Contents

Medical Microbiology (BMI2603)

Learning unit 0

Welcome

Welcome to Medical Microbiology. We hope this course will broaden your understanding of microbiology, and help you further your studies.

0.1 Welcome note

Welcome to Medical Microbiology

Young girl in Bangladesh infected with smallpox (1973). The WHO International Commission officially certified that smallpox had been eradicated from the world in 1979. (http://en.wikipedia.org/wiki/File:Child_with_Smallpox_Bangladesh.jpg.

This image comes from the Public Health Image Library (PHIL) of the Centers for Disease Control and Prevention, with identification number #3265. Image credit: CDC/James Hicks.)

Welcome to Medical Microbiology, BMI2603.

Medical microbiologists study **microorganisms of medical interest**. They research how pathogens cause disease in humans, how diseases spread, and the effects they have on the human body. They are also concerned with how to treat, control and eradicate pathogenic agents. Most medical microbiologists work in laboratories that identify disease-causing organisms and make treatment recommendations to a physician. They may also be involved in identifying and controlling epidemics and outbreaks of disease.

The most effective way to control a pathogen is to eradicate it. To date only one human pathogen, *Variola minor,* the virus that causes smallpox, has been eradicated worldwide. In the case of most pathogens eradication is impossible, and therefore diagnosing, treating and controlling infections is important.

Medical microbiology also includes the study of **beneficial microbes** as a source of antibiotics or as probiotics that provide health benefits to the host, for instance by inhibiting the growth of pathogens.

This module is a 12-credit course, which means that you will need to devote at least **120 study hours** to it. You will need to prepare a portfolio. The myUnisa website will be the main teaching medium for this module. Please visit this site frequently to interact with fellow students and to participate in discussions about certain topics that we will be covering. Try to be online at least once a week. I will say more about the structure of the course in the next sections.

0.2 Course information

0.2.1 How this course is organised

Before you begin studying the first study unit, I would like to give you some details about the module as a whole. Study unit 1 introduces a number of central concepts in medical microbiology. Study units 2 to 4 cover some relevant bacteria, and study units 5 to 7 deal with some important viruses. Study unit 8 deals with transmissible spongiform encephalopathies and study unit 9 with fungi. The last unit, study unit 10, introduces medical parasitology.

- Unit 1: Introduction to medical microbiology
- Unit 2: Staphylococcus
- Unit 3: Mycobacteria
- Unit 4: Vibrio
- Unit 5: Rotavirus
- Unit 6: Papillomavirus
- Unit 7: Retrovirus
- Unit 8: Prion diseases
- Unit 9: Fungal diseases
- Unit 10: Medical parasitology

0.2.2 Textbook

The prescribed textbook for BMI2603, which you will be using in conjunction with the online material, is:

Brooks, G.F., Carroll, K.C., Butel, J.S., Morse, S.A. & Mietzner, T.A. (2010). *Jawetz, Melnick, & Adelberg's medical microbiology* (25th edition). McGraw Hill Medical, USA.

ISBN: 9780071624961

In the study guide I will refer to the textbook as Brooks.

The textbook is a comprehensive guide to medical microbiology. You do not have to study the whole textbook: the online study material will guide you in terms of what you need to learn. If you find a topic particularly interesting, you are more than welcome to do further reading about it. Note that if you purchase the latest edition of the textbook, you may find that the pages in the study guide do not directly correlate to the pages in this latest edition of the textbook. However, I am sure that you will find it easy to locate the relevant section, rather than the specific pages, in the newer textbook.

0.2.3 Purpose of this module

In this module we focus on the capacity of microorganisms to cause disease – the term for this is **pathogenicity**. This trait is relatively rare among microbes – which is fortunate, considering the enormous number of microorganisms that exist!

Even so, there are more pathogenic organisms than we could cover adequately in a semester module. This second-level module sets out to introduce you to the field of medical microbiology by concentrating on a few examples that are of particular relevance to the South African situation.

Different pathogens such as bacteria, viruses, fungi, protozoa and helminths vary in their aetiology, in how they are diagnosed, and in how they are treated – in medical microbiology, there is no "one size fits all." However, this module lays a solid foundation that you can build on throughout your career.

I hope that you will find the module interesting and useful, and that you will be able to apply the knowledge not only in your career, but also in understanding your own health better.

0.2.4 Outcomes of the module

This module is designed to introduce you to concepts such as

- pathology
- epidemiology
- diagnosis
- treatment
- prevention
- control
- immune responses

in a way that will help you understand them and apply them in relation to any other organisms you may encounter in future. Always try to see how these concepts apply to your own geographic location.

0.3 Distance learning

Distance learning is very different from learning in a contact situation. Once you have received your study material, please plan how you will approach and complete this module. Draw up a reasonable study schedule for the whole module. Remember to include the assignment due dates

as given in Tutorial Letter 101.

0.3.1 Independent study

A crucial element in understanding and learning the basics of medical microbiology is the ability to express your ideas both orally and in writing. Only when you have tried this for yourself will you understand the full value of this exercise.

Assessment measures an aspect of your success. For this module there is both formative (ongoing) and summative (final) assessment in the form of assignments and examinations. These are mainly in the form of written work. Your reflections on your learning are therefore also an important part of your studies. Since the focus in this module is on understanding and applying the concepts of medical microbiology, assessment will focus on the competencies you need to display.

Work through your study guide, making use of the guidelines in the next section. Construct mind maps and make your own summaries of the objectives and content of chapters of the textbook. Restrict summaries to one page. Additional textbooks and articles give alternative views or provide more insight into issues under discussion, and are optional additional reading.

Be focused. Build up your own study and exam preparation **portfolio** (consisting of your assignments, activities, reflections, summaries, self-evaluations and notes) throughout your academic and/or experiential learning. **The lecturer will not assess this portfolio, but you will need to prepare it in order to be able to complete the assignments and ultimately pass the final examination**. It is also very important to use this portfolio, in combination with your assignments and subsequent feedback (tutorial letters), for your **exam preparation**. The advantage is that by doing this you take part actively in your learning, you set goals, you evaluate your own progress through reflective actions, and you evaluate your ability to realise the learning outcomes, thus becoming a more independent and self-directed learner.

What is a portfolio? A portfolio is a folder or file in which you gather and compile additional and/or summarised information during the year as you work through the study guide and textbook. This portfolio will help you to prepare for the examination by focusing on the most important facts and issues.

Your portfolio should comprise:

- answers to each activity in each study unit
- a mind map/summary of each study unit
- your returned assignments (or a copy you made prior to submitting your assignment)
- your reflections on each study unit
- extra reading material taken from the internet, additional books, and medical and/or scientific journals
- a new vocabulary of words or glossary of new terms defined in your own words

To help you, in the next sections I provide some study skills guidelines you may find helpful.

0.3.2 Improved study skills

It is critical that you think independently and learn to look beyond the study guide and textbook. I have included a number of additional references in this study guide, and I really encourage you to consult them. In addition, as a more advanced distance-education student, you need to learn how to search for research/scientific articles via the internet.

0.3.2.1 How to search for research/scientific articles

Google has created an additional search engine under “Advanced search”, called Google Scholar. This has its own advanced search function. If you state your subject query in four to six words and press “Search enter”, a variety of websites relating to the query will appear. This has the advantage of allowing you to access most of the journal references from any internet site in addition to myUnisa. Some journals, however, such as *ScienceDirect*, can be accessed only through a tertiary academic institution such as Unisa. To access this journal:

1. Go to Unisa online at http://www.unisa.ac.za/
2. Click on Library at the top of the page.
3. In the maroon area on the top of the page, click on “Search for information resources”.
4. Follow the guidelines if you are a first-time user.
5. Click on the option “A–Z list of the names of all electronic resources” on the right-hand side of the page.
6. Various links for databases will now appear on your screen. Click on any database to do a search. For biotechnology I recommend *ScienceDirect*, *Nature* or Springer Link.
7. Once you have entered one of these databases, you can search for scientific articles by typing keywords in the “Search” box. Use specific keywords. If you type in just a single, general word, you will usually get too much information, and it won’t necessarily relate to the topic you are looking for.
8. You will need to do some independent searches yourself for your portfolio, assignments and exam preparation.

Contact the Unisa Library at +27 12 4293206 if you have any difficulties or if you need assistance, or consult the library website for the telephone number of your local branch library.

0.3.3 Skimming, scanning and study-reading strategy (SSS strategy)

The SSS strategy is one of a number of strategies you may find helpful. The three techniques in the SSS strategy are

- **s**kimming,
- **s**canning and
- **s**tudy-reading.

Skimming

1. **Page through and explore**. First, read the section, chapter or unit quickly, forming a rough idea of the content. Concentrate on headings and subheadings, any words or phrases in bold or italics, text in boxes, tables and illustrations, and – in the case of a chapter or unit – introductions and summaries. The objectives set for a unit or chapter are important. (Think of how you would page through a magazine. When starting a new study unit, scan it and concentrate on the concepts that catch your eye.)
2. **Make a cursory survey**. As you read, ask yourself: What key terms occur in this section, unit or chapter? Stop when you identify a key term, read carefully what is said about it, and mark it so that you will be able to find it again easily later when you need to. Your key question at this point is: **Where**?

Scanning and reflecting

3. **Scan** the section, chapter or unit.
4. **Start a mind map** (either for the whole section, unit or chapter or for parts of it). You are looking for items and concepts while reading the information in the section or chapter in a more evaluative way. Reflect on relationships between concepts. The question now is: **What**? What is the meaning and the purpose? Visualisation is important, and at this point you begin to write down key concepts.
5. **Deeper reflection**. As you work through the prescribed activities of the section, unit or chapter, keep returning to the mind map to fill in the detail. Reflect on the value and meaning of categories, concepts, motivations, variables and key terms.

Study-reading

6. **Study-read**. There is a close relationship between this stage and stages 2, 4 and 5. Read carefully, thoroughly and thoughtfully. During this stage you link the key terms and concepts you have already identified, and this is where the mind map and summaries are important. (Remember to put your detailed mind map in your portfolio.) Pause while reading, consolidate what you remember and consider how new information fits in with what you already have.

7. **Activity-based approach**

   Whenever you get to an activity in your study guide, complete it in full on loose pages which you then insert in your portfolio, grouped together per section and study unit. Supplement this with your own notes. (You do not need to submit activities or the portfolio to the lecturer, but these are essential for exam preparation.)

