INFECTION and IMMUNITY
GRADUATE ENTRY PROGRAMME

SEMESTER ONE 2007

PARASITOLOGY PRACTICAL 5 – Dr TW Jones

DIAGNOSIS OF PROTOZOAL INFECTIONS

Objectives

After this class I expect you to be able to:

1. Identify the rhizopod, ciliate and flagellate protozoa by their type of movement in wet-film preparations.
2. Identify and distinguish between trophozoites and cyst forms of *Balantidium* in stained smear preparations.
3. Identify *Trichomonas* in stained smear preparations.
4. Identify *Babesia divergens* in stained smear preparations.
5. Distinguish between *Babesia divergens* and *B. major*
6. Identify certain stages in the life cycle of tissue-cyst forming protozoa from gross pathology and histological sections
7. Describe the relative merits and demerits of methods used for the diagnosis protozoal infections

This class requires you to make observations on a variety of materials and to answer questions related to that material. The format and type of the question used in this class are typical of those that will be used in new format spot examination so it is important that you record your observations and answers a permanent way so that you can use them as a revision aid.

Wet films

Wet film preparations are a quick and simple way of detecting protozoa in fluids such as blood or culture media. They are mostly used to detect motile protozoa and you should be able to identify each of the main protozoan groups based on the way they move.
For this class you are provided with suspensions of ciliates, flagellates and Rhizopods. Place a small drop of each protozoan suspension onto a glass microscope slide and cover it with a coverslip (wet-film preparation). Examine the preparation using the low (x10 objective) and medium (x40 objective) power of the microscope. DON'T use the oil immersion objective with wet-films. Now do the following:

a. Describe the movement of each group based on
   i. Direction
   ii. Speed
b. Answer the following questions
   i. How would you distinguish between the three groups based on movement?
      1. Rhizopods (amoeba) move very slowly using pseudopodia
      2. Ciliates move quickly in straight lines
      3. Flagellates move slowly usually in different directions
   ii. Which group of protozoa is missing and why?
      1. Sporozoa – too small and type of movement (gliding) not easily seen under a microscope, mostly intracellular
   iii. Which of the following adjustments to the microscope can be used to improve your ability to see the organisms in a wet-film

1. Adjust the light level – has little effect on contrast and can make it too dark to see anything
2. Adjust the field diaphragm – has no effect on contrast makes the field of view too small
3. Adjust the condenser focus – has little effect on contrast
4. Adjust the condenser diaphragm – this is the one that can be used to increase contrast

Stop and think - what do you think are the practical limitations for using wet-films for the diagnosis of protozoan parasites?

i. Wet-films need to be examined soon after preparation
ii. Need a microscope
iii. Not a permanent record

Stained films

Stained films are an extension of the wet-film technique in that a the film will have been processed in some way to preserve its contents and stained to make them easier to detect the components in the original material which can be gut contents, blood or culture fluid. The commonest stain used for protozoa belong to the Romanovsky group e.g. Giemsa's, Leishman's, Field's stains. All these stains colour DNA purple-blue, RNA pink-red, cytoplasm blue. Because of the small size of protozoa it is likely that you will need to use the ×100 oil immersion objective to see the fine details of most of the protozoa. As always you should use the lowest powered objectives first to find an area of interest and then switch to the highest power. Be careful to avoid getting oil on the lower powered objectives. Also PLEASE wipe the oil from the slide once you have finished and return it to the slide tray. You should now practice identification of protozoa in stained films using the following materials

1. **Gut and faecal smears (*Balantidium*)**
    a. Before you start, think about which cells etc you might expect to find in a smear from gut contents or faeces – and how they might react with the a Romanovsky stain
    b. Now look at the two Balantidium preparations of trophozoite and cysts forms and identify the parasitic material based on the staining pattern and their size in relation to what other things you might expect to see

    Stop and think - Which features would you use to distinguish between the trophozoite and cyst form and how are these related to the parasite's life cycle?

    i. Trophozoite – oval, cialia on outer surface (looks like a fuzzy layer), found predominantly in the gut – feeding and reproduction
    ii. Cyst – round, thick wall on outside, predominantly in faeces - infective form

2. **Blood films (*Babesia*)**
    a. Before you start, think about which cells etc you might expect to find in a blood smear and how they might react with the stain
    b. Now look at the Babesia preparation and identify the very small Babesia parasite inside the rbcs.
    c. Why do you think Babesia are so small compared to Balantidium?
        i. Need to live inside cells
    d. Why do you think this species of Babesia is called "divergens"?
        i. The pairs are at a very pronounced angle to one another - divergent
    e. Now look at the photograph showing B.divergens and B.major – based on the evidence in front of you what features could you use to tell them apart
        i. Size – major slightly larger than divergens
        ii. Location – divergens at the periphery of the cell, major in the middle
        iii. Relationships of the pairs - divergens usually widely apart, major together

