ASP ADVANCED COURSE
The Australian Society for Parasitology Inc.
ANU KIOLOA COASTAL CAMPUS, NSW

Concepts in Parasitology

A two-week parasitology course for ECRs and postgraduates



Diagnostic Parasitology

Practicals

Lecturer: Prof Peter O'Donoghue

Department of Parasitology
School of Chemistry and Molecular Biosciences
The University of Queensland

Diagnostic Parasitology

Intended Learning Outcomes:

The diagnosis of parasitic infections is a holistic integrated science involving inductive and deductive inference using multiple clinical/paraclinical parameters covering host-parasite biology, morphology, physiology, biochemistry and immunology. Accurate diagnosis is mandatory for effective treatment and control.

It essential that students get to know the parasites themselves in order to understand the ways in which they interact with their hosts and cause disease, as well as to understand the logic behind different diagnostic techniques.

By applying fundamental parasitology knowledge in a paraclinical context, students will develop their skills to:

- differentially diagnose the major parasitic groups in host tissues, fluids and fomites;
- deduce patterns and modes of transmission from their sites of infection;
- indicate their virulence/pathogenicity for different hosts and demographic groups;
- identify boundaries to their zoogeographic distribution and abundance;
- assess their significance with respect to human and animal health and welfare; and
- suggest appropriate management strategies (treatment, prevention, control).

This one-day course is divided into four modules (short lecture immediately followed by practical/workshop)

Lecture 1: Principles of diagnosis
(terminology, techniques, requirements, outcomes, OH&S)

Practical 1: Results (dry lab using paper-based calculations/exercises)
(medical testing, interpretation, manipulation, consequences)

Lecture 2: Working with faeces
(techniques, concentration, counts, culture)

Practical 2: Coprology (wet lab using faecal samples)
(centrifugal flotation, faecal egg count, microscopy of preserved samples)

Lecture 3: Working with blood
(techniques, concentration, counts, culture)

Practical 3: Haematology/Serology (wet lab using blood samples)
(smears, haematocrits, haemagglutination, microscopy of preserved samples)

Lecture 4: Working with tissue
(techniques, concentration, counts, culture)

Practical 4: Histology (wet lab using tissues samples)
(squashes, digests, microscopy of preserved samples)

Parasites range greatly in size - covering seven orders of magnitude. Some adult tapeworms are several metres in length (10^{1}) while some protozoa are only several microns in size (10^{-6}). Despite such size variation, most parasite developmental stages used for diagnostic purposes (cellular and subcellular features) are microscopic in size, thus parasitologists need to develop good microscopy skills. Optimal illumination systems should be established for stained specimens, while the examination of unstained specimens requires suboptimal or special illumination to introduce contrast into the specimen (e.g. diffraction, phase-contrast, differential interference-contrast). Please read the following instructions (even if you think you know how to drive a microscope properly).

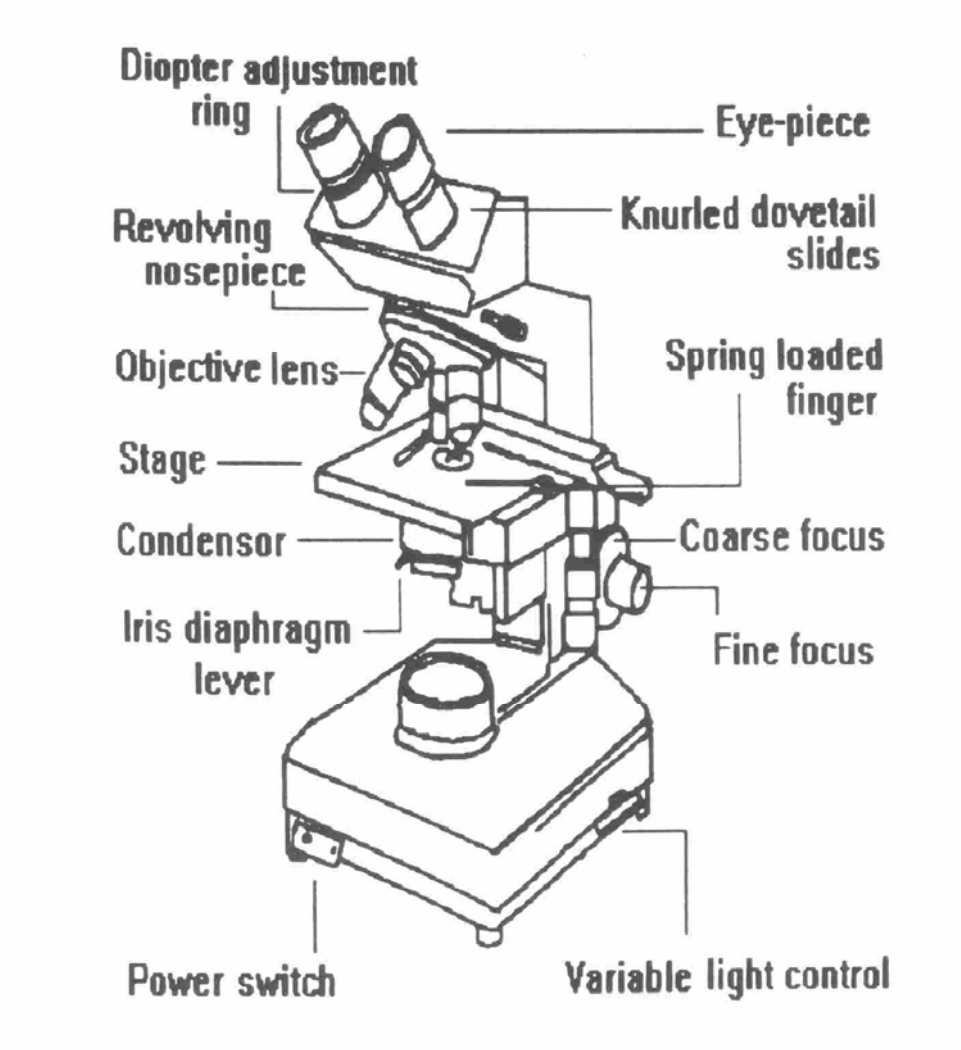
Microscopy

The light microscope is one of the most important tools available in the study of microorganisms. The amount of information one can get from a light microscope depends not only on the type of microscope, but also to a large degree on how the microscope is set up and how well it is cared for. You can also see more detail through the microscope by eye than revealed by photomicroscopy (photos do not do most parasites justice).

When you carry your microscope, always use both hands. Grasp the microscope arm firmly with one hand and lift it carefully. Place your other hand under the base of the microscope for support as you carry it. Keep the microscope vertical to ensure that the ocular lens does not fall out. **Each time** you use your microscope, clean the optical system (ocular lens, objectives, condenser lens) before and after use. Clean the oil immersion lens last so that you do not transfer oil onto the other lenses. Use lint-free Kim wipes to clean the lenses. DO NOT USE FACIAL TISSUES. Avoid touching any of the optical system with your fingers, as skin oils can be difficult to remove. When using oil immersion, always double check that it is the oil immersion (100x) lens that you are lowering into the oil. It is clearly identifiable by a black band around its base. The other objectives are not designed to be used in oil and may be damaged if used in this way. If you accidentally lower the wrong lens into oil, clean it immediately with Kim wipe tissue. When you have finished with your microscope for the day and have cleaned it properly, swing the lowest power objective into position. This is to prevent the other two longer objectives from being accidentally lowered into the condenser.

Setting up the binocular light microscope for oil immersion microscopy

a) Identify the various parts of your microscope using following figure. Ask instructors if you are unsure.
b) To obtain optimal conditions of illumination for the microscope, use the following procedure:
    1. Switch on the microscope and adjust the voltage control dial to high.
    2. Lower the stage by means of the coarse adjustment and then swing the 10x objective into place.
    3. Raise the condenser as far as it will go. The plane surface of the condenser should be almost in line with the level of the stage. Ensure that the condenser is properly in its mount.
    4. Place a microscope slide containing a specimen in the spring loaded specimen holder. NOTE: The specimen must be on the upper side of the slide. Take care to release the spring loaded holder carefully, so as not to break or damage the slide. If any fragments of slide fall onto sliding surfaces of the microscope, damage may result.
    5. Focus on the slide using coarse and then fine focus adjustment knobs.
    6. Looking through the binocular tube, adjust the inter-pupillary distance between the sliding eyepieces until binocular vision is obtained; and then focus both eyepieces to suit your eyes (use the fine focus adjustment knob to focus the specimen for the eye using the non-adjustable eyepiece turret, then use the adjustable eyepiece turret to focus the specimen for the other eye).
    7. Close the iris diaphragm completely and then open it until the field is just fully illuminated. This does not mean that the iris diaphragm needs to be fully open.
    8. If the intensity of light is too great it should be decreased by turning the voltage control dial. .
    9. Without altering the focus, bring the 40x lens into position. Focus on the slide using fine focus only. Again adjust the iris diaphragm to give a well illuminated field.
    10. Without changing the focus, swing the 40x lens away so that the 100x objective is the next objective to come into position. To use the oil immersion (100x) objective, place a drop of oil on the slide and carefully swing the oil immersion objective into place (it should end up in the oil). Provided you have not shifted the course focus, the field should be visible and only in need of fine focusing. It is important to be slow and cautious in turning the fine focus to ensure that breakage of the slide and damage to the lens system does not occur. Remember the extremely short working distance available to you when using the oil-immersion objective. If the specimen is weakly stained or has a low number of cells, it can be very easy to miss. If you have lifted the objective out of the oil, you have definitely gone too far. Try focusing on the smear with low power again before repeating the above step. If you still can't find anything, try another field. If this doesn't work, ask your instructors.
c) Always wipe off immersion oil from the objective lens after viewing, using lint-free Kim wipe tissues. Wipe the lenses in the following order: 10x first, then 40x, then 100x.



Eyepiece: View specimens through the 10X eye-pieces after you have adjusted their distance apart for your eyes using the knurled dovetail slides and focussed both lenses for binocular vision using the diopter adjustment ring.

Objective lens: You can change the power of magnification by using different objective lenses fitted onto the revolving nosepiece. Most microscopes are fitted with 10x, 20x, 40x and 100x objective lenses (giving final magnification of 100x, 200x, 400x and 1,000x).

Condenser: This is a system of lenses whose function is to concentrate light on the object. It contains an iris diaphragm which can be opened or closed to vary the amount of illumination.

Variable light control: This rotating or slide control permits adjustment of brightness.

Stage: This flat plate supports the microscope slide containing the specimen. The slide is held in place by a spring-loaded lever and the whole stage is moved by means of two rotating knobs.

Course focus: This is a large serrated screw which is attached to the upper part of the limb. Its function is to alter the distance between the object under examination and the objective. If stiff, report it to a tutor.

Fine focus: This is a smaller serrated screw which is attached to the limb. Its function is to finely adjust the distance between the object under examination and the objective.

PARASITE DIAGNOSIS ≠ PARASITE TAXONOMY

Diagnostic parasitology seeks to identify the aetiological (causative) agent of disease thus enabling appropriate management (treatment/control) options. Diagnosis is based on a sound working knowledge of the taxonomy of living organisms, but the identification/characterization/classification of individual parasite taxa (species/strains/genotypes) is not the primary aim. Often, the identity of the actual parasite species is not required by the clinician, e.g. knowledge that gastro-intestinal nematodes have been implicated as the cause of neonatal scours may be sufficient to commence treatment with an anthelmintic.

Diagnostic parasitology is not a dry esoteric exercise conducted by technicians/scientists in remote laboratories, but rather a detailed reasoning exercise conducted by the clinician who interacts with the patient and is ideally cognizant of most relevant facts and uses a variety of technologies and support services to gain further knowledge to make a differential diagnosis.

While the mental processes involved in clinical reasoning and differential diagnosis are difficult to categorize (involving many cognitive and metacognitive processes), there are certain things that are obviously known to the clinician which can be used to make some common sense predictions.

- The clinician should always know the host species involved - medical practitioners should recognize their patients as humans, and veterinary practitioners should be able to identify the animal species they are working on. This simple information already allows to clinician to consciously/subconsciously access information on host specificity, host range, host distribution, host susceptiblity/resistance, etc.

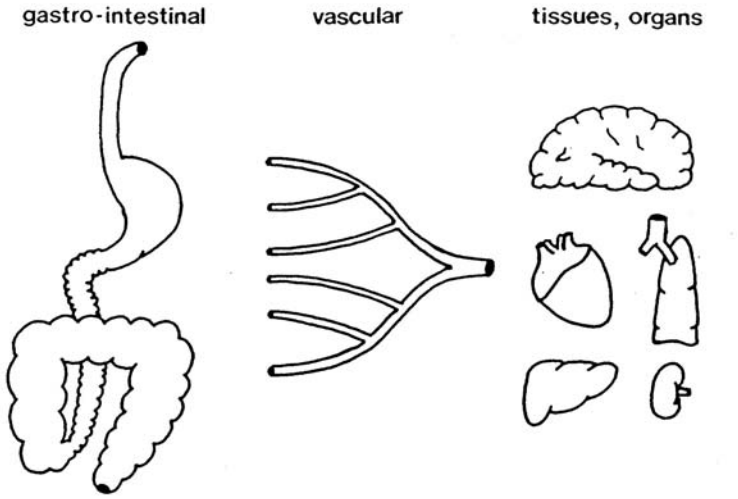
- The clinician should be able to determine why the patient has presented – symptoms can be described, signs can be observed. This allows an assessment of how serious and urgent the situation may be, as well as helps to pin-point the tissue/organ systems involved. Most parasites have predilection sites of infection and exhibit tissue trophism and this information helps to facilitate diagnosis, to assess disease development and progression, and to predict possible outcome (prognosis).

- Samples can then be collected for further examination, and it is a poor clinician who does not know that they are up to their armpits in faeces, urine, vomitus, sputum, blood, tissue aspirates, tissue biopsies, etc. In selecting specific samples, the clinician is seeking further information to help rule in or rule out certain aetiological agents. It is also up to the clinician to request the most appropriate tests to be conducted (e.g. full blood count, differential, haematocrit, liver enzymes, worm egg count, etc.).

However self-evident, all of this host-parasite information helps in the clinical reasoning process so that the clinician can make a working diagnosis to begin disease management. In addition to providing detailed information about the specific case in hand, it also allows the clinician to predict the mode of transmission of the parasite so that preventive measures can be implemented to avoid further cases. Clinicians not only have a responsibility for their individual patients, but also a broader responsibility for the general community to identify disease clusters, prevent outbreaks, recommend control strategies, etc.

As a broad generalization, there are three major integrated (common-sense) concepts:

THERE ARE THREE MAIN SITES OF INFECTION!
THERE ARE THREE MAIN MODES OF TRANSMISSION!
THERE ARE THREE MAIN TYPES OF PARASITIC DISEASE!



enteritis, diarrhoea	fever, anaemia	lesions, malfunction
faecal-oral transmission	vector-borne transmission	predator-prey transmission

Knowledge of the site of infection helps explain the clinical signs and predicts the mode of transmission:
e.g. many parasites in the gut cause enteritis and diarrhoea and are transmitted by faecal-oral route
many parasites in the circulation cause fever and anaemia and are vector-borne
many parasites in the viscera cause lesions and organ malfunction and are transmitted by carnivorism

THERE ARE THREE MAIN PARASITE ASSEMBLAGES!

Many types of organisms have adopted a parasitic mode of existence; that is, they require a host for their own survival. Three major groups of parasites are recognized: protozoa (belonging to the kingdom Protista), and helminths and arthropods (belonging to the kingdom Animalia, or Metazoa).

PROTOZOA
amoebae flagellates sporozoa ciliates

HELMINTHS
nematodes cestodes trematodes

ARTHROPODS
arachnids insects

PROTOZOA are eukaryotic organisms (with a membrane-bound nucleus) which exist as structurally and functionally independent individual cells (including those species which are gregarious or form colonies). None have adopted multicellular somatic organisation characteristic of metazoan organisms. Instead, protozoa have developed relatively complex subcellular features (membranes & organelles) which enable them to survive the rigours of their environments. Four main groups of protozoa are recognized on the basis of their locomotion using specialized subcellular and cytoskeletal features:

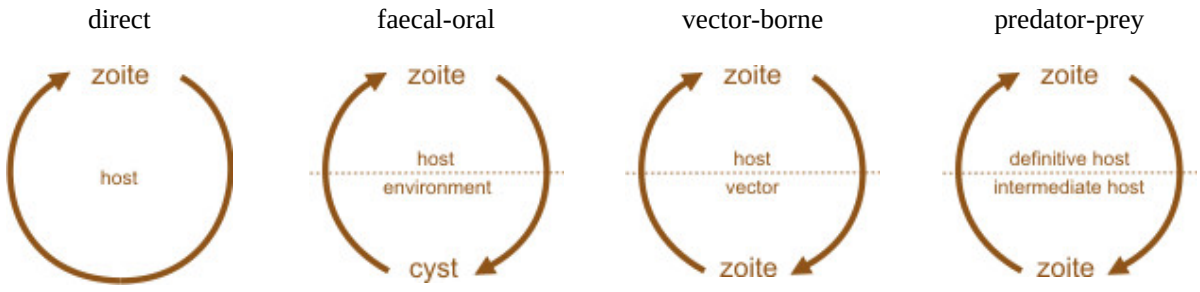
- **Amoebae** use pseudopodia to creep or crawl over solid substrates. Pseudopodia (or 'false feet') are temporary thread-like or balloon-like extensions of the cell membrane into which the protoplasm streams. Similar amoeboid motion has been observed in cells of many life-forms, esp. phagocytic cells.

- **Flagellates** use elongate flagella which undulate to propel the cell through liquid environments. Flagella are 'whip-like' extensions of the cell membrane with an inner core of microtubules arranged in a specific 2+9 configuration (this configuration is conserved throughout eukaryotic biology).

- **Ciliates** use numerous small cilia which undulate in waves allowing cells to swim in fluids. Cilia are 'hair-like' extensions of the cell membrane similar in construction to flagella but with interconnecting basal elements facilitating synchronous movement.

- **Sporozoa** form non-motile spores as transmission stages. Recent studies have shown that many pre-spore stages move using tiny undulating ridges or waves in the cell membrane imparting a forward gliding motion, but the actual mechanisms involved are not yet known.

Most protozoa have enormous reproductive potential because they have short generation times, undergo rapid sequential development and produce large numbers of progeny by asexual or sexual processes. These characteristics are responsible for many protozoan infections rapidly causing acute disease syndromes. Within hosts, feeding trophozoites may be found intracellularly (within host cells) or extracellularly (in hollow organs, body fluids or interstitial spaces between cells). While trophozoites are ideally suited to their parasitic mode of existence, they are not very resistant to external environmental conditions and do not survive long outside of their hosts. To move from host-to-host, protozoan parasites use one of four main modes of transmission: direct, faecal-oral, vector-borne and predator-prey transmission.



HELMINTHS are multicellular eukaryotic invertebrates with tube-like or flattened bodies exhibiting bilateral symmetry. They are triploblastic (with endo-, meso- and ecto-dermal tissues) but the platy-helminths (flatworms) are acoelomate (do not have body cavities) while the nemat-helminths (roundworms) are pseudocoelomate (with body cavities not enclosed by mesoderm). Parasitic helminths are an almost universal feature of vertebrate animals; most species have worms in them somewhere. Three major assemblages of parasitic helminths are recognized:

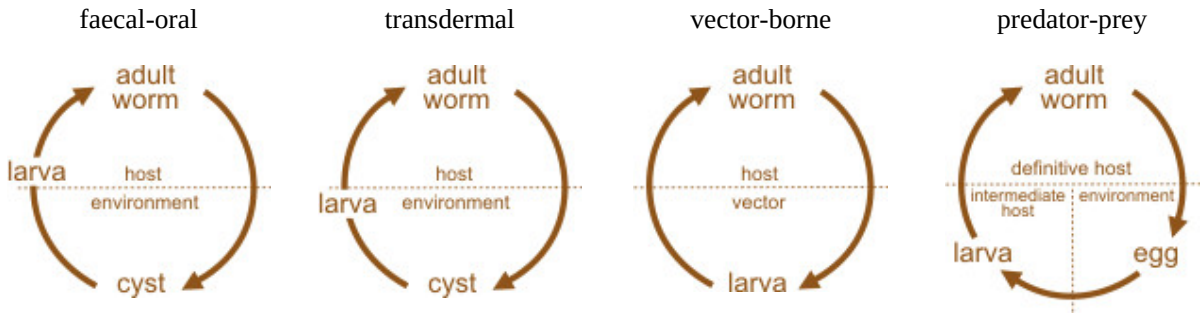
- **Nematodes** (roundworms) have long thin unsegmented tube-like bodies with anterior mouths and longitudinal digestive tracts. They have a fluid-filled internal body cavity (pseudocoelum) which acts as a hydrostatic skeleton providing rigidity (so-called 'tubes under pressure'). Longitudinal muscles produce a sideways thrashing motion. Adult worms form separate sexes with well-developed reproductive systems.

- **Cestodes** (tapeworms) have long flat ribbon-like bodies with an anterior holdfast organ (scolex) and numerous segments. They do not have a gut and all nutrients are taken up through the tegument. Segments exhibit slow body flexion produced by longitudinal and transverse muscles. All tapeworms are hermaphroditic and each segment contains both male and female organs.

- **Trematodes** (flukes) have small flat leaf-like bodies with oral and ventral suckers and a blind sac-like gut. They exhibit elaborate gliding or creeping motion over substrates using compact 3-D arrays of muscles. Most species are hermaphroditic (individuals with male and female reproductive systems) although some blood flukes form separate male and female adults.

Helminths do not proliferate within their hosts, but grow, moult, mature and produce offspring which are voided to infect new hosts. The severity of infection is often dependent on the number of infective stages taken up. Worms develop slowly compared to other infectious pathogens so any resultant diseases are slow in onset and chronic in nature. Most helminth infections are well tolerated by their hosts, although subclinical infections have been associated with loss of condition. Other helminths cause serious diseases characterized by high morbidity and mortality. Clinical signs of infection vary considerably. Larval and adult nematodes lodge, migrate or encyst within tissues resulting in obstruction, inflammation, oedema, anaemia, lesions and granuloma formation. Infections by adult cestodes are generally benign, but the larval stages penetrate and encyst within tissues leading to inflammation, space-occupying lesions and organ malfunction. Adult flukes usually cause obstruction, inflammation and fibrosis in tubular organs, but the eggs of blood flukes can lodge in tissues causing extensive granulomatous reactions and hypertension.

Helminths form three main life-cycle stages: eggs, larvae and adults. Nematodes produce eggs that embryonate *in utero* or outside the host. The emergent larvae undergo 4 moults before they mature as adults. Cestode eggs release oncospheres which are ingested by intermediate hosts where they penetrate tissues and become encysted. When eaten by definitive hosts, they excyst and form adult tapeworms. Trematodes have more complex life-cycles where 'larval' stages undergo asexual amplification in snail intermediate hosts. Eggs hatch to release free-swimming miracidia which actively infect snails and multiply in sac-like sporocysts to produce numerous rediae. These stages mature to cercariae which are released from the snails and either actively infect new definitive hosts or form encysted metacercariae on aquatic vegetation which is eaten by definitive hosts. The four main modes of transmission by which the helminths infect new hosts are faecal-oral, transdermal, vector-borne and predator-prey transmission:



ARTHROPODS form a huge assemblage of small coelomate animals with "jointed limbs" (hence the name arthro-pods). They exhibit segmentation of their bodies (metamerism) which is often masked in adults because their 10-25 body segments are combined into 2-3 functional groups (called tagmata). They exhibit varying degrees of cephalization whereby neural elements, sensory receptors and feeding structures are concentrated in the head region. Arthropods possess a rigid cuticular exoskeleton consisting mainly of tanned proteins and chitin. The exoskeleton is usually hard, insoluble, virtually indigestible and impregnated with calcium salts or covered with wax. The exoskeleton provides physical and physiological protection and serves as a place for muscle attachment. Skeletal plates are joined by flexible articular membranes and the joints are hinges or pivots made from chondyles and sockets. Most parasitic arthropods belong to two classes: the 6-legged insects, and the 8-legged arachnids

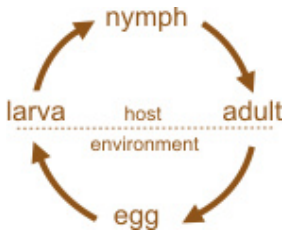
- **Insects** have 3 distinct body parts, commonly called the head, thorax and abdomen. The head has 2 antennae and the thorax has 6 legs arranged in 3 bilateral pairs. Many insect species also have 2 pairs of wings attached to the thorax. Parasitic insect species include fleas, flies and lice which actively feed on host tissues and fluids at some stage in their life-cycles

- **Arachnids** have 2 body parts known as the prosoma (or cephalothorax) and opisthosoma (or abdomen). The cephalothorax has 8 legs arranged in 4 bilateral pairs and arachnids do not have wings or antennae. Important parasitic assemblages include the ticks and mites which bite into tissues and feed off host fluids.

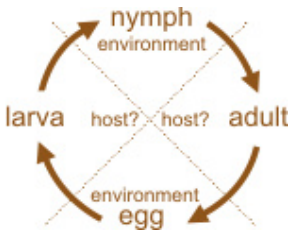
Adult arthropods are generally small in size, most are visible but some remain microscopic. Arthropod sexes are separate and fertilization is internal. A wide range of mating behaviours, insemination and egg production strategies are involved. In most species, the egg develops into a larva: i.e. a life-cycle stage that is structurally distinct from the adult and must undergo metamorphosis (structural reorganization) before becoming an adult. This metamorphosis may be complete (involving major changes during a pupation stage) or incomplete (involving gradual changes in nymph stages). For example, the grub-like larval stages of flies and fleas form cocoon-like pupae where they undergo complete metamorphosis and emerge as radically-different adult insects. In contrast, the larval instars (or nymphs) of lice, ticks and mites undergo incomplete metamorphosis through a series of moults gradually becoming more adult-like in appearance

Arthropods are involved in nearly every kind of parasitic relationship, either as parasites themselves or as hosts/vectors for other micro-organisms (including viruses, bacteria, protozoa and helminths). They are generally ectoparasitic on, or in, the skin of vertebrate hosts. Many species are haematophagous (suck blood) while others are histophagous (tissue-feeders) and bite or burrow in dermal tissues causing trauma, inflammation and hypersensitivity reactions. Infestations are transmitted from host-to-host either by direct contact or by free-living larvae or adults actively seeking hosts.

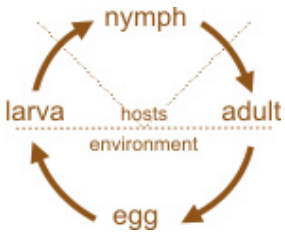
direct



host-seeking
(larva or adult parasitic)



host-seeking
(all feeding stages parasitic)



Workshop 1: Interpretation of Diagnostic Tests

A variety of medical and veterinary tests are used to diagnose diseases. How good they are depends on many factors, both technical and biological. These tests are not 100% perfect for a variety of reasons; such as:

- test cannot identify recently acquired infections (e.g. lag in test parameter formation);
- test cannot differentiate between chronic infection and previous exposure (e.g. antibodies persist);
- test cannot detect low dose infections (e.g. test below detectable threshold); and
- test cannot identify specific infections (e.g. cross-reactivity between parasites).

It is therefore important that users of any particular test know how good it is (as determined by objective quantitative assessment). Test efficacy is quantified by comparing results with disease status in a reference population (disease status being the 'gold' standard). Regrettably, gold standard tests are not always available so test efficacy may be poorer than reported as they were compared against semi-flawed tests. Bayes' theorem is used to determine test accuracy, sensitivity, specificity, and predictive values. These parameters (usually expressed as percentages) are included in the product information accompanying all commercial test kits

Four outcomes are possible when testing the population: as shown in the following 2x2 matrix:

		DISEASE		
		Present	Absent	
TEST	Positive	A	B	A+B
	Negative	C	D	C+D
		A+C	B+D	A+B+C+D = N

Test results (and consequences):

A = true positive: test diagnoses disease, facilitating treatment.
B = false positive: test falsely diagnoses disease, resulting in unnecessary treatment.
C = false negative: test falsely rules out disease, allowing disease progression, death
D = true negative: test rules out disease, suggesting other cause.

Prevalence (proportion positive or diseased at a particular point in time)

Disease prevalence = (A+C)/N [total diseased over total population]
Test prevalence = (A+B)/N [total test-positive over total population]

Accuracy (most relevant when choosing test)

Test accuracy = (A+D)/N [total true results over total population]

Sensitivity (probability of positive test in diseased person)

Test sensitivity = A/(A+C) [true positives over total diseased]
Ideally, test will have low rate of false negatives, thus a negative test often rules out disease
[SNNOUT = SeNsitive test, Negative test rules OUT diagnosis]
When a disease is very serious and missing it will have dire consequences, select a sensitive test.

Specificity (probability of negative test in non-diseased person)

Test specificity = D/(B+D) [true negatives over total non-diseased]
Ideally, test will have low rate of false positives, thus a positive test often rules in disease
[SPPIN = SPecific test, Positive test rules IN diagnosis]
When a disease is suggested by other data, select a specific test to rule in a diagnosis.

Predictive values (probability of disease in test-positives, probability of non-disease in test-negatives)

Positive Predictive Value (PPV) = A/(A+B) [true positives over total positives]
Negative Predictive Value (NPV) = D/(C+D) [true negatives over total negatives]

Exercise 1. Calculation of IHAT sensitivity and specificity

Many tests have been developed to diagnose *Toxoplasma* infections. Early tests were based on the inoculation of necropsy or biopsy tissue samples into laboratory mice and then examining their brains for tissue cysts several weeks after inoculation.

Some regard these mouse inoculation tests (MIT) as the most accurate and sensitive tests as they amplify and recover parasites from host tissues. They have been used as the 'gold standards' in the assessment of a variety of less invasive ante-mortem tests, including the indirect haemagglutination test (IHAT).

The efficacy of the IHAT* was assessed against the MIT in a cohort of 100 patients permitting lymph node biopsy and serum sampling. The results are tabulated below:

patient	MIT	IHA titre
1	+	1/512
2	+	1/4096
3	-	<1/64
4	+	1/64
5	+	1/256
6	-	<1/64
7	+	1/1024
8	-	<1/64
9	+	<1/64
10	-	<1/64
11	-	1/64
12	+	1/2048
13	+	1/128
14	+	1/4096
15	-	<1/64
16	+	1/1024
17	+	<1/64
18	+	1/512
19	-	1/128
20	+	1/2048
21	+	1/128
22	-	<1/64
23	+	1/1024
24	-	<1/64
25	+	1/256
26	+	<1/64
27	+	1/4096
28	+	1/2048
29	-	<1/64
30	+	1/512
31	+	1/1024
32	+	1/256
33	+	1/2048

patient	MIT	IHA titre
34	+	1/4096
35	-	<1/64
36	+	1/512
37	-	1/64
38	+	1/1024
39	+	1/256
40	+	1/128
41	-	<1/64
42	+	1/128
43	-	<1/64
44	-	<1/64
45	+	1/4096
46	+	1/64
47	-	1/64
48	+	1/64
49	+	1/2048
50	+	1/64
51	+	1/1024
52	-	<1/64
53	-	<1/64
54	+	1/512
55	+	1/256
56	+	1/64
57	+	1/1024
58	+	1/512
59	-	<1/64
60	+	1/128
61	+	1/4096
62	-	<1/64
63	-	<1/64
64	+	1/1024
65	+	1/512
66	-	1/128

patient	MIT	IHA titre
67	-	1/64
68	-	<1/64
69	+	1/4096
70	+	1/256
71	+	1/512
72	+	<1/64
73	-	1/128
74	+	1/2048
75	+	1/256
76	+	1/256
77	+	1/1024
78	+	<1/64
79	+	1/4096
80	+	1/128
81	+	1/64
82	+	1/1024
83	+	1/512
84	-	1/64
85	+	1/2048
86	+	1/128
87	-	<1/64
88	+	1/1024
89	-	<1/64
90	+	1/4096
91	+	1/64
92	+	1/2048
93	+	1/1024
94	+	1/512
95	-	<1/64
96	+	1/64
97	-	<1/64
98	+	1/256
99	+	1/128
100	+	1/2048

*The manufacturers of the IHAT kit recommend that titres >1/64 be regarded as positive, and titres <1/64 (together with borderline titres = 1/64) be regarded as negative.