8. **Understanding what you read**

   Take the time to note new vocabulary words. Consult a dictionary to understand the meaning of new words. You could compile a page for each study unit, and add it to your portfolio.

0.3.4 Managing your self-paced study time

If you are an average student, you need to devote at least **120** study hours to this module (however, this time may vary substantially). You should therefore plan to devote at least **8** study hours per week to the module, in which case you should complete it in **15** weeks. If you have registered for more than one module, you need to plan time for each module accordingly.

I advise you to keep a study schedule or diary, so that you have a clear idea of the time you have available for study. This will help you to manage your studies within the time you have available, and balance study with work and family life. In Tutorial Letter 101 you will find a list of due dates for the assignments, so enter these in your diary. Divide the large assignments into a series of smaller tasks to complete one step at a time.

In order to manage your workload, study frequently and regularly. Establish a routine in an environment with low noise and good lighting. Reward yourself after a productive session.

0.3.5 Academic specialist guidance

If you need help, please contact the staff of the Department of Life and Consumer Sciences who are responsible for this module.

0.4 Plagiarism

Never try to pass off other people's work (and that includes Unisa study material) as your own. If you want to quote other people's words and ideas or Unisa study material in your own answers, you must use quotation marks and acknowledge your source. (Use the Harvard method.) If you are unsure about the correct way of acknowledging sources, contact Unisa's Library Information Desk.

Students who fail to acknowledge quotations or who draw on lecture notes and other sources without acknowledgment or who copy someone else's answers may be refused permission to write the examination, or may be penalised in the assignment.

0.5 In conclusion

After reading this general introduction you should have a better understanding of what the module involves and how you should approach your studies in Medical Microbiology.

Learning unit 1

Introduction to microbiology

1.1 Introduction

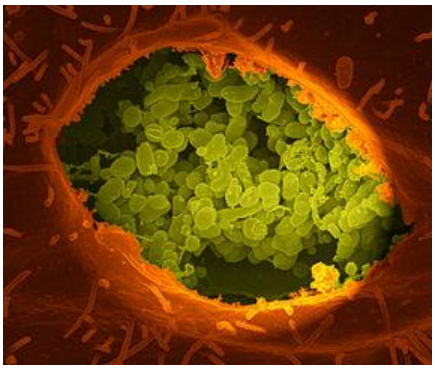

Figure 1.1: *Coxiella burnetii,* an obligate intracellular pathogen, growing within a cell (false colour image) (http://en.wikipedia.org/wiki/File:Coxiella_burnetii,_the_bacteria_that_causes_Q_Fever.jpg)

Figure 1.2: Agar plate with growing yeast colonies (http://commons.wikimedia.org/wiki/File:Yeast_agar_plate-01.jpg)

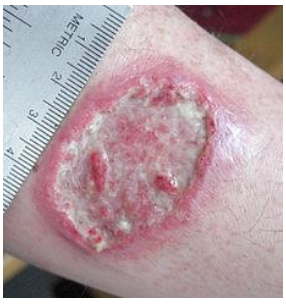

Figure 1.3: Leishmaniasis ulcer (http://en.wikipedia.org/wiki/File:Leishmaniasis_ulcer.jpg)

Medical microbiology is the study of

- medically important microorganisms,
- their transmission,
- the effect they have on the human body, and
- the diagnosis, treatment and prevention of the diseases they cause.

In this study unit we will explore

- how diseases are spread,
- the infectious process,
- the different types of pathogens that can infect humans and
- some techniques used to diagnose infection.

To complete this study unit you will need to refer to sections of chapters 1, 9 and 47 of Brooks.

1.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- discuss the history of medical microbiology
- discuss modes of transmission and the spread of infection
- explain the pathogenicity of microorganisms
- discuss diagnosis and diagnostic techniques
- discuss nosocomial infections

1.3 History of medical microbiology

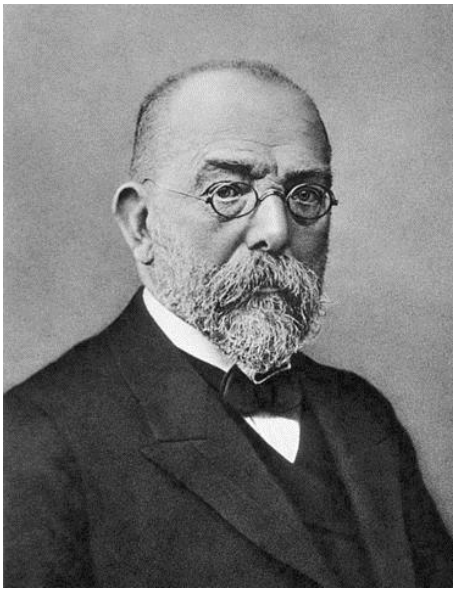

Figure 1.4: Robert Koch (http://en.wikipedia.org/wiki/File:Robert_Koch_BeW.jpg)

Many people have contributed to the field of medical microbiology. As early as the 3rd century BC, Hippocrates was conducting experiments and clinical examinations. Around 55 BCE the Roman philosopher Lucretius discussed disease in his poem "De Rerum Natura", and in the 16th century CE, Fracastoro developed on Lucretius's ideas by proposing that invisible living creatures were the cause of disease. Most people at the time, however, believed that illness was caused by supernatural forces or poisonous vapours, or was due to an imbalance of the four humours that were thought to be inside the body.

A number of scientists working in the 18th and 19th centuries provided the first direct evidence that

microorganisms could cause disease in plants or animals. Agostino Bassi (1773–1856), M. Berkeley (1803–1889) and H. de Bary (1831–1888) demonstrated that microorganisms could cause disease in silkworms, potato crops and cereal crops, respectively.

Louis Pasteur (1822–1895) demonstrated that microorganisms did not arise spontaneously in media, but were introduced from the environment. He also established that microorganisms can be inactivated by heat and pressure, and therefore that food could be preserved by a process that we know as pasteurisation. After reading the work of Louis Pasteur, Joseph Lister (1827–1912), a surgeon, started to employ a number of aseptic techniques in his surgery. He sterilised the surgical instruments with heat, and used phenol in surgical dressings. These techniques resulted in fewer post-operative infections, providing strong indirect evidence that microorganisms play a role in disease. Pasteur also developed vaccines against chicken cholera, anthrax and rabies.

Robert Koch (1843–1910) was the first person to demonstrate directly that bacteria cause disease. Koch initially worked on anthrax and demonstrated a direct relationship between exposure to *Bacillus anthracis* and the disease anthrax. He developed what we know as Koch's postulates, which are a list of criteria to verify what particular agent is responsible for a specific disease. He then worked on *Mycobacterium tuberculosis*, and proved that it was the causative agent of tuberculosis.

1.3.1 Activity 1.1

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

Briefly describe how Pasteur and Koch contributed to the field of medical microbiology.
What are Koch’s postulates? Discuss the following statement: Koch's postulates are sufficient, but not necessary, to establish causation of a disease. Give examples. In your discussion, include how asymptomatic carriers of disease or non-culturable organisms affect the postulates.

1.3.2 Feedback on activity 1.1

Koch’s postulates can be found in table 9-1 on page 146 in Brooks.

If Koch’s postulates are valid for a particular disease, then this proves that a particular microorganism is responsible for the disease. However, if they cannot be proved, this does not necessarily mean that that disease is not caused by that specific microorganism.

Did you remember to mention instances where Koch’s postulates can be applied to establish that a particular organism is directly responsible for disease? Did you also mention that it is impossible to grow some pathogens in culture, and therefore their association with diseases cannot be established using Koch’s postulates?

1.4 Diversity of pathogens

A pathogen is a biological agent that can cause disease in a host. The host may be a plant, an animal, a fungus or another microorganism. Pathogens may be

- cellular, as in the case of bacteria, fungi and protozoa, or they may be
- non-cellular, as in the case of viruses and prions.

Pathogens can be divided into

- obligate pathogens and
- accidental or incidental pathogens.

Obligate pathogens can only survive and reproduce **within a host**. They cause disease, although the degree of illness may vary.

Accidental or **incidental** pathogens can survive **without causing disease in a host**. Many bacteria fall into this group, living either as part of normal flora and sometimes causing disease, or living in other hosts or reservoirs and causing disease when suitable conditions and susceptible hosts present themselves.

Bacterial pathogens can be divided into

- opportunistic pathogens and
- primary pathogens.

Primary pathogens can cause infection and disease in otherwise healthy people who have intact immune systems.

Opportunistic pathogens seldom cause disease in individuals with intact natural defences and immune systems.

Many bacteria are present as part of the normal human skin and mucous membrane flora. Most of the time these microbes are harmless, and may even be beneficial: for example they may prevent other, more pathogenic, bacteria from colonising a site. However, under certain conditions these opportunistic bacteria can cause disease. These conditions include

- a compromised immune system (e.g. from disease, surgery or malnourishment)
- when competing bacteria have been removed (e.g. through use of broad-spectrum antibiotics)
- when bacteria enter sites where they are not normally found

1.4.1 Activity 1.2

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

In your own words, explain what the following terms mean:

- obligate pathogen
- accidental pathogen
- primary pathogen
- opportunistic pathogen

Can a microorganism fall into more than one of these categories simultaneously? Give reasons for your answer.

1.5 Important terms

Epidemiology is the study of the frequency, distribution, causes and effects of health and disease in populations.

Pathology is the study and diagnosis of disease through the examination of organs, tissues, and bodily fluids.

Pathogenicity is the ability of a pathogen to produce disease in a host organism.

Virulence refers to the degree of pathogenicity of an organism, and is related to the ability of the organism to invade and damage the tissues of the host.

1.6 The infectious process

Recommended reading: the section on the infectious process on pages 147–148 in chapter 9 of Brooks.

The infectious process involves the interaction of three factors:

- pathogen,
- host, and
- environment.

Very few of the millions of microorganisms in existence are harmful to humans. Generally, once a microorganism has established infection it will multiply, and may spread within the host. Some pathogens are non-invasive, and do not spread beyond the initial site of infection.

The virulence of an organism depends on

- its invasiveness
- its toxigenicity
- the dosage
- the portal of entry

The host's immune condition will either help resist infection or make it more susceptible. A host which has previously been infected by a pathogen will have some degree of resistance to re-

infection. Immunisation against a specific pathogen also provides relevant antibodies. On the other hand, a weakened immune response increases the likelihood of infection. Environmental conditions, for example overcrowding or poor sanitation, also influence the infectious process.

