Parasitology & Mycology – Review

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MMC
Principle of diagnosis Lab Dx of Microbial diseases

- Demonstration of causative agents by microscopy commonly practiced for parasitic diseases & fungal diseases
- Isolation and identification of causative agent by artificial culture - (C/S) commonly practiced for bacterial diseases.
- Detection of antibody or antigen from blood or other body fluids practiced for viral diseases & some bacterial & parasitic diseases
- Detection of Nucleic acid segment (DNA or RNA)
Steps involved in Lab Dx of Microbial diseases

- **Specimen**: according to site of infection
- **Microscopic Examination**:  
  - Light microscopy / DGM / FM / EM  
    - Unstained preparation  
    - stained smear -Gram, AFB, Fluorescence
- **Isolation & identification**: Bacteriological, fungal, protozoal Culture, Cell culture for viruses  
  - Identification by standard biochemical & serological test
- **Immunological test**: detection of Ag or Ab by LAT, ELISA etc
- **Detection of gene**: NA based techniques by PCR etc
- **Special tests**: Toxigenicity tests
Formula for Lab Dx of Parasitic diseases

• Almost all parasitic diseases can be diagnosed by M/E examination of appropriate specimen and finding some structure of the parasites.

• Specimen: depends on site of infection
  • Blood, stool, HVS, BM, etc

• Protozoal structure
  • Cyst, trophozoite, Oocyst, special - LD bodies, schizont etc

• Metazoal structure
  • Ova, larva, segments
Examples of Lab Dx of Parasitic diseases by M/E

- **Amoebiasis**
  - By M/E of stool for Cyst or trophozoite, Liver aspirate for trophozoite

- **Giardiasis**
  - By M/E of stool for Cyst or trophozoite

- **Trichomoniasis**
  - By M/E of vaginal discharge for trophozoite

- **Malaria**
  - By M/E of PBF for schizont, merozoite, gametocytes etc (MP)

- **Kala-azar**
  - By M/E of BM, Spleen or LN for LD bodies

- **Cryptosporidiasis**
  - By M/E of ZN stained smear of stool for Oocyst
Dx of Parasitic diseases by other tests

• Kala-azar -
  By culture & immunological tests

• Malaria -
  • by immunological test by detecting Ag/Ab

• Hydatid disease
  By immunological tests
  Casoni’s test (traditional)
  Indirect haemagglutination test (IHA) - modern

• Filariasis -
  By detecting antibody - Occult filariasis (CFT, IFAT)
  By detecting antigen - classical filariasis (ICT)
Lab Dx of kala-azar

Principle:

Kala-azar can be diagnosed by demonstration of the parasite (LD bodies) from the BM, Spleen or L.node aspirate and by detection of antibody and antigen by immunological tests and the organism can also cultured if facilities are available.

Steps:

- **Specimen:** Bone marrow/ spleen / lymphnode aspirate or serum
- **M/E:** leishman stained smear shows LD bodies
- **Culture:** can be cultured in NNN medium at 22°C for 1-2 weeks
- **Serological tests:**
  - **Specific:** IFAT, DAT, ICT, CFT for detection of antibody
  - **Nonspecific:** Aldehyde test for detection of high Globulin.
What is black water fever? Why it is so called?

A complication of P. falciparum malaria where colour of urine becomes black due to intravascular hemoysis.
Lab Dx of malaria

Principle:
Malaria can be diagnosed by demonstration of the parasite (ring form, schizonts, gametocytes etc) from the Peripheral blood film, and by detection of antigen by immunological tests.

Steps:
Specimen: Blood
M/E : leishman / Giemsa stained smear of thick and thin blood films demonstrates the parasites and species can also be identified by seeing the morphology.
Culture : rarely done
Serological tests:
Specific : ICT for detection of antigen / antibody
What is protozoa? 5 common protozoa disease in Our country? What are rare protozoal disease in BD

- The word Protozoa is derived from “Proto”-primitive, “Zoo” -life  i.e. Primitive life or first life.