Questions:

1. Complete the 2x2 matrix:

		Reference Standard (MIT)		
		Positive	Negative	
IHA TEST	Positive	57	3	60
	Negative	13	27	40
		70	30	100

2. According to the MIT, what is the prevalence of infection?

   MIT prevalence = 70/100 = 70%

3. What is the seroprevalence of infection according to the IHAT?

   (remember, titres >1/64 are positive, titres <1/64 and =1/64 are negative)

   IHAT seroprevalence = 60/100 = 60%

4. How many false positive IHAT results occurred? What are the implications for these patients?

   3 false positives - needless treatment

5. How many false negative IHAT results occurred? What are the possible consequences?

   13 false negatives - disease to death

6. What is the sensitivity of the IHAT?

   sensitivity = 57/70 = 0.814

7. What is the specificity of the IHAT?

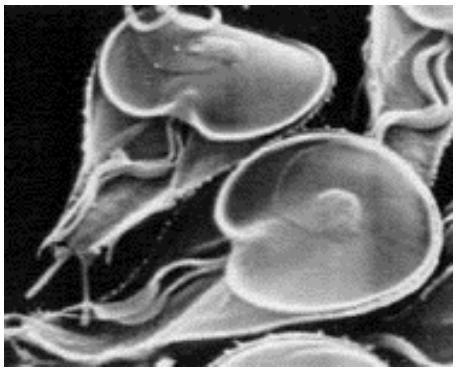
   specificity = 27/30 = 0.90

8. What are the positive and negative predictive values?

   PPV = A/(A+B) = 57/60 = 0.95
   NPV = D/(C+D) = 27/40 = 0.675

Exercise 2. Calculation of predictive values for *Giardia* testing.

A new enzyme immunoassay has been developed for *Giardia* infections which cause diarrhoea. In 512 faecal specimens sent to a diagnostic pathology laboratory by a local general practice clinic, the new test was compared with the reference standard test. The new test identified *Giardia* in 32 of the 33 *Giardia*-positive specimens and wrongly identified 14 *Giardia*-negative specimens as being positive.



Questions:

9. Complete the following table:

		Reference Standard		
		Positive	Negative	
TEST	Positive	32	14	46
	Negative	1	465	466
		33	479	

10. Calculate the sensitivity and specificity of the new test.

    Sensitivity = A/(A+C) = 32/33 = 0.97
    Specificity = D/(B+D) = 465/479 = 0.97

11. Calculate the positive and negative predictive values in this population of specimens.

    PPV = A/(A+B) = 32/46 = 0.70
    NPV = D/(C+D) = 465/466 = 0.998

12. How could you explain the difference in predictive values despite the similarity in sensitivity and specificity.

    PPV (probability that patient with positive test has *Giardia*) is low because there are too many false positives, while NPV (probability that patient with negative test does not have *Giardia*) is high because there are few false negative)

13. If patient X had the new test and it was negative, calculate the probability that patient X had *Giardia*.

    1 in 466 = 0.002

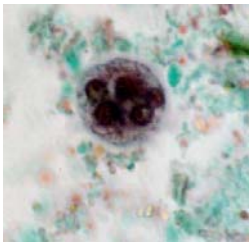
Workshop 2: Coprology (working with faeces)

In the next hour, you should complete the following three activities:

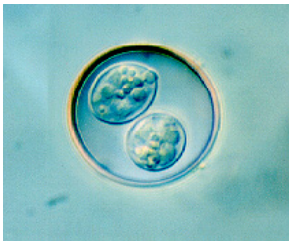
a) wet lab – conduct a centrifugal flotation of chicken faecal slurry for coccidial oocysts
b) wet lab – conduct a worm egg count using a McMaster/Whitlock chamber
c) dry lab – examine a range of gastroenteric parasites by light microscopy

Most gut parasites produce encysted/encapsulated stages (protozoal cysts and worm eggs) which are released into the environment to facilitate horizontal transmission between hosts. These stages are often produced in abundance and are very resistant to external environmental conditions – they can contaminate food and water supplies for some time. Once ingested, the cysts/eggs release their infectious cargoes to begin a new cycle of infection.

worm egg (*Ancylostoma*)



protozoal cyst (*Entamoeba*)



protozoal oocyst (*Isospora*)

Hosts react to gastro-intestinal disturbances by developing gastroenteritis (inflammation of the gut) often culminating in diarrhoea. It is important that diagnosticians score the matrix under test by characterizing the faecal sample on the basis of size (volume/weight), frequency, consistency, contents, colour and odour. Many diarrhoeic samples can be categorized as either small bowel watery malabsorptive diarrhoea or large bowel dysenteric bloody diarrhoea.

Faecal samples taken from clinically affected hosts are examined for the presence of protozoal cysts and/or worm eggs to help facilitate differential diagnosis. Diagnostic procedures include:

- fresh wet mounts (for live motile organisms)
- concentration techniques (for worm eggs and protozoal (oo)cysts)
    - sedimentation (in water, formalin-ether, iodine-trichrome)
    - floatation (in saturated sugar/salt solutions)
- permanent mounts (fixed stained smears of sample/concentrates)
- cultures (filter paper/Baermann funnel, inoculation into axenic/monoxenic tubes/plates)
- copro-antigens tests (DFA, EIA, dipstick)
- molecular techniques (extract DNA, PCR)
- sticky-tape test (pinworm)
- colonoscopy/endoscopy
- medical imaging (X-ray, ultrasound, CT, MRI)

Considerable practice is required to confidently identify parasite stages in faecal material by light microscopy. Novices frequently have problems with poor size perspective, wrong magnification, loss of plane of focus, lack of contrast, presence of air bubbles, artefacts and organic contaminants. Remember, you are generally working over two orders of magnitude between 100x and 1,000x magnification - worm eggs are relatively big (circa 100 μm), protozoa are smaller (circa 10 μm), whereas bacteria are very small (circa 1 μm). If the faecal preparation is unstained, you need to introduce contrast into your specimen (by lowering condenser, closing diaphragm, using phase-contrast or using differential interference contrast). However, if the specimen is stained, you need to optimize the illumination pathway for your microscope.

A range of common gastro-intestinal parasites have been selected for closer examination, but this list is illustrative only and by no means comprehensive. The parasites and some of their characteristics are tabulated below:

Code	Parasite Genus	Diagnostic stage	Notes	Endogenous stages	Notes
PROTOZOAN PARASITES					
F1	*Entamoeba*	cyst	round, 4 nuclei	trophozoite	amoeboid
F2	*Giardia*	cyst	oval, 2 nuclei	trophozoite	2 nuclei
F3	*Balantidium*	cyst	round, macronucleus	oval	macronucleus
F4	*Eimeria*	oocyst	oval, 4 sporocysts	schizonts/gamonts	clusters
F5	*Cryptosporidium*	oocyst	round, 4 sporozoites	meronts/gamonts	epicellular
F6	*Nosema*	spores	oval, refractile	sporonts	clusters
F7	*Trichomonas*	no cyst stage	STI	trophozoites	flagellated, + UM
HELMINTH PARASITES					
F8	*Enterobius*	eggs	asymmetrical	adult/gut DH	perianal pruritis
F9	*Trichuris*	eggs	bipolar plugs	adult/gut DH	prolapse
F10	*Ascaris*	eggs	mamillated	larvae/lungs, adult/gut	obstruction
F11	*Toxocara*	eggs	mamillated	larvae/tissues, adult/gut	larval migrans
F12	*Ancylostoma*	eggs	elliptical,	larvae/lungs, adult/gut	blood-sucker
F13	*Strongyloides*	larvae	eggs hatch in vivo	adult/gut DH	+ free-living cycle
F14	*Taenia*	eggs	striated wall	adult/gut DH	larva/tissues IH
F15	*Fasciola*	eggs	dark, operculated	adult/liver DH	larvae/snail IH

DH = definitive host, IM = intermediate host; UM = undulating membrane; STI = sexually-transmitted infection

a) Wet lab – Faecal concentration by centrifugal floatation

Infectious micro-organisms voided with host faeces contaminate the environment and are transmitted to new hosts via the ingestion of contaminated food and/or water. Many of the stages voided into the external environment are well protected for their free-living sojourn by being encysted within membranous casings which are relatively impervious to external environmental influences. Most exogenous stages are non-feeding and live on their own food reserves but they can live for long periods of time under the right conditions by being dormant with low metabolic requirements.

Helminths produce eggs, protozoa form cysts or oocysts, fungi form spores, bacterial cells have cell walls external to their plasma membranes and viruses form protein coats and sometimes membranous envelopes. These stages are designed to resist external environmental conditions and to survive passage to a new host. Within that host, they often must transit the acidic environment of the stomach until they reach the haven of the intestines. Given the right biochemical triggers (usually featuring bile salts, pH and carbon dioxide), they will excyst or initiate infection in the new host.

Worm eggs and protozoal (oo)cysts may sediment in water but they are quite buoyant in more viscous solutions, such as saturated sugar or heavy metal salt solutions (which have higher specific gravities). These solutions can therefore be used to harvest/concentrate eggs and (oo)cysts from faecal samples.

TECHNIQUE

- Add two drops (~ 200 μL) of mixed faecal suspension to a 10 mL centrifuge tube.
- Fill the tube to 10 mL with saturated magnesium sulphate solution (specific gravity 1.34)
- Centrifuge tube for 2 minutes at 2,000 rpm
- Carefully remove the tubes from the centrifuge (do not tilt, jar or disturb them in any way)
- Take a sample of meniscus from tube with bacteriological loop, place on slide and coverslip
- Examine under a light microscope (with condenser wound down) looking for oocysts
  [you are working on the right focal plane when you see small air bubbles trapped under the coverslip]

b) Wet lab – Worm egg count using a McMaster chamber

The diagnosis of gastro-intestinal nematode infections is frequently made by the examination of faecal samples for eggs shed by gravid female worms. In some instances, the concentration of eggs in the faeces correlates well with the intensity of infection by worms in the gut, thereby giving an indication of the severity of infection at that particular point in time. The differential diagnosis of individual worm species is often not possible because the eggs of many worm genera are similar in size, shape and appearance. Nonetheless, different groups of worms can be identified by differences in egg morphology.

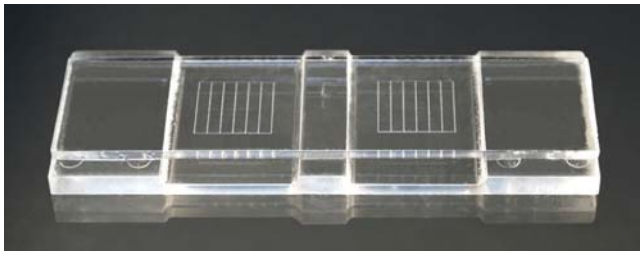
Various diagnostic tests have been developed for diagnostic use; including:

- direct mounts (MIF = merthiolate-iodine-formalin)
- faecal smears (various histochemical stains)
- concentration by sedimentation (eggs are denser than water and sink)
- concentration by floatation (eggs float in solutions with high specific gravity)
- Baermann filtration (for lungworm larvae)

The most accepted and convenient technique used by diagnostic labs specializing in work on domestic ruminants is the McMaster technique which is a quantitative method for enumerating the number of eggs per gram of faeces. The faecal egg count (fec) is a floatation concentration technique which uses heavy metal salt solutions of high specific gravity (SG~1.3) to float eggs from faecal matter so they can be readily detected by low power microscopy. They are used routinely for health surveillance monitoring, disease diagnosis, treatment strategies and drug resistance monitoring.

TECHNIQUE

- Weigh out 1 g of ruminant faeces and place in small mixing jar (e.g. plastic urine jars)
- Add 30 mL of saturated magnesium sulphate solution, and mix thoroughly using dipsticks
- Cut narrow tip off a 1 mL plastic disposable pipette and use broad pipette to fill one chamber on a McMaster/Whitlock counting slide (chamber volume = 0.5 mL)
- Examine under a light microscope at medium magnification
- Count the number of worm eggs which float to the surface beneath the etched area on the slide
- Try to identify worm genera on basis of egg morphology (see pictorial guide on next page)



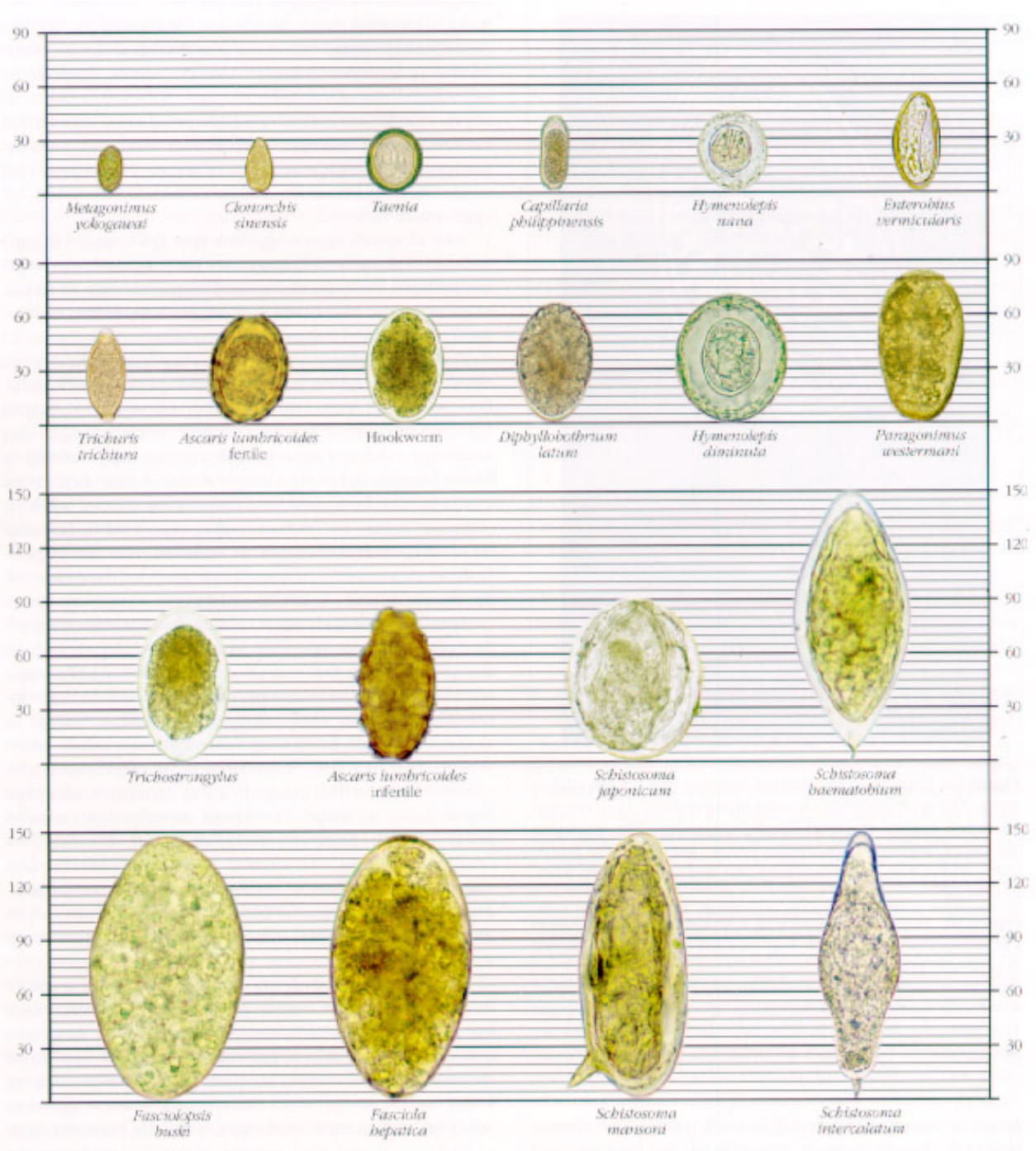
- Calculate the number of eggs per gram of faeces (epg). One chamber is 0.5 mL of an initial volume of 30 mL which contained 1 g of faeces, therefore epg = your count x 60.
  [Work it out on a piece of paper to make sure you understand it!]
- To increase the accuracy of the epg estimation, you could use more faeces, count more chambers and average the results. You must ensure that the faecal slurry is well mixed especially at the time of filling chambers as that will determine how accurate and similar your counts are.

INTERPRETATION: More eggs per gram of faeces suggests more worms in the gut, but:

- fecundity per worm may be reduced as intensity of infection increases, and
- infection may be in prepatent period (before egg laying commences), and
- host may be suppressing reproduction of nematodes

Therefore epg is an indication not an exact measure of what is going on inside the animal. Still, lots of eggs always means lots of worms. As a clinical guide, farmers are recommended to drench their animals if worm egg counts are greater than 1,000 epg.

Pictorial guide to representative helminth eggs (to scale)



FOR INFORMATION ONLY

Drench Resistance Testing

Nematode populations may be managed by a variety of anthelmintic drugs. At the turn of the century, various metal compounds (lead, tin, copper) were used to reduce worm burdens but they exhibited high toxicity to their hosts. They have now given way to synthetic compounds manufactured by the pharmaceutical industry. A very successful group of compounds called the benzimidazoles (or white drenches) were developed in the 60's followed by another group called the levamisoles (or clear drenches). The first group acted upon microtubules causing worm paralysis and expulsion while the second group blocked cholinergic neurotransmission causing paralysis and expulsion. Regrettably, worm populations have now developed resistance against both groups of drugs. A third group of compounds called the avermectins (ivermectin in particular) was developed in the 70's and they inhibited neurotransmission in worms. There are numerous reports of drench resistance against avermectins overseas and several in Australia.

Producers are therefore faced with difficult choices – which drench to use and will it be effective? A subsidiary industry has now emerged which screens flocks for drench resistance and recommends the most appropriate drug to use to control worm populations. These programs are called different names in different states e.g. Worm-Check, Worm-Buster, etc. Basically, they involve drenching test groups of sheep with white, clear, combination and/or avermectin drenches (plus an untreated control group) and then testing 10-14 days later to see if faecal egg counts have been reduced by at least 95% for the drench to be declared effective.

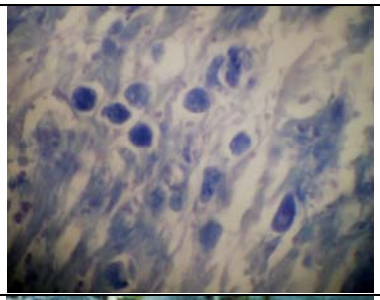
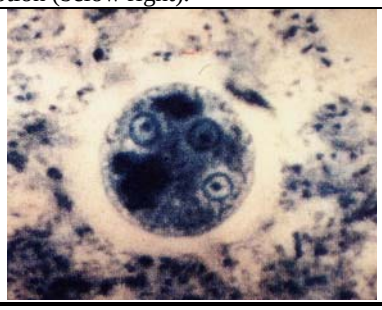
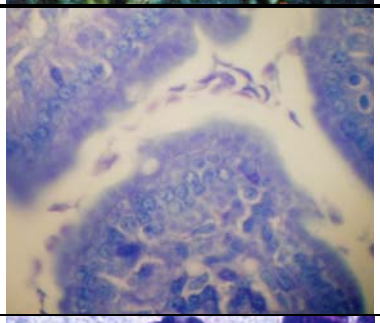
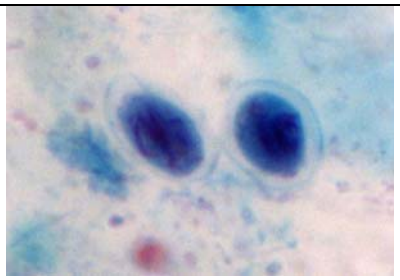
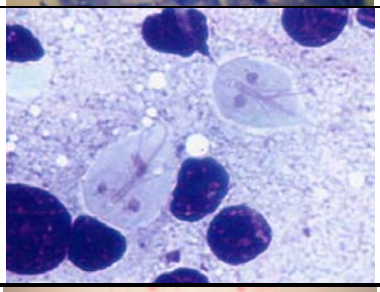
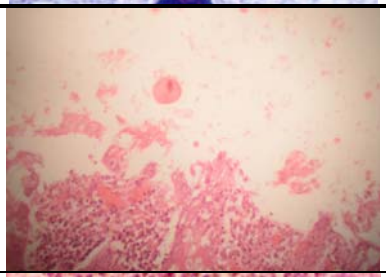
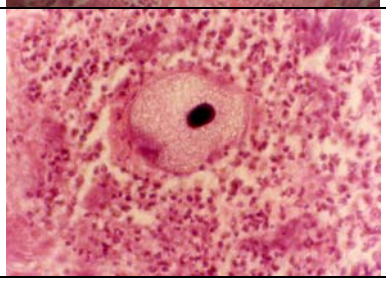
The following serves as a blank guide to drench resistance testing (provided for your information only).
Identify 4 groups of animals to act as:

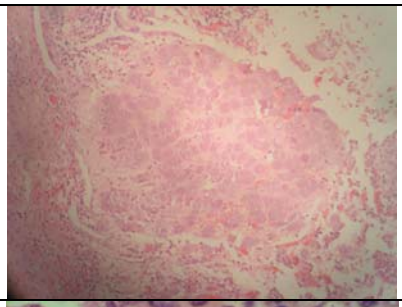
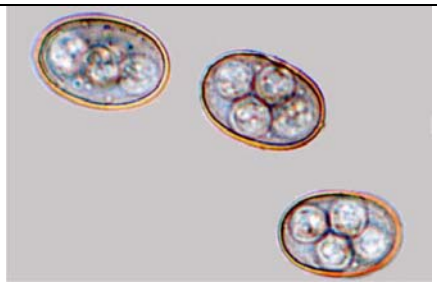
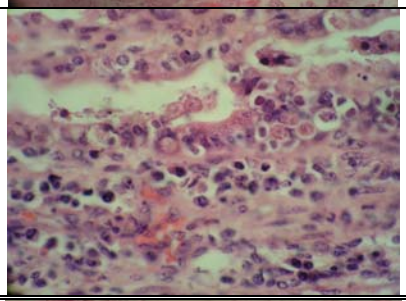
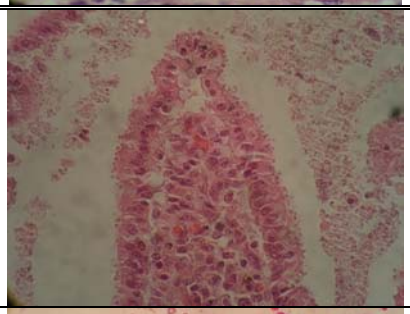
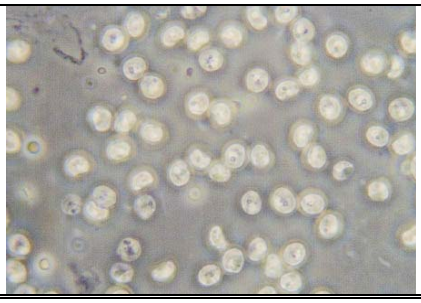
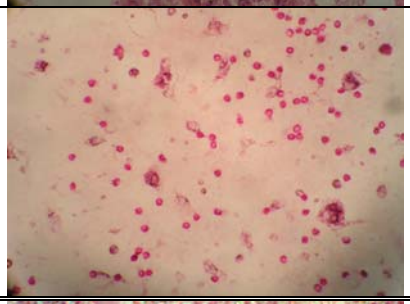
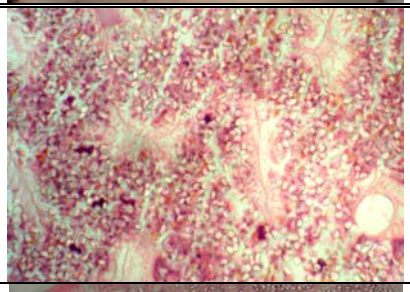
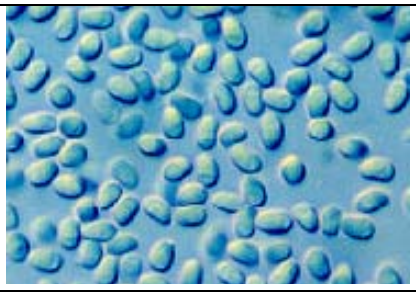
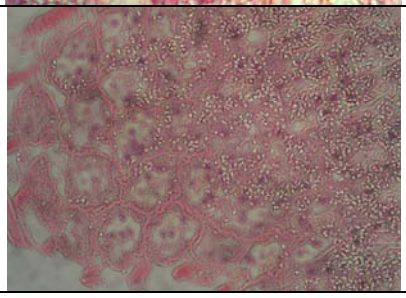
- group C = untreated controls
- group BZ = white benzimidazole drench
- group LV = clear levamisole drench
- group AV = avermectin drench

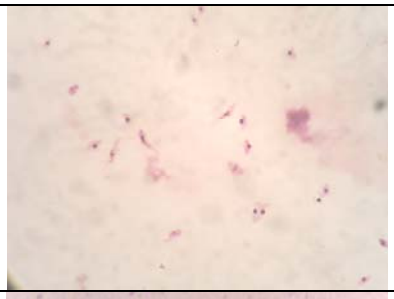
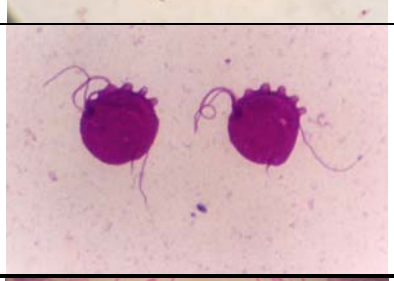
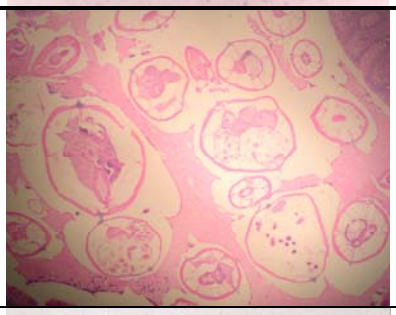
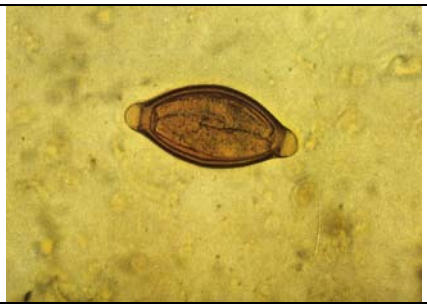
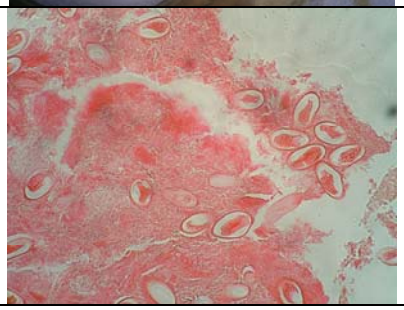
Approx. two weeks after treatment, collect faecal samples from animals in each group and conduct faecal egg counts (in duplicate). Compare the treated (t) and control (c) groups for evidence of drug resistance:

	untreated	treated		
Replicate	C (control)	BZ (white)	LV (clear)	AV (avermectin)
1				
2				
Average (X)				
% reduction = 100(1-Xt/Xc)				

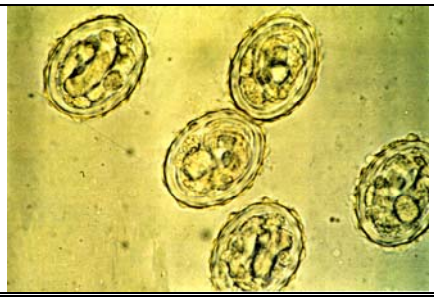
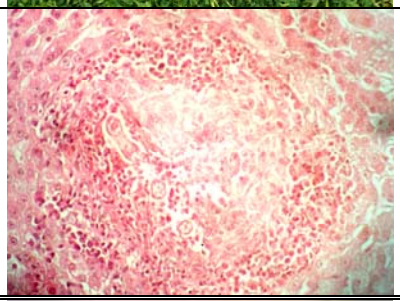
c) Dry lab – Examination of slide set by light microscopy (work with your neighbours)

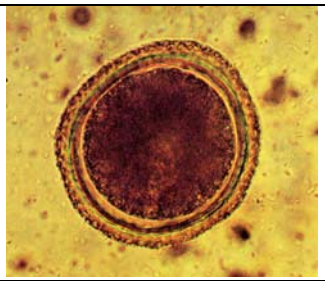
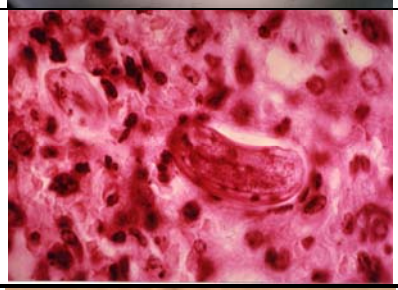
F1. (amoeba) ***Entamoeba histolytica*** trophozoites human large intestine stained section	Scan slide at low power looking for vacuolated areas containing basophilic trophozoites (right); examine invasive foci at higher power noting tissue destruction. The diagnostic stage in coprology is the spherical cyst with four vesicular nuclei (below). Examination of fresh wet faecal mounts sometimes reveals pleomorphic trophozoite stages exhibiting amoeboid motion (below right).	
		
F2. (flagellate) ***Giardia duodenalis*** trophozoites dog small intestines stained section	Examine the intervillous spaces in the intestines looking for numerous flattened (not rounded) trophozoites at the mucosal surface (right). The diagnostic stage for coprology is the ovoid cyst, often with a tangential axostyle and surrounding 'halo' (below). Examination of gut content or culture material reveals pyriform trophozoites with 2 nuclei (below right).	
		
F3. (ciliate) ***Balantidium coli*** trophozoites pig colon stained section	Scan the epithelial surface of the colon at low power looking for large eosinophilic trophozoites within erosive mucosal lesions (right). High power reveals the trophozoites to have a relatively enormous macronucleus (below right). The coprological diagnostic stage is the round cyst with conspicuous macronucleus (below).	
		

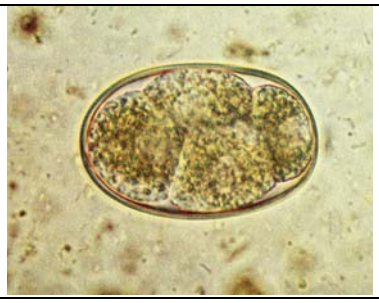
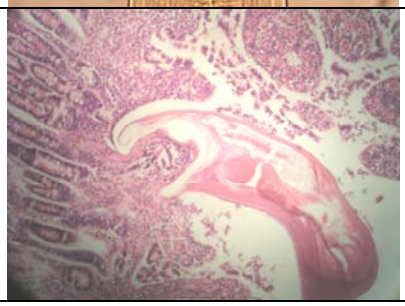
F4. (apicomplexan: coccidian) ***Eimeria* sp.** schizonts kangaroo small intestines stained section	Examine the gut mucosa looking for large patches of 'blue grapes' (clusters of basophilic schizonts filled with zoites) (right) or 'pink grapes' (developing oocysts with eosinophilic wall-forming bodies) (below right). Faecal examination of patent infections reveals oocysts with a typical 1:4:2 configuration (each oocyst with 4 sporocysts, each sporocyst with 2 sporozoites).	
		
F5. (apicomplexan: coccidian) ***Cryptosporidium parvum*** schizonts/gamonts various host tissues stained section	Scan the mucosal surface at high power to find patches of small round endogenous stages adherent to the epithelial cells (right). The diagnostic stages in coprology are small round oocysts which are refractile/phase-bright in faecal floats (below) and acid-fast in faecal smears stained with modified Ziehl-Neelson stain (below right)	
		
F6. (microsporan) ***Nosema apis*** spores bee gut stained smear/section	Examine the tissues at medium power magnification but with the condenser wound down to introduce diffractive contrast into the highly refractile microsporan spores distributed throughout the tissues (right, below right). High power observation reveals huge numbers of small ovoid spores characteristic for microsporan parasites (below).	
		