1.7 Transmission and the spread of infection

Recommended reading: the section on transmission of infection on page 147 in chapter 9 of Brooks.

Another crucial element in the infectious process is the **spread of infection**. Illness is not only spread from obviously sick individuals.

- In some instances, individuals (carriers) are infected and transmit disease, yet remain healthy;
- some diseases are contagious during incubation;
- and still other diseases are acquired from animal or environmental sources.

It is advantageous for microorganisms to produce asymptomatic or mild disease in some individuals, as this enhances the possibility of transmission from one person to another. Some diseases are primarily diseases of animals, and only occasionally infect humans.

Linked to the spread of infection is the **mode of transmission**. In order for a disease to be infectious, it must be **transmitted** from host to host. Routes of transmission include

- direct contact (including sexual transmission)
- indirect contact
- air-, food- and water-borne transmission
- animal vectors
- transplacental (mother-to-child) transmission

The skin and mucous membranes are the primary defence against infectious agents. To cause disease, microorganisms need to pass these barriers. The most common portals of entry are

- the respiratory tract (upper and lower airways)
- the mouth
- the genital area
- the urinary tract
- damaged skin (cuts, burns etc.)

The symptoms of the illness (runny nose, diarrhoea, cough, genital discharge) also often promote the transmission of the disease.

In this module we will consider examples of pathogens that use each of these modes of transmission.

1.7.1 Activity 1.3

Do the following activity and add it to your portfolio.

(a) Think of illnesses you have had. How do you think you became infected? Try to think beyond the obvious colds that you caught from people around you – have you had malaria or tick bite fever? Diarrhoea? Do you always wash your hands before eating, or did you eat something that was bad?
(b) Discuss how infection spreads. In your discussion, include examples of diseases that are spread by each mode of transmission.

1.7.2 Feedback on activity 1.3

Modes of transmission include

- direct contact (including sexual transmission)
- indirect contact
- air-, food- and water-borne transmission
- animal vectors
- transplacental (mother to child) transmission

You should have been able to think of at least one disease that is spread by each mode of transmission. Some examples would be

direct contact – pink eye

indirect contact – flu

By what modes of transmission are the following diseases spread?

- HIV
- malaria
- intestinal worms
- cholera

(Diseases may spread by more than one mode of transmission.)

1.8 Bacterial pathogenicity

Recommended reading: pages 148–157 in chapter 9 of Brooks.

Bacteria are **haploid**, and reproduce by binary fission (one cell splits into two identical daughter cells). The binary fission process does not allow for the exchange of genetic material. However, bacteria have developed mechanisms for exchanging genetic information through the transfer of **extrachromosomal genetic elements** (e.g. plasmids). The genes associated with virulence factors are often encoded on extrachromosomal genetic elements, and if these are transferred between bacteria, the bacteria receiving the genetic material will gain the genes for those virulence factors.

What are virulence factors?

Essentially they are elements that **enhance a bacteria's ability to cause infection and disease**. They include

- adherence factors
- invasive factors
- toxins
- enzymes
- antiphagocytic factors
- bacterial secretion systems

1.8.1 Activity 1.4

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, define pathogenicity.
(b) A number of factors contribute to the virulence of bacterial pathogens. How do these factors increase the virulence of bacteria?

1.8.2 Feedback on activity 1.4

The factors that contribute to the virulence of bacterial pathogens are described on pages 148–154 in chapter 9 of Brooks.

After discussing the factors that contribute to the virulence of bacterial pathogens, you should understand how each factor can increase the virulence of a bacterium. For example, antiphagocytic factors will stop the immune cells from phagocytosing the bacterium and killing it. If it is not killed, it is able to reproduce and cause further disease.

1.9 Diagnosis

Recommended reading: chapter 47 in Brooks, especially the sections entitled: "Communication between physician and laboratory", "Diagnosis of bacterial and fungal infections" and "Diagnosis of infection by anatomic site".

It is not always important to know exactly which pathogen is causing the illness, and a doctor may prescribe an antibiotic or other medication that will target the organism most likely to be causing the infection. In some cases, however, it may be essential to know exactly which organism is causing the disease, especially if

- the illness can be caused by a variety of pathogens that would all require different treatment,
- the current treatment is not working or
- a very serious and contagious pathogen is thought to be causing the illness.

A medical microbiologist doing diagnostic work will work with various **samples** that have been obtained by a doctor.

Depending on the illness specimens may be

- tissue samples
- blood
- urine
- cerebrospinal fluid
- respiratory secretions
- gastrointestinal tract specimens
- genital discharge

Laboratory results often depend on the quality of the specimens. This depends on the specimen collected, its age, transport to the laboratory and how it is treated once it gets there. The techniques used to study the sample will depend on the symptoms the patient is experiencing. Read the section on blood specimens on pages 716–717 in Brooks to learn more about specimen collection and analysis.

1.9.1 Diagnostic procedures and techniques

When a patient presents with an unknown disease, the treating physician needs to obtain a history and use it to make a tentative diagnosis. There are several reasons for this.

- Many microorganisms grow slowly, so commencement of treatment cannot always wait for a definitive diagnosis.
- The specimen must be taken from a relevant site and in an appropriate manner, and this can only occur with the aid of a detailed history.
- Techniques for diagnosing a pathogenic organism vary depending on whether a bacterium, a virus, a fungus or a parasite is involved, as there is no single diagnostic technique for all of these, and so the laboratory needs guidelines on the sort of organism that may be causative.
- Many tests are highly specific (not to mention costly!)

A range of tools and techniques may be used to identify microorganisms, including

- microscopy
- cultures
- serology
- antigen and antibody detection
- biochemical analysis
- molecular techniques

Click on http://virology-online.com/general/Tests.htm for some valuable additional information on diagnostic methods used in virology. I have included this purely for interest, so it is not essential reading.

1.9.2 Activity 1.5

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, describe why it is important for the person doing the diagnostic testing to know the patient's symptoms.
(b) Describe how blood specimens are obtained and examined to diagnose infection (refer to pages 716–717 in chapter 47 of Brooks).
(c) Distinguish between direct and indirect diagnostic methods.
(d) Briefly discuss

- common microscopy and staining techniques
- simple culture methods
- antigen detection (EIA/ELISA)
- latex agglutination
- molecular techniques (the use of nucleic acid probes, PCR, real-time PCR and reverse transcriptase PCR)

1.10 Treatment

Once an illness has been diagnosed and the infectious agent identified, the patient needs to be treated. Some infections will resolve on their own as the body's immune system fights them, while it may be necessary to treat other infections with antimicrobial drugs.

- Bacterial infections are usually treated with **antibiotics**,
- viral infections are treated with **antiviral compounds** and
- fungal infections are treated with **antifungals**.

A medical microbiologist may make drug recommendations based on the strain of microbe and its antibiotic resistances.

- Parasites are treated with a variety of **antiparasitic drugs**.

Immunisation with **vaccines** is an effective way of preventing illness caused by pathogens. Vaccines work by exposing the body to elements of the pathogen, and allowing the immune system to become alert to that pathogen. This strengthens the body's defence against the pathogen, so if the individual ever becomes infected, their immune system is able to attack and destroy the pathogen before it causes disease.

1.11 Hospital infections

Hospital-acquired infections (**nosocomial infections**) are infections which were **not** present (or in the incubation phase) at the time the patient was admitted, but instead **are acquired while the patient is in hospital**. Some hospital-acquired infections become apparent only after the patient has been discharged. Nosocomial infections may be acquired from

- exogeneous sources (e.g. from other patients or from the environment), or they may be acquired
- endogenously (from a different site within the same patient).

The possibility of infection is enhanced by the patient's reduced resistance.

There are many routes of transmission, including

- food supplies – inadequate hygiene standards can lead to food-borne infections
- water and air supplies – organisms such as *Legionella* may colonise ducting and be transmitted through air-conditioning systems or water pipes
- air supply – other respiratory diseases can be transmitted through air-conditioning or theatre air supplies
- fomites – inanimate objects can become contaminated and transmit diseases

Because of the high antibiotic use in hospitals, antibiotic-resistant strains of bacteria have developed, and may be difficult to treat. The most common nosocomial infections are infections of the urinary tract, surgical site or pneumonia.

Infection can be controlled by sanitation of surfaces, hand washing or changing of gloves between patients, changing gowns regularly, sterilisation of equipment and isolation of infective patients.

1.12 Conclusion

Review the outcomes of this module, and think about whether you have achieved them.

Do you understand how diseases are transmitted, resulting in the spread of infection, and what makes an organism pathogenic? Can you describe various diagnostic techniques that are used to identify the cause of infection?

Learning unit 2

Staphylococcus

2.1 Introduction

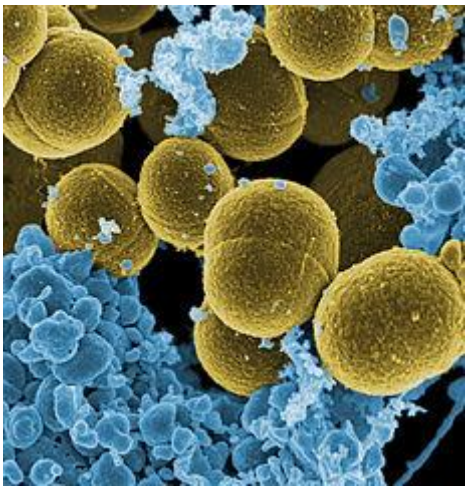

Figure 2.1: *Staphylococcus aureus* bacteria, false colour added (http://www.niaid.nih.gov/topics/antimicrobialResistance/Pages/aureusBacteria.aspx)

The staphylococci include a number of species of bacteria. Many of the species form part of the normal flora of human skin or mucous membranes, and do not cause disease. Other species are pathogenic and cause disease in humans and animals through infection or toxin production. The most common reason for food poisoning is due to staphylococcal toxins, which accumulate in improperly stored food.

In this study unit we will look at some of the pathogenic staphylococci. To complete the study unit, you will need to refer to chapter 13 of Brooks. The following website has additional information on the content of this study unit: http://pathmicro.med.sc.edu/fox/strep-staph.htm.

