

DIAGNOSIS OF PARASITIC INFECTIONS

Unit 2

2.1 Specimen in which parasites are found

1. Faeces/Stool

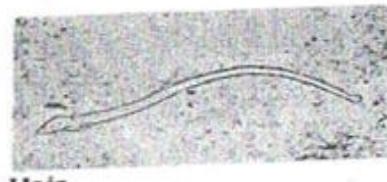
Protozoan parasites, depending on type of parasite

- ❖ Cyst
- ❖ Amoeba
- ❖ Flagellate
- ❖ Ciliate
- ❖ Oocyst

Faeces/Stool (Cont'd.)

- ❖ For some parasites both their cyst and amoeba, or cyst and flagellate or cyst and ciliate may be found

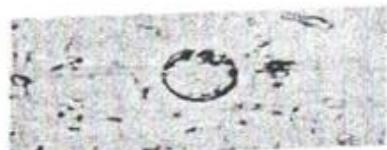
Structures found in faeces that may be confused with parasites



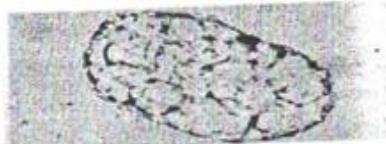
Hair



Vegetable fibres



Blastocystis hominis



Starch cell



Yeasts and spores



Charcot Leyden crystals



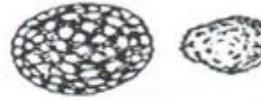
Air bubbles



Muscle fibres



Fat globules



Pollen grains

1

Plate 13.2 Structures which may be found in faeces
and can be confused with parasites.

Cheethrough, 1987

Faeces/Stool (Cont'd.)

Helminth parasites, depending on the type of parasites

- ❖ Egg
- ❖ Segment
- ❖ Egg capsule
- ❖ Worm
- ❖ Larva

Faeces/Stool (Cont'd.)

- ❖ For some parasites both their egg and segment or egg and capsule or egg and worm may be found

Specimen in which parasites are found (Cont'd.)

2. Blood

- ❖ Protozoan parasites
- ❖ Trophozoite
- ❖ Schizont
- ❖ Gametocyte
- ❖ Trypomastigote
- ❖ Helminth parasites (Nematodes)
- ❖ Microfilaria

Specimen in which parasites are found (Cont'd.)

3. Urine

- ❖ Protozoan parasites
- ❖ Flagellate (rarely)
- ❖ Helminth parasites
- ❖ Egg
- ❖ Microfilaria (rarely)

Specimen in which parasites are found (Cont'd.)

4. Sputum

- ❖ Helminth parasite
- ❖ Egg
- ❖ Flukes (occasionally)

Specimen in which parasites are found (Cont'd.)

5. Cerebrospinal fluid

- ❖ Protozoan parasites
- ❖ Trypomastigote
- ❖ Amoeba

Specimen in which parasites are found (Cont'd.)

6. Bone marrow

- ❖ Protozoan parasites
- ❖ Amastigote
- ❖ Toxoplasm

Specimen in which parasites are found (Cont'd.)

7. Lymph gland Aspirate

- ❖ Protozoan parasites
- ❖ Trypomastigote
- ❖ Amastigote
- ❖ Toxoplasm

Specimen in which parasites are found (Cont'd.)

8. Liver Aspirate

- ❖ Protozoan parasites
- ❖ Amoeba
- ❖ Amastigote
- ❖ Toxoplasm

Specimen in which parasites are found (Cont'd.)

9. Spleen aspirate

- ❖ Protozoan parasites
- ❖ Amastigote
- ❖ Toxoplasm

Specimen in which parasites are found (Cont'd.)

10. Skin

- ❖ Protozoan parasites
- ❖ Amastigote
- ❖ Helminth parasites
- ❖ Microfilaria
- ❖ Larva in ulcer fluid
- ❖ Egg on perianal skin

Specimen in which parasites are found (Cont'd.)

11. Muscle

- ❖ Helminth parasites
- ❖ Larvae

Specimen in which parasites are found (Cont'd.)