F7. (flagellate) ***Tritrichomonas foetus*** trophozoites bull preputial cavity stained smear	Carefully scan the smear at medium power to find small elongate trophozoites (right). Examine at high power to reveal anterior flagella and recurrent flagellum forming an undulating membrane running longitudinally down the length of the trophozites (below right). This parasite does not form a cyst stage, and is only transmitted venereally.	
	no cyst stage (transmission requires intimate body contact) (venereal rather than faecal-oral)	
F8. (pin-worm) ***Enterobius vermicularis*** adults human appendix stained section	Scan the slide at low power to locate sections through worms with distinct cuticle (note lateral spikes) (right). Female worms (below right) emerge from the anus and attach characteristic asymmetrical eggs (below right) to the skin where they cause perianal pruritis. Infections are best diagnosed by the "sticky-tape test" (imagine it done to you!).	
		
F9. (whip-worm) ***Trichuris trichiuria*** adult worm ex human intestine whole mount	Examine the worm at various magnifications noting details of head (thin anterior end), tail (thick posterior end), spicule/vulva, cuticle and internal organ systems (right). The diagnostic stage for coprology is the detection of characteristic ovoid eggs with protruding bipolar plugs in faecal samples (below, below right).	
		

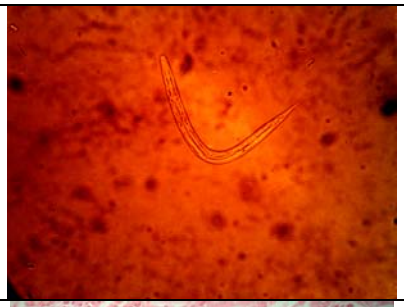
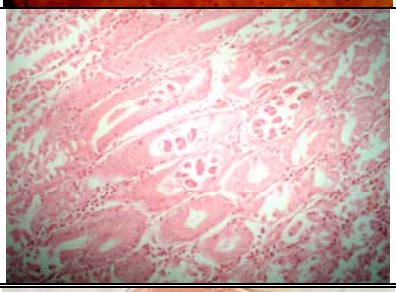
<table>
<tr><td rowspan="2">F10. (round-worm)

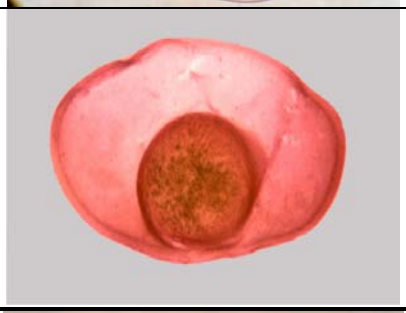
Ascaris lumbricoides
eggs
human faeces
wet mount</td><td>Carefully examine the wet mount for round ascarid eggs with thick pitted walls (below). The adults are large whitish roundworms (right) which may entangle causing intestinal obstruction. Larvae undergo an obligatory pulmonary migration causing transient lesions in the lungs (below right)</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">F11. (round-worm)

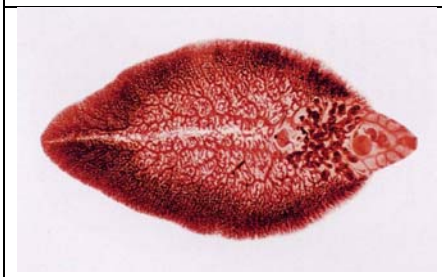
Toxocara canis
adult worm
ex dog/cat intestines
whole mount</td><td>Locate the head of the worm and note details of the buccal cavity, anterior oesophagus and presence of lateral alae (wings) (right). These worms lay distinctive round eggs with unsegmented contents and rough brown shells (below). Larvae also undergo a pulmonary migration and some may become dormant in host tissues (hypobiosis) (below right).</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">F12. (hook-worm)

Ancylostoma duodenale
adult worm
ex human intestines
whole mount</td><td>Examine the head and buccal cavity of this adult worm at medium to high power to observe the characteristic hooks (right) with which the worms bite host tissues (below right) to feed on blood. The diagnostic stage for coprology is the elliptical egg with thin wall and multicellular blastomere (non-larvated) contents (below right).</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
</table>

<table>
<tr><td rowspan="2">F13. (thread-worm)

Strongyloides stercoralis
larva
human faeces
whole mount</td><td>Wind down the condenser to introduce diffraction patterns into the specimen and carefully search the slide for small rhabditiform larva (right) as the eggs usually hatch in the gut. The worms may cause serious infections by burrowing into the gut mucosa (below right) causing catarrhal inflammation (and sometimes super-infection).</td><td>  </td></tr>
<tr><td>eggs not found in faeces
(usually hatch in vivo)</td><td>  </td></tr>
<tr><td rowspan="2">F14. (cestode (tape-worm))

Taenia solium
scolex (head)
ex human gut
whole mount</td><td>Examine the amazing attachment devices (hooks and suckers) on the scolex (head) of this tapeworm (right). Amazingly, the adults cause little disease. Round eggs with thick striated walls and hexacanth embryos are passed in faeces (below) and release oncospheres which form encysted larva (metacestodes) known as cysticerci (below right) in pig (and sometimes human) tissues.</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">F15(trematode (fluke))

Fasciola hepatica
miracidia from eggs
ex cow faeces in water
stained mount</td><td>Scan the slide looking for miracidial stages with prominent eyespots (right). These stages are released from eggs into water. They infect snails and undergo massive asexual amplification releasing cercariae (below right) which swim to water plants and form encysted metacercariae. Adult flukes (below) live in biliary spaces in the liver and pass eggs to the gut to be voided with faeces.</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
</table>

Workshop 3: Haematology (working with blood)

In the next hour, you should complete the following four activities:

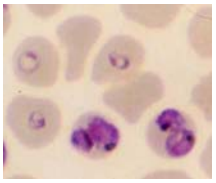
a) wet lab – prepare thin blood smear
b) wet lab – harvest buffy coat by microhaematocrit tube centrifugation
c) wet lab – conduct serological test (IHAT) for antibodies against *Toxoplasma*
d) dry lab – microscopy of blood parasites

Many parasites infect the blood of vertebrate hosts and rely on blood-sucking invertebrate vectors for their transmission between hosts. Invertebrate vectors include insects (flies, lice, fleas, bugs), arachnids (ticks, mites), and crustaceans (copepods). These vectors may act as definitive hosts (in which the parasites undergo sexual reproduction), intermediate hosts (in which the parasites only undergo asexual reproduction) or paratenic hosts (in which the parasites are simply transported without undergoing reproduction). The vectors are parasites in their own right, taking their nutrition by feeding on host fluids/tissues. Various types of blood parasites are transmitted by vectors; including kinetoplastid flagellates (trypanosomes), adelerione coccidia (haemogregarines), haematozoa (haemosporidia, piroplasms), as well as microfilarial stages of filarial nematodes.

Blood from vertebrate hosts is a lovely place to live (rich in nutrients and oxygen) provided the parasite can survive host homeostatic mechanisms (e.g. clotting) and immune defences (e.g. innate phagocytic and inflammatory responses and adaptive humoral and cell-mediated responses). Blood contains many cell types to infect: erythrocytes or red blood cells (anucleate in mammals, nucleate in birds, reptiles, fish); leucocytes or white blood cells (granulocytic neutrophils, basophils, eosinophils and agranulocytic lymphocytes and monocytes); as well as a viscous fluid medium (plasma) rich in sugar, proteins, etc. Many protozoan parasites specialize in infecting red blood cells in which they grow and divide, but with the consequence of lysing the cell thus contributing to host anaemia. Other parasites occur extracellularly in the plasma deriving nutrients at the expense of the host.

Samples of blood can be readily collected from the vasculature of many animals by peripheral venepuncture and dispensed into containers with or without anticoagulants. The cellular components of blood can be visualized for microscopy by staining blood films, and even harvested by various concentration techniques. Serum can be harvested from clotted blood and plasma from non-clotted blood by centrifugation and then examined for host antibodies or parasite antigens or parasite DNA. Various methods have been developed to examine blood samples, including:

- wet smears (for motile parasites)
- permanent stained blood films (thick/thin smears)
- concentration techniques
    - microhaematocrit tube centrifugation (buffy coat)
    - Knott's concentration (lyse rbc)
    - membrane filtration (e.g. Nucleopore)
    - gradient centrifugation (e.g. Hypaque, Ficoll)
- culture (*in vitro*, *in vivo*)
- immunoserology (host antibodies, parasite antigens)
- molecular techniques (extract DNA, PCR)



While many commercial antibody, antigen and DNA test kits have recently become available for testing blood samples, many of them are expensive and require special equipment or technical expertise. Diagnostic laboratories in many countries still rely on screening blood samples by conventional light microscopy of stained films. All parasitologists should become competent in producing good blood films and examining them by high power oil-immersion microscopy.

A range of blood parasites have been selected for closer examination, but this list is illustrative only and by no means comprehensive. The parasites and some of their characteristics are tabulated below:

Code	Parasite	Hosts	Blood stage	Notes	Endogenous stages	Notes	Vector
PROTOZOAN PARASITES							
B1	*Trypanosoma brucei*	vertebrates	trypomastigote	plasma, UM, kinetoplast	none	-	tsetse fly
B2	*Hepatozoon*	vertebrate IH	gamont	leucocytes	schizonts	viscera	arthropod DH
B3	*Haemogregarina*	reptile IH	gamont	erythrocytes	schizonts	viscera	leech, arthropod DH
B4	*Plasmodium falciparum*	human IH	rings/schizonts/gamonts	erythrocytes, pigment	schizonts	liver	mosquito DH
B5	*Plasmodium malariae*	human IH	rings/schizonts/gamonts	erythrocytes, pigment	schizonts	liver	mosquito DH
B6	*Plasmodium vivax*	human IH	rings/schizonts/gamonts	erythrocytes, pigment	schizonts	liver	mosquito DH
B7	*Hepatocystis*	bat IH	gamonts	erythrocytes, pigment	schizonts	liver	dipteran DH
B8	*Haemoproteus*	bird IH	gamonts	erythrocytes, pigment	schizonts	RE cells (viscera)	dipteran DH
B9	*Leucocytozoon*	bird IH	gamonts	white/red cells	schizonts	liver, then RE cells	blackfly DH
B10	*Babesia bigemina*	mammal IH	merozoites, gamonts	erythrocytes	none	-	tick DH
B11	*Babesia bovis*	mammal IH	merozoites, gamonts	erythrocytes	none	-	tick DH
B12	*Theileria*	mammal IH	merozoites, gamonts	erythrocytes	schizonts	lymphocytes	tick DH
HELMINTH PARASITES							
B13	*Dirofilaria immitis*	dog/cat DH	microfilaria	plasma	adults	heart	mosquito IH
B14	*Wuchereria bancrofti*	human DH	microfilaria	plasma	adults	lymphatics	mosquito IH
B15	*Loa loa*	human DH	microfilaria	plasma	adults	subcutaneous tissues	deer fly IH

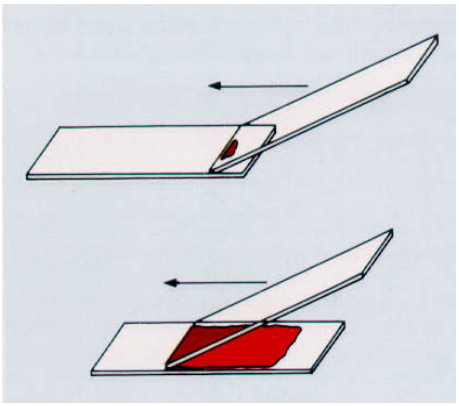
DH = definitive host, IM = intermediate host; UM = undulating membrane

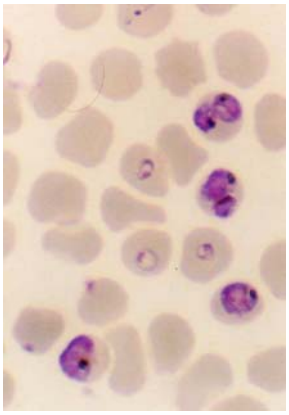
a) Wet lab - prepare blood smears

Stained blood smears are the mainstay of haematology – they are permanent mounts which can be examined both for haematological information (red/white cell numbers/characteristics) as well as foreign bodies/organisms (including haemo-parasites). A range of protozoa form developmental stages which are located within the blood of their hosts: including extracellular trypomastigotes of trypanosomes and intracellular meronts and/or gamonts of haemogregarines, haemosporidia and piroplasms. In contrast, fewer helminths form blood stages, mainly the filarial nematodes which produce extracellular microfilariae. All haemo-parasites utilize blood-sucking invertebrates (especially mosquitoes, flies and ticks) as vectors for horizontal transmission between hosts.

The preparation of a good blood smear requires considerable practice – trained haematologists just seem to know exactly how much blood to use, what angle to hold the spreader, how much pressure to exert during smearing, etc. The objective is to make a cell monolayer that tapers/feathers out along the slide. The cells can then be stained with Romanowsky stains (usually Giemsa) which highlights nuclei in both host and parasite cells. Thin blood smears are examined under a compound light microscope at high power (1,000 x magnification = 100 x objective multiplied by 10 x eyepiece) using immersion oil (care should be taken when rotating the objective lens nose-piece to ensure oil is not smeared on the other lenses). Clean up oil spills with tissue paper dipped in ethanol.

- Place a very small drop of blood (in anticoagulant) at one end of a clean glass slide using dipstick/pipette
- Place another slide just in front of the blood drop at angle of 45-60°
- Gently draw back until blood touches slide and streams to either side
- Gently drag out blood smear by pushing scraper slide the length of the first slide (as shown in diagram) (let the weight of the slide do the work, do not exert any pressure)
- You should end up with a thin monolayer blood smear which feathers out towards the end of the side (the common mistake is to use too much blood which does not feather out)
- Repeat until you obtain the perfect smear





The slide would now be fixed in methanol and stained with Giemsa, but we do not have enough time to process our own slides, and the blood sample we have used does not have any parasites in it for OHS reasons. Nonetheless, the technique used is as follows:

- fix smears by flooding with 100% methanol for 1 min, remove excess, airdry
- stain for 15 mins in 1:7 dilution of stock Giemsa stain* in phosphate buffer**
- rinse gently in tap water to remove surplus stain
- airdry (do not mount with coverslip, slides last longer and do not bleach, they can be examined as wet oil mounts and oil can be removed by soaking in xylene)

*stock Giemsa stain:

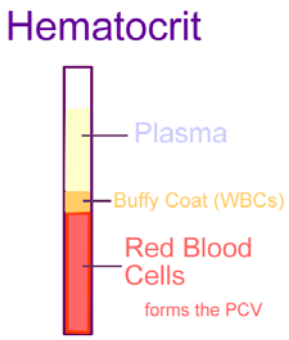
0.4 g Azure II-eosin; 0.2 g Azure II; 0.2 g Azure B-eosinate; 50 mL glycerol; 50 mL methanol

**phosphate buffer pH 7.2-7.4

5.47 KH_2PO_4 (MW136.09); 3.8 g Na_2HPO_4 (MW 141.96); 1,000 mL distilled water

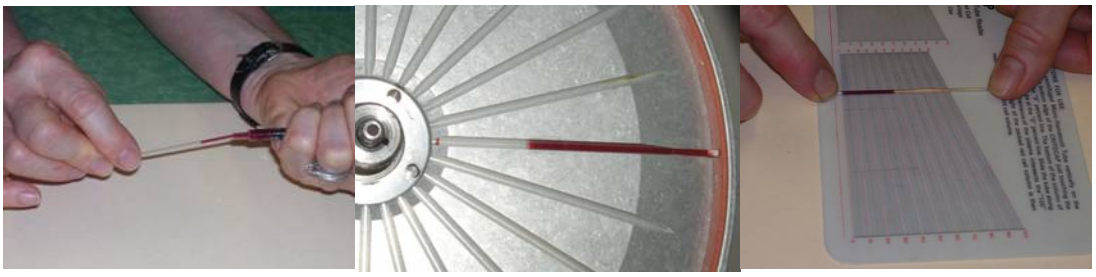
b) Wet lab - harvest buffy coat by microhaematocrit tube centrifugation

The percentage volume of blood occupied by red blood cells is called the packed cell volume (PCV) or haematocrit (normally around 30% in healthy individuals). Erythrocytes are the heaviest cells in blood by virtue of their haemoglobin content (full of iron) therefore they will sediment under gravity (which can be accelerated by centrifugation). Microhaematocrit tube centrifugation is an easy and reliable technique to measure PCVs as well as to separate white blood cells which form a white buffy coat on top of the sedimented red cells. Extracellular haemo-parasites such as trypanosomes and microfilariae accumulate in the buffy coat layer and intra-erythrocytic parasites such as haemosporidia and piroplasms are often found in the upper portion of the red cell layer (infected cells are less heavy).



TECHNIQUE

- fill ~75-90% of a microhaematocrit centrifuge tube with non-clotted blood (in anticoagulant) by dipping one end in sample and allowing capillary action to fill tube (regulate flow by tilting tube from vertical to horizontal)
- hold finger over open end to tube to stop blood from leaking out
- plug bottom of tube by plunging end into plasticine tray
- place tube in microhaematocrit centrifuge (with plugged end facing out)
- centrifuge for 3-5 minutes
- remove tube and record haematocrit (PCV) using graduated scale



- examine buffy coat at low power under light microscope by rolling tube over stage (looking for motility e.g. wriggling trypomastigotes/microfilariae)
- score tube just above buffy coat layer using diamond pencil/glass cutter
- carefully snap tube using fingers
- touch cut end tube to glass slide to expel buffy coat
- coverslip and examine under light microscope

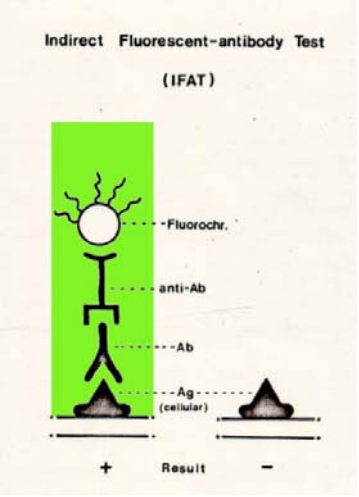
c) Wet lab – *Toxoplasma* IHAT (indirect haemagglutination test)

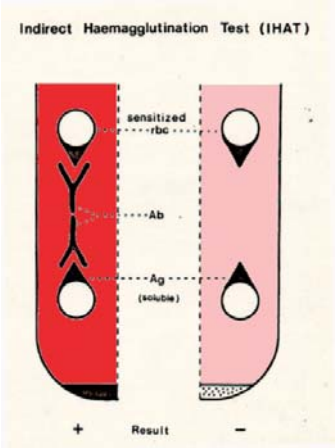
The differential diagnosis of infectious diseases is frequently complicated by the nonspecific nature of any disease symptoms (such as diarrhoea or fever), confounding clinical parameters (haematology and blood biochemistry), difficulties in detecting organisms in test samples (few present and irregular occurrence) and their variable characteristics (pleomorphy, virulence, growth requirements, drug sensitivity, etc.).

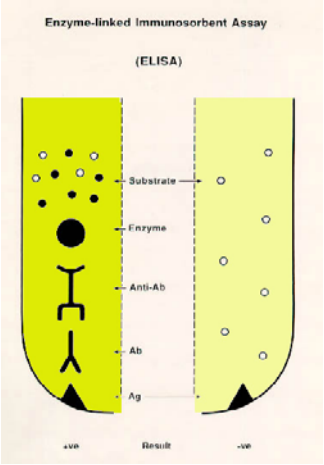
Recourse is therefore often made to the indirect demonstration of infections using immunoserological techniques to provide presumptive evidence of infection. Serum samples are tested for the presence of host antibodies formed against microbial antigens.

Animals respond to most infectious diseases by forming antibodies against the infecting pathogen as part of their immunological defenses. Antibodies (also called gamma-globulins or immunoglobulins) are produced by plasma cells (transformed B lymphocytes) and are secreted into the blood stream to circulate through the body. When they come into contact with the relevant antigen, they bind to it and tag it for destruction. The presence of specific antibodies is therefore frequently used as an indicator of infection, particularly for diseases which have nonspecific symptoms or clinical signs (fever, diarrhoea).

A range of immunoserological tests have been developed to demonstrate these antibody-antigen interactions; including immunodiffusion, complement fixation, haemagglutination, fluorescent-antibody labelling, enzyme and radio immunoassays.







All rely on host antibodies recognizing and binding to antigenic epitopes specific to individual pathogens. The antigens are immobilized on substrates, incubated with test and control samples, an indicator system is added and the results read qualitatively (positive or negative) or quantitatively (end-point titre). It is particularly important to quantitate the amount of antibody present as this provides an indication of the severity of infection and the immunocompetence of the host. The concentration of many other chemicals present in blood (hormones, electrolytes, drugs, etc) can be measured in absolute terms and expressed in specific units (μg/mL, etc). However, measuring the concentration of antibodies is much more difficult due to their variable specificity, cross-reactivity, highly reactive nature, and strong binding affinity to detection systems.

Maths to the rescue! An accurate indication of the amount of antibody present can be obtained by serially diluting the blood to the point it no longer tests positive. The last positive dilution is then called the end-point titre and this indicates the quantity of antibody present. For example, an end-point titre of 1/100 indicates there was enough specific antibody present to elicit a positive reaction when the blood was diluted 100 times.

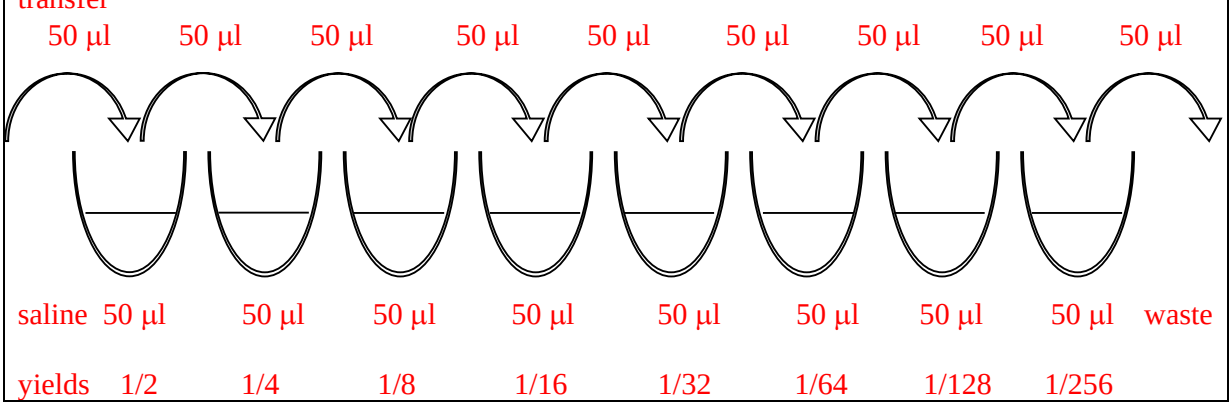
Note that this reciprocal notation represents a fraction not a ratio; 1/10 means 1 in 10 (while 1:10 means 1 as to 10). A 1/10 dilution is therefore made by adding 1 part blood (usually serum) to 9 parts diluent (usually physiological saline) to give a 1 in 10 dilution. The most commonly used series are doubling dilutions beginning with a 1/2 dilution (i.e. 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, etc). Serial dilutions are usually made on 96-well plastic microtitre plates (wells arranged in 8 rows and 12 columns).

The results of serological tests are best read objectively and quantitatively; that is, without subjective interpretation by individual operators and with measurement of a related parameter, such as spectrophotometric absorbance (optical density), intensity of fluorescence, or degree of haemolysis. Various instruments have been developed to measure test results, compare and adjust them to standards and reference controls, and then calculate and present the results. Regrettably, the test results can be influenced by many factors which affect the integrity of the relationships between parameters (such as edge effects, detectable levels, accuracy, interference, competition, nonspecific background reactions, cross-reactivity with other microbes, reactions against vaccines previously given, poor test sensitivity and specificity). Nonetheless, the end-point titre (= last positive dilution) of any particular sample can be given with a high degree of confidence. Interpreting the significance of the test results requires thorough knowledge of the kinetics (onset and duration) and dynamics (intensity) of the host response to infection. Longitudinal samples are obtained to determine whether antibody titres in a particular individual remain stable or whether they are increasing or decreasing (plot titre over time). The results provide strong presumptive evidence on the status of infection within that individual, thus allowing appropriate therapy.

Question: The serological test you are using requires 50 µl of each titration in each test well.
How would you physically make a doubling dilution series from 1/2 to 1/256?
(use a diagram to illustrate your answer)

Answer:

Serial titration method (need only use one 50 µl micropipette and two tips)
- add 50 µl of diluent to each of 8 consecutive wells
- then add 50 µl of whole blood to first well (giving 100 µl of 1/2 dilution)
- then take 50 µl of 1/2 dilution and add to next well (giving 100 µl of 1/4 dilution)
- repeat another 6 times



INDIRECT HAEMAGGLUTINATION TEST

Toxoplasma gondii is a tissue cyst-forming sporozoan parasite which has been detected in a wide range of vertebrate hosts (including humans) in association with clinical disease. The parasite undergoes asexual development in various cell types (acute phase of infection) culminating in the formation of tissue cysts (chronic phase of infection). Many infections are asymptomatic but acute infections may cause flu-like symptoms. Tragically, acute infections may be transmitted transplacentally in pregnant females causing spontaneous abortion, stillbirth or congenital abnormalities such as hydrocephalus, brain calcification, mental retardation and chorioretinitis. Numerous tests have therefore been developed for the diagnosis of infections. The indirect haemagglutination test (IHAT) was developed several decades ago and has been used in mass screening programmes. The principle of the IHAT is based on the ability of specific antibody to agglutinate particles bearing appropriate epitopes. In this case, *Toxoplasma* antigens have been coated (tanned) onto sheep red blood cells which will consequently agglutinate in the presence of anti-*Toxoplasma* antibody in test serum.

PROCEDURE:

NOTE: With all projects using animal serum, exercise caution. Dispose of used containers and pipette tips in appropriate containers. Clean up any spills immediately with alcohol solution. Report any injuries immediately.

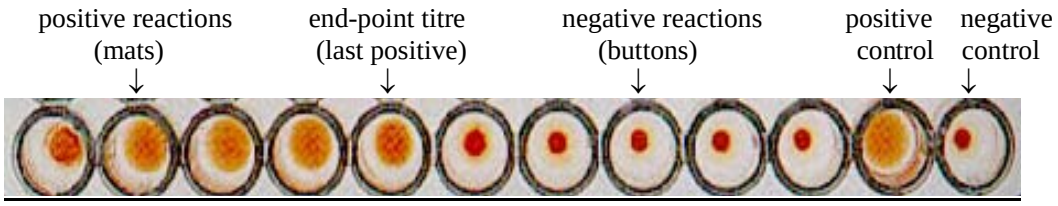
PRACTICE USING YOUR MICROPIPETTE: Trainees always insist that they know how to use a micropipette. However, they then go on to demonstrate that they do not and thus consequently bugger up the test by pipetting wrong volumes and filling wells with evil air-bubbles. Remember that when depressing the thumb lever, you will encounter two stop positions: the first is the position to use to take up the required volume of sample or diluent (in this case 50 µl), the second is the position to use to expel the fluid from the micropipette tip (over enthusiastic expulsion results in air bubbles so work slowly and carefully). Practice titrating some diluent across your microtitre plate.

1. Working in pairs, each student add 50 µl phosphate-buffered saline (PBS) diluent to each well in one row of a microtitre plate (e.g. A1-A12)
2. Add 50 µl of test serum to first well (e.g. A1) giving 100 µl of 1/2 dilution
3. Serially titrate each serum through to last well of each row giving 50 µl dilutions from 1/2 to 1/4096 (transfer 50 µl between wells)
4. Add 50 µl of diluent to the 1/32 dilution of each serum (e.g. A5) and discard 50 µl thereby producing a second 1/64 dilution

Take plate to tutor to check before adding indicator system (i.e. sensitized red blood cells)

5. With tutor, add one drop (15 µl) of unsensitized red cells to first 1/64 dilution
6. With tutor, add one drop (15 µl) of sensitized red cells to 1/64-1/1024 dilutions
7. Gently tap side of plate to distribute cells throughout well (do not swirl)
8. Place plate in safe vibration-free location and leave undisturbed for as long as possible (1-2 hours)
9. Read plates by visual inspection (negative results are represented by a compact button of cells at the bottom of the well, positive results by a smooth mat of cells covering the bottom of the well, and the end-point titre as the last serum dilution giving a positive reaction). The manufacturers regard titres <1/64 as negative, titres >1/64 as positive and borderline titres = 1/.64 as negative.
10. Record your test serum titre and note the cumulative class results

Examples of haemagglutination appearance



Questions:

1. If the objective of the test is the agglutination of sensitized red blood cells in the presence of antibody, why are positive reactions observed as mats of cells and negative reactions as buttons of cells?

   positive mats are cross-linked and held in suspension (antibodies bivalent),
   negative buttons are gravity sediments

2. Is it important to determine the end-point titre of the test sera or simply to record them as positive or negative?

   end-point titres good for differentiating acute/chronic, recent/previous infections
   or for longitudinal studies

3. What does a positive reaction in the 1/64 well receiving unsensitized cells denote? Does this influence the test result? How could this complication be nullified? *

   non-specific agglutination = false positive, therefore adsorb agglutinins

4. Does the IHAT detect any specific class of antibody? How could the test be modified to differentiate between IgG and IgM antibody reactions?**

   detects all classes, selectively reduce IgM with 2ME to record IgG titres

5. One advantage of the IHAT over IFAT (indirect fluorescent antibody tests) and ELISA (enzyme-linked immunosorbent assays) techniques is that it is not restricted in use to particular animal species. Why?

   IHAT does not need anti-species serum

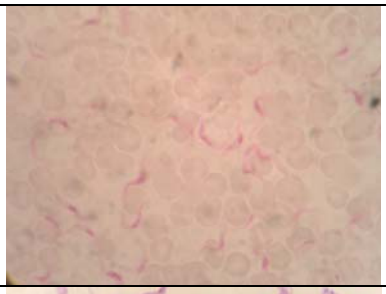
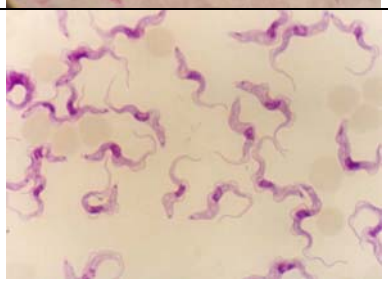
6. Because tanned red cells become fragile, the IHAT reagents have relatively short shelf-lives. What other particles could be substituted for the red cells?