2.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- list the major pathological staphylococcal species and the diseases they cause
- identify and discuss sources of infection and conditions which contribute to disease
- discuss the enzymes and toxins produced by staphylococci
- identify the various diagnostic tests that could be used to identify staphylococcal infections
- explain the ability of staphylococci to become resistant to antibiotics
- describe what MRSA is
- identify practices that could be useful in preventing the spread of disease caused by staphylococci

A note on bacterial classification and nomenclature

Bacterial nomenclature is revised periodically as new species are discovered and as new scientific information results in the revision of existing names.

A good source of up-to-date nomenclature is the website http://www.bacterio.cict.fr, which is regularly updated to comply with changes published in the *International Journal of Systematic and Evolutionary Microbiology*.

2.3 Genus *Staphylococcus*

Recommended reading: the whole of chapter 13 in Brooks

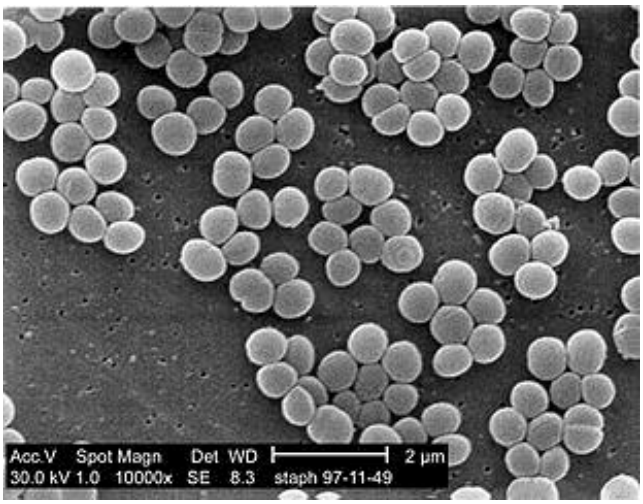

Figure 2.2: Scanning electron micrograph of *Staphylococcus aureus*, showing the grape-like clustering that is common to the *Staphylococcus* species (http://uk.wikipedia.org/wiki/%D0%A4%D0%B0%D0%B9%D0%BB:Staphylococcus_aureus_01.jpg. This image comes from the Public Health Image Library (PHIL) of the Centers for Disease Control and Prevention, with identification number #6486.)

Staphylococci are gram-positive, spherical bacteria. They usually occur in grape-like clusters (this is where the name "staphylococcus" comes from: the Greek word *staphyle* means "bunch of grapes"), but can also occur as single cells, pairs or tetrads. There are over 40 species in the genus *Staphylococcus*. The main human pathogens are *S. aureus*, *S. epidermidis* and *S. saprophyticus*.

S. aureus is a significant human pathogen, and disease ranges in severity from food poisoning and minor skin infections to life-threatening infections.

S. epidermidis may be associated with infections of implanted devices such as prostheses, shunts and catheters, especially in immunocompromised patients.

S. saprophyticus is a common cause of urinary tract infections.

Staphylococci are easily cultured on most types of bacteriological media, and grow best at 37 °C. The colonies formed are round, smooth, raised and glossy. Different degrees of haemolysis may

be observed for *S. aureus*. Haemolysis can be seen in the image below, where the lighter regions around the colonies are due to the lysing of the red blood cells in the media.

Figure 2.3: *Staphylococcus aureus* growing on Columbia horse blood agar (http://commons.wikimedia.org/wiki/File:Staphylococcus_aureus_agar_sangre,_acercamiento.jpg. Image credit: Nathan Reading)

2.3.1 Activity 2.1

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) Describe some characteristics of staphylococci. Include shape, size, clustering, gram stain and motility.
(b) What is haemolysis?
(c) Describe *S. aureus* in terms of spores, motility, growth characteristics and antigenic structures.
(d) *S. epidermidis* and *S. saprophyticus* are coagulase-negative, and *S. aureus* is coagulase-positive. What does the production of coagulase have to do with pathogenicity?

2.4 Pathology and pathogenesis

For more information on the pathogenesis of staphylococcus infection, go to http://www.textbookofbacteriology.net/staph_2.html.

Staphylococci are found as normal flora of the human skin, respiratory and gastrointestinal tracts. Infection most commonly occurs where host resistance is lowered, or when skin is broken (for example as a result of eczema, surgical incisions or the insertion of intravenous devices) or mucous membranes are damaged. Warm, moist conditions also promote staphylococcal infections.

Staphylococcus aureus colonises skin, and can be found in the nose or throat of up to 40% of healthy people. *S. aureus* causes a variety of infections in humans and animals, ranging from minor skin infections such as pimples to impetigo, boils and abscesses to food poisoning and potentially fatal conditions such as pneumonia, meningitis, toxic shock syndrome (TSS), bacteraemia, and sepsis.

Staphylococcus epidermidis is a commensal of the skin and may cause opportunistic infections when implanted devices such as prostheses and catheters or foreign bodies are present. In addition to the very young and the elderly, immune-suppressed patients are more likely to develop these infections.

Staphylococcus saprophyticus is part of the normal vaginal flora and may be involved in infections of the urinary tract in sexually active young women.

2.4.1 Activity 2.2

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) List the three major staphylococcal pathogens that are of clinical importance, and describe the diseases they cause.
(b) What is bacteraemia?

2.4.2 Feedback on activity 2.2

The three major staphylococcal pathogens of clinical importance described in the textbook are *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus.*

You should have mentioned what diseases they cause and who they typically infect.

2.5 Virulence factors and toxins

Disease originating from staphylococci is caused partly by the **rapid multiplication and spread** of the bacteria in the host tissues, and partly by the **range of extracellular substances** the bacteria produce. Some of these substances are **enzymes**, and others are **toxins**. Many of these toxins are coded by plasmids contained within the bacterium.

Some of the important **enzymes** produced by staphylococci are

- catalase: produced by all staphylococci
- coagulase (clots plasma and coats the bacterial cell to inhibit phagocytosis) and clumping factor
- deoxyribonuclease (degrades DNA): produced by *S. aureus*
- hyaluridonase (breaks down hyaluronic acid and assists in spread of the bacteria)
- staphylokinase (dissolves fibrin and aids in spread of the bacteria)
- lipase (digests lipids)
- β-lactamase (drug resistance)

Toxins produced by staphylococci include

- exotoxins (exoproteins): a group of toxic proteins (e.g. α-lysin, β-lysin, γ-lysin and δ-lysin) produced by *S. aureus*
- Panton-Valentine leucocidin: a two-component toxin produced by *S. aureus*
- exfoliative or epidermolytic toxins: two different toxic proteins, one heat-stable and one heat-labile, produced by *S. aureus*
- toxic shock syndrome toxin (TSST-1): produced by many strains of *S. aureus*
- enterotoxins: one or more are produced by approximately 50% of *S. aureus* strains and are an important cause of food poisoning. There are multiple types (A–E, G–J, K–R, U and V).

Follow the links below for useful additional information about enzymes and toxins:

- http://www.textbookofbacteriology.net/staph_3.html
- http://www.textbookofbacteriology.net/staph_4.html

2.5.1 Activity 2.3

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) List three enzymes produced by *S. aureus*. Describe their catalytic activity.
(b) What toxins are produced by *S. aureus*? Describe the roles they play in disease.

2.5.2 Feedback on activity 2.3

The enzymes and toxins produced by staphylococci are described in the section, “Enzymes and toxins” on page 187 of Brooks.

2.6 Diagnosis

Depending on the disease involved, specimens for diagnostic use can be collected from one or more of the following sources:

- pus from an open lesion, abscess, burn etc.
- sputum
- aspirate from trachea
- vomit
- faeces
- blood
- spinal fluid

Swabs from the anterior walls of both nostrils and the perineum are used to identify carriers of *S. aureus*. Once specimens have been taken, one or more of several techniques may be used to identify the disease-causing agent. In the case of *Staphylococcus* these techniques include

- microscopy (smear)
- culture
- catalase test
- coagulase test

Other tests, such as antibiotic susceptibility testing, and molecular techniques, such as PCR, pulsed-field gel electrophoresis and multilocus sequence typing, are typically used to follow the spread of specific clones of *S. aureus*.

Figure 2.4: The figure shows an antibiotic sensitivity test of *Staphylococcus aureus* on an agar plate. The disc in the centre contains an antibiotic that diffuses into the media. If the bacteria are sensitive to the specific antibiotic, they will not be able to grow close to the disc. (http://www.flickr.com/photos/nathanreading/6855355785/ Image credit: Nathan Reading. Licence: http://creativecommons.org/licenses/by/2.0/deed.en)

2.6.1 Activity 2.4

Do the following activity and add it to your portfolio.

Briefly describe the laboratory techniques (microscopy, culture, catalase test and coagulase test) used to test for staphylococcal infection.

2.6.2 Feedback on activity 2.4

The laboratory techniques are explained on page 189 of your textbook, in the section, “Diagnostic laboratory tests”.

For more information on the laboratory protocols, follow this link: http://www.microbelibrary.org/about/51

The microbelibrary.org website is searchable, and includes excellent pictures of the results of this and other tests, as well as other resources.

2.7 Antibiotic susceptibility and MRSA

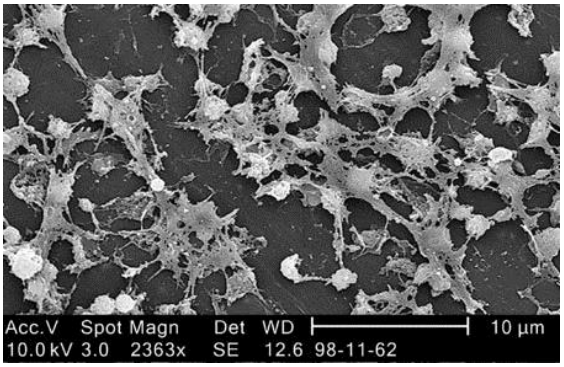

Figure 2.5: This electron micrograph depicts large numbers of *Staphylococcus aureus* bacteria, which were found on the luminal surface of an indwelling catheter. Of importance is the sticky-looking substance woven between the round cocci bacteria, which was composed of polysaccharides, and is known as “biofilm”. This biofilm has been found to protect the bacteria that secrete the substance from attacks by antimicrobial agents such as antibiotics. (http://commons.wikimedia.org/wiki/File:Staphylococcus_on_catheter.png)

Staphylococci are variably sensitive to many antimicrobial agents, and may become difficult to treat if they develop resistance to a number of antibiotics. In recent times, strains of *S. aureus* have developed which are resistant to the antibiotic methicillin and other common beta-lactams such as oxacillin, penicillin and amoxicillin.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is usually treated using antimicrobials of the glycopeptide type, such as vancomycin or teichoplanin. However, more recently, some *S. aureus* strains have started to show either intermediate or full resistance to vancomycin and glycopeptides, resulting in vancomycin-resistant *S. aureus* (VRSA) and glycopeptide-resistant *S. aureus* (GRSA). Typically, MRSA strains have been found primarily in hospitals, but more recently, in addition to these hospital-associated MRSA strains, some community-acquired resistant strains (CA-MRSA) have been observed. Approximately 1% of the population is colonised with MRSA.

