

Microbiological Stool Examination: Overview

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INTRODUCTION

Stool (faeces) is an important body substance which has to be checked for the presence of disease-causing microorganisms [1-4].

Definitions

Diarrhoea: Is defined as an increase in the frequency, fluidity or the volume of the bowel movement, which is relative to the usual habit of an individual. The passage of three or more motions a day is considered as diarrhoea.

Dysentery: It is defined as the passage of blood and mucus stained stools, which is often associated with abdominal cramps and tenesmus.

Gastroenteritis: Is defined as an inflammation of the mucus membranes of the stomach and the intestines, resulting in diarrhoea which is associated with vomiting.

Examination of the stool for bacteria

Bacterial agents which are responsible for diarrhoea [1]

1. Gram positive
 - *Staphylococcus aureus*
 - *Clostridium perfringens*
 - *Clostridium difficile*
 - *Bacillus cereus*
2. Gram negative
 - *Vibrios*
 - (i) *Vibrio cholera*
 - (ii) *Vibrio parahaemolyticus*
 - (iii) Other halophilic vibrios
 - *Escherichia coli*(ETEC, EPEC)
 - *Salmonella*
 - (i) *S. enteritidis*
 - (ii) *S. typhimurium*
 - *Shigella spp*
 - *Campylobacter jejuni*
 - *Yersinia enterocolitica*

Bacterial agents which are responsible for dysentery [1]

1. *Shigella spp* (*sh. dysenteriae, sh. flexneri, sh. boydii and sh. sonnei*)
2. *Escherichia coli* (EIEC and EHEC)
3. *V. parahaemolyticus*
4. *Campylobacter jejuni*
5. *Salmonella spp*

Notes on pathogens

1. *Staphylococcus aureus*.

S. aureus produces six types of enterotoxins (A to F). The type

A is most often incriminated in outbreaks of staphylococcal food poisoning. Acute staphylococcal food poisoning is caused by the ingestion of preformed toxin contaminated food (often dairy products). The incubation period is 2-6 hours and it is associated with an acute onset of nausea and vomiting, sometimes followed by diarrhoea [1,2].

2. *Bacillus cereus*

It is an aerobic, spore forming, gram positive bacillus. It produces two types of toxins, one which resembles the LT of *E. coli* and the other which resembles staphylococcal enterotoxin. It causes two distinct clinical manifestations, one which is characterized by diarrhoea and abdominal pain (incubation period of 6-15 hours), which is similar to gastroenteritis that is caused by *E. coli* and the other which is characterized by nausea and vomiting, which resembles Staphylococcal food poisoning (short incubation period of 1-4 hours). Food poisoning is caused by the ingestion of preformed toxin which is usually present in fried rice or other cereals which have been cooked and stored at warm temperatures [1].

3. *Clostridium perfringens*

This organism is widely distributed in soil and it is found in the faeces of humans and animals. Gastroenteritis which is caused by *C. perfringens* is characterized by diarrhoea and abdominal cramps, following the ingestion of contaminated food (poultry products and meat). Most of cases are caused by the type A strains which produce an enterotoxin. (incubation period of 6-12 hours) [3,4].

4. *Clostridium difficile*

It is associated with antibiotic associated diarrhoea and pseudo-membrane colitis. The common antibiotics which are involved are lincomycin, clindamycin, ampicillin and the cephalosporins. There is an overgrowth of antibiotic-resistant *C. difficile* due to the suppression of the normal cut flora [1].

5. *Vibrio cholera*

The classical as well as the *EITor* biotypes of *V. cholerae* cause cholera. The severe dehydration, vomiting, abdominal pain and acidosis which are associated with cholera are due to the action of the exotoxin, CT(cholera toxin), which activates cAMP, leading to the outpouring of fluid and electrolyte-diarrhoea. In severe cases, the typical rice water stools (without faecal matter) are passed, necessitating urgent fluid replacement therapy to prevent collapse and death. *V. Parahaemolyticus* causes invasive diarrhoea and it is responsible for a majority of the food poisoning cases in many parts of the world, including Asia, Africa, Europe and America. The foods which are responsible include raw seafood (fish and shell fish). The mechanism of the diarrhoea which is produced by it is not well understood. It produces a heat labile enterotoxin like the

one which *E.coli* produces [5,6].