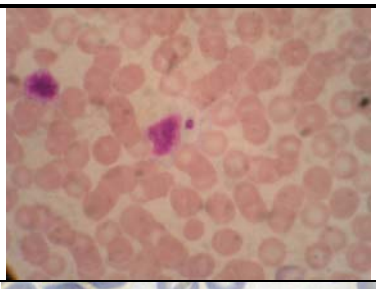
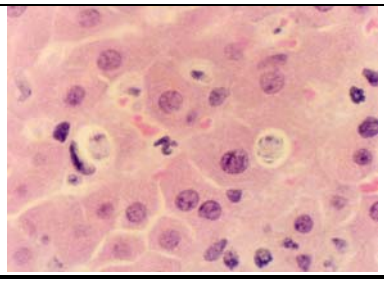
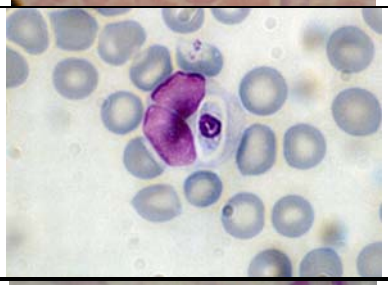
   latex agglutination (LAT), fixed cells (MAT)

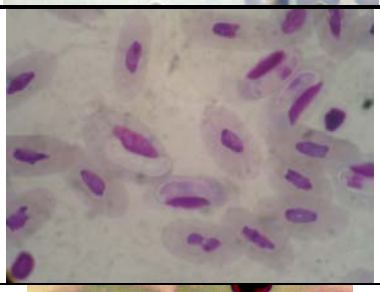
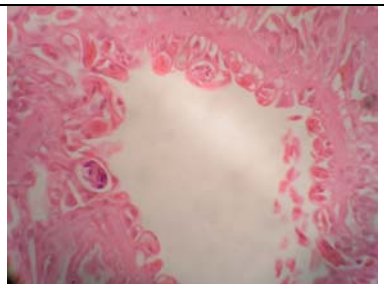
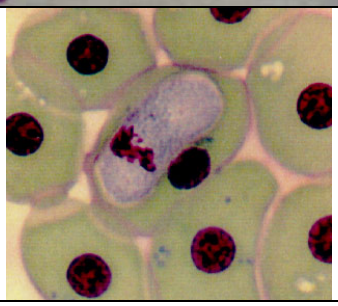
*HINT: think in terms of natural agglutinins and immunoabsorbents
**HINT: think in terms of reducing agents (e.g. 2ME)

d) Dry lab – Examination of slide set by light microscopy (work with your neighbours)

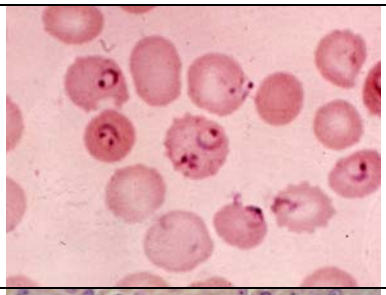
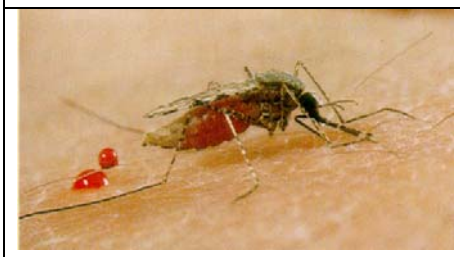
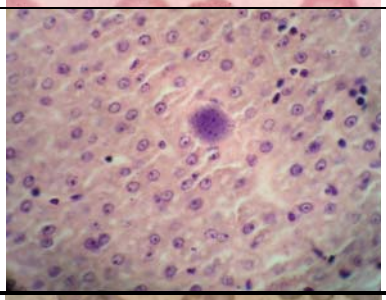
<table>
<tr>
<td rowspan="2">B1. (flagellate)

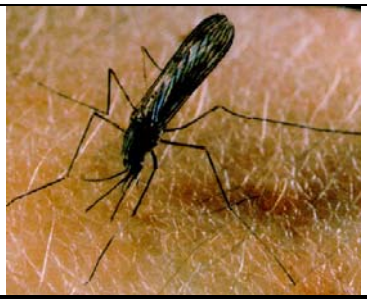
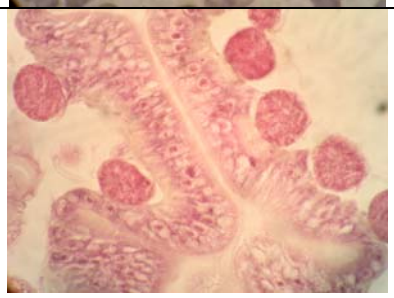
Trypanosoma brucei
trypomastigotes
cow blood
stained smear</td>
<td>Scan smear at medium power looking for elongate wavy trypomastigotes located between red blood cells (right). Examine at high power noting basophilic nucleus, posterior kinetoplast and long undulating membrane (below right). Infections are transmitted by blood-sucking tsetse files (below) by salivarian transmission (anterior station).</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td>  </td>
</tr>
<tr>
<td rowspan="2">B2. (sporozoan)

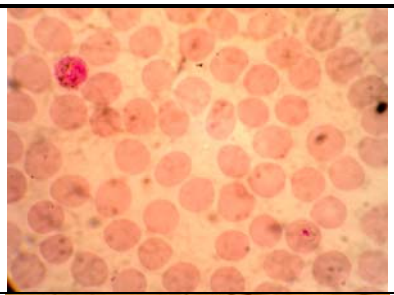
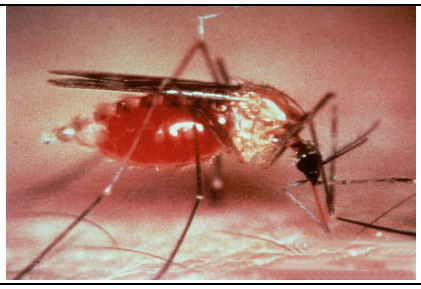
Hepatozoon muris
gamont
mouse blood
stained smear</td>
<td>Examine slide at medium power to find pale vacuolated bodies in or adjacent to white blood cells, mainly neutrophils (right). High power reveals them to be pale nucleated gamonts (below right). Most infections are transmitted by the ingestion of infected ticks and parasites multiply in visceral organs, esp. liver (below), before infecting blood cells.</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td>  </td>
</tr>
<tr>
<td rowspan="2">B3. (sporozoan)

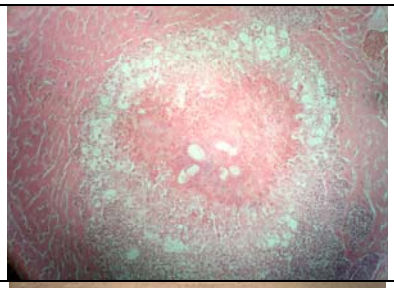
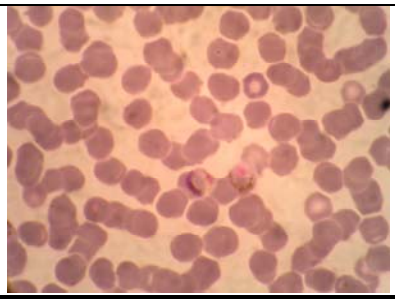
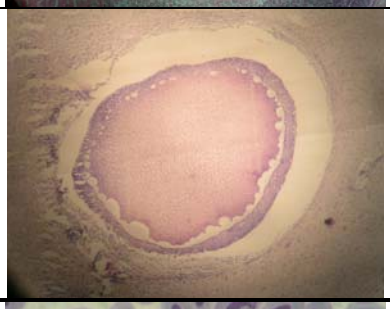
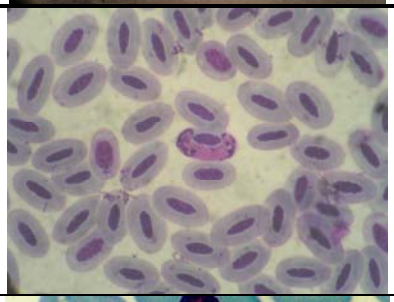
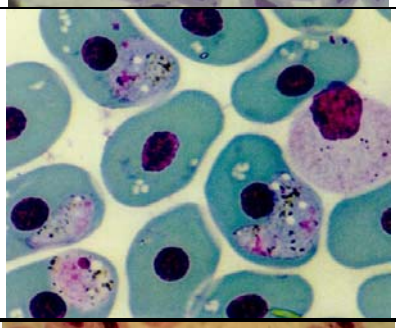
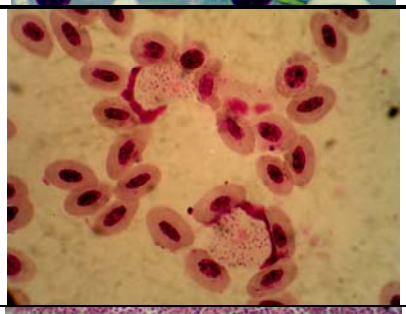
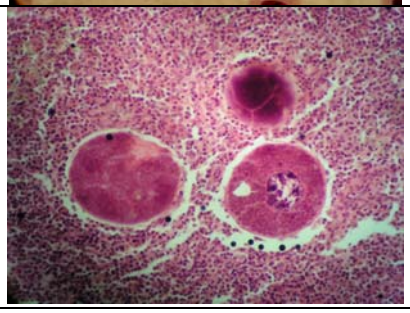
Haemogregarina boigae
gamont
brown tree snake blood
stained smear</td>
<td>Note the nucleated red blood cells (indicating non-mammalian host). Examine cells with 2 nuclei to reveal elongate cresentic intra-erythrocytic gamonts (right, below right). Infections are transmitted by the bite of leeches and arthropods and parasites multiply in visceral organs, esp. lungs (below) before invading blood cells.</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td>  </td>
</tr>
</table>

<table>
<tr>
<td rowspan="2">B4. (haemosporidian)

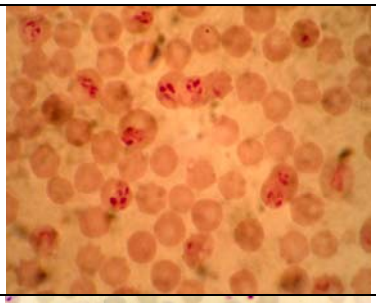
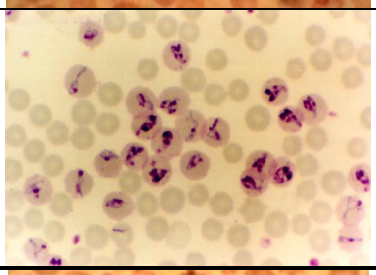
Plasmodium falciparum
rings/schizonts/gamonts
human blood
stained smear</td>
<td>Examine infected erythrocytes under oil immersion noting trophozoites, ring forms, pleomorphic schizonts (right), haemozoin (brown) pigment, and occasional ‘banana’ gametocytes. Infections are transmitted by mosquito bite (below), exoerythrocytic schizogony occurs in the liver (below right) and then blood stages cause malignant tertian malaria..</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td>  </td>
</tr>
<tr>
<td rowspan="2">B5. (haemosporidian)

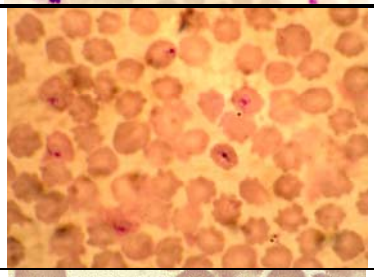
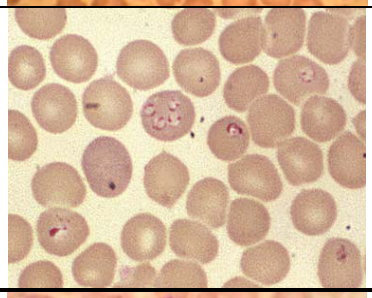
Plasmodium malariae
rings/schizonts/gamonts
human blood
stained smear</td>
<td>Scan smear for infected erythrocytes containing trophozoites and schizonts (right) which cause benign quartan malaria Mosquitoes (below) act as vectors – in fact they are definitive hosts in which oocyst formation occurs on the exterior of the mosquito gut (below right) before sporozoites are released to invade the salivary glands.</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td>  </td>
</tr>
<tr>
<td rowspan="2">B6. (haemosporidian)

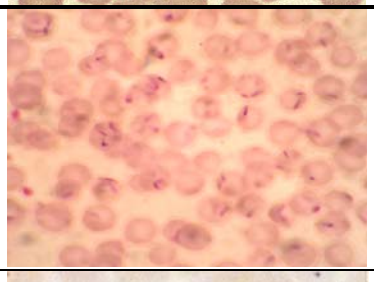
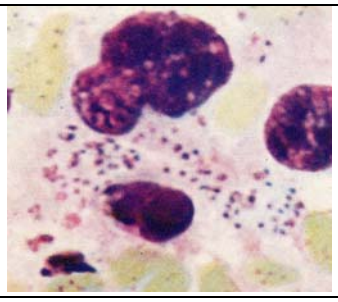
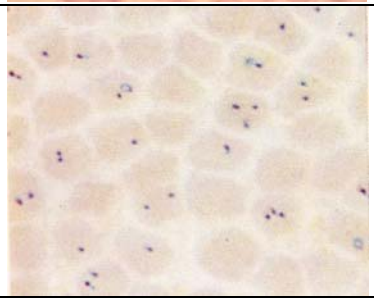
Plasmodium vivax
rings/schizonts/gamonts
human blood
stained smear</td>
<td>Screen smear carefully for intra-erythrocytic stages (right) including ring forms, schizonts and some gamonts; which cause benign tertian malaria. Infections are transmitted by mosquitoes (below) which inject small rod-like sporozoites (below right) with their saliva. Once infected, mosquitoes stay infected for life.</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td>  </td>
</tr>
</table>

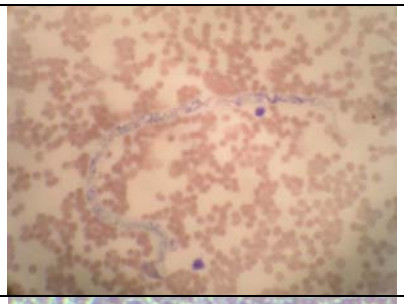
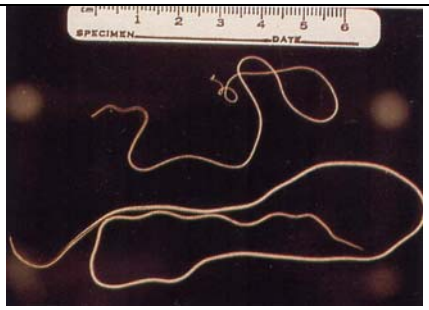
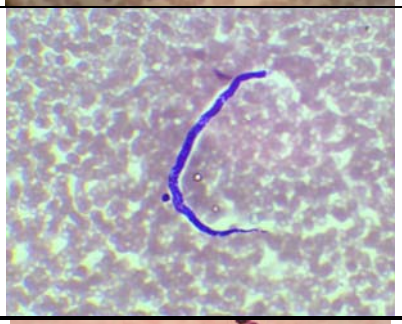
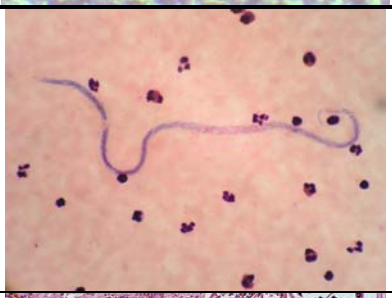
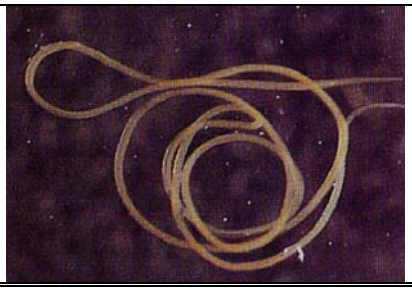
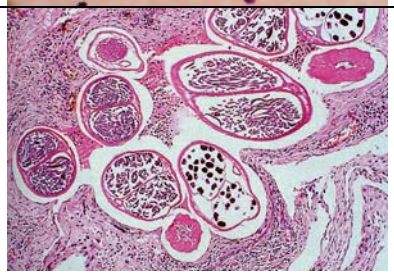
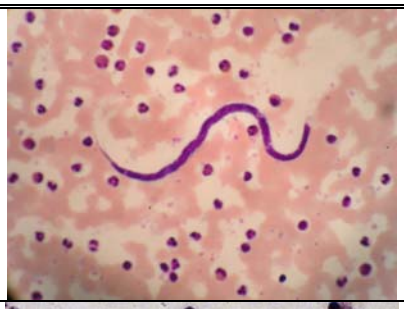
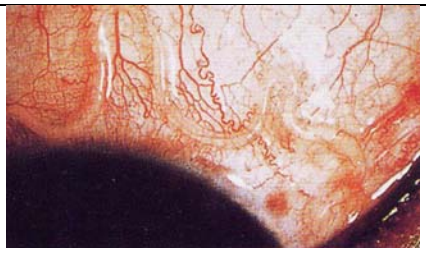
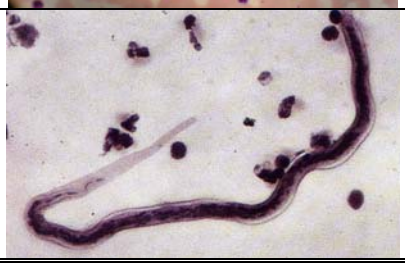
B7. (haemosporidian) ***Hepatocystis pteropi*** schizonts bat liver stained section	Use low power to survey the liver for vacuolated schizonts (right). These exoerythrocytic stages grow and coalesce into large merocysts filled with colloidal fluid and rimmed by merozoites (below right). Merozoites are eventually released to invade red blood cells where they grow into gametocytes (below).	
		
B8. (haemosporidian) ***Haemoproteus columbae*** gamonts bird blood stained smear	Examine smear at high power looking for round to crescentic gamonts adjacent to host cell nucleus (note prominent haemozoin pigment) (right, below right). Infections are transmitted by hippoboscids (louse flies) (below) and parasites multiply in reticulo-endothelial (RE) cells in various organs before forming intraerythrocytic gamonts.	
		
B9. (haemosporidian) ***Leucocytozoon simondi*** gamonts duck blood stained smear	Scan the smear to find 'exploded' white blood cells which are actually gamonts with lateral spindle nuclei (right) Note absence of haemozoin pigment. Infections are transmitted by simuliids (blackflies) (below) and parasites multiply in RE cells forming large megaloschizonts (with vacuoles and cytomeres) in various organs (below right)	
		

<table>
<tr><td rowspan="2">B10. (piroplasm)

Babesia bigemina
merozoites
cow blood
stained smear</td><td>Scan erythrocytes looking for intra-erythrocytic basophilic piroplasms (right, below right). Note size, shape, colour and juxtaposition of merozoites - look for paired (bigeminate) bodies joined at an acute angle – this species forms large merozoites. Infections are transmitted by ixodid ticks (below) in which trans-stadial and trans-ovarian transmission occur (making ticks infected for generations!)</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">B11. (piroplasm)

Babesia bovis
merozoites
cow blood
stained smear</td><td>Examine slide at high power looking for pyriform merozoites inside erythrocytes (right, below right). Look for paired bodies joined at an obtuse angle – this species forms small merozoites. Infections are transmitted by Rhipicepahlus (Boophilus) ticks (below) and cause tick fever, anaemia, jaundice and haemoglobinuria.</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">B12. (piroplasm)

Theileria buffeli
merozoites
buffalo blood
stained smear</td><td>Use oil immersion to look for small intra-erythrocytic rod-round merozoites (right, below right). These parasites are transmitted by the bite of Rhipicephalus ticks and they undergo substantial amplification as macro- then micro-schizonts in lymphocytes in lymph glands (below) before invading red blood cells.</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
</table>

B13. (filarial worm) ***Dirofilaria immitis*** microfilaria dog blood stained smear	Search the slide at low power looking for elongate larvae (microfilariae) located between red cells (right). Examine at medium power to note that the microfilariae are not ensheathed and have a tapered anterior end and a blunt posterior end (below right). Infections are mosquito-borne and long thin adult worms (below) lodge in the heart and pulmonary artery.	
		
B14. (filarial worm) ***Wuchereria bancrofti*** microfilaria human blood stained smear	Examine smear at low power to locate elongate microfilariae (right). Examine at medium power to observe sheath and absence of nuclei in tail (*Brugia* forms shorter mf, also sheathed but with two nuclei in tail). Infections are transmitted by mosquitoes and adult worms (below, below right) live in lymphatics and cause elephantiasis in groin and lower limbs.	
		
B15. (filarial worm) ***Loa loa*** microfilaria human blood stained smear	Screen the blood film to find long microfilariae (right). Examine at medium power noting presence of sheath and nuclei to the tip of the tail (below right). Infections are transmitted by tabanid flies and adult worms move through subcutaneous connective tissues causing transient swellings, and sometimes crossing the conjunctiva of the eye (below)	
		

Workshop 4: Histopathology

In the next hour, you should complete the following three activities:

a) wet lab – tissue squash preparation
b) wet lab – digest skin scraping
c) dry lab – microscopy of tissue parasites

Many parasites are not content to dwell in the luminal spaces of tubular organs or the blood stream of their hosts – instead, some actively invade the deeper tissues and organs of their hosts either transiently (as migrating stages) or for prolonged intervals (as encysted stages). Many protozoa form multiplicative stages (trophozoites/tachyzoites/bradyzoites/cystozoites/amastigotes, meronts/schizonts, cysts/pseudocysts) in host tissues; many being temporary and linked to acute disease syndromes although some can persist and cause chronic lesions and/or disease. Many helminth parasites have juvenile (larval) or adult (immature and mature) developmental stages which actively penetrate tissues and lodge in internal organs, some even becoming dormant (hypobiotic). Some geohelminths infect their hosts by transdermal penetration of the skin resulting in larval migrans. Other nematodes exhibit an obligatory pulmonary migration through the lungs before maturing as adults in their final predilection sites. All cestodes form encysted larval stages (metacestodes) in the tissues of intermediate hosts, and most trematodes infect their hosts when cercariae penetrate host tissues. All ectoparasites (fleas, lice, flies, ticks, mites) feed on host fluids/tissues by penetrating the skin, and some even invade subdermal tissues for some or all of their life-cycles.

The diversity and abundance of tissue parasites begs the obvious question: how do parasites encapsulated in host tissues for prolonged periods of time manage to get to other hosts? Several tissue parasites exhibit vertical transmission between hosts (from mother to offspring) by transplacental or transmammary transmission, but the majority exhibit horizontal transmission by exploiting the food chain, that is, stages in the tissues of prey animals (usually herbivorous intermediate hosts) are eaten by predatory animals (usually carnivorous definitive hosts). Historically, humans as omnivores can act as intermediate or definitive hosts for various parasites (although these days few humans are eaten by carnivores). To complete the life cycle, predators usually void parasite eggs, larvae or cysts which contaminate pastures or water supplies where they are taken up by prey animals. The resultant predator-prey transmission is quite successful even though two hosts are involved together with free-living stages. Such is the power of food chains in Nature!

In addition to stool and blood samples, various host tissues and other fluids/secretions can be collected ante-mortem and examined for parasitic infections, including sputum, vomitus, skin scrapings, fluid aspirates and tissue biopsies. If the patient dies or is humanely euthanized, samples can be collected at post-mortem from virtually any internal organ or tissue. A variety of techniques have been developed to examine tissues, including:

- macroscopic examination (gross pathology)
- microscopic examination (histopathology)
- histochemistry (staining and immunolabelling)
- concentration (tissue digests)
- culture (*in vitro, in vivo*)
- molecular techniques (extract DNA, PCR)

Most diagnostic laboratories have trained pathologists who examine gross and histological samples to make a provisional diagnosis based on the detection of parasite developmental stages or characteristic lesions. Their work is now being complemented by an increasing range of immunological and molecular biological techniques to demonstrate specific indicator molecules (proteins, nucleic acids). Pathologist reports, however, may often be non-definitive so the clinician must assess each case on the basis of all information available (presentation/symptomatology, history, physical examination, other laboratory reports, responses to empirical treatment, etc).

A range of tissue parasites have been selected for closer examination, but this list is illustrative only and by no means comprehensive. The parasites and some of their characteristics are tabulated below:

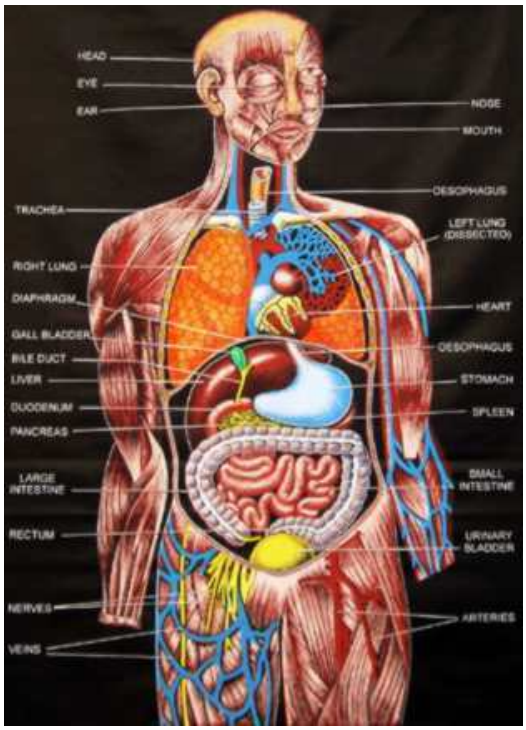
Code	Parasite Genus	Hosts	Diagnostic stage	Notes	Life-cycle
PROTOZOAN PARASITES					
T1	*Trypanosoma cruzi*	mammals	amastigotes	pseudocysts in tissues	epimastigotes in reduviid bug vectors
T2	*Leishmania*	mammals	amastigotes	macrophages/skin	promastigotes in sandfly vectors
T3	*Toxoplasma*	vertebrate IH	zoites/cysts	macrophages/brain	oocysts in cat DH
T4	*Sarcocystis*	vertebrate IH	zoites/cysts	endothelia/muscle	sporocysts in carnivore DH
T5	*Thelohania*	crustacea	spores/cysts	muscle	cycle unknown
T6	*Kudoa*	fish	spores/cysts	muscle	cycle unknown
HELMINTH PARASITES					
T7	*Trichinella*	carnivores	larva	muscle	larva/adults in carnivores
T8	*Onchocerca*	mammals	microfilaria	subcutaneous	adults in mammals, mosquito vectors
T9	*Echinococcus*	mammals	hydatid cyst	viscera	adult tapeworm in dog DH
T10	*Schistosoma*	mammals	egg granulomas	gut/bladder, viscera	asexual multiplication in snail vectors
ARTHROPOD PARASITES					
T11	*Ctenocephalides*	mammals	adult flea	skin	eggs/larvae/pupa in bedding/ground
T12	*Pediculus*	mammals	nymph/adult lice	hair	all stages on host
T13	*Culex/Anopheles*	animals	adult mosquitoes	skin	females blood-sucking
T14	*Ixodes*	mammals	larva/nymph/adult ticks	skin	1, 2 or 3 hosts
T15	*Sarcoptes*	mammals	larva/adult mites	tunnels in skin	all stages in host

DH = definitive host, IM = intermediate host; UM = undulating membrane

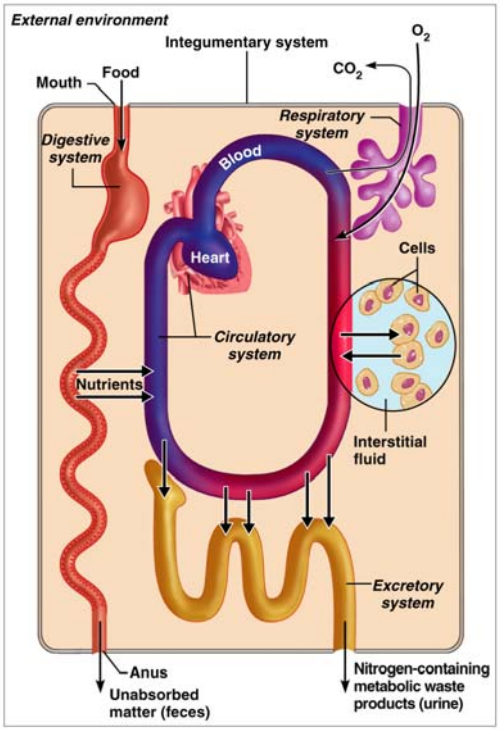
a) Wet lab - Tissue squash preparation

It is beneficial to adopt an integrated anatomical-physiological approach to body structure and function. Rather than simply resort to a catalogue of host tissue/organ systems, it is better to understand how they all interact together both structurally and functionally. A problem in one tissue may have local, focal or systemic consequences involving other adjacent or distant systems.

Anatomy (structure)



Physiology (function)



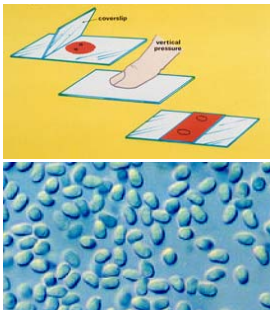
Parasites may migrate or sequester in host tissues as they feed and grow, and while they elicit host responses, they have developed sophisticated mechanisms to avoid potentially fatal host reactions (immunological and pathological). It therefore behoves clinicians to think laterally and collect samples of host tissues/fluids that may assist in the differential diagnosis of parasitic infections, such as:

sputum	urine	aspirates	biopsy	necropsy
lungs	bladder	CSF	liver, spleen	any organ
		bone marrow	lymph nodes	any tissue
		skin lesions	skin lesions	any fluid
		urine	bladder mucosa	
		vaginal swabs	rectal mucosa	

Samples can be examined as centrifugal sediments, filtrates, touch impressions, squash preparations, teased tissues or cut sections with or without staining (e.g. trichinoscopy examines squash muscle preparations for encapsulated *Trichinella* larvae). In this exercise, we will examine yabbie tissues for microsporan infections.

TECHNIQUE

- Cut a small segment from the crayfish tissue (size of pinhead)
- Place in drop of fluid on glass slide and chop into small pieces
- Examine under compound light microscope (with condenser wound down to introduce contrast into unstained tissues)
- Look for refractile cysts inside muscle fibres
- now place coverslip on tissue and carefully squash preparation with finger
- Re-examine under light microscope (with condenser wound down)
- Looking for refractile cysts in tissues and refractile spores in fluids



b) Wet lab - Skin scraping digest

Skin can be home to many pathogens, including viruses, bacteria, fungi, protozoa, helminths and ectoparasitic arthropods. Skin infections/infestation are frequently diagnosed on the basis of their appearance, but many pathogens may cause lesions similar in appearance, and they are frequently masked by host inflammatory (rubor/calor/tumor/dolor = red/hot/swollen/painful) or pathological processes (sloughing, shedding, crusting, etc.).

A technique used to aid differential diagnosis involves the alkaline digestion of skin scrapings to reveal pathogenic micro-organisms – especially mites and fungi. Skin samples are collected by scraping lesions with the flat of a scalpel and then digesting the material in dilute potassium hydroxide (which digests skin, keratin and hair, but not mites or fungi). The resultant slurry is examined under a light microscope for the presence of infectious agents.

Sarcoptes scabiei is a parasitic mite that lives within the subcutaneous tissues, causing the condition known as scabies in humans (similar mites causing mange in animals). The mite is distributed worldwide, and can affect all socioeconomic groups. Scabies mites are oval, straw-coloured, very small (0.2-0.4 mm in length), covered with fine lines and several long hairs. The mites have no eyes, and they have short and thick legs, with the first two pairs of legs stalked. The immature stages are comprised of a six-legged larval stage, followed by 2 nymphal stages that have eight legs, and each stage resembles the adult mite. The entire life cycle of the mite occurs over 10-17 days.