- Common protozoa disease in BD
  - Amoebiasis
  - Giardiasis
  - Malaria
  - Kalazar
  - Trichomoniasis

- Rare protozoal diseases
  - Sleeping sickness
  - Chagas disease
  - Cryptosporidiasis

Toxoplasmosis - ??
Examples of Lab Dx of Helminthic diseases by M/E

- **Filariasis**
  By M/E of PBF for larva (microfilaria)

- **Intestinal helminthes**
  By M/E of stool for ova or larva

- **Enterobiasis**
  By M/E of Peri-anal swab for ova ?

- **Taenia saginata or solium**
  By M/E of stool for segments or ova
  What are the differences between ova of T.saginata & T.solium?

- **Diphylobothriasis**
  By M/E of stool for ova or larva

- **Schistosomiasis**
  By M/E of urine or stool ?
How many species of plasmodium are there? Which spp. are found in Our country?

- *Plasmodium vivax* – benign tertian malaria
- *Plasmodium falciparum* – malignant tertian malaria
- *Plasmodium ovale* – oval tertian malaria
- *Plasmodium malariae* – quartan malaria
- *P. vivax* and *P. falciparum* are found in Bangladesh
Which spp. of plasmodium is most dangerous and why?

- Plasmodium falciparum – is most dangerous because
  - It infects both young & old RBCs
  - Parasitaemia is heavy
  - Pernicious malaria/ cerebral malaria occurs
  - Mortality is high
  - Frequently drug resistant (Resistant to chloroquine)
How anaemia occurs in Kala-azar?

Following mechanisms are involved

- Bone marrow depression due to infiltration by parasites.
- Hemolysis
- Hypersplenism
What are organs for motility of protozoa? examples

- Protozoa are motile by
  - Pseudopodia – Entamoeba histolytica
  - Flagella – Giardia, Trichomonas, Leishmania
  - Cilia – Balantidium coli
Which are the infective form following protozoa?

- E. histolycia - Quadrinucleate cyst
- G. lamblia - Cyst
- Trichomonas vagialis - trophozoites (Cyst absent)
- L. donovani - Promastigote
- Plasmodium - Sporozoites
- Toxoplasma - ?
No man can be a good teacher unless he has feelings of warm affection towards his pupils and a genuine desire to impart to them what he himself believes to be of value.

Bertrand Russel
What is the mechanism of megaloblastic anemia by D. latum?

- *D. latum* interferes with fat absorption by inhibiting intrinsic factor
Men learn while they teach.

Seneca

To be proud of learning, is the greatest ignorance.

Jeremy Taylor
Which parasite is found within RBC? Which are found in Macrophage & which are found in plasma?

Ans: Malarial parasite (MP) is found within RBC, L.donovani within Macrophage & T.bruci, T.cruzi, microfilaria in plasma
Name common helminthic disease in BD

- Nematodes are common in our country
  - Ascariasis - commonest
  - Hook worm infestation* -
  - Enterobiasis
  - Trichiuriasis
  - Filariasis
Name helminthic disease uncommon in our country?

- **Cestodes**
  - Taenia saginatum, Taenia solium
  - Diphylobothrium latum
  - Echinococcus granulosus (Hydatid cyst)
  - H.nana

- **Trematodes**
  - Schistosomes- S.japonicum, S.mansoni, S.heamtoobium
  - Paragonimus westermani - lung flukes
  - Fasciola hepatica, Clorchis sinensis, fasciolepsis buski
Which are the infective form following Helminths?

- Ascaris lumbricoides - Mature ova
- Hook worms - Filariform larva
- S. stercoralis - Filariform larva
- Enterobius vermicularis - Ova
- Trichuris trichiura - Ova
- W. bancrofti - 3rd stage larva
How can U diagnose Cryptosporidium in Lab? Whom it causes diarrhoea?

- Cryptosporidium causes persistent diarrhoea in immunocompromised persons (AIDS patients).
- It can be diagnosed in the lab by modified Acid fast staining of stool, where oocysts can be found.
Which cysts commonly found in the stool of child of BD? What is difference in their pathogenesis? What is character of stool?