6. *E.coli*

The strains of *E.coli* which are recognized to cause diarrhoeal diseases include enterotoxigenic *E.coli* (ETEC) and enteropathogenic *E.coli* (EPEC). ETEC produces a heat-labile enterotoxin (LT) and a heat stable enterotoxin (ST), resulting in mild to severe diarrhoea in the developing countries. It is an important cause of traveller's diarrhoea. EPEC have been implicated in diarrhoea in infants, that neither produce toxins nor do they invade the gut mucosa. The exact mechanism of their action is not known. They adhere to the mucosal cells of the small intestine and multiply [5,6].

7. *Shigella species*

S.dysenteriae, *S.flexneri*, *S.bodyii* and *S.sonnei* cause bacillary dysentery. The dysentery which is caused by the *Shigella* species is referred to as shigellosis. The *Shigella spp* causes at least 50% of the cases of bloody diarrhoea in young children and adults in the developing countries. *S.dysenteriae* type 1 is particularly virulent, causing epidemic and endemic dysentery. It is highly infectious and resistant to the commonly available antibiotics [2].

8. *Salmonellae*

Acute gastroenteritis which is caused by the *Salmonella spp* is characterized by self limiting fever and diarrhoea. The incubation period is 12-36 hours. A large majority of the outbreaks are caused by, *S.typhimurium* and *S. enteritidis*. The human infection is usually caused by the consumption of animal foods or food products. The infection leads to salmonella food poisoning and sometimes septicaemia may develop [2].

9. *Campylobacter jejuni*

C.jejuni is the common cause of enteritis in the developing countries. It occurs in the intestinal flora of many animals, especially poultry, which probably serves as the major source of the infections in humans. The milk and waterborne outbreaks of diarrhoea have also been documented. The disease is sometimes associated with vomiting and bloody mucoid stools [1,2].

10. *Yersinia enterocolitica*.

Y. enterocolitica has been identified as an important cause of diarrhoea. The sources of the infection are birds and animals. Foodborne outbreaks have also been reported. The predominant symptom in young children is acute watery diarrhoea. In older children and young adults, there is pain in the abdomen and fever.

Examination of the stool for parasites [7]

1. Protozoa

- *Entamoeba histolytica*
- *Giardia lamblia*
- Intestinal Coccidian Parasites
 - (i) *Cryptosporidium parvum*
 - (ii) *Cyclospora*
 - (iii) *Isospora*
- *Balantidium Coli*

2. Helminthes

- Nematodes:
 - (i) *Ascaris lumbricoides*
 - (ii) *Trichuris trichuria*

(iii) *Hookworm*

- *Ancylostoma duodenale*
- *Nectar americanus*

(iv) *Strongyloides stercoralis*

• Cestodes:

(i) *Taenia spp*

- *T. saginata*
- *T.solium*

(ii) *Hymenolepsis nana*

(iii) *Enterobius vermicularis*

Notes on pathogens

1. *Entamoeba histolytica*

It is endemic in many parts of the tropical and sub-tropical areas. It is transmitted by the faeco-oral route. The cysts which contain four nuclei are indicative of an infective stage in humans. It produces amoebic dysentery which is characterized by large, flask shaped ulcers. It may get complicated into amoebic liver abscess and amoebic lung abscess [7].

2. *Giardia lamblia*

The infection with *Giardia lamblia* may lead to explosive watery diarrhoea, foul smelling stools and steatorrhoea. In endemic areas, young children are more frequently infected than the adults, particularly those who are malnourished. It produces a severe form of infection in immuno-compromised individuals, particularly in those with AIDS [7].

3. *Coccidian parasites*

Cryptosporidium parvum, *Isospora. Belli* and *Cyclospora* have been recognized as important agents which are responsible for the watery diarrhoea in immunocompromised patients, particularly in those with HIV-AIDS [7].