You have been provided with an Eppendorf tube containing a skin scraping from a pig infested with *Sarcoptes scabiei* var. *suis* (this subspecies is not considered to be zoonotic, i.e. it is not infectious to humans; but nonetheless we will take all precautions – wear gloves, clean spills with 100% alcohol, discard used plastic-ware appropriately, wash hydroxide spills under running tap water)

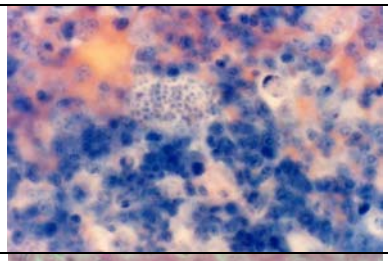
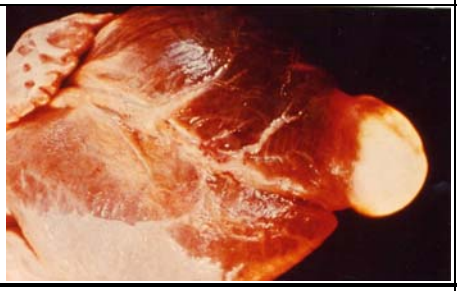
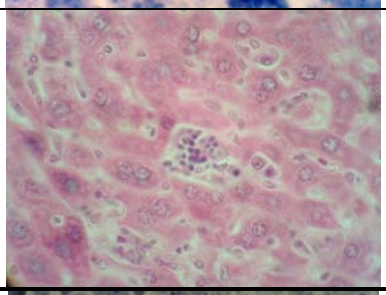
TECHNIQUE

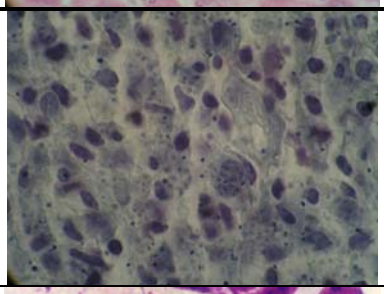
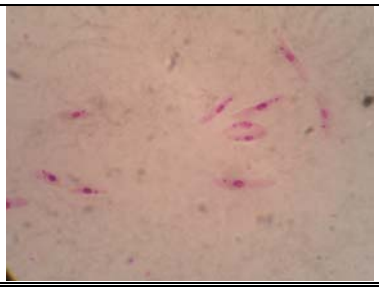
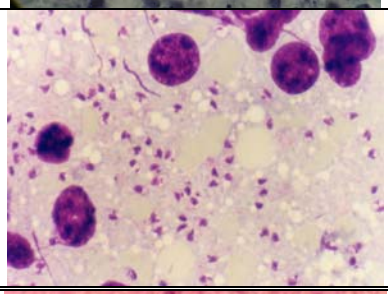
- Add 1 mL of 5% potassium hydroxide to Eppendorf tube containing skin scraping and cap tube
- Place Eppendorf tube in rack in hot water bath for 20 minutes
- Remove tube and centrifuge in microfuge for 1 min
- Remove and discard supernatant
- Add one drop of sediment to microscope slide and coverslip
- Examine under light microscope at 100-400x magnification (looking for mites and eggs)

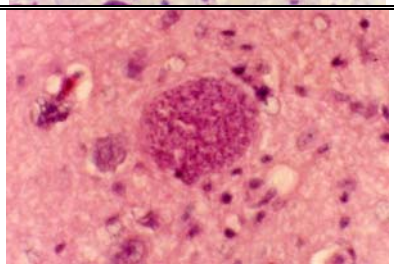
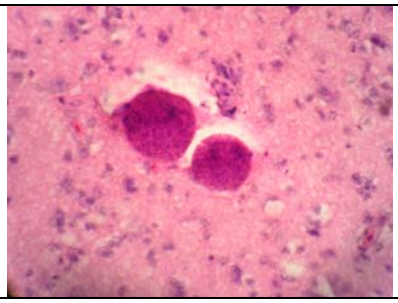
eggs	adults
	

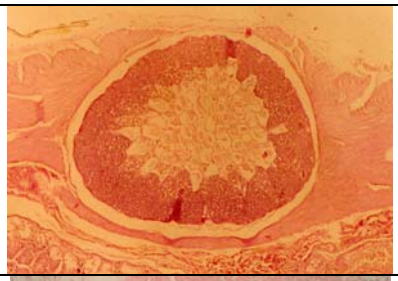
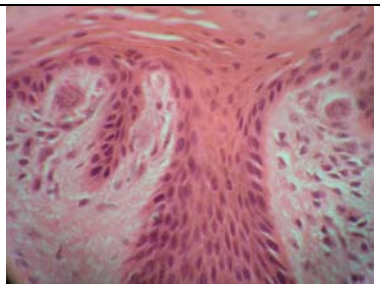
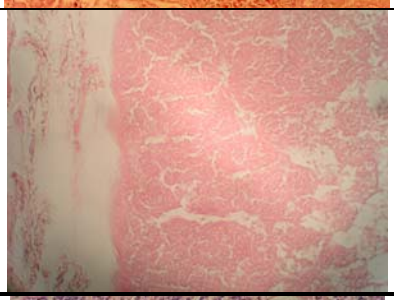
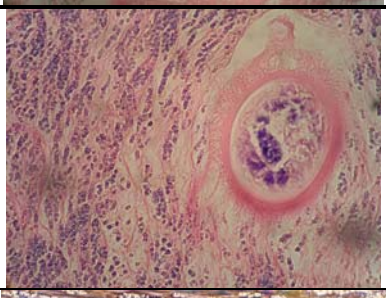
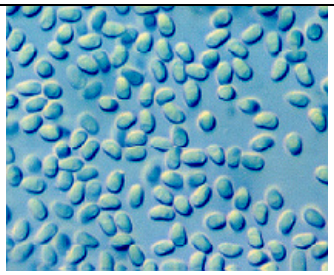
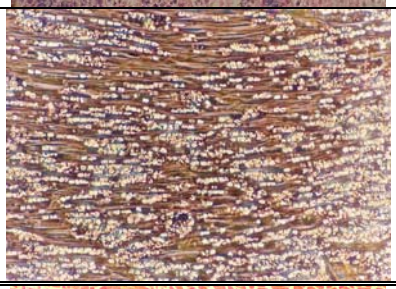
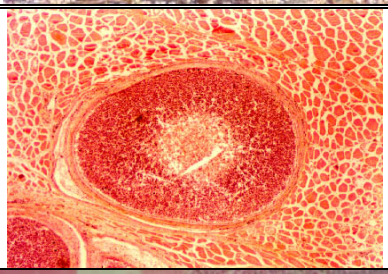
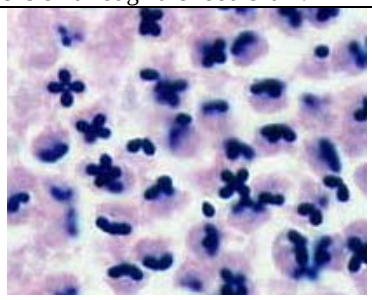
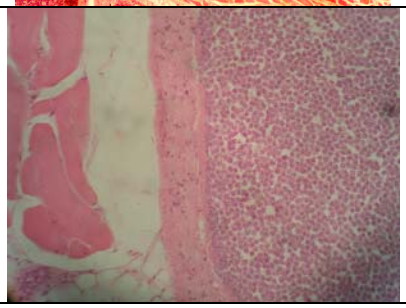
c) <u>Dry lab – Examination of slide set by light microscopy</u> (work with your neighbours)

<table>
<tr><td rowspan="2"><u>T1</u>. (flagellate)

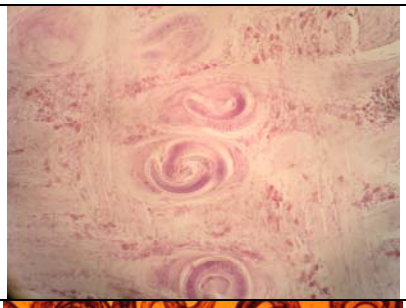
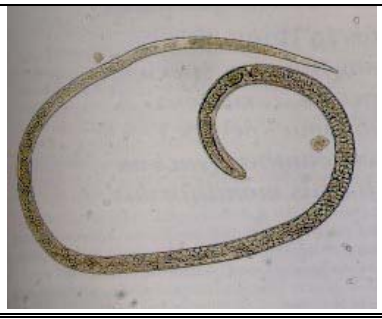
Trypanosoma cruzi
amastigotes
visceral tissue
stained slide</td><td>Search tissues at high power looking for multinucleate colonies (pseudocysts) of amastigotes within host cells (right, below right). These parasites are transmitted by reduviid bugs (stercorarian transmission) and they cause Chagas disease characterized by necrotic tissue lesions and severe inflammation (heart below).</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2"><u>T2</u>. (flagellate)

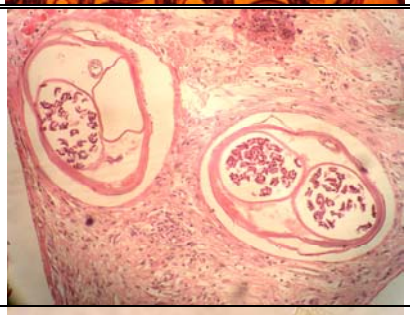
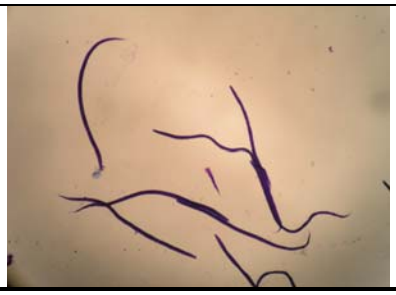
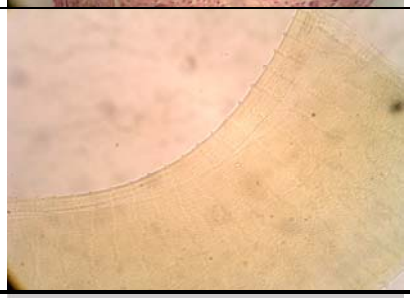
Leishmania tropica
amastigotes
human skin
stained slide</td><td>Examine the section at high power looking for thousands of tiny amastigotes with small purple nuclei ringed by cytoplasm (right, below right). Compare the size of the host and parasitic cells. The parasite is one of the smallest known eukaryotic cells. It is transmitted by sandflies in which elongate flagellated promastigotes develop (below).</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2"><u>T3</u>. (sporozoan)

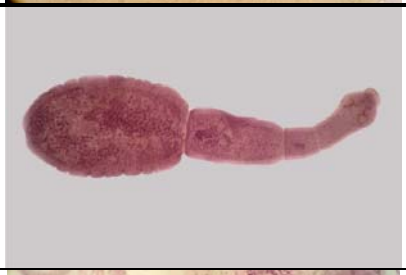
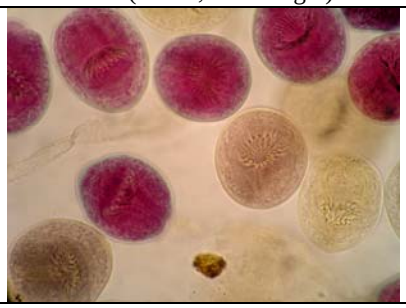
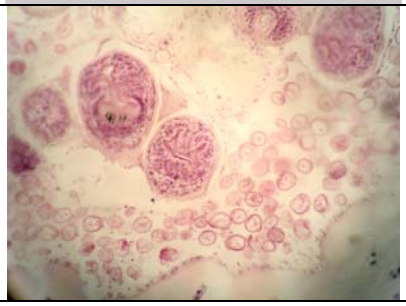
Toxoplasma gondii
cyst/bradyzoites
owl brain
stained slide</td><td>Carefully scan the brain tissue looking for basophilic cysts located in white matter (evident as collection of numerous small blue bradyzoite nuclei bound by thin membranous cyst wall) (right, below right). Prior to cyst formation, tachyzoites (below) proliferate in macrophages throughout the body. Infections are transmitted by oocysts from cats, eating cysts in meat and (tragically) congenitally.</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
</table>

T4. (sporozoan) ***Sarcocystis gigantea*** cyst/cystozoites sheep oesophagus stained slide	Look at the slide before you put it on the microscope to see the macroscopic cyst (right). Now use medium power to examine the cyst wall and contents (below right). Precyst development involves a series of schizont generations in arteriole and capillary endothelial cells (below). Infections are transmitted when sporocysts from predators are taken up by prey.	
		
T5. (microsporidian) ***Thelohania parastaci*** spores yabbie muscle stained slide	With condenser wound down for contrast, examine muscles noting presence of numerous refractile microspores (right, below right) causing condition known as cotton-tail (ignore conspicuous trematode metacercariae bounded by thick walls). Yabbies become infected when spores (below) evert their polar tubes and inject their infective sporoplasms.	
		
T6. (myxozoan) ***Kudoa thyrsites*** cyst/spores tuna muscle stained slide	Look at the slide macroscopically to see the large cyst (right). Examine at medium power noting fibrous wall composition (below right) and hundreds of multicellular spores (with four distinct basophilic polar capsules) (below). The source of infection is speculative but may involve actinospore stages in marine worms as well as ascension through the food chain.	
		

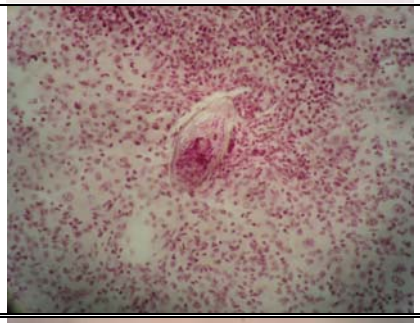
<table>
<tr><td rowspan="2">T7. (nematode)

Trichinella spiralis
larvae
human muscle
stained slide</td><td>Examine the section at medium power noting the presence of numerous coiled larvae within muscle ‘nurse’ cells (right, below right). Adult worms (below) live in the intestinal mucosa and release larvae which coil up in tissues (the host is thus both the definitive host and intermediate host). Infections are transmitted horizontally by carnivorism.</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">T8. (filarial nematode)

Onchocerca volvulus
adult in nodule
human subcutaneous tissue
stained slide</td><td>Look for sections through large worms in subcutaneous tissues (right). The adults (with cuticular ridges, below right) coil up in nodules and release numerous larvae (unsheathed microfilariae) (below) into connective tissues where they are taken up by pool-feeding blackfly vectors. Infections can cause pruritic rash, oedema, pachydermia, hanging groin and river blindness</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">T9. (cestode)

Echinococcus granulosus
adult tapeworm
ex dog gut
stained whole mount</td><td>Examine the small dog tapeworm noting the armed scolex (suckers and hooks) and small number (3-5) of proglottids (right). The adults pass eggs in dog faces and when these are consumed by other animals (incl. humans), they form large encysted larval stages (hydatid cysts) in viscera. The cysts contain hydatid ‘sand’, i.e. numerous protoscoleces (below, below right).</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
</table>

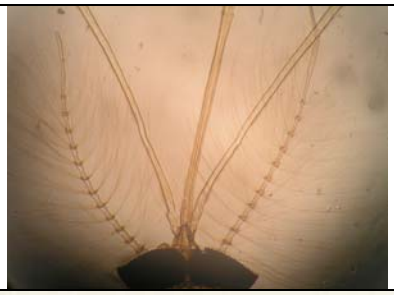
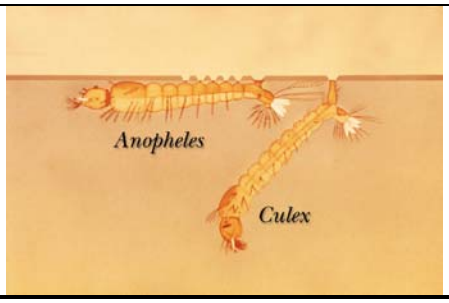
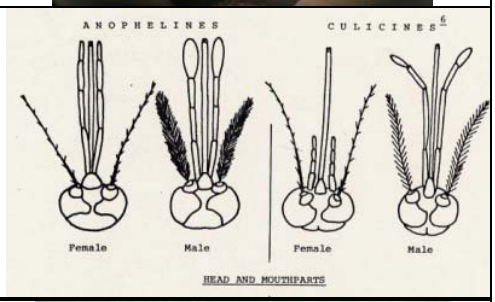
<table>
<tr><td rowspan="2">T10. (trematode)

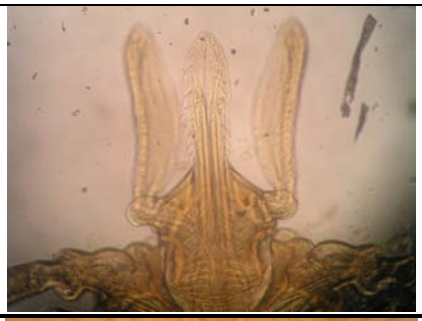
Schistosoma mansoni
adult trematodes
ex mouse mesenteric veins
stained whole mount</td><td>The blood flukes are unusual in that they are not hermaphroditic but have separate sexes (long thin female in gynaecophoric canal of stouter male) (right). The adults live in blood vessels and release eggs (below right) which penetrate the urinary or rectal mucosa. Eggs release miracidia which infect aquatic snails and undergo massive multiplication releasing cercariae (below) to infect vertebrate hosts.</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">T11. (flea)

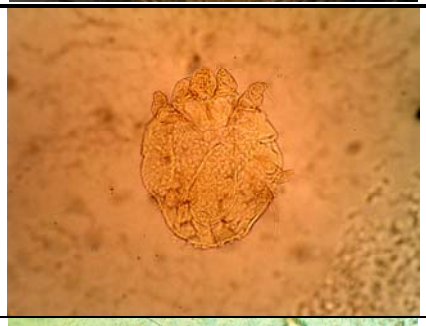
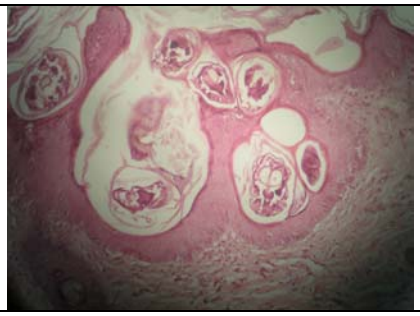
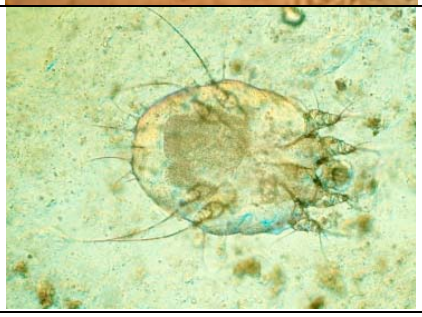
Ctenocephalides felis
adult flea
ex dog/cat skin
whole mount</td><td>Examine the adult flea noting presence of pronotal and genal combs with >5 spines, sloping frons (head), long powerful hindlegs and numerous numerous spines (right). Adult fleas are blood feeders (below right). They lay eggs which hatch to release grub-like larvae (below) which feed on adult scat and then undergo complete metamorphosis by pupation.</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td rowspan="2">T12. (lice)

Pediculus humanus
adult louse
ex human hair
whole mount</td><td>Examine adult louse noting pointed head (typical for sucking lice), antennae pointing forward, and enlarged claws on legs for grasping hairs (right). Adult lice feed on blood (below right) and cement eggs (nits) to hairs (below). The eggs hatch releasing nymphs which moult several times before forming adults (gradual metamorphosis).</td><td>  </td></tr>
<tr><td>  </td><td>  </td></tr>
</table>

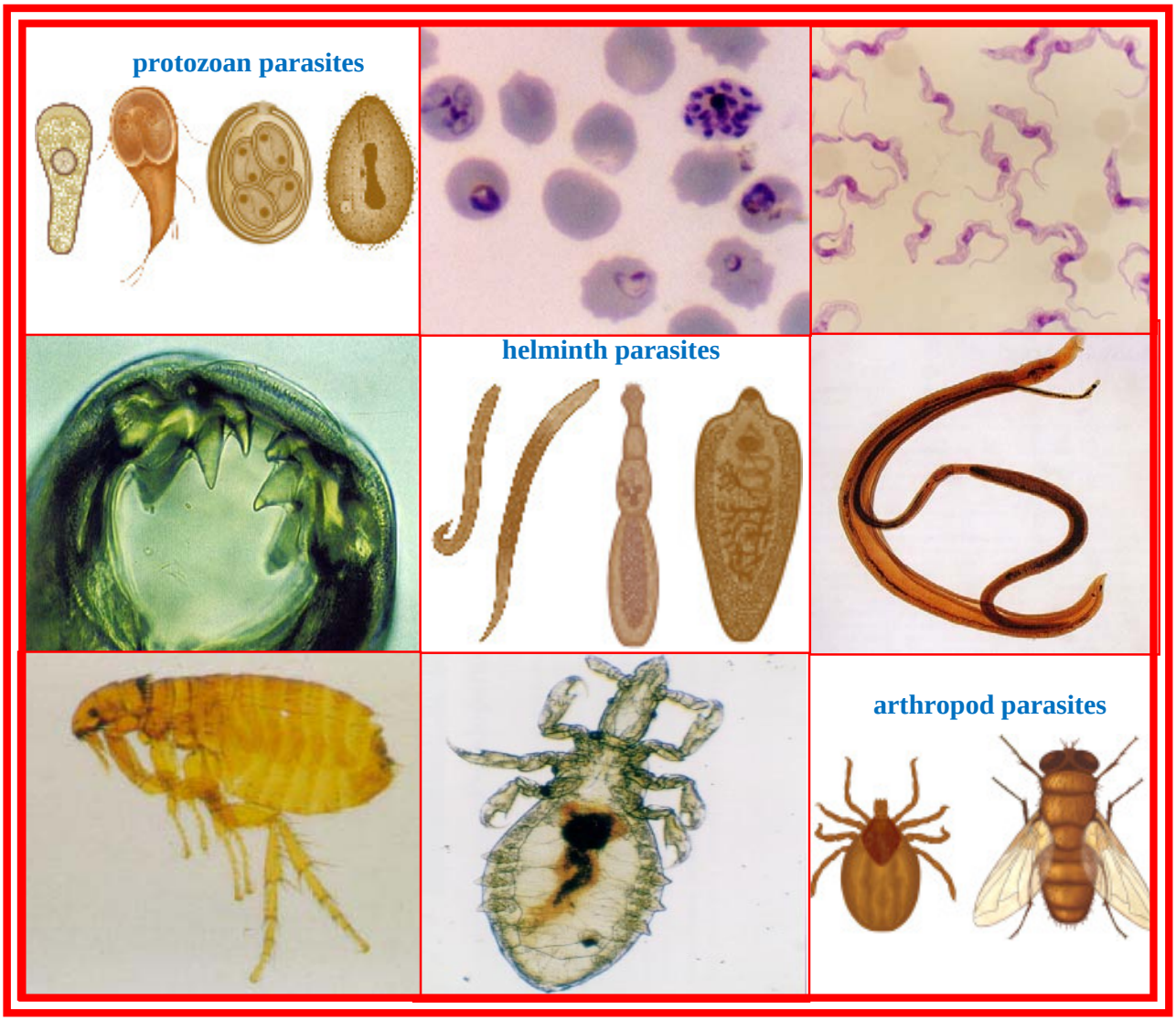
<table>
<tr>
<td rowspan="2">T13. (dipteran insect)

Anopheles annulipes
adult head
free-living
whole mount</td>
<td>Mosquitoes can be identified on the basis of structural elements and behaviour. Adult Anopheles mosquitoes have two long feathery antennae, a long proboscis, and long palps (right) - compared to the short palps of Culex mosquitoes (below right). Anopheline larvae (wrigglers) do not have a breathing tube in contrast to culicine larvae (below).</td>
<td>  </td>
</tr>
<tr>
<td> 
</td>
<td> 
</td>
</tr>
<tr>
<td rowspan="2">T14. (tick)

Ixodes holocyclus
adult tick
ex bandicoot
whole mount</td>
<td>Use low power to examine the dorsal surface of the tick noting the presence of a scutum (lined in females) and capitulum. Examine the ventral surface noting the conspicuous groove in front of anus (right). Ticks have barbed mouthparts (below right) to attach and feed on blood (below). Ticks have 1, 2 or 3-host cycles depending on how many hosts larvae, nymphs and adults feed on.</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td>  </td>
</tr>
<tr>
<td rowspan="2">T15. (mite)

Sarcoptes scabiei
adult mite with egg
ex pig skin
whole mount</td>
<td>Lower the condenser and scan to slide to find the very small round mites (right). Use high power to note the prominent dorsal spines and how coxae 1 and 2 are separated from coxae 3 and 4 (legs 3 and 4 are short and do not extend beyond body margin) (below right). The mites live in tunnels in the skin (below) causing intense pruritis known as scabies in humans and mange in animals.</td>
<td>  </td>
</tr>
<tr>
<td>  </td>
<td>  </td>
</tr>
</table>

Diagnostic Parasitology



The Australian Society for Parasitology Inc.

PARASITOLOGY

DIAGNOSIS (D^x)

DIAGNOSIS OF WHAT?

- INFECTION (presence of parasites)
  - stage: incubation, latent, pre-patent, patent
  - type: cryptic, occult, ectopic…
  - consequences: asymptomatic, subclinical, clinical
- DISEASE (perturbation in structure/function = pathology)
  - onset: fast / slow (acute / chronic)
  - duration: transient / prolonged (acute / chronic)
  - manifestations: symptoms / signs
  - severity: mild to fatal

Who makes diagnoses?

- doctors
- nurses
- healthcare workers
- scientists
- technicians
- patients
- family
- community
- public

Diagnosticians

Clinicians / Practitioners (medical / veterinary)

supported by:
- health-carers (e.g. nurses)
- laboratories (e.g. scientists / technicians)

"D^x is central intellectual activity of medicine!"

process to turn data about patient into names of diseases
- serves as a guide to action / intervention (M^x, T^x)
- helps foretell future (prognosis)

Educational continuum

Primary: Literacy + Numeracy (3Rs)
Secondary: Natural Sciences / Social Sciences

Tertiary (health sciences):
- disciplinary
- specific
- compartmentalized
- hypothetico-deductive logic
- preclinical
- health
- problem-based L
- student/system-centred

⇒ SCIENTISTS

Quaternary (medicine):
- multidisciplinary
- holistic
- integrated
- clinical reasoning
- clinical
- sickness
- case-based L
- patient-centred

⇒ PRACTITIONERS

Clinical reasoning

Clinical reasoning, aka clinical judgment, detective work, problem solving, decision making, critical thinking…

Process by which clinicians:
- collect cues;
- analyse information;
- come to an understanding of patient problem;
- plan / implement interventions;
- evaluate outcomes; and
- reflect on situation.

Not a linear process, but conceptualised as a series of linked and ongoing clinical encounters

Philosophically,

Clinical reasoning is a complex logic chain/loop using inductive and deductive inference, and falsification

LOGIC
(= reasoned argument)
premises → conclusion

Inductive reasoning
particular case → generalization
(belief in 'uniformity of Nature')

Deductive reasoning
general → particular
(if … and …, then …)

Falsification
prove conjecture true / false (Dx by exclusion)

Dx process

Difficult to teach clinical reasoning
- quite intuitive, experiential, reflective…

Nevertheless, begin with basic systematic process:

P	presentation	- main complaint
H	history	- medical, morbid state
D	data	- physical examination
		- laboratory tests

Presentation

often pain, trauma, functional abnormality
(severe enough to seek intervention)

general health assessment

- vitals (begin with first-aid DR ABC)
- then systematic [VINDICATE PECS]
(vascular, infectious/inflammatory, neoplastic, degenerative, intoxication/idiopathic/iatrogenic, congenital/genetic, allergic/autoimmune, traumatic, endocrine/metabolic, psychological, ethico-legal, clinical, society/population)

History

obtain medical history (conditions, drugs, allergies…)
(family, environment…)

determine pre-morbid state as basis for comparison

assess morbidity

- begin with functional enquiries
(cardiovascular, respiratory, gastrointestinal, neurological, musculoskeletal)
- then details [NILDOCAR]
(nature, intensity, location, duration, onset, concomitant, aggravating, relieving)

Data

gather 1^0 data - physical examination (hands-on)
- inspect (look)
- palpate (feel)
- percuss (listen)
- auscultate (listen)
- quantitate (measure)

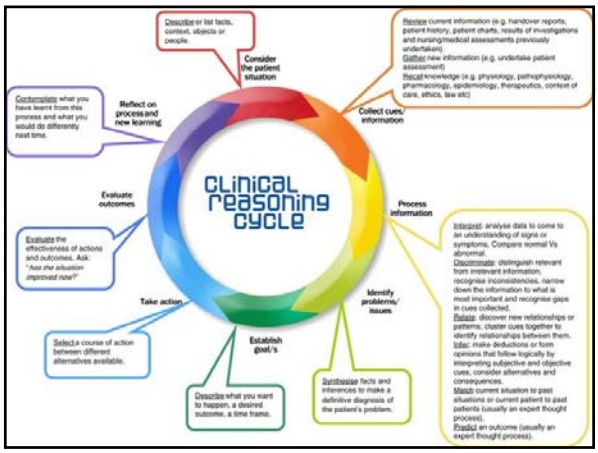
then seek 2^0 data - laboratory tests (remote)
- biopsy / necropsy
- test matrix
  - stool, blood/fluids, tissues
- test procedure
  - coprology, haematology, biochemistry, serology, microbiology, histopathology…

DDx

- use current information to shortlist possible Dx
- select most likely ("working Dx")

- consider other possible Dx
- decide on discriminating tests
- form Mx plan (surgical, pharmacological…)

- use further data to check Dx ("final Dx")
- follow-up
- repeat cycle as required



Why is Dˣ so difficult?

- most clinical manifestations are not specific
- huge number of manifestations (~ 5,000)
- huge number of diseases (~ 5,000)
- what is normal / abnormal?
- unreliable / incomplete data
- multiple pathology (> 1 disease involved)
- variable incidence (rare → common)
- human error
- imperfect technologies
- poor test accuracy, sensitivity, specificity

- some symptoms simply inexplicable!

Main causes of pathology

- hypoxia (oxygen deprivation)
  [ischaemia, anaemia]
- physical agents
  [trauma, burns, radiation, electric shock]
- infectious agents
  [viral, bacterial, fungal, parasitic]
- chemical agents
  [drugs, poisons, toxins, metabolites]
- immunological reactions
  [hypersensitivity, immunopathology]
- genetic derangements
  [gene defects]
- nutritional imbalances
  [protein deficiencies, lipid excesses]

Parasitism

Parasites need hosts in order to live by:

- attaching / invading
- feeding / growing
- multiplying / reproducing
- transitioning between hosts

All while surviving / avoiding host responses:

- physiological
- immunological
- pathological

Parasite pathogenicity

Endoparasites cause disease by:

- stealing nutrients
  (ingestion, absorption..)
- destroying cells
  (ingestion, lysis…)
- migrating through tissues
  (tunneling, tracking..)
- lodging in tissues
  (obstruction, space-occupying lesions..)
- releasing metabolites
  (E/S products, anticoagulants, toxins..)
- provoking host reactions
  (inflammation, hypersensitivity..)

Parasite pathogenicity

Ectoparasites cause disease by:

- harassing hosts
  (altered behaviour..)
- feeding on blood
  (anaemia, dermatitis…)
- injecting toxins
  (producing host reactions..)
- irritating skin
  (causing pruritis..)
- invading skin
  (causing trauma, dermatitis, hypersensitivity..)
- invading tissues
  (causing trauma, inflammation, hypersensitivity..)

