School of Chemistry & Molecular Biosciences,
Faculty of Science

LABORATORY NOTES

PARA3002

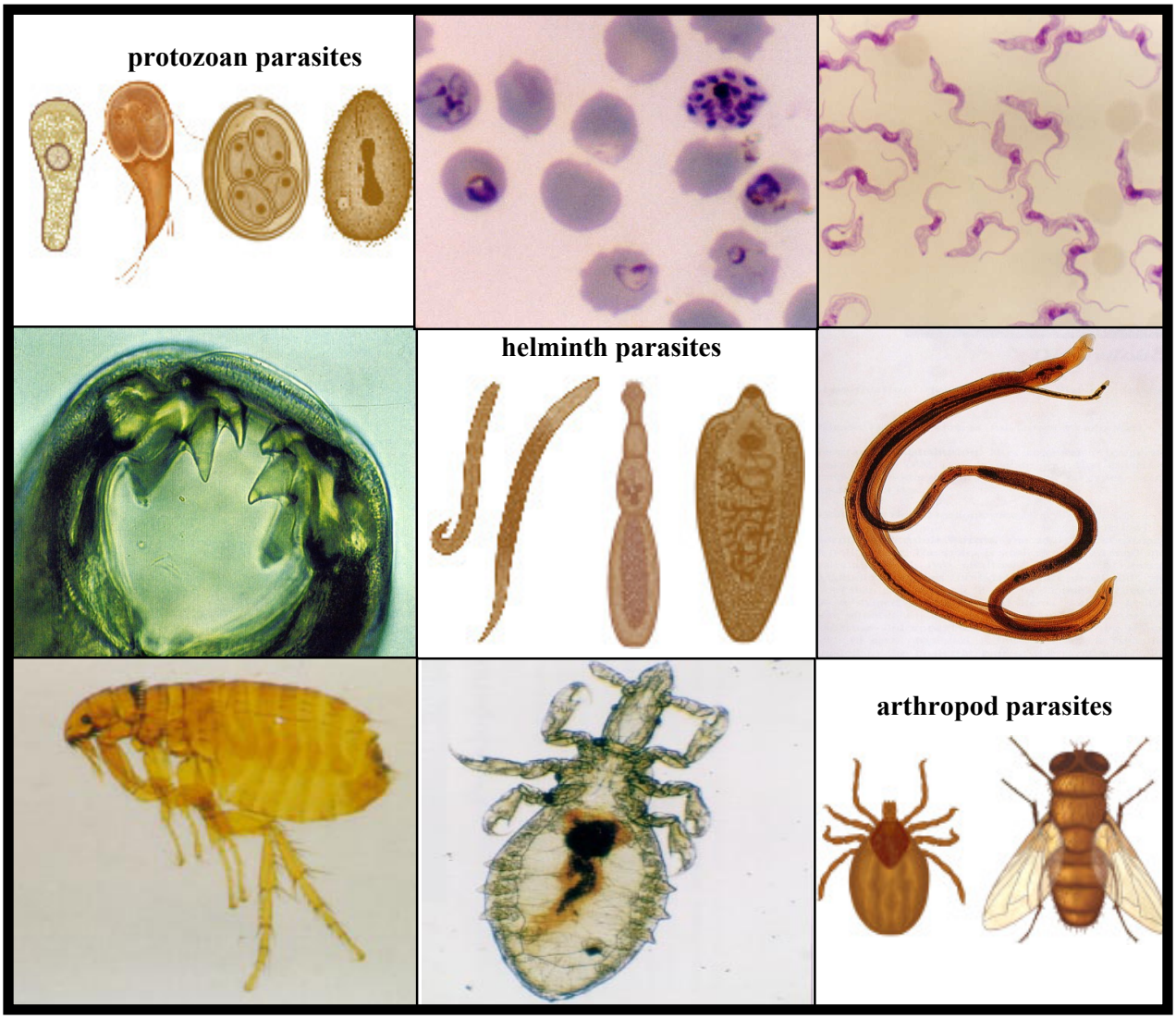
Biomedical parasitology

Instructor Copy

scmb.uq.edu.au

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

PARA3002: Biomedical Parasitology



The University of Queensland

Table of contents *page*

COURSE PROFILE

Students are directed to the Electronic Course Profile (ECP), SI-Net and Blackboard websites for all current and relevant details pertaining to this course, especially assessment requirements

www.courses.uq.edu.au/

Course: PARA3002

Biomedical Parasitology

Preamble

There are three types of infectious organisms in medicine: bacteria, viruses and parasites. Biomedical Parasitology deals with the three main types of parasites: the protozoa, helminths (worms) and arthropods. Parasites have always threatened the lives and well-being of humankind. Such threats have not diminished substantially with time. Indeed, the re-emergence over the last 10 years of parasitic diseases like malaria, schistosomiasis, trypanosomiasis, pediculosis (infection with head lice) and the development of drug-resistance in roundworms and arthropods have shocked many governments and international health agencies into action. Parasitism is by far the most common way of life on earth. So the ubiquity of parasites in medicine ensures demand for graduates with knowledge in biomedical parasitology. Indeed, despite the optimism of the 18th and 19th centuries that science would rid humankind of parasites, parasites are just as great a threat today as they were then. In Biomedical Parasitology students use contemporary techniques in molecular, cellular & population biology for the diagnosis, treatment & control of parasites. The evolution of resistance to drugs in parasites, vaccines and novel therapies are also covered.

Teaching Staff:

Professor Peter O'Donoghue	Email: p.odonoghue@uq.edu.au
Professor Stephen Barker	Email: s.barker@uq.edu.au
Associate Professor Thomas Cribb	Email: t.cribb@uq.edu.au

Objectives:

This course has been designed to teach you to:

1. evaluate conventional and modern methods of diagnosing parasitic infections
2. understand the molecular bases of resistance to anti-parasitic chemicals and drugs
3. appreciate that molecular techniques are critical to contemporary research on and control of parasites
4. identify factors involved in the resurgence of parasitic diseases
5. understand the importance of a deep understanding of the life-cycle of a parasite in order to control that parasite

Assessment

Theory examination (worth 60% of total) (end-of-semester examinations period)

The theory examination is scheduled for two hours during the central examinations period and students are required to answer eight short-answer questions. The short-answer questions will test student's understanding on broad issues rather than test for specific recall. For example, questions will ask students to compare/contrast information about different parasites to test relational thinking.

Practical examination (worth 20% of total) (end-of-semester examinations period)

The practical examination is scheduled for one hour in the School laboratories during the central examinations period. Students will play musical microscopes by attending 15 workstations and spending three minutes at each identifying the parasite present and answering some simple questions about the specimens. The practical exam tests the differential diagnostic capabilities of the students and then tests their broader parasitological knowledge (specific, general and contextual). Students are required to identify 5 protozoan, 5 helminth and 5 arthropod parasites.

Assignment (Parasitological Review) (worth 20% of total)

Students work on their assignments for the first 9 weeks of semester (submission due date is the Friday immediately before the mid-semester break). Students are required to review the relevant scientific literature in order to write a 1,500 word prose report (essay) on “how a particular parasite causes disease” (students select a parasite of interest from an accompanying list). The assignment will require selecting and reading pertinent literature (research papers, reviews, texts and accredited websites) and then creating and editing a systematic and logical answer to the question of pathogenesis. Students will be tutored on what an appropriate response would be and the academic rubric (marking matrix) is available on the course Blackboard web site. Reports are to be submitted as hard-copies with attached coversheet to the School office and an electronic copy is to be uploaded through the Turn-it-in plagiarism-detection software available through the course Blackboard website. Strict penalties apply for late submissions.

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.

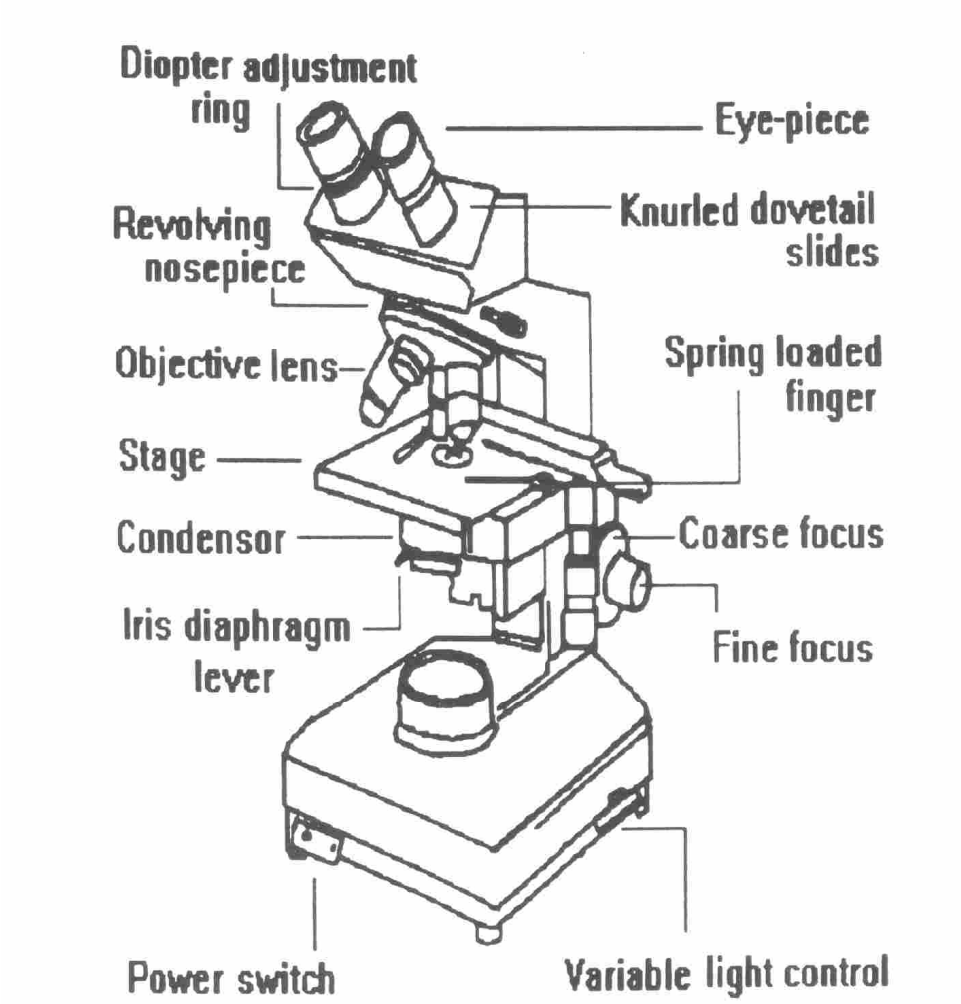
Always use both hands when you carry your microscope. Grasp the microscope arm firmly with one hand and lift it carefully. Place your other hand under the base of the microscope for support as you carry it. Keep the microscope vertical to ensure that the ocular lens does not fall out. **Each time** you 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 **only** the Kim wipes at the ends of each bench to clean the lenses. DO NOT USE FACIAL TISSUES. Avoid touching any of the optical system with your fingers. Skin oils can be difficult to remove. When using oil immersion, always check and double check that it is the oil immersion (x100) 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) Read the paragraph above about care of your microscope **before** you start.
b) Identify the various parts of your microscope using following figure. Ask your tutor if you are unsure.

c) 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 distance between the sliding eyepieces until perfect binocular vision is obtained.
   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 tutor.

d) Always wipe off immersion oil from the objective lens after viewing, using **Kim wipe tissues** which are provided at end of bench. 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 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.

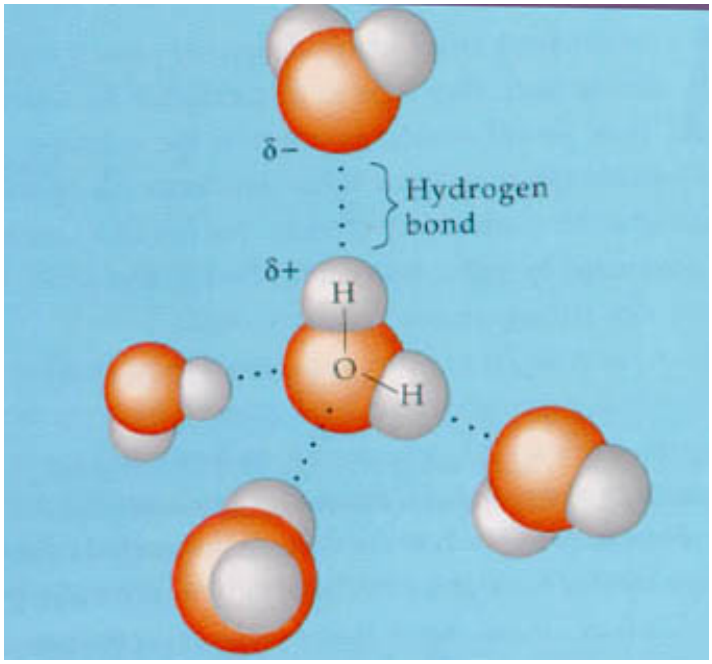
Course focus: This is a large serrated screw which is attached to the upper part of the limb. Its function is to alter the distance between the object under examination and the objective. If stiff, report it to a tutor.

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.

WEEK 1:

PRACTICAL

Wet Lab – DISSECTION



dihydrogen monoxide

the blue planet

HOST-PARASITE ASSOCIATIONS (AQUATIC EXEMPLAR)

Water is one commodity that we take for granted. Turn on a tap and it magically appears ready for consumption or use. Its origin is no big deal. It periodically falls from the sky – sometimes so profusely that flooding results. It runs downhill in various water courses, occasionally becomes trapped behind impoundments and ultimately empties at the coast into the ocean. We are surrounded by water and use it daily in many different ways. It is essential to life as we know it – prokaryote or eukaryote, microscopic or macroscopic, biome or planet. All cells are composed of around 70-95% water. Chemically, H_2O exhibits many unique properties.

- polarity: charged ends contribute to cohesion (binding) and adhesion (wetting)
- thermal bank: high specific heat due to kinetic energy (acts to stabilize temperature)
- three physical states: both gaseous and solid states expand (ice floats)
- ionic: H^+ OH^- pH = negative log of hydrogen ion concentration = $-\log[H^+]$
- solvent: dissolves salts, sugars, proteins (fluid of life)

Despite our aquatic dependency, we have different conceptions of water quality. Mountain streams are considered pristine, deltas rich, rain water clean, flood waters dirty, spring water pure, industry effluent polluted. Water authorities are concerned about the quantity and quality of:

- drinking water
- surface, ground and raw water
- sewage and domestic effluents
- waste water and effluents
- boiler and cooling water
- aquaculture
- swimming pools and spas

This practical will examine abiotic and biotic parameters associated with local pond water used to culture crayfish for local restaurants:

- abiotic - physicochemical (temperature, pH, conductivity)
- biotic - microscopic (protista, invertebrates)
  - macroscopic (arthropods)

Several native species of crayfish have been introduced into aquaculture facilities; including yabbies (*Cherax destructor*), marron (*C. tenuimanus*), redclaw or tropical blue crayfish (*C. quadricarinatus*) and gilgies (*C. quinquecarinatus*). Most species demonstrate good growth characteristics and are suitable for intensive culture. However, various internal parasites and ectocommensal fouling organisms may severely reduce production by causing:

- mortality (death)
- morbidity (disease)
- production losses (reduced growth)
- lesions (tissue cysts)

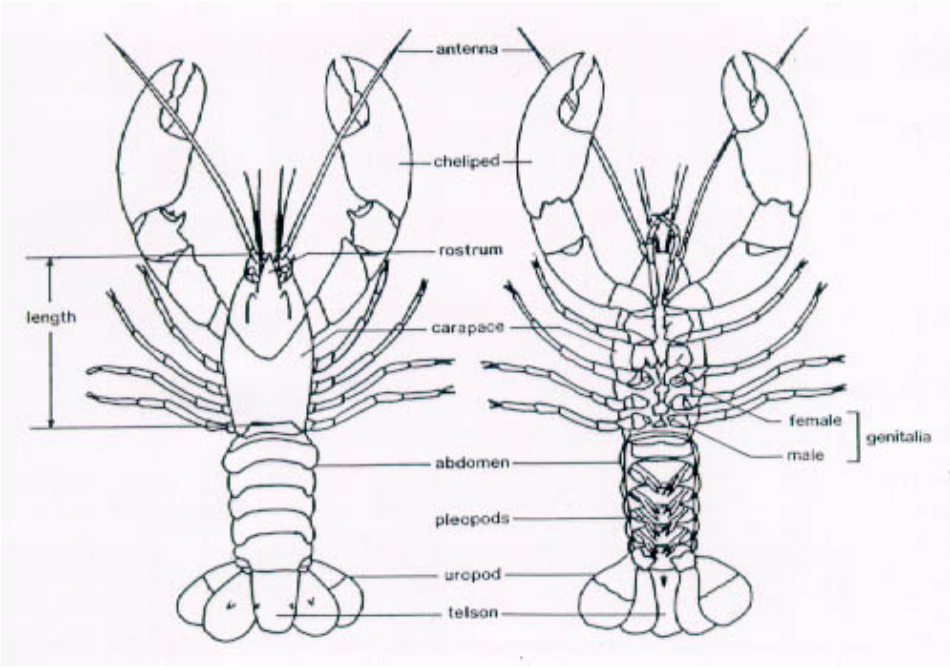
The presence and density of many of these organisms is determined by inter-species relationships and environmental (water) quality. For example, filter-feeding ciliate communities are dependent on bacterial populations which in turn are dependent on organic content. Waters enriched by agricultural run-off or the over-supply of fish food will exhibit blooms of fouling organisms. Microparasites exhibiting direct transmission may show a ‘crowd’ effect (i.e. incidence dependent on host density) and macroparasites exhibiting cyclic transmission between different host species may show over-dispersion (some hosts with most parasites).

Materials (students to work in pairs):

- lab coats
- dissection instruments (scalpel, forceps, scissors)
- plastic dishes and petri dishes
- dissecting microscope
- glass slides and coverslips
- compound microscope
- thermometer
- pH and conductivity meter

Methods

- collect a redclaw crayfish from one of the numbered barrels
- place it in a plastic dish and cover with water from the barrel
- kill redclaw by pithing with scalpel immediately behind rostrum
- place dead crayfish in large petri dish keeping it immersed in water
- measure water temperature, pH and conductivity
- examine the water for motile organisms by visual and microscopic examination
- record organisms present (should include bacteria, algae and micro-invertebrates)
- remove redclaw pleopods with forceps and place in water in small petri dish
- remove carapace covering gills with scissors
- remove gill filaments with forceps and place in water in small petri dish
- examine pleopods and gills under dissecting microscope
- place 2 pleopods in drop of water on slide and coverslip
- place 2 gill filaments in drop of water on slide and coverslip
- examine preparations under compound microscope at 100-400X magnification
- prepare a squash preparation of tail musculature and examine for microsporidian spores
- record and draw organisms present



Types of organisms you are likely to encounter in or on the crayfish:

Protozoa

Thelohania/Pleistophora	(microsporidia forming cysts in muscles)
Vaginicola/Cothurnia/Pyxicola	(upright loricate peritrichous ciliates fouling gills/pleopods)
Lagenophrys	(flat peritrichous ciliates fouling gills)
Vorticella/Epistylis/Zoothamnium	(stalked peritrichous ciliates fouling gills/pleopods)
Acineta/Tokophrya/Sphaerophrya	(suctorian ciliates fouling gills)

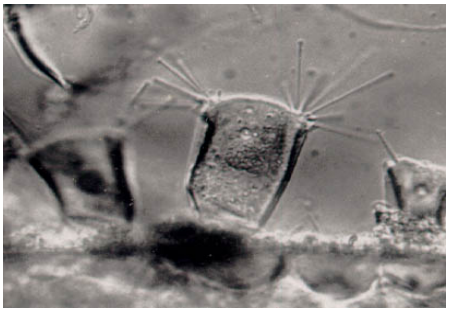
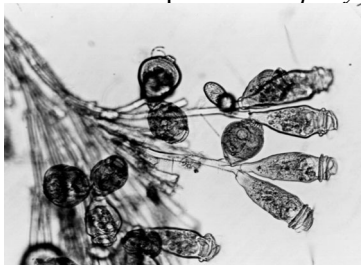
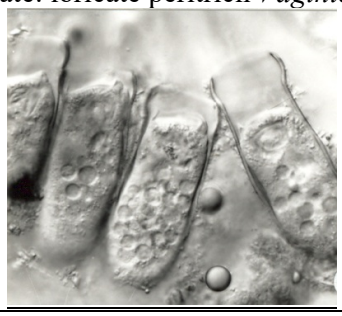
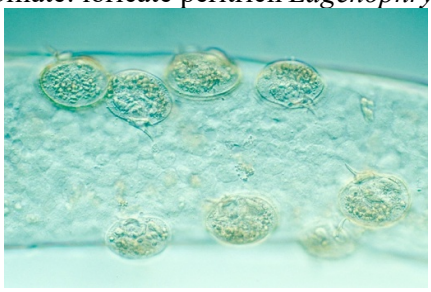
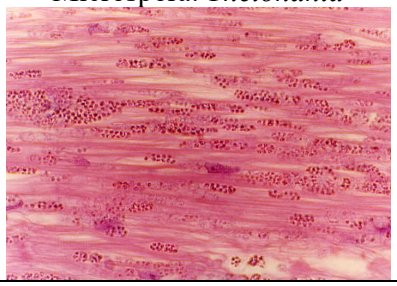
Flatworms

Temnocephala/Craspedella	(free-living flatworms attaching eggs to crayfish)
Microphallus	(trematodes forming metacercariae in muscles)

Keep notes on what you find and in your practical write-up, indicate what the significance of your observations may be for aquaculture producers and consumers!

Freshwater Invertebrates

Protozoa	animacules	
Porifera	sponges	
Cnidaria	hydroids, jellyfish	
Turbellaria	flatworms	
Nemertea	proboscis worms	
Gastrotricha	gastrotrichs	
Rotatoria	rotifers	
Nematoda	nematodes	
Nematomorpha	horsehair worms, gordian worms	
Tardigrada	water bears	
Bryozoa	moss animalcules	
Annelida	aquatic earthworms, polychaetes, leeches	
Gastropoda	snails, limpets	
Pelecypoda	clams, mussels	
Crustacea	Eubranchiopoda	fairy, tadpole & clam shrimps
	Cladocera	water fleas
	Copepoda	copepods
	Ostracoda	seed shrimps
	Mysidacea	opossum shrimps
	Isopoda	aquatic sow bugs
	Amphipoda	scuds, sideswimmers
	Decapoda	crayfish, shrimp
	Hydracarina	water mites
Insecta	Collembola	springtails
	Plecoptera	stoneflies
	Ephemeroptera	mayflies
	Odonata	dragonflies, damselflies
	Hemiptera	bugs
	Megaloptera	alderflies, dobsonflies, fishflies
	Trichoptera	caddis flies
	Coleoptera	beetles
	Diptera	flies, mosquitoes, midges

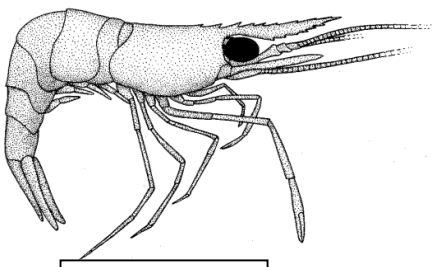
Temnocephalan: *Craspedella* 	Temnocephalan: *Diceratocephala* 
Ciliate: suctorian *Tokophrya* 	Ciliate: suctorian *Acineta* 
Ciliate: stalked peritrich: *Epistylis* 	Ciliate: stalked peritrich *Vorticella* 
Ciliate: loricate peritrich *Vaginicola* 	Ciliate: loricate peritrich *Lagenophrys* 
Trematode: *Microphallus* 	Microspora: *Thelohania* 

Crayfish fouled with temnocephalan eggs

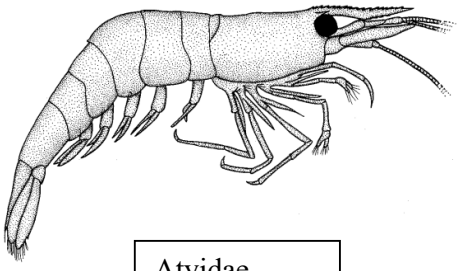
Crayfish on right with cotton tail (muscles packed with microsporidia)

Examination of wild freshwater shrimps/prawns

The crayfish (family Parastacidae) that you examined first in this practical were from a crayfish farm and the extent to which they were infected/fouled by symbionts may reflect the intensive culture conditions. As an extra exercise, you should now examine a freshwater shrimp collected from a natural system to explore the extent to which it has external and internal symbionts. There are (probably) two types of freshwater prawns provided – representatives of the families Atyidae and Palaemonidae. Both have almost transparent tissues when alive (which allows internal parasites to be detected relatively easily). The two families are distinguished by the length of the claws, long in Palaemonidae (and often much bigger animals) and short in the Atyidae.

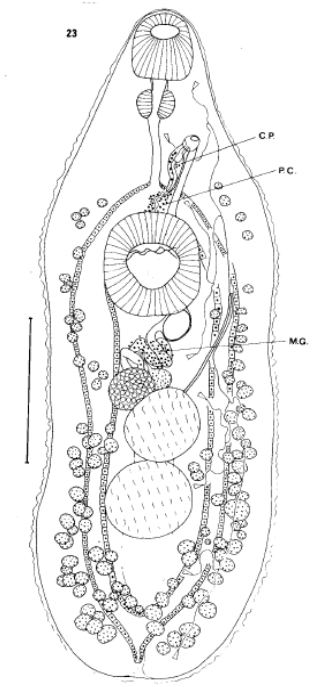


Palaemonidae

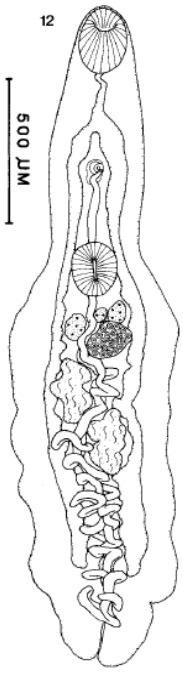


Atyidae

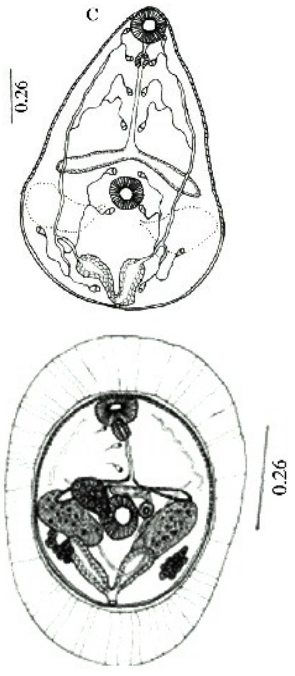
These shrimps should be dissected in the same way as the crayfish with the distinction that as soon as the animal is killed it should be subjected to careful examination as a whole animal (it is small enough and transparent enough to allow this. With careful examination it is likely that you will detect multiple internal and external symbionts on the shrimps. The groups of symbionts that you may find are broadly similar to those outlined for the crayfish above. However, you should look specifically for, and try to identify, three types of trematode metacercariae as follows:



Opecoelidae

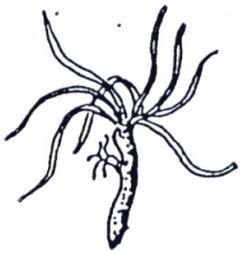


Gorgoderidae

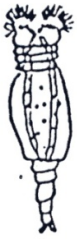


Microphallidae

POND LIFE I.D.



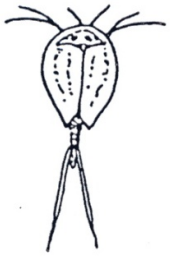
Hydra



Rotifera

Freshwater Mussel

Fairy Shrimp



Tadpole Shrimps

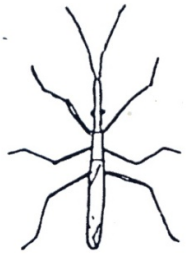
Clam Shrimps

Crab

Screech Beetle

Water Scavenger

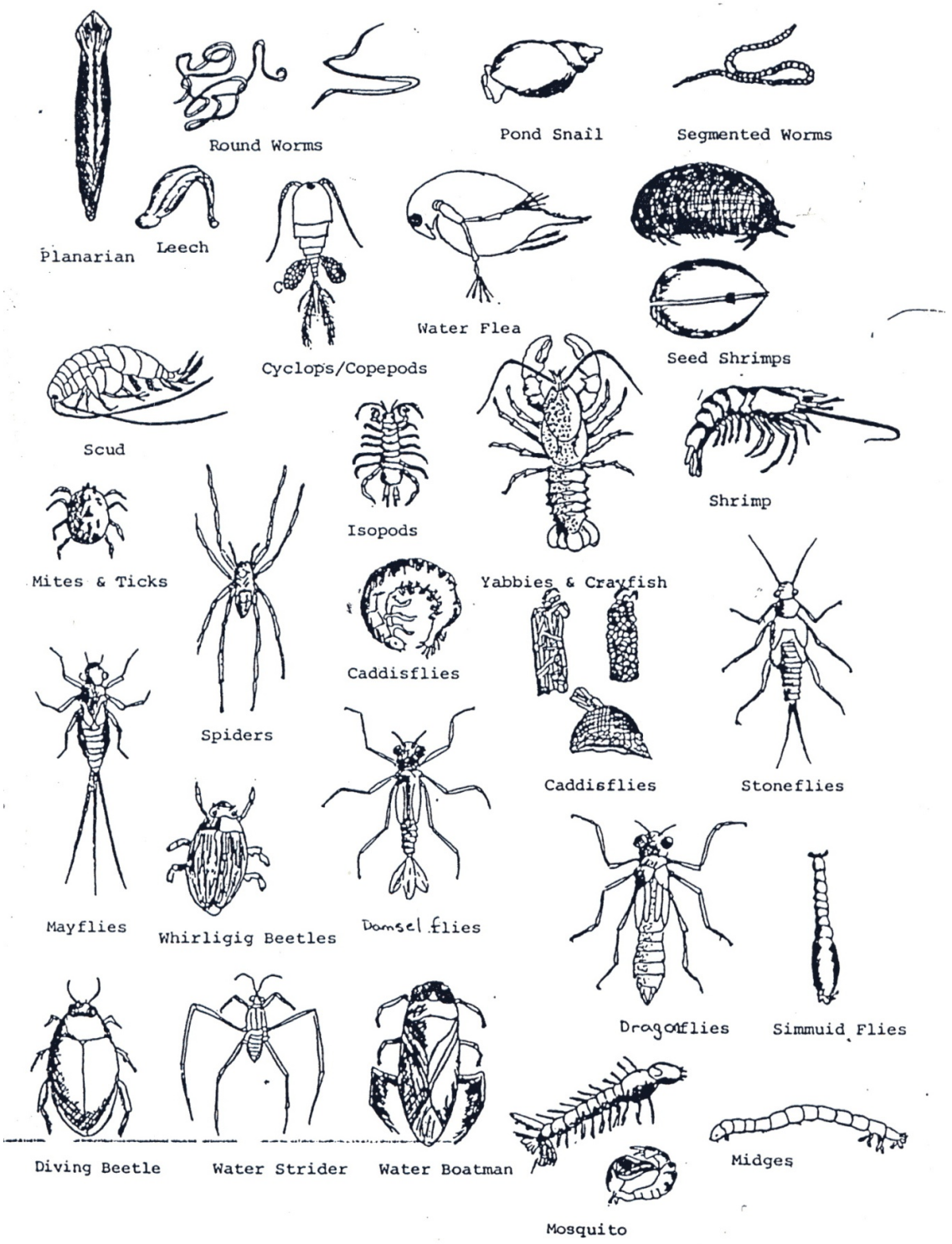
Lesser Water Boatman



Marsh Treader

Water Scorpian

POND LIFE I.D.


Round Worms
Pond Snail
Segmented Worms
Planarian
Leech
Water Flea
Seed Shrimps
Cyclops/Copepods
Scud
Shrimp
Isopods
Mites & Ticks
Yabbies & Crayfish
Spiders
Caddisflies
Caddisflies
Stoneflies
Mayflies
Whirligig Beetles
Damsel flies
Dragonflies
Simmuid Flies
Diving Beetle
Water Strider
Water Boatman
Midges
Mosquito

WEEK 2: TUTORIAL

HOW CAN WE CONTROL PARASITIC INFECTIONS?

Three main strategies are utilized to control infectious diseases:

- drugs (to cure/curb/prevent infection);
- vaccines (to protect against infection/disease); and
- environmental management (to prevent transmission).

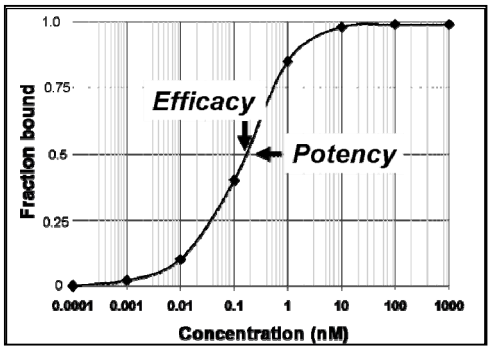
DRUGS

Throughout history, various substances have been endowed with medicinal qualities and folklore herbal remedies have long been used by humans (and even by some animal populations). Many drugs come from plants, particularly those species that have developed chemical defenses against pests, pathogens and disease. Scientists are continuing to identify the active ingredients in herbal remedies and they are constantly attempting to create synthetic analogues to overcome problems in supply and demand. Drugs may kill parasites ('-cidal' activity) or inhibit their growth or reproduction ('-static' activity). However, because parasites are eukaryotic organisms just like their hosts, the drugs may also act on the hosts. Anti-parasitic drugs must therefore exhibit a selective toxicity for the parasite many times greater than that for the host (selectivity index ≥ 30). Parasites also belong to several disparate assemblages, so no single formulation exhibits broad-spectrum activity against all parasites. Indeed, anti-protozoal drugs generally do not work against helminths or arthropods, anthelmintics often do not work against protozoa or some arthropods, and acaricides and insecticides do not work against protozoa or most helminths. Drugs may be used:

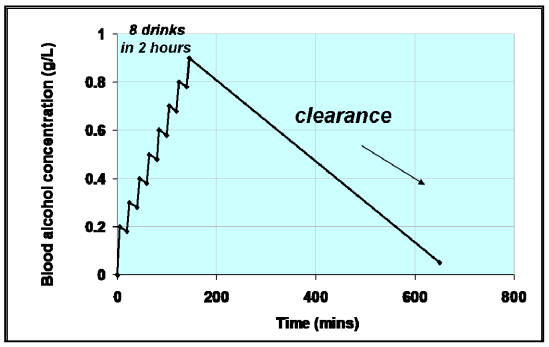
- therapeutically to treat infections (chemotherapy), or
- prophylactically to prevent infection (chemoprophylaxis).