The control of these bacteria therefore presents an ongoing challenge. Diagnostic laboratories are important for identifying outbreaks of MRSA. Typically the bacteria are cultured from blood, urine, sputum or other body fluid samples, and antibiotic susceptibility testing is performed. More rapid techniques to characterise MRSA have been developed and include quantitative PCR procedures, which can be used to quickly recognise MRSA strains. Another frequent laboratory assay is the latex agglutination test, which can be used to detect modified penicillin-binding proteins that allow *S. aureus* to be resistant to some antibiotics.

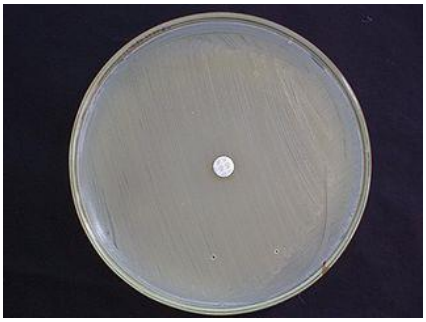

Figure 2.6: Antibiotic susceptibility test showing MRSA resistant to the antibiotic oxacillin, which is present on the disc (http://en.wikipedia.org/wiki/File:Muller_Hinton_agar_with_MRSA.jpg)

Visit http://www.textbookofbacteriology.net/staph_5.html for more information on antibiotic resistance and MRSA.

2.7.1 Activity 2.5

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) How does CA-MRSA differ from hospital-associated MRSA?
(b) Discuss antibiotic susceptibility testing of staphylococcal isolates.

2.7.2 Feedback on activity 2.5

You can find the information about CA-MRSA at the end of the section on epidemiology and control. You should have mentioned that CA-MRSA strains appear to be more virulent then MRSA strains, and have caused infections in young patients that are not at risk of acquiring MRSA.

Antibiotic susceptibility testing is described in the section on treatment. You should have mentioned that because many staphylococcal isolates are drug resistant, any isolates causing serious disease should be tested for drug susceptibility to aid in the selection of effective drugs for treatment. You should also have mentioned which drugs should not be used because of the development of resistance, and which drugs may be effective to use in certain instances.

2.8 Epidemiology

Staphylococci are ubiquitous in humans. Sources of infection include shedding from contaminated lesions, and transfer occurs via hands, clothing or other objects. Infection can also occur via the respiratory system. Cross-infection from infected lesions to other parts of the body is another important source of infection. Coagulase-negative staphylococci are found on the skin. They are opportunistic, and are typically a problem only in immunocompromised individuals. *S. aureus* is carried harmlessly in the nostrils of 30 to 40% of healthy individuals, and is also commonly found on the skin. *S. aureus* may be primary or opportunistic.

Skin infections are promoted by warm, moist conditions or broken skin (through conditions such as eczema, surgical incisions, or intravenous devices). Cleanliness, good hygiene and aseptic technique in the treatment of lesions can reduce the spread from infected lesions, but it is not possible to prevent the spread from the skin and respiratory system of all carriers.

MRSA is of particular concern in the hospital environment. MRSA-infected patients need to be isolated, and aseptic management of wounds needs to be strictly observed in order to ensure that hospital workers do not become carriers of the organisms. Despite these precautions, many staff and patients may be asymptomatic carriers of resistant staphylococci.

Staphylococci which have been shed into the environment may remain dormant for months unless killed by heat, light or disinfection.

2.8.1 Activity 2.6

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, discuss sources and modes of staphylococcal infection.
(b) Describe measures which should be taken to prevent the spread of staphylococcal infections in hospitals, especially in areas that are high risk, for example, the neonate section, intensive care units, operating theatres and cancer chemotherapy wards.

2.9 Conclusion

Review the outcomes of this module and think about whether you have achieved them. Can you list the major pathological staphylococcal species, their characteristics and the diseases they cause? Are you able to state the diagnostic tests that can be used to diagnose staphylococcal infections? Do you understand how staphylococci become resistant to antibiotics and what MRSA is?

Learning unit 3

Mycobacteria

3.1 Introduction

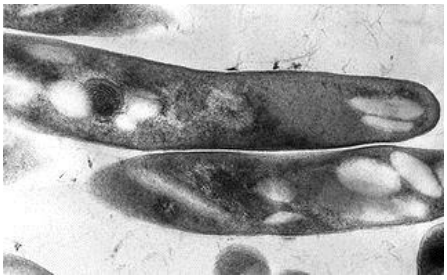

Figure 3.1: TEM of *Mycobacterium tuberculosis*, the causative agent of tuberculosis (http://en.wikipedia.org/wiki/File:Mycobacterium_tuberculosis_01.jpg. Originally from the CDC (http://phil.cdc.gov/phil/home.asp Public Health Image Library). Image credit: CDC/Eliz)

There are over 125 mycobacterial species. They are ubiquitous and can be found living in water (including tap water) and various food sources. Many are harmless saprophytes, several are opportunistic pathogens, and a few, like *Mycobacterium tuberculosis* and *Mycobacterium leprae*, are always pathogenic. In this study unit we will focus on *Mycobacterium tuberculosis* and *Mycobacterium leprae*, the bacteria responsible for tuberculosis and leprosy respectively. We will also consider at a few common opportunistic mycobacteria.

To complete the study unit, you will need to refer to chapter 23 of Brooks. The following websites also have further information on this topic: http://pathmicro.med.sc.edu/fox/mycobacteria.htm and http://textbookofbacteriology.net/tuberculosis.html.

3.1 Learning outcomes

Upon completion of this learning unit you should be able to:

- give the names of some common mycobacteria
- describe the features of *M. tuberculosis*
- discuss tuberculosis infections in terms of pathogenesis, pathology, diagnosis, treatment, epidemiology and control
- describe the features of *M. leprae*
- identify the major types of leprosy and describe the treatment of this condition
- discuss common opportunistic mycobacteria in terms of
    - different classification systems
    - pathogenesis, diagnosis and treatment

3.3 Genus *Mycobacterium*

Recommended reading: the whole of chapter 23 of Brooks

Mycobacteria are rod-shaped (straight or slightly curved), aerobic, nonmotile and do not have a capsule. They are neither strictly gram-positive nor gram-negative, but are usually considered gram-positive, as they lack an outer cell membrane. Mycobacteria are typical acid-fast bacteria. When grown in liquid they form mould-like pellicles, hence their name (the Greek word *myco* means mould).

Mycobacterium species can be grouped in several ways: one important division is between the **tuberculosis** (tubercle) mycobacteria and **non-tuberculosis** (environmental) mycobacteria. A distinction can also be made between **rapid** and **slow** growers (note that even the fast growers have a slow growth rate when compared with other bacteria).

Members of the *M. tuberculosis* complex, which includes *M. tuberculosis*, *M. bovis* and several closely related variants, are the causative agents of tuberculosis, a disease affecting humans and other animals. *M. leprae* causes the disfiguring disease leprosy. Around six million people worldwide suffer from this disease, which is most widely prevalent in Africa and India. Humans and armadillos are the only natural hosts of *M. leprae*. *Mycobacterium aviumintracellulare* (also referred to as *M. avium* complex or MAC) is an environmental mycobacterium that causes a range of diseases in immunocompromised individuals. This complex is particularly problematic in AIDS patients.

3.3.1 Activity 3.1

Do the following activity and add it to your portfolio.

(a) What is the difference between gram-positive and gram-negative bacteria?
(b) Look up acid-fast staining. (Use the index in the textbook.)

- What is Ziehl-Neelsen stain?
- How does acid fastness affect gram staining?
- Are most bacteria acid fast?

For interest, go to http://www.bacterio.cict.fr and find the page on mycobacteria.

3.4 *Mycobacterium tuberculosis* complex

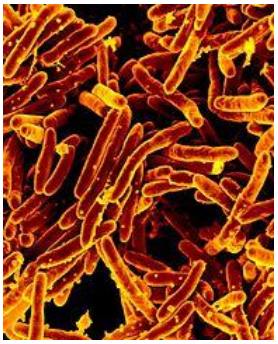

Figure 3.2: Scanning electron micrograph of *Mycobacterium tuberculosis* (http://commons.wikimedia.org/wiki/File:Mycobacterium_tuberculosis_MEB.jpg)

Mycobacterium tuberculosis complex contains all the mycobacterium species that can cause tuberculosis in humans or other animals. These include

- *Mycobacterium tuberculosis*
- *Mycobacterium africanum*
- *Mycobacterium bovis*
- *Mycobacterium microti*

3.5 Morphology and growth

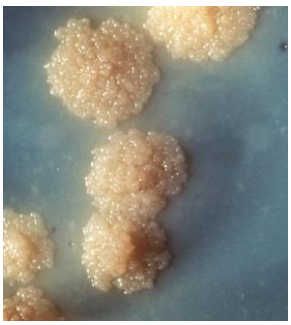

Figure 3.3: This is a close-up of a *Mycobacterium tuberculosis* colony, showing its typical morphologic characteristics when grown on solid media. (http://en.wikipedia.org/wiki/File:TB_Culture.jpg)

When a patient is diagnosed with tuberculosis, it is important to establish whether the tuberculosis is caused by the *M. tuberculosis* bacillus or another mycobacterium. Mycobacterium species can be identified by looking at a number of traits, such as

- growth rate
- colony morphology
- pigmentation
- biochemical profiles
- molecular methods

Other distinguishing characteristics can also be used – for example, *M. tuberculosis* does not grow at 25 °C or at 41 °C, while other members of the genus do.

3.5.1 Activity 3.2

Do the following activity and add it to your portfolio.