12. Rectal Scraping

Protozoan parasites

❖ Amoeba

Helminth parasites

❖ Egg

Specimen in which parasites are found (Cont'd.)

12. Rectal Scraping

Protozoan parasites

❖ Amoeba

Helminth parasites

❖ Egg

Methods used in diagnosis

1. Microscopic examination

- ❖ For majority of intestinal, urinary and blood parasites in stained or unstained preparations

- ❖ The preparations may be viewed directly or following concentration (when the parasites are few)

2. Cultural techniques

- ❖ Few parasitic infections are diagnosed routinely by cultural techniques
- ❖ For example stool or faecal culture (Coproculture) to enable distinguishing of nematode specimens;
- ❖ the method is necessary for identifying e.g. hookworm specimens (*Ancylostoma sp.*; *Necator sp.*, *Oesophagostomum sp.*).

2. Cultural techniques (Cont'd.)

- ❖ Culture of the faecal specimen leads to hatching of the eggs which develop to the L₃ larval stage when the worms can be identified on the basis of their unique larval features.

3. Immunodiagnostic techniques

□ These are used when:

- Parasites are in the tissues of internal organs and cannot be easily removed for examination
- Parasites can be found in specimens only in certain stages of an infection (e.g. in the acute, and not in the chronic stage)
- Parasites are present only intermittently or in too few numbers to be easily detected in specimens

3. Immunodiagnostic techniques (Cont'd.)

- ❖ The techniques used are generally complex or time-consuming
- ❖ Parasitic diseases for which immunodiagnosis is of particular value include:
 - Trypanosomiasis
 - Leishmaniasis

3. Immunodiagnostic techniques (Cont'd.)

- Amoebic Liver abscess
- Toxoplasmosis
- Hydatid disease
- Filariasis (Occult and chronic infections)

3. Immunodiagnostic techniques (Cont'd.)

- Trichinellosis
- Toxocariasis
- Schistosomiasis (chronic stage)

3. Immunodiagnostic techniques (Cont'd.)

***Immunodiagnostic techniques are based on the
detection of:***

- ❖ Antibody in a person's serum, produced in response to a particular parasitic infection.
- ❖ The antibody may persist for a long period in the serum after an infection has ended hence the tests do not indicate whether the infection is current or long ago.
- ❖ Antibody tests need to be interpreted with care.

3. Immunodiagnostic techniques (Cont'd.)

- ❖ Antigen, which is excreted by parasites and can be found in serum, urine, cerebrospinal fluid, faeces or other specimens.
- ❖ Antigen tests provide evidence of present infection and are therefore of greater value than antibody tests in clinical diagnosis of parasitic infections.

3. Immunodiagnostic techniques (Cont'd.)

- ❖ In a few parasitic infections the demonstration of IgM antibody helps to indicate whether an infection is a recent one, e.g. Toxoplasmosis.

3. Immunodiagnostic techniques (Cont'd.)

- ❖ Testing for a rising antibody titre can be of value in indicating whether an infection is an active one and in some sensitive tests, changes in antibody titre can indicate whether a patient is responding to treatment.

3. Immunodiagnostic techniques (Cont'd.)

- ❖ Interpretation of antibody test results needs care because **false positive** reactions may occur due to cross-reactions with other human and animal parasitic antigens.
- ❖ Different helminthes share many antigens and hence species-specific diagnosis is not always possible. **False negative** results may occur due to an excess of antigen removing the antibody

Antibody/Serological Tests

- ❖ These include:
- ❖ Rapid Slide Agglutination Test (RSA test)
- ❖ Gel or Cellulose Acetate Precipitin Test (GCAPT)
- ❖ Indirect Haemagglutination Test (IHT)
- ❖ Complement Fixation Test (CFT)
- ❖ Indirect Fluorescent Antibody Test (IFAT) and
- ❖ Enzyme-linked Immunosorbent Assay (ELISA)

Antibody/Serological Tests (Cont'd.)

- ❖ The choice of technique depends on a number of factors, including the
 - Parasitic infection under investigation
 - The number of tests
 - The availability of reagents
 - Sensitivity and specificity of the test system and
 - The ability and facilities available in the laboratory.

