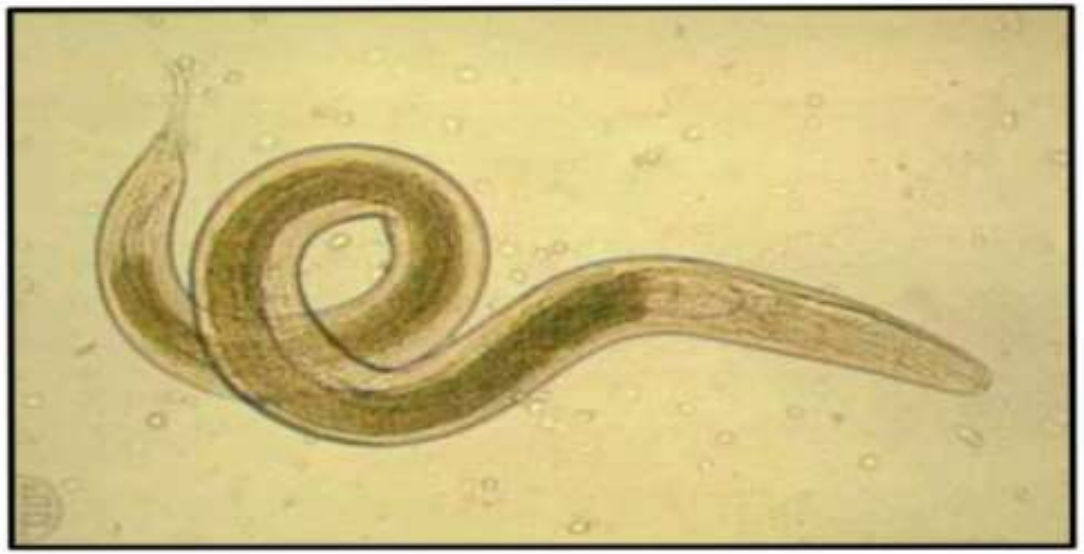
Cyst



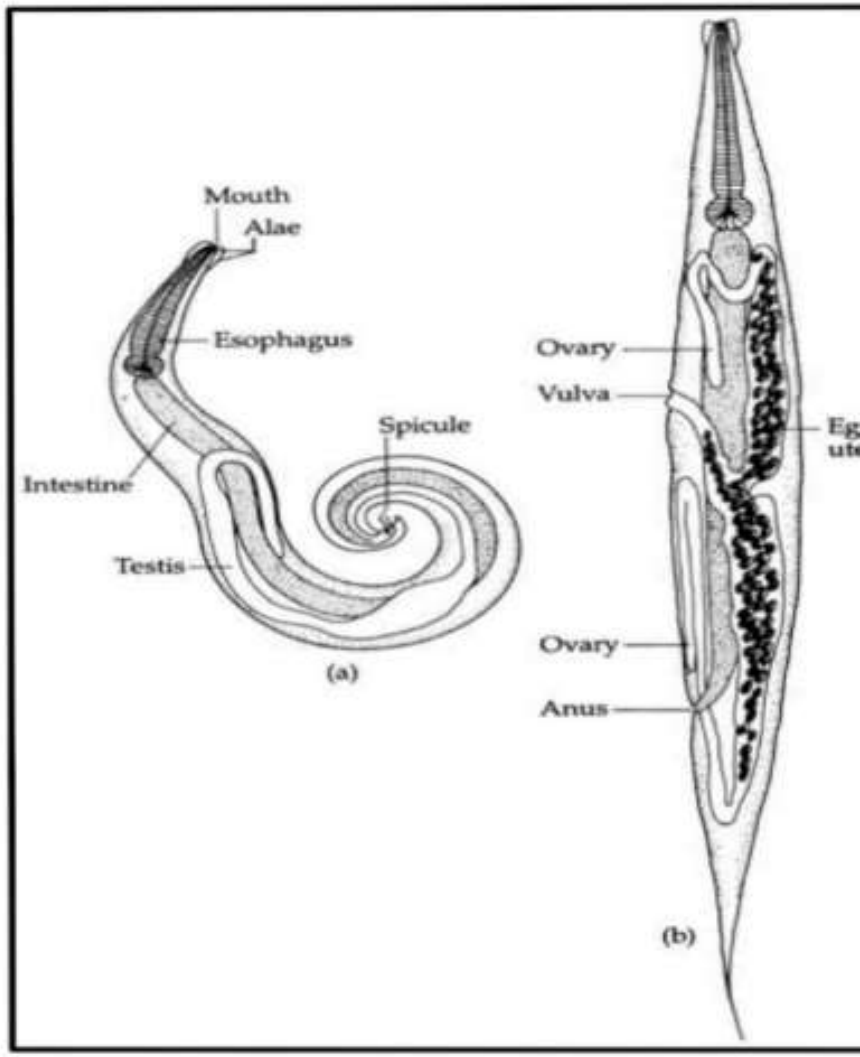


Enterobius Vermicularis

Worm

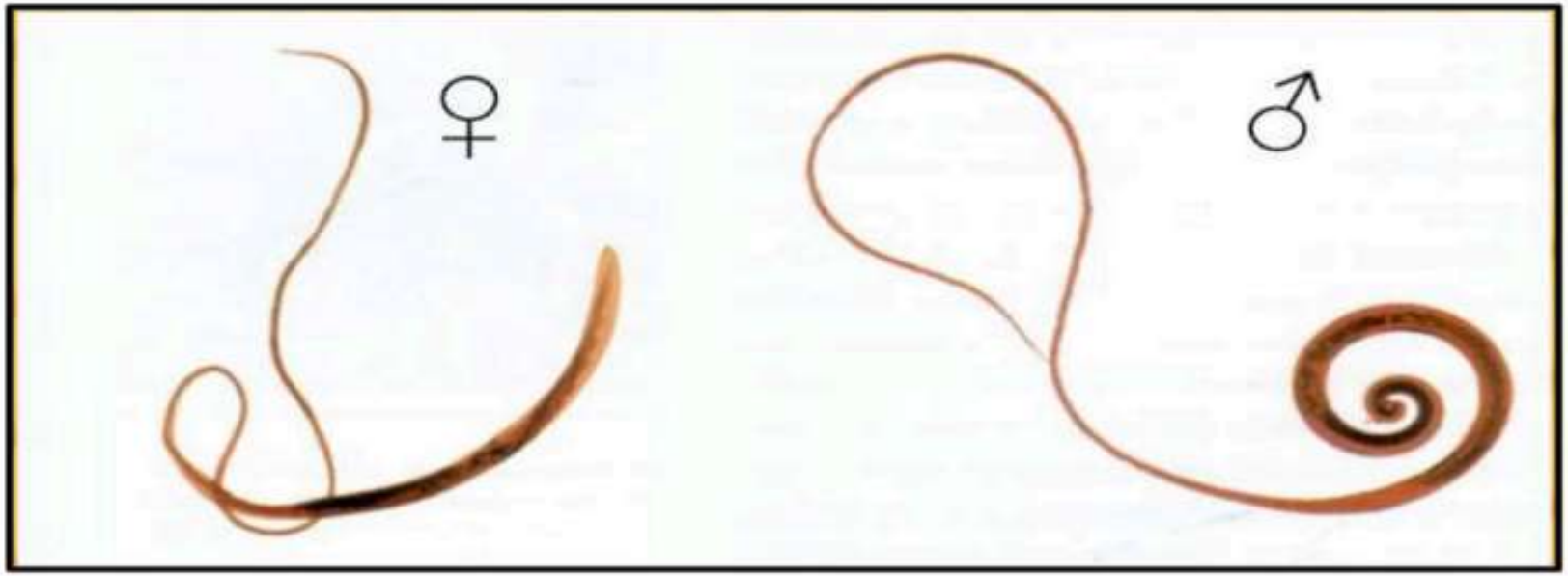


Egg

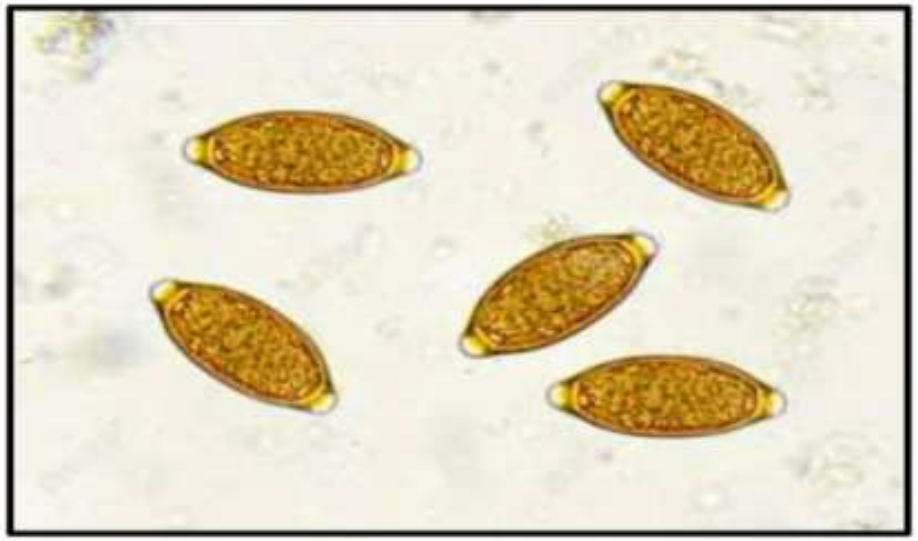


Trichuris Trichiura