In your own words, describe *M. tuberculosis* in terms of

- motility
- spore and capsule formation
- shape
- growth characteristics
- growth response to various media
- environmental requirements (including temperature and atmospheric preferences)
- reaction to physical and chemical agents

3.6 Pathogenesis and pathology

Humans are vulnerable to infection by *M. tuberculosis* when they inhale organisms that have been released into the environment by infected people. Infection with this bacterium results in pulmonary tuberculosis. Infected people give off mycobacteria in microscopic droplets (< 25 µm in diameter) when they sneeze, cough or speak.

Primary infection usually occurs when these infectious droplet nuclei are inhaled, and lodge in lung alveoli. When in the lungs the bacilli are engulfed by the alveolar macrophages, but the macrophages are incapable of digesting the bacterium. Instead, the bacilli multiply inside the macrophages, and form a lesion.

The lesions that develop can take several forms. The lesions are classified as either

- exudative or
- productive.

Exudative lesions occur as a result of acute inflammatory reaction. The exudate secreted includes polymorphonuclear leukocytes and monocytes. These lesions are common in lung tissue, and cause symptoms similar to pneumonia. They may heal with the development of necrotic tissue, or the lesions may develop into the second form, the **productive** lesion. In productive lesions there is no exudate. Instead, macrophages surround the foci of infection and form granulomas, which consist of several zones:

- The central area contains multinucleated giant cells containing bacilli
- The mid zone contains epithelioid cells
- The outer zone consist of fibroblasts, lymphocytes and monocytes

The interaction between the components of the immune system and the bacteria elicits a range of responses. Inside the granulomas, most bacilli die from acidosis and anoxia. Not all bacilli die, however. Some become dormant and can later be reactivated, causing post-primary infection.

Reactivation may occur many years later.

At a later stage of infection some bacilli can be carried via the lymphatics to the regional lymph nodes, where additional infection foci develop, forming a primary complex (also called a Ghon complex). If the bacilli then enter the bloodstream they can be distributed to all organs (miliary distribution). Bacilli may also be swallowed and enter the stomach and intestines, forming secondary infections.

3.6.1 Activity 3.3

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) Make your own notes on the pathogenesis and pathology of the *M. tuberculosis* complex.
(b) Compare primary and post-primary (reactivation) tuberculosis.
(c) In addition to pulmonary tuberculosis, *M. tuberculosis* infections can manifest in other places in the body. In your own words, discuss other forms of tuberculosis.

3.6.2 Feedback on activity 3.3

When discussing primary and post-primary (reactivation) tuberculosis, did you remember to include notes on the site of infection, the progress of the disease, the immune system responses, the involvement of the lymph nodes, and the outcomes?

When describing how tuberculosis infections can occur in other places in the body, did you remember to include notes on miliary tuberculosis? Did you remember to mention the spread of infection through the lymphatic channels and bloodstream, resulting in the infection of other organs?

3.7 Diagnosis

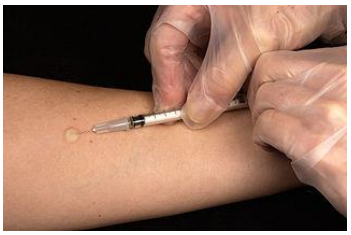

Figure 3.4: This technician is in the process of carrying out a Mantoux tuberculin skin test on the recipient's forearm. This test is used to determine whether someone has been exposed to *Mycobacterium tuberculosis*.
(http://commons.wikimedia.org/wiki/File:Mantoux_tuberculin_skin_test.jpg)

The tuberculin test was the earliest form of diagnostic test for tuberculosis, and is used to assess people for latent TB infection. In the tuberculin (Mantoux) test, a purified protein derivative (PPD) is injected just under the skin (see the image above). If the injected individual has never been in contact with mycobacteria there will be no reaction at the site of the injection. If the individual has had a primary TB infection, induration (hardening), oedema (swelling) and erythema (reddening)

will develop within 24 to 48 hours. A positive reaction does not mean that the individual has an active TB infection, as they may have cleared the primary infection, but will be at risk of reactivation of the primary infection.

Other diagnostic techniques can be divided into the following categories:

- microscopy
- culture methods
- molecular methods
- drug susceptibility testing

Drug susceptibility testing is important when choosing which drugs to use for treatment if antibiotic resistance is suspected.

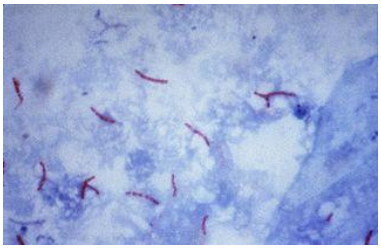

Figure 3.5: This photomicrograph reveals *Mycobacterium tuberculosis* bacteria using acid-fast Ziehl-Neelsen stain. (http://en.wikipedia.org/wiki/File:Mycobacterium_tuberculosis_Ziehl-Neelsen_stain_02.jpg)

3.7.1 Activity 3.4

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) Discuss the laboratory diagnosis of tuberculosis, with reference to

- collection and treatment of specimens
- the microscopic techniques used (remember to discuss Ziehl-Neelsen staining and fluorescence microscopy)
- cultures (appropriate media, culture temperatures, incubation periods)
- molecular methods (include nucleic acid techniques such as PCR along with molecular probes)

(b) Discuss the tuberculin reactivity test. What does a positive tuberculin test indicate about the presence or absence of disease? How does past vaccination with bacillus Calmette-Guérin (BCG) affect the tuberculin test?

3.7.2 Feedback on activity 3.4

With regard to the tuberculin reactivity test you should have mentioned that a positive result does not indicate active disease, but it does indicate that the individual has been exposed to TB. If they do not currently have an active infection, they will be at risk of reactivation of the infection.

Vaccination with BCG may give a positive result for three to seven years after vaccination.

3.8 Treatment

Tuberculosis is typically treated using drugs.

The two main **first-line** drugs used against *M. tuberculosis* are isoniazid and rifampin. Three other first-line drugs are pyrazinamide, ethambutol and streptomycin.

Second-line drugs are either more toxic for the patient or less effective against the bacteria (or both), and are used only if treatment with the first-line drugs is failing, or if the bacteria are known to be drug resistant.

The specific choice of drug depends on the World Health Organization (WHO) treatment category of the patient. Treatment with drugs is generally lengthy, and initial treatment is usually between six and nine months.

The development of drug resistance through mutation is of enormous concern in the treatment of tuberculosis. To counteract this, successful treatment requires the simultaneous use of at least two drugs, and generally a four-drug cocktail is recommended if drug-resistant bacteria are suspected.

3.8.1 Activity 3.5

Do the following activity and add it to your portfolio.

(a) Multidrug-resistant tuberculosis is a growing problem. Discuss how such strains arise, and the treatment options for individuals who have this disease. What can be done to reduce the likelihood of such strains emerging? What are the long-term implications of multidrug resistance?
(Although you will find all the information you need in the textbook, you will find additional information on the WHO website.)

(b) What is XDR-TB? What is the clinical outcome of treatment for somebody infected with XDR-TB?

3.9 Epidemiology and control

Early detection and **effective treatment** are crucial to stop the spread of the disease. This may involve proactively searching for potentially infected persons, as well as close monitoring of anyone who has had contact with infected individuals and, if they show symptoms, treating them before they spread the illness. To reduce the chance of the development of multidrug-resistant bacteria, treatment regimens must be strictly adhered to.

Anybody exposed to an infectious source will be susceptible to primary infection, although disease occurs in only a small percentage of infected people. Genetics, age (elderly people and infants are more susceptible), malnutrition, immunological status and other coexisting illnesses (e.g. diabetes) will all influence the development of the disease in the individual.

Take note of how **socioeconomic** factors affect the incidence of the disease:

- reducing overcrowding lowers the chance of infection, while
- factors which lower host resistance, such as starvation, infection or suppressed immunity, contribute towards disease manifestation.

According to the WHO, the risk of developing tuberculosis is estimated to be between 12 and 20 times greater in people living with HIV than among those without HIV infection.

The spread of TB is controlled through a number of measures:

- Effective detection and treatment of all cases of TB.
- Asymptomatic tuberculin-positive people who are at risk for reactivation of active disease (whether because of age or other contributing factors) may be given treatment to reduce the chances of reactivation.
- Vaccination of infants, most commonly using BCG (bacillus Calmette-Guérin, an attenuated bovine bacterium), is practised in many countries. The efficacy varies, but studies have shown effective protection of children for a limited period of time. BCG is contraindicated for HIV-positive individuals, and alternative vaccines are under investigation.
- HIV/AIDS is a major risk factor for the development of tuberculosis, and HIV-positive people need to be monitored to receive treatment timeously.
- *M. bovis* infections have been reduced through monitoring of cattle herds for tuberculin reactivity and treating ill cattle. The pasteurisation of milk has also prevented the transmission of *M. bovis* to humans through milk products.

3.10 Other mycobacteria

3.10.1 *Mycobacterium leprae*

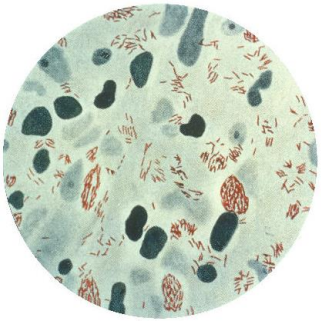

Figure 3.6: A photomicrograph of *Mycobacterium leprae*, the small red rods, taken from a leprosy skin lesion (CDC, US Government public domain, 1979) Public Health Image Library (PHIL) #2123. http://en.wikipedia.org/wiki/File:Mycobacterium_leprae.jpeg)

M. leprae causes leprosy, a disfiguring disease that can result in permanent injury to the skin, nerves, limbs and eyes. *M. leprae* is thought to be transmitted from human to human, probably via nasal secretions and shed skin. Leprosy is not very contagious. Incubation is slow, and there may be no symptoms for between 2 and 40 years (average 5 to 7 years). Lesions tend to form on cooler tissues such as skin, nose, superficial nerves, ears, eyes, larynx, pharynx and testicles.

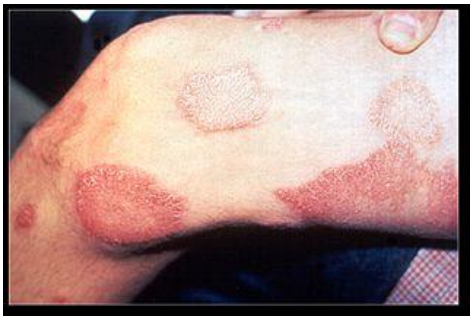

Figure 3.7: Thigh with demarcated cutaneous lesions of leprosy (http://commons.wikimedia.org/wiki/File:Leprosy_thigh_demarcated_cutaneous_lesions.jpg)

There are two major types of disease, namely **lepromatous** and **tuberculoid**, and several intermediate forms. Disease progress varies from **benign and non-progressive (tuberculoid or TT leprosy)** to **malignant and progressive in lepromatous leprosy (LL)**.

Diagnosis involves scrapings of skin and nasal mucosa or skin biopsies, and microscopy following acid-fast staining. PCR techniques may also be used, but no serological testing is conducted.

Leprosy is curable with treatment. Treatment involves one or more drugs, with the **sulfones** being the first-line drugs for both forms of leprosy. Treatment with drugs is generally over a number of months, but may need to extend over a number of years to clear the infection completely. Drug therapy is often only a small part of the overall treatment, with a range of other interventions being required, for example the correction of deformities, as well as counselling.

M. leprae is very slow growing, and is difficult to culture on bacteriological media. It is generally grown in the footpads of mice.

3.10.2 Environmental mycobacteria

Other names for this group of organisms include non-tuberculous mycobacteria, opportunistic mycobacteria, atypical mycobacteria and “mycobacteria other than tuberculosis” (MOTT). These organisms can cause a range of diseases, including chronic pulmonary disease; skin, tissue, bone and joint infections; catheter-related infections and disseminated infections. The non-tuberculous mycobacteria can be subdivided into the rapid growers (growth in ≤ 7 days), photochromogens, scotochromogens and non-chromogens, based on differences in pigment production.

Pathogenesis

Environmental mycobacteria are opportunistic pathogens which generally only produce overt disease in persons whose immune response is poor. HIV/AIDS patients, children, and people recovering from illness or injury are most often affected.

Diagnosis and treatment

Diagnosis of non-tubercular mycobacteria may be done through a number of methods to identify which species is causing disease. These methods include

- culture conducted at a range of temperatures (some species will only grow at certain temperatures) using diverse media or additives; growth rate, colony morphology and the presence of pigmentation can help to identify the species
- microscopy: acid-fast staining to ascertain whether it is a mycobacterium does not differentiate species
- biochemical testing
- molecular methods, including molecular probes, HPLC and PCR-based techniques

The molecular methods are replacing the traditional approaches, as results are obtained far more quickly (in days rather than weeks).

Drugs and/or surgery may be used during treatment. The choice of treatment depends on the site of infection, the specific mycobacterium involved, and on whether or not the patient has complicating factors such as HIV.

3.10.3 Activity 3.6

Do the following activity and add it to your portfolio.

(a) In your own words, discuss the two main types of leprosy. Include notes on numbers of bacteria, immune response, disease progress and treatment options.
(b) Describe what photochromogens, scotochromogens and non-chromogens are. Give the names of some organisms in each group, and describe their impact on humans (you may find tables 23-1 and 23-2 in Brooks helpful).
(c) Make notes on the non-tubercular disease caused by *M. avium*. Discuss the reservoir of the bacteria and the common symptoms of the disease (refer to table 23-1 in Brooks).
(d) Molecular probes provide a rapid and sensitive method to identify mycobacteria. What are molecular probes?

3.10.4 Feedback on activity 3.6

Molecular probes used for the diagnosis of TB are discussed at the end of the section, “Culture, identification and susceptibility testing” on page 294 of the textbook. DNA probes for specific rRNA sequences are often used. These probes will bind to DNA sequences only if they are present in the bacterial DNA, and this binding can be detected. As the different species of mycobacteria have different rRNA sequences, the probes can be used to distinguish the different species.

3.11 Conclusion

Review the outcomes of this module and think about whether you have achieved them.

To sum up all you have covered in this study unit, draw up a table as a way of comparing *M. tuberculosis*, *M. leprae* and *M. avium*. Include any characteristics that can be used to distinguish between *Mycobacterium* species – this could include shape, motility and presentation.

	M. tuberculosis	*M. leprae*	*M. avium*
Motility			
Spore formation			
Capsule formation			
Shape			
Colony morphology			
Growth temperature			
Growth characteristics			
Environmental requirements			

Learning unit 4

Vibrio

4.1 Introduction

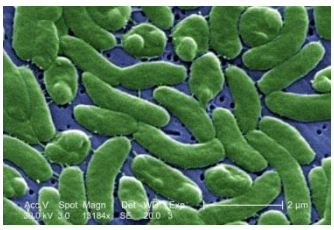

Figure 4.1: False colour scanning electron micrograph of *Vibrio vulnificus* bacteria (Obtained from the CDC (http://phil.cdc.gov/phil/home.asp Public Health Image Library). Image credit: CDC/James Gathany (PHIL #7815) http://en.wikipedia.org/wiki/File:Vibrio_vulnificus_01.png)

There are more than 100 *Vibrio* species, many of which are found in aquatic environments. Only a few species are pathogenic. Disease-causing strains are typically associated with gastroenteritis and wound infection, and some may cause bacteraemia. *V. cholerae* is generally transmitted via contaminated water, and is the causative agent of cholera. *V. parahaemolyticus* and *V. vulnificus* are also important pathogens of humans, usually associated with the eating or handling of contaminated seafood.

To complete this study unit, you will need to refer to chapter 17 of Brooks. You will find more information on this topic on the following website:
http://www.textbookofbacteriology.net/themicrobialworld/cholera.html

4.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- list some common vibrios
- describe *V. cholerae* and discuss the different pathogenic serotypes
- discuss cholera infections in terms of
    - pathogenesis and pathology
    - diagnosis, treatment, epidemiology and control
- explain what *V. parahaemolyticus* is and discuss its pathology and treatment
- explain what *V. vulnificus* is and discuss its pathology and treatment

4.3 Genus *Vibrio*

Recommended reading: the section on vibrios on pages 235–238 in chapter 17 of Brooks

Vibrios are short, gram-negative rods. They are often curved. They possess a single polar

flagellum, and so are motile. They are facultatively anaerobic. The genus can be divided into

- halophilic species, which require salt to grow, and
- non-halophilic species, which do not require salt to grow.

V. vulnificus and *V. parahaemolyticus* are halophilic.

V. cholerae and many others are non-halophilic. However, the non-halophilic species are salt-tolerant to some degree.

The species most relevant to medical microbiologists are *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus*. A few other species sometimes cause opportunistic infections.

V. cholerae is water- and food-borne. It is responsible for **cholera**, a disease that causes abundant watery diarrhoea, often accompanied by vomiting. Together these can lead to dehydration and, if left untreated, may lead to death.

V. parahaemolyticus is acquired primarily through eating infected seafood. It causes acute **gastroenteritis**.

V. vulnificus may cause **septicaemia** (when infected, raw shellfish are consumed), or **cellulitis** (when a wound becomes contaminated with the bacterium).

Figure 4.2: Yellow coloured (sucrose fermenting) colonies of *Vibrio cholerae* on TCBS agar (http://en.wikipedia.org/wiki/File:Vibrio_cholerae_on_TCBS_agar.jpg)

4.4 *Vibrio cholerae*

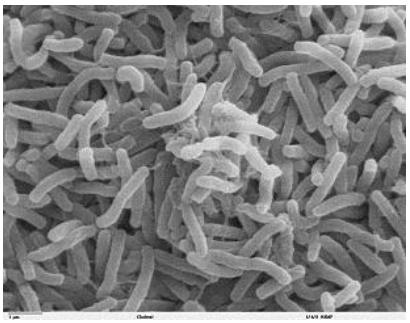

Figure 4.3: Scanning electron microscope image of *Vibrio cholerae* (http://en.wikipedia.org/wiki/File:Cholera_bacteria_SEM.jpg)

V. cholerae lives freely in freshwater environments, and a number of strains cause cholera in

humans. It was first isolated as the cause of cholera by Filippo Pacini in 1854.

There are many different **strains** of *V. cholerae.* They are differentiated primarily according to the type of **O antigen** they produce. The O antigen is a lipopolysaccharide that is found in the outer membrane of gram-negative bacteria that helps stabilise the overall membrane structure. As the composition of the O chain varies from strain to strain, it confers serological specificity.

The pathogenic effect of a particular *V. cholerae* depends on which O antigens it possesses. There are at least 139 different O antigens in the species. Classical epidemic cholera is caused by the O1 antigen. A different serogroup, bearing antigen O139, has more recently emerged as a new epidemic strain. Strains bearing other antigens do not cause cholera, but may cause diarrhoea or cholera-like symptoms.

The O1 strains can be further subdivided into three subtypes (Ogawa, Inaba and Hikojima), and two biotypes (classic and El Tor).

4.4.1 Activity 4.1

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, describe *V. cholerae* in terms of

- shape
- motility
- reaction to gram-staining
- tolerance to salt
- growth requirements
- culture characteristics
- metabolic processes
- temperature
- pH range

(b) What are O antigens? In your own words, describe the role they play in bacterial pathogenicity. (You may have to refer to other sections of the textbook or additional resources to answer this question fully.)

(c) What is meant by serological specificity?

(d) Would a bacterium such as *Bacillus cereus,* a gram-positive bacterium that causes watery diarrhoea and vomiting, have O antigens? Give reasons for your answer.

(e) What are H antigens? Compare H antigens and O antigens. Why are H antigens not used in the differentiation of *V. cholerae* strains?

4.4.2 Feedback on activity 4.1

If you look up O antigens in the index of the textbook, you will see that you are referred to pages 26 and 27. The O antigen is highly immunogenic in vertebrate animals, and is found on some gram-negative bacteria.

The O antigens are serologically specific because the different strains of *V. cholerae* have different O antigens which interact with different antibodies against each different type of O antigen. This allows the different strains to be distinguished from one another by means of a serological test.

Most vibrios have the same H antigen, and so it cannot be used to differentiate the strains.

4.4.3 Pathogenesis and pathology

Cholera infections are generally acquired from drinking water containing *V. cholerae* that may have been contaminated with the faeces of an infected person.

V. cholerae is susceptible to acid, so when water is the mode of transmission, a large dose (around 10^{10} bacilli) must be ingested in order for infection to occur, unless a person is achlorhydric or on antacids. When the mode is food, a much smaller number of organisms (~ 100 to 10 000) may be infective, since the food acts as a buffer against the stomach's acidity. *V. cholera* is non-invasive, and the organisms remain in the intestinal tract. They cause disease by binding to the microvilli of the intestinal epithelial cells, where they reproduce.

More than half of people infected with *V. cholerae* do not develop symptoms. For people who display symptoms (nausea, vomiting, diarrhoea and abdominal cramps), the incubation period is 1 to 4 days. Without treatment, the mortality rate is between 25 and 50%.

4.4.4 Diagnosis

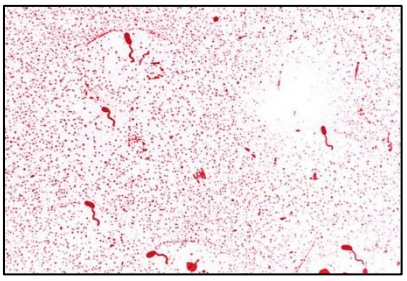

Figure 4.4: *Vibrio cholerae* with a Leifson flagella stain (digitally colourised) (http://en.wikipedia.org/wiki/File:Vibrio_cholerae_01.jpg. Original image from the CDC Public Health Image Library (PHIL #1034). Image credit: CDC/Dr Edwin P. Ewing, Jr)

When a patient presents with profuse vomiting, nausea and “rice-water” stool, strain-specific diagnosis is of far less importance than urgent treatment. If cholera is prevalent in an area, laboratory diagnosis may be bypassed altogether. In the case of sporadic or imported cases, however, a range of tests are utilised to isolate the causal organism.

4.4.5 Activity 4.2

Do the following activity and add it to your portfolio.

In your own words, discuss laboratory diagnosis of *V. cholerae*. Refer to

- collection and treatment of specimens
- the microscopic techniques used (include reference to dark-field and phase contrast microscopy)
- cultures (appropriate media, culture temperatures, incubation periods)

4.4.6 Treatment

V. cholerae is susceptible to many antimicrobial agents, which are generally given orally. In the absence of treatment, mortality from full-blown cholera may be as high as 50%. Patients must receive fluids and electrolytes to replace those lost through diarrhoea and vomiting. Death usually occurs as a result of dehydration and nutrient loss.

4.4.7 Epidemiology and control

V. cholerae is only pathogenic to humans, and is able to live freely in aquatic environments. Humans who become infected do not always show symptoms, and these individuals may act as reservoirs of the disease. Crowded living, poor hygiene, inadequate sewage disposal and insufficient access to clean drinking water all contribute to the spread of infection. Cholera pandemics have caused the death of millions of people since the 19th century.

Cholera epidemics are controlled through education and improvement of sanitation of food and water. Infected individuals need to be educated about hygiene so they do not spread the disease. Cholera vaccines can also be administered as an additional tool in the control of cholera outbreaks.

For more background information about cholera epidemics, consult http://textbookofbacteriology.net/cholera_2.html.

4.4.8 Activity 4.3

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

Conduct your own research on recent cholera outbreaks in the Southern African region and in Haiti. (A good source of information on the Haitian outbreak can be found at http://www.un.org/News/dh/infocus/haiti/UN-cholera-report-final.pdf.)

(a) Are the same serotypes/biotypes responsible for the Southern African and Haitian outbreaks?
(b) In your own words, discuss ways of controlling cholera. Include remarks on the use of vaccines.
(c) How do epidemics caused by the classic cholera strain differ from those caused by El Tor cholera? Why do you think the El Tor strain is more prevalent today? Refer to carrier:case ratios.

4.5 Other vibrios

Other vibrios cause a range of diseases that affect humans. *V. parahaemolyticus* and *V. vulnificus* can cause gastroenteritis and wound infections. Several other vibrios, such as *V. alginolyticus*, *V. fluvialis* and *V. mimicus* cause a range of diseases, from diarrhoea to wound and ear infections.

4.5.1 *Vibrio parahaemolyticus*

V. parahaemolyticus causes acute gastroenteritis characterised by nausea, vomiting, cramps, fever and diarrhoea that may be watery or bloody. These symptoms appear 12 to 24 hours after consumption of contaminated raw seafood, and generally subside after approximately 1 to 4 days. Wound infections and infections of the eyes and ears may occur, and are associated with aquatic environmental exposure or with handling of contaminated seafood.

Symptoms typically disappear within 3 days, but can continue for up to 10 days in immunocompromised individuals. Treatment is often not necessary, although fluid and electrolyte replacement may be required.

V. parahaemolyticus is generally identified by its oxidase-positive growth on blood agar.

4.5.2 *Vibrio vulnificus*

V. vulnificus is a free-living bacterium that can infect humans who either work with or consume contaminated seafood. It can cause wound infection, gastroenteritis and bacteraemia. The bacteria are identified by culturing on standard or TCBS media, where they form blue-green sucrose-negative colonies. The disease often progresses rapidly and needs to be treated with antibiotics.

An outbreak of *V. vulnificus* occurred in New Orleans after Hurricane Katrina, and resulted in a number of deaths.

4.5.3 Activity 4.4

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) *V. parahaemolyticus* can be differentiated from *V. cholerae* in a laboratory using a culture medium (TCBS medium). In your own words, discuss what TCBS medium is, and how you would distinguish the two bacteria on that medium.
(b) Discuss the various clinical syndromes caused by *V. vulnificus*. Include notes on pathogenesis and disease progress.
(c) Make notes on the epidemiology, treatment and control of *V. vulnificus*.

4.5.4 Feedback on activity 4.4

You will find the answers to the questions on other vibrios in the section, "*Vibrio parahaemolyticus* and other vibrios" on page 238 in the textbook.

4.6 Conclusion

Review the outcomes of this module and think about whether you have achieved them.

To sum up all you have covered in this study unit, compare the three species of vibrios that have the most significant effect on humans: *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*.

Include notes on

- identification
- salt tolerances and requirements
- growth characteristics on culture
- pathogenesis and pathology (including toxins)
- diagnosis
- treatment and control

Learning unit 5

Rotavirus

5.1 Introduction

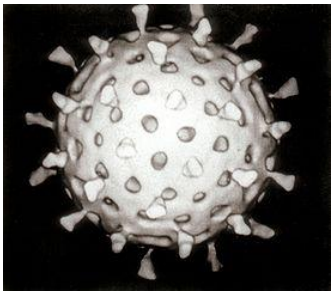

Figure 5.1: Computer-assisted reconstruction of a rotavirus particle (http://en.wikipedia.org/wiki/File:Rotavirus_Reconstruction.jpg)

Rotavirus is the most frequent cause of severe diarrhoea among infants and young children, and is estimated to be involved in at least half a million deaths a year. The symptoms of rotavirus infection include vomiting, watery diarrhoea, and low-grade fever. Dehydration is the most common reason for death related to rotavirus infection.

To complete the study unit, you will need to refer to chapter 37 of Brooks. You will find more information on this topic on these websites: http://pathmicro.med.sc.edu/virol/rotaviruses.htm and http://virology-online.com/viruses/Diarrhoea2.htm

5.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- summarise the classification, structure and replication of Reoviridae
- describe the classification of rotaviruses, including groups and serotypes
- discuss the morphology and physical characteristics of rotaviruses
- discuss the pathology and clinical features of rotavirus infections
- describe the diagnosis of rotavirus infections
- explain the epidemiology of rotavirus infections
- discuss treatment and control options for rotavirus infections

5.3 Reoviridae

Recommended reading: the sections on reoviruses and rotaviruses on pages 507–512 in chapter 37 of Brooks

Reoviruses are RNA viruses that infect a number of species, ranging from plants and insects to birds and mammals. The family name comes from "*r*espiratory *e*nteric *o*rphan", because the earliest isolates could not be associated with disease; even today, although various reoviruses trigger the development of antibodies in humans, there is often still no clear link between the virus and illness. This ambiguity does not apply to the *Rotavirus* genus, which is proven to cause gastroenteritis and is acknowledged as the most important cause of severe infantile gastroenteritis worldwide.

5.3.1 Classification

The family Reoviridae is divided into 15 genera, of which only 4, *Orthoreovirus*, *Rotavirus*, *Coltivirus* and *Orbivirus*, contain species able to infect humans and animals. As I said earlier, microorganism classification undergoes periodic revision as new scientific information becomes available, so while Brooks et al state that there are 15 genera in the Reoviridae family, the latest data from the International Committee on Taxonomy of Viruses may differ.

For your own interest, visit the following website: http://ictvonline.org/index.asp

For the most recent release year, look up reoviruses.

- How many sub-families are listed?
- How many genera are listed?
- Which sub-family contains rotavirus?

5.3.2 Structure and composition

Reoviruses are non-enveloped (rotaviruses acquire a temporary envelope during replication, but it is soon lost). They are icosahedral, with a double-layer capsid surrounding a central core, which consists of the genome as well as enzymes required for transcription and capping of the viral RNA.

The genome is split into linear segments, the number and size of which vary depending on the genus. There are 11 segments of double-stranded RNA, totalling between 16 and 27 kbp.

5.3.3 Replication

In general, virus replication strategies differ depending on

- whether the virus is DNA or RNA
- whether it is single or double stranded
- the polarity (or sense) of the strand

The following website clearly sets out the different RNA replication strategies, with examples: http://pathmicro.med.sc.edu/mhunt/RNA-HO.htm

Before reoviruses can replicate, they must attach to and enter an appropriate host cell. To ensure this, specific cell attachment proteins (a component of the outer capsid) on the virus interact with specific receptors on the cell surface. In most reoviruses, this interaction causes a conformational change in the capsid, enabling the reovirus to enter the cell through endocytosis. Since reoviruses are double-stranded RNA, their replication cycle occurs entirely within the host cell cytoplasm. Refer to figure 37-3 in Brooks for an illustration of the rotavirus replication cycle.

This University of Calgary animation illustrates reovirus replication step by step: http://www.mcb.uct.ac.za/tutorial/calgary_files/Reoviridae.swf

5.3.4 Activity 5.1

Do the following activity and add it to your portfolio.

Write a one-page essay describing the steps involved in the replication of reoviruses. Be sure to refer to attachment to receptors, penetration of the cell, uncoating in lysosomes, replication of the virus, particle maturation and cell lysis.

5.4 Rotaviruses