Disease

Severity influenced by:

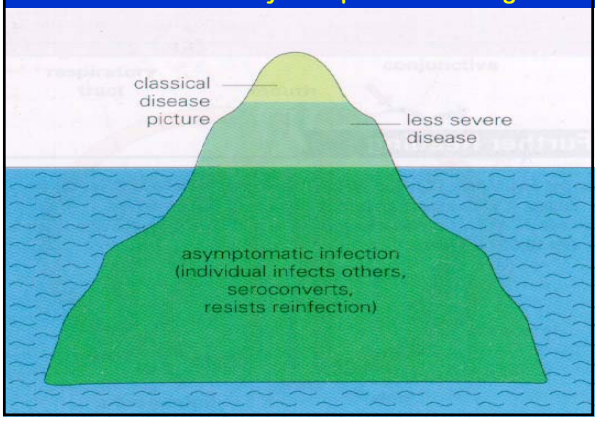
- number of parasites
- size of parasites
- sex of parasites
- stage of development
- site of infection
- duration of infection
- metabolic requirements
- growth rates
- asexual multiplication rates
- sexual reproductive rates

Host + Parasite ≠ Disease

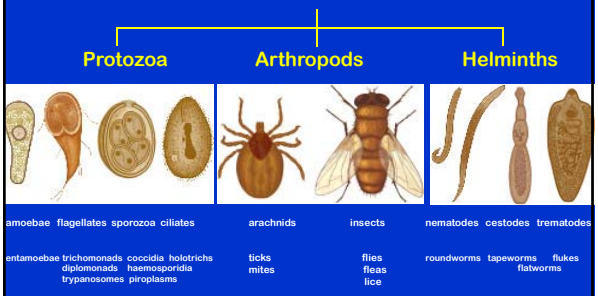
Outcome of infection dependent on various host-parasite interactions, especially:

- parasite pathogenicity
  - high pathogenicity causes morbidity/mortality
  - low pathogenicity tolerated (commensalism?)
- host responses
  - over-reaction causes pathology (immunopathology)
  - under-reaction fails to clear infection

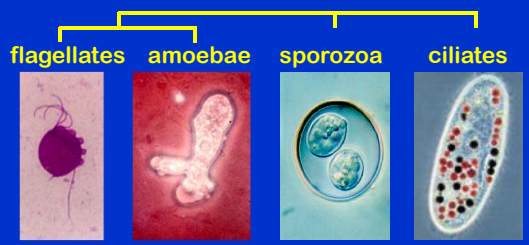
Disease is merely the tip of the iceberg



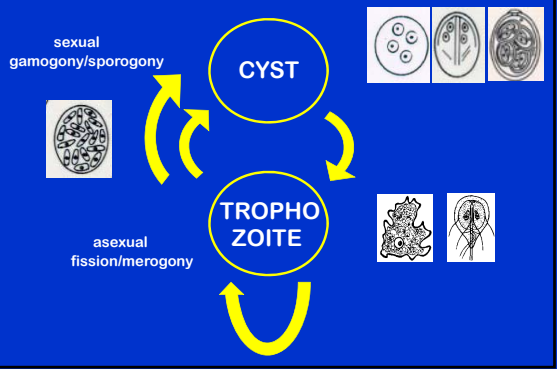
PARASITE ASSEMBLAGES

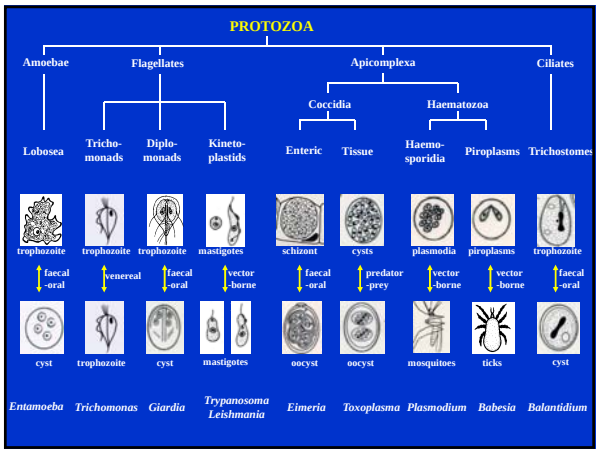


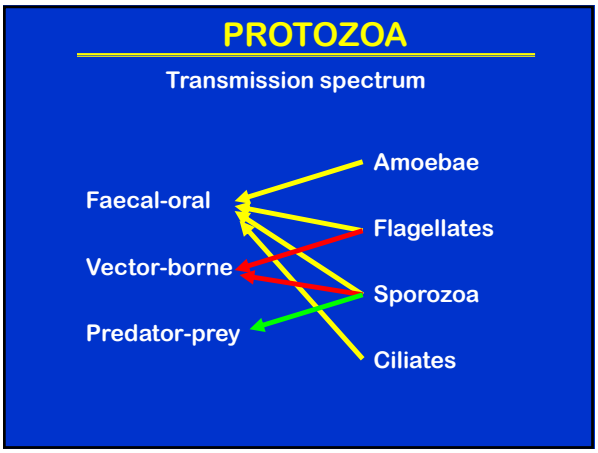
PROTOZOA (unicellular eukaryotes)

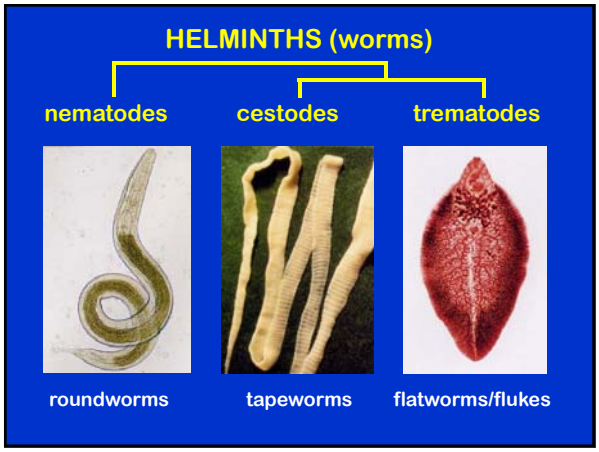


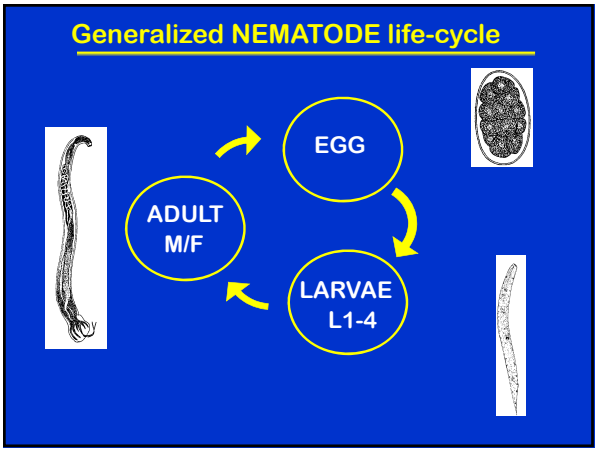
Generalized PROTOZOAN life-cycle

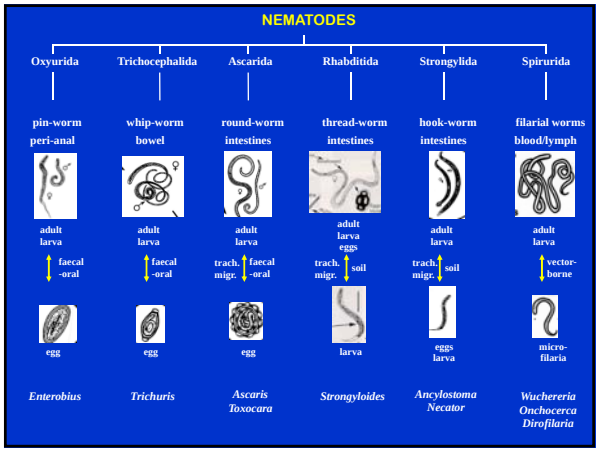


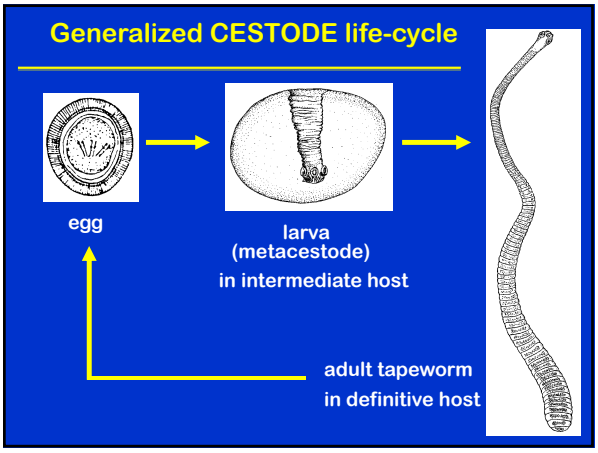

PROTOZOA
Amoebae
Flagellates
Apicomplexa
Ciliates
Coccidia
Haematozoa
Lobosea
Tricho-monads
Diplo-monads
Kineto-plastids
Enteric
Tissue
Haemo-sporidia
Piroplasms
Trichostomes
trophozoite
trophozoite
trophozoite
mastigotes
schizont
cysts
plasmodia
piroplasms
trophozoite
faecal -oral
venereal
faecal -oral
vector -borne
faecal -oral
predator -prey
vector -borne
vector -borne
faecal -oral
cyst
trophozoite
cyst
mastigotes
oocyst
oocyst
mosquitoes
ticks
cyst
Entamoeba
Trichomonas
Giardia
Trypanosoma
Leishmania
Eimeria
Toxoplasma
Plasmodium
Babesia
Balantidium

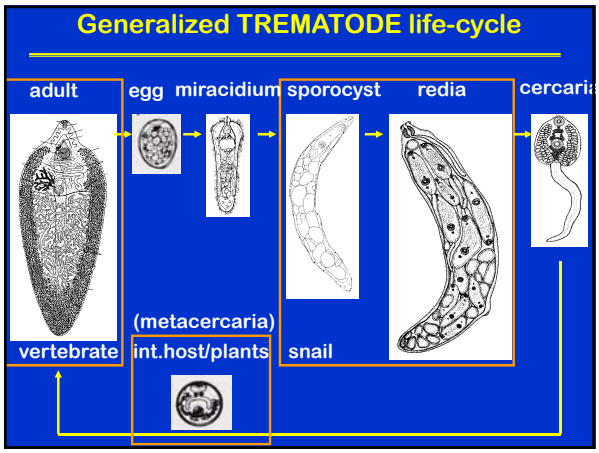

PROTOZOA
Transmission spectrum
Faecal-oral
Vector-borne
Predator-prey
Amoebae
Flagellates
Sporozoa
Ciliates

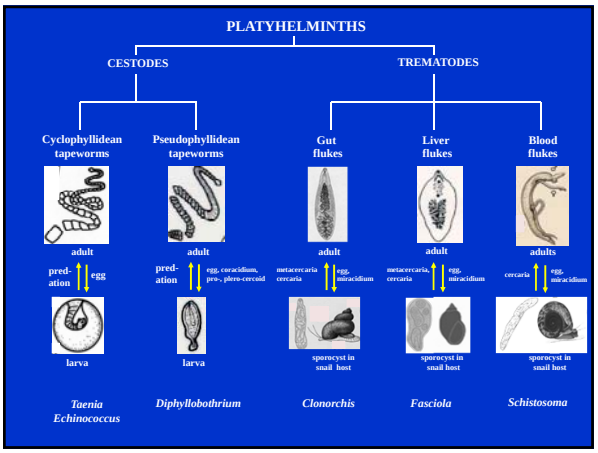

HELMINTHS (worms)
nematodes
cestodes
trematodes
roundworms
tapeworms
flatworms/flukes

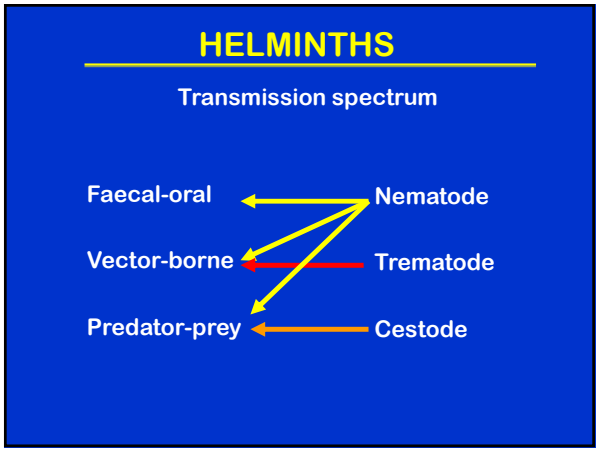

Generalized NEMATODE life-cycle
EGG
ADULT M/F
LARVAE L1-4

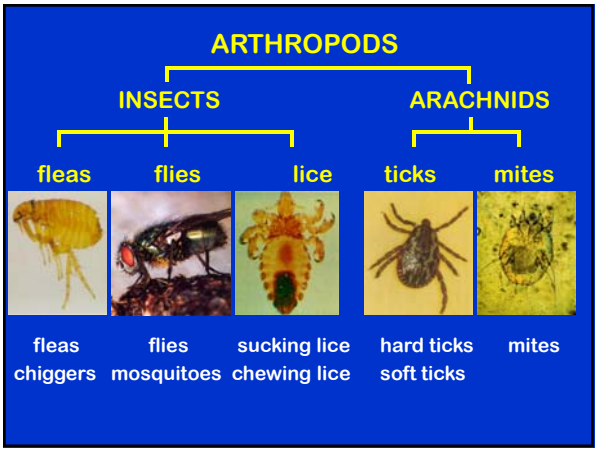

NEMATODES
Oxyurida
Trichocephalida
Ascarida
Rhabditida
Strongylida
Spirurida
pin-worm
peri-anal
whip-worm
bowel
round-worm
intestines
thread-worm
intestines
hook-worm
intestines
filarial worms
blood/lymph
adult larva
adult larva
adult larva
adult larva eggs
adult larva
adult larva
faecal -oral
faecal -oral
trach. migr.
faecal -oral
trach. migr.
soil
trach. migr.
soil
vector-borne
egg
egg
egg
larva
eggs larva
micro-filaria
Enterobius
Trichuris
Ascaris
Toxocara
Strongyloides
Ancylostoma
Necator
Wuchereria
Onchocerca
Dirofilaria

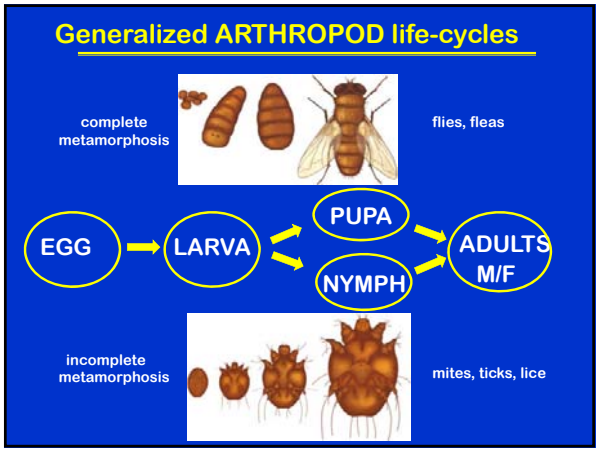

Generalized CESTODE life-cycle
egg
larva
(metacestode)
in intermediate host
adult tapeworm
in definitive host

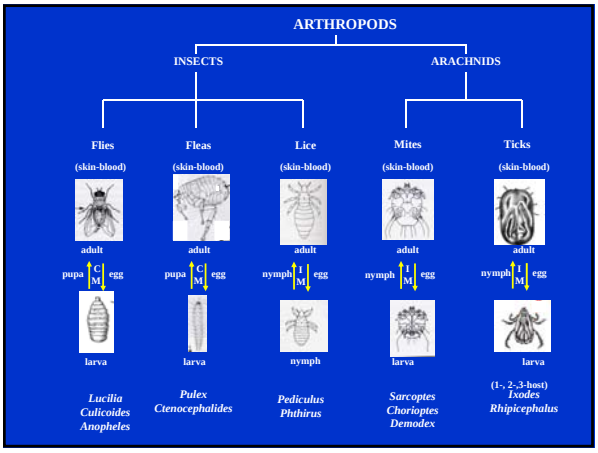

Generalized TREMATODE life-cycle
adult
egg
miracidium
sporocyst
redia
cercaria
(metacercaria)
vertebrate
int.host/plants
snail


PLATYHELMINTHS
CESTODES
TREMATODES
Cyclophyllidean tapeworms
Pseudophyllidean tapeworms
Gut flukes
Liver flukes
Blood flukes
adult
pred-ation
egg
egg, coracidium, pro-, plero-cercoid
metacercaria cercaria
egg, miracidium
sporocyst in snail host
cercaria
larva
Taenia
Echinococcus
Diphyllobothrium
Clonorchis
Fasciola
Schistosoma


HELMINTHS
Transmission spectrum
Faecal-oral
Vector-borne
Predator-prey
Nematode
Trematode
Cestode


ARTHROPODS
INSECTS
ARACHNIDS
fleas
flies
lice
ticks
mites
fleas
chiggers
flies
mosquitoes
sucking lice
chewing lice
hard ticks
soft ticks
mites


Generalized ARTHROPOD life-cycles
complete metamorphosis
flies, fleas
EGG
LARVA
PUPA
NYMPH
ADULTS M/F
incomplete metamorphosis
mites, ticks, lice


ARTHROPODS
INSECTS
ARACHNIDS
Flies
Fleas
Lice
Mites
Ticks
(skin-blood)
adult
pupa
egg
nymph
larva
Lucilia
Culicoides
Anopheles
Pulex
Ctenocephalides
Pediculus
Phthirus
Sarcoptes
Chorioptes
Demodex
(1-, 2-, 3-host)
Ixodes
Rhipicephalus

ARTHROPODS

Transmission spectrum

~~Faecal-oral~~

~~Vector-borne~~

~~Predator-prey~~

Insects → Direct (host-finding)

Arachnids →

CLINICIANS

Working with patients

Can make some common-sense deductions/inferences/predictions

Knowledge integration

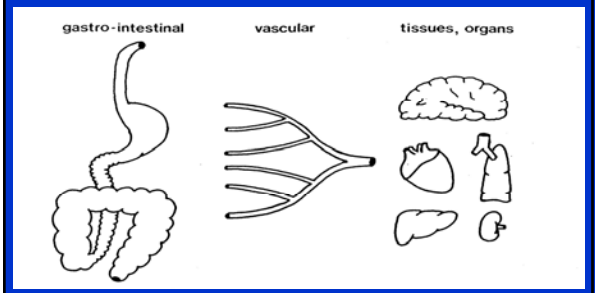
HOST IDENTITY:
- medical
- veterinary

SIGNS OF DISEASE:
- enteric
- vascular
- visceral

SITE OF INFECTION:
- gut
- blood
- tissues

TRANSMISSION CYCLE:
- faecal-oral
- vector-borne
- predator-prey

Site of infection, symptomatology, transmission



enteritis diarrhoea	anaemia fever	lesions malfunction
faecal-oral	vector-borne	predator-prey

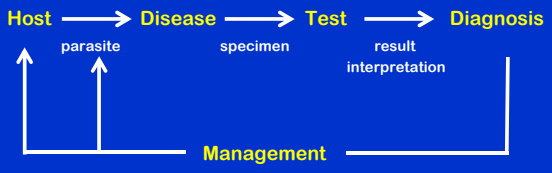
Clinical Review

Site	Symptoms	Transmission	PROTOZOA	HELMINTHS	ARTHROPODS
Gut	diarrhoea, blockage, anaemia	faecal-oral	amoebae diplomonads coccidia Ciliates	round-, pin-, whip-,thread-, hook-worms tapeworms enteric flukes	-
Blood	anaemia, fever, ischaemia	vector-borne	trypanosomes haemosporidia Piroplasms	filiarial worms blood flukes	-
Tissues	lesions dysfunction inflammation	predator-prey	cyst-forming coccidia microspora	hydatids cysticerci liver flukes Trichinella	-
- skin	lesions	direct	-	-	flies fleas lice mites ticks

Parasitology

TEST PERFORMANCE

Diagnostic Testing



Multi-step process
Numerous factors can influence test integrity
Consequences can be negligible to dire
Best practice should be enshrined in legislation

OH&S requirements

Biological samples (universal precautions…)

- method/size/number/time of collection
- appropriate container(s)
- characteristics (content/consistency/…)
- preservation
- transport
- storage
- disposal

Tests not *ad hoc*, but recommended/regulated

- Professional bodies (societies, organizations)
  (best practice, WHO, CDC…)
- State government legal regulations
  (health depts, hospitals, pathology labs…)
- Federal government national codes
  (NHMRC, TGA, OHS compliance)
- NATA accreditation
  (accreditation, value-added)

⇒ SOP (standard operating procedures)
⇒ QA/QC (quality assurance/control)

Parasitology diagnostic tests

Indirect indication of disease
- symptomatology (fever, inflammation, wheeze, etc…)

Direct detection of parasites
- macroscopic examination (visible characters)
- microscopy (live/fixed, contrast/stained, fluorescence)
- culture (*in vitro*, *in vivo*, xeno-D^{x})
- imaging (X-ray, ultrasound, CT, MRI)

Indirect demonstration of parasite products/host responses
- haematology (FBC, ESR, differential, etc…)
- biochemistry (plasma/serum, liver/muscle/gut, etc…)
- serology (host antibodies, parasite antigens)
- molecular biology (parasite proteins/DNA/RNA)

Types of tests

Subjective (observation)
- find/identify parasite stages
  (e.g. microscopy)

Objective (independent measurement)
- colorimetric reading
  (e.g. ELISA, pepsinogen assay…)

Hybrids (bit of both)
- visual assessment of indicator system
  (e.g. IHAT, PCR amplicon…)

Ideal characteristics of diagnostic test

- safety consideration
- cost efficient
- time efficient
- long-lived reagents
- ease of performance
- reproducibility
- accuracy
- specificity
- sensitivity

Sensitivity

Definitions:
1. the state or quality of being sensitive
2. the smallest concentration of a substance that can be reliably measured by a given analytical method
3. the probability that a person having a disease will be correctly identified by a clinical test

- not how few parasites can be detected (ng/mL, parasitaemia…)
  ⇒ limit of detection (LOD)
- diagnostic test sensitivity is a measure of inclusion (true positive rate)

Specificity

Definitions:
1. the quality or state of being specific
2. the probability that a person who does not have a disease will be correctly identified by a clinical test

- not how specific a test is for a parasite genus/species/strain/serotype/genotype
  ⇒ test cross-reactivity (presence/absence)
- diagnostic test specificity is a measure of exclusion (true negative rate)

EFFICACY OF TEST

		INFECTION STATUS: infected	INFECTION STATUS: not infected	
TEST	positive	A true +	B false +	A+B
	negative	C false -	D true -	C+D
		A+C	B+D	N

TEST ACCURACY	=	(A+D) / N
TEST SENSITIVITY	=	A / (A+C)
TEST SPECIFICITY	=	D / (B+D)
POSITIVE PREDICTIVE VALUE	=	A / (A+B)
NEGATIVE PREDICTIVE VALUE	=	D / (C+D)

Test parameters

Gold Standard — Disease Status

Diagnostic Test		Present (D+)	Absent (D-)	Total
	Positive (T+)	a	b	a+b
	Negative (T-)	c	d	c+d
	Total	a+c	b+d	N

- Sensitivity = Pr(T+|D+) = **a/(a+c)**
  --Sensitivity is **PID** (Positive In Disease)
- Specificity = Pr(T-|D-) = **d/(b+d)**
  --Specificity is **NIH** (Negative In Health)

Consequences of misdiagnosis

<u>Poor sensitivity</u>
unacceptable number of false negatives

- no treatment → disease progression → death

<u>Poor specificity</u>
unacceptable number of false positives

- unnecessary treatment → side effects → cost

Probability

In the 1980s, blood screening in Florida found that 22 people who had donated blood tested positive for AIDS. Once notified of the test results, seven of these donors committed suicide.

The AIDS test has a very high sensitivity [99.9%] and specificity [99.99%].

The prevalence of infection (for people with low-risk behaviour) is around 1 in 10,000.

Probability

Q. What is the probability that someone who tests positive for AIDS is infected?

consider 10,000 people

infection	1 +	9,999 -
test	sens 1 +	spec 1 +
	true +	false +

p = 0.5

What is acceptable?

Table 3. Trial 8. comparison of prediction of parasitaemia by ICT, slide and reported symptoms in 334 patients, and axillary temperature in 244 patients with parasitaemia, using parasitaemia detected by ICT and/or slide as standard[a]

Test/symptom	Sensitivity (%)	Specificity (%)	PPV[b] (%)	NPV[c] (%)
ICT	97.9 (94.5–99.3)[d]	–	–	–	97.2 (92.5–99.1)
Slide (restained)	74.9 (68.1–80.7)	–	–	–	73.9 (68.9–79.9)
Slide (in-vivo)	60.0 (52.7–66.9)	–	–	–	64.1 (57.2–66.9)
Fever (F)	95.4 (91.1–97.7)	16.5	11.0–24.0)	61.6 (55.8–67.1)	71.9 (53.0–85.6)
Chills (C)	86.2 (80.3–90.5)	38.8 (30.8–47.5)	66.4 (60.2–72.1)	66.7 (55.2–76.5)
Headache (H)	79.3 (73.0–84.8)	17.5 (11.6–24.8)	57.4 (51.5–63.3)	37.5 (26.0–50.3)
F + C	82.6 (76.3–87.5)	47.5 (39.0–56.1)	68.8 (62.4–74.6)	66.0 (55.8–75.0)
F + H	74.9 (68.1–80.7)	32.4 (24.8–40.9)	60.8 (54.3–67.0)	43.9 (37.6–58.4)
F + C + H	66.7 (59.0–73.7)	54.7 (46.0–63.1)	67.3 (60.0–73.7)	53.5 (45.0–61.9)
F + (C +/or H)	91.3 (86.2–94.7)	25.2 (18.4–33.4)	63.1 (57.2–68.7)	67.3 (52.8–79.3)
Axillary temperature ≥ 37.0 °C	67.5 (58.4–75.5)	45.8 (36.8–55.1)	56.1 (47.7–64.1)	57.9 (47.3–67.8)
Axillary temperature ≥ 37.5 °C	55.8 (47.5–63.9)	86.2 (78.7–91.4)	71.0 (57.9–81.4)	58.6 (51.5–65.6)
Axillary temperature ≥ 38.0 °C	23.6 (16.6–32.7)	93.3 (86.9–96.9)	70.4 (61.3–78.6)	54.4 (47.3–61.3)

[a] Recently treated patients excluded.
[b] PPV = positive predictive value.
[c] NPV = negative predictive value.
[d] Figures in parentheses are 95% confidence limits.

ICT = immuno-chromato-graphic test)

What is acceptable?

Malaria Antigen Detection - RDTs

Feature	PfHRP-2 tests	pLDH tests
Sensitivity/ Specificity*	Sensitivity 92-100% Specificity 85- 100%	Sensitivity *P.f.* 88-98% *P.v.* 89-94% Specificity *P.f.* 93-99% *P.v.* 99-100%
Commercial cost/test**	Approximately US$ 0.60 –1.00	Approximately US$ 2.50
Commercial products	1) PATH falciparum Malaria IC Strip test – Program for Appropriate Technology in Health 2) MAKROmed™ 3) Orchid ®	1) OptiMAL® - Flow, Inc. 2) Binax NOW ®ICT Malaria - Binax, Inc.

* Compared to microscopy, results from multiple studies
** Varies by size of order and vendor

RDT = rapid diagnostic test

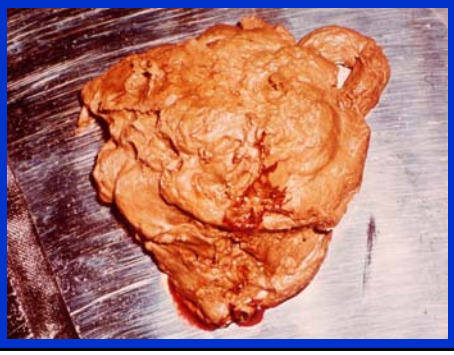
What is acceptable?

Comparison Test	Sample Size SNAP 3Dx Test/Reference Test +/+	−/+	+/−	−/−	Total	Sample Type	Relative Sensitivity and Specificity 95% Confidence Limit	Kappa Statistic
PetChek® Heartworm	152	3	0	157	312	Serum/ Plasma/ Whole Blood	Sen., 98% (95% CL 94%–100%) Spec., 100% 95% CL 97%–100%)	0.98
Heartworm Necropsy	54	8	0	0	62	Serum/ Plasma	Sen., 87% (95% CL 76%–93%)	N/A
E. canis IFA/Western blot	79	1	0	164	244	Serum	Sen., 99% (95% CL 92%–100%) Spec., 100% 95% CL 97%–100%)	0.99
B. burgdorferi IFA/Western blot	171	14	0	170	355	Serum	Sen., 92% (95% CL 88%–96%) Spec., 100% 95% CL 97%–100%)	0.92

Comparison Test	Sample Size SNAP® Giardia Test/Reference Test +/+	−/+	+/−	−/−	Total	Sample Type	Relative Sensitivity and Specificity 95% Confidence Limit	Kappa Statistic
Immunofluorescence microscopy	74	4	1	144	223	Fecal	Sen., 95% (95% CL 87%–98%) Spec., 99% 95% CL 96%–100%)	0.95
Microplate ELISA	75	3	0	145	223	Fecal	Sen., 96% (95% CL 88%–99%) Spec., 100% 95% CL 97%–100%)	0.97

Coprololgy

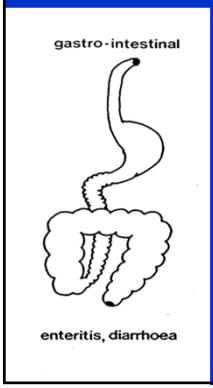
(working with faeces)



Alimentary tract

- anorexia (loss of appetite)
- diarrhoea (frequency & consistency)
- vomiting (regurgitate)
- oedema (swollen tissues)
- dehydration (water loss)
- abdominal pain (local, referred)
- anaemia (reduced haematocrit)
- hypoalbuminaemia (reduced albumin)
- eosinophilia (increased eosinophils)
- plasma pepsinogen (stomach damage)
- liver enzymes (liver damage)

Site of infection and symptomatology



structure	function
•stomach	produce molecular soup
•small intestines	absorb nutrients
•large intestine	retain water

excessive evacuation of too fluid faeces

(frequency + volume + consistency)

Diarrhoea

- Definition:
  - Imprecise…
  - Fluid stools
  - 3+ bowel movements/day?
  - Increased stool weight?
- Need to consider:
  - frequency, volume, consistency, colour, content, smell

Whole gut transit time	Type of stool	Description	Pictorial representation
Long transit (e.g., 100 hours)			
	Type 1	Separate hard lumps, like nuts, hard to pass	
	Type 2	Sausage shaped but lumpy	
	Type 3	Like sausage but with cracks on its surface	
	Type 4	Like sausage or snake, smooth and soft	
	Type 5	Soft blobs with clear-cut edges (passed easily)	
	Type 6	Fluffy pieces with ragged edges, a mushy stool	
	Type 7	Watery, no solid pieces	Entirely liquid
Short transit (e.g., 10 hours)			

General Syndromes

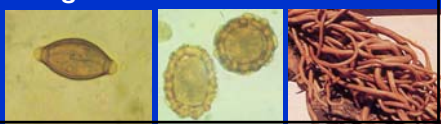
- Small intestine diarrhoea (non-inflammatory)
  - leucocytes absent
  - mucus rare
  - blood rare
  - voluminous
  - little pain
  - no fever
- viruses, *Vibrio cholerae, E. coli* (ETEC, EPEC), *Staphylococcus, Bacillus, Clostridium perfringens, Giardia, Cryptosporidium, Isospora, Cyclospora*
- Large intestine diarrhoea(inflammatory)
  - leucocytes present
  - mucus present
  - blood present
  - normal volume
  - severe pain (LLQ)
  - fever may be present
- *Shigella, Salmonella, Yersinia, Campylobacter, Clostridium difficile, E. coli* (EHEC, EIEC), *Aeromonas, Vibrio parahaemolyticus, Entamoeba*

GI-PATHOGENS

- bacteria (boring)
- viruses (vile)
- parasites (rule!)