Worm

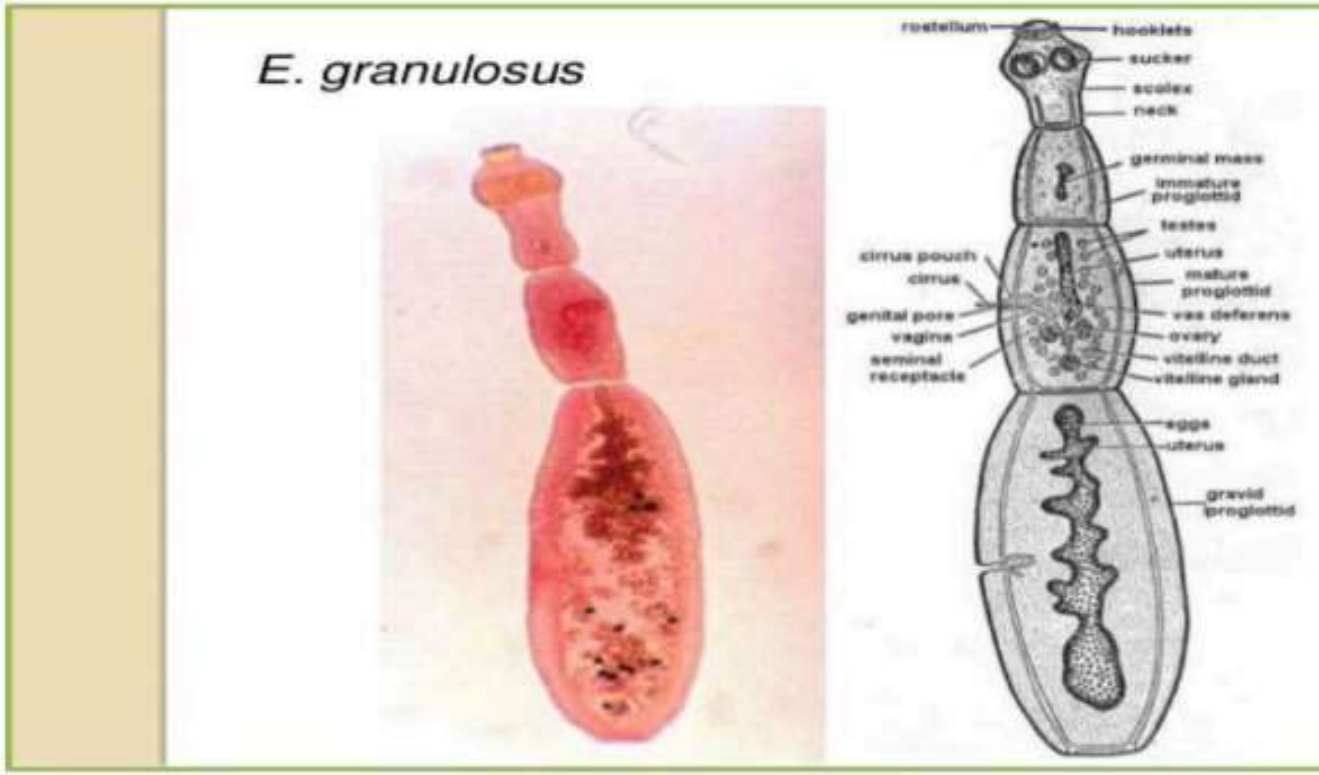


Egg



Echinococcus granuloses

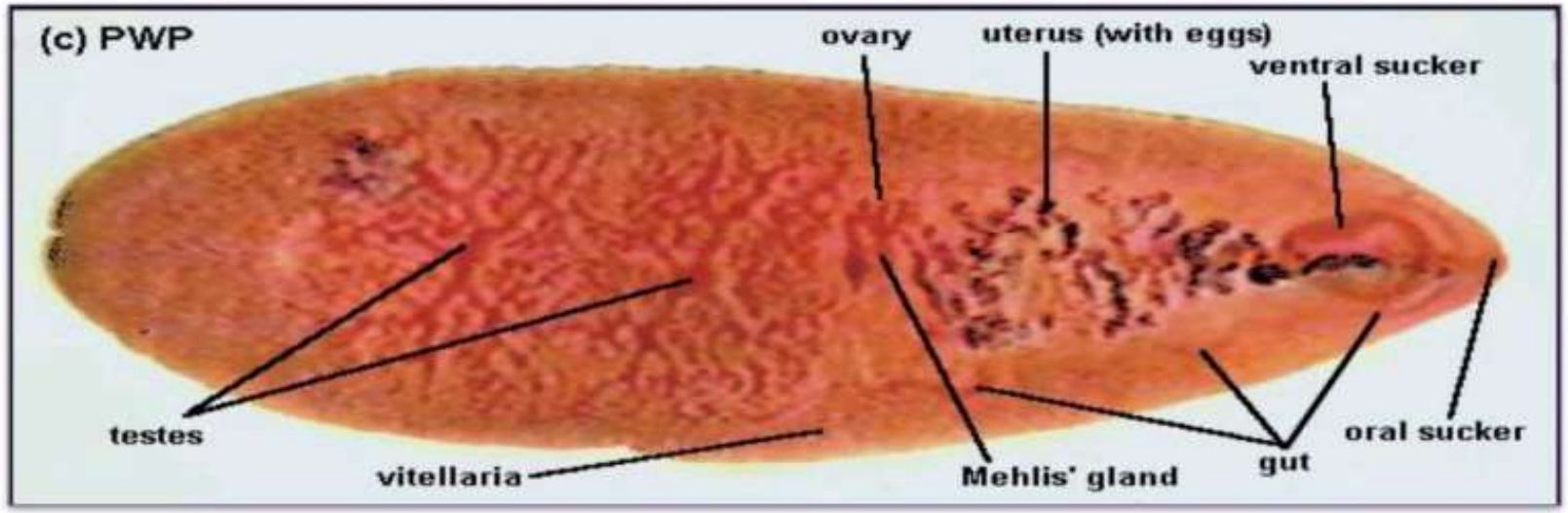
Ova



Worm

fasciolosis buski

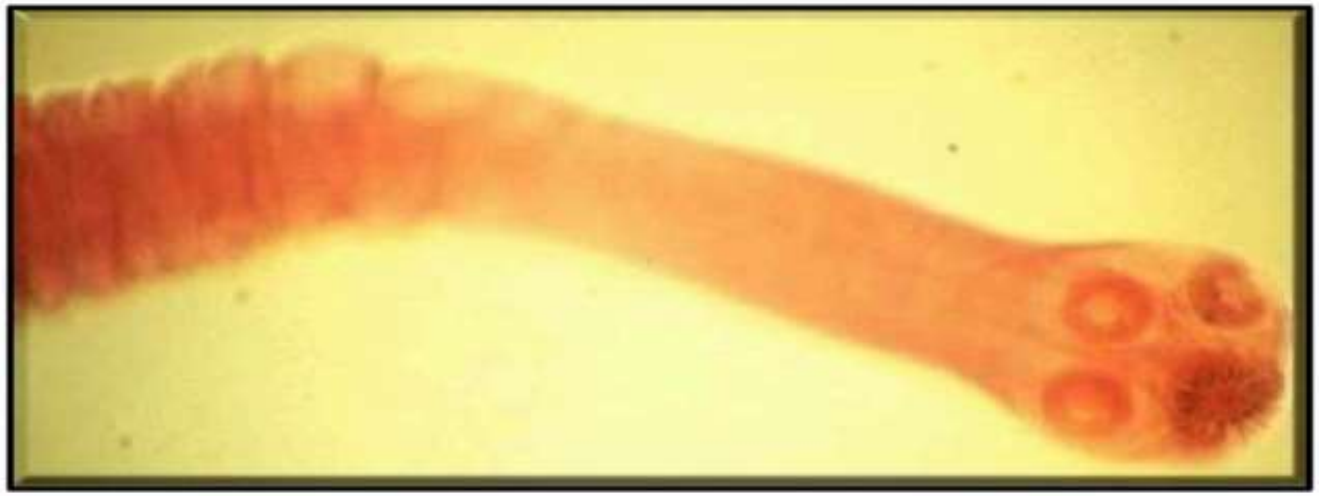
Worm

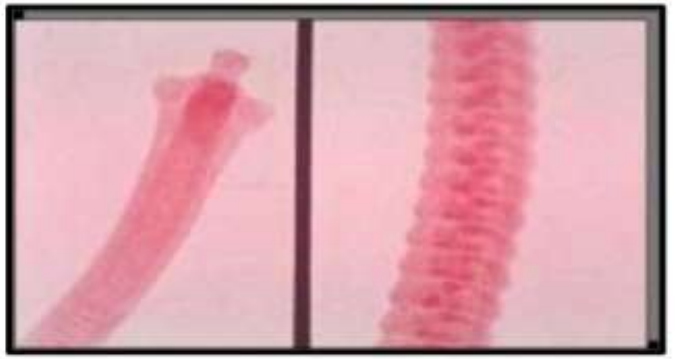