Antibody/Serological Tests (Cont'd.)

- ❖ The ELISA has become a standard technique in the diagnosis and epidemiological studies of the important parasitic diseases. It can be used to detect antibody or antigen.
- ❖ ELISA can be used to diagnose malaria amoebiasis, schistosomiasis, onchocerciasis, toxoplasmosis, echinococcosis, trypanosomiasis, leishmaniasis and trichinellosis.

Antibody/Serological Tests (Cont'd.)

- ❖ ELISA tends to be more sensitive and specific than other serological tests, provides a quantitative result using a single serum dilution and can be automated in a central immunodiagnostic laboratory.

Sources of Antigens for Serological Tests

- ❖ Usually, most laboratories prepare their own antigens from parasites grown in cultures or developed in laboratory animals.
- ❖ Only a few antigens are available from commercial sources but these may be very expensive and have only short shelf-lives

Sources of Antigens for Serological Tests (Cont'd.)

- ❖ Two new techniques which are developing rapidly and expected to provide specific antigens for serological tests are
 - Monoclonal techniques and
 - Recombinant techniques

Monoclonal Antibodies

- ❖ These are made by fusing antibody producing lymphocytes (B lymphocytes) from an immunized mouse, with a mouse myeloma cell line.
- ❖ Clones of the resultant hybridomas retain both the capacity to produce the particular antibody made by the lymphocyte and also the capacity for indefinite growth *in vitro*.

Monoclonal Antibodies (Cont'd.)

- ❖ This allows the production *in vitro* of large quantities of a range of antibodies, each being usually against a narrow range of antigens.
- ❖ If the antigens which are useful in immuno-diagnosis can be identified, the appropriate monoclonal can be used to extract and purify it, e.g. by affinity chromatography.

Recombinant DNA (DNA probes)

- ❖ This technique involves, firstly, extracting and fragmenting the DNA from one organism and hybridizing (incorporating) this with DNA fragments from another organism.

Recombinant DNA (Cont'd.)

- ❖ The resulting hybrids are then reinserted into the latter organism which is known as the vector.
- ❖ This is usually a virus or a bacterial plasmid, nearly always from *E. coli*, which is able to replicate itself very rapidly, thereby producing large quantities of the proteins.
- ❖ For example, the DNA coding for an antigenic protein in a parasite could be inserted into *E. coli*, which, by its rapid replication, would yield large quantities of this protein.

Antigen Test

- ❖ Monoclonal techniques are also used to develop highly reactive and specific antibody reagents, especially for ELISA (Sandwich assay).

Other Tests for identification of some parasites

- ❖ Biochemical tests to detect isoenzyme and genetic characteristics.
- ❖ Enzyme electrophoresis

Definition of Terms for Diagnostic Stages

- ❖ **Amastigote:** the non-flagellate, intracellular, morphologic stage in the development of certain haemoflagellates, resembling the typical adult form of Leishmania.
- ❖ **Oocyst:** the encysted or encapsulated ookinete (the fertilized form of the malarial parasite in the body of a mosquito); it is formed by fertilization of a macrogamete by a microgamete and develops into an oocyst.

Definition of Terms for Diagnostic Stages (Cont'd.)

- **Trophozoite:** the active, motile, feeding stage of a protozoan organism.
- **Trypomastigote:** a morphological stage in the development of certain haemoflagellates, resembling the typical adult form of *Trypanosoma* and characterized by having a flagellum

Definition of Terms for Diagnostic Stages (Cont'd.)

- ❖ **Schizonts:** the stage in the development of the malarial parasite, *Plasmodium*, following the trophozoite, whose nucleus divides into many smaller nuclei, followed by the division of the cytoplasm to surround each nucleus,
- ❖ thus forming a merozoite (merozoites).
- ❖ **Microfilaria:** the prelarval stage of the Filarioidea in the blood of humans and in the tissues of the vector.

Definition of Terms for Diagnostic Stages (Cont'd.)

- ❖ **Toxoplasm**: the immature stage of *Toxoplasma* sp. that may be found in stained preparations of body fluids such as lymph node/gland, liver or spleen aspirates or bone marrow.