Stop and think –

- Why we are more likely to use stained films rather than wet-films for the diagnosis of Babesia?
    - Babesia too small to be seen at limit of magnification used for wet-films
    - Babesia do not move
- Name another blood protozoan that could be easily diagnosed using the wet-film technique
    - Trypanosomes

3. **Culture fluid films (*Trichomonas*)**
    a. This is a fluid culture system so how do you think this affects your ability to can detect any protozoa
    b. Look carefully at the preparation and find the small purple-blue Trichomonas organisms
    c. Compare what you can see with the diagram on the board showing the morphological features of *Trichomonas* – how many of these can you identify in the stained smear. Explain why you might not be able to see all of them.
        i. Depends on how well the preparation has been made e.g. drying before staining
        ii. Depends on the orientation of the parasite on the slide when it dried

Stop and think - based on your experience up to now – list the factors that would influence your choice of wet-films vs stained smears for the diagnosis of parasitic protozoa?

i. Wet-film quick and easy good for large, motile protozoa
ii. Stained films good for permanency, small protozoa, revealing intracellular details

Stained tissue sections

Protozoa such as the coccidia that develop inside cells cannot be detected using either wet or stained film techniques so these stages can only be found in stained histological sections of target organs such as gut, muscle or brain. Such sections can also provide information on pathological changes associated with parasite infection. However, these preparations can only usually be provided post-mortem. Look at the following preparation as an example of the use of a stained section – you will look at other examples of stained sections in the next practical class

Section of cerebellum from a dog infected with *Toxoplasma*

a. Before you start, think about the structure of the cerebellum and the range of tissues and cells that you might expect to see in a section
b. Now look for small round pink bodies embedded in the cerebellum tissues – these are bradyzoite cysts. These will be visible at lower levels of magnification. A closer look should show that each cyst contains a large number of very small blue dots – each one is the nucleus of an individual bradyzoite. Now answer the following questions
    i. Bradyzoite cysts are often found in clusters rather than spread throughout the tissue – why might this be?
        1. During asexual reproduction in the tachyzoite stage neighbouring cells become infected from an initial infection giving rise to a focus of infected cells
    ii. What impact do you think these structures might have had on the health of the dog?
        1. CNS symptoms – this dog was admitted to the Hospital for Small Animals with severe fitting
    iii. Based on what you know about Toxoplasma, how do you think the dog became infected with the parasite?
        1. Probably eating meat containing bradyzoite cysts
    iv. How likely is it that the dog could have passed the infection on to other dogs?

1. Not at all unless the other dogs ate bits of the dog. However if they were fed the same diet then they might also be infected

Gross pathological changes

A few protozoa can be associated with definitive gross pathological changes in the host which can be seen with the naked eye or very low level magnification with a hand lens. These can then be followed up with stained histological sections to confirm the presence of the parasite

Start by looking at the photograph of a **sheep oesophagus infected with *Sarcocystis* -** the white blister-like structures are caused by infection with Sarcocystis and are called Sarcocysts. Based on what you know about Sarcocystis from the lectures answer the following questions

a. Which group of parasitic protozoa does Sarcocystis belong to?
   - The coccidia
b. What other names might be used for these structures?
   - Sarcocysts
c. What is the importance of the Sarcocysts to the parasite's survival?
   - Contain bradyzoites – the infective stage for the next host
d. Which other hosts are likely to be involved in the parasite's life cycle?
   - A carnivore such as a dog or cat

Now look at the stained section from an infected oesophagus
First of all look at the slide by eye – the large clear area is the inside of a large sarcocyst - try estimating the diameter of the cyst remembering that the whole Sarcocyst is derived from a single living host muscle cell – **what other name might be given to the Sarcocysts**

- Bradyzoite cysts

Now use the microscope to examine the structure of the wall area of the cyst making sure that you can see the parasite material is located around the periphery of the cyst appearing as a thick purple-pink layer – these are the bradyzoites – **what is the role of the bradyzoite stage in the epidemiology of infection with *Sarcocystis*?**

- Infection of the next host

Look at other parts of the section and see if you can find smaller cysts which do not have an unstained central area – **why do you think different sized cysts develop in the same host tissue?**

- Large cysts develop on the outside of organs where there is room to expand, smaller ones are constrained by the muscle

Stop and think - all the techniques that you have looked at today are designed to detect the presence of the parasite in the host. Which factors do you think affect the reliability of these techniques for the routine diagnosis of infection with parasitic protozoa? Which other techniques can you think of that you might be able to use for the diagnosis of parasitic protozoa that does not depend on demonstrating the presence of the organism?

i. Some require the parasite to be present in large numbers e.g. wet-film
ii. Some require biopsy or are only available post-mortem
iii. Other methods – PCR or serological methods based on antibody or antigen detection e.g. ELISA