egg

Hymenolepis Nana

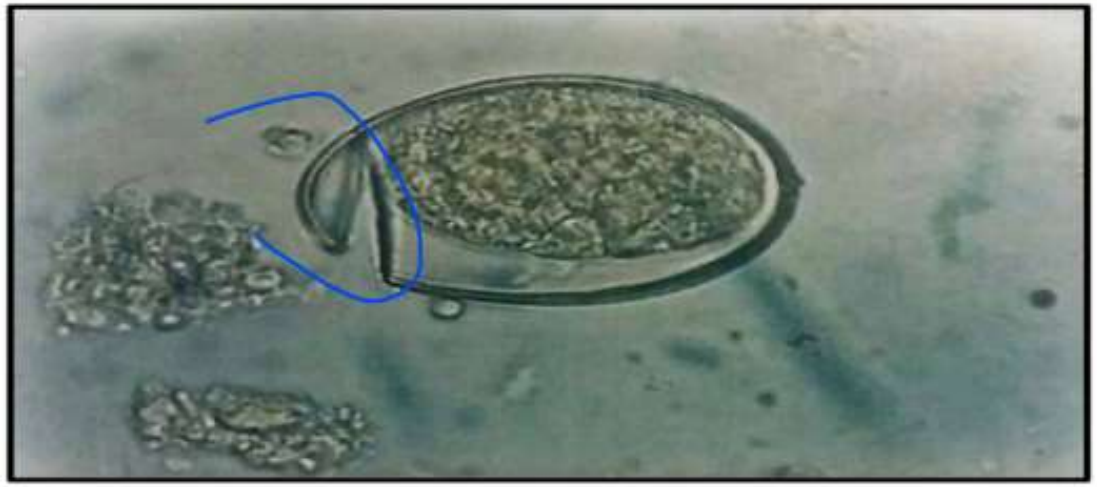
Ova



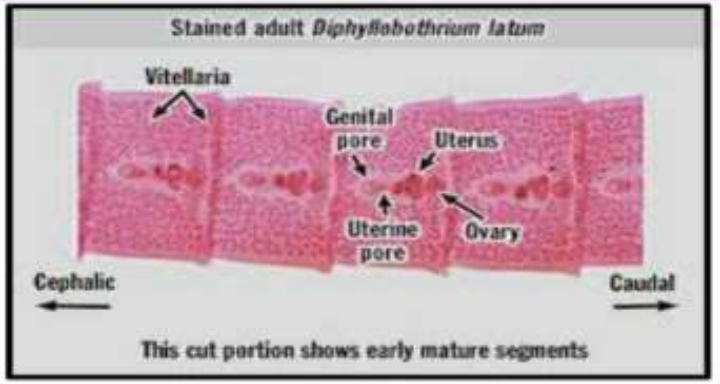


Worm

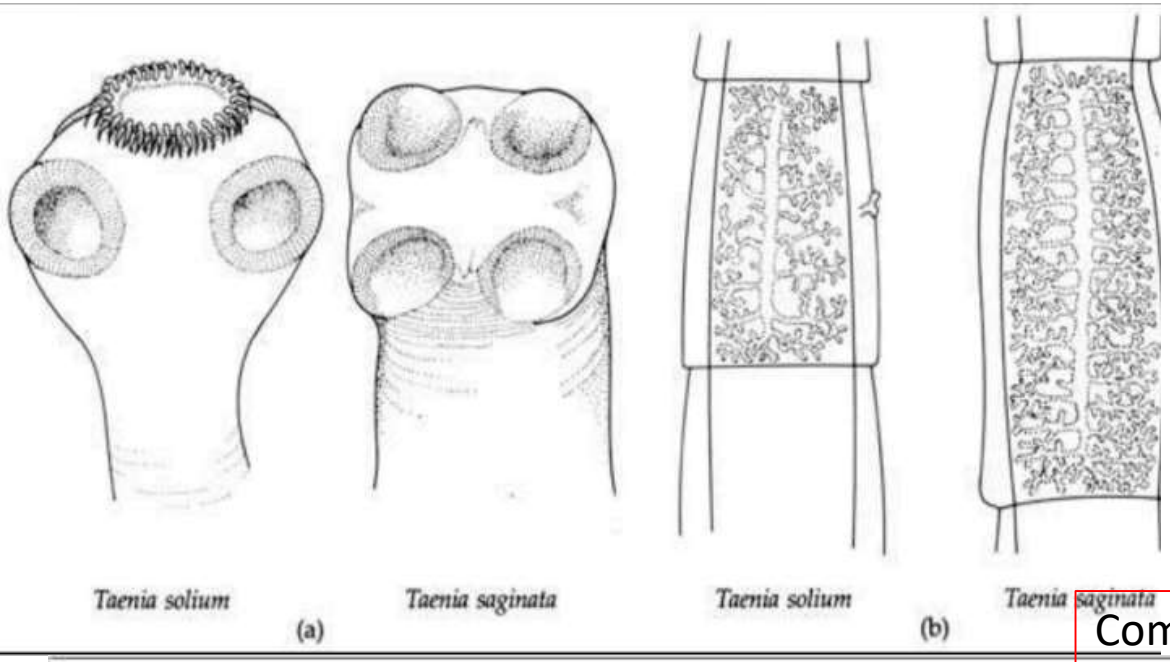
Diphyllobothrium latum



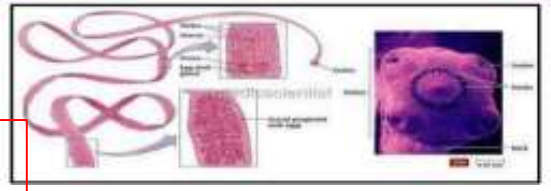