A huge range of prescription and nonprescription drugs are produced by multinational companies under a staggering number of names (one international non-proprietary name, but with several regional non-proprietary names and many proprietary brand names). Drugs come in a vast array of topical preparations (liniments, lotions, ointments, dips, shampoos, washes, pour-ons, spot-ons, collars, creams, sprays, powders, aerosols), oral formulations (tablets, pills, capsules, bolus, liquids, emulsions) or parenteral formulations (liquids in ampoules/vials, or solid implants, for subcutaneous, intramuscular, intravenous or intraperitoneal administration). Pharmacological modes of action may involve selective activity on parasite DNA synthesis (alkylation, purine, cofactor), protein synthesis (inhibition, translation), energy metabolism (electron transport, reduction), neurotransmission (blockers, inhibition), membrane function (vacuoles, permeability), microtubule function (paralysis), or hem(oglobin) interaction (disruption).

Pharmaco-dynamics (PD) is the study of the biochemical and physiological effects of drugs on the body (or on infective microorganisms within the body) and the mechanisms of drug action and the relationship between drug concentration and effect. The majority of drugs either mimic or inhibit normal physiological/biochemical processes or inhibit pathological processes. Drugs may act as stimulants, depressants, toxins or substitutes in their chemical interactions with enzymes, structural proteins, carrier proteins, ion channels, hormones, neuromodulators or neurotransmitters. Many drugs act as ligands which bind to receptors influencing cellular processes, either resulting in enhanced action (agonist), blocked action (antagonist), or even opposite action (inverse agonist). For drugs to work, they must reach specific target concentrations. Many factors affect drug concentrations; such as patient size, age, genetic disposition, physiology, metabolism, etc. There may also be undesirable side-effects including: cell mutation (carcinogenic activity); metabolic disturbances, physiological damage and abnormalities. The therapeutic window is the amount of drug between the amount that gives an effect (effective dose) and the amount that gives more adverse effects than desired.



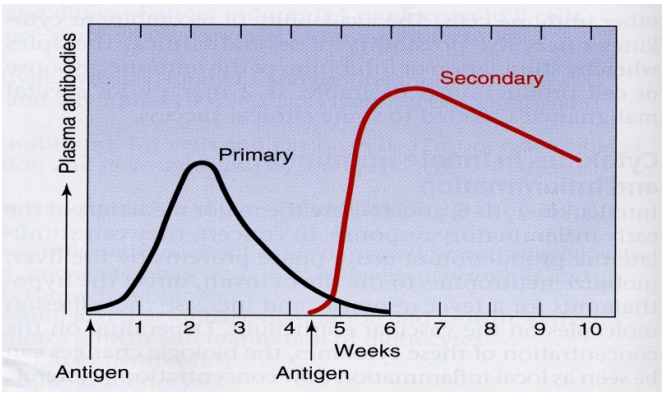
Pharmaco-kinetics (PK) is a branch of pharmacology dedicated to the determination of the fate of substances administered externally to a living organism (applies mainly to drugs, but in principle concerns other substances such as nutrients, metabolites, hormones, toxins, etc). PK considers four main areas: the extent and rate of absorption, distribution, metabolism and excretion (ADME scheme). Pharmacokinetic analyses are performed by noncompartmental or compartmental methods. Noncompartmental methods estimate the exposure to a drug by estimating the area under the curve of a concentration-time graph while compartmental methods use kinetic models to describe and predict the concentration-time curve. Bioanalytical methods are necessary to construct a concentration-time profile. Chemical techniques are employed to measure the concentration of drugs in biological matrix, most often plasma or urine. It is very important that concentrations of drugs can be accurately determined (and predicted) in order to maximize therapeutic benefits and minimize potential side-effects.



The efficacy of many drugs may be compromised by the development of drug-resistance by parasite strains, whereby they develop mechanisms to alter the drug target, alter drug uptake or even inactivate drugs. This gives these strains an advantage as they survive to reproduce and become disseminated throughout host populations despite drug treatment. Many factors may act to induce drug-resistance, including environmental factors (such as exposure to sub-lethal doses) and genetic factors (such as variable recombination and/or high mutation rates). Drug residues may also accummulate in the food chain so most livestock industries mandate with-holding periods whereby drug application prior to slaughter is prohibited. Some drugs also persist in the environment and may exert deleterious effects on other organisms. Ideally, all drugs should have registered "terms of use", including intended application, contra-indications, drug composition, mode of action, target species (pathogen and host), dosages, course and route of administration, side effects, storage and disposal information (usually detailed on product inserts). Successful chemotherapeutic treatment may cure the patient of current infection, but it does not impart any protection against re-infection. Patients go back to their homes and their old habits and often quickly become re-infected. To stop this from happening, patients require regular chemoprophylactic treatment, or vaccination, or re-education about parasite transmission.

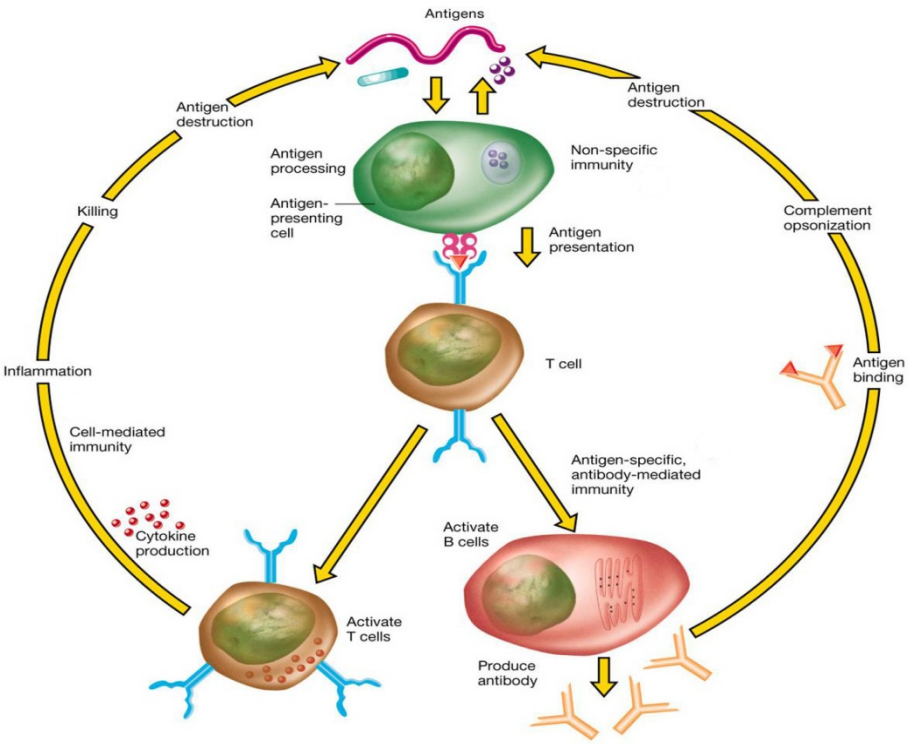
VACCINES

In the course of infection, host immune responses develop to combat the invading pathogens. Nonspecific defences such as the skin and inflammation provide general barriers to infection, but other immune responses are more specific, identifying and destroying particular invaders. Many consider there to be three lines of immunological defence to infection. The first line involves nonspecific barriers to infections: both physical barriers, such as the skin and mucous membranes; and chemical barriers, such as mucus, lysozyme and gastric juices. The second line of defence involves nonspecific innate immunity, whereby inflammatory responses deliver cells (phagocytes and natural killer cells) and antimicrobial proteins (complement, interferon) to the site of infection to combat invading organisms. The four cardinal signs of inflammation involve rubor (redness), calor (heat), tumor (swelling), and dolor (pain) due to the increased blood flow at the site of infection. The third line of defence involves specific adaptive (or acquired) immunity, whereby cytotoxic cells and antibodies are produced to specifically attack the invading pathogens. These specific responses involve effector and memory cells, the latter facilitating more efficient responses when the host is exposed to secondary challenge infections. Vaccination aims to prime the adaptive immune system to antigens of a particular microbe so that a first infection induces a secondary response.



Adaptive immune responses are highly specific and are reliant on lymphocytes. These cells circulate throughout the blood and lymph and are found in high numbers in lymphatic tissues. They develop from pluripotent stem cells in the bone marrow; B cells mature in marrow and T cells mature in the thymus. B and T cells recognize antigens via specific surface antigen receptors; B cell membrane antibodies and T cell receptors. Self-tolerance develops as cells bearing receptors for native molecules are destroyed or rendered nonresponsive. Major histocompatibility complex (MHC) molecules are crucial to antigen presentation. Class I MHC molecules located on all nucleated cells present antigens to cytotoxic T cells while class II MHC on macrophages and B cells present antigens to helper T cells. When an antigen binds to a particular lymphocyte, it is activated to produce numerous identical copies (clonal selection). The primary immune response (first exposure to an antigen) results in clones of short-lived infection-fighting effector cells as well as clones of long-lived memory cells. Subsequent exposure to the same antigen activates the memory cells and the resultant secondary immune responses are faster, stronger and often protective (providing the basis for vaccination).

Adaptive immunity involves both cell-mediated (T-cell) and humoral (B-cell) responses. Cell-mediated responses provide excellent defence against intracellular pathogens because infected cells are identified and destroyed. Helper T cells interact with antigen-class II MHC complexes on macrophages through T cell receptors and the cell surface protein CD4. Contact stimulates the helper T cells to grow and divide as does interleukin-1 secreted by the macrophages. The helper T cells secrete interluekin-2 which activates other cells in the immune system; including cytotoxic T cells (stimulated to become active killer cells), B cells (stimulated to become antibody-producing plasma cells) as well as additional helper T cells (stimulated to divide more rapidly and increase cytokine production). Antigens from intracellular pathogens are gathered in nucleated cells of the body and presented on the surface by class I MHC proteins. They are recognized by cytotoxic T cell receptors and the cell surface protein CD8. Cytotoxic T cells are stimulated by contact and by interleukin-2 to release the protein perforin which punctures the target cell, allowing water and ions to rush in causing swelling and lysis. Humoral responses are effective against extracellular pathogens. B cells have antibody receptors in their plasma membranes specific for particular antigens. Successful binding stimulates B cell proliferation producing clones of plasma cells and memory cells. Plasma cells secrete antibodies in increasing amounts peaking 10-17 days after activation. Antibodies flow through the body's fluids and tag foreign cells and molecules for destruction via neutralization, agglutination, precipitation and complement fixation.



While adaptive/acquired immune responses may provide protection against re-infection, they only do so against the particular parasite species (or strain) that elicited the initial response. This high specificity means that no single vaccine will give broad-spectrum protection because separate vaccines will have to be developed against each individual parasite species/strain. The degree of immunity may also vary, ranging from sterile immunity (pathogens eliminated) to partial immunity (clinical disease prevented) to concomitant (premunitive) immunity (some pathogens persist but hosts are protected against super-infection). The duration of protection may also vary, with protection lasting for years or waning after months.

Many vaccination strategies have been proposed for infectious diseases, including anti-infection (prevent infection); anti-disease (prevent disease if not infection); and anti-transmission (prevent further dissemination). Most parasites have complex life-cycles involving sequential developmental stages which may be quite different from each other, may infect different tissues, and may elicit different immune responses. This complicates the process of finding parasite immunogens (antigens that also provoke protective responses). Vaccines may be developed from live organisms (attenuated strains), whole killed organisms (fixed), subcellular fragments (extracts from lysed organisms), toxoids (inactivated toxins that still induce protective antibodies), or artificially synthesized antigens (produced using various molecular biological approaches). Vaccines need to be monitored for reversion to virulence (in the case of live organisms), allergic or hypersensitivity reactions (particularly those involving adjuvants), and contamination (by non-target microbes or genes). Vaccines must also be extensively tested *in vitro* (in tissue culture) and *in vivo* (in laboratory animal models) before they undergo clinical trials (often using double-blind placebo-control protocols). The moral and ethical dilemmas involved in confirming vaccine efficacy by challenging vaccine recipients with clinical infections are numerous, particularly for virulent diseases with compromised treatment options. Despite the promise of modern technology to produce vaccines against parasites, few have been successfully developed because we know so little about host-parasite immunological interactions, and we are constantly discovering mechanisms by which parasites evade host immune responses.

MANAGEMENT

Parasites are transmitted between hosts by both horizontal (e.g. venereal, faecal-oral, water-borne, food-borne, predator-prey, air-borne, vector-borne,) and vertical (transplacental, transmammary) routes of transmission. Infections can therefore be prevented by interrupting transmission cycles by manipulating the environment and host behaviours. Management strategies to control parasitic infections include changing:

- physical conditions (e.g. sanitation, water purification, sewage disposal, food hygiene, etc.);
- biological entities (e.g. control of vectors, animal reservoirs, breeding sites, etc.); and
- sociological behaviours (improved hygiene, healthcare, nutrition, housing, food safety, etc.).

Increasing urbanization of human populations has led to the emergence of many so-called ‘crowd diseases’ associated with our close proximity in contaminated environments. Improved hygiene and sanitation has done much to reduce the incidence of many parasitic infections. The provision of clean drinking water by removing contaminants (by sedimentation, flocculation and filtration) and disinfection (by chlorination, ozone treatment or heating) has greatly reduced the impact of water-borne diseases. The development of sewers and sewage treatment works has helped reduce faecal contamination of the environment and led to cleaner and safer food and water supplies. Food security has become a priority in many countries, not only to secure a stable supply but also to ensure it is safe to eat.

Many strategies have been utilized to control arthropod vectors; including insects (mosquitoes, flies, fleas and lice), arachnids (ticks and mites) and molluscs (snails). Physical barriers and chemical repellants have been used to protect hosts, quarantine measures have been used to isolate infected individuals, and people have been educated to avoid vector contact. Vector populations have also been reduced by eliminating breeding sites (drain swamps, avoid puddles, clear land), manipulating reproduction (sterile male release, *Wohlbachia* male killing), poisoning vectors (chemical control using sprays, traps) and biological control using predators (wasps), parasitoids (fungi), and pathogens (viral, bacterial, protozoan and helminths infections).

Public health officials are constantly monitoring disease occurrence and developing interventions for outbreak situations. While governments and health organizations do much good, the greatest impediment to parasite control appears to be the difficulty in changing people’s behaviour. Many people persist in activities which perpetuate parasite transmission and survival because such behaviours are perceived to be cultural norms (e.g. eating rare meat, using night-soil fertilizer, etc.). Effective parasite control programs involve concerted education campaigns using local, national and international resources and as much media exposure as possible.

WEEK 3: TUTORIAL

BUG QUIZ

(1). Name five different modes of transmission for parasites!

horizontal [faecal-oral, predator-prey, vector-borne, direct contact, venereal, air-borne]
vertical (transplacental, transmammary)

(2). Name six different groups of vectors!

arthropods	insects (flies, fleas, lice)
	arachnids (ticks, mites)
	crustacea (copepods)
molluscs	gastropods (snails)

(3). What type of hosts are the vectors?

definitive (e.g. mosquito, sexual development of *Plasmodium*)
intermediate (e.g. snail, asexual development of *Schistosoma*)
paratenic (e.g. small fish, metacercaria of *Clonorchis*)(second/third intermediate hosts)

(4). Can vectors be parasites in their own right?

yes (all except snails – no vampire snails known)

(5). What effects can they have on their hosts?

focal	pain (annoyance, irritation, severe behavioural problems)
	trauma (lesions, structural damage)
	dermatosis (inflammation, ulceration, itching)
	allergy (hypersensitivity reactions)
systemic	anaemia (mild-severe blood loss)
	toxicosis (serum exudation, ascending paralysis)
	other infections (viral, bacterial, rickettsial,spirochaete, protozoal, helminths)

(6). How can we reduce contact with vectors?

Physical/mechanical separation

barriers (screens, nets)
avoidance (diurnal cycles, education)
quarantine (isolation)
habitat alteration (puddles, paddies, burning, burying, ploughing, etc.)
repellants (plants, chemicals)

(7). Can we make hosts more resistant to vectors?

Yes. Breeding programs (tick resistant *Bos indicus* cattle)

(8). Is vaccination an option (given the success of many anti-toxins)?

Qualified yes! Anti-toxin immuno-therapy
Vaccination immuno-prophylaxis
Case for vaccination (acquired protection?????)
Success of some vaccination programs (tick gut antigens)

(9). How can we control vector populations?

kill them	chemical control (poisons)
	physical control (traps, removal)
	biocontrol (predators/parasitoids/pathogens)
reduce habitat	(drain swamps, avoid puddles, clear land, etc.)
reduce reproduction	(sterile male release)
	(*Wohlbachia* rickettsial proteobacteria
	- male killing
	- feminization
	- parthenogenesis
	- cytoplasmic incompatability)

(10). What do anti-vector compounds target?

drugs target DNA synthesis, protein synthesis, energy metabolism, membrane function, microtubule function, neurotransmission

insecticides/acaricides target:
- neurotransmitters (sodium channels, chloride channels, AChE)
- cuticle (growth regulators)
- ecdysis (juvenile hormones)

molluscicides (snail baits/pellets)
- metal salts
- metaldehydes
- AChE inhibitors

WEEK 4: PRACTICAL

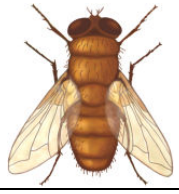
PARASITIC ARTHROPODS

The practical involves 3 activities which you should complete over the next three hours:

- dry practical (examine the bench set of parasite samples provided)
- wet practical (perform your own parasite diagnostic test with samples provided)
- spot-quiz (formative assessment item similar in structure and content to final practical exam)

Today's practical is focussing on arthropod parasites – multicellular arachnids and insects which infest humans and other animals where they suck blood or eat host tissues causing irritation, hypersensitivity, lesions and clinical disease. The severity of disease depends on the numbers of ectoparasitic stages and infections may be acute or chronic in presentation.

Arthropods have "jointed limbs" and exhibit varying degrees of body segmentation and cephalization. They have a rigid exoskeleton and therefore must moult in order to grow. Some undergo complete metamorphosis (where larval stages are completely different from adult stages) while others undergo incomplete metamorphosis (larval/nymph stages similar to adults). Thousands of arthropods occur as free-living aquatic or terrestrial species while hundreds have adopted a parasitic mode of existence; including insects and arachnids:

insects (6 legs)			arachnids (8 legs)	
fleas	flies	lice	ticks	mites
				

Objectives:

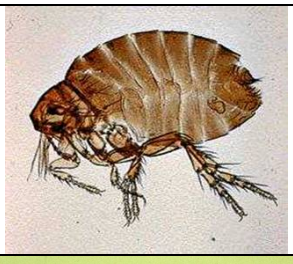
- To review the key morphological features of parasitic insects (fleas, lice, flies)
- To review the key morphological features of parasitic arachnids (mites, ticks)
- To examine different stages in the developmental cycles of the parasites
- To illustrate the pathological consequences of infections within host tissues

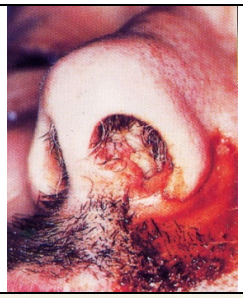
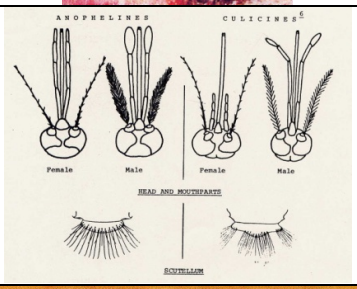
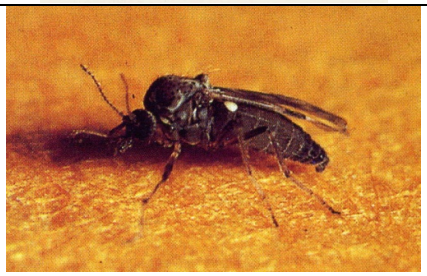
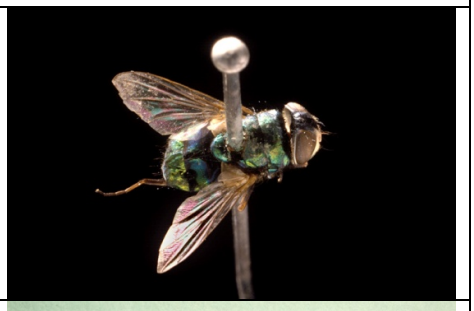
Overview:

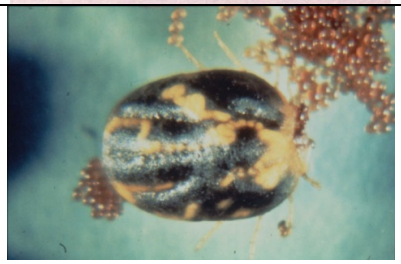
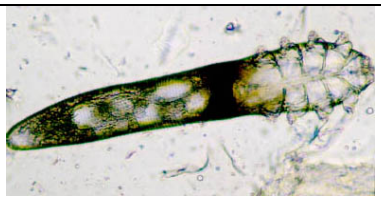
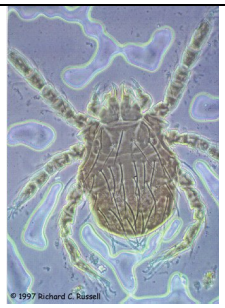
Parasite group	Main distinguishing features	Site of infection	[Parasitic stages]
fleas	laterally compressed body, powerful legs	skin	egg-larva-pupa-[adult]
chewing lice	body flattened, broad rounded head	skin	egg-[nymphs-adult]
sucking lice	body flattened, long pointed head	skin	egg-[nymphs-adult]
Psychodidae	sand flies	skin	egg-larva-pupa-[adult]
Culicidae	mosquitoes	skin	egg-larva-pupa-[adult]
Calliphoridae	blowflies/maggots	skin/tissues	egg-[larva]-pupa-adult
Oestridae	bot flies/maggots	skin/tissues	egg-[larva]-pupa-adult
ixodids	hard ticks, terminal capitulum	skin	egg-[larva-nymph-adult]
argasids	soft ticks, subterminal capitulum	skin	egg-[larva-nymph-adult]
mange mites	astigmata, pierce skin at base of hairs	skin	egg-larva-[nymph-adult]
scabies mites	astigmata, tunnel in skin	skin	egg-larva-[nymph-adult]

DRY PRACT: BENCH SETS

Examine each sample provided. Draw a sketch of what you see. Annotate your drawing.

Slide 1. ***Ctenocephalides felis*** (cat flea – adult female) cat fur whole mount	Examine specimen. Note presence of pronotal and genal combs with >5 spines, head (frons) sloping, twice as long as high, powerful hindlegs, numerous spines.	
Slide 2. ***Ctenocephalides felis*** (cat flea - larvae) bedding whole mount	Examine mount of larva noting elongate shape, lack of eyes, presence of spines. These stages live in the bedding eating debris and blood-rich adult flea faeces.	
Slide 3. ***Echidnophaga gallinacea*** (sticktight flea – adult female) chicken skin whole mount	Examine whole flea mount noting small compact body, blunt truncated frons, absence of combs, and relatively elongate mount parts.	
Slide 4. ***Bovicola (Damalinia) ovis*** (sheep body louse) sheep skin whole mount	Examine whole mount noting rounded head (chewing lice), prominent antennae, elongate body. Infestations cause skin irritation which is aggravated by host rubbing and biting with resultant abrasions and fleece loss.	
Slide 5. ***Pediculus capitis*** (human head louse) human hair whole mount	Examine whole mount noting pointed head (sucking lice), antennae pointing forward, enlarged claws on legs for grasping hairs. Adult lice cement eggs (nits) to hairs.	
Specimen 6. ***Lucilia cuprina*** (green bottle fly) adult free-flying	Examine adult fly with magnification lens noting metallic green body. white face, thorax with 4 rows of bristles and black legs (except for green thighs of first pair).	

Specimen 7. ***Lucilia cuprina*** (green bottle fly) larva (maggot, bot) sheep breech strike	Examine larva in cavity block noting size, colour, smooth surface and pattern of posterior spiracles. These stages rip and eat flesh.	
Specimen 8. ***Sarcophaga* sp.** (flesh fly) larva (maggot, bot) carcase	Examine larva noting ridged appearance and sunken posterior spiracles.	
Specimen 9. ***Culex* sp.** (mosquito) adult female free-flying head	Note two long feathery antennae, long proboscis, and two short palps (cf. long palps of *Anopheles*).	
Slide 10. ***Culicoides brevitarsus*** (biting midge) adult free-flying	Examine specimen noting humped thorax, slender abdomen, long feathery antennae, hairy wings, usually spotted, short mouth-parts.	
Specimen 11. ***Chrysomyia bezziana*** (screw-worm fly) adult free-flying	Adult fly with green abdomen, no bands, face orange-buff, squamae waxy white, well developed mouthparts.	
Slide 12. ***Ixodes holocyclus*** (scrub/paralysis tick) adult ex dog	Examine dorsal surface of specimen noting presence of scutum (lined in females) and capitulum. Examine ventral surface noting presence of anal groove in front of anus.	

Specimen 13. ***Rhipicephalus sanguineus*** (brown dog tick) adult ex dog	Examine adult ticks noting shorter mouthparts, leg colour and arrangement (dark legs spaced apart in U-shape), hexagonal basis capitulum.	
Slide 14. ***Rhipicephalus (Boophilus) microplus*** (cattle tick) adult female ex cow	Examine inornate tick noting presence of eyes on scutum but absence of festoons. The palpi are ridged and the basis capitulum has lateral projections.	
Slide 15. ***Demodex canis*** (dog follicle mite) adult ex dog	Examine long vermiform (worm-like) mite with 4 pairs of short stumpy legs located anteriorly.	
Slide 16. **trombiculid mite** (chigger mite) larva ex human	Examine the 6-legged larval stage which is parasitic in the skin. Note the claws on the legs and the body setae (hairs).	
Slide 17. ***Sarcoptes scabiei*** (scabies mite) adult ex pig	Examine the small round mites noting dorsal spines and coxae 1 and 2 separated from coxae 3 and 4 (legs 3 and 4 are short and do not extend beyond body margin).	

WET PRACT: DIAGNOSTIC SAMPLES

Human skin can be home to many pathogens, including viruses, bacteria, fungi, protozoa, helminths and ectoparasitic arthropods. Skin infections 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.).

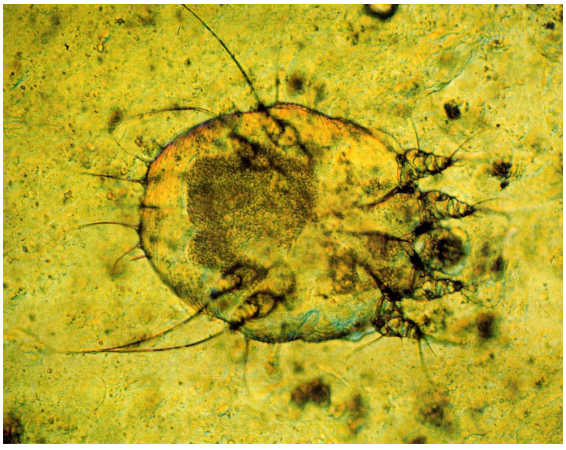
A technique used to aid differential diagnosis involves the alkaline digestion of skin scrapings to reveal pathogenic micro-organisms – especially mites and fungi. Skin samples are collected by scraping lesions with the flat of a scalpel and then digesting the material in dilute potassium hydroxide (which 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.

Human scabies is usually diagnosed by the appearance of the rash and the presence of burrows. Burrows can be revealed by applying topical tetracycline, which is retained by the burrows and fluoresces under a Woods lamp. They can also be localized with ink. Scabies is confirmed by the demonstration of mites (adults, nymphs or eggs) in skin scrapings.

Each pair of students will be 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 and glasses, close your lab coats, disinfect and clean spills with 100% alcohol, discard used plastic-ware in autoclave bins, discard used glassware in sharps bins, wash hydroxide spills under running tap water, do not burn/scald yourself in the hot water baths, report all incidents).
Prepare the material as follows:

- Add 1 mL of 5% potassium hydroxide to the Eppendorf tube containing the 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 undigested mites and egg shells)
-

eggs	adults
	

SPOT QUIZ

Examine the specimens and answer the attached questions
(Allow 3 minutes per specimen; this is the time allowed in the exam)

QUESTIONS	ANSWERS
1. Ectoparasite collected from young school girl in Brisbane. (a) Identify the parasite (scientific & common names). (4 marks) (b) What do the parasites eat? (3 marks) (c) What are the eggs commonly known as? (3 marks)	[slide] *Pediculus capitis* (head louse) blood nits
2. Parasite recovered from clothing of sheep shearer. (a) Identify the type of parasite. (4 marks) (b) What do the parasites eat? (4 marks) (c) Would this parasite cause lesions on humans? (2 marks)	[slide] *Bovicola ovis* (sheep louse) skin/blood no
3. Parasite caught in shag-pile carpet at home. (a) What is the parasite genus? (4 marks). (b) What 4 stages occur in the life cycle? (4 marks) (c) What are the normal hosts? (2 marks)	[Slide] *Ctenocephalides* egg, larva, pupa, adult dogs and cats
4. Parasite removed from behind the ear of a cow. (a) Name the genus of parasite! (3 marks) (b) How many hosts are required to complete the life cycle? (3 marks) (c) What life cycle stages are formed? (4 marks)	[slide] *Rhipicephalus (Boophilus)* one host egg-larva-nymph-adult
5. Parasite recovered from behind the ear of a young child. (a) Identify the parasite genus! (4 marks). (b) What developmental stage is present? (4 marks) (c) What can be the consequences of infestation? (2 marks)	[slide] *Ixodes* adult female paralysis
6. Skin scraping from elderly patient. (a) Identify the parasite genus. (4 marks) (b) What disease does the parasite cause? (3 marks) (c) Are the parasites zoonotic? (3 marks)	[slide] *Sarcoptes* scabies no
7. Head of insect caught inside home in Cairns. (a) What type of insect is it? (3 marks) (b) Identify the genus. (4 marks) (c) What three features can you see? (3 marks)	[slide] mosquito *Anopheles* antennae, proboscis, palps

WEEK 5: TUTORIAL

POOP Quiz

(1). Name three modes of transmission used by parasites!

horizontal (faecal-oral, vector-borne, predator-prey, direct contact, venereal, air-borne)
vertical (transplacental, transmammary)

(2). In the last week, how many times have you been to the toilet?

range 3-14 (begs the question, what is normal?

(3). List ten vernacular names for faeces!

poo, shite, number twos, pat, stool, dung, droppings, brown mullet, turd, crap, etc.

(4). List five physical characteristics of faeces.

appearance (form), consistency, weight/volume, colour, contents, odour, frequency,
cf. senses (sight, sound, smell, taste, touch)

(5). List five chemical characteristics of faeces.

hydrocarbons [= carbon (C) biomass, hydrogen (H) metabolites [pH], oxygen (O) metabolites]
nitrogenous (N) metabolites, phorphorus (P) metabolites, electrolytes, etc.