4. *Balantidium coli*

It is an uncommon parasite which is found in humans. It commonly infects pigs and it has a world-wide distribution. It produces dysentery which is transmitted by the ingestion of infective cysts in food and water or from hands which are contaminated with pig faeces [7].

5. *Ascaris lumbricoides*

It has a world-wide distribution and its spread occurs by the faecal pollution of the environment. A person becomes infected by ingesting its infective eggs through contaminated food or by eating with contaminated hands [7].

6. *Trichuris trichiura*

It is common in the moist, warm climates. Its infection is spread by ingesting its infective eggs through contaminated food or by eating with contaminated fingers. In children, it can cause chronic diarrhea and intestinal ulceration with blood and mucus [7].

7. *Hookworm*

Its infection is caused by *A.duodenale* and *Nectar americanus*, with *Ancylostoma* as the predominant species. The infection is spread by the faecal pollution of the soil. The infection occurs when the infective filariform larvae penetrate the skin. Hookworm resides in the intestine and sucks blood, leading to iron deficiency anaemia and chronic blood loss [7].

8. *Strongyloides stercoralis*

It is endemic in many tropical and sub-tropical countries. The infection occurs by the penetration of skin by the infective filariform larvae. In immuno-competent hosts, it is generally asymptomatic or it produces minimal symptoms. But in immuno-compromised hosts, it produces a potentially life threatening infection called the hyperinfection syndrome [7].

9. *Taenia Spp.*

Two species, namely *T.saginata* and *T.solium*, are responsible for the infections in humans. The infection is mainly transmitted by eating raw or insufficiently cooked beef and pork meat respectively. *T.solium* is not as widely distributed as *T.saginata*, but it can produce a serious infection called neurocysticercosis, which causes epilepsy and other central nervous disorders [7].

10. *Hymenolepis nana*

It is transmitted by ingesting its eggs from food or water or from hands which are contaminated with infected faeces. The eggs are infective when they are passed in the faeces. An internal auto-infection is a common problem which is caused by *H.nana* [7].

11. *Enterobius vermicularis*

It has worldwide distribution, with children being more commonly infected than adults. Its transmission is by the ingestion of infective eggs. The eggs are deposited on the anal skin usually during the night hours. An autoinfection is common in children because the eggs cause intense irritation and scratching in the infected anal area [7].

Examination of the stool for viruses

1. *Rota virus*
2. *Norwalk virus*
3. *Adenovirus*
4. *Astrovirus*
5. *Calicivirus*
6. *Coronavirus*

Rotavirus

This virus is now considered as the most common cause of diarrhoea in infants and young children. The disease peaks at the ages of six months to two years. The mode of the infection is believed to be the faeco-oral route. The incubation period is 2-4 days. Vomiting is a predominant early symptom, which often precedes diarrhoea. The stools are watery and they are associated either mild fever or respiratory symptoms [1,4,6].

Other viruses have also been frequently incriminated in producing diarrhoea and vomiting in infants and young children [1,4,6].

Laboratory examination of faeces

Collection of the stool specimen

The faeces for the microbiological examination should be collected during the acute stage of the diarrhoeal disease [1,4,6,7,8].

- Ask the patient to pass the stool sample in a clean, dry, disinfectant free, suitable, wide-necked container or a plastic cup with a tight fitting lid.
- About 20-40 grams of well-formed stool or 5-6 table spoonfuls of watery stool will suffice for a routine examination.
- The ingestion of some medicines prior to the collection of the faecal sample may interfere with the detection of the

micro-organisms. These include tetracyclines, sulfonamides, antiprotozoalagents, laxatives, antacids, castor oil, magnesium hydroxide, barium sulphate, bismuth kaolin compounds, hypertonic salts, etc. These should not be taken 1-2 weeks before the examination of the stool sample.

- All the specimens must be properly labelled with the patient's name, age, sex, and the date of the sample collection.

Note

- Do not keep the specimen at warm temperatures. Try to keep it in cool places.
- Prevent the drying of the specimen.
- Prevent its contamination with urine or dirt particles.
- Multiple stool examinations are required before the presence of an infection is ruled out.
- The stool should not be collected from bed-pans which contain disinfectants.