- protozoa — small — multiplicative — acute

- worms — big — cumulative — chronic



Diagnostic tests

- macroscopic (characteristics)
- microscopic (cysts, eggs, larvae)
  - wet mount (saline, iodine, methylene blue)
  - concentration techniques
    - sedimentation (formalin-ether, iodine-trichrome)
    - floatation (saturated salt/sugar)(FEC)
  - permanent stained smears (fixed, stained)
    - trichrome, iron haematoxylin, acid-fast, …
- copro-antigen (DFA, EIA, dipstick)
- molecular biology (DNA extraction, PCR)
- culture (filter paper, Baermann, …)
- sticky tape test (pinworm)
- endoscopy/colonoscopy

Sedimentation / Floatation

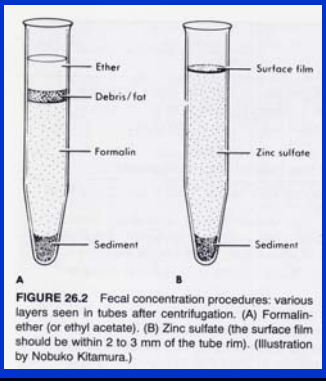


FIGURE 26.2 Fecal concentration procedures: various layers seen in tubes after centrifugation. (A) Formalin-ether (or ethyl acetate). (B) Zinc sulfate (the surface film should be within 2 to 3 mm of the tube rim). (Illustration by Nobuko Kitamura.)

protozoal (oo)cysts
helminth eggs
(filled with E reserves)

heavier than water, thus sediment

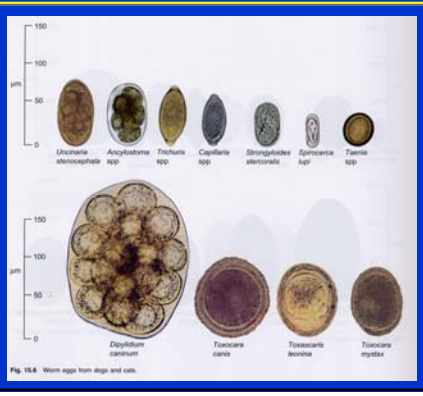
buoyant in viscous media, thus float
dep on SG (1.1-1.4)
- $MgSO_4$
- $ZnSO_4$
- sugar/sucrose
- KI

Helminth eggs

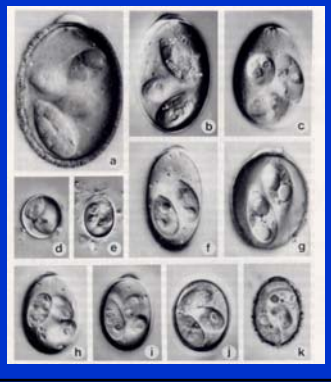
morphometric characterization

- size
- shape
- appearance
- wall
- adornments
- contents

Helminth eggs



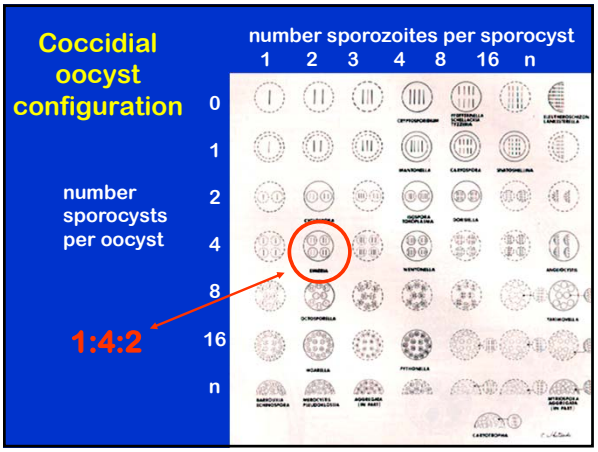
Protozoal cysts



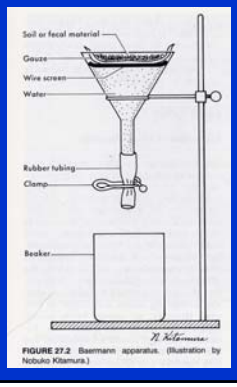
morphometric characterization

- size
- shape
- appearance
- wall
- adornments
- contents

Coccidial oocyst configuration

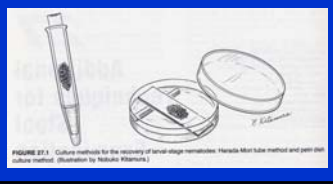


Faecal culture

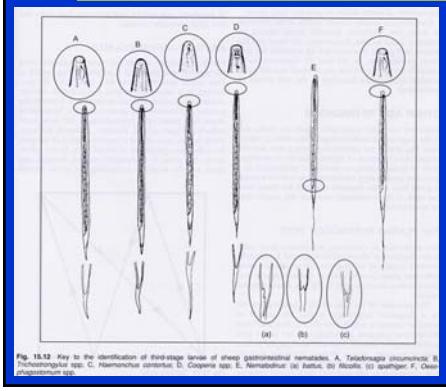


helminth eggs hatch, release larvae

migrate out of faeces



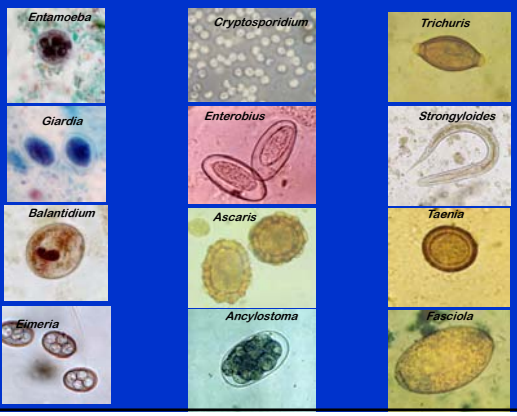
Larval identification



larval morphology

heads & tails

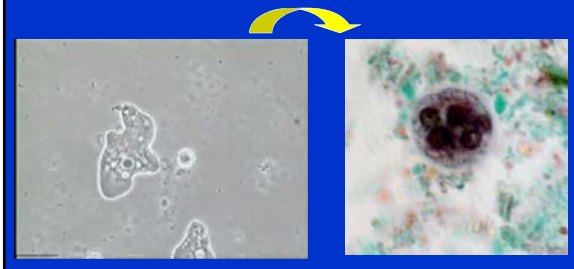
PARASITES IN FAECES



G-I PROTOZOA

AMOEBAE		
Entamoeba	diarrhoea	faecal-oral
FLAGELLATES		
Giardia	diarrhoea	faecal-oral
SPOROZOA		
Isospora	diarrhoea	faecal-oral
Cryptosporidium	diarrhoea	faecal-oral
Cyclospora	diarrhoea	faecal-oral
CILIATES		
Balantidium	diarrhoea	faecal-oral

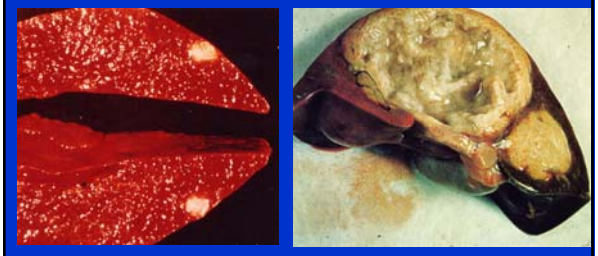
AMOEBA - *Entamoeba histolytica*



trophozoite (feeding stage) (multiplicative)

cyst (environmentally-resistant)

Abscess formation

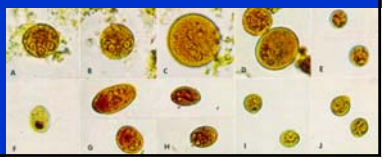


space-occupying pustular lesions

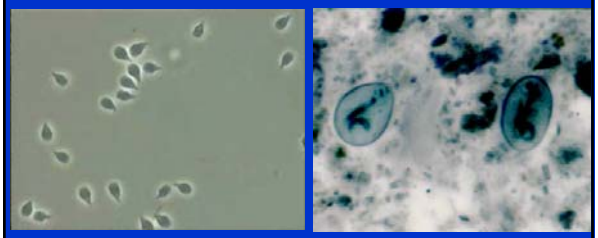
Diagnosis

Entamoeba histolytica	enteropathogen
Entamoeba dispar	nonpathogenic '*histolytica*'?
Entamoeba polecki	sometimes pathogenic
Dientamoeba fragilis	sometimes pathogenic
Entamoeba coli	nonpathogenic
Entamoeba hartmanni	nonpathogenic
Endolimax nana	nonpathogenic
Iodamoeba butschlii	nonpathogenic

cyst size
no. nuclei
chromatin pattern



FLAGELLATE - *Giardia duodenalis*



trophozoites
(feeding stages)
(multiplicative)

cysts
(environmentally-
resistant)

Action of parasite

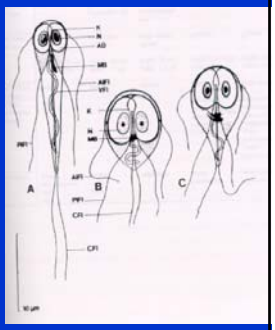
- physical blanketting
- chemical action
- malabsorption
- epithelial turnover
- villous atrophy

HOST SPECIFICITY

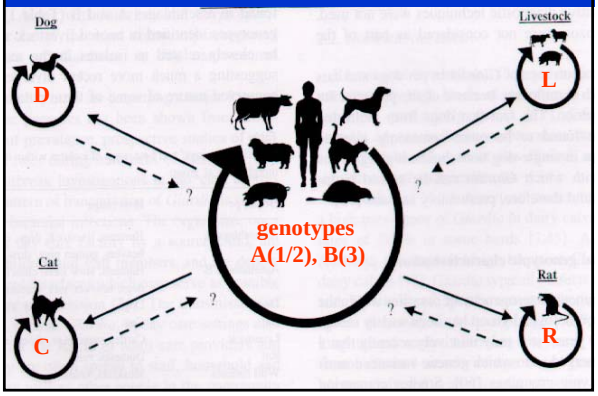
Three morphotypic groups

- *G. agilis (gracilis)*
  amphibia, birds, reptiles
- *G. muris (ardae)*
  rodents, birds, reptiles
- *G. duodenalis*
  (syn. intestinalis, lamblia)
  mammals, birds, reptiles

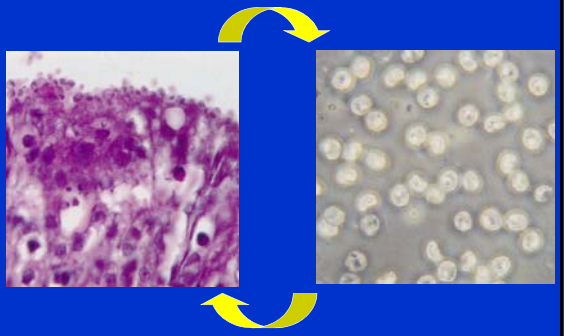
zoonotic potential?!!!



Molecular characterization (rDNA, GDH)



Enteric coccidia - *Cryptosporidium* spp.

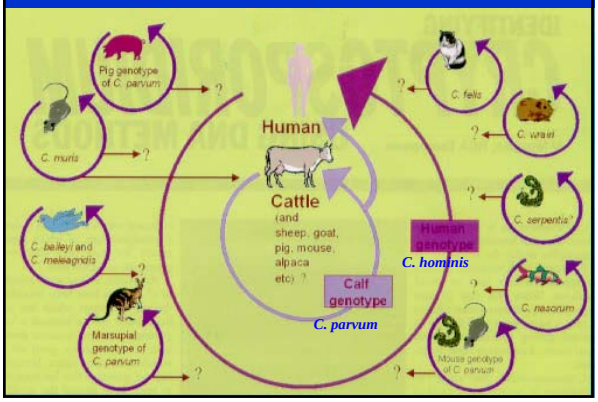


endogenous stages

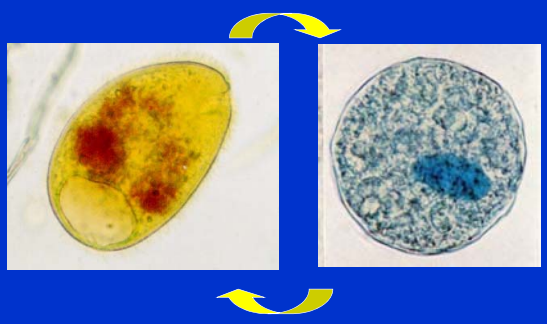
exogenous oocysts

parasite carpet

Genetic characterization



Ciliate: *Balantidium coli*

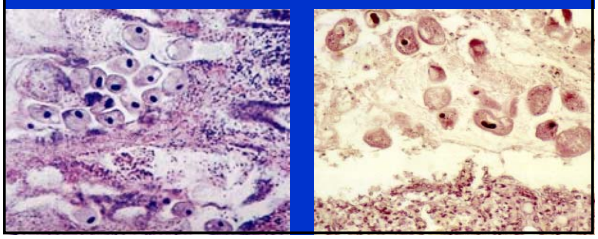


trophozoite in colon

cyst in faeces

Balantidiasis

- infections in humans, monkeys and pigs, esp. in tropics
- most infections asymptomatic
- some cause dysentery-like syndrome (diarrhoea, tenesmus, nausea, vomiting, anorexia, headache, insomnia, weakness)
- some infections involve tissue invasion (mucosal sloughing, haemorrhage, abscess formation, necrosis)



G-I NEMATODES

FAECAL-ORAL (eggs ingested)

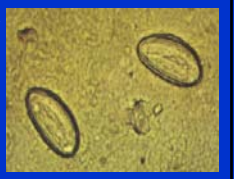
Enterobius (pinworm)	pruritus
Trichuris (whipworm)	malnutrition
Ascaris (roundworm)	obstruction

CONTACT (larvae penetrate skin)

Ancylostoma (hookworm)	anaemia
Necator (hookworm)	anaemia
Strongyloides (threadworm)	variable

PINWORM - *Enterobius vermicularis*

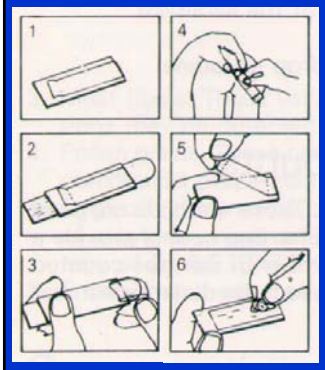
egg ingested

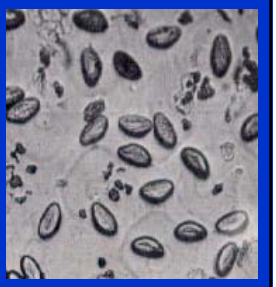


adult worms (feeding stages) (cumulative)

eggs (environmentally-resistant)

peri-anal sticky tape test





pruritus
irritability
insomnia

WHIPWORM - *Trichuris trichiura*

adult worms

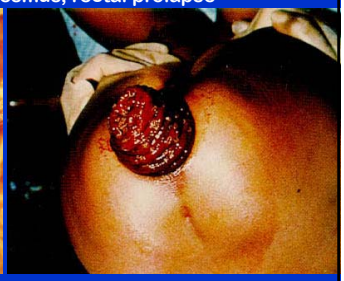
(feeding stages)
(cumulative)

eggs

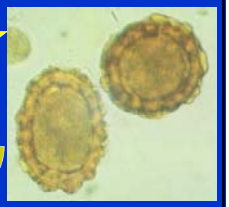
(environmentally-resistant)

Pathogenesis

- diarrhoea
- allergy
- mechanical damage to mucosa
- tenesmus, rectal prolapse



ROUNDWORM - *Ascaris lumbricoides*



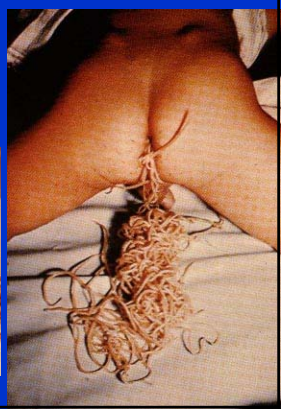
adult worms
(feeding stages)
(cumulative)

eggs
(environmentally-resistant)

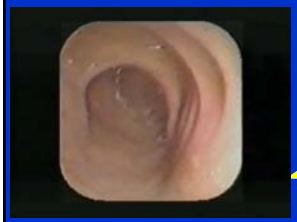
Pathogenesis

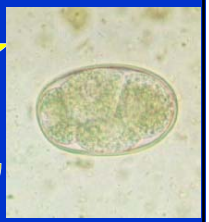
- mechanical blockage

(gut obstruction)



Old World hookworm - *Ancylostoma duodenale*
New World hookworm - *Necator americanus*



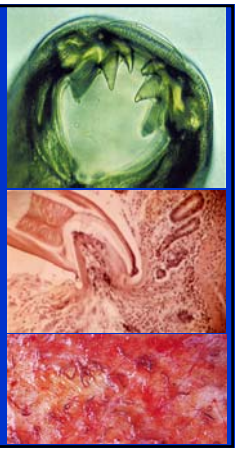


adult worm
(feeding stage)
(cumulative)

egg
(hatches exogenously)
(larvae penetrate skin)

Pathogenesis

- mucosal necrosis
  (ingested plug)
- diarrhoea
  (black-red stools)
- blood loss
  (direct ingestion)
  (haemorrhage)
  (iron deficiency anaemia)



Strongyloides (threadworm)

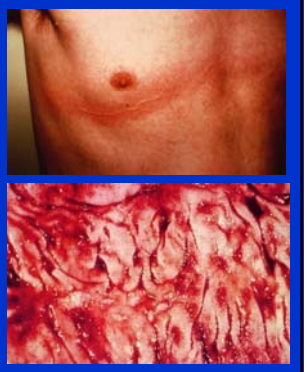
hermaphroditic female 5-7 mm
embedded in small intestine mucosa

larvae in faeces

numerous taxa ranging from free-living to parasitic
in cattle, sheep, horses, pigs, dogs, cats and humans

Pathogenesis

- asymptomatic
- peripheral eosinophilia
- cutaneous lesions
  (larva currens)
- pulmonary damage
  (pneumonitis)
- intestinal disease
  (mucosal damage)
- hyperinfection syndrome
  (auto-infection)



Clinical Review

Site	Symptoms	Transmission	PROTOZOA	HELMINTHS	ARTHROPODS
Gut	diarrhoea, blockage, anaemia	faecal-oral	amoebae diplomonads coccidia ciliates	round-, pin-, whip-,thread-, hook-worms tapeworms enteric flukes	-

Mx

- chemotherapy
  - anti-protozoals
  - anthelmintics
- treat symptoms/signs
  - dehydration (fluid, electrolytes)
  - gut motility (anti-diarrhoeal agents)
- preventive measures
  - improve hygiene, reduce contamination
    - personal/familial/community
    - food/water/environment

Parasitology re-defined!

I am a COPROLOGIST
proud to study ..IT.
Not Information Technology
nor International Trade
More like Internal Trafficking
of digesta previously made.

I revel in the baseness,
the beauty of the beasts,
the cunning little parasites
indulging in my feasts.

Haematology

(working with blood)

BLOOD SAMPLES

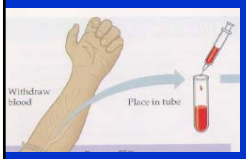
Usually taken by needle venepuncture (often using vacutainer)

Containers with coagulants
- gold or striped red/black top: with clot activator and gel
- red top: with clot activator
- orange or striped grey/yellow top: with thrombin

Containers with anticoagulants
- green top: with heparin or lithium heparin
- light green or striped green/gray top: for plasma
- purple or lavender top: with EDTA
- grey top: with fluoride and oxalate
- light blue top: with citrate reversible anticoagulant
- dark blue top: with sodium heparin
- pink top: with EDTA
- black top: used for ESR

Other
- white top: no additives
- light yellow top: with sodium polyanethol sulfonate
- tan top: with heparin/EDTA

Blood composition



without anticoagulant

serum

clot (cells + fibrinogen)

with anticoagulant

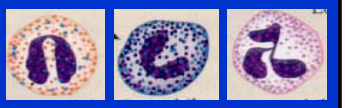
plasma (serum + fibrinogen)
white blood cells
red blood cells

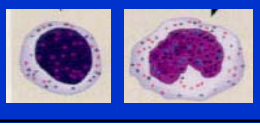
BLOOD CELLS

circulating cells divided into:

- erythrocytes (red blood cells)

- leucocytes (white blood cells)
  - granular
    - eosinophils
    - basophils
    - neutrophils
  - agranular
    - lymphocytes
    - monocytes

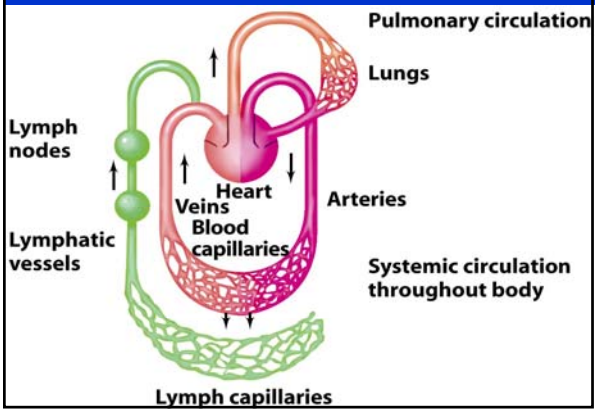




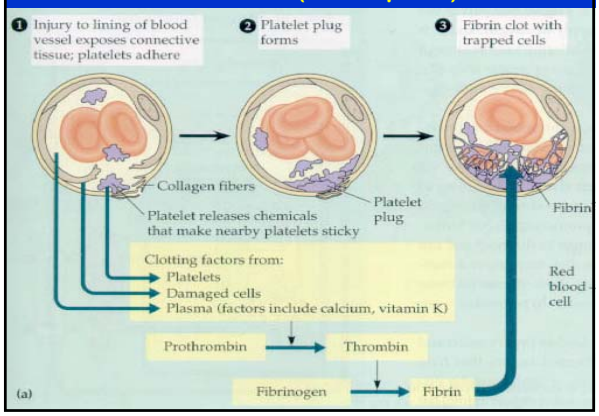
PLASMA CONSTITUENTS

• water	solvent
• ions	Na, K, Ca, Mg, Cl, HCO_3 (osmotic balance, pH buffering, regulation of membrane permeability)
• proteins	albumin (osmotic balance, pH)
	fibrinogen (clotting)
	gamma-globulins (humoral immunity)
• gases	O_2 and CO_2 (respiration)
• nutrients	glucose, fatty acids, vitamins, etc
• metabolic waste	urea, lactic acid, etc
• hormones	various

CV system (containment) (but leaks)



CV SYSTEM (self-repairs)



Clinical haematology

Haematology = study of blood

- for disturbances in:
  - vascular characters (blood vessels)
  - cellular characters (red/white blood cells)
  - subcellular characters (platelets/bodies/haem)
  - acellular characters (protein, fibrinogen, etc)
- for presence of parasites
  - intracellular
  - extracellular
  - extravascular

Clinical Haematology - erythrocytes

- RBC concentration (millions per μl)
- PCV = packed cell volume = haematocrit (%)
- RBC diameter (μm)
- MCV = mean corpuscular volume (fl)
- Hb = haemoglobin (g/dl)
- MCH = mean corpuscular Hb (pg)
- MCHC = mean corpuscular Hb concentration
- ESR = erythrocyte sedimentation rate (mm/h)
- reticulocytes (%)
- nucleated erythrocytes (%)
- fragility test (% saline → haemolysis)

ERYTHROCYTE CHARACTERS

- size (anisocytosis, macrocytic, microcytic)
- shape (poikilocytes, leptocytes, spherocytes, target cells, acanthocytes, spherocytes, schistocytes)
- distribution (single, Rouleau, agglutination)
- colour (normochromic, hypochromic, polychromasia)
- abnormal structures (Howell-Jolly, Heinz, Pappenheimer bodies, basophilic stippling, nucleated red cells, inclusion bodies, nuclear fragmentation, parasites)

Clinical Haematology - leucocytes

- total WBC (no./μl)
- differential count (% types) (lymphocytes, monocytes, neutrophils, basophils, eosinophils)
- differential absolute count (no./μl)
- platelets = thrombocytes (no./μl)

LEUCOCYTE CHARACTERS

- abundance
- types
- abnormalities (nuclear degeneration; hyper/hypo-segmentation; toxic changes such as azurophilic granules, vacuolation, foaminess, basophilia, Dohle bodies; giant bizarre forms)
- cytoplasmic inclusions (phagosomes; inclusions; bacteria; parasites)

Impact of parasites

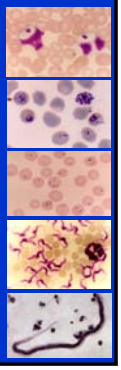
- compromise blood function (gas, nutrients, ..) (anaemia, haemolysis, erythrophagocytosis)
- disturb blood delivery (vascular changes) (ischaemia, cytoadherence, DIC)
- release toxins (haemozoin pigment)
- cause range of haematological abnormalities
- burden often quantitated as % parasitaemia

HAEMO-PARASITES

PROTOZOA	
Dactylosomatidae	*Dactylosoma, Babesiosoma, Haemohormidium, Haematractidium*
Haemogregarinidae	*Haemogregarina, Hepatozoon, Cyrilia, Desseria, Hemolivia, Karyolysus*
Lankesterellidae	*Lankesterella, Lainsonia, Schellackia*
Plasmodiidae	*Plasmodium, Hepatocystis, Mesnilium, Polychromophilus, Nycteria*
Haemoproteidae	*Haemoproteus, Haemocystidium, Parahaemoproteus, Simondia, Haemamoeba*
Leucocytozoidae	*Leucocytozoon, Akiba*
Babesiidae	*Babesia, Sauroplasma, Serpentoplasma*
Theileridae	*Theileria, Cytoauxzoon*
Trypanosomatidae	*Trypanosoma, Sauroleishmania*
NEMATODA	
Onchocercidae	*Onchocerca, Dirofilaria, Wuchereria, Mansonella, Brugia, Setaria, Loa*

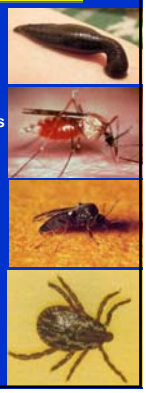
HAEMO-PARASITES

	meronts	gamonts
PROTOZOA		
Dactylosomatidae	rbc	rbc
Haemogregarinidae	viscera	rbc
Lankesterellidae	RE cells	RE cells
Plasmodiidae	liver	rbc
Haemoproteidae	RE cells	rbc
Leucocytozoidae	RE cells	wbc
Babesiidae	rbc	rbc
Theileridae	wbc	rbc
Trypanosomatidae	extracellular	
NEMATODA		
Onchocercidae	extracellular	



HAEMO-PARASITES

PROTOZOA	
Dactylosomatidae	leeches
Haemogregarinidae	leeches, arthropods
Lankesterellidae	mosquitoes, mites, leeches
Plasmodiidae	mosquitoes
Haemoproteidae	midges, louse flies
Leucocytozoidae	black flies
Babesiidae	ticks
Theileridae	ticks
Trypanosomatidae	flies, leeches
NEMATODA	
Onchocercidae	mosquitoes, midges, flies

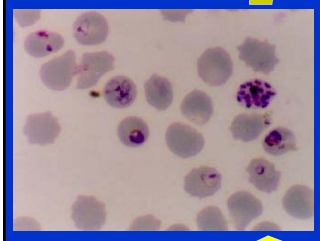


PARASITE ASSEMBLAGES

Protozoa | Arthropods | Helminths

Protozoa	Arthropods	Helminths
amoebae, flagellates, sporozoa, ciliates	arachnids, insects	nematodes, cestodes, trematodes
entamoebae, trichomonads, diplomonads, trypanosomes, coccidia, haemosporidia, piroplasms, holotrichs	ticks, mites	flies, fleas, lice
		roundworms, tapeworms, flatworms, flukes

Plasmodium spp. (malaria)

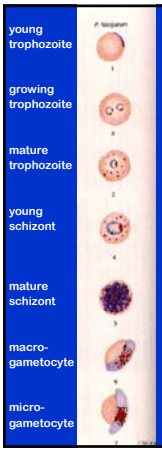


zoites-schizonts → salivarian transmission → mosquitoes

MALARIA

species	*P. falciparum*	*P. malariae*	*P. ovale*	*P. vivax*
malaria	malignant tertian	benign quartan	benign tertian	benign tertian
erythrocyte cycle	48 hrs	72 hrs	48 hrs	48 hrs
exoerythrocyte cycle	9 days	14-15 days	9 days	8 days
gametocytes	crescent	ovoid	ovoid	ovoid
frequency	~40%	~10%	<1%	~50%
distribution	worldwide in 'tropics'	scattered in 'tropics'	tropical Africa	worldwide in 'tropics'
	recrudescent persistent erythrocytic forms		relapsing persistent exoerythrocytic forms	

Plasmodium falciparum



- accounts for ~40% of all cases
- invade all ages of RBC (thus high parasitaemia)

- onset 8-12 days
  - vague symptoms for 3-4 days (aches, pains, headache, fatigue, anorexia)
  - then acute onset (fever, severe headache, nausea, vomiting, epigastric pain)
  - then periodicity <48 hours (fever, chills)
  - resolution 2-3 weeks but recrudescence

- schizogony in vessels in organs
- severity may not correlate with parasitaemia
- complications caused by plugging -> ischaemia
  - cerebral malaria (comatose)
  - bilious remittent fever (hepatomegaly)
  - dysentery (malabsorption diarrhoea)
  - algid malaria (circulatory collapse)
  - blackwater fever (haemoglobinuria)

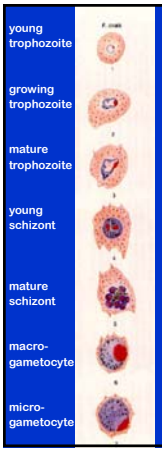
Plasmodium malariae

- accounts for ~10% of all cases
- infect mature RBC (thus parasitaemia reduced)

- incubation period 27-40 days
  - vague symptoms for 3-4 days (headache, photophobia, muscle aches, anorexia)
  - then regular periodicity (severe paroxysm, longer cold stage, more severe symptoms during hot stage)
  - spontaneous recovery but prolonged recrudescence

- proteinuria common
- nephrotic syndrome in children

Plasmodium ovale



- accounts for ~1% of all cases
- infects reticulocytes (young RBC) (parasitaemia 2-5%)

- clinically similar to *P. vivax* (but less severe and relapses less frequently)

- incubation period 7-10 days
  - vague symptoms for 3-4 days (headache, photophobia, muscle aches, anorexia)
  - then steady or irregular low-grade fever
  - then paroxysms (regular 48 hour cycle)
  - spontaneous recovery after 6-10 paroxysms although relapses can occur after weeks/months/years

- splenomegaly during first few weeks
- leukopenia usually present
- severe complications rare

Plasmodium vivax

- accounts for ~50% of all cases
- infects reticulocytes (young RBC) (parasitaemia 2-5%)

- clinically similar to *P. ovale* (but more severe and relapses more frequently)

- incubation period 7-10 days
  - vague symptoms for 3-4 days (headache, photophobia, muscle aches, anorexia)
  - then steady or irregular low-grade fever
  - then paroxysms (regular 48 hour cycle)
  - slow irregular recovery over 3-8 weeks but relapses occur after weeks/months/years

- splenomegaly during first few weeks
- leukopenia usually present
- severe complications rare but can include cerebral malaria

How malaria kills!

Malaria is one of history's GREAT FEVERS!
Pathology caused by:

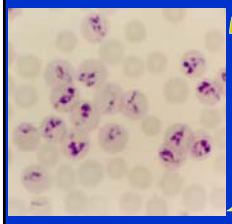
- haemolysis
- erythrophagocytosis

} anaemia

- accumulation of haemozoin
- cytoadherence
- DIC

} ischaemia

Babesia spp. (tick fever)



tick-borne

piroplasms

tick vectors

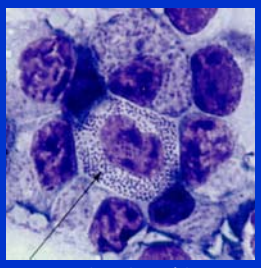
Transmission enigma

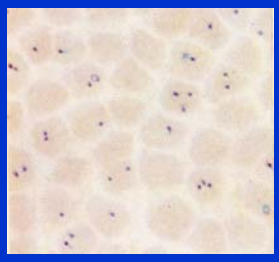
How can ticks act as vectors?