(6). List five biological entities you may find in faeces.

viruses, bacteria, fungi, protozoa, helminths
smallest 10 nm to largest (10 m)

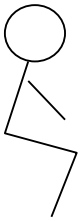
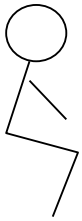
(7). Name three protozoan parasites transmitted by faeces.

cysts: *Entamoeba, Giardia, Balantidium*
oocysts: *Isospora*

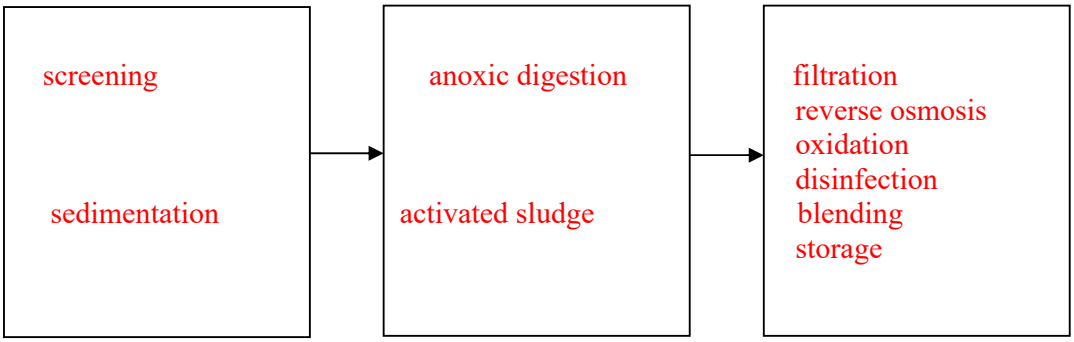
(8). Name two nematode parasites transmitted by faeces.

eggs: *Ascaris, Trichuris*, *Enterobius*
larvae: *Strongyloides, Ancylostoma/Necator*

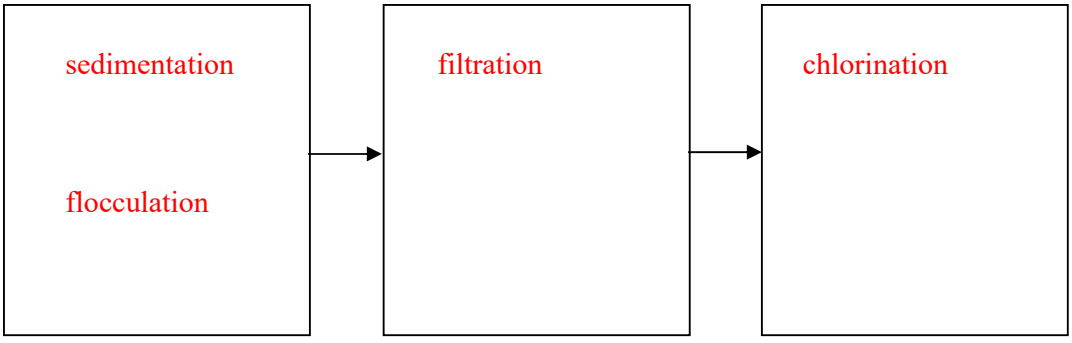
(9). Faecal collection

nomad (hunter/gatherer)	farmer (homestead)	villager (dwellings)	urbanite (towns/cities)
			
indiscriminate	pots night soil	cess pools (aerobic) septic tanks (anaerobic)	sewers treatment works

(10). Faecal treatment

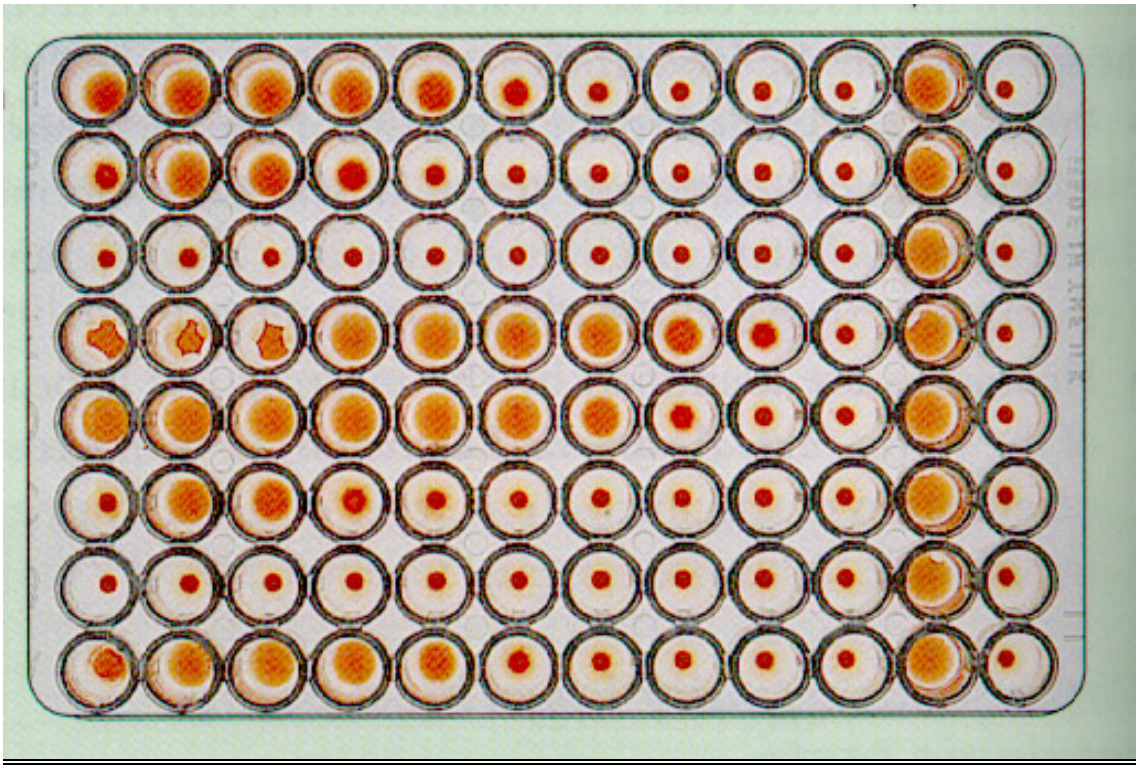


then water treatment



WEEK 6: PRACTICAL

Serological testing



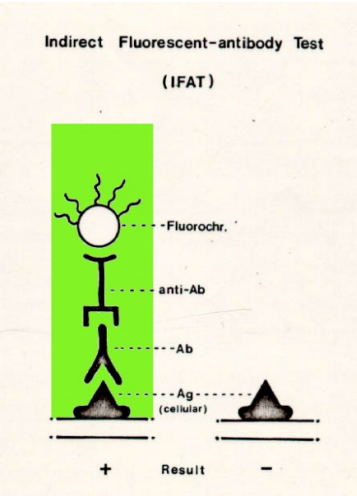
Serological Tests

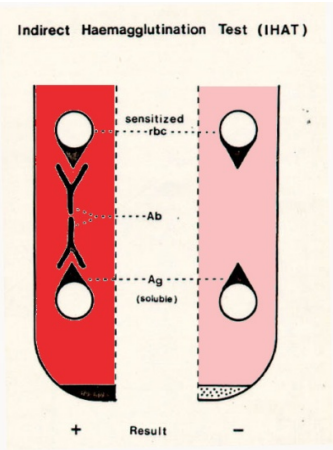
The differential diagnosis of infectious diseases is frequently complicated by the nonspecific nature of any disease symptoms (such as diarrhoea or fever), confounding clinical parameters (haematology and blood biochemistry), difficulties in detecting organisms in test samples (few present and irregular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

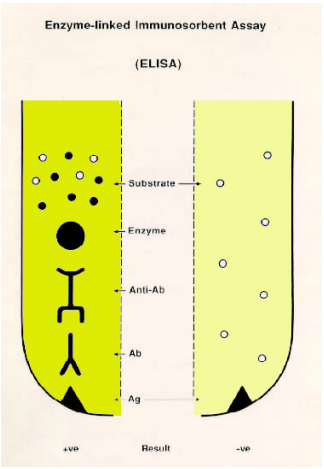
Recourse is therefore often made to the indirect demonstration of infections using immunoserological techniques to provide presumptive evidence of infection. Serum samples are tested for the presence of host antibodies formed against microbial antigens.

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.

Mathematics came 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 still elicit a positive reaction when the blood was diluted 100 times. It is common for antibody titres during active acute infections to be in the vicinity of 1/500 to 1/1000.

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)

What is the minimum amount of blood and diluent needed to make this series?

Answer:

Serial titration method (need only use one 50 µl micropipette and two tips)

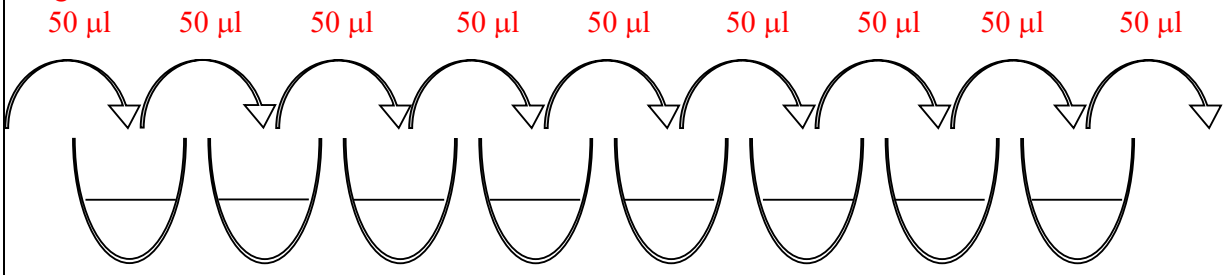
- add 50 µl of diluent to each of 8 consecutive wells

- then add 50 µl of whole blood to first well (giving 100 µl of 1/2 dilution), mix

- then take 50 µl of 1/2 dilution and add to next well (giving 100 µl of 1/4 dilution)

- repeat another 6 times

diagram



saline	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl	waste
yields	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	

In total, need 8 x 50 µl diluent = 400 µl (= 0.4 mL), and 50 µl of blood

INDIRECT HAEMAGGLUTINATION TEST FOR *TOXOPLASMA*

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.

Materials: **per pair**

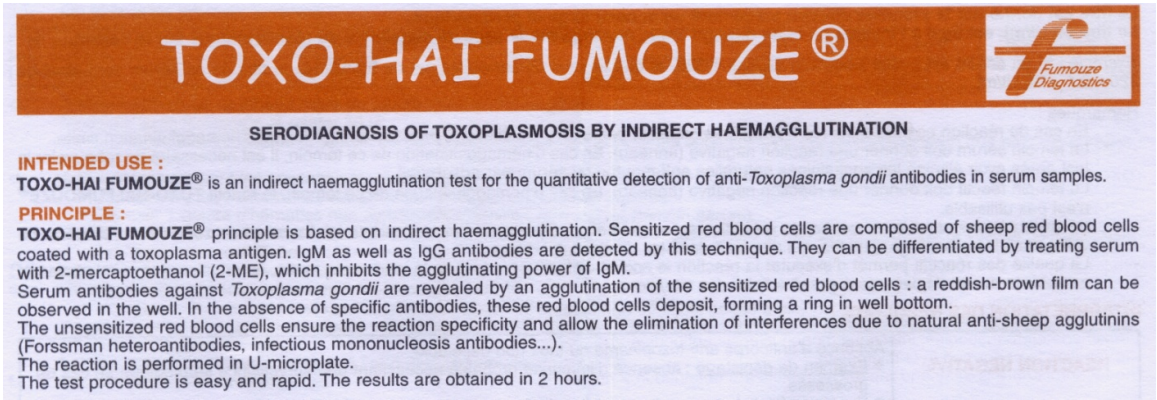
round bottom microtitre plate
50 ml phosphate-buffered saline (PBS) pH 7.2
2 x 100 µl animal serum

Materials: **per bench**

Single channel 50 µl pipettors + tips
200 ml alcohol cleaning solution

Materials: **per tutor**

Sensitized and unsensitized sheep red blood cells with drop tubes (15 µl)
Positive and negative control sera



TOXO-HAI FUMOUZE®

Fumouze Diagnostics

SERODIAGNOSIS OF TOXOPLASMOSIS BY INDIRECT HAEMAGGLUTINATION

INTENDED USE :
TOXO-HAI FUMOUZE® is an indirect haemagglutination test for the quantitative detection of anti-*Toxoplasma gondii* antibodies in serum samples.

PRINCIPLE :
TOXO-HAI FUMOUZE® principle is based on indirect haemagglutination. Sensitized red blood cells are composed of sheep red blood cells coated with a toxoplasma antigen. IgM as well as IgG antibodies are detected by this technique. They can be differentiated by treating serum with 2-mercaptoethanol (2-ME), which inhibits the agglutinating power of IgM.
Serum antibodies against *Toxoplasma gondii* are revealed by an agglutination of the sensitized red blood cells : a reddish-brown film can be observed in the well. In the absence of specific antibodies, these red blood cells deposit, forming a ring in well bottom.
The unsensitized red blood cells ensure the reaction specificity and allow the elimination of interferences due to natural anti-sheep agglutinins (Forssman heteroantibodies, infectious mononucleosis antibodies...).
The reaction is performed in U-microplate.
The test procedure is easy and rapid. The results are obtained in 2 hours.

PERFORMANCE CHARACTERISTICS :
TOXO-HAI FUMOUZE® reagent is composed of red blood cells sensitized with a mixed toxoplasma antigen made up of both toxoplasma endogenous and membrane constituents. It ensures sensibility and specificity to the reaction.
So, the results of the test evaluations show a sensitivity of 99.2 % and a specificity of 97.7 %.
Moreover, when this test is combined with TOXOLATEX FUMOUZE®, the correlation is between 98.2 (18) and 99.8 % (17), depending on the used reference technique.
During the "Réévaluation de 40 trousses de réactifs pour la détection des anticorps anti-toxoplasmose de type IgG" by Medicine Agency in 1998 march (19), 40 different sera have been titrated with TOXO-HAI FUMOUZE® :
- 9 negative sera were found negative ;
- No negative reaction for 7 limit positive sera tested ;
- 24 positive sera were found positive.

The french nomenclature of medical biology acts specifies 2 tests must be realized simultaneously for the serological diagnostic of toxoplasmosis. Therefore, the overall interpretation of this serology will have to be done according to the results of the different used techniques. In any case, diagnosis should be made using the results of this test together with the other clinical, epidemiological and laboratory findings.

PROCEDURE:

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

PRACTICE USING YOUR MICROPIPETTE: Each year, students assure the tutors that they know how to use a micropipette. However, they then go on to demonstrate to us 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. If problems persist, see your tutor!

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

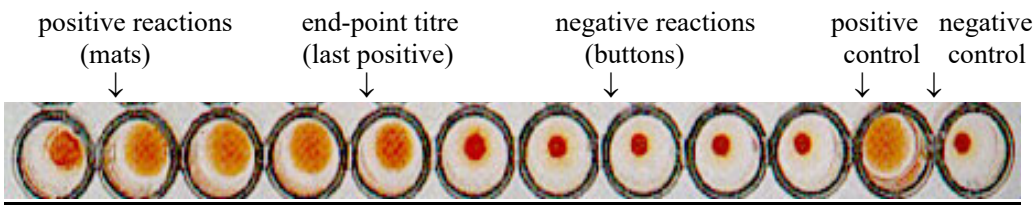
7. Gently tap side of plate to distribute cells throughout well (do not swirl)

8. Place plate on top shelf of bench divider and leave undisturbed for as long as possible (1-2 hours) before reading [complete accompanying questions during this time]

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 on the backboard 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)

Medical Testing, Bayes' Theorem, Test Sensitivity and Specificity

A variety of medical tests are used to diagnose diseases. How good they are depends on many factors, both technical and biological. Remember that all serological tests provide indirect presumptive evidence of infection by demonstrating the presence and amount of antibody against a particular pathogen. These tests are not 100% perfect for a variety of reasons; such as:

- acute infection (host recently infected and antibodies not yet formed)
- chronic infections (host infected and cured years ago but antibodies persist)
- low dose infections (host immune response so low it cannot be measured)
- cross-reactivity (antibodies cross-react against other antigens)

It is therefore important that users of any particular test know how good it is (as determined by objective quantitative assessment). Efficacy is quantified by comparing test results with known disease status in a reference population (disease status being the 'gold' standard). Regrettably, gold standard tests are not always available so serological test efficacy may be poorer than reported. 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
Test prevalence = (A+B)/N

Accuracy (most relevant when choosing test)

Test accuracy = (A+D)/N

Sensitivity (probability of positive test in diseased person)

Test sensitivity = A/(A+C)
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)
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 true positive/negative test results in diseased/nondiseased patients)

Positive Predictive Value (PPV) = A/(A+B)
Negative Predictive Value (NPV) = D/(C+D)

IHAT sensitivity and specificity

A variety of 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

Questions:

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

   MIT prevalence = 70/100 = 70%

8. 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%

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

   3 false positives - needless treatment

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

    13 false negatives - disease to death

11. What is the sensitivity of the IHAT?

    sensitivity = 57/70 = 81.4%

12. What is the specificity of the IHAT?

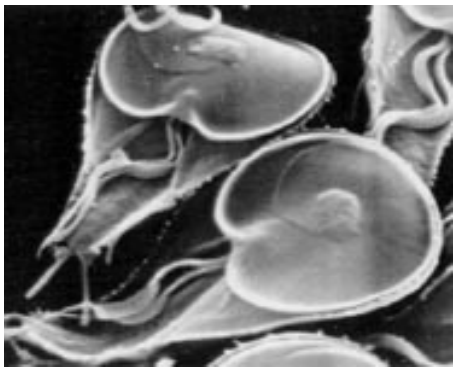
    specificity = 27/30 = 90%

13. If the manufacturers of the IHAT reclassified borderline titres = 1/64 as positive instead of negative, what impact would this have on test sensitivity and specificity? (recalculate these parameters)

    sensitivity = 65/70 = 93%
    specificity = 22/30 = 73%

***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:

14. Complete the following table:

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

15. 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

16. 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

17. 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)

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

1 in 466 = 0.002

WEEK 7: TUTORIAL

DRIP QUIZ

(1). What is unique about dihydrogen monoxide!

H_2O asymmetyrical, polar charge contributes to cohesion (binding) and adhesion (wetting)

(2). Name the 3 physical states of water (notice anything unusual?).

- gas (water vapour, steam) (>100ºC)
- liquid (fluid) (>0ºC, <100ºC)
- solid (ice) (<0ºC) (ice less dense than liquid and floats, forming thermal blanket)

(3). Why is water the fluid of life?

universal solvent, dissolves salts, sugars, proteins, nucleotides (but not fat)
75-90% of cells made up of water

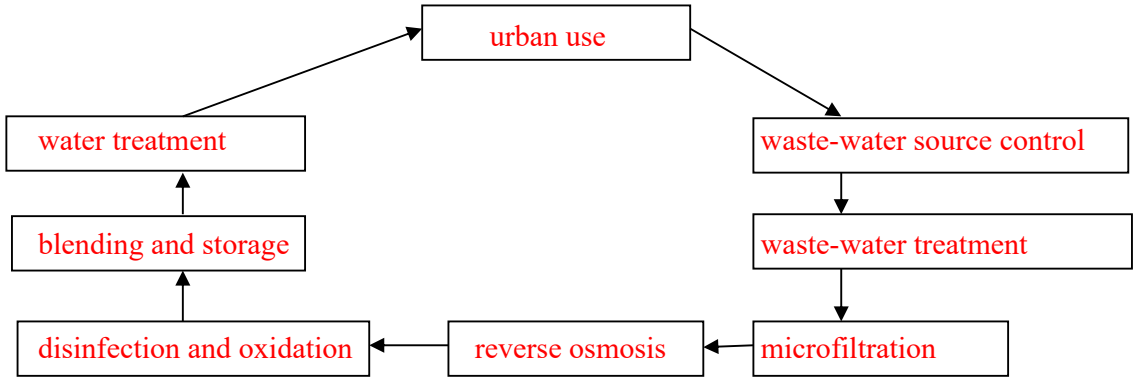
(4). What parameters are measured to indicate water quality?

- physical (pH, turbidity, temp, hardness, conductivity (total dissolved solids))
- chemical (N, P, S compounds, electrolytes, gases, metals, inorganic pollutants)
- biological (viruses, bacteria, fungi, protista/algae, metazoa)

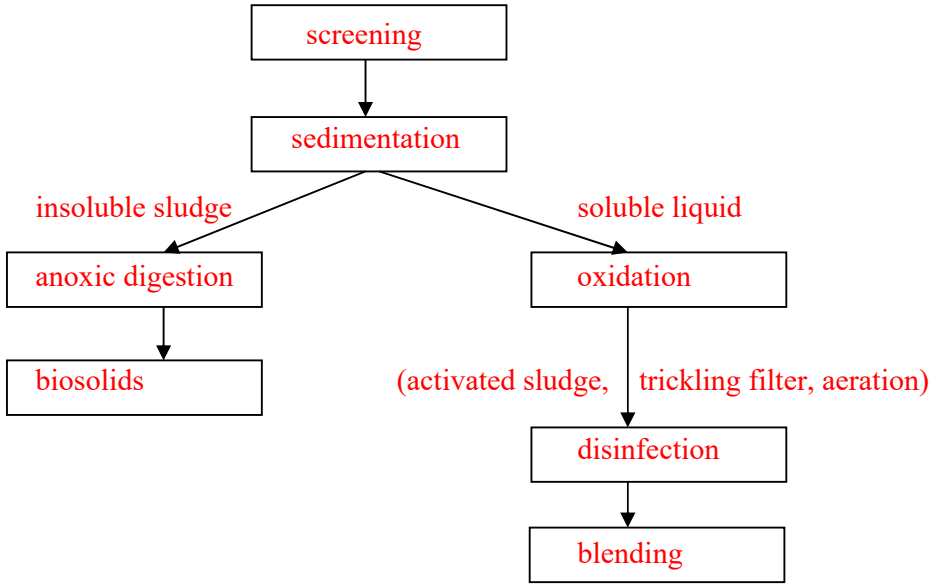
(5). What are the four major sources of water contamination?

- human waste (sewage)
- animal waste (agricultural run-off)
- domestic waste (effluent)
- industral waste (effluent)

(6). Develop a flow-chart for recycled water?



(7). Develop a flow-chart for waste-water treatment?



(8). Name four processes used to decontaminate drinking water (i.e. to remove contaminants).

- sediment (gravity settle, reservoirs, tower tanks)
- flocculation/coagulation (alum)
- filter (sand, membrane, micro)
- adsorb (carbon)

(9). Name three processes used to disinfect drinking water (i.e. to kill contaminants).

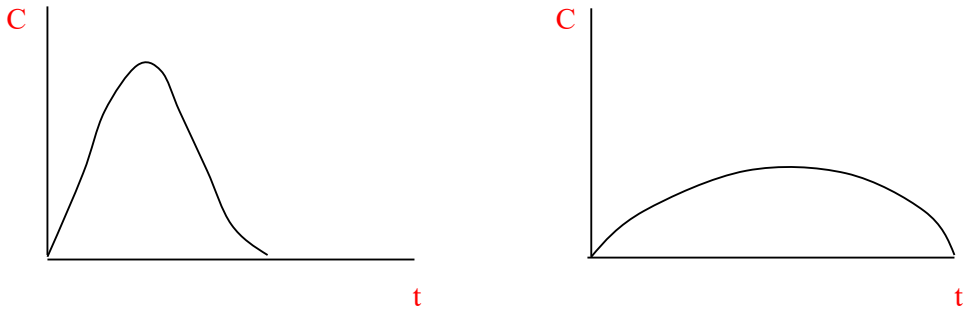
- heat (boil, solar)
- chemicals (chlorine, chloramine, chlorine dioxide, ozone)
- energy sources (UV, gamma, X-ray, high energy electron sources)

(10). Watson's Law states that K = C.t

where K = disinfection index
C = concentration of disinfectant
t = time of contact

What does K actually represent?

Total exposure to disinfectant (AUC of PK concentration - time curve)



WEEK 8: PRACTICAL

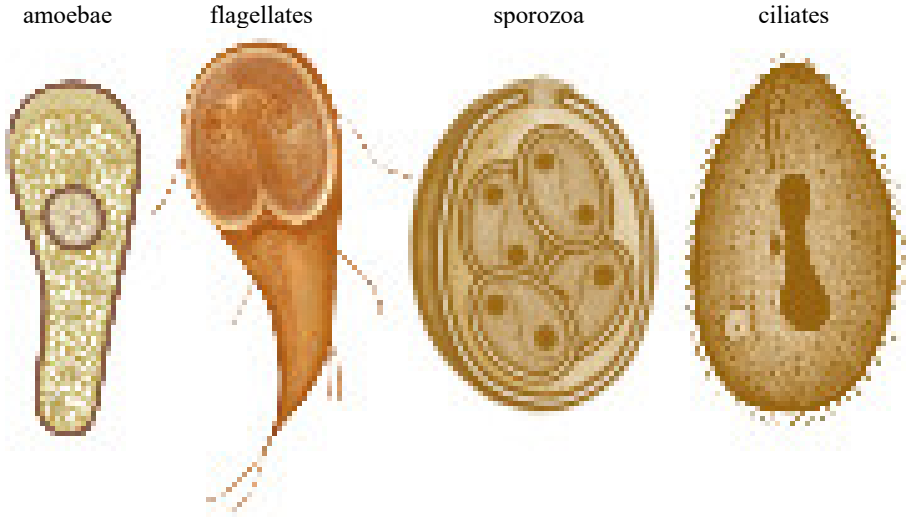
PARASITIC PROTOZOA

This practical involves 3 activities which you should complete over the next three hours:

- dry pract (examine the bench set of parasite samples provided)
- wet pract (perform your own parasite diagnostic test with samples provided)
- spot-quiz (formative assessment item similar in structure and content to final practical exam)

Today's practical is focussing on protozoan parasites – single-celled eukaryotic cells which infect humans and other animals where they invade or attach to a variety of host cells and quickly proliferate in number. Infections generally cause acute transient disease ranging from mild to severe in intensity.

Protozoan parasites are diverse in their form and function; indeed modern classification systems recognize many new phyla of protista. Nevertheless, most references texts on medical and veterinary parasitology use conventional classification systems based on parasite morphology, as practitioners need to look at the parasites in order to diagnose infections. Four major assemblages are recognized:



Objectives:

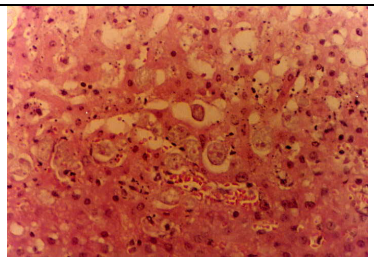
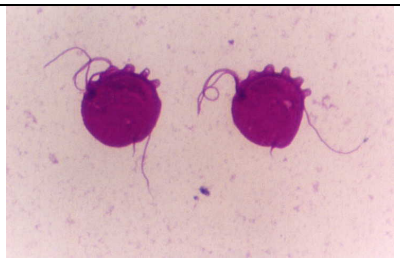
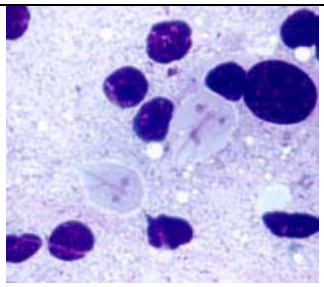
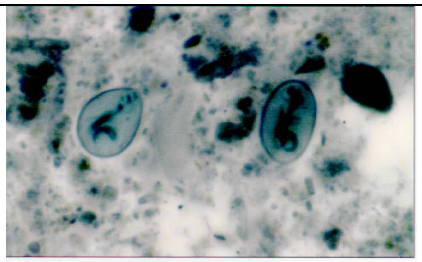
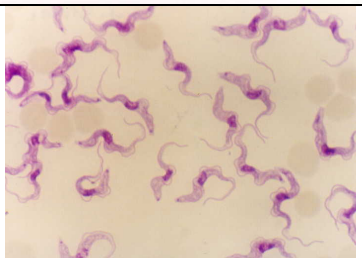
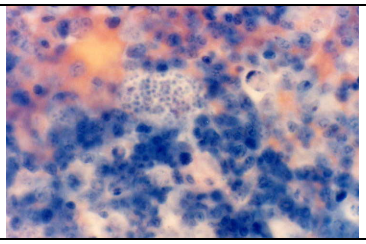
- To review the key morphological features of parasitic amoebae, flagellates, sporozoa and ciliates
- To examine different stages in the developmental cycles of the parasites
- To illustrate the pathological consequences of infections within host tissues
- To provide an understanding of the modes of transmission of the parasites

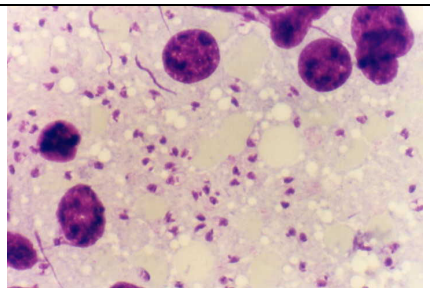
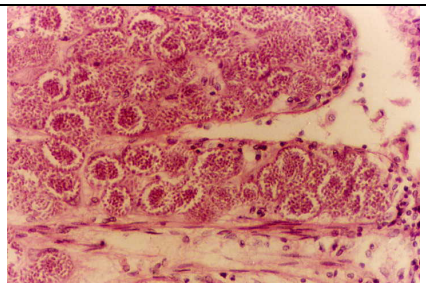
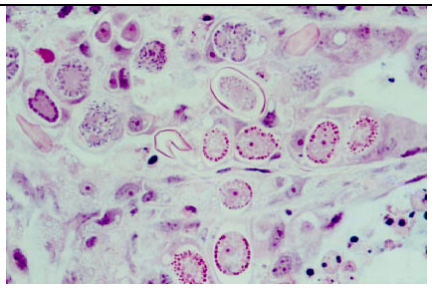
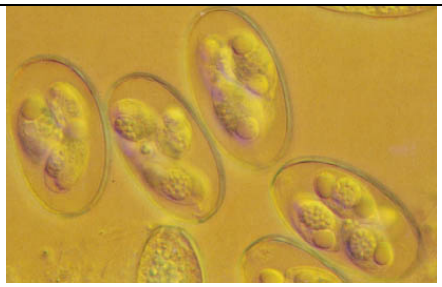
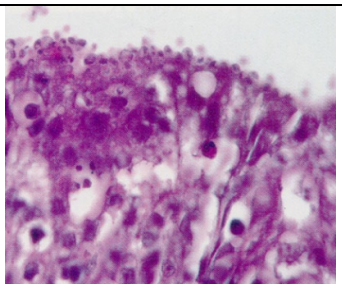
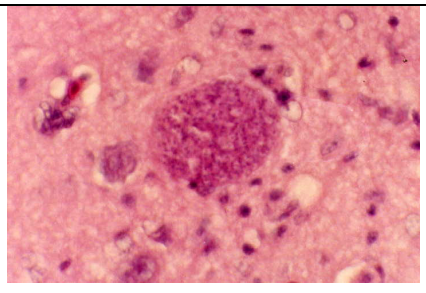
Overview:

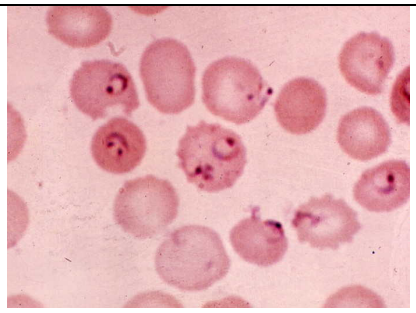
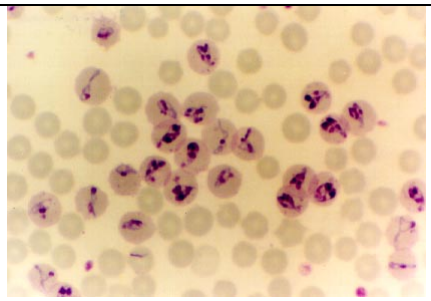
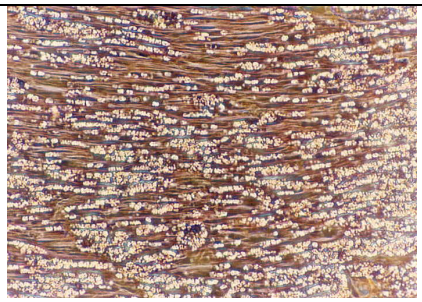
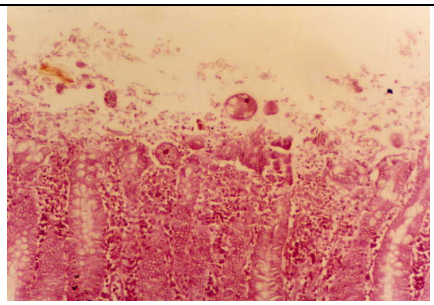
Parasite group	Main distinguishing features	Site of infection	Transmission
entamoebae	amoeboid trophozoites	enteric	faecal-oral
trichomonads	prominent undulating membrane	enteric	venereal
diplomonads	bilateral symmetry, 2 nuclei	enteric	faecal-oral
kinetoplastids	kinetoplast, undulating membrane	blood/tissues	vector-borne
enteric coccidia	oocysts, sporocysts, sporozoites	intestines	faecal-oral
cyst-forming coccidia	dormant tissue cysts	muscle, brain	predator-prey
haemosporidia	small intraerythrocytic organisms	blood cells	vector-borne
piroplasms	pear-shaped zoites	blood cells	vector-borne
microspora	unicellular spores, polar tubes	muscles, gut	direct ?
ciliates	macro- and micro-nuclei, cilia	gut, skin, gills	faecal-oral

DRY PRACT: BENCH SETS

Set up your own compound microscope and look at each sample provided in your bench set [make sure your microscope is set up for your eyes (binocular vision, both eyepieces focussed)]. Draw a sketch of what you see (neither what the text book shows nor what you imagine to be present). [WYSIWYG = what you see is what you get]. Annotate your drawing.

P1. ***Entamoeba histolytica*** trophozoites hamster liver stained section	Scan slide by eye noting pale staining lesions within tissues; examine edge of lesion at 40x to find trophozoites (large cells with vesicular nuclei) eating into normal tissue, many are located in white shrinkage artefacts.	
P2. ***Tritrichomonas foetus*** trophozoites cow uterine culture stained smear	Examine organisms noting robust shape due to culture, anterior flagella, recurrent flagellum with prominent undulating membrane and longitudinal axostyle protruding from posterior end.	
P3. ***Giardia duodenalis*** trophozoites mouse intestinal culture stained smear	Examine pink trophozoites noting pear-shaped body, bilateral symmetry, lateral and caudal flagella, longitudinal ventral groove and two prominent nuclei (giving characteristic appearance of organism staring back at you)	
P4. ***Giardia duodenalis*** cysts calf faeces stained smear	Locate small oval cysts using 40x objective (negatively stained = background stained dark, cysts pale). These cysts are very resistant to environmental conditions and survive most methods of sewage and water treatment.	
P5. ***Trypanosoma brucei*** trypomastigotes cow blood stained smear	Find thin trypomastigotes located between red blood cells, note wavy appearance, long undulating membrane, small posterior kinetoplast, central nucleus	
P6. ***Trypanosoma cruzi*** amastigotes human skin/liver/spleen stained section	Examine colonies (called pseudocysts) of amastigotes (small nucleated cells) within host cells; these non-flagellated cells divide and destroy their host cells causing necrotic tissue lesions and severe inflammation.	