Rectal swabs

Only when it is not possible to obtain faeces, should a specimen be collected by using a cotton wool swab. The swab should be inserted in the rectum for about 10 seconds. Care should be taken to avoid unnecessary contamination of the specimen with bacteria from the anal skin [7,8].

The adhesive tape method

This is useful for the detection of the eggs of *E.vermicularis*. The eggs can be collected by wrapping a strip of clear adhesive tape (e.g. cello tape, scotch tape) around the anus. After collecting the eggs, the tape should be stuck lengthways, face down on a microscope slide. Alternatively, an anal or perianal specimen can be collected by using a National Institute of Health (NIH) swab [7,8].

Transport of the specimen

- The specimen must reach the laboratory within 30 minutes of passing of the stool, since the motile organisms, for example, *Vibrio* and amoebic trophozoites are heat sensitive and they can die or become unrecognizable after that period.
- Transport media such as the Cary-Blair medium can be used for *Salmonella*, *Shigella* and *Yersinia*.
- When cholera is suspected, about 1 ml of specimen should be transferred into 10 ml of alkaline peptone water, which will act as an enrichment as well as transport medium.
- When worms or tapeworm segments are present, these should be transferred to a container of physiological saline and sent to a laboratory for identification [1,4,6].

Macroscopic examination

Various points which have to be noted are:

- **Consistency:** formed, unformed (soft), loose or watery. The cysts have been mostly found in the formed stools, while trophozoites have been most abundantly found in watery stools.
- The presence of blood, mucus or pus.
- The presence of worms, e.g. *Enterobius Vermicularis*, *Ascaris*, tapeworm segments, e.g. *Taenia* species.
- Colour (white, yellow, brown or black).
- Normal faeces appear brown and formed or semiformal. Infant faeces are yellow-green and semiformal [1,5,8,9].

Microscopic examination

Methylene blue preparation

Place a small fleck of the stool specimen or the rectal swab together with a small flake of mucus in a drop of 0.05% methylene blue solution on a clean glass slide and examine it for cellular exudates as follows [1,2,4]:

- clumps of pus cells of > 50 cells per high power field along with macrophages and erythrocytes are typical of shigellosis.
- A smaller number of pus cells of <20 per high power field are found in salmonellosis and in infections which are caused by invasive *E.coli*.
- Few leucocytes (< 5 cells per high power field) are present in cholera, EPEC and ETEC and viral diarrhoea.

Wet mount

The simplest way of examining a bacterial suspension for motile bacteria is by doing a wet mount. Place a small drop of suspension on a slide, cover it with a coverslip and examine microscopically for motile organisms by using the 10X and the 40X objectives. Also make sure that the iris diaphragm of the condenser is sufficiently closed, to give a good contrast [2].

| Type of motility | Organisms |
|----------------------------------|------------------------------|
| Sluggish | <i>E.coli</i> |
| Actively motile | <i>Salmonella</i> spp |
| Dartingly motile | <i>Vibrio cholera</i> |
| Tumbling | <i>Campylobacter</i> spp |
| Falling leaf | <i>Giardia lamblia</i> |
| Actively motile with pseudopodia | <i>Entamoeba histolytica</i> |

[Table/Fig-1]: Types of motility

Hanging drop preparation

Placing a drop of suspension on a cover glass and inverting this over a cavity slide or a normal slide which is supported on a ring of plasticine can also be used for observing motile organisms [1,2].

Basic fuschin smear

Make a thin smear of the specimen on a slide, stain it with Basic fuschin and examine the slide by using a 100X objective. This has been shown to be a sensitive method for the presumptive diagnosis of *Campylobacter* spp. It appears as small, delicate, spiral curved bacteria or s-shaped forms [10,11].