- if feeding stages leave hosts to moult?
  - trans-stadial transmission (larva - nymph - adult)
- if all feeding stages stay on same host (1-host ticks)?
  - trans-ovarian transmission (female - eggs)

Theileria spp. (East Coast fever)

leucocytes infected first — then erythrocytes





merozoites in schizont

trophozoites/merozoites

Trypanosoma brucei

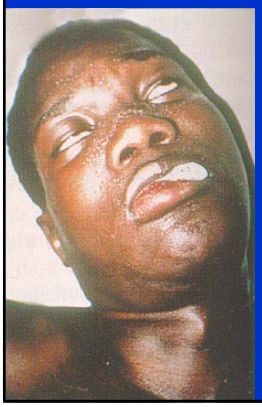
Old World/African trypanosomiasis

trypomastigotes

salivarian transmission

tsetse fly (*Glossina*)

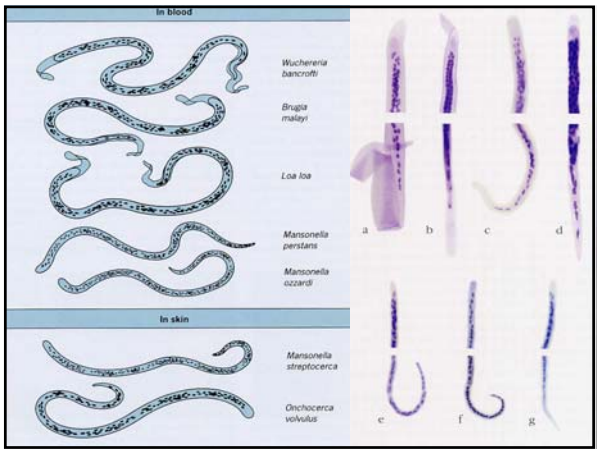
Sleeping sickness



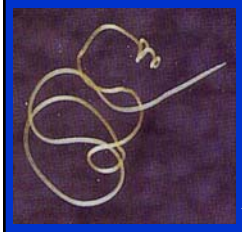
- trypanosomes multiply
- cyclic fevers
- macroglobulinaemia (due to antigen variation)
- microvascular damage
- coagulopathy
- perivascular inflammation
- penetrate blood-brain barrier
- encephalitis, coma

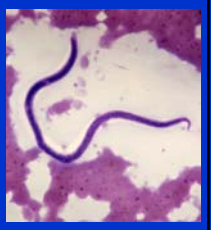
FILARIAL NEMATODES

Onchocerca	blindness, skin lesions Africa, Central America	adults subcutaneous mf in tissues	blackfly
Wuchereria	Bancroftian filariasis (elephantitis) tropics	adults in lymphatics mf in blood	mosquito
Brugia	Malayan/Timorian fil. (elephantitis)	adults in lymphatics mf in blood	mosquito
Loa	Calabar swellings Central/West Africa	adults subcutaneous mf in blood	tabanids
Mansonella	skin lesions Central America	adults in dermis mf in blood	sandfly/blackfly
Dirofilaria	pulmonary lesions widespread	adults in heart mf in blood	mosquito



Wuchereria/Brugia





adults 40-100 mm
in lymph vessels

microfilariae 230-260 μm
in blood

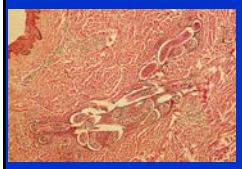
transmitted by mosquitos
cause lymphatic filariasis (elephantiasis)

Disease

acute manifestations
- recurrent adenolymphangitis
  (inflammation of lymph ducts)
- fever, nausea, headaches, rash, eosinophilia

chronic manifestations
- lymphoedema (elephantiasis)
- tropical pulmonary eosinophila (TPE) rarer

Loa/Mansonella



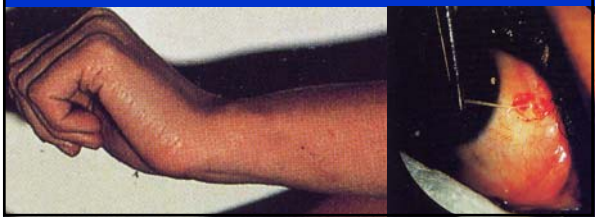
adults 60-90 mm
in skin

microfilariae 200-300 μm
in blood

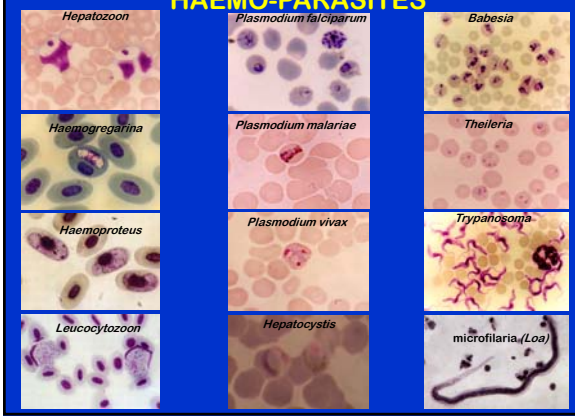
transmitted by tabanid/sand/blackflies/midges
cause painful swellings

Disease

- painful oedematous Calabar swellings
  transient (disappear and reappear elsewhere)
- pruritis, fever, eosinophilia
- sometimes encephalitis, myocardial fibrosis
- adults may cross conjunctiva, surgical removal



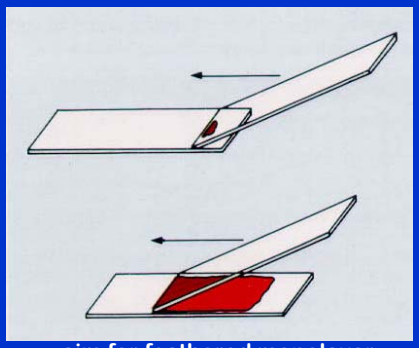
HAEMO-PARASITES



BLOOD EXAMINATION

- wet smear (motile mf, tryps)
- permanent stained blood films
  - thick/thin, Giemsa/haematology
- concentration procedures
  - microhaematocrit centrifiugation (buffy coat)
  - Knott's concentration (lyse rbc)
  - membrane filtration (5 um Nucleopore)
  - gradient centrifugation (Hypaque, Ficoll)
- culture (in vitro, in vivo)
- immunoserology
  - Ab (CFT, IHAT, IFAT, ELISA, RIA)
  - Ag (immunochromatography, EIA)
- molecular (DNA extraction, PCR amplification)

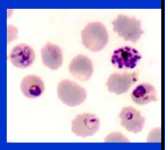
Most microscopic techniques rely on thin blood smear preparation



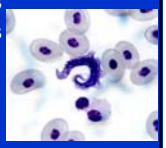
aim for feathered monolayer

Staining smears

Romanowsky stains
- basophilic nuclei
- polychromatic granules



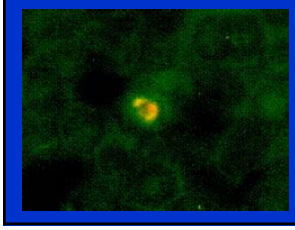
Variants
- Diff-Quik - good for host cells
- Wright - good for host cells
- Giemsa - good for parasites
- Leishman - good for tryps

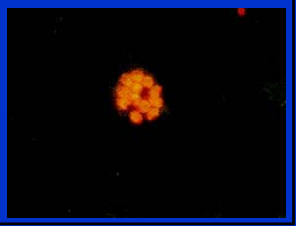


⇒ watch pH of stain [6.8-7.2]

Fluorochrome stains

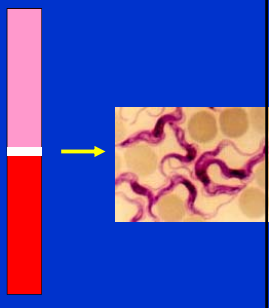
e.g. acridine orange (AO) for malaria
Microscopy with ultraviolet (UV) light source
UV-excited AO emits photons





Capillary tube centrifugation

- fill haematocrit tube
- plug ends
- centrifuge (10')
- measure PCV
- harvest buffy coat (score and snap)
- examine for motile parasites (tryps/mf)



Concentration

Knott's test (for microfilaria)

- clarify blood by hypotonic lysis of RBC (use dilute formalin)
- pellet remaining cells by centrifugation
- visualize by methylene blue staining
- examine wet preparations for mf

Immunoserology

Provides presumptive evidence of infection by demonstration of:

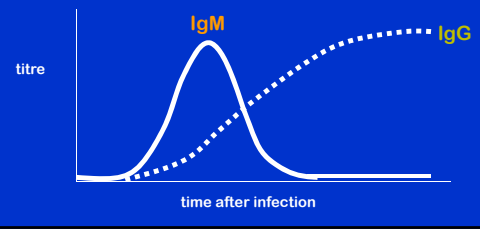
- host antibodies
- parasite antigens

Useful for:

- antemortem diagnosis
- detecting carriers (asymptomatic)
- differentiating acute and chronic infections

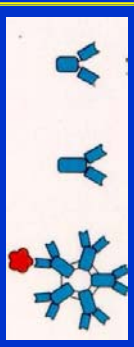
Antibody kinetics and dynamics

- early transient IgM response (coincident with acute phase)
- late persistent IgG response (coincident with chronic phase)

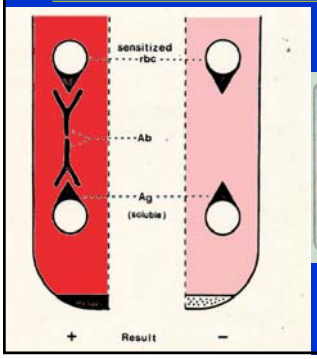


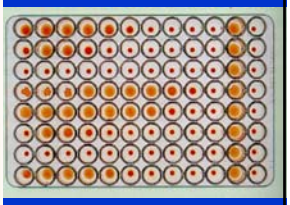
SEROLOGICAL TESTS

- precipitin tests
- immunodiffusion
- immunoelectrophoresis
- complement fixation
- agglutination tests
- immunofluorescence
- enzyme immunoassays
- radio immunoassays

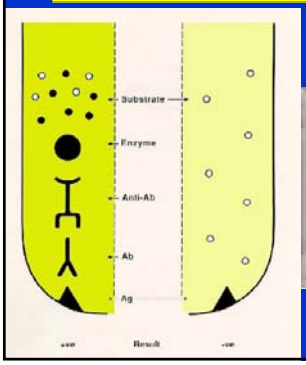


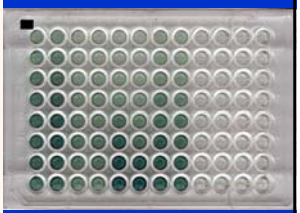
IHAT
Indirect Haem-Agglutination Test



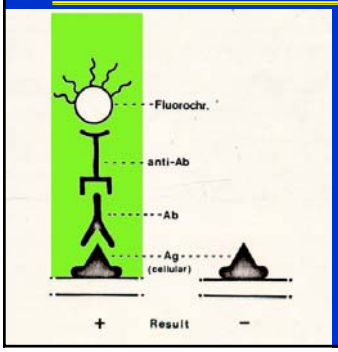


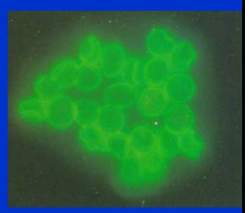
ELISA
Enzyme Linked Immuno-Sorbent Assay





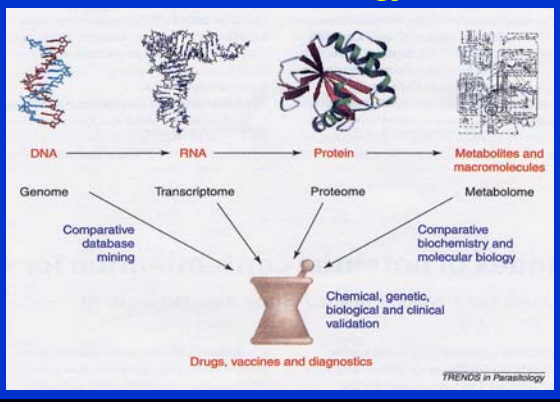
IFAT
Indirect Fluorescent-Antibody Test





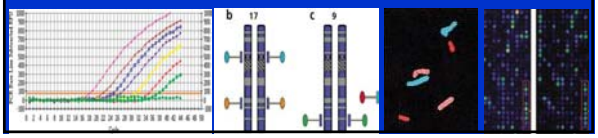
Disease	Organism	CDC Test	Specimens
Amebiasis	*Entamoeba histolytica*	EIA	Serum
Babesiosis	*Babesia microti* *Babesia* sp. WA1	IFA	Serum
Baylisascariasis	*Baylisascaris procyonis*	Immunoblot	Serum, CSF
Chagas disease	*Trypanosoma cruzi*	IFA	Serum
Cysticercosis	Larval *Taenia solium*	Immunoblot	Serum, CSF
Echinococcosis	*Echinococcus granulosus*	EIA, Blot	Serum
Filariasis	*Wuchereria bancrofti* and *Brugia malayi*	EIA	Serum
Leishmaniasis	*Leishmania braziliensis* *L. donovani* *L. tropica*	IFA	Serum
Malaria	*Plasmodium falciparum* *P. malariae* *P. ovale* *P. vivax*	IFA	Serum
Paragonimiasis	*Paragonimus westermani*	Blot	Serum
Schistosomiasis	*S. mansoni* *S. haematobium* *S. japonicum*	FAST-ELISA Blot	Serum
Strongyloidiasis	*Strongyloides stercoralis*	EIA	Serum
Toxocariasis	*Toxocara canis*	EIA	Serum, vitreous

Molecular biology

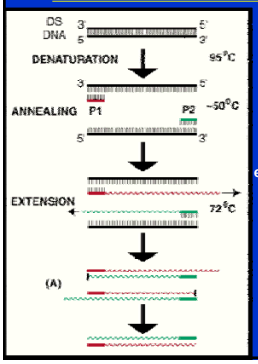


Molecular pathology

- PCR (polymerase chain reaction)
  (incl. RT-PCR, Q-PCR, RAPD, RFLP…)
- FISH (fluorescent *in situ* hybridization)
- SKI (spectral karyotyping imaging)
- DNA microarrays



Polymerase Chain Reaction

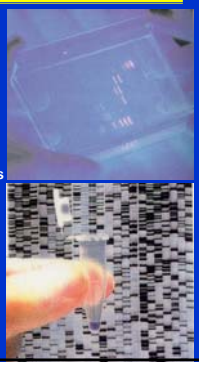


specific primers

DNA amplification

amplicon electrophoresis

± DNA sequencing

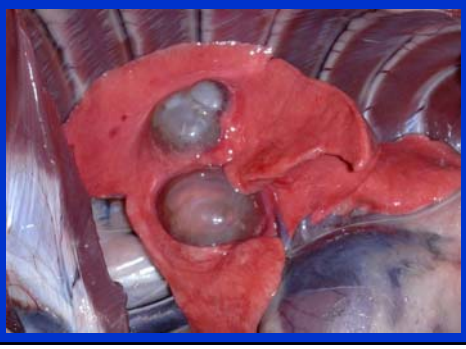


Clinical Review

Site	Symptoms	Transmission	PROTOZOA	HELMINTHS	ARTHROPOD
					-
Blood	anaemia, fever, ischaemia	vector-borne	trypanosomes haemosporidia piroplasms	filarial worms blood flukes	-

Histology

(working with tissues)



SAMPLES

Ante-mortem (host alive)
- tissue biopsies
- skin scrapings
- aspirates
- sputum
- swabs

Post-mortem (host dead/euthanized)
- any tissue/organ/fluid
- worm counts (GIN)
- gut digest (immature/hypobiotic)
- lung/perfusion (lungworms)
- brain smears (piroplasms)….

PROCEDURES

- gross pathology (macroscopic)
- histopathology (microscopic)
  - frozen/fixed sections
  - histochemical staining
  - immuno-labelling
- concentration
  - luminal content (counts, …)
  - tissue digest (larvae, arthropods)
- culture (*in vitro, in vivo*)
- molecular (extract DNA, PCR)

HISTOLOGICAL PREPARATION

• fixation	(predominantly formalin)
• dehydration	(graded ethanol series)
• clearing	(xylene/toluene…)
• embedding	(paraffin wax)
• sectioning	(microtome)
• enrobing	(slides)
• staining	(aqueous/alcoholic stains)
• dehydrating	(ethanol)
• clearing	(xylene…)
• mounting	(resins…)

- preparative artefacts
- interpretive artefacts (2D→3D)
- serial sections (avoid giving histologists worms!)

BIOPSY MATERIAL

- suspect tissue
- lesions/lumps/cysts/ulcers

- examine
  - fresh mounts
  - impression/teased/squash preparations
  - histology (fixed/frozen sections)
  - skin, muscle, liver, lung, lymph nodes…
  - intestinal/bladder mucosa, cornea…
- filariae, *Trichinella*, metacestodes, fluke eggs…
- amoebae, kinetoplastids, *Toxo*, microspora…
- ectoparasites (fleas, flies, mites)

ASPIRATES

- fluids/lavages/washings, lumps/cysts/abscesses

• sigmoidoscopy	(duodenal)
• bronchoscopy	(broncho-alveolar lavage)
• fine needle	(peritoneum, pleura, lymph nodes, spleen, liver, lung, bone marrow, spinal fluid, eye…)

- threadworm, filariae, hydatids…
- amoebae, kinetoplastids, Crypto, Toxo…

SPUTUM

- pneumonia, pneumonitis, Loeffler's syndrome

- expectorated (postural drainage)
- induced (nebulizer)
- BAL

- wet mount (mucolytic NaOH)
- stained (methenamine silver, Giemsa)
- immuno-labelling (fluorochromes)

- nematode larvae, fluke eggs, metacestodes,
- amoebae, Crypto, microspora, PCP

UROGENITAL SAMPLES

- haematuria, cystitis, vaginitis, urethritis, prostatitis, infertility

- urine
- vaginal/prostatic/preputial swabs

- examine
  - fresh preparations (cytology)
  - concentrates (sediments, filtrates)
  - cultures (in-pouch)

- fluke eggs…
- trichomonads…

CULTURE

- *in vitro* (*ex vivo*)
  - axenic (sterile)
  - xenic e.g. monoxenic, feeder organism(s) (slopes/plates, tubes/liquid/pouches)
  - tissue culture

- *in vivo* (animal inoculation)
  - hamsters, guinea-pig
  - lab rats, lab mice

- xenodiagnosis
  - allow uninfected vectors to feed on patients

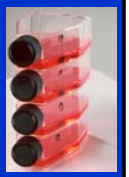
CULTURE

- amoebae (*Entamoeba, Acanthamoeba, Naegleria*)
  [Balamuth's egg yolk infusion, Locke-egg-serum, TYI-S-33, TYSGM-9, peptone-yeast-glucose]
- trichomonads (*Trichomonas*)
  [casein hydrolysate-serum, CPLM, Diamond's TYM]
- haemoflagellates (*Trypanosoma*, *Leishmania*)
  [NNN, Evan's, NIH, 4N, Yaeger's, USAMRU blood agar]
  [hamster, guinea-pigs, lab rats/mice]
- apicomplexans (*Toxoplasma*, *Plasmodium*)
  [Human Foreskin Fibroblast, RPMI + rbc, MEM + rbc]
  [lab mice]
- Chagas disease (*Trypanosoma cruzi*)
  [reduviid bugs]
- Trichinosis (*Trichinella*)
  [lab rats]

CULTURE

- can only culture relatively few parasites

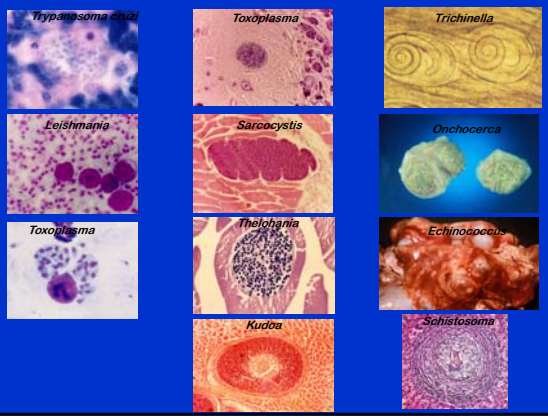
- often require actual or surrogate hosts



- slowly learning *in vitro* culture requirements

- cultures have good utility in research labs
- but application to diagnostics superseded by molecular techniques
  - to detect parasite molecules (esp. PCR), or
  - to grow parasite molecules (biotechnology)

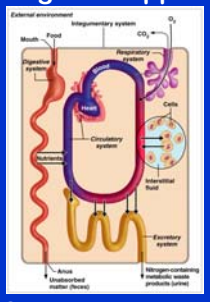
PARASITES IN TISSUES



Clinical Pathology

Systems approach

Integrated approach



structure and function

PATHOLOGY

structural/functional changes

need to know your pathogen

and how the host will react

Nervous system

- anorexia (loss of appetite)
- depression (reduced vitality)
- stupor coma (unconscious)
- seizures (convulsions)
- ataxia (loss of movement)
- paresis (partial paralysis)
- paralysis (motor/sensory)
- dysmetria (unable to direct motions)
- muscle weakness/tremor (innervation)
- erratic behaviour (motor, cognitive)

CNS

Forebrain (cerebrum, diencephalon)	Brainstem (midbrain, pons, medulla oblongata, vestibular nuclei)	Cerebellum	Spinal cord
depression, stupor, coma, convulsive seizures, mild hemiparesis	depression, stupor, coma, reduced consciousness, hemiparesis, tetraparesis, head tilt, twisting, turning or circling ataxia	ataxia, wide-based stance, dysmetria, intention tremor, muscle weakness	sensory dysfunction (hypersthesia, hyposthesia), paresis, paralysis, spinal reflex abnormalities, muscle atrophy
P: *Toxoplasma* P: *Plasmodium* P: *Encephalitozoon* N: *Toxocara* N: *Dirofilaria* C: *Cysticercus* C: *Coenurus* T: *Schistosoma* A: *Chrysomya*	P: *Toxoplasma* P: *Plasmodium* P: *Encephalitozoon* N: *Dirofilaria* C: *Cysticercus* C: *Coenurus* T: *Schistosoma*	P: *Toxoplasma* P: *Plasmodium* P: *Encephalitozoon* N: *Toxocara* C: *Cysticercus* T: *Schistosoma* A: *Chrysomya*	P: *Toxoplasma* N: *Angiostrongylus* N: *Toxocara* N: *Ancylostoma* C: *Coenurus* T: *Schistosoma*

Respiratory system

- anorexia (loss of appetite)
- sneezing (URT clearance)
- coughing (LRT clearance, wet/dry)
- nasal discharge (mucus)
- tachypnea (rapid respiration)
- dyspnea (difficult breathing)
- oesonophilia (increased eosinophils)

Difficulty breathing

dyspnoea at rest

obstruction of passages	filled alveoli (pneumonia/oedema)	compressed alveoli (emphysema)
inspiratory	mixed	expiratory
Filaroides *Oestrus* *Schistosoma*	*Angiostrongylus* *Toxoplasma* *Pneumocystis*	*Dictyocaulus* *Metastrongylus* *Muellerius*

Irritated mucous membranes

sneezing | coughing

nasal cavity | pharynx/larynx | trachea | bronchi(oles)

Oestrus *Lingatula*

Mammonogamus *Rhinoestrus*

Capillaria *Filaroides*

larval migration *Dictyocaulus* *Muellerius*

PARASITE ASSEMBLAGES

Protozoa | Arthropods | Helminths

amoebae flagellates sporozoa ciliates

entamoebae trichomonads coccidia holotrichs diplomonads haemosporidia trypanosomes piroplasms

arachnids

ticks mites

insects

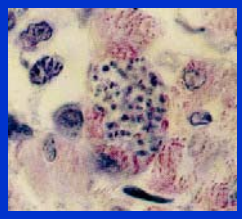
flies fleas lice

nematodes cestodes trematodes

roundworms tapeworms flukes flatworms

Trypanosoma cruzi

New World/American trypanosomiasis



amastigotes

stercorarian transmission

reduviid bug

Chagas disease

acute disease

amastigotes multiply in tissues

- Romana's sign
- hepatosplenomegaly
- lymphadenopathy
- cardiac arrhythmia

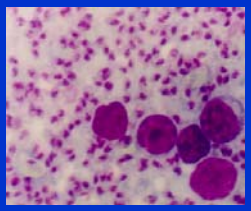
chronic disease

amastigotes throughout tissues

- cardiomyopathy
- myositis
- 'mega-organ syndrome'

Leishmania spp.

cutaneous/visceral leishmaniasis



amastigotes

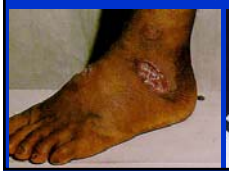
vector-borne transmission

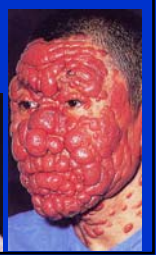
sandflies

Leishmaniasis

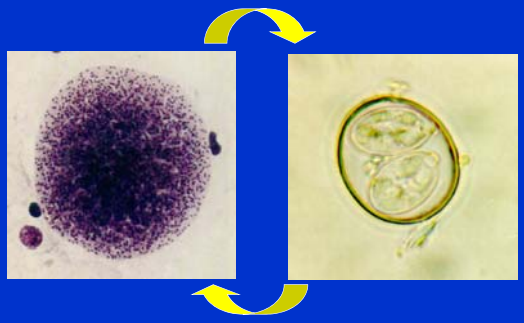
Three types of clinical disease

- cutaneous/oriental sore (*L. tropica/mexicana*)
  skin lesions, sores, ulceration
- mucocutaneous/espundia (*L. braziliensis*)
  destructive nasopharyngeal lesions
- visceral/kala azar (*L. donovani*)
  hepatosplenomegaly, oedema, anaemia





Toxoplasma gondii



tissue cysts — prey-predator — oocysts

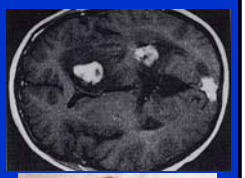
TOXOPLASMOSIS

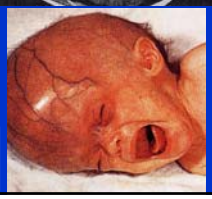
ACUTE (tachyzoite multiplication)
- mild flu-like, lymphadenopathy

CHRONIC (cyst formation)
- space-occupying lesions
- functional deficits??
- reactivation in immuno-compromised patients

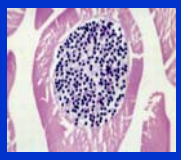
CONGENITAL (transplacental)
- abortion (1st trimester)
- stillbirth (2nd trimester)
- abnormalities (3rd trimester)

NB: mother must become infected during pregnancy

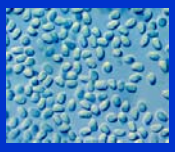




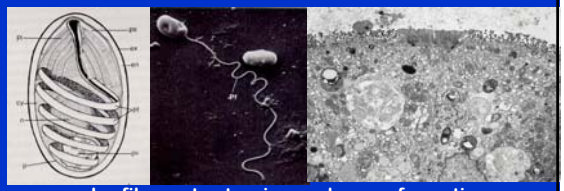
Enterocytozoon (microsporan)



prey-predator
?



cysts in tissues — spores in faeces

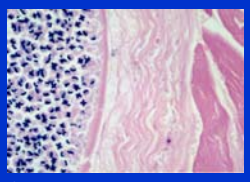


polar filament extrusion and spore formation

Kudoa (myxozoan)

prey-predator

myxospores in fish — actinospores in oligochaetes



myoliquefaction

Trichinella (muscle worm)

adult worm 1-4 mm
under small intestinal epithelium

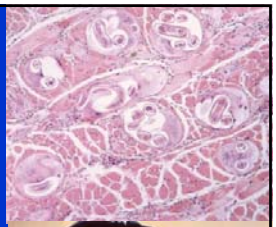
larvae 100 µm
in muscle cells

important parasite in many parts of the world
(except Aust.)

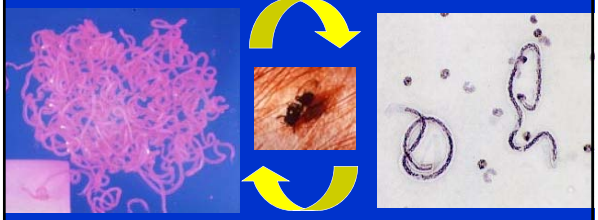
Pathogenesis

intestinal phase
- mucosal irritation

muscle invasion phase
- inflammation, fever
- facial oedema
- muscle damage
- myocarditis
- neurological complications



Onchocerca (filarial worm)



adult worm up to 50 cm
in subcutaneous nodules

microfilaria 220-360 μm
in tissues

O. volvulus pathogenic in humans
several pathogenic species in animals

Pathogenesis

- Subcutaneous nodules

River blindness
- anterior/posterior lesions (mf in cornea/retina)

Echinococcus granulosus (hydatid tapeworm)

predator-prey

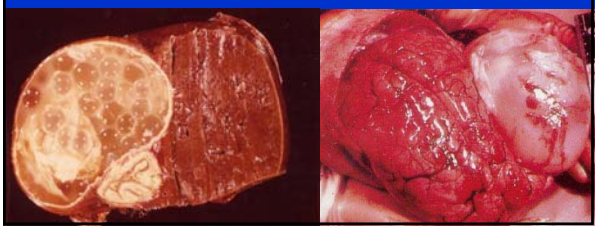
adults 2-6 mm
in small intestines of dogs

eggs 30-43 μm
passed in dog faeces

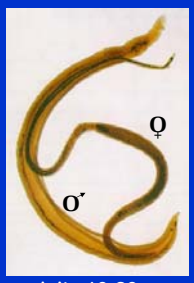
hydatid disease caused by larval metacestodes

Pathogenesis

- space-occupying cysts
- organ enlargement accompanied by various signs (abdominal distention, epilepsy, blindness, etc.)



Schistosoma spp. (blood fluke)



snail vector

adults 10-20 mm
in blood vessels

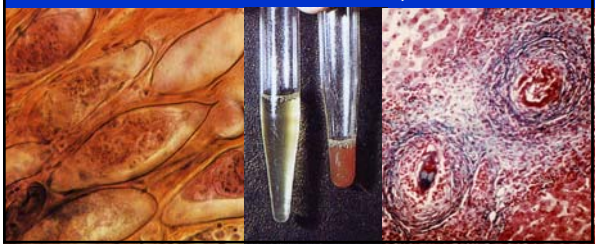
non-operculated eggs
passed in faeces/urine

cause bilharzia (fibrosis, portal hypertension)

Pathogenesis

acute phase
- eggs pass through tissues
- haematuria, pyrexia, lymphadenopathy, diarrhoea, oesinophilia ('Katayama' syndrome)

chronic phase
- eggs trapped in tissues
- inflammation→granulomas
- portal hypertension, hepatosplenomegaly, ascites, varices



Ectoparasites and Skin

- pruritis (itching)
- erythema (patchy inflammation)
- scales (flakes)
- crusts (scabs)
- excoriations (peeling)
- alopecia (hair loss)
- nodules (lumps)
- papules (pimples)
- vesicles (fluid-filled)
- pigmentation abnormalities (patches)
- lichenification (reddish eruption)
- anaemia (reduced haematocrit)

Ctenocephalides

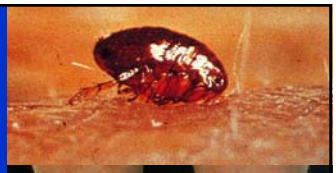
adult flea biting skin

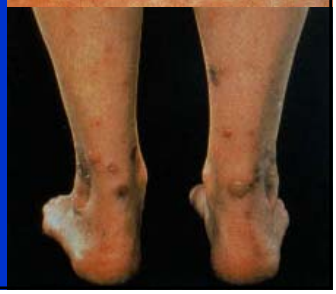
ectoparasite transmission

larvae in bedding

PATHOGENICITY

- blood loss
- tissue trauma
- pain
- itching
- dermatitis
- ulceration
- allergic reactions
- hypersensitivity
- secondary infections





Pediculus

adult lice on skin

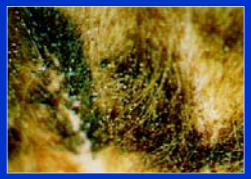
ectoparasite transmission

eggs (nits) attach to hairs

Pediculus – impact of infection

- attach to hair (esp. back of neck and behind ears)
- infestations associated with crowding

- bites cause red papules
- intense pruritis
- dermatitis
- secondary infection



DIPTERA: adults parasitic

Blood-feeders	Diseases transmitted
sand flies (*Phlebotomus*)	*Leishmania* (kala azar)
tsetse flies (*Glossina*)	*Trypanosoma* (sleeping sickness)
mosquitos (*Anopheles, Culex*)	*Plasmodium* (malaria)
black-flies (*Simulium*)	*Onchocerca* (nodule worm)
midges (*Culicoides*)	arboviruses
horse flies (*Tabanus*)	*Anaplasma*
buffalo flies (*Haematobia*)	intense irritation

DIPTERA: larvae parasitic (myiasis)

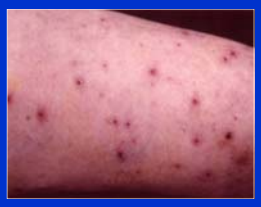
Flies	Tissues
• screw-worm (*Chrysomya*)	mucous membranes
• blow flies (*Lucilia*)	body/breech
• flesh flies (Sarcophaga)	wounds
• skin bot flies (*Dermatobia*)	skin
• cattle grubs (*Hypoderma*)	skin
• stomach bots (*Gasterophilus*)	stomach

Ixodes

adult ticks on skin — ectoparasite — eggs laid on ground

Ixodes – impact of infection

- anaemia
- inflammation
- dermatosis
- scrub itch
- ulceration
- toxicosis (serum blisters)
- ascending paralysis (due to toxins)
- secondary infections

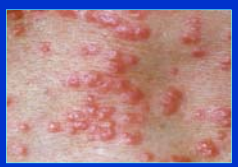


Sarcoptes

adult mites in skin — ectoparasite transmission — eggs laid in skin tunnels

Sarcoptes – impact of infection

- irritation
- intense itching
- dermatitis, rash
- exudate, vesicles, crusts
- hair loss
- thickened skin
- septic pustules
- weight loss
- emaciation
- secondary infections



Clinical Review

Site	Symptoms	Transmission	PROTOZOA	HELMINTHS	ARTHROPOD
Gut	diarrhoea, blockage, anaemia	faecal-oral	amoebae diplomonads coccidia ciliates	round-, pin-, whip-,thread-, hook-worms tapeworms enteric flukes	-
Blood	anaemia, fever, ischaemia	vector-borne	trypanosomes haemosporidia piroplasms	filarial worms blood flukes	-
Tissues	lesions dysfunction inflammation	predator-prey	cyst-forming coccidia microspora	hydatids cysticerci liver flukes *Trichinella*	-
- skin	lesions	direct	-	-	flies fleas lice mites ticks