P7. ***Leishmania tropica*** amastigotes hamster spleen stained impression smear	Smear contains thousands of tiny amastigotes with small purple nuclei ringed by cytoplasm, note swollen host spleen cells packed with developing amastigotes. Compare host and parasite nuclei sizes (both eukaryotes)..	
P8. ***Eimeria* sp.** schizonts kangaroo intestine stained section	Locate masses of developing schizonts (asexual stages) in mucosal layer (evident as multinucleated aggregates similar to bunches of grapes). Excessive parasite proliferation causes severe disease.	
P9. ***Eimeria* sp.** gamonts and oocysts sheep intestines stained section	Note basophilic gamonts (sexual stages, most macrogamonts) in submucosa. Lower condenser to see membranes around developing oocysts (some with prominent eosinophilic wall-forming bodies).	
P10. ***Eimeria tenella*** oocysts chicken faeces wet mount	Examine fully sporulated oocysts of chicken coccidia noting characteristic eimeriid 1:4:2 configuration; ie. one oocyst containing four sporocysts, each sporocyst with 2 sporozoites.	
P11. ***Cryptosporidium muris*** endogenous stages mouse gut stained section	Using 40x objective, examine brush border (luminal surface) of gut epithelium looking for small basophilic bodies which are parasite meronts and gamonts adherent to surface of host cells.	
P12. ***Toxoplasma gondii*** cysts mouse brain stained section	Scan brain tissue using 10-20x objective looking for small parasitic cyst located in white matter (evident as collection of numerous small blue bradyzoite nuclei) bound by thin membranous cyst wall.	

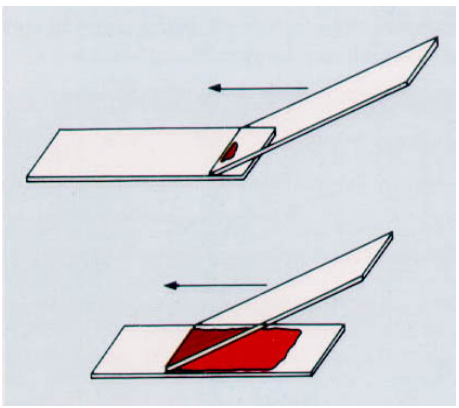
P13. ***Plasmodium falciparum*** ring stages & schizonts human blood stained smear	Look for any intraerythrocytic basophilic bodies and note characteristic 'signet-ring' stages (immature trophozoites) and multinucleate polymorphic stages (schizonts) with brown haemozoin pigment granules.	
P14. ***Babesia bigemina*** merozoites cow blood stained smear	Scan erythrocytes for small basophilic piroplasms (1-4 per cell), note size, shape, colour and juxtaposition of merozoites; look for twin bodies (bigeminate bodies!) – this species forms large merozoites.	
P15. ***Thelohania* sp.** microspores yabby muscle stained section	With condenser wound down for contrast, examine muscles noting presence of numerous refractile microspores causing condition known as cotton-tail (ignore conspicuous trematode metacercariae bounded by thick walls);	
P16. ***Balantidium coli*** trophozoites pig/human colon stained section	Examine large pink ciliate with conspicuous blue macronucleus located near the mucosal surface; note the severe erosion of the colonic mucosa, profuse lymphocytic infiltrates and blood released from numerous haemorrhages.	

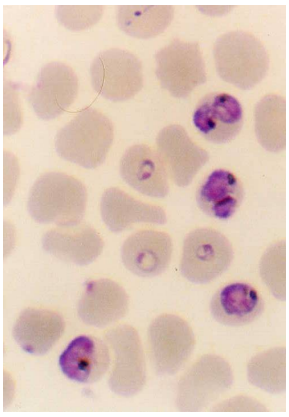
WET PRACT: DIAGNOSTIC SAMPLES

Your tutors have set up three work stations: one for blood, one for faeces, and one for tissues. Attend each station and conduct the following three activities.

Thin blood smear

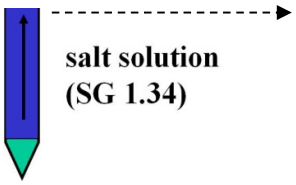
- Place a small drop of blood (in anticoagulant) at one end of a clean glass slide.
- Use another slide to make a thin blood smear as shown in the diagram.

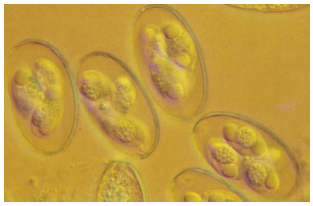




Centrifugal floatation

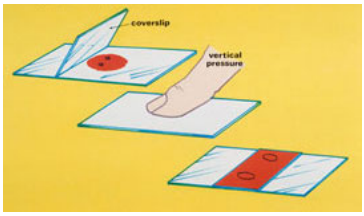
- Add one drop of mixed faecal suspension to a 10 ml centrifuge tube.
- Fill the tube to 10 ml with saturated magnesium sulphate solution (specific gravity 1.34)
- Centrifuge for 2 minutes at 2,000 rpm
- Remove meniscus from tube with bacteriological loop, place on slide and coverslip
- Examine under a light microscope (with condenser wound down) looking for oocysts

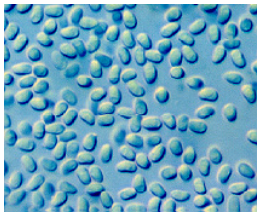




Tissue squash preparation

- Cut a small segment from the crayfish tissue (size of pinhead)
- Place in drop of water on glass slide and chop into small pieces
- Coverslip and carefully squash preparation with thumb
- Examine under light microscope (with condenser wound down) looking for refractile microspores





SPOT QUIZ

Examine the specimens and answer the attached questions
(Allow 3 minutes per specimen; this is the time allowed in the exam)

Question 1.

Blood smear from cow with neurological signs.
(a) Name the parasite genus (4 marks) — *Trypanosoma*.....
(b) Name the developmental stages present? (3 marks) — trypomastigote.....
(c) How are infections transmitted between hosts? (3 marks) — tsetse fly vector.....

Question 2.

Faecal smear from diarrhoeic cat.
(a) Name the parasite genus present (3 marks) — *Giardia*.....
(b) What host tissues are infected (4 marks) — small intestinal mucosa.....
(c) Are the parasites infectious to man (3 marks) — some genotypes are!.....

Question 3.

Faecal smear from diarrhoeic chicken.
(a) Name the parasite genus present (3 marks) — *Eimeria*.....
(b) Name the developmental stages present (4 marks) — oocysts/sporocysts.....
(c) What is the disease commonly called (3 marks) — .coccidiosis.....

Question 4.

Blood smear from human patient with high fever.
(a) Name the parasite genus present (4 marks) — *Plasmodium*.....
(b) How are infections transmitted between hosts (3 marks) — mosquito vector.....
(c) Is this parasite endemic in Australia (3 marks) — no.....

Question 5.

Histological section of horse muscle.
(a) Name the parasite genus (3 marks) — *Sarcocystis*.....
(b) Are these developmental stages infectious to other horses (3 marks) — no.....
(c) How are infections transmitted between hosts (4 marks) — via dogs (predator-prey cycle).....

WEEK 9: LEVEL 3 TEACHING-FREE WEEK

NOTE: Assignment is due by 4pm on Friday 23/9/2016

WEEK 10: TUTORIAL

WORMY QUIZ

(1). Name ten different types of worms!

Worm-like invertebrate phyla
- earthworms (annelids)
- bristleworms (polychaetes)
- bootlace worms (nemerteans)
- arrow worms (chaetognathans)
- phallus worms (priapulids)
- jaw worms (gnathostomulids)
- acorn worms (hemichrodates)
- velvet worms (onychophorids)
- horseshoe worms (phoronids)
- peanut worms (sipunculids)
- **roundworms (nematodes)**
- **flatworms (platyhelminths) incl. tapeworms (cestodes) & flukes (trematodes)**

Common name "worm' also given to large range of insects:
railroad worms, wood worms, glow worms, blood worms, inch worms,
canker worms, meal worms, silk worms, woolly bear worms

(2). List some defining characteristics of platyhelminths!

triploblastic (3 body layers)	consequences
ecto/meso/endo-derm	flat body, small size (for osmosis)
acoelomate (no body cavity)	3D musculature ('squirmers')
metamorphic development	egg-larva-adult

(3). List some defining characteristics of nematodes!

triploblastic (3 body layers)	consequences
ecto/meso/endo-derm	tubular body (hydrostatic skeleton)
pseudocoelomate (body cavity)	longitudinal musculature ('thrashers')
metamorphic development	egg-larva-adult

(4). List some attributes of worm eggs!

numerous (not all survive), environmentally resistant, broadcast in environment
contain developing stages (embryos, larvae), limited food reserves, may get caught in tissues

(5). List some attributes of worm larvae!

variable habitats (free-living/parasitic), immature stages (moult, encysted), some feeding,
infective/transmissive stages, cause pathology (penetration, migration, lodgement, encystment)

(6). List some attributes of adult worms!

endoparasites (shelter, food, transport), feeding (passive, active, voracious),
mature stages (fertility, fecundity), (asexual parthenogenesis, sexaul hermaphrodites/sexes),
cause pathology (host nutrients/cells/tissues, trauma, lesions, inflammation, ...)

(7). Name four nematode groups?

Pin worms (oxyurids)
Whip worms (trichocephalids)
Round worms (ascarids)
Thread worms (rhabditids)
Hook worms (strongylids)
Filarial worms (spirurid)

(8). Name two cestode groups!

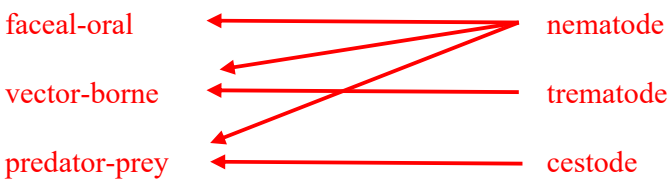
Cyclophyllidea (terrestrial)
Pseudophyllidea (aquatic)
Trypanorhyncha
Diphyllidea
Tetraphyllidea
Caryophyllidea
Litobothridea
Spatheobothridea
Nippotaenidea
Aporidea
Proteocephalta

(9). Name two trematode groups!

Monogenea (one-host)
- mono-opisthocotylids
  - gyrodactylids, dactylogyrids, monocotylids
- poly-opisthocotylids
  - polystomatids, diclidophorids, hexastomatids

Digenea (two-hosts)
- echinostomes
- paramphistomes
- opisthorchids
- plagiorchids
- strigeatids

(10). Identify the three main modes of transmission!



WEEK 11: PRACTICAL

PARASITIC NEMATODES

This practical involves 4 activities which you should complete over the next three hours:

- dry pract (examine the bench set of parasite samples provided)
- demonstration material (examine additional samples set up on side benches)
- wet pract (perform your own parasite diagnostic test with samples provided)
- spot-quiz (formative assessment item similar in structure and content to final practical exam)

Today's practical is focussing on one group of helminth parasites – the nematodes, worms which infect humans and other animals. Many nematodes infect the gastro-intestinal tract while others may invade tissues and organs causing a variety of disease conditions. Nematode infections are cumulative (intensity based on transmission success – 'eat one egg, get one worm') rather than multiplicative (worms do not amplify numbers in tissues, nonetheless, they reproduce and shed offspring). Heavy infections are associated with chronic disease syndromes that worsen with continued exposure.

The nematodes are commonly known as the round-worms due to their body shape, as distinct from the platyhelminths (or flat-worms). Nematodes 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 ('tubes under pressure'). Worms use longitudinal muscles to produce a sideways thrashing motion. Adult worms form separate sexes with well-developed reproductive systems. All nematodes form three different life-cycle stages: eggs, larvae and adults. Adult worms infect definitive hosts (in which sexual development occurs) whereas larval stages may be free-living or parasitize definitive, intermediate or paratenic hosts.

Many nematode assemblages are recognized; six in particular having representatives of major significance to human health:

Oxyurida (pin-worms)	Trichocephalida (whip-worms)	Ascarida (round-worms)	Rhabditida (thread-worms)	Strongylida (hook-worms)	Spirurida (filarial worms)
					

Objectives:

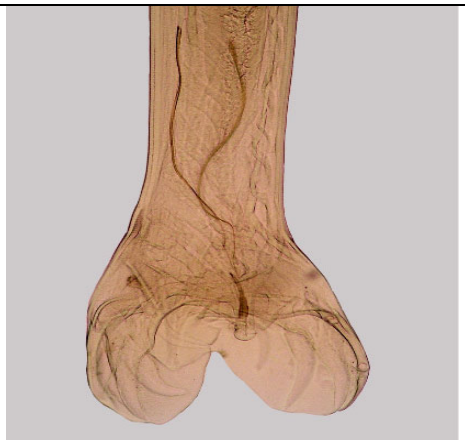
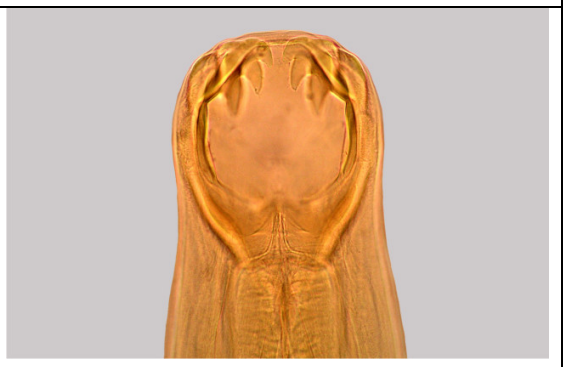
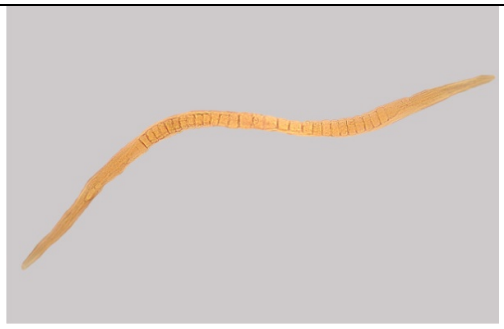
- To review the key morphological features of parasitic nematodes
- To examine different stages in the developmental cycles of the parasites
- To illustrate the pathological consequences of infections within host tissues
- To provide an understanding of the modes of transmission of the parasites

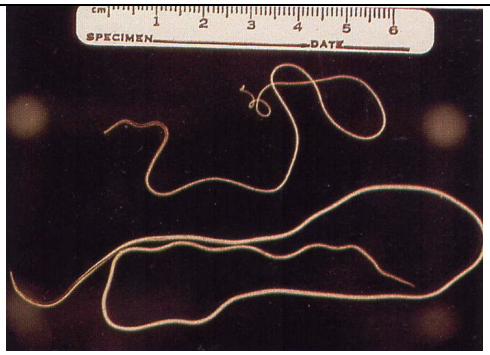
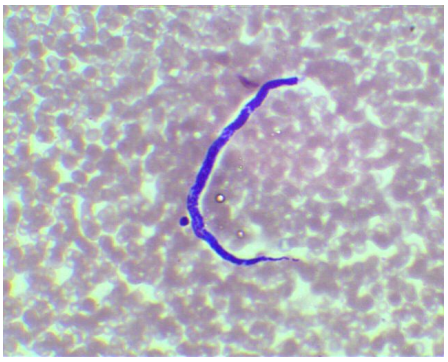
Overview:

Parasite group	Main distinguishing features	Site of infection	Transmission
Oxyurida	pin body, asymmetrical peri-anal eggs	colon (eggs glued)	faecal-oral
Trichocephalida	whip body, bipolar eggs	colon (eggs shed)	faecal-oral
Ascarida	large round body, larval tracheal migration	intestines (eggs shed)	faecal-oral
Rhabditida	thread body, larval tracheal migration, parthenogenesis, free-living stages	intestines (larvae shed)	faecal-percutaneous
Strongylida	male with bursa	intestines (eggs shed)	faecal-percutaneous
Spirurida	filarial body, microfilarial larvae	tissues	predator-prey or vector-borne

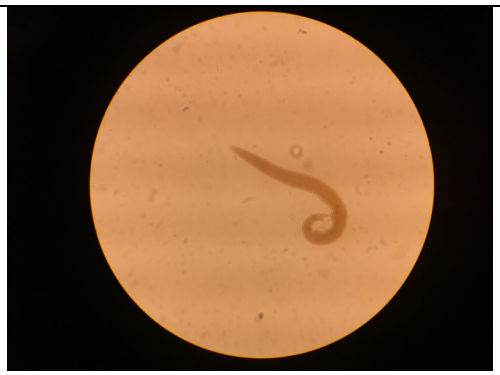
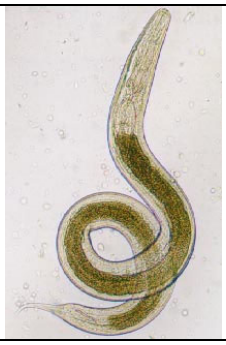
DRY PRACT: BENCH SETS

Take a slide from the bench set and take it to your microscope. Find the parasite, try to recognise the key features mentioned in the notes below, and make a sketch with labels. The act of making a sketch will help you remember what you saw much better than just staring at it.

Order Strongylida (the bursate nematodes or "strongyles")		
N1. adult strongyle male worm posterior end to show bursa (not a species to learn) ex: intestines slide mount	All strongyles have a bursa (although it may be highly reduced in the Metastrongyloidea). Look for a pair of spicules (sometimes protracted [=stuck out], sometimes retracted) and the bursa with rays in it. What is it for?	
N2. ***Ancylostoma caninum*** (dog hookworm) adult worm (head) ex: intestines, dog slide mount	Dog hookworm buccal capsule. Note strongly sclerotised buccal capsule and teeth. These nematodes feed on blood and cause significant anaemia. This makes sense in terms of the impressive buccal capsule and teeth. Another species of *Ancylostoma* is an important parasite of humans	
N6. **Strongylids** filariform larvae (L3) faecal sample human slide mount	This slide has a lot of third stage larvae which are "ensheathed, non-feeding and infective". Where they occur and what they do depends on what superfamily they are from. We are looking at them here to see how big they are and how difficult it is to identify them to species. If you look carefully, on some specimens you may see evidence of the loose cuticle of the 2nd stage larva still retained. If you can find a worm that is broken in the middle then this is more obvious.	

Order Spirurida (including the filarial worms)		
N8. ***Dirofilaria immitis*** dog heartworm anterior end, adult female ex: dog heart slide	Note the lack of a buccal capsule or any really distinctive features. If not for the site in the host (dog heart), this nematode would be quite difficult to identify.	
N9. ***Dirofilaria immitis*** microfilariae dog blood thin smear of peripheral blood slide, Giemsa stain	You may need to search quite a while to find the microfilariae in this slide – on some there are not many. Once you find them, however, they are unmistakeable. This is the stage that is taken up by the mosquito intermediate host. What happens in the mosquito? Some microfilariae occur in the lymph rather than the blood.	
Order Ascaridida (the round-worms)		
N10. ***Toxocara canis*** dog roundworm ex: intestine of puppy anterior end, adult slide mount	This shows the three large lips that are characteristic of the Order Ascaridida in general. Specific characteristics of this species are the broad cervical (neck) alae (flaps) that you can see. These lead to the common name of "arrow worms" sometimes given to this species. Another feature of this species is the fact that there is a distinct ventriculus or glandular area at the back end of the oesophagus.	

Order Rhabditida (the thread-worms)		
N11. ***Rhabdias bufonis*** (toad lung-worm) parthenogenetic female ex: lungs, cane toad slide mount	This parasite is quite large. Look for the simple, straight oesophagus and the reproductive system full of embryonating eggs. The intestine often appears as a dark streak down the side of the worm (dark because it is full of blood). The eggs pass up the respiratory tree and then out with the faeces. They hatch en route so 1st stage larvae appear in faeces. When they reach the ground they develop into free-living males and females (shown on next slide).	
N12. ***Rhabdias bufonis*** (toad lung-worm) free-living adults (left, male) (middle, mature female) (right, old female) slide mount	The adult male worm is probably nearly black but it is completely different from the parasitic female. Note the small irregular area close to the posterior end of the worm where the spicules protrude. The mature female worm is completely different from the parasitic female and just contains some winding gonads with little else evident. The old female worm has finished reproducing, her offspring have embryonated inside her, hatched and have grown at her expense so that she is now just a shell containing perhaps 3-5 large larvae. These larvae will be infective for the toad by penetration of the skin.	
N13. ***Strongyloides*** threadworm parasitic female ex: sheep intestines slide mount	These are some of the smallest and most featureless nematodes there are. The parasites are all females and they are characterised by the very long straight oesophagus, the winding gonads, and, I suppose, the absence of males. They do not make good slides.	
N14 ***Pneumonema teliquae*** lizard lung-worm ex: blue-tongue lizard slide mount	This specimen is a rhabditid from the lungs of the blue-tongue lizard and it is common here in Brisbane. The biology of this species is comparable to that of *Rhabdias*. The morphology is very striking, however. The cuticle in the anterior body is beset with enormous thorn-like spines.	

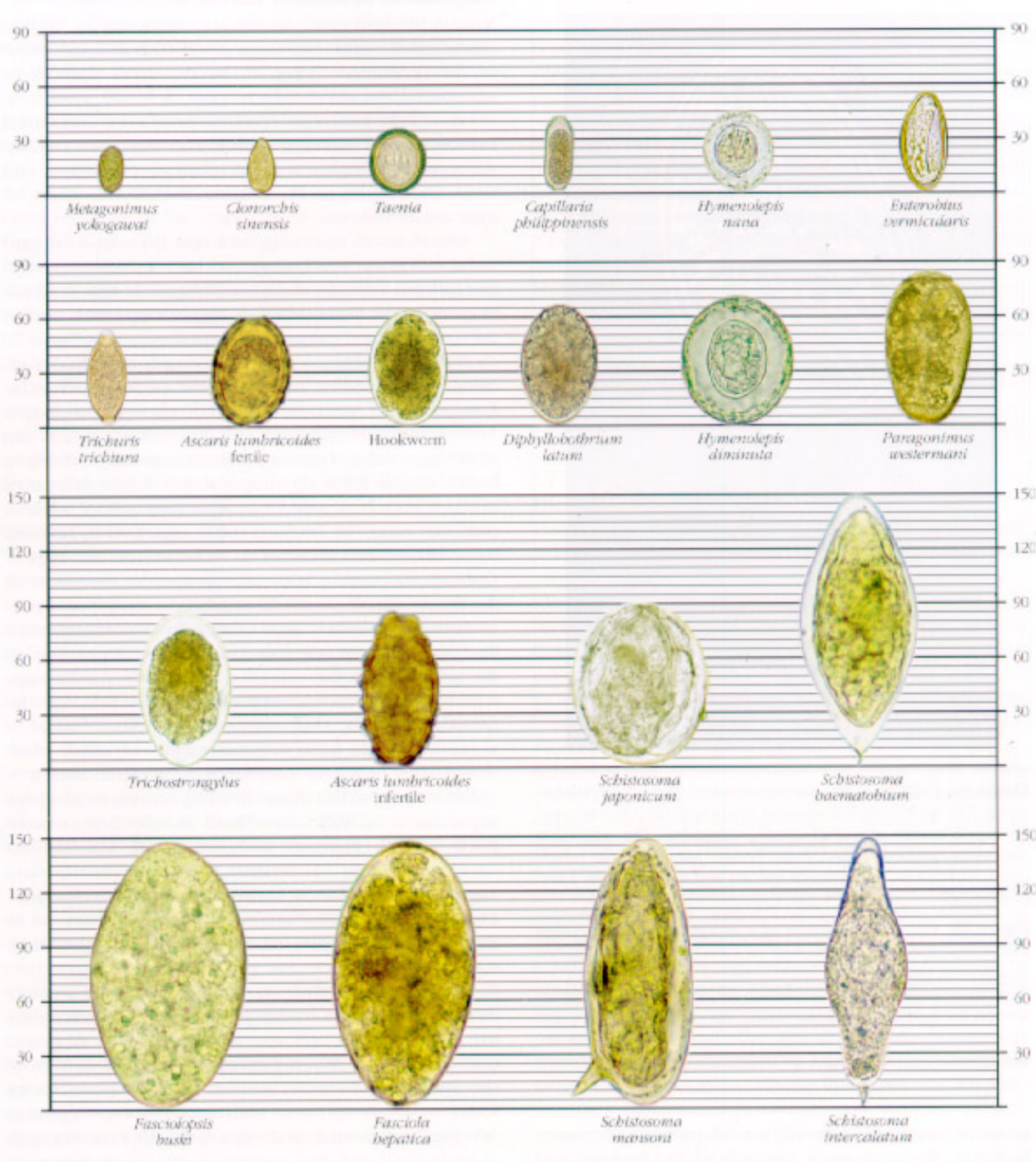
Order Oxyurida (the pin-worms)		
N15. ***Syphacia* sp.** **(**pin-worm) adult male native bush rat, lower intestine slide mount	This is another quite small nematode and features are difficult to see. It has a very long pointed tail – thus, a pin-worm. The oesophagus has a characteristic bulb posteriorly that is used for grinding its food. Remember that these tend to live in the lower gut and feed on gut contents. Finally, you can probably justly make out a spicule demonstrating that this is, indeed, a male.	
N16 ***Syphacia* sp.** **(**pin-worm) adult female native bush rat, lower intestine slide mount	Female pinworms resemble the males in the pointy tail and oesophageal bulb but here you can make out developing eggs (in most specimens) and there is no spicule.	
Order Trichocephalida (the whip-worms)		
N17. ***Trichuris* sp.** (whip-worm) adult males and females ex: large intestine, dog slide mount	You can tell the sexes apart by the presence of eggs in the female. The uterus opens at the vulva close to the junction between pharynx and intestine (more-or-less where the worm gets thick). Both sexes have an elongate stichosome pharynx (very long and narrow anterior end of the worm). The gut is a very narrow tube surrounded by quite large gland cells (stichocytes) which give a sort of "concertina" appearance. Males have a single terminal spicule and eversible spiny spicule sheath.	

SIDE-BENCH DEMONSTRATIONS

On one of the side-benches you will find a variety of nematode specimens that you should look at. Some of them illustrate parasites of medical importance. Some demonstrate parasites of veterinary or wildlife importance that we have not emphasized in lectures but are included to illustrate the richness of parasitic nematodes.

WET PRACT: DIAGNOSTIC SAMPLES

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.

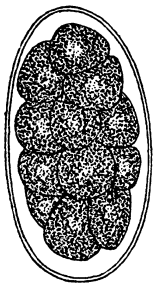


Nematode egg counting – the floatation technique

Purpose

Estimation of the size of the infection (number of worms) is an important diagnostic tool for determining the ecology of infections of parasitic worms and, directly, of when humans or animals need to be treated. In today's prac we will determine "faecal epgs" for samples of faeces from sheep.

Direct examination

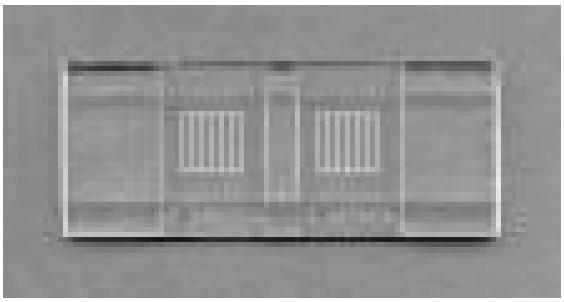


Wear gloves. You are provided with (fairly) fresh faecal material from sheep infected with strongyle nematodes. Take a smear of the faeces and mount it with a drop of water under a cover-slip and examine for the presence of helminth eggs. You may well find cestode or trematode eggs but the dominant eggs are likely to be those of nematodes. Sketch and attempt to identify any eggs that you find. The goal here is just find an egg or two and to distinguish it from all the other material present in the faeces. The drawing opposite is of a typical strongyle egg.

Determining faecal eggs per gram (epg)

The basis of the technique is to take a known mass of faeces and to use a flotation technique to concentrate the eggs and allow them to be counted accurately. Our method is as follows:

Method. Weigh 2 g of faeces and add 60 ml of saturated Magnesium sulphate solution. Use a bottle and stirring beads to form an even slurry. You may need to strain out the larger particulate matter with the plastic strainers provided. While keeping the slurry evenly mixed, pipette some of the slurry into the chamber (chamber volume = 0.15 ml) of the Whitlock counting slide. Count the number of eggs which float to the surface beneath the etched area on the slide.



Calculation of epg. 1 chamber-full is 0.5 ml of an initial volume of (about) 60 ml which contained 2 g of faeces. Therefore *epg* = count x 60. [Work it out on a piece of paper to make sure you understand it!]

i.e. multiply the count by 60 to get eggs per gram.

To increase the accuracy of the calculation, increase the number of counts made, and average the results. The extent to which the slurry is well mixed will determine how accurate and similar your counts are.

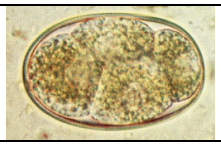
There is an extensive literature on the topic of maximising the efficiency of these counts.

Interpretation

- the more worms, the more eggs per gram can be expected
- but, fecundity per worm tends to reduce as total infection increases
- and, infection may be in prepatent period
- 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.

SPOT QUIZ

1. Found in the intestine of a dog in Brisbane.	
(a) Identify to Order and Genus (4 marks).	Strongylida, *Ancylostoma*
(b) What is the main way that the dog may be harmed by this parasite (3 marks)?	Blood-sucking/anaemia
(c) Sketch the egg that would pass in the faeces of the dog (3 marks).	
2. Blood smear from a dog from Townsville. Scan the slide and find the parasite or parasites present	
(a) Identify all parasites found to Order and genus (total 4 marks for each parasite species).	Spirurida: *Dirofilaria immitis* – no other parasites present – but they could have been. The point is that mixed infections are common
(b) What is the name of the life cycle stage or stages seen here (2 marks).	microfilaria
(c) Are there any other hosts in the life cycle(s)? If so name them (2 marks).	Yes, mosquito
(d) Name any parasites of humans comparable to this species. (2 marks).	*Wuchereria*, *Brugia* – but they are not actually in the heart.
3. This collection of worms was taken from the small intestine of a puppy that died in Brisbane	
(a) Identify the parasite to Order and genus (4 marks).	*Toxocara canis*
(b) Is this an adult or a larval worm (2 marks)?	Adult.
(c) How is the puppy likely to have become infected (2 marks).	Transplacental migration.
(d) What related parasite species normally infects humans (2 marks)?	*Ascaris lumbricoides*
4. These worms were expelled from the gut of an African child following drug treatment.	
(a) Identify to order and genus (4 marks)	Trichocephalida, *Trichuris*
(b) How would the child have become infected? (3 marks).	Faecal/oral; ingesting eggs from contaminated food or water
(c) Sketch what the egg of this parasite would look like (3 marks).	
5. Faecal smear from a human freshly arrived in Brisbane following an overseas holiday	
(a) What human parasite is indicated by the presence of this egg (4 marks).	Hookworm – *Ancylostoma* or *Necator*
(b) Does this egg hatch in the open or does it need to be eaten by a definitive host?	Hatches and develops to infective L3.
(c) What, if any, is the role of intermediate hosts in the life cycle of this parasite?	Usually no intermediate host. Rarely paratenic hosts.

19 Random questions. If you can answer these then you are well on-track.

1. In general, how do you known an adult ascaridid nematode when you seen one?
   Big white worm from intestines

2. In general, how do you known an adult strongyle nematode when you see one?
   Smaller white worm from gut, males with bursa

3. Outline the principles of doing a faecal epg.
   Quantitate eggs in faeces, weigh aliquot, put in known volume of flotation medium, mix, put aliquot in chamber, count eggs, calculate epg.

4. What is a tracheal migration?
   Larvae move from blood to air spaces in lungs, ascend bronchial tree to trachea, swallowed, down to gut.

5. Name two different nematodes that have one.
   Ascaris, Ancylostoma

6. Name three nematodes that humans are infected by that are related to poor hygiene.
   Ascaris, Trichuris, Enterobius

7. Name two nematodes that infect humans that are transmitted through other animals.
   Loa, Onchocerca, Wuchereria, Angiostrongylus

8. What is so unusual about the life cycle of *Trichinella spiralis*.
   Completes life-cycle in one host which acts first as definitive host and then as intermediate host

9. Why is human pinworm relatively common in Australia?
   As elsewhere, poor hygiene of children – just having good sewerage does not control it.

10. Why are hookworm and *Ascaris* relatively rare in Australia?
    Sanitation, sewage treatment, wear shoes

11. Why is *Wuchereria bancrofti* essentially absent from Australia?
    Mosquito control

12. What are two special features of the life cycle of *Strongyloides*?
    Auto-infection, parthenogenesis and free-living stages

13. How could you tell that the free-living female of a *Rhabdias* was the same species as the parasitic female from the lungs of a toad?
    molecular biology (e.g. matching DNA sequences) or experimental infections

14. What does faecal epg stand for?
    Eggs per gram

15. What is the point of doing a faecal epg on a bunch of sheep?
    Determine level of infection in flock overall (overdispersion means that some hosts have most parasites so we cannot just look at one animal)

16. What are some limitations of the faecal epg?
    Poor species recognition, assume fecundity constant (it is not), time

17. What is a microfilaria?
    Fully embryonated L1 of filarioids

18. Do all nematodes moult?
    Yes, most moult 4 times

19. Write an 8-page essay in which you contrast the epidemiology of human hookworm, human filariasis, enterobiasis and ascariasis drawing attention to issues of human behaviour and the interplay of the immune system and submit it by 5 p.m. today OR nominate your favourite colour.
    BLUE!

WEEK 12: TUTORIAL

DOOF Quiz

(1). Name five different methods of cooking food!

dry-heat (with/without fat)
- saute/frying
- roasting/baking
- grilling/broiling

moist heat
- poach/steam
- boiling/microwaving
- braising/stewing

(2). Name five different methods of food preservation!

chemical (salting/brining, pickling/seasoning, marinating/fermenting, sugaring (jams/syrup)
storage (canning, bottling, jugging, potting,
temperature (refrigerating, freezing,
dehydration (drying, smoking, vacuum-packing,
combination (freeze-drying,

(3). Where do apples come from?

trees/farms/shops
seasonal/cold storage

(4). How do they get to your dinner table?

farm – harvester - processor - warehouse - wholesaler - market – retailer - consumer

(5). Can they transmit parasites to you?