Culturing the specimen

MacConkey's Agar

This is useful, non-selective medium for use for general purposes and an added advantage is the formation of pink coloured colonies

| Bacteria | Motility | Gas formation | Acid formation | Indole test | MR test | VP test | Citrate | Urease | H2S |
|-----------------------|----------|---------------|----------------|-------------|---------|---------|---------|--------|-----|
| <i>Escherichia</i> | + | + | + | + | + | - | - | - | - |
| <i>Salmonella</i> spp | + | - | + | - | + | - | + | - | + |
| <i>Shigella</i> spp | - | - | + | d | + | - | - | - | - |
| <i>Vibrio</i> | + | - | + | + | - | - | - | - | - |

[Table/Fig-2]: Biochemical tests for identification of Bacteria

MR=Methyl red test

VP=voges-proskauer test

d=different spp shows variable reactions

in case of lactose fermenters and colourless colonies in case of nonlactose fermenters. Also, it inhibits most of the gram positive organisms and the swarming growth of *Proteus*, which may pose a problem in mixed cultures. *Salmonella*, *Shigella* and *Vibrio* form colourless colonies on this medium as these are nonlactose fermenters. *E.coli* which are lactose fermenters, form pink coloured colonies [10,11].

Xylose lysine deoxycholate (XLD) agar

This selective medium has been recommended for the isolation of *Salmonella* and particularly *Shigella* from faecal samples [10,11].

Shigella forms pink-red colonies because it does not ferment xylose and lactose.

Salmonella also forms pink coloured colonies with black centres because of hydrogen sulphide production.

Thiosulphate citrate bile salt sucrose (TCBS) agar

This is an excellent, selective medium for the primary isolation of *V.cholerae*. Prior enrichment in alkaline peptone water is recommended unless the specimen contains large number of *Vibrio* bacteria in the acute stage. On TCBS, *Vibrio* produces large yellow coloured colonies because of sucrose fermentation [10,11].

Sorbitol MacConkey's agar

This MacConkey's agar contains sorbitol instead of lactose. *E.coli* 0157 produces colourless colonies on this medium because it does not ferment sorbitol. Most of the other *E.coli* strains and other enterobacteria ferment sorbitol and produce pink colonies. So, this medium is useful for screening 0157 *E.coli* [10,11].

Slide agglutination test

The isolation of *Salmonella* and *Vibrio* can be done by doing the slide agglutination test. A loopful of growth from the culture is emulsified in two drops of saline on a slide. One emulsion acts as a control to show that the strain is not autoagglutinable. In case of *Salmonella*, the 'o' antiserum is added to one drop of bacterial emulsion on the slide. A prompt agglutination denotes the presence of the *Salmonella* group [8,9].

| <i>Salmonella</i> spp | 'o' antiserum | 'H' antiserum |
|-----------------------|---------------|---------------|
| <i>S. typhimurium</i> | 4-B | i |
| <i>S. enteritidis</i> | 9-D | d |

[Table/Fig-3]: Slide agglutination test

Similarly for *V.cholera*, the *cholera* o subgroup I serum is tested. If it is found to be positive, the agglutination may be repeated by using the specific Ogawa and Inaba sera for serotyping.

Stool examination for parasites [7,12]

1. **Saline wet mount:** It is used to detect worms, bile stained eggs, larvae, protozoan trophozoites and cysts. In addition, it can reveal the presence of RBCs and WBCs.
2. **Iodine wet mount:** It is used to stain the glycogen and nuclei of the cysts. A cyst is appreciated better in an iodine preparation, but the motility of the trophozoite is inhibited in the iodine preparation.

Procedure

- Place a drop of saline on the left half of the slide and one drop of iodine on the right half.
- With an applicator stick, pick up a small portion of the specimen (equivalent to the size of a match head) and mix it with a saline drop.
- Similarly, pick up a similar amount and mix with a drop of iodine.
- Put the cover slip separately on both and examine under the microscope.
- The ova, cysts, trophozoites and adult worms can be identified as per their characteristic features.

Concentration techniques [7,8,13,14]

If the number of parasites in the stool specimens is low, the examination of a direct wet mount may not reveal them and hence the stool should be concentrated. Eggs, cysts and larvae can be recovered after the concentration procedure, whereas trophozoites can get destroyed during this procedure. This makes a direct wet mount examination obligatory as the initial phase of the microscopic examination.