Egg



Worm



Taenia saginata

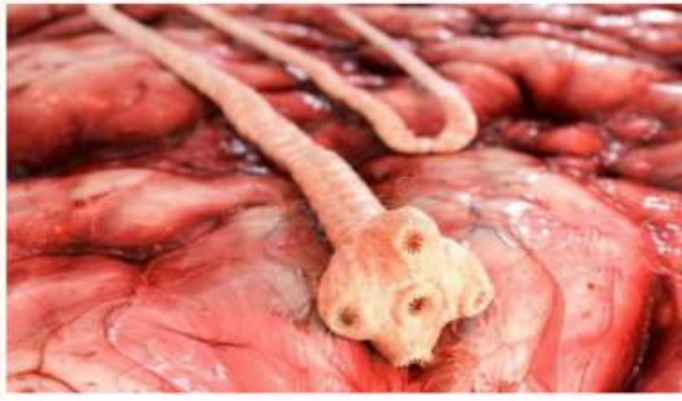


Common in the far east, China, and Filipina, bcz of eating pork

Taenia solium

ɛnia Solium

Taenia saginata



52) **Patient presents with a history of watery diarrhea of several weeks' duration and the microscopic examination of stool sample showing as the picture. Which of the following is the cause?**

A) Giardia lamblia.
B) Clostridium difficile.
C) Entamoeba histolytica.
D) Yersinia enterocolitica.
E) Enterotoxigenic Escherichia coli.

A

30. T.C.B.S media is selective for

Answer: Vibrio Cholera

Regarding Salmonella, it is:
Answer: H2S positive, Urease negative, Citrate positive, Indol negative.

BEST WISHES