- Cysts of *E. histolytica*, *Giardia lamblia*
- *E. histolytica* causes lesion in the large intestine and forms ulcer by invasion. Stool contains pus cells, RBC & cysts or trophozoites of *E. h.*
- *G. lamblia* causes lesion in the small intestine and do not invade but by attachment with cup like dis interferes with absorption of fat. Stool contains fat cells but no pus cells or RBC, & cysts or trophozoites of *G. L*
Which parasites causes anaemia & What type of anaemia? With mechanism

- **Hook worm** - Microcytic, hypochromic & Dimorphic anaemia.
- **Plasmodium** - Hemolytic anaemia
- **L. donovani** - Normocytic anaemia
- **Diphylobothrium latum** - megaloblastic anaemia
Between T. saginata & Solium which one is more dangerous and why? How can U differentiate them? What are the difference in their ova?

- **T. solium** is dangerous than T. saginata because Cysticercus celulosae (larval stage of T. solium) occurs in man.

- Can be differentiated by N/E and M/E of segments.
  - T. saginata - longer, solium - broader
  - Rostellum & hooks - present in T. solium
  - Difference in genital organs

- No difference in their ova
T. solium gravid segment

T. saginata gravid segment

T. solium with hooks

T. saginata with no hooks
Which helminthic infection occur following ingestion of meat and fish & vegetable?

- Improperly cooked beef- *T. saginata*
- Improperly cooked pork *T. solium*
- Fish - *Diphylobothrium latum*
- Raw Vegetable-
  - *E. histolytica*
  - *Giardia*
  - *Ascaris lumbricoides, Trichuris trichiura*
Which are vector bone parasitic disease?

- Mosquito-
  - Malaria, Filaria
- Sandfly -
  - Kalazar
- Tse Tse fly-
  - Trypanosoma brucei
- Reduvid bug
  - Trypanosoma cruzi
- Deer fly/ Black fly
  - Onchocerca volvulus
What is the role Eosinophil & IgE in combating helminthic infestation?

- Help to expel helminths by ADCC mediated by IgE & Eosniophil.
Which parasitic infection occurs with fondling of dogs? How? What type of host is man? Lab diagnosis? What will happen if explore it?

- **Echinococcus granulosus**
- By ingestion of egg present in dogs faeces
- Man is accidental intermediate host, Dog is the definitive host.
- Can be diagnosed by immunological test
  - Casonis tes, IHA (Indirect haemagglutination test)
  - Possibility of anaphylactic reaction and multiple cyst in other parts of body
Which nematodes have heart lung migration?

- *Ascaris lumbricoides*
- Hook worms
  - *Ankylostoma duodenale* & *Necator americana*
- *Strongyloides stercoralis*
Which one is the commonest helminthic infection in BD? What are the clinical problems that occur with ascariasis?

- Due to Adults & Larva
- Adults
  - Spoliative action - PEM, Vitamin A deficiency (night blindness)
  - Mechanical - Obstruction in bile duct, Intestine
  - Toxic - Typhoid like fever, rash
- Larva
  - Loeffler's syndrome - Pneumonitis
Which ova can be found in the stool of Bangladeshi children?

A.L

AD

T.T
How can differentiate between *P.vivax* and *P.falciparum*?

- By the morphology in RBC & shape of gametocytes.
Which one infective form of E.histolytica? Why trophozoites can not cause infection?

- Infective form – Quadrinucleate cyst.
- Trophozites can not cause infection because they are destroyed by the gastric juice.
What is definitive and Intermediate host? Where Man is intermediate host?

- Definitive host – where parasite passes adult for or sexual stage of life cycle.
- Intermediate host – larval form or asexual stage of multiplication takes place.
- Man is the intermediate host in following cases-
  - Malaria
  - Hydatid cyst
- Man is both definitive & Intermediate host
  - Taenia solium
  - Trichenella spirilim
How can you classify fungi morphologically?

- **Morphological classification of fungi**
  - **Yeast** – *Cryptococcus neoformans*
    - rounded structure
  - **Mould** – *Dermatophytes*
    - Branching structure with hyphae & mycelia
  - **Yeast like** – *Candida albicans*
    - possess pseudohyphae
  - **Dimorphic** – *Deep fungi*
    - yeast within human tissue (37°C)
    - Mould in environment 25°C
Name the dermatophytes. Why they so called? Where they infect?