The concentration procedures can be grouped under 2 categories:

1. **Sedimentation procedures:** In which the eggs and cysts settle down at the bottom.
2. **Flotation procedures:** In which the eggs and cysts float at the surface due to the specific gravity gradient.

The basic disadvantage of the sedimentation technique is that the examination of the sediment is often difficult due to the presence of excessive faecal debris that may mask the presence of the parasites. The basic disadvantage of the flotation technique is that not all eggs and cysts float in the flotation procedures.

Two commonly used concentration techniques are the formalin-ether and the saturated salt solution techniques.

The formal ether sedimentation technique [7,8,15]

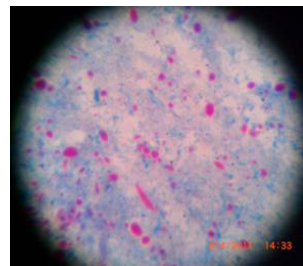
Procedure

- Transfer half a teaspoonful of faeces into 10 ml of water in a glass container and mix it thoroughly.
- Place 2 layers of gauze in a funnel and strain the contents into a 15 ml centrifuge tube.
- Centrifuge for 2 minutes at about 500 g.
- Discard the supernatant and resuspend the sediment in 10 ml of physiological saline. Centrifuge at 500 g and discard the supernatant.
- Resuspend the sediment in 7 ml of 10% formaldehyde (1 part of 40% formalin in 3 parts of saline).
- Add 3 ml of ether (or ethyl acetate).
- Close the tube with a stopper and shake vigorously to mix. Remove the stopper and centrifuge at 500g for 2 minutes.

- Rest the tube on a stand. Four layers now become visible; the top layer consists of ether, the second layer is a plug of debris, the third layer is a clear layer of formalin and the fourth layer is the sediment [Table/Fig-2].
- Detach the plug of debris from the side of the tube with the aid of a glass rod and pour off the liquid, leaving only a small amount of formalin for the suspension of the sediment.
- With a pipette, remove the sediment and mix it with a drop of iodine. Examine under the microscope.

The saturated salt flotation technique [7,8,9]

- Place about one millilitre of faeces in a container which is flat bottomed and which has a diameter of less than 1½ inches and a capacity of about 15-20 ml.
- Add a few drops of saturated salt solution (specific gravity 1.200) and stir it to make a fine emulsion.
- Add more salt solution so that the container is nearly full, stirring the solution continuously.
- Remove any coarse matter which floats up.
- Place the container on a levelled surface. Do the final filling by using a dropper until a convex meniscus is formed.
- A glass slide 3"x 2" is carefully laid on the top of the container so that the centre is in contact with the fluid.
- This preparation is allowed to stand for 20 minutes after



[Table/Fig-4]: Modified AFB stain showing oocyst of *C. parvum*

which the glass slide is quickly lifted, turned over smoothly to avoid the spilling of the fluid and it is examined under the microscope after putting a coverslip on it.

Staining procedures [12,13]

The modified acid fast staining is a simple and effective method for the diagnosis of coccidian parasites in the stool sample by using 1% H₂SO₄ as a decolourizer.

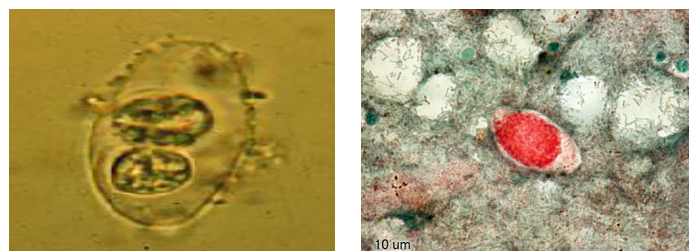
In unstained wet preparations, the small oocysts of *C. parvum* are difficult to differentiate from those of yeasts and other small spherical structures which are found in faeces.

The oocyst of *C. parvum*

Small, round to oval pink red stained bodies which measure 4-6µm.

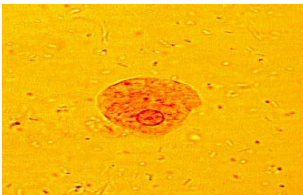
The oocyst of *Isospora belli*

It is oval, measuring 20-22 µm in diameter and it usually shows a granular zygote. Occasionally, the oocyst may show two sporocysts (each with four sporozoites).



[Table/Fig-5(A)] Oocyst of *I. belli* showing two sporocysts (each with four sporozoites) in wet preparation; (B) Pink red coloured oocyst of *I. belli* showing a zygote ON modified Z/N stain

Oocyst of *I. belli* showing two sporocysts (each with four sporozoites).



[Table/Fig-6]: Trophozoite of *E. histolytica*



[Table/Fig-8]: Trophozoite of *G. lamblia*



[Table/Fig-7]: Cyst of *E. histolytica*

B- Pink red coloured oocyst of *I. belli* showing a zygote

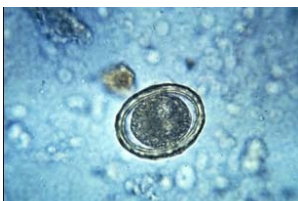
Morphological features of the common parasites/eggs/ova/cysts

Entamoeba histolytica trophozoite

It measures 12-60 μ , it is asymmetric, it shows purposeful directional motility and it has a single spherical nucleus, a single central karyosome and delicate and evenly distributed chromatin.

Entamoeba histolytica cyst

It is spherical and it measures 10-20 μ . It is a mature cyst with four nuclei, with a compact, centrally located karyosome; the chromatin is delicate. Some cysts may have chromatoid bars. This is the infective stage of the parasite

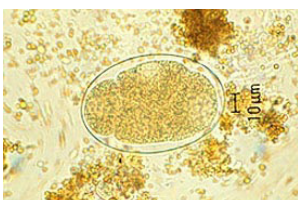


[Table/Fig-10]: Fertile egg of round worm

shaped, with tapering ends. It is actively motile like a falling leaf and it has 2 centrally placed nuclei and uniform granular cytoplasm.

Giardia lamblia cyst

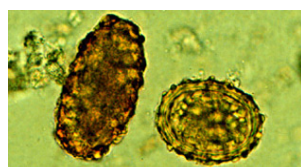
It is oval, 8-12 μ long and 7-10 μ wide, and its nucleus has 4 karyosomes, which tend to be eccentrically placed. There is a clear space between the cell wall and the cytoplasm. Four median bodies are present.



[Table/Fig-12]: Eggs of hookworm

Fertile egg of roundworms

It measures 60 \times 45 μ and is round or ovoid with a thick shell. It is



[Table/Fig-11]: Unfertilized and fertile egg of round worm



[Table/Fig-13]: Eggs of *E. vermicularis*

covered by a thick albuminous coat, its inner cell is in various stages of cleavage and it is brown in colour.

Decorticated egg of roundworm

The albuminous coat is lost. All other features are the same as in a fertile egg.

Infertile egg of roundworm

It measures 90 \times 40 μ , it is elongated, its shell is often thin and its internal material is a mass of globules.

Hookworm egg

They are oval and ellipsoid and they measure 60 \times 40 μ . Their shells are thin walled, smooth and colourless. Their internal cleavages are well developed at the 4-8 cell stage, which pull away from the shell, leaving an empty space.

Threadworm egg (*E. vermicularis*)

It is a planoconvex, elongate and an asymmetric egg which measures 55 \times 26 μ . Its shell is thin and smooth. Fully developed larvae are seen in the eggs.

Whipworm egg (*Trichuris trichura*)

It is elongate and barrel shaped, with polar hyaline plugs. It measures 54-22 μ . Its shells is yellow to brownish and its plugs are colourless.

Tapeworm egg (*Taenia spp*)

It is spherical, it measures 31-43 μ and it has a thick shell with prominent radial striations. An embryonated oncosphere which possesses 3 pairs of hooklets within the shell is diagnostic of the genus. Species identification on the basis of morphology is not possible.



[Table/Fig-14]: Eggs of *Trichuris trichura*



[Table/Fig-15]: Eggs of *Taenia spp*

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