Yes. Not in fruit, but on fruit from contaminated water used for washing.

(6). Where does steak come from?

Cow/farm/butcher
not seasonal, but cold storage

(7). How does it get to your dinner table?

farm – saleyard – abattoir – processor - wholesaler - market - retailer - consumer

(8). Can it transmit parasites to you?

Yes. Parasites in meat (e.g. *Toxoplasma*, hydatids, *Trichinella*, etc.

(9). Who checks produce?

farmer/harvester
processor/warehouse
wholesaler/retailer
consumer (you)
inspectors (food/meat/produce)
industry/government (local, state, national, exporter)

(10). Should it be legislated?

Already is through food hygiene legislation
(covering production, processing, storage, preparation, cooking, sale)
Notifiable diseases schedules

WEEK 13: PRACTICAL

PARASITIC FLATWORMS

This practical involves 4 activities which you should complete over the next three hours:

- dry pract (examine the bench set of parasite samples provided)
- demonstration material (examine additional samples set up on side benches)
- wet pract (perform your own parasite diagnostic test with samples provided)
- spot-quiz (formative assessment item similar in structure and content to final practical exam)

Today's practical is focussing on the platyhelminths (flatworms), comprising the cestodes (tape-worms) and trematodes (flukes). Many flatworms species are parasitic in animals in their adult and/or larval stages. Infections may cause a range of diseases, typically slow in onset and chronic in nature.

Cestodes have long flat ribbon-like bodies with a single anterior holdfast organ (scolex) and numerous segments. They do not have a gut and all nutrients are taken up through the tegument. All tapeworms are hermaphroditic and each segment contains both male and female organs. Cestode eggs released from gravid segments embryonate to produce 6-hooked embryos (hexacanth oncospheres) which are ingested by intermediate hosts. The oncospheres penetrate host tissues and become metacestodes (encysted larvae). When eaten by definitive hosts, they excyst and form adult tapeworms. Infections by adult cestodes are generally benign as they are not invasive, but the larval stages penetrate and encyst within tissues leading to inflammation, space-occupying lesions and organ malfunction.

Trematodes have small flat leaf-like bodies with oral and ventral suckers and a blind sac-like gut. Most species are hermaphroditic (individuals with male and female reproductive systems) although some blood flukes form separate male and female adults. Trematodes have 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 or in other animals which in both cases are eaten by definitive hosts. 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.

CESTODA	
Cyclophyllidea (terrestrial 2-host life-cycles)	Pseudophyllidea (aquatic 3-host life-cycles)
	

TREMATODA	
echinostomatid fasciolids (liver flukes)	strigeatid schistosomes (blood flukes)
	

Objectives:

- To review the key morphological features of parasitic flatworms
- To examine different stages in the developmental cycles of the parasites
- To illustrate the pathological consequences of infections within host tissues
- To provide an understanding of the modes of transmission of the parasites

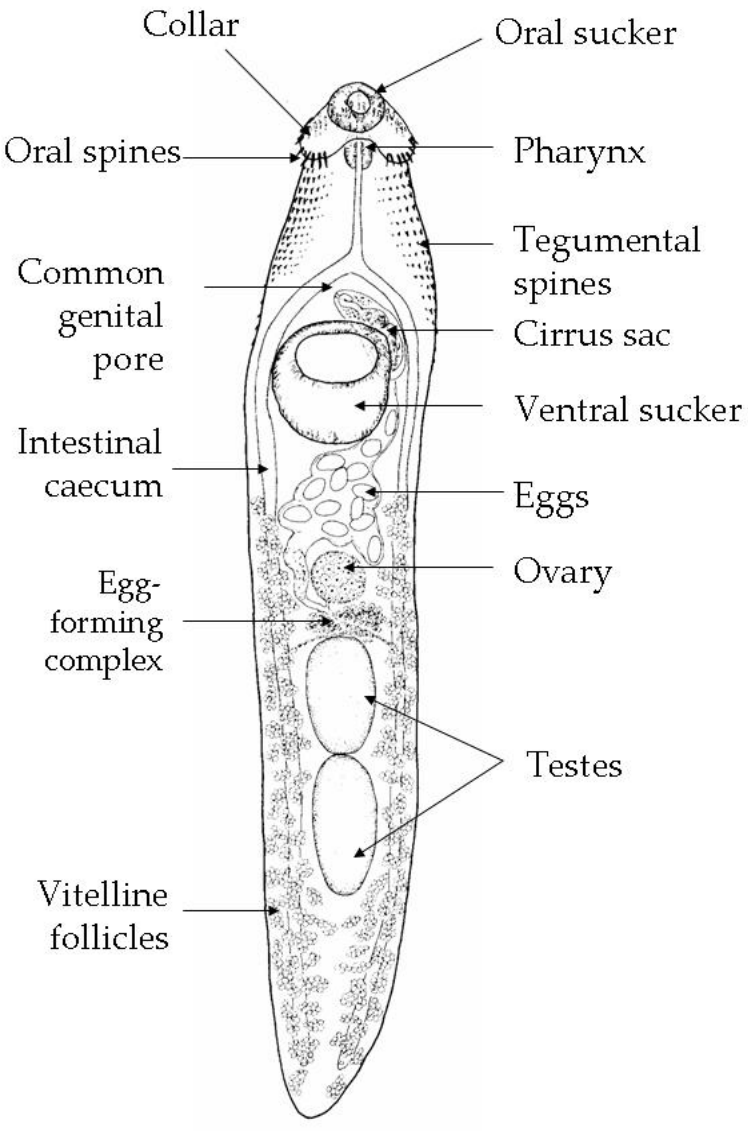
Overview:

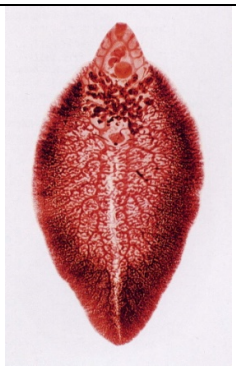
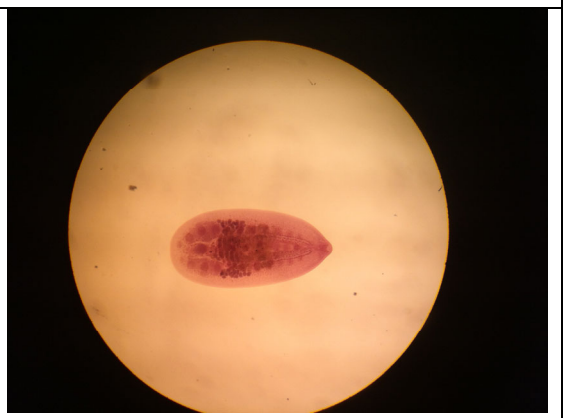
Parasite group	Main distinguishing features	Site of infection	Transmission
Cyclophyllidea	scolex with suckers, larval cysts	intestines	predator-prey
Pseudophyllidea	scolex with bothridia, cercoid larvae	intestines	predatory-prey
Fascolids	liver flukes, metacercariae	liver	snail vectors
Schistosomes	separate sexes, eggs in tissues	veins	snail vectors

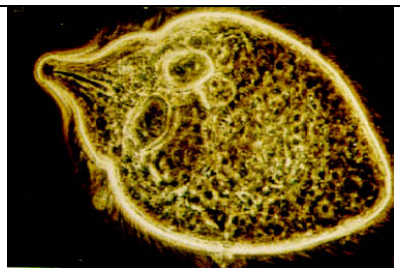
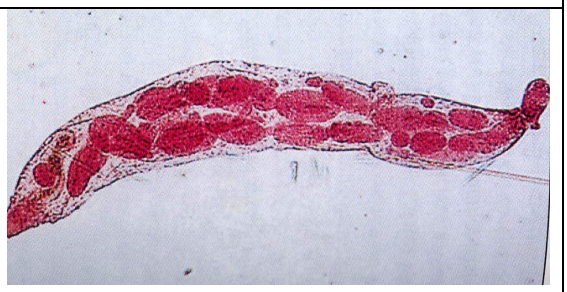
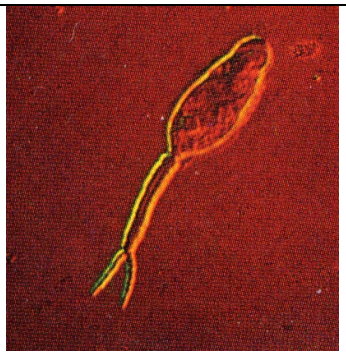
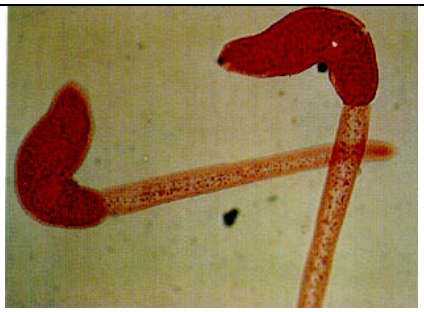
DRY PRACT: BENCH SETS

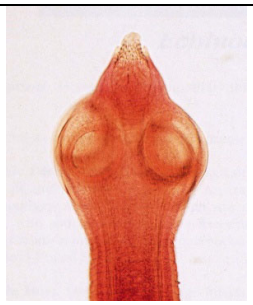
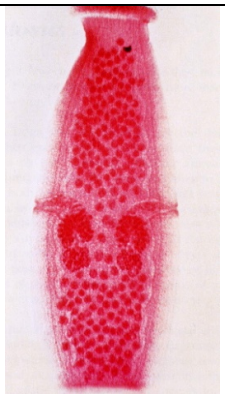
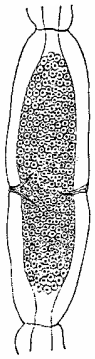
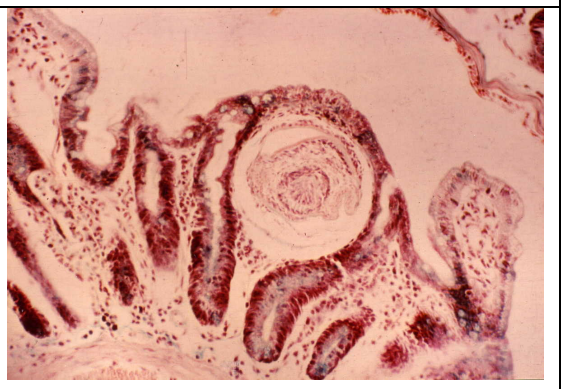
Take a slide from the bench set and take it to your microscope. Find the parasite, try to recognise the key features mentioned in the notes below, and make a sketch with labels. The act of making a sketch will help you remember what you saw much better than just staring at it.

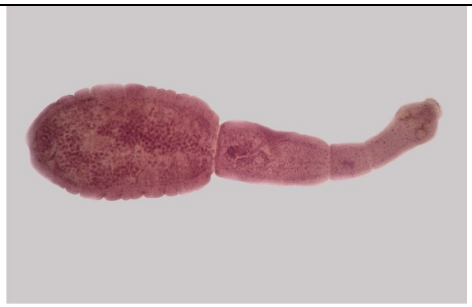
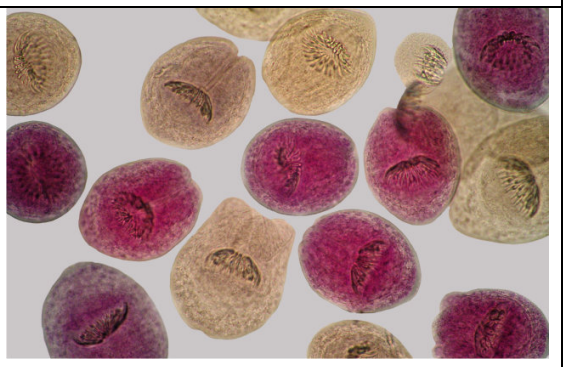
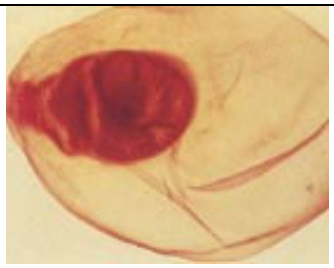
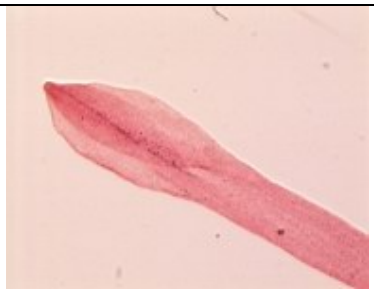
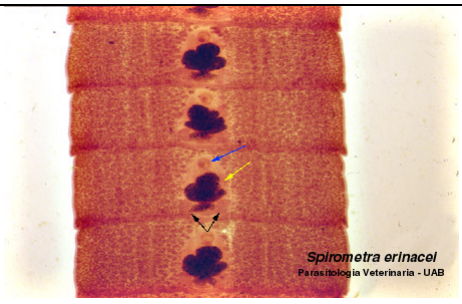
TREMATODES	
D1. ***Echinoparyphium* sp**. echinostome trematode adult fluke ex: intestine, cormorant slide mount	Echinostomatids are common parasites of birds and some mammals. They are transmitted as metacercariae in the tissues of molluscs and fishes. The adult flukes are a nice size to examine under a compound microscope and their morphology is relatively straightforward. Examine and draw the specimen using a full A4 sheet. Identify the features noted below and consider the biological function.



D2. ***Fasciola hepatica*** (liver fluke) adult worm ex: sheep liver slide mount	These flukes are large and economically important in agriculture (and occasionally in humans). Apart from its size, this digenean is typical of trematodes except that all body organs are highly branched (testes, ovary, gut & vitellarium). It undergoes asexual amplification in snails and then forms metacerceriae on vegetation.	
D3. ***Schistosoma* sp.** (blood fluke) adult worms ex: mesenteric veins, mouse slide mount	These flukes live in blood vessels of birds and mammals. They differ from other trematodes in that the sexes are separate - the larger male carries the long slender female in the gynaecophoric canal. Oral and ventral suckers are visible on the male but are less easy to distinguish on the female. This genus does not occur in humans in Australia but elsewhere it is by far the most important trematode parasite of people. Schistosomes are common in Australian water birds.	
D4. ***Notocotylus* sp.** (rat fluke) adult worm ex: intestine, water rat slide mount.	This family of trematodes has very minor economic significance. We are examining the specimen with a view to exploring morphological diversity within this group. Notocotylids have no ventral sucker and have only an oral sucker or a pharynx (it is not easy to tell which it is). They also have large glands on their ventral surface. Try to relate the morphology that you see to what we saw in the echinostomatids.	

LIFE CYCLE STAGES		
D5. ***Fasciola hepatica*** (liver fluke) Miracidium stage ex: water slide mount	This is what hatches from the egg. It is usually no more than 100 µm long, ciliated, and has penetration glands to allow it to enter the molluscan intermediate host, and germinal cells which it uses to reproduce asexually. How is this important?	
D6. ***Fasciola hepatica*** (liver fluke) Redia and sporocyst stages ex: snail tissues slide mount	These are generations in the digenean life-cycle that reproduce asexually in the mollusc. Typically they are simple elongate sausage-shaped bodies filled with the developing next generation. A redia has a mouth, a pharynx and a short gut. A sporocyst has no organs whatsoever and must absorb all its food. What sort of next generation is being produced (rediae or cercariae)? How is this important?	
D7. ***Schistosoma* sp.** (blood fluke) Cercaria stage ex: water slide mount	The is the final point of asexual reproduction in the mollusc. The cercaria is the larva of the sexual adult that occurs in the vertebrate definitive host. It metamorphoses by losing its tail and turning first into a metacercaria, and then an adult. A few trematodes (e.g. schistosomes) develop to an adult directly without an intervening metacercarial stage. Schistosomes have distinctive fork-tailed cercariae. How is this important?	
D8. ***Fasciola hepatica*** (liver fluke) Cercaria stage ex: water slide mount	This cercaria has a simple (unforked) tail. This cercaria encysts on grass (forming a metacercaria) from where it is ingested by the sheep. How is this important?	

CESTODA - Cyclophyllidea		
C1. ***Dipylidium caninum*** (dog tapeworm) adult, head (scolex) ex: dog, intestines slide mount	A typical cyclophyllidean scolex with four suckers, a spiny, protrusible rostellum, and segmentation posteriorly	
C2. ***Dipylidium caninum*** (dog tapeworm) adult, mature segment and gravid segment ex: dog, intestines slide mounts	The mature segment has two sets of reproductive organs; there are genital pores on both sides of the segment. Look for testes and a cirrus-sac in the male system. In the female system look for the copulation canal (opening with the cirrus-sac), the ovary, the restricted vitellarium immediately behind the ovary, and (perhaps) a simple sac-like uterus extending anteriorly from the ovary on each side of the segment. The gravid segment is filled with paruterine organs (specialised structures found only in some cestodes) which each hold several eggs. Most of the rest of the reproductive system has been obliterated although you will probably still be able to see the genital pores on either side of the segment. See if you can see the characteristic six hooks in the oncosphere in the eggs. (Not easy!)	 
C3. ***Hymenolepis diminuta*** (dwarf tapeworm) cysticercoids stage ex: body cavity, beetle slide mount	A typical tapeworm metacestode. This is equivalent to what is found in the body cavity of the flea for *Dipylidium*. You can see the retracted scolex within a capsule (plus some host reaction tissue surrounding it). None of the segmentation has developed yet. How did this get here?	

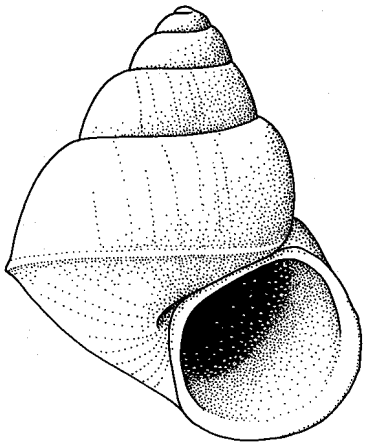
CESTODA - Cyclophyllidea		
C4. ***Echinococcus granulosus*** (hydatid tapeworm) adult ex: dog, intestines slide mount	This specialised tapeworm has all the features that you have just seen in *Dipylidium* but in just three or four segments. The larva of this tapeworm is the hydatid. This is by far the most important cestode in Australia	
C5. ***Echinococcus granulosus*** (hydatid tapeworm) hydatid sand, hydatid cyst ex: sheep, viscera slide mount	. On this slide you will find a few "grains" of hydatid sand from the wall of a hydatid cyst. Each contains 8-20 inverted protoscoleces of *Echinococcus*. When the dog eats the hydatid cyst each protoscolex will turn inside out and attach to the intestine of the dog. You can already make out the rostellar spines and suckers on the scolex.	
C6. ***Taenia ovis*** metacestode stage ex: sheep, muscle slide mount	This is a metacestode from the muscle of a sheep. The sheep becomes infected by eating AN EGG from dog faeces. The dog becomes infected by eating the sheep.	
CESTODA - Pseudophyllidea		
C7. ***Spirometra erinacei*** adult, head (scolex) ex: dog, intestines slide mount	Scolex is simple with bothria (= shallow grooves) but no true suckers.	
C8. ***Spirometra erinacei*** adult, segments ex: dog, intestines slide mount	Reproductive system (not visible on all slides): ovary bilobed towards posterior end of body; testes and vitellarium follicular throughout body of worm; uterus filled with yellow eggs. . On segments genital pores are ventral rather than marginal as in the cyclophyllideans (and thus much harder to see).	

SIDE-BENCH DEMONSTRATIONS

On one of the side-benches you will find a variety of platyhelminth specimens that you should look at. Some of them illustrate parasites of medical importance. Some demonstrate parasites of veterinary or wildlife importance that we have not emphasized in lectures but are included to illustrate the richness of parasitic flatworms.

WET PRACT: Trematode infections of *Posticobia brazieri*

We have collected some freshwater snails from tributaries of the Brisbane River. The Brisbane River and tributaries supports a diverse range of water life that, in turn, hosts a highly diverse range of parasites. This snail, ***Posticobia brazieri*** (family Hydrobiidae), is tiny, but it is known to harbour at least **17 species** of trematodes (a conservative estimate). Six species have had their life-cycles fully described - the rest are unknown or only suspected. *Posticobia brazieri* is probably the mollusc best known for its digenean parasites in Australia.

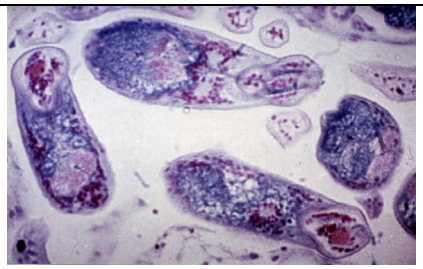


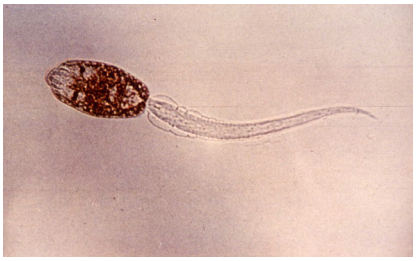
Your task is to dissect some of these snails **until you find an infection of trematode asexual stages**. We do not know how many snails you will need to dissect –it may be one – it may be many (it will vary because of the **prevalence** in the population and through chance – whether you are lucky or not). I strongly encourage you to persist until you find an infection.

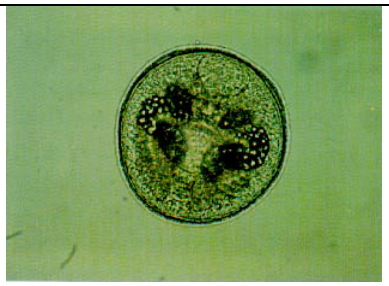
What to do.

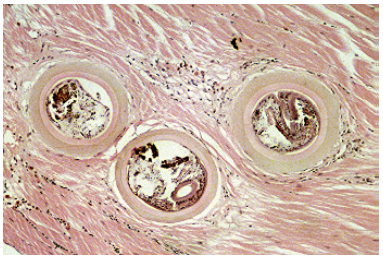
The snail is small (smaller than a match head). Take 5 of these and place them in an excavated block or small Petri dish with a little saline and then dissect them under a dissecting microscope. Try to dissect the snail carefully by working your way into the snail by breaking bits of shell off the aperture progressively. In such a small snail this is not easy! Ultimately, however you go about it, you need to get inside the snail shell and examine the soft tissues. Fortunately, usually if the snail is infected at all it will be **heavily** infected with trematodes. If it is not infected you are likely to see sperm if it is a male or developing eggs if it is a female. For the first few snails that you dissect, mount some of the snail tissue on a slide to try to understand and interpret what you are looking at.

The asexual parasite stages that you will find will be either **rediae** (with a pharynx and a gut) or **sporocysts** (without a pharynx and gut) or **cercariae** (the tailed juvenile) of trematodes. You may also come across metacercariae (encysted spheres) in (or on) some specimens. Notice how heavy the infections in the snails are. The snail is probably castrated by the infection. Can you tell where the infections are localised? Usually the digestive gland or gonad or both are affected.









SPOT QUIZ

1. Found in the liver of a sheep in New South Wales	
(a) Identify to genus and species.	*Fasciola hepatica*
(b) What does this parasite feed on as an adult?	blood
(c) What does the cercaria feed on when it emerges from the snail?	nothing
(d) What is the name of the stage that enters the snail?	miracidium
2. Found at autopsy in blood vessels of an Australian in Darwin. The unfortunate person was female, had recently travelled in the United Sates and Africa, and had died in a car crash.	
(a) Identify to genus (and family to make clear that you know what it is)	Schistosomatidae, *Schistosoma*
(b) What is the name of the life cycle stage that would have entered the person	cercaria
(c) Because this was an adult parasite (in case you were wondering) we could look for its eggs or larvae somewhere. Where would we look and what would we look for?	eggs in faeces/urine
(d) Discuss the significance of the epidemiological data that you have for the person who had this infection.	acquired in Africa
3. In a few well-chosen lines explain how to independently focus eye-pieces on a high-power microscope. establish binocular vision (move eye-pieces apart to suit individual inter-pupilary distance) focus each eye-piece (one using microscope focus controls, other using eye-piece focus ring)	
(a) How does the process change for a dissecting microscope?	same
(b) Why is this process important?	establish best image (not blurred) avoid head-aches
4. This was dissected from the tissues of a freshwater snail.	
(a) To what group of parasites does this belong? (Be as specific as possible.)	digenean trematodes, flukes
(b) What is the name of the life cycle stage?	redia or sporocyst
(c) How do you know it is that stage? (mark for previous correct answer will be lost along with 4 more marks if this is answered incorrectly).	simple sac (possibly with a pharynx and a gut if it is a redia) containing embryos of the next generation
(d) What is the biological significance of this stage in the life cycle of this group of parasites?	reproduction! The asexual generations allow a single trematode egg to lead to huge numbers of cercariae.
5. Crikey! I found this in the local creek.	
(a) What is it? Identify in terms of life cycle stage and the taxonomic group of parasites to which it belongs.	fork-tailed cercaria, Trematoda
(b) What is the forked structure called?	tail
(c) What is it used for?	swimming
(d) What is the front end used for?	penetrating vertebrate skin
(e) What should you keep away from this? Yourself, your pet dog, armadillo or duck?	duck!

6. This was found in the intestine of a dog. The dog was killed in car crash in Darwin and had just immigrated from Africa via north America.	
(a) Obviously it is a platyhelminth, but what Class?	Cestoda
(b) The morphology is so distinctive that obviously it belongs to genus	*Dipylidium*
(c) We all know that dogs get infections with this kind of thing by eating things. In this case, what things	fleas
(d) Do you think it possible that the infection rendered the dog more easily hit by a car? Why or why not?	Not especially. Adult cestodes are usually not very pathogenic.
7. This was found in the intestine of a dog, my dog, Nigel. When the vet opened him up he found THIS in the small intestine. (Pretty crowded in there with hookworm and *Toxocara* too.)	
(a) Identify to genus and species.	*Echinococcus granulosus*
(b) What did the dog eat to become infected?	hydatid cyst in an animal like a sheep or kangaroo
(c) If you had eaten the same thing would you have become infected?	no.
(d) Each year, some Australian people become infected by the larva of this parasite[1]. How do these infections occur?	ingestion of eggs from dog faeces
8. This parasite was found in the intestine of a … cat; O.K., I admit it, it was a dog. It was just down from an *Ancylostoma*, up from a *Toxocara*, and across from a *Taenia*. Or maybe it WAS a *Taenia*! What a nightmare!	
(a) Identify to genus and order if possible.	Pseudophyllidea, *Spirometra*
(b) What is distinctive about the Scolex of this species?	no suckers, no spines
(c) Outline the basic life-cycle of this parasite.	egg to copepod to tadpole/frog to dog/cat
9. I had to have my dog, Nigel, put down. This was because he had killed our pet sheep, Arthur. When we looked in Arthur we found lots of these in Arthur's liver.	
(a) Identify the scientific name of the parasite.	*Echinococcus granulosus*
(b) Identify the life-cycle stage of the parasite (you need to refer to two specific terms here).	hydatid cyst, hydatid sand, protoscoleces
(c) How did Arthur get this infection?	ate eggs from dog faeces
(d) Do you think the infection could have made Arthur more susceptible to attack by Nigel?	Maybe yes. That would have been in the "interests" of the parasite.
10. And that's not all that was in him. His (Arthur the sheep's) muscle was ABSOLUTELY riddled with THESE things.	
(a) What are they taxonomically?	Cestoda, *Taenia ovis*
(b) What are they in terms of life cycle stage?	metacestodes, specifically, cysticerci
(c) How did the sheep (Arthur) become infected?	ate eggs from dog faeces
(d) Where would these become adults? (Yes, giving the game away here, they are larvae.)	in the intestines of a dog
(e) Outline three ways in which you could work out the true identity of this parasite.	experimental infection, sequence matching, matching morphology of rostellar hooks

Random questions

1.	Does *Echinococcus granulosus* occur in Australia.	yes
2.	Does *Fasciola hepatica* occur in Australia.	yes
3.	How do humans become infected by schistosomes?	skin penetration by cercariae in water
4.	What is a metacercaria.	encysted (tail-less) cercaria
5.	What is a miracidum?	ciliated larva released from trematode egg
6.	What is a scolex?	head of tape-worm
7.	What is a sporocyst (in a trematode life cycle)?	asexual multiplicative larval stage
8.	Where would you look for the egg of a *Fasciola hepatica*?	faeces (sheep/cow)
9.	Where would you look for the egg of a human-infecting *Taenia*?	faeces (human)
10.	Why are tapeworms segmented?	reproductive compartments (hermaphrodites)

11. Draw simple sketches representing each life-cycle stage of:
a nematode, a cestode and a trematode.

nematode cycle
egg - larvae (L1-L4) - adult

cestode cycle
egg - metacestode - adult

trematode cycle
egg-miracidium-sporocyst-redia-cercaria-(metacercaria)-adult

Welcome to:

"PARA-SITE: an interactive multimedia electronic resource dedicated to parasitology",

developed as an educational initiative of the ASP (Australian Society of Parasitology Inc.) and the ARC/NHMRC (Australian Research Council/National Health and Medical Research Council) Research Network for Parasitology.

PARA-SITE was designed to provide basic information about parasites causing disease in animals and people. It covers information on: parasite morphology (fundamental to taxonomy); host range (species specificity); site of infection (tissue/organ tropism); parasite pathogenicity (disease potential); modes of transmission (spread of infections); differential diagnosis (detection of infections); and treatment and control (cure and prevention).

PARA-SIGHT life-cycle diagrams and photographs illustrating:

> developmental stages

> host range

> sites of infection

> modes of transmission

> clinical consequences

PARA-CITE textual description presenting:

> general overviews for each parasite assemblage

> detailed summaries for specific parasite taxa

> host-parasite checklists

Developed by Professor Peter O'Donoghue, School of Chemistry & Molecular Biosciences

Artwork & design by Lynn Pryor The School of Biological Sciences

Published by: Faculty of Science, The University of Queensland, Brisbane 4072

ISBN 978-1-8649999-1-4

http://parasite.org.au/

Foreword

In developing this resource, we considered it essential that students get to know the parasite assemblages 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 and various treatment and control strategies. By learning basic parasitological information in a clinical context, it is hoped students will develop their skills to:

- diagnose the major parasitic groups in host tissues and fomites;
- deduce their modes of transmission from their sites of infection;
- indicate their pathogenicity for different host groups;
- identify boundaries to their distribution and abundance;
- recommend appropriate treatment and control strategies; and
- assess their significance with respect to human and animal health and welfare.

The impetus for this work was provided by the apparent absence of textbooks giving the right degree and mix of biological and clinical information. Many general biology texts only give cursory information on a small range of parasites while most clinical texts concentrate on a few of the most serious parasitic diseases of medical and veterinary significance. At the other end of the spectrum, there are many specialist texts dealing exclusively with individual parasitic groups and the information presented can be overwhelming to students. This electronic resource was designed in an attempt to find some common middle ground between the generalist and specialist texts available.

This resource has a distinctly Australian flavour as it was based on published accounts of parasites in Australian hosts (host-parasite checklists, parasite-host checklists and associated bibliographies are attached). This does not mean that the resource is only pertinent to students of Australian parasitology but rather that locally available parasites are used as examples. In many cases, the parasites are cosmopolitan species which are found worldwide (particularly in humans and their domestic and companion animals) whereas other examples are restricted to endemic species found only in Australia (especially in our unique native animals). Other parasite species do not occur naturally in Australia but are introduced as unwanted guests in international travellers, imported livestock or zoo animals. Knowledge of all these different parasites is essential for their differential diagnosis, treatment and control.

Introduction to Parasitology

Parasitism is the most common way of life; more than 50% of all animal species are parasites. Parasites occur in all animal species and they may have a profound effect on the health of people, domestic animals and wildlife. Parasitology is the study of parasitism; a multidisciplinary subject covering many topics including morphology, taxonomy, biology, behaviour, life-cycles, pathogenesis, epidemiology, ecology, physiology, biochemistry, genetics and molecular biology, as well as the diagnosis, immunology and treatment of infections.

Parasites live at the expense of their hosts whereas other symbiotes may be mutualists (living in mutual benefit with host) or commensals (living without benefit or detriment to host). Parasites may infect the gastrointestinal tracts or circulatory systems of their hosts, they may invade different tissues and organs or they may live on the external surfaces of their hosts. Many infections may be asymptomatic whereas others may cause acute (transient) or chronic (persistent) clinical diseases ranging markedly in severity (mild to fatal). Parasitic infections may cause mortality (foetal, neonatal, adult death), morbidity (disease manifest by enteritis, fever, anaemia, etc.), production losses (reduced meat, milk, fibre production), and tissue lesions (reduced marketability of product). Despite many advances in parasite treatment and control, infections still persist due to many factors, including urbanization (crowding together); more intensive farming systems, greater translocation of animals, further land and marine development, inadequate effluent disposal, emergence of parasite drug resistance, and spread of vector insecticide resistance.

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

helminths

arthropods

Protozoa: Over 10,000 species of single-celled protozoa have been described in the gut, blood or tissues of vertebrate and invertebrate hosts. Parasitic flagellates cause enteric diseases such as giardiasis, urogenital diseases such as trichomoniasis, systemic diseases such as sleeping sickness, and tissue diseases such as Chaga's disease and kala azar. Parasitic amoebae cause dysentery, meningoencephalitis and corneal lesions. Spore-forming sporozoa cause many serious diseases: Apicomplexa cause coccidiosis, malaria and tick fevers; Microspora parasitize fish and insects; and Ascetospora cause seasonal mortalities in oysters. Parasitic ciliates cause diarrhoea or lesions in humans and animals while commensal species cause serious fouling problems in aquaculture.

Helminths: Around 50,000 species of multicellular helminths (worms) have been described from a wide range of hosts. Roundworms (nematodes) cause much morbidity and mortality in humans and animals throughout the world. Serious infections include filiariases, hookworm and threadworm diseases. Larval and adult tapeworms (cestodes) may be found in many vertebrate hosts. Some species do not cause clinical disease whereas others may cause severe weight loss, diarrhoea, abdominal pain or space-occupying lesions. Flukes (trematodes) include many important species such as sheep liver fluke and human schistosomes or blood flukes.

Arthropods: Thousands of arthropods are parasitic at some stage in their life-cycles. Many cause serious diseases and limit agricultural productivity. Parasitic insects include biting and sucking lice which may cause skin lesions or anaemia, fleas which may cause allergic dermatitis, and various flies which suck blood as adults or produce larvae which feed on host tissues. Parasitic arachnids include ticks which feed on blood and may cause anaemia or paralysis and mites which feed on skin and may cause mild itching, hair loss or severe mange.

Overview

Three general environments are available for life as we know it: terrestrial, aquatic and biotic. By definition, parasites are those animals which occupy the last niche, i.e. live in or on another species, their **host**. **Parasitism** is a form of **symbiosis**, an intimate relationship between two different species. There is a **biochemical interaction** between host and parasite; i.e. they recognise each other, ultimately at the molecular level, and host tissues are stimulated to react in some way. This explains why parasitism may lead to disease, but not always. It is often a life-long relationship for the parasite, which cannot survive without its host.

While it is often claimed (even by definition) that a parasite must damage its host in some way (to distinguish parasitism from **commensalism** and **mutualism**), in practice this can be impossible to establish, because we know so little about most symbiotic relationships; certainly, many human parasitic infections are asymptomatic (which is not the same as non-pathogenic).

Parasitism must have arisen very early in the history of life on Earth, when primordial micro-organisms learnt to survive inside other cells which they had invaded either passively (e.g. by phagocytosis) or actively (e.g. by penetration). When multicellular organisms with alimentary tracts appeared, they would have inevitably (accidentally or intentionally) eaten free-living microorganisms (and, later, free-living helminths). Ingested animals that managed to survive in this new environment would have appreciated the nutrient-rich environment; energy saved in looking for food could then be diverted to proliferating and resisting the host's efforts to dislodge them. With time, these parasites became so adapted to life in the host, they "forgot" how to survive outside. However, to succeed, they still needed to produce offspring that could negotiate the outside world to find new hosts.

Not surprisingly, all parasitic animals have free-living counterparts to which they are clearly related, and the greatest diversity of parasites is still found within the alimentary tracts of "higher" animals. As host species diverged with evolution, they "carried" with them their parasites. It is virtually the rule today that parasitic protozoa and helminths found in any vertebrate species have almost identical relatives in related vertebrates, and most of them are exquisitely host-specific. For example, the two common amoebae of the human colon, *Entamoeba histolytica* and *E. coli*, have almost identical relatives within a wide range of vertebrate hosts. There is even *E. moshkovskii*, a species that has been found only in sewers, which probably evolved from parasitic species! *E. gingivalis* occurs only in the human mouth, and has lost its cystic stage, presumably because trophozoites are so efficient at transferring between hosts. The same occurs with helminths, e.g. the roundworm of the human small intestine, *Ascaris lumbricoides*, has counterparts in pigs, dogs, cats, flying foxes, elephants, dolphins and many other mammals.

Once established in the host intestine, some parasites "learned" to invade the gut mucosa and deeper tissues, or to survive in the guts of predators that consumed their original hosts. Involvement of invertebrate "micropredators" in such life-cycles could then have led to parasite transmission via blood or tissue ingestion. Other parasites, in their infective stages, developed the ability to invade via the skin. It is not too difficult to conceptualise how complex life-cycles, utilizing a range of different hosts, might have arisen. Many examples of "missing links" in parasite evolution can still be found today, although far more are well-and-truly extinct. It is misleading to think of extant protozoan or helminth species as "primitive", for they have been evolving as long as all other species, including *Homo sapiens*, and utilise sophisticated survival mechanisms that we are only beginning to understand.

Parasitism clearly has advantages over independent existence, for parasites greatly outnumber free-living animals, both in terms of individuals and species; from an evolutionary viewpoint, it is the ultimate life-style. The obvious benefit to the parasite is that its host provides, *gratis*, a relatively stable, nourishing home. The energy saved in seeking food, shelter and transport is then concentrated on reproducing and evading host defence mechanisms, which are provoked in virtually every case, although not always obviously.

Medical Parasitology is the study of those organisms which parasitise humans. According to the definition above, parasites could include the **viruses**, **bacteria**, **fungi**, **protozoa** and **metazoa** (multicellular organisms) which infect their host species. However, for historical reasons (and because they are NOT classed as animals), the first three have been incorporated into the discipline of **Microbiology**.

Parasitology claims those **protozoa** (unicellular animals), **helminths** (worms) and **arthropods** (insects and arachnids) whose existence depends on the availability of host animals, i.e. they are **obligate** parasites. Some

rare parasites are called **facultative**, because they can survive and reproduce without a host, but very few that infect humans belong to this group (e.g. free-living amoebae). While we could argue about whether certain insects and mites are "temporary parasites" or "micro-predators", insects as a group belong to the discipline of **Entomology**, while ticks and mites are the concern of **Acarology**. Another crude way of distinguishing these is to label them **ectoparasites** (living on the host body surface), in contrast to the **endoparasites** (which live inside the host). The major contribution of insects in Parasitology is as vectors of several infections, although several are true parasites in their own right.

The disciplines also differ in ways other than taxonomic boundaries. In Microbiology, while morphology or staining properties (e.g. with Gram's stain) are important in the basic categorisation of the organisms, species identification generally depends on culturing and identifying specific enzymatic reactions, antigenic configurations or DNA sequences; i.e. the test-tube is important. In Parasitology, morphological recognition remains foremost, so that parasites (or their vectors) are still identified on characteristic shapes and sizes; i.e. the microscope rules supreme. Subspeciation or strain-typing is less well-developed, and may depend on molecular configurations or host-specificity. Culture has been a basic tool in Microbiology almost from its inception, and cell-culture is especially important in Virology (where viruses are not observed directly, but initially recognised by their effects on cultured cells). In Parasitology, culture was for a long time virtually impossible for most organisms, including protozoa.

Nevertheless, in recent years, technical advances have allowed the *in vitro* cultivation of increasing numbers of parasite species, including even some helminths, although this is a procedure still in its infancy and used largely in research, rather than for routine clinical diagnosis. Advances in molecular biology are revolutionising all the biological sciences, including Parasitology. However, the organisms still must be identified initially on their morphology, and this is the basis of most parasite diagnoses made in clinical pathology laboratories.

Every known species (living and extinct) is assigned a unique combination of **genus** and **species** names which, by convention, are printed in italics or underlined. Infections with parasites are often indicated by the abbreviated genus name plus the suffix **-osis**. Some authorities use the suffix **-iasis** if the infection causes disease, but this distinction is often meaningless or impossible to establish. Purists argue that -osis belongs to species names derived from Greek, while those with Latin parentage deserve -iasis (it becomes tricky if you don't know the name's origins). Either can be used, depending on which sounds better (although a recent international convention aims to standardise all this), and we must be tolerant of the many exceptions, e.g. tuberculosis (mycobacteriosis), malaria (plasmodiosis), elephantiasis (lymphatic filariasis, or filariosis). If more than one parasite belongs in the genus, then the species name may be added to qualify the infection, *e.g.* schistosomiasis mansoni (not italicised).

Life-cycles

While parasites are adapted to living in or on their hosts, they can only survive by producing offspring capable of finding new hosts. The key to understanding their dispersal through the world is through knowledge of their **life-cycles** or modes of **transmission**, involving many aspects of parasite biology, reproduction and epidemiology.

Protozoa, in their motile, feeding, growing, asexually-multiplying forms are known as **trophozoites** (*trophe* = nutrition; *zooite* = minute animal) These are adapted for existence in the host and, generally, are unable to survive the rigours of life outside. Under appropriate conditions, which we do not yet understand, some trophozoites of gut protozoa coat themselves in a protective shell and shut down metabolically, to become **cysts**. These are designed to survive in the outside world long enough to reach new hosts. In the most highly-evolved protozoa (apicomplexans), which are obligate intracellular parasites, asexual division of the trophozoite (**schizogony**; *schizein* = to divide, or split; *-gony* = reproduction) leads to the generation of many **merozoites** (*meros* = piece, segment) which then invade other host cells. Eventually, instead of undergoing further schizogony, merozoites undergo sexual reproduction (**gamogony**) developing into either **macrogametocytes** (female) or **microgametocytes** (male). Fertilisation results in the formation of a zygote, termed an **oocyst** (= egg-cyst), which is designed to survive in the outside world so that it may infect another host. The ripe (sporulated) oocyst contains infective "seeds" known as **sporozoites**, which arise during its maturation (**sporogony** = generation of spores).

The metazoan parasites (multi-celled, *i.e.* worms and arthropods) generally are **dioecious**, i.e. adults occur as separate males and females. Tapeworms and most flukes are the exceptions (**hermaphrodites**). After

copulation, females produce fertile eggs, each containing an embryo. This undergoes embryonation developing into a juvenile or **larva** which will hatch out under suitable conditions. The egg may be the infective stage, or larvae may develop in the outside world to infectivity, or larvae may develop further in one or more intermediate hosts before they are able to reinfect their definitive hosts. Because their larvae must develop outside the host, adult helminths cannot multiply directly within a host (in stark contrast to protozoa which can proliferate to large numbers).

Many parasites complete their developmental cycle in a single host species (**monoxenous** life-cycles) while others require multiple host species (**heteroxenous** life-cycles). When multiple hosts are involved, the **definitive host** is that species in which the adult (or sexual) form of the parasite occurs, whereas the **intermediate host** is the species which supports the development and/or multiplication of the non-sexual, or larval (for helminths), stages of the parasite. Intermediate hosts which physically carry the infective stage from one host to another are also termed **vectors**; they are **mechanical** vectors if they simply transmit the parasite (unchanged and non-multiplied), and **cyclical** vectors if they also function as true intermediate hosts that support essential development and/or proliferation of the parasite.

Intermediary hosts may be optional in some helminth life-cycles; the parasite might not undergo essential development in them, although it may increase in size. These **paratenic hosts** carry parasites through food chains to the definitive host, ensuring successful transmission even when the hosts are thinly dispersed through the environment. Some parasites exhibit low specificity for their definitive and/or intermediate hosts and so can develop in a range of animal species. A **zoonosis** is a human infection caused by an organism which occurs naturally in other animals, known as **reservoirs** of infection. Most parasite life-cycles that are known have only been worked out quite recently; i.e. within the last 100 years. Information is therefore fragmentary and many ambiguities exist. We could argue about whether the mosquito genus *Anopheles* or the primate species *Homo sapiens* is the definitive host for malaria parasites as gamogony in initiated in the human but culminates in fertilization in the mosquito.

Host-specific parasites are very particular about which species they will use; this can apply to definitive as well as intermediate hosts. Host-specificity is determined by a complex of factors, some obvious and others still obscure. The first requirement is that the prospective host shares its environment with the parasite (**ecological** specificity); e.g. parasites of dolphins might not have much luck infecting humans who don't live near the sea (although modern food transport networks have changed this!). Secondly, host behaviour must expose it to the parasite (**ethological** specificity); e.g. people who eat dolphin food (fish) may acquire parasites intended for dolphins. Finally, once the parasite comes into contact with the host, it must recognise appropriate cues and feel comfortable within its new surroundings (**physiological** specificity); e.g. if a parasite of dolphins thinks it is in a large fish or a dolphin when it arrives in the human gut, it may then behave accordingly. Obviously, this last determinant of host-specificity is the one we understand least.

Parasites interact with host secretions and surfaces and membranes: they must recognise and respond to molecular configurations (receptors/ligands). Detection of subtle variations in metabolites allows them to follow road-maps; they need to make critical changes in behaviour and development according to changes in host physiology/behaviour (neural/endocrine cues?); and they must be satisfied with their diet (host intestinal contents, blood and/or tissues). Clearly, all these combinations are unique for each host species, and vary even among individuals within a species, within an individual host throughout its own life-cycle and even throughout a 24-hour day. Likewise, each population of parasites is heterogeneous, so some individuals succumb very easily if in the wrong host ("losers") whereas others persist and may come close to full development ("pioneers"). This is the driving force of evolution, and parasites are the most rapidly evolving animals.

Epidemiology

This is the descriptive and analytical study of how diseases or infective organisms are distributed in human populations. A parasite is **endemic** to a geographical region if it is sustained by transmission amongst people living there. An infection maintained in animal populations is **enzootic** (which must apply to all zoonoses), although this term is going out of fashion. An infection acquired locally (usually in an endemic region) is **autochthonous**. Infected people who bring an organism into a non-endemic area are labelled **imported** cases; should the parasite then transfer to another person in that region, the secondary case becomes an **introduced** infection. If the parasite establishes in the new population, it becomes newly-endemic.

The level of infection in a population is measured by prevalence and incidence. **Prevalence** refers to the prevailing level of a condition within a defined population, and is best applied to conditions without a clearly

identifiable onset, such as most helminthic infections, chronic toxoplasmosis, Chaga's disease (or malignant, degenerative or metabolic diseases). It is measured by a single study of a population over a brief time-period (cross-sectional survey). **Incidence** refers to the number of new cases acquired per unit of population per unit of time, and is more meaningful for acute, short events (incidents), with an identifiable beginning (or end!) e.g. many viral infections, acute malaria (or deaths, or accidents). It can be measured only by monitoring a population over a sufficient period or time (longitudinal study) and determining the rate increase or decrease. Obviously, the incidence, prevalence and duration of a particular condition are closely and simply inter-related. An **epidemic** occurs when the incidence of new cases significantly exceeds the usual rate; if the disease is protracted, this will be reflected by an increase in prevalence as well.

Quantitation of infection

Infective organisms have been categorized as either **microparasites**, which multiply directly within the host (all the microbes, plus protozoa) or **macroparasites**, which generally cannot multiply in the host; their numbers depend on how many infective eggs or larvae are taken on board. Ectoparasites do not happily fit into this classification, for they are clearly “macro”, but often can multiply to huge populations on the one host. However, their development may be considered “external”, as they usually reside outside the host on the surface. The term “infestation”, sometimes used for macroparasitic infections, is going out of fashion, but can be applied to contaminated inanimate objects, e.g. a house infested with fleas, or bushland infested with ticks.

Infection with microparasites is an all-or-none situation; you either have measles, influenza, bubonic plague, toxoplasmosis, giardiasis, etc., or you don’t. It is not often possible, or necessary, to quantify reliably the **intensity** of such an infection (number of organisms on board a host). In many instances, the severity of disease is not reliably related to the numbers of parasites detectable in blood, tissues or secretions (a notable exception is malaria, in which the percentage of infected red cells can be estimated and sometimes is important clinically). In the case of helminths and arthropods, which are generally visible macroscopically as discrete individuals, the number of organisms is meaningful, because it can be measured and does influence the likelihood or severity of disease. Therefore, in epidemiological studies of macroparasitic infections, their intensity becomes important, in addition to incidence and prevalence. Virtually all population studies have shown that the intensity of infection does not follow a normal distribution, but exhibits an “aggregated” or “overdispersed” pattern: a small number of hosts harbour most of the parasites, whereas most individuals carry few or no parasites (characterised mathematically as a “negative binomial distribution”).

Clinical and pathological considerations

While, by definition, a parasite should evoke a host reaction, there need not be any obvious adverse effects because, in the great majority of cases, infected individuals exhibit little evidence of disease. In many cases, it can be difficult or impossible to determine whether an organism is a parasite or commensal (e.g. many intestinal protozoa, and worms). However, other parasite infections do cause serious disease, to such an extent that they become major public health problems. It is generally assumed that, the longer a parasite and its host species have co-evolved, i.e. have had time to adapt to each other, the less pathogenic the infection becomes. On the other hand, infections with parasites that are poorly adapted to humans, i.e. zoonoses, are more likely to cause serious disease. However, there are many exceptions to these “rules”. Remember, in the clinical world, we see only those individuals who develop disease; there may be many more who remain well, even though infected. Apart from host factors, the major important determinant here is parasite **virulence**, i.e. capacity to induce disease, including the inter-related factors of invasiveness (motility, enzyme secretion, presence of specific tissue receptors, induction of phagocytosis), fecundity (rate of producing offspring), means of egress from host, stimulation and/or suppression of immunity and inflammation, production of exo- and endo-toxins and resistance to host defences.

Such virulence-determinants often correlate directly with the parasite’s capacity to survive and reproduce, but they also may adversely affect host survival and fecundity. This applies a pressure on host populations that selects out more resistant individuals; it has even been argued that parasites serve to improve the fitness of their host species (Red Queen Hypothesis), and were a major influence in the evolution of sex! However, genetic changes that increase resistance to infection often handicap the host in other ways, generating a dynamic equilibrium between protection against infection and susceptibility to other diseases (**balanced polymorphism**). There is no doubt that infectious organisms exert a powerful and continuous evolutionary pressure on host populations (and *vice versa*).

In the field of infectious diseases, it is conceptually important not to confuse aetiological agents with their effects on the host. An **infection** occurs when an **organism**, i.e. the parasite, is found in its host. Some experts don't like to label this an infection, unless there is evidence of a response in host tissues; this applies particularly to commensal organisms, which normally occur on human skin or in the gastrointestinal tract, but which cause disease only when they breach the surface barriers. Infection is a host-organism interaction; it cannot exist without a host. Presence of infective organisms in the environment, e.g. in food, on fomites or in water, is not infection, but contamination (or "infestation"). We should not talk about "infected water supplies", for example.

Moreover, an infection is not the same as a **disease**, which is a pathological change in the host, i.e. abnormalities induced in tissues by direct mechano-chemical damage and/or release of toxins and/or inflammatory mediators. **Illness** occurs when the host suffers the effects of the disease and becomes a patient, i.e. complains of **symptoms** (subjective, felt by the patient) which interfere with normal life, and perhaps manifests clinical **signs** (objective, detectable by the doctor), always with psychosocial undertones and ramifications. This is summarised as follows:

ORGANISM ⇔ INFECTION ⇔ DISEASE ⇔ ILLNESS

Where you start in the above sequence depends on whether you are the parasite or the patient! The illness is what the patient complains about to the doctor (often with judicious prompting), the disease is what the clinician may detect on physical examination (and the pathologist confirms in laboratory tests or at autopsy), and the causative organism (or its products, or antibodies to it) is what the diagnostic laboratory usually seeks and identifies. In any particular patient, all of these apparent components might be totally unrelated, so that linking them together becomes a major and still unresolved difficulty, even in some very common infections. Such distinctions may seem pedantic, but their appreciation helps in understanding stages in the evolution of an infectious disease, and is important to minimise confusion. Many people are infected; in fact, every one of us has at some time harboured at least one parasite species, and most of the world's people carry many parasites most of the time. However, relatively few are diseased, and not all of them suffer illness. Infections without illness are called **subclinical** or **asymptomatic**. Note that this does not mean being free of disease.

If you conclude, from the foregoing discussion, that infection with parasites is a normal state in humans, you would be right, partly. To be able to distinguish normal from abnormal, and to know when to intervene and when simply to reassure, are good reasons for medical students to be aware of and understand human parasitic infections. Seemingly innocuous parasitoses can turn nasty unpredictably, whereas harmless species with exotic names, when they appear in patients' pathology reports, can generate panic in medical practitioners.

The interval between **exposure** to infection and the onset of illness is known as the **incubation**, **latent** or **pre-patent** period or phase. Some authorities define this period as the time from exposure to the time of becoming infective to others, but not all agree with this. Others define the latent period as the time from exposure to the first occurrence of recognisable specific manifestations, be they symptoms, signs, positive serology or other laboratory findings; if for symptoms, then it is called the incubation period. With many parasitic infections in endemic areas, these definitions may be of little use clinically, because people are repeatedly being exposed to infection.

An infection is **patent** when direct evidence of the organism can be detected, e.g. in the patient's faeces, blood or secretions, regardless of whether symptoms have appeared. Some infections may be patent but subclinical; others may cause illness, yet not be patent. However, the individual who has patent infection is essential to transmission of the organism, because it can then be transferred directly to other hosts, to vectors, or into the environment, where it may need to develop through stages to infectivity. Obviously, the detection of patency depends on the sensitivity of the test being used to identify the organism.

Often, indirect evidence of infection is the best that can be offered by the diagnostic laboratory, in the form of circulating antibodies generated against antigens of the infecting organism. Apart from the issues of **specificity** and **sensitivity**, another difficulty common to all antibody tests is distinguishing between ongoing, active infection and recently resolved or past infection. In other cases, serology may be even less adequate, for the simple reason that a test has not been developed, and the infection is known as **cryptic**. In some infections, specific monoclonal antibodies can be used to identify parasite antigens, and the polymerase chain reaction is becoming increasingly available to identify nucleotide sequences from infective organisms, although the limitations of these technological advances have not been well-established.

Usually, disease results predominantly from the host's efforts to deal with the parasite, involving immunological and other less well understood responses to an organism which refuses to go away, and which utilises effective strategies to avoid being damaged. The principles (and even details) of host responses to infecting organisms apply equally to microbial and parasitic infections and, as we learn more about the precise mechanisms, the more difficult it becomes, in the clinical context, to justify the separation of these groups of pathogens. Obviously, viruses may succumb more readily than worm larvae to protective mechanisms involving antibodies, complement, lymphocytes, phagocytes and other effector cells and molecules, but all infections initially trigger similar basic repertoires of responses. A major discriminating influence is whether the organisms are intra- or extra-cellular, which partly determines the class of MHC molecules with which they interact. The minute parasitic protozoa that multiply in host cells have much in common with viruses, so that host responses to these infections and the resulting diseases can be so similar that, clinically, they may be indistinguishable. Patients have only a limited range of symptoms to complain about, so that generally it is impossible to diagnose the causative organism from the clinical features. However, a careful history, taken to evaluate the likelihood of exposure to specific parasites, often narrows the range of options (differential diagnosis), and indicates the specimens which should be sent to the laboratory for definitive diagnosis.

Parasitology in perspective

Parasitic infections of humans can be studied from many angles: we can focus on the parasites, their hosts, the environments they share and the ways in which they interact. People working in this field come from numerous backgrounds, including zoology, physiology, biochemistry, immunology, molecular biology, pharmacology, ecology, economics, anthropology, sociology, engineering, agriculture, education, mathematics and, of course, human and veterinary medicine. Irrespective of background, it can be very helpful to compartmentalize and consider parasitological information under the following headings:

- **AETIOLOGY** (causative organisms): colloquial and scientific (binomial) species name, broad group e.g. amoeba, nematode; stages occurring in humans (larva, cyst, trophozoite etc.); approximate size/shape.

- **LIFE-CYCLE** (summary of biology): Hosts – definitive, intermediate, paratenic; anatomical locations and sites of multiplication; development and survival in intermediate host/environment.

- **EPIDEMIOLOGY** (dispersal in populations): distribution, prevalence, demographics, transmission; ecological determinants, *i.e.* geography, climate, vectors, human behaviour and resources

- **PATHOGENESIS** (dispersal within host; mechanisms of disease): sites affected; mechanical and/or chemical damage; local and systemic host responses (acute and chronic); effectiveness of immunity

- **CLINICAL MANIFESTATIONS** (how patient affected): logical extension of knowledge on pathogenesis; know mainly which organ system(s) involved and how manifests; symptoms and signs.

- **DIAGNOSIS** (how detected): specimens required; collection, preservation, transport, tests, reliability of results (sensitivity, specificity, predictive values); safety aspects, disposal

- **TREATMENT** (therapy): Is it necessary? effective? safe? Drugs, modes of action; contra-indications, side-effects; compliance; susceptibility/resistance

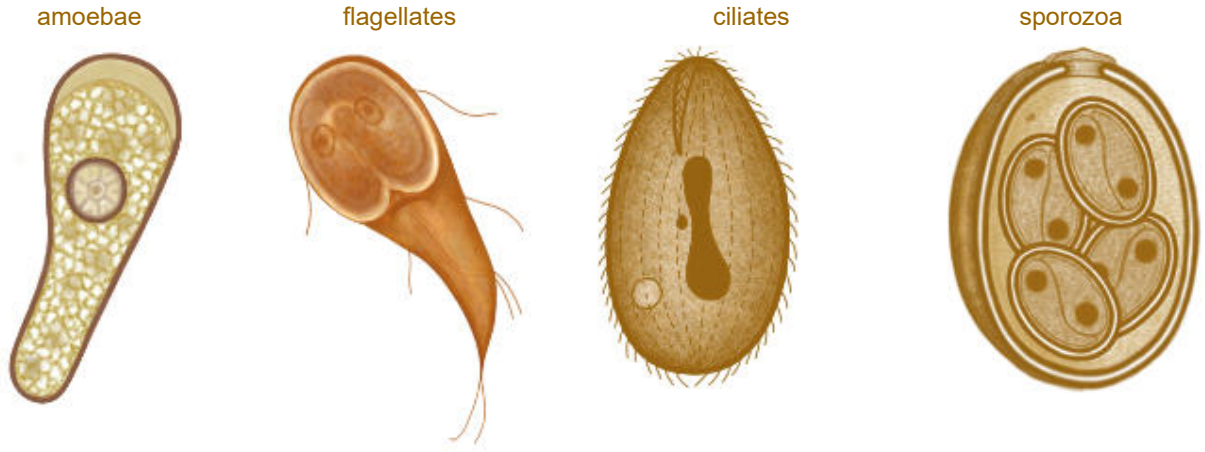
- **PREVENTION/CONTROL** (prophylaxis/intervention/management): public health; chemoprophylaxis; interruption of transmission; education; screening, vaccination; environment/food/water contamination

Protozoan Parasites

The name ‘proto-zoa’ literally means ‘first animals’ and early classification systems grouped the protozoa as basal members of the animal kingdom. However, they were recognized as a discrete assemblage on the basis of their unicellularity and were assigned to the taxon Protozoa (but still invariably figured as the trunk of the animal tree of life). Members of the subkingdom Protozoa are quite disparate; indeed the taxon has never been considered a natural assemblage of organisms but rather one of convenience. More recently, the protozoa have been classified together with several algal and fungal groups in the kingdom Protista (protozoa representing the motile protists). Irrespective of contemporary classification systems, most parasitological texts continue to use the name protozoa for historical reasons.

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. Most protozoa are microscopic organisms, only a few grow to a size large enough to be visible to the naked eye. As unicellular eukaryotes, protozoa display all the same essential life activities as higher metazoan eukaryotes: they move about to survive, feed and breed.

Four main groups of protozoa are recognized on the basis of their locomotion using specialized subcellular and cytoskeletal features:



- Amoebae use pseudopodia (singular: pseudopodium) 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, especially phagocytic cells (e.g. human macrophages).
- Flagellates use elongate flagella (singular: flagellum) 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 (2 single central microtubules surrounded by 9 peripheral doublets). This configuration is conserved throughout eukaryotic biology, many organisms produce flagellated cells (e.g. human spermatozoa).
- Ciliates use numerous small cilia (singular: cilium) 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. Ciliated cells are found in specialized tissues and organs in many other higher life-forms (e.g. human bronchial epithelial cells).
- Sporozoa (‘spore-formers’) were originally recognized not on the basis of their locomotion, but because they all formed non-motile spores as transmission stages. Recent studies, however, 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.

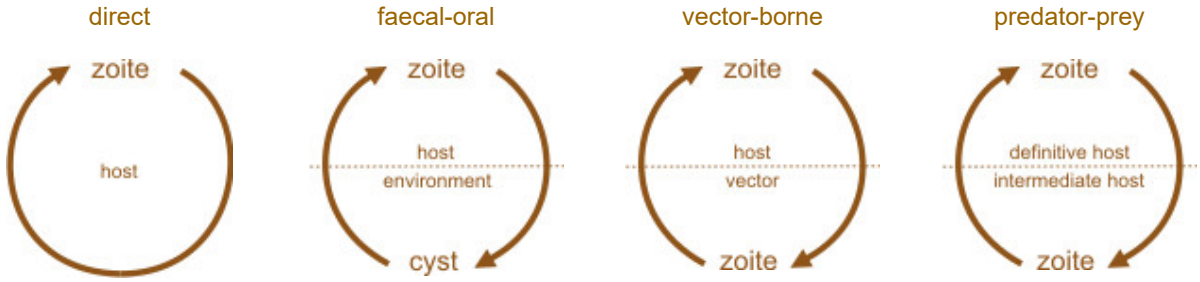
Biodiversity

Protozoan biodiversity (or species richness) includes counts (or estimates) of some 32,000 extant (living) species and another 34,000 extinct (fossil) species (especially foraminifera). Of those alive today, some 21,000 species occur as free-living organisms in aquatic or terrestrial environments, whereas the remaining 11,000 species are parasitic in vertebrate and invertebrate hosts. There are approximately 6,900 flagellate species (1,800 parasitic, 5,100 free-living), 11,550 amoebae species (250 parasitic, 11,300 free-living), 7,200 ciliate species (2,500 parasitic, 4,700 free-living) and 5,600 sporozoan species (all parasitic).

Life-cycles

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. Parasites may multiply by asexual division (fission/splitting or internal/endogenous budding) or sexual reproduction (formation of gametes and fertilization to form zygote, or unique process of conjugation where ciliates exchange micronuclei).

Protozoan developmental stages occurring within hosts generally consist of feeding trophozoites, and they 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.



> direct transmission of trophozoites through intimate body contact, such as sexual transmission (e.g. *Trichomonas* spp. flagellates causing trichomoniasis in humans and bovine infertility in cattle).

> faecal-oral transmission of environmentally-resistant cyst stages passed in faeces of one host and ingested with food/water by another (e.g. *Entamoeba histolytica*, *Giardia duodenalis* and *Balantidium coli* all form faecal cysts which are ingested by new hosts leading to amoebic dysentery, giardiasis and balantidiasis, respectively).

> vector-borne transmission of trophozoites taken up by blood-sucking arthropods (insects or arachnids) and passed to new hosts when they next feed (e.g. *Trypanosoma brucei* flagellates transmitted by tsetse flies to humans where they cause sleeping sickness, *Plasmodium* spp. haemosporidia transmitted by mosquitoes to humans where they cause malaria).

> predator-prey transmission of zoites encysted within the tissues of a prey animal (e.g. herbivore) being eaten by a predator (carnivore) which subsequently sheds spores into the environment to be ingested by new prey animals (e.g. tissue cysts of the sporozoan *Toxoplasma gondii* being ingested by cats, and tissue cysts of the microsporan *Thelohania* spp. being ingested by crustaceans).

Taxonomic overview

Flagellates and amoebae are considered to be closely related, because some amoebae form transient flagellated stages (to aid in dispersal) and some flagellates exhibit intermittent amoeboid motion. Two groups of flagellates are recognized on the basis of the presence or absence of chloroplasts:

Phytoflagellates with chloroplasts derive energy by photosynthesis. Most are free-living aquatic organisms and some exhibit periodic blooms (e.g. red tides). Others contain potent neurotoxins and cause paralytic shellfish poisoning.

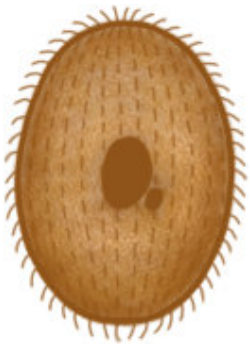
Zooflagellates without chloroplasts derive energy by the absorption of nutrients or the ingestion of food particles. Many species occur as free-living aquatic organisms whereas others live in insects and some vertebrates as symbiotes, commensals or parasites (several species cause major human diseases such as sleeping sickness, Chagas disease, kala azar and diarrhoea).

Two groups of amoebae are recognized on the basis of the types of pseudopodia formed with or without regular microtubule arrays:

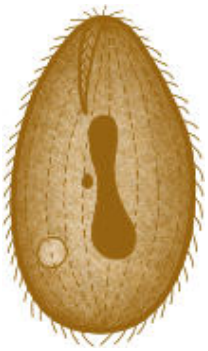
Rhizopod amoebae produce broad lobopodia, fine filopodia or net-like reticulopodia which do not contain regular microtubule arrays. Many aquatic species contribute to water quality by consuming bacteria and algae whereas terrestrial species contribute to soil health via nutrient cycling. Some species, such as foraminifera, build unique tests (shells) which contribute to fossil records.

Actinopod amoebae form radial axopodia which are stiffened by internal arrays of microtubules arising from an organizing centre. All species are free-living planktonic organisms, marine species known as radiolaria, and freshwater species known as heliozoa (or sun animacules).

The ciliates are regarded to be quite separate from other groups, more because they possess 2 types of nuclei (vegetative macronuclei and reproductive micronuclei) than because they possess cilia. Three groups are recognized on the basis of their patterns of somatic (body) and buccal (oral) ciliature:



Lower holotrichs have simple body and oral ciliature. Most are free-living aquatic species but some are highly specialized symbionts aiding cellulose digestion in herbivores.



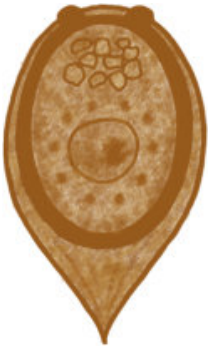
Higher holotrichs have simple body ciliature but more specialized oral ciliature forming membranelles. Most occur as free-living aquatic organisms but some live as commensals or parasites in a range of animals.

Spirotrichs have reduced body ciliated but well developed oral ciliature forming an adoral zone of membranelles. The majority are bactivores living in aquatic and terrestrial habitats.

All sporozoa are obligate parasites, they form temporary non-motile spores which contain infective cells. Four major groups are recognized on the basis of different spore morphology:

Apicomplexan parasites form distinctive oocysts containing infective sporozoites. Many species occur only in invertebrates whereas others may infect vertebrates causing severe diseases (such as malaria, tick fever, diarrhoea or abortion).

Microsporan parasites form unicellular spores containing coiled polar tubes used to infect host cells. Most species infect invertebrates (especially insects) although some form cysts in vertebrates (mainly fish).



Haplosporidian parasites form unicellular spores without polar filaments in the tissues of aquatic invertebrates. They cause significant morbidity and mortality in oysters throughout the world.

Paramyxean parasites form unique spore-within-spore arrangements within the tissues of bivalves and polychaetes. They cause QX and Aber disease in oysters

Helminth Parasites

The word ‘helminth’ is a general term meaning ‘worm’, but there are many different types of worms. Prefixes are therefore used to designate types: platy-helminths for flat-worms and nemat-helminths for round-worms. All 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 flatworms are acoelomate (do not have body cavities) while the roundworms are pseudocoelomate (with body cavities not enclosed by mesoderm). In contrast, segmented annelids (such as earthworms) are coelomate (with body cavities enclosed by mesoderm).

Many helminths are free-living organisms in aquatic and terrestrial environments whereas others occur as parasites in most animals and some plants. Parasitic helminths are an almost universal feature of vertebrate animals; most species have worms in them somewhere.

Biodiversity

Three major assemblages of parasitic helminths are recognized: the Nemathelminthes (nematodes) and the Platyhelminthes (flatworms), the latter being subdivided into the Cestoda (tapeworms) and the Trematoda (flukes):

> 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’). Worms use longitudinal muscles to produce a sideways thrashing motion. Adult worms form separate sexes with well-developed reproductive systems.

> Cestodes (tapeworms) have long flat ribbon-like bodies with a single anterior holdfast organ (scolex) and numerous segments. They do not have a gut and all nutrients are taken up through the tegument. They do not have a body cavity (acoelomate) and are flattened to facilitate perfusion to all tissues. 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 do not have a body cavity (acoelomate) and are dorsoventrally flattened with bilateral symmetry. 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.

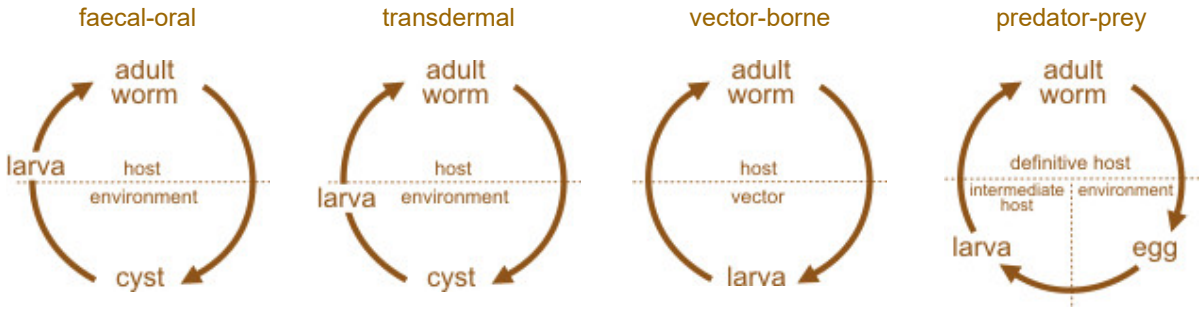
Life-cycles

Unlike other pathogens (viruses, bacteria, protozoa and fungi), helminths do not proliferate within their hosts. Worms grow, moult, mature and then produce offspring which are voided from the host to infect new hosts. Worm burdens in individual hosts (and often the severity of infection) are therefore dependent on intake (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. Although most helminth infections are well tolerated by their hosts and are often asymptomatic, subclinical infections have been associated with significant loss of condition in infected hosts. Other helminths cause serious clinical diseases characterized by high morbidity and mortality. Clinical signs of infection vary considerably depending on the site and duration of infection. 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 as they are not invasive, 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. Adult worms infect definitive hosts (those in which sexual development occurs) whereas larval stages may be free-living or parasitize invertebrate vectors, intermediate or paratenic hosts.

- Nematodes produce eggs that embryonate in utero or outside the host. The emergent larvae undergo 4 metamorphoses (moults) before they mature as adult male or female worms.
- Cestode eggs released from gravid segments embryonate to produce 6-hooked embryos (hexacanth oncospheres) which are ingested by intermediate hosts. The oncospheres penetrate host tissues and become metacestodes (encysted larvae). 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 emerge from the snails and either actively infect new definitive hosts or form encysted metacercariae on aquatic vegetation or inside second intermediate hosts which are, in both cases, eaten by definitive hosts.

Helminth eggs have tough resistant walls to protect the embryo while it develops. Mature eggs hatch to release larvae either within a host or into the external environment. The four main modes of transmission by which the larvae infect new hosts are faecal-oral, transdermal, vector-borne and predator-prey transmission:



> faecal-oral transmission of eggs or larvae passed in the faeces of one host and ingested with food/water by another (e.g. ingestion of *Trichuris* eggs leads directly to gut infections in humans, while the ingestion of *Ascaris* eggs and *Strongyloides* larvae leads to a pulmonary migration phase before gut infection in humans).

> transdermal transmission of infective larvae in the soil (geo-helminths) actively penetrating the skin and migrating through the tissues to the gut where adults develop and produce eggs that are voided in host faeces (e.g. larval hookworms penetrating the skin, undergoing pulmonary migration and infecting the gut where they feed on blood causing iron-deficient anaemia in humans).

> vector-borne transmission of larval stages taken up by blood-sucking arthropods or undergoing amplification in aquatic molluscs (e.g. *Onchocerca* microfilariae ingested by blackflies and injected into new human hosts, *Schistosoma* eggs release miracidia to infect snails where they multiply and form cercariae which are released to infect new hosts).

> predator-prey transmission of encysted larvae within prey animals (vertebrate or invertebrate) being eaten by predators where adult worms develop and produce eggs (e.g. *Dracunculus* larvae in copepods ingested by humans leading to guinea worm infection, *Taenia* cysticerci in beef and pork being eaten by humans, *Echinococcus* hydatid cysts in offal being eaten by dogs).

Taxonomic overview

Two classes of nematodes are recognized on the basis of the presence or absence of special chemoreceptors known as phasmids: Secernentea (Phasmidea) and Adenophorea (Aphasmidea). While many different orders are recognized within these classes, the main parasitic assemblages infecting humans and domestic animals include one aphasmid order (Trichocephalida) and 6 phasmid orders (Oxyurida, Ascaridida, Strongylida, Rhabditida, Camallanida, and Spirurida).

Trichocephalid 'whip-worms' have long thin anterior ends which they embed in the intestinal mucosa of their hosts. They have simple life-cycles where infections are acquired by the ingestion of eggs and emergent larvae moult and mature to adults in the gut. *Trichuris* infections in humans may cause inflammation, tenesmus, straining and rectal prolapse.

Oxyurid 'pin-worms' have small thin bodies with blunt anterior ends. They have simple life-cycles, but with an unusual modification. Female worms emerge from the anus of their hosts at night and attach eggs to the skin. This causes peri-anal itching and eggs are transferred by hand to mouth. Infections by *Enterobius* cause irritability and sleeplessness in humans, especially children.

Strongyle 'hookworms' have dorsally curved mouths armed with ventral cutting plates or teeth which they embed in host tissues to feed on blood. They have complex life-cycles where larvae develop in the external environment (as 'geo-helminths') before infecting hosts by penetrating the skin. Once inside, they undergo pulmonary migration before settling in the gut to feed. Heavy infections by *Ancylostoma* and *Necator* cause severe iron-deficient anaemia in humans, especially children.

Rhabditid 'thread-worms' have tiny bodies which become embedded in the host mucosa. Their life-cycle includes parasitic parthenogenetic females producing eggs which may hatch internally (leading to auto-infection) or externally (leading to transmission of infection or formation of free-living male and female adults). Super-infections by *Strongyloides* may cause severe haemorrhagic enteritis in humans.

Ascarid 'roundworms' have large bodies with 3 prominent anterior lips. Their life-cycles involve a stage of pulmonary migration where larvae released from ingested eggs invade the tissues and migrate through the lungs before returning to the gut to mature as adults. *Ascaris* infections in humans cause gastroenteritis, protein depletion and malnutrition and heavy infections can cause gut obstruction.

Spirurid 'filarial worms'occur as long thread-like adults in blood vessels or connective tissues of their hosts. The large female worms release live larvae (microfilariae) into the blood or tissues which are taken up by blood-sucking mosquitoes or pool-feeding flies and transmitted to new hosts. *Onchocerca* infections cause nodules, skin lesions and blindness in humans, while those of *Wuchereria* cause elephantitis.

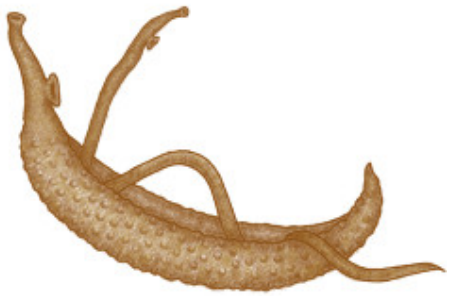
Spirurid 'guinea worms' infect host tissues where the large females cause painful blisters on the feet and legs. When hosts seek relief by immersion in water, the blisters rupture releasing live larvae which infect copepods that are subsequently ingested with contaminated drinking water. The 'fiery serpents' mentioned in historical texts are thought to refer to *Dracunculus* infections.

Two subclasses of cestodes are differentiated on the basis of the numbers of larval hooks, the Cestodaria being decacanth (10 hooks) and the Eucestoda being hexacanth (6 hooks). Collectively, 14 orders of cestodes have been identified according to differences in parasite morphology and developmental cycles. Two orders have particular significance as parasites of medical and veterinary importance.

Cyclophyllidean cestodes have terrestrial 2-host life-cycles where adult tapeworms develop in carnivores (scolex with 4 suckers and sometimes hooks) while larval metacestodes form bladder-like cysts in the tissues of herbivores. The larvae of *Taenia* spp. cause cysticercosis in cattle, pigs and humans, while those of *Echinococcus* cause hydatid disease in humans, domestic and wild animals.

Pseudophyllidean cestodes have aquatic 3-host life-cycles, involving the sequential formation of adult tapeworms in fish-eating animals (scolex with 2 longitudinal bothria), procercoid larval stages in aquatic invertebrates (copepods) and then plerocercoid (spargana) stages in fish e.g. *Diphyllobothrium* in humans, dogs and cats being transmitted through copepods and fish.

Two major groups of trematode–like worms are recognized on the basis of their structure and development. Monogenea have complex posterior adhesive organs and direct life-cycles involving larvae called oncomiracidia; they are external parasites of aquatic vertebrates, mainly fishes. The true Trematoda have oral and posterior suckers and heteroxenous life-cycles where adult worms infect vertebrates and larval miracidia infect molluscs to proliferate and produce free-swimming cercariae. Monogeneans are important in wildlife and aquaculture parasitology, but none infect humans. Numerous species of ‘true trematodes’ infect humans. Classification in the group is not obvious but can be most conveniently considered here in terms of the blood flukes (schistosomatoids) which include species in mammals, birds, reptiles and fishes, and ‘everything else”.



Fasciolid (liver fluke) trematodes are broadly representative of most trematodes. This particular group live as adults in hepatic bile ducts of mammals where they may cause fibrotic ‘pipestem’ disease. The parasites proliferate in freshwater snails and mammals become infected by ingesting metacercariae attached to aquatic vegetation. Several *Fasciola* spp. cause hepatic disease in domestic ruminants and occasionally in humans.

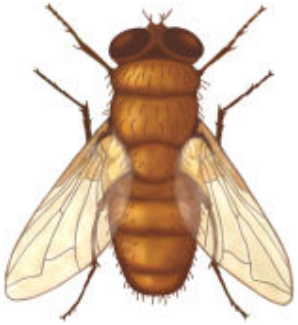
Bird and mammal schistosomes (blood flukes) are unusual in that the adults are not hermaphroditic but form separate sexes which live conjoined in mesenteric veins in mammals. Female worms lay eggs which actively penetrate tissues to be excreted in urine/faeces or they become trapped in organs where they cause granuloma formation. Miracidia released from eggs infect aquatic snails and produce fork-tailed cercariae which actively penetrate the skin of their hosts. Several *Schistosoma* spp. cause schistosomiasis/bilharzia in humans.

Arthropod Parasites

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.

The main arthropod assemblages include crustaceans (crabs, lobsters, crayfish, shrimp), arachnids (spiders, scorpions, ticks, mites) and insects (beetles, bugs, earwigs, ants, bees, termites, butterflies, moths, crickets, roaches, fleas, flies, mosquitoes, lice). Most parasitic arthropods belong to 2 main classes: the 6-legged insects, and the 8-legged arachnids

insects



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.

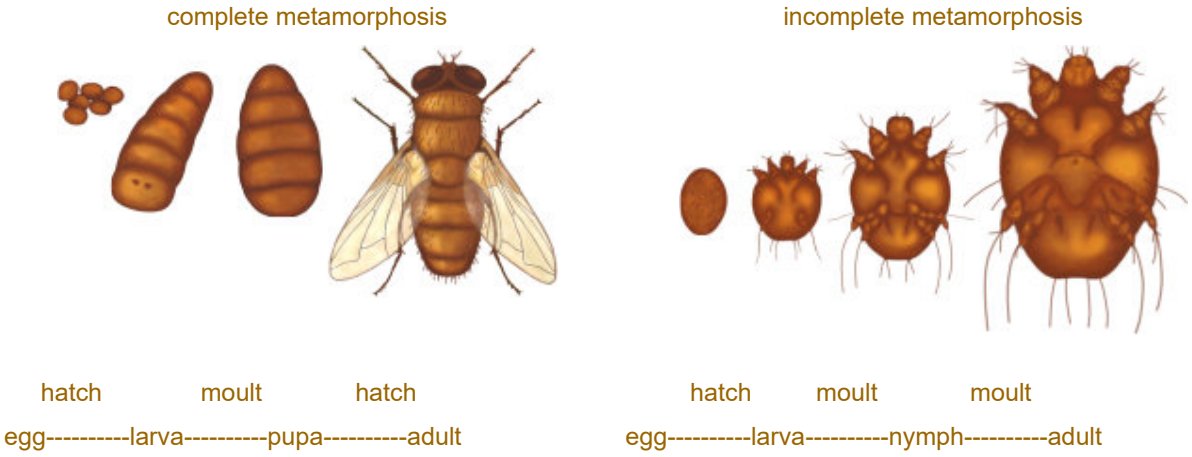
> Arachnids have 2 body parts known as the prosoma (or cephalothorax) and opisthosoma (or abdomen). The cephalothorax has 8 legs arranged in 4 bilateral pairs and arachnids do not have wings or antennae. Important parasitic assemblages include the ticks and mites which bite into tissues and feed off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

Biodiversity

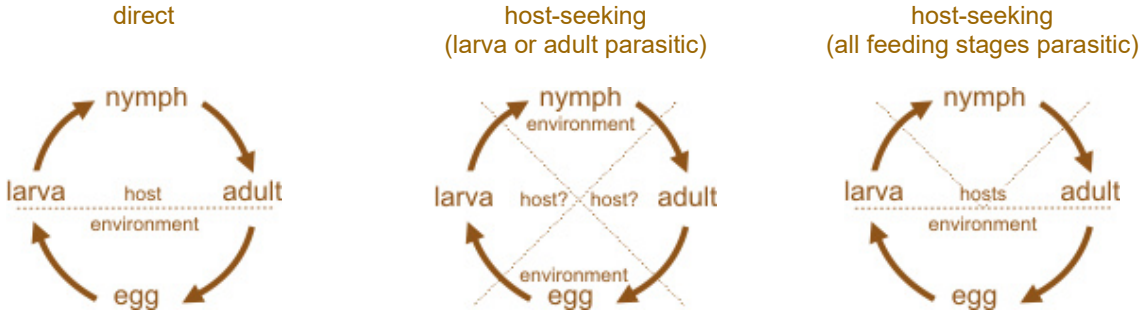
Collectively, arthropods account for a substantial share of global biodiversity, both in terms of species richness and relative abundance. There are over 1,000,000 species of insects and over 50,000 species of arachnids. They are very successful and adaptable organisms and are capable of forming large populations due to their rapid and fertile reproduction rates. Many species are also able to withstand adverse environmental conditions by undergoing periods of developmental arrest (diapause). The protection afforded by their exoskeletons allows them to colonize many habitats and they overcome the problem of growing larger in a non-expandable exoskeleton by undergoing periodic moulting (or ecdysis) which is mediated by hormones. Developmental stages between moults are referred to as instars. Moulting is a complex process and its timing is mediated by many environmental and physiological cues. It involves detachment of the hypodermis from the procuticle, partial resorption of the old cuticle, production of a new epicuticle, dehiscence (splitting) of the old cuticle, emergence of the animal, stretching and expansion of the new cuticle by air and/or water intake, and then sclerotization of the new cuticle.

Life-cycles

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 transmission of infective stages occurs when hosts come into close contact with each other or share quarters, bedding or clothing. Larvae, nymphs or adults may cross from one host to another, while eggs or pupae may contaminate shared environments. Insects (fleas and lice) and arachnids (mites) rely on close contact between hosts.

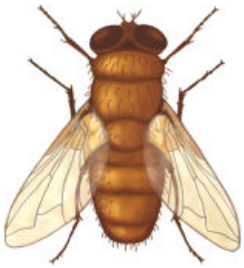
> Many adult insects actively seek hosts in order to feed or lay eggs. Winged insects (mosquitoes, flies) fly to new hosts to feed while fleas jump onto passing hosts. Some adult flies (botflies) do not feed on their hosts but deposit eggs from which larvae emerge and feed on host tissues and exudates.

> Tick larvae actively seek hosts by climbing vegetation and questing for passing hosts. Some species complete their life-cycle on the same host (one-host ticks) while others detach after feeding and drop to the ground to moult before seeking new hosts as nymphs or adults (two-host or three-host ticks).

Taxonomic overview

Insects exhibit extraordinary biodiversity, both in terms of species richness (numbers of species) and relative abundance (population sizes). Most parasitic species belong to three main groups: the jumping fleas (Siphonaptera); the winged flies (Diptera); and the wingless lice (Phthiraptera).

Fleas are bilaterally-flattened wingless insects with enlarged hindlimbs specially adapted for jumping (up to 100 times their body length). Jumping feats are accomplished using elastic resilin pads which expand explosively when uncocked from the compressed state. Fleas undergo complete metamorphosis whereby grub-like larvae form pupae from which adult fleas emerge. The larvae are not parasitic but feed on debris associated mainly with bedding, den or nest material, whereas the adult stages are parasitic and feed on host blood. There are some 2,500 flea species, most parasitic on mammals (especially rodents) and some on birds. They vary in the time spent on their hosts ranging from transient feeders (rodent fleas) to permanent attachment (sticktight fleas and burrowing chigoes).



Flies and mosquitoes are winged insects with two pairs of wings attached to the thorax and a well-developed head with sensory and feeding organs. They undergo complete metamorphosis involving a pupation stage. Different species vary in their feeding habits, both as adults (parasitic or free-living) and larvae (parasitic or free-living). There are over 120,000 species belonging to 140 families. Two main suborders are recognized on the basis of structural differences, Nematocera (adult stages parasitic, larval stages often free-swimming) and Brachycera (adult stages parasitic or free-living, larvae stages often predaceous).

Lice are small wingless insects, dorsoventrally flattened, with reduced or no eyes and enlarged tarsal claws for clinging. All lice undergo gradual metamorphosis and there are no free-living stages. Eggs are cemented to hair/feathers whereas nymphs and adults cling to hair/feathers. Two orders of lice are recognized on the basis of their mouthparts: the Mallophaga (chewing/biting lice) with some 3,000 species infesting birds and mammals; and the Anoplura (sucking lice) with 500 species found on mammals

Many non-spider arachnids (subclass Acari) are found as parasites on animal or plant hosts. They belong to two main groups: the macroscopic ticks and the microscopic mites. Many species are important in human and animal medicine as causes of disease or as transmission vectors for other pathogens.

Ticks are epidermal parasites of terrestrial vertebrates that may cause anaemia, dermatosis, paralysis, otoacariasis and other infections (transmit viral, bacterial, rickettsial, spirochaete, protozoal and helminth pathogens). They feed mainly on blood and their mouthparts are armed with small backward-facing teeth to aid in attachment. All ticks undergo gradual/incomplete metamorphosis whereby larval and nymphal instars resemble adults. The integument is relatively thick and respiration occurs via spiracles (usually only one pair) and trachea. Two major families of ticks are recognized on the basis of many morphological features: the Ixodidae (hard ticks with a tough cuticle and a large anterodorsal scutum) with some 650 species that infest mammals, birds and reptiles; and the Argasidae (soft ticks with a leathery integument and no scutum) with 160 species that infest mainly birds and some mammals.

Mites are microscopic arachnids which undergo gradual or incomplete metamorphosis. Adults and nymphs have 4 pairs of legs whereas larvae have 3 pairs. Over 30,000 species of mites have been described, many are free-living species, some are plant parasites while others are parasitic on terrestrial and aquatic hosts. Most parasitic species feed on skin debris or suck lymph, some burrow into the skin, some live in hair follicles, and some in the ear canals. Their mouthparts are variable in form but the hypostome is never armed with teeth. The integument is usually thin and three orders are recognized on the basis of their respiratory systems: the Mesostigmata with respiratory spiracles (stigmata) near the third coxae; the Prostigmata (Trombidiformes) with spiracles between the chelicerae or on the dorsal hysterosoma; and the Astigmata (Sarcoptiformes) without tracheal systems as they respire through the tegument.

TAXONOMIC CLASSIFICATION OF PARASITES

Definitive hosts (support sexual development of parasite) listed where appropriate;
otherwise: IH = intermediate host (asexual development); PH = paratenic (transport) host

Kingdom: **Protista** (unicellular eukaryotes)

Subkingdom: **Protozoa** (motile protists)

Phylum: **Amoebozoa** (locomotion by pseudopodia, asexual development)

Subphylum: Conosa (archamoebae & mycetozoa, many flagellated forms, flagellar root with microtubular cone)
Class: Archamoebae (amoebae (no flagellates), cysts rounded, uni-/multi-nucleate, amitochondriate)
Family: Entamoebidae (uninucleate amoeboid forms, symbiotic in digestive tract of vertebrates)
Entamoeba mammals, colon (liver/brain), direct (faecal-oral)

Subphylum: Lobosa (with lobose pseudopodia)
Class: Discosea/Flabellinea (flattened forms, protoplasmic flow polyaxial)
Order: Dactylopodida (tapering finger-like subpseudopodia (= dactylopodia), most do not form cysts)
Family: Paramoebidae (digitiform subpseudopodia, parasomes (Nebenkorper) near nucleus, scales on cell surface)
Paramoeba fish, skin/gills, direct (water)
Family: Vexilliferidae (long slender subpseudopodia, spiny appearance, many with glycostyles on cell surface)
Neoparamoeba fish, gills, direct (water)
Order: Centramoebida (finely-tapering subpseudopodia (= acanthopodia), most form cysts)
Family: Acanthamoebidae (trophozoites flattened, prominent subpseudopodia, cysts stellate)
Acanthamoeba human, CNS, direct (water)
Family: Balamuthiidae (large vesicular nucleus, cysts rounded)
Balamuthia mammals, CNS, direct (soil/water)

Phylum: **Heterolobosea** (diverse group, incl. amoebo-flagellates, most form cysts or clusters of fruiting bodies)
Order: Schizopyrenida (no fruiting bodies)
Family: Vahlkampfidae (eruptive limax amoeboid form cylindrical, most form temporary flagellated stages)
Naegleria human, CNS, opportunist (normally free-living)

Phylum: **Mastigophora** (locomotion by flagella, asexual development)
Class: Zoomastigophora (zooflagellates, without chloroplasts)
Order: Diplomonadida (with 1-2 karyomastigonts (each with 4 basal bodies/flagella associated with nucleus))
Family: Hexamitidae (2 karyomastigonts arranged in binary axial symmetry)
Giardia vertebrates, small intestines, direct (faecal-oral)
Hexamita, Spironucleus vertebrates, small intestines/organs/skin, direct (faecal-oral, water-borne)

Phylum: **Parabasalia** (anaerobic flagellates, with parabasal body supporting Golgi cisternae or dictyosome)
Class: Trichomonada (simple parabasalids, some with reduced mastigonts, some with several karyomastigonts)
Order: Trichomonadida (with 3-5 anterior flagella, and single recurrent flagellum)
Family: Monocercomonadidae (simplest forms, costa absent, most without undulating membrane, some aflagellate)
Histomonas birds, caeca/liver, direct (+ nematode PH)
Dientamoeba humans/rodents, gut, direct (faecal-oral)
Family: Trichomonadidae (stout axostyle, costa present, supporting undulating membrane)
Trichomonas mammals/birds, gut/urogenital tract, direct

Phylum: **Euglenozoa** (flagella inserted in anterior pocket, some heterotrophs, some autotrophs (with chloroplasts))
Order: Kinetoplastida (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)
Suborder: Trypanosomatina (single anterior flagellum, non-emergent/emergent, often forming undulating membrane)
Family: Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)
Trypanosoma brucei vertebrates, blood, indirect (fly vectors)
Trypanosoma cruzi vertebrates, tissues, indirect (bug vectors)
Leishmania vertebrates, tissues, indirect (sandfly vectors)
Suborder: Bodonina (mastigotes with two heterodynamic flagella, one trailing)
Family: Bodonidae (free-living bactivores, sometimes symbiotic on fish)
Ichthyobodo (= *Costia*) fish, gill/skin, direct (water)

Phylum: **Apicomplexa** (with apical complex, all parasitic, sexual development (gamogony))

Class: Conoidasida (with conoid)
Subclass: Coccidiasina (small intracellular gamonts)
Order: Eucoccidiorida (cyclic merogony (schizogony), gamogony, sporogony)
Suborder: Adeleina (syzygy, 1-4 microgametes)
Family: Haemogregarinidae (ookinete, gamonts in blood cells, invertebrate vectors)
Haemogregarina reptiles/amphibia/fish, tissues/blood, indirect (leech/arthropod vectors)
Hepatozoon mammals/birds/reptiles, tissues/blood, indirect (ingestion of arthropod vector)
Family: Klossiellidae (zygote inactive, sporocysts formed (rather than oocysts))
Klossiella mammals, kidney, direct
Suborder: Eimeriorina (no syzygy, many microgametes)
Family: Cryptosporidiidae (epicellular location, attachment organelle present, oocysts with 4 naked sporozoites)
Cryptosporidium vertebrates, intestines/stomach/lungs, direct (faecal-oral)
Family: Eimeriidae (monoxenous, endogenous intracellular merogony and gamogony, exogenous sporogony)
Caryospora birds/reptiles/mammals, gut, direct (faecal-oral)
Cyclospora mammals/reptiles, gut, direct (faecal-oral)
Cystoisospora (= mammalian *Isospora*) mammals, intestines, direct (faecal-oral + predator-prey)
(C. felis, C. belli, C. canis, C. ohioensis, C. rivolta, C. suis)
Eimeria non-carnivorous vertebrates, intestines + tissues, direct (faecal-oral)
Epieimeria fish, gut/intestines, direct (faecal-oral, water)
Globidium mammals, gut+tissues, direct?
Goussia fish, gut, direct (faecal-oral, water)
Isospora (+ *Atoxoplasma*) birds, intestines, direct (faecal-oral)
Family: Lankesterellidae (heteroxenous, merogony in tissues, sporozoites in blood cells, leech/arthropod vectors)
Lankesterella (+ Atoxoplasma) birds/reptiles/amphibia, blood, indirect (invertebrate vector)
Family: Dactylosomatidae (merogony in erythrocytes of cold-blooded vertebrates, vectors unknown)
Schellackia reptiles, blood, indirect (tick vectors?)
Family: Sarcocystidae (heteroxenous, oocysts with two sporocysts, tissue cyst formation in intermediate host)
Subfamily: Sarcocystinae (metrocytes present in cysts, simple/elaborate cyst walls)
Frenkelia birds/mammals/reptiles, gut/tissues, indirect (predator-prey)
Sarcocystis mammals/birds/reptiles, gut/muscles, indirect (predator-prey)
Subfamily: Toxoplasmatinae (metrocytes not present, thin cyst walls)
Besnoita mammals/reptiles, gut/tissues, indirect (predator-prey)
Hammondia mammals, gut/tissues, indirect (predator-prey)
Neospora dogs/herbivores, gut/muscles/CNS, indirect (predator-prey)
Toxoplasma cats/vertebrates, gut/muscles/CNS, indirect (predator-prey + vertical)

Class: Aconoidasida (without conoid)
Order: Haemospororida (pleomorphic stages in blood of vertebrates, insect vectors. motile zygote (ookinete))
Family: Plasmodiidae (schizogony in tissues then blood cells, gamonts in blood cells)
Billbraya, Fallisia, Haemocystidium, Hemolivia, Hepatocystis, Johnsprentia, Pirhemocyton
Plasmodium mammals/birds/reptiles, liver/erythrocytes, indirect (mosquito vectors)
Haemoproteus birds, endothelia/erythrocytes, indirect (hippoboscid fly vectors)
Leucocytozoon birds, tissues/leucocytes, indirect (simuliid fly vectors)
Order: Piroplasmidora (pear-shaped stages in blood cells of vertebrates, tick vectors)
Family: Babesiidae (merogony in erythrocytes, trans-stadial + trans-ovarian transmission in ticks)
Babesia mammals, erythrocytes, indirect (ixodid tick vectors)
Family: Theileriidae (merogony in leucocytes then erythrocytes, trans-stadial transmission in ticks)
Theileria ruminants, leucocytes/erythrocytes, indirect (ixodid tick vectors)
Family: Haemohormidiidae (merogony in blood cells of fish, amphibians, reptiles, birds)
Haemohormidium fish/amphibia/reptiles/birds, blood, indirect (vectors unknown)

Phylum: **Ciliophora** (with cilia, nuclear dualism, pellicular alveoli, reproductive conjugation)
Subphylum: Intramacronucleata (microtubules occur inside macronuclear envelope during division))

Class: Litostomatea (simple mouths, special somatic kineties)
Subclass: Trichostomatia (endosymbionts, holotrichous ciliation)
Order: Vestibuliferida (distinct oral depression (= vestibulum))
Family: Balantidiidae (monoxenous symbiotes, in vertebrates, sometimes histophagous)
Balantidium pigs/primates, large intestine, direct (faecal-oral)

Class: Phyllopharyngea (cytopharynx with leaf-like phyllae)
Subclass: Phyllopharyngia (cyrtos, ventral cilia)
Order: Chlamydodontida (body dorsoventrally flattened, ventral cilia thigmotactic)
Family: Chilodonellidae (reniform body dorsoventrally flattened, two fields of dorsal ciliary rows)
Chilodonella fish, gills/skin, direct (water)

Class: Oligohymenophorea (distinct oral ciliature, comprising right paroral membrane and 3 left membranelles)
Subclass: Scuticociliatia (with scuticum or scuticovestige)
Order: Philasterida (short paroral dikinetid membrane)
Family: Uronematidae (membranelles aligned with long axis, anterior pole non-ciliated)
Uronema fish, tissues, opportunist (normally free-living)
Subclass: Hymenostomatida (right paroral dikinetid plus 1-3 left polykinetids)
Order: Hymenostomatida (preoral suture, somatic monokinetids)
Suborder: Ophryoglenina (with organelle of Lieberkuhn (watchglass organelle))
Family: Ichthyophthiriidae (monoxenous ectoparasites, form encysted tomonts which release swarmers/theronts)
Ichthyophthirius, Cryptocaryon fish, skin/gills, direct (water)
Suborder: Tetrahymenina (organelle of Lieberkuhn absent)
Family: Tetrahymenidae (pyriform body, longitudinal ciliary rows)
Tetrahymena fish, skin/gills/organs, direct (water)
Subclass: Peritrichia (lacking somatic kineties, oral cilia extend from infundibulum)
Order: Mobilida (mature trophont mobile, aboral holdfast organelle)
Family: Trichodinidae (stout cylindrical body, posterior adhesive disc with denticular ring)
Trichodina fish, skin/gills, direct (water)

Kingdom: **Fungi**
Division: Microsporidia (form unicellular spores, with coiled polar tubes, amitochrondriate, all parasitic)

Class: **Microsporea** (polar filament well-formed, oval spores)
Order: Microsporida (polaroplast present)
Suborder: Apansporoblastina (sporophorous vesicle absent)
Family: Nosematidae (all stages diplokaryotic)
Nosema insects (bees), tissues, direct?
Family: Unikaryonidae (all stages unikaryotic, in cell cytoplasm or in parasitophorous vacuole)
Encephalitozoon mammals, tissues, direct?
Enterocytozoon mammals, gut, direct?
Suborder: Pansporoblastina (sporophorous vesicle present)
Family: Glugeidae (all stages unikaryotic, numerous sporoblasts formed in vesicles)
Glugea fish, tissues, direct?
Pleistophora fish, muscles, direct (water)
Trachipleistophora fish, tissues, direct?
Pseudoloma fish, nervous system, direct (water, carnivorism
Family: Thelohaniidae (meronts diplokaryotic, spores unikaryotic, 8 spores formed in each vesicle)
Thelohania crustaceans/insects, tissues, direct?

Kingdom: **Animalia** (multicellular eukaryotic animals)

Phylum: **Cnidaria** (diverse group with stinging cells; including sea anemones, corals, jellyfish, hydrozoa, myxozoa)

Subphylum: **Myxozoa** (form multicellular valved spores with polar filaments)
Class: Myxosporea (spores with 1-2 sporoplasms, 1-6 polar capsules)
Order: Bivalvulida (spores with two valves)
Suborder: Platysporina (polar capsules in sutural plane)
Family: Myxobolidae (spores flattened, suture forms elevated ridge, one polar capsule smaller than the other)
Myxobolus fish, tissues, direct + indirect?
Order: Multivalvulida (radially symmetrical spores, 3-7 valves, 3-7 polar capsules grouped together at apex)
Family: Kudoidae (four valves and polar capsules, mainly histozoic in muscles of marine fish)
Kudoa fish, muscles, direct/indirect?
Family: Trilosporidae (three valves and polar capsules, coelozoic/histozoic in marine fish)
Unicapsula fish, muscles, direct/indirect?

Phylum: **Nemathelminthes** (unsegmented, pseudocoelomate worms, free-living/symbiotic species)

Class: **Nematoda** (roundworms, hydrostatic skeletons, longitudinal musculature, sexual dimorphism, spicules present)

Subclass: Secernentea (= Phasmidia) (phasmids present, amphids anterior, setae absent on females, single testis in males, cuticle 2-4 layers)

Order: **Rhabditida** (small worms, commonly with 6 lips, tripartite muscular oesophagus)

Superfamily: Rhabditoidea (free-living/parasitic in invertebrates/lower vertebrates)
Family: Strongyloididae (threadworms, parasitic parthenogenetic females, free-living sexual generations)
Strongyloides mammals/birds, small intestines, direct (faecal-oral + transmammary)
Family: Rhabdiasidae (protandrous hermaphrodite (male becomes female), parasitic and free-living generations)
Rhabditis, Pelodera animals, skin, direct (faecal-oral, transdermal)

Order: **Strongylida** (slender worms, oesophagus lacking bulb, well-developed copulatory bursa in males)

Superfamily: **Trichostrongyloidea** (hair-like trichostrongyles, found in gut, infection by ingestion of L3)
Family: Trichostrongylidae (lips reduced/absent, females lay thin-shelled eggs in morula stage, direct cycles)
Trichostrongylus herbivorous mammals/birds, gut, direct (faecal-oral)
Ostertagia ruminants, abomasum, direct (faecal-oral)
Telodorsagia ruminants, abomasum, direct (faecal-oral)
Haemonchus ruminants, abomasum, direct (faecal-oral)
Cooperia ruminants, small intestines, direct (faecal-oral)
Nematodirus ruminants, small intestines, direct (faecal-oral)
Hypostrongylus pig, stomach, direct (faecal-oral)
Family: Dictylocaulidae (lung worms, direct cycle, infection by ingestion of L3)
Dictylocaulus ruminants/equids/camelids, lungs, direct (faecal-oral)
Family: Ollulanidae (head with spiral coil, female tail with cusps, viviparous (develop to L3 in uterus))
Ollulanus cat/fox/pig, stomach, direct (ingestion of vomitus)
Family: Heligmosomidae (adults filiform, reddish in colour, direct cycle)
Nippostrongylus rodents, small intestines, direct (percutaneous)

Superfamily: **Strongyloidea** (strongyles, large buccal capsules, often with teeth/leaf crown, infection by ingestion of L3)
Family: Strongylidae (three pairs of branches in dorsal ray, equid hosts,)
Subfamily: Strongylinae (large strongyles, red-worms, globular buccal capsules)
Strongylus equines, caecum/colon, direct (faecal-oral)
Triodontophorus equines, caecum/colon, direct (faecal-oral)
Subfamily: Cyathostominae (small strongyles, cylindrical buccal capsule)
Cyathostomum, Poteriostomum equines, caecum/colon, direct (faecal-oral)
Family: Chabertiidae (nodular worms, two pairs of branches in dorsal ray)
Chabertia ruminants, caecum/colon, direct (faecal-oral)
Oesophagostomum ruminants/pigs/humans, caecum/colon, direct (faecal-oral)
Family: Stephanuridae (kidney-worm, in pigs)
Stephanurus pig, kidneys, direct (faecal-oral, transdermal) + earthworm PHs
Family: Syngamidae (gapeworm, in trachea of birds and mammals)
Syngamus birds, trachea, direct (faecal-oral) + earthworm/mollusc PHs

Superfamily: **Ancylostomatoidea** (hookworms, large buccal capsule bent dorsally, armed with teeth/cutting plates, infection usually by percutaneous/transdermal penetration of infective L3)
Family: Ancylostomatidae (single family)
Ancylostoma humans/dogs/cats, small intestines, direct (faecal-oral, transdermal)
Necator humans/pigs, small intestines, direct (faecal-oral, transdermal)
Uncinaria dogs/foxes/cats, small intestines, direct (faecal-oral)
Bunostomum ruminants, small intestines, direct (transdermal, faecal-oral)
Globocephalus pigs, small intestines, direct (faecal-oral)
Gaigeria sheep, small intestines, direct (transdermal)

Superfamily: **Metastrongyloidea** (lungworms, small buccal capsule, bursa reduced, indirect cycles with molluscan IH)
Family: Metastrongylidae (infection of pigs by ingestion of earthworm/molluscan IH carrying L3)
Metastrongylus pigs, lungs, indirect (molluscan IH)
Family: Protostrongylidae (infection of ruminants by ingestion of earthworm/molluscan IH carrying L3)
Protostrongylus, Muellerius sheep/goats, lungs, indirect (mollusc IH)
Parelaphostrongylus cervids, brain, indirect (snail IH)
Elaphostrongylus cervids, muscles, indirect (snail IH)
Family: Angiostrongylidae (no buccal cavity, infection of vertebrates by ingestion of earthworm/molluscan IH)
Aelurostrongylus cats, lungs, indirect (mollusc IH) + PHs
Angiostrongylus dog, pulmonary artery, indirect (mollusc IH) + PHs
Parastrongylus (formerly *Angiostrongylus*) rat, pulmonary artery, indirect (mollusc IH) + PHs
Family: Filaroididae (direct cycle, infection of carnivores by ingestion of L1)
Filaroides dogs/mustelids, lungs, direct (faecal-oral)
Oslerus dogs, trachea, direct (faecal-oral)

Order: **Ascaridida** (large round-worms, mouth opening surrounded by three large lips, numerous caudal papillae)

Superfamily: Ascaridoidea (ascarids, eggs thick-shelled, direct cycle but larvae undertake hepato-pulmonary migration)
Family: Ascarididae (large pale roundworms, in terrestrial mammals)
Ascaris humans/pigs, small intestines, direct (faecal-oral)
Parascaris horses, small intestines, direct (faecal-oral)
Toxascaris dogs/foxes/cats, small intestines, direct (+ PHs)
Toxocara dogs/cats/bovids, small intestines, direct (vertical + faecal-oral) + PHs
Bayliascaris dogs/raccoons, small intestines, direct (faecal-oral) + PHs
Family: Anisakidae (large stout worms, in marine mammals/fishes/birds)
Anisakis dolphins/whales, gut, indirect (copepod IH) + fish PHs

Superfamily: Heterakoidea (preanal sucker anterior to cloaca in males, direct cycle, infection by ingestion of eggs)
Family: Heterakidae (worms with lateral alae, oesophagus with rounded terminal bulb)
Heterakis birds, caeca, direct (faecal-oral) + earthworm PHs
Family: Ascaridiidae (slender club-shaped oesophagus without rounded terminal bulb)
Ascaridia birds, small intestines, direct (faecal-oral) +earthworm PHs

Order: **Oxyurida** (small pin-worms, pointed tails, oesophagus with terminal bulb, males with single spicule, haplodiploidy (haploid males develop parthenogenetically, diploid females develop from fertilized eggs))

Superfamily: Oxyuroidea (common in mammals, birds, reptiles, amphibians)
Family: Oxyuridae (direct cycle, females deposit sticky eggs around anus, infection by ingestion of egg)
Oxyuris horse, large intestines, direct (faecal-oral)
Enterobius humans, large intestines, direct (faecal-oral)
Passalurus rabbits, large intestines, direct (faecal-oral)
Syphacia rodents, large intestines, direct (faecal-oral)

Order: **Spirurida** (oesophagus divided into anterior muscular and posterior glandular portions, never with bulb, coiled tail in males, two spicules invariably dissimilar, indirect cycles, arthropod IHs)

Superfamily: **Spiruroidea** (two prominent trilobed lips (pseudolabia), infect oesophagus/stomach (crop/gizzard))
Family: Habronematidae (pharynx with dorsal and ventral tooth, indirect cycle involving ingestion of fly)
Habronema, Draschia horses, stomach, indirect (muscid flies IH)
Family: Gongylonematidae (anterior cuticle covered with large bosses or irregular scutes arranged in 8 rows)
Gongylonema cattle/sheep, oesophagus, indirect (beetle/cockroach IH)
Family: Tetrameridae (extravagant sexual dimorphism, females swollen, coloured bright red)
Tetrameres birds, proventriculus, indirect (water fleas/grasshoppers IH)
Family: Acuariidae (with peculiar anterior grooves/ridges (cordons) or extravagant cuticular projections, in birds)
Acuaria, Cheilospirura, Dispharynx birds, gizzard, indirect (water fleas/grasshoppers/beetles IH)
Family: Thelaziidae (hexagonal mouth, lacking lips, conspicuous transverse anterior striations, live on surface of eye)
Thelazia cattle/horses, conjunctiva, indirect (muscid flies IH)
Oxyspirura birds, eye, indirect (cockroaches IH)
Family: Physalopteridae (two large lateral pseudolabia, armed with teeth, lips with basal collar, caudal alae on males)
Physaloptera cats, stomach, indirect (crickets IH) + vertebrate PHs

Family: Spirocercidae (stout pink-red worm, well-developed buccal capsule, with 6 rudimentary lips)
Spirocerca dog, oespohagus/aorta, indirect (beetle IH) + vertebrate PHs
Ascarops, Physocephalus pigs, stomach, indirect (beetles IH) + vertebrate PHs
Cylicospirura, Cyathospirura cats/foxes/dasyurids, stomach, indirect (beetle IH)
Family: Gnathostomidae (swollen anterior head bulb, covered with rows of hooks, two lateral lips, four cervical sacs)
Gnathostoma cats/pigs, stomach, indirect (copepod IH) + vertebrate PHs

Superfamily: **Filarioidea** (tissue-dwelling filarial parasites, lack lips, infect subcutaneous/intermuscular tissues, blood vessels or lymphatic systems of hosts, indirect cycles with arthropod IH)
Family: Filariidae (numerous anterior papillae and cuticular ridges, lay eggs with L1 already fully formed)
Parafilaria horses/cattle, connective tissue, indirect (muscid flies IH)
Stephanofilaria cattle, skin, indirect (buffalo flies IH)
Family: Onchocercidae (adults loose in tissues or in nodules, viviparous (live birth of L1 microfilariae))
Onchocerca humans/cattle/horses, connective tissue, indirect (ceratopogonid/simuliid IH)
Dirofilaria dogs/cats/humans, heart, indirect (mosquito IH)
Dipetalonema/Acanthocheilonema dogs/camelids/humans, connective tissue, indirect (fleas/lice IH)
Wuchereria humans, lymphatics, indirect (mosquito IH)
Brugia humans/cats, lymphatics, indirect (mosquito IH)
Setaria sheep/cattle/horses, peritoneum/eye/scrotum, indirect (mosquito IH)
Loa humans, subcutaneous, indirect (fly IH)
Mansonella humans, body cavities, indirect (midges IH)

Order: **Camallanida** (conspicuous phasmids, L1 with dorsal prominence/tooth, ovoviviparous, L1-L3 in copepod)
Family: Camallanidae (buccal capsule well-developed, with pair sclerotized valves, male with caudal alae)
Camallanus fish, intestines, indirect (copepod IH)
Family: Dracunculidae (buccal capsule reduced, female highly enlarged, filled with L1)
Dracunculus humans, subcutaneous tissues, indirect (copepod IH)

Subclass: **Adenophorea** (= Aphasmidea = Enoplea) (phasmids absent, amphids behind lips, setae present, usually 2 testes in males, cuticle 4 layers)

Order: **Enoplida** (single (or no) spicule, stichosome oesophagus, L1 with stylet in rudimentary buccal capsule)
Family: Trichuridae (whipworms, sudden transition in width, slender anteriorly, barrel-shaped eggs with polar plugs)
Trichuris mammals, caeca, direct (faecal-oral)
Family: Capillariidae (gradual transition in width, in gut/respiratory tract of mammals/birds, eggs with polar plugs)
Capillaria (Eucoleus?) mammals/birds, various tissues, direct (faecal-oral) + earthworm PH
Pseudocapillaria fish, intestines, direct (water) + indirect oligochaete PHs
Family: Trichinellidae (males with copulatory pseudobursae, spicules absent, viviparous, juveniles and adults can occur in same host, juveniles intracellular in skeletal muscle nurse cell)
Trichinella mammals, small intestines/muscles, direct (carnivorism)

Phylum: **Acanthocephala** (thorny-headed worms, pseudocoelomate, unsegmented, anterior retractable proboscis with numerous hooks, lack gut, indirect cycles, eggs with acanthor, acanthella develops in arthropod IH (or PH))

Class: Archiacanthocephala (oval thick-shelled eggs, body wall lacunar canals dorsal & ventral (or just dorsal))
Order: Oligacanthorhynchida (proboscis subspherical, short rows of several hooks, protonephridial organs present)
Family: Oligacanthorhynchidae (single family)
Macracanthorhynchus pig, small intestines, indirect (beetle IH)
Oncicola cats/foxes/dingoes, small intestines, indirect (beetle IH + bird PHs)

Class: Palaeacanthocephala (elongate eggs, sometimes with polar thickenings, body wall lacunar canals lateral)
Order: Polymorphida (trunk wrinkled, proboscis bulbose/cylindrical, with numerous hooks in alternating rows)
Family: Polymorphidae (spinose trunk, proboscis bulbous, double-walled proboscis receptacle)
Polymorphus ducks, small intestines, indirect (copepod IH)

Phylum: **Platyhelminthes** (flatworms, acoelomate, free-living/parasitic, most parasites hermaphroditic, prominent attachment organs)

Class: **Cestoda** (tapeworms, gut absent, anterior scolex, proglottid segments, heteroxenous, predator-prey cycles)

Subclass: Eucestoda (larvae hexacanth (with six hooks))

Order: **Cyclophyllidea** (terrestrial species, scolex with four suckers, often bearing hooks, eggs release oncospheres)
Family: Taeniidae (tapeworms of carnivores/humans, scolex often armed, proglottids with unpaired reproductive organs and single genital pore, fluid-filled cystic metacestodes)
Taenia carnivores/omnivores, intestines/tissues, indirect (predator-prey)
Echinococcus dogs/omnivores, gut/tissues, indirect (predator-prey)
Multiceps dog/herbivores, muscle/brain, indirect (predator-prey)
Family: Anoplocephalidae (tapeworms of hoofed animals, scolex unarmed, cysticerci in arthropods)
Anoplocephala, Anaplocephaloides, Equinia, Moniezia, Thysaniezia
herbivores, intestines, indirect (soil mite/insect IH)
Family: Dipylidiidae (armed scolex, proglottids with paired reproductive organs and two lateral genital pores)
Dipylidium dog/cat, small intestines, indirect (flea/louse IH)
Family: Dilepididae (tapeworms of dog/cat and fowl, armed scolex, genital pores alternate, cysticercoid larva)
Amoebotaenia birds, small intestines, indirect (earthworms IH)
Choanotaenia birds, small intestines, indirect (beetle/fly IH)
Family: Davaineidae (tapeworms of birds, large rostellum with hammer-shaped hooks and spiny suckers)
Davainea birds, small intestines, indirect (terrestrial mollusc IH)
Raillietina birds, small intestines, indirect (ants/beetles/cockroaches IH)
Family: Hymenolepididae (tapeworms of birds/rodents/humans, slender strobilia, 1-4 testes, cysticercoid larva)
Hymenolepis mammals/birds, small intestines, indirect (arthropod/annelid/mollusc IH)

Order: **Diphyllobothriidea** (= **Pseudophyllidea**) (aquatic host species, unarmed scolex, with two grooves (bothria), genital organs and pores centrally placed, indirect cycles with two IH)
Family: Diphyllobothriidae (eggs release coracidium, more than one IH (procercoid in copepods, pleroceroids in frogs and other aquatic vertebrates) and often PHs)
Diphyllobothrium piscivorous mammals, small intestines, indirect (copepod IH-1/fw fish IH-2)
Spirometra carnivores, small intestines, indirect (copepod IH-1/frogs IH-2)
Family: Bothriocephalidae (eggs, hexacanth coracidia, procercoid larvae in copepods)
Bothriocephalus fish, intestines, indirect (copepod IH-1)

Class: **Trematoda** (flukes, most with dorsoventrally-flattened bodies, sac-like gut)

Subclass: **Digenea** (two or more hosts (one a mollusc), cycle involves larval miracidium, sac-like sporocyst/redia stages in snail, cercariae/metacercariae)

Order: **Echinostomatiformes** (adult with scales/spines, acetabulum near oral sucker, rediae with appendages, cercariae without eyespots, metacercariae in open or in IH-2)
Family: Fasciolidae (large leaf-shaped flukes, in herbivores, conical anterior end, ventral sucker at level of shoulders)
Fasciola mammals, liver, indirect (freshwater snail IH)
Fasciolopsis man/pig, intestines, indirect (planorbid snail IH)
Family: Echinostomidae (slender worms, collar of peglike spines, in piscivores, two IH (snails and fishes/frogs))
Echinostoma birds/mammals, gut, indirect (snail IH-1, molluscs/planaria/fish/tadpoles IH-2)

Order: **Paramphistomiformes** (thick fleshy worms, acetabulum near posterior end, rediae with appendages, cerceriae with two eyespots, metacercariae in open)
Family: Paramphistomidae (rumen flukes, conical shape, water snail IH)
Paramphistomum, Calicophoron, Orthocoelium
cattle/sheep, rumen/reticulum, indirect (planorbid snail IH)
Gastrodiscoides pig/humans, large intestine, indirect (snail IH)

Order: **Opisthorchiformes** (medium-sized flukes, often spinose, no cirrus sac, rediae without appendages, cerceriae with two eyespots, metacercariae in second IH)
Family: Opisthorchidae (delicate leaf-shaped flukes, in bile ducts of fish-eating mammals, two IH (snails and fish))
Opisthorchis piscivorous mammals, liver, indirect (fw snail IH-1, fw fish IH-2)
Clonorchis carnivores, liver, indirect (fw snail IH-1, fw fish IH-2)
Metorchis cat/dog/fox/seal, liver, indirect (snail IH-1, fish IH-2)

Family: Heterophyidae (tiny pyriform flukes, in intestines of mammals/birds, two IH (snails and fishes/frogs))
Heterophyes carnivores, intestines, indirect (fw snail IH-1, fw fish IH-2)
Metagonimus dogs/cats/pigs/humans, small intestines, indirect (snail IH-1, fish IH-2)

Order: **Plagiorchiformes** (adult with spines, midventral acetabulum, cercariae with two eyespots, not furcate, metacercariae in second IH)
Family: Dicrocoeliidae (small lancet-like flukes, eggs ingested by snails, no redial stage, two-three IH)
Dicrocoelium ruminants, liver, indirect (terrestrial snail IH-1, ant IH-2)
Family: Troglotrematidae (thick oval flukes, scale-like spines, miracidia, snail IH-1, crustacean/insect IH-2)
Paragonimus carnivores, lungs, indirect (fw snail IH-1, fw crustaceans IH-2)

Order: **Strigeiformes** (adult with spines, midventral acetabulum, rediae without appendages, brevifurcate cercariae with two eyespots)
Family: Schistosomatidae (blood flukes, cylindrical bodies, in blood vessels of alimentary/urinary tract, separate sexes, male with gynaecophoric canal to hold female)
Schistosoma mammals/birds, blood vessels, indirect (fw snail IH)
Trichobilharzia, Austrobilharzia birds, blood vessels, indirect (snail vectors)

Class: **Monogenea** (monoxenous ectoparasites, sac-like gut, direct cycle, oncomiracidium with 3 ciliary bands)

Order: Monopisthocotylea (hermaphroditic, viviparous (sometimes hyperviviparity (Russian nested dolls))
Family: Gyrodactylidae (small worms, posterior haptor with pair of large central hooks and 16 small marginal hooks)
Gyrodactylus fish, skin/fins/gills, direct (water)
Family: Dactylogyridae (small worms, posterior haptor with pair of large ventral hooks and 14 small marginal hooks)
Dactylogyrus fish, gills, direct (water)
Family: Capsalidae (large worms, haptor with pair anterior suckers and two pairs of posterior hooks)
Benedenia fish, skin/gills, direct (water)

Phylum: **Arthropoda** (coelomate metameric invertebrate animals, chitinous exoskeleton, segmented body, jointed limbs, moults (ecdyses) between instars, metamorpohosis common)

Class: **Insecta** (three body regions (head, thorax, abdomen), six legs, single pair antennae, many with wings, free-living and parasitic species)

Order: **Phthiraptera** (lice, small wingless insects, permanent obligate ectoparasites, dorsoventrally flattened, stout legs and claws, incomplete metamorphosis (eggs, nymphs, adults))

Suborder: **Anoplura** (sucking lice, narrow pointed head, pierce skin and feed on fluids (solenophagy))
Family: Haematopinidae (short-nosed lice, ectoparasites of domestic animals, claws on ends of legs of similar size)
Haematopinus horses/cattle/pig, skin/hair, direct
Family: Linognathidae (long-nosed lice, claws on first leg smaller than those on other legs)
Linognathus ruminants/dogs/foxes, skin/hair, direct
Solenoptes cattle, skin/hair, direct
Family: Pediculidae (head/body/pubic lice of primates)
Pediculus primates, skin/hair, direct
Phthirus humans, skin/hair, direct

Suborder: **Mallophaga** (= wool-eating) (chewing lice, broad rounded head, feed on keratin, host/site specific)
Superfamily: Ischnocera (without maxillary palps, prominent filiform antennae, keratin feeders (hairs/feathers))
Family: Trichodectidae (parasitize mammals, 3-segmented antennae, single claw on tarsi)
Trichodectes dogs, skin/hair, direct
Bovicola (= *Damalinia*) sheep/cattle, skin/fleece/hair, direct
Werneckiella (= Damalinia) horses, skin/hair, direct
Felicola cats, skin/hair, direct
Family: Philopteridae (parasitize birds, five-segmented antennae, paired claws on tarsi)
Lipeurus, Goniocotes birds, skin/feathers, direct
Superfamily: Amblycera (with maxillary palps, large rounded heads, 4-segmented antennae in antennal grooves)
Family: Menoponidae (parasitize birds)
Menopon, Menacanthus birds, skin/feathers, direct
Family: Boopiidae (parasitize mammals/marsupials)
Heterodoxus dogs/macropods, skin/hair, direct

Order: **Hemiptera** (true bugs/aphids/scale insects, mouthparts with stylet-like mandibles/maxillae, gradual metamorphosis)
Suborder: Heteroptera (some plant-feeders, some predatory on other arthropods, some blood-feeders on vertebrates)
Family: Cimicidae (small wingless bugs, incl. bed-bugs, blood feeders on animals)
Cimex mammals/birds, skin, direct
Family: Reduviidae (large winged cone-nose/kissing/assasin bugs, incl. triatome bugs, blood feeders on animals)
Triatoma mammals, skin, direct

Order: **Siphonaptera** (fleas, wingless (=-aptera), adults feed on blood ("siphon-), laterally compressed, third pair of legs adapted for jumping, complete metamorphosis with vermiform larvae, pupation in silk cocoons)
Family: Pulicidae (parasites of mammals)
Pulex humans/dogs/cats, skin, direct
Echidnophaga mammals/birds, skin, direct
Ctenocephalides dogs/cats/humans, skin, direct
Xenopsylla rats/dogs/cats/humans, skin, direct
Spilopsyllus rabbit, skin, direct
Family: Tungidae (chigoes/jiggers/chiggers/chique/sand fleas, females burrow under skin, enclosed in sinus)
Tunga mammals, skin, direct

Order: **Diptera** (flies, midges, mosquitoes, with single pair of membranous forewings (diptera), hindwings modified into halteres, complete metamorphosis with vermiform larvae)

Suborder: **Nematocera** (small midges/mosquitoes, long filamentous segmented antennae (= nemato-cera), aquatic life-cycles (larval/pupal stages associated with water), female adults require blood meal before they can lay eggs)
Family: Culicidae (mosquitoes, elongate mouthparts form proboscis, slender wings with scales on veins/margins)
Subfamily: Culicinae (scutellum with trilobed posterior margin, scaly abdomen, larva with prominent air-tube)
Culex, Aedes, Mansonia mammals/birds, skin, direct
Subfamily: Anophelinae (scutellum rounded or straight, addominal sternites lack scales, larva lacks air-tube)
Anopheles mammals/birds, skin, direct
Family: Ceratopogonidae (small biting midges/sand flies, narrow spotted wings, maritime species associated with mangroves/swamps; native species associated with freshwater; introduced species associated with dung)
Culicoides (incl. *Lasiohelea*) mammals/birds, skin, direct
Family: Simuliidae (small black flies/buffalo gnats, characteristic humped backs, wings not patterned or hairy)
Simulium, Austrosimulium mammals/birds, skin, direct
Family: Psychodidae (moth flies/sandflies, incl. phlebotomines, characteristically hairy bodies and wings)
Phlebotomus/Sergentomyia/Lutzomyia mammals/birds, skin, direct

Suborder: **Brachycera** (large tabanid/March flies, with stout and fewer antennal segments (= brachy-cera), females with slashing-sponging mouthparts to pierce skin and feed on pool of blood (telmophagy))
Family: Tabanidae (large stout horse/deer/March flies, often brightly coloured, painful bite, daytime feeders)
Chrysops, Tabanus mammals, skin, direct

Suborder: **Cyclorrhapha** (Muscomorpha) (small-medium sized flies, short pendulous antennae composed of 3 segments usually with feather-like arista, sponging/biting mouthparts, some cause larval myiases)
Family: Glossinidae (tsetse flies, biting mouthparts, characteristic proboscis bulb, both sexes blood-feeders)
Glossina mammals, skin, direct
Family: Hippoboscidae (flat/louse flies, leathery abdomen, piercing mouthparts, strong claws on feet)
Melophagus sheep, skin/fleece, direct
Hippobosca mammals, skin, direct
Family: Muscidae (house/bush/stable/buffalo flies, nuisance flies, synanthropic (associated with human activity))
Subfamily: Muscinae (with sucking mouthparts adapted to feeding on decaying organic matter)
Musca mammals, nonparasitic
Subfamily: Stomoxinae (with elongate biting mouthparts adapted to blood feeding)
Stomoxys mammals, skin, direct
Haematobia (= Lyperosia) bovines, skin, direct
Family: Calliphoridae (blow flies, often metallic, larvae cause myiases (flystrike/screw-worm infestation))
Lucilia (primary), *Calliphora* (secondary), *Chrysomyia* (secondary/primary)
mammals, skin/subcutaneous tissues, direct
Cochliomyia (primary screw-worm) *Chrysomyia* (Old World screw-worm)
mammals, skin/subcutaneous tissues, direct
Cordylobia (tumbu fly) mammals, skin/subcutaneous tissues, direct
Family: Sarcophagidae (flesh flies, not metallic, breed in excrement/carrion/decomposing organic matter)
Sarcophaga mammals, skin/subcutaneous tissues, direct

Family: Oestridae (large hairy bot-flies, third larval stage or bot resemble small sausages, larvae cause myiases)
Subfamily: Cuterebrinae (skin bot flies)
Dermatobia cattle/humans, skin, direct
Subfamily: Oestrinae (head maggots)
Oestrus sheep, nasal sinuses, direct
Subfamily: Hypodermatinae (cattle grubs, ox warbles, heel flies)
Hypoderma cattle, subcutaneous tissues, direct
Subfamily: Gasterophilinae (stomach bots)
Gasterophilus equines, stomach, direct

Class: **Arachnida** (spiders, scorpions, ticks, mites, body divided into two parts, head (capitulum, gnathosoma) and body (idiosoma), 4 pairs of legs (except for larvae with 3 pairs), no antennae, incomplete metamorphosis)

Order: **Acarina** (Acari) (ticks & mites, segmentation inconspicuous/absent, sac-like body, mouth and appendages on capitulum)

Suborder: **Ixodida** (= **Metastigmata**) (ticks, macroscopic, spiracles/stigmata posterior to legs, hypostome toothed, exposed, obligate blood-feeding ectoparasites of vertebrates)
Family: Argasidae (soft ticks, lack dorsal scutum, capitulum covered by body, hide in cracks/crevices)
Argas birds, skin, direct
Ornithodorus, Otobius mammals, skin, direct
Family: Ixodidae (hard ticks, with dorsal scutum, capitulum projects anteriorly, attach and feed on 1, 2 or 3 hosts)
Ixodes mammals/birds, skin, direct
Rhipicephalus (= Boophilus) mammals, skin, direct
Haemaphysalis mammals, skin, direct
Amblyomma (*Aponomma*) mammals/reptiles, skin, direct
Boophilus mammals, skin, direct
Dermacentor mammals, skin, direct

Suborder: **Mesostigmata** (gamesid mites, legs grouped anteriorly, spiracles/stigmata between second and fourth legs)
Family: Macronyssidae (large blood-sucking ectoparasites, only protonymph and adults feed, relatively long legs)
Ornithonyssus birds, skin/feathers, direct
Family: Dermanyssidae (large blood-feeding ectoparasites, greyish-white bodies becoming red when engorged)
Dermanyssus mammals/birds, skin/feathers, direct
Family: Halarachnidae (obligate parasites in respiratory tracts or ears of mammals)
Pneumonyssoides dogs/primates, nasal passages/sinuses, direct
Raillietia mammals, ears, direct
Family: Rhinonyssidae (parasites of nasopharynx of birds)
Sternosoma birds, nasal passages, direct
Family: Varroidae (bee mites, flat button shape, red-brown colouration, suck haemolymph)
Varroa bees, cuticle, direct

Suborder: **Prostigmata** (Trombidiformes) (mites with spiracles/stigmata on capitulum, distinct setae on body/legs)
Family: Demodecidae (small follicle mites, elongate cigar-shaped body, 4 pairs stumpy legs at front of body)
Demodex mammals, hairs, direct
Family: Cheyletidae (predatory and parasitic mites, body with waist, palps enlarged, legs terminate in combs)
Cheyletiella dogs/cats/rabbits, skin, direct
Family: Psorergatidae (body circular, legs regularly spaced, long posterior setae, legs with inward-curved spines)
Psorergates sheep, skin, direct
Family: Trombiculidae (only larval stages parasitic, nymphs and adults free-living)
Eutrombicula, Neotrombicula, Guntheria
mammals/birds, skin, direct

Suborder: **Astigmata** (Sarcoptiformes) (mange mites, without spiracles, respire through body surface, first two pairs of legs separated from posterior pairs, lack claws, with sucker-like modifications)
Family: Psoroptidae (non-burrowing mites, oval bodies, third and fourth pairs of legs project beyond body margin)
Psoroptes ruminants/horses/rabbits, skin, direct
Otodectes cats/dogs/foxes/ferrets, ear, direct
Chorioptes horses/sheep/cattle, skin, direct
Family: Sarcoptidae (burrowing mites, circular bodies, third and fourth legs do not project beyond body margin)
Sarcoptes humans/dogs, skin, direct
Notoedres cats/rabbits/rats, skin, direct
Trixacarus guinea pigs, skin, direct

Family: Knemidocoptidae (burrowing scaly leg and face mites, round body, no dorsal spines, short stubby legs)
Knemidocoptes, Neocnemidocoptes birds, skin, direct
Family: Ctyoditidae (respiratory parasites of birds, chelicerae absent, palps fused to form sucking organ)
Cytodites birds, air sacs, direct
Family: Listrophoridae (parasitic on fur-bearing mammals, distinct dorsal shield, legs modified for grasping hairs)
Lynxacarus cats, skin, direct
Lepoacarus rabbits/hares, skin, direct
Myocoptes mice, skin/hair, direct
Family: Atopomelidae (fur and feather mites)
Chirodiscoides guinea pigs, skin, direct
Family: Laminosioptidae (small mites, smooth elongated body, few setae, affect muscles of birds)
Laminosioptes birds, subcutaneous tissues, direct

Subphylum: **Crustacea** (chitinous cuticle, gills, 2 pairs antennae, mouthparts comprise pair mandibles and 2 pairs maxillae, segments with pair biramous extremities (podia), metamorphosis involving larval nauplius/zoea)

Class: **Copepoda** (elongate body, thorax with 7 somites (first few fused with head to form cepahalothorax), gradual metamorphosis with series of copepodod instars succeeding naupliar instars, some ectoparasitic forms)
Order: Cyclopoida (antennules short with 10-16 articles, buccal cavity open)
Family: Lernaeidae (females insert anterior attachment organ into host tissues, develop paired egg sacs, free-swimming naupliar stage, five copepodid stages and adults on hosts)
Lernaea fish, gills, direct (water)
Order: Poecilostomatoida (simple parasitic forms of fishes to bizarre symbiotic forms of invertebrates)
Family: Ergasilidae (antennae modified into powerful organs of prehension, parasitic in freshwater and marine fishes)
Ergasilus fish, gills, direct (water)

Class: **Branchiura** (head with flattened bilobed cephalic fold, antennae reduced, carapace expands laterally to form respiratory alae, blood suckers on fish)
Order: Argulidea (single order)
Family: Argulidae (fish lice, discoid body, attaches using hooks/suckers/barbs, stylus inserted to feed on blood)
Argulus fish, skin/gills, direct

Subphylum: **Pentastomatida** (tongue worms, crustacean-related parasites, lost virtually all appendages, elongate, segmented, anterior end with mouth and two pairs of tiny claws (penta-stome appearance))
Order: Porocephalida (mouth between or below anterior hooks, hooks with fulcrum, vuvla near posterior)
Family: Porocephalidae (parasites of snakes)
Porocephalus snakes, respiratory passages, indirect (rodent IH)
Family: Linguatulidae (parasites of mammals)
Linguatula mammals, respiratory passages, indirect (mammalian IH)