- Epidermophyton – skin
- Microsporum – skin & hair
- Trichophyton – skin, hair & nail
- They are called dermatophytes because they have affinity for dermis.
What is taenia infection? Give examples

- Infection by dermatophytes are known as Taenia infection. According to site they are as follows-
  - Taenia capitis – (head)
  - Taenia – corporis (body)
  - Taenia barbi (beard)
  - Taenia unguium – (fingers)
  - Athlets foot – (Ankle)
• What is dimporphism and why this occurs?
  • Two morphological forms at different temperature.
  • Mold - saprophytic - free living - at 25°C for existence
  • Yeasts - in the hosts parasitic at 37°C

• What are the fungal spores?
  • Are means of reproduction. Both asexual & sexual.

• Where fungi lives?
  • Mostly in the environment
  • candida - in the host as normal flora

• Are fungal disease communicable?
  • No
• How can diagnose a fungal infection? What is the role of KOH?

• Mostly by M/E after dissolving in alkaline solution (KOH or NAOH), Calciflor white- a fluorescent dye can stain the cell wall and helpful in tissues.

• Culture

• Serology

• DNA probe- coccidiodes immitis, Histoplasmosis, Blastomycosis
• Why antibacterial agents do not act on fungi?
  • Target structures like cell wall, etc are absent..
• What is the significance of ergosterol in CM of fungi?
  • Is the basis for antifungal drugs.
• Why bacteria do not multiply in fungal media?
  • Due to high acidity of the medium and antimicrobial agents like chloramphenicol & chlorhexidine
How can you clinically classify fungal diseases?

- Clinical classification:
  - Superficial - dermatophytes
  - Subcutaneous - *Sporothrix shinckii*
  - Systemic – *Histoplasmosis*
  - Opportunistic – *Candidiasis, Cryptococcosis, Aspergillosis*
What are systemic fungal infections?

- **Coccidiodes - C.immitis** - Granulomatous lesion of Bones, CNS
- **Histoplasmosis - H.capsulatum** (inside macrophage) - Liver, spleen
- **Blastomycosis - B.dermatitidis** - skin
- **paracoccidioides - P.brasiilensis** - lung & disseminated
What are opportunistic fungal infections?

• Opportunistic

• Candidiasis - Candida albicans - mouth, vagina, & disseminated

• Cryptococcosis - C. neoformans, capsulated, pneumonia, meningitis

• Aspergillosis - A. fumigatus - mold, fungus ball in the lung, Allergic bronchopulmonary aspergillosis (ABPA)

• Pneumocystis carinii - yeast like, pneumonia in AIDS patients.
Which one is capsulated fungus? What is its structure? Disease? How can be diagnosed?

- Cryptococcus neoformans
- It is a yeast
- opportunistic infection in CNS, lungs
  - Pneumonia, meningitis
- Capsule can be demonstrated by India ink preparation also by immunological test (Ag detection)
How can you diagnose a ringworm infection? Role of KOH? Temp & time of culture? How species are identified?

- Can be diagnosed by M/E of infected specimen after dissolving in keratolytic solution like KOH, NAOH, then by culture in Sabrauds dextrose agar media.
  - Specimen – Skin scrapping, hair plucking, nail shaving
  - M/E – shows fungal structures like hyphi, mycelia etc.
  - KOH dissolves keratin layers
  - Culture incubated at 25 oc or room temp for 2-3 weeks.
  - Species is identified by M/E of growth, for microconidia & macroconidia
Check list (Parasitology & Mycology)

• Name of common protozoal diseases with their causative agents, Definitive host, Intermediate host, Mode of transmission and infection.
• Name of common helminthic diseases with their causative agents, Definitive host, Intermediate host, Mode of transmission and infection.
• Lab diagnosis of malaria, kala-azar, Giardiasis, Intestinal and Hepatic amoebiasis, Filariasis, AL, AD, Enterobiasis, Hydatid disease.
• Pathogenicity of AL, AD, EV, EH, LD, Malaria, Kalaazar, Filaria
• Morphological forms of fungi with examples
• Name of superficial fungal infection and fungi & lab diagnosis (Use of KOH in M/E), macroconidia & microconidia.
• Candidiasis in details
• Deep fungi - names
• Cryptoccus neoformans
Best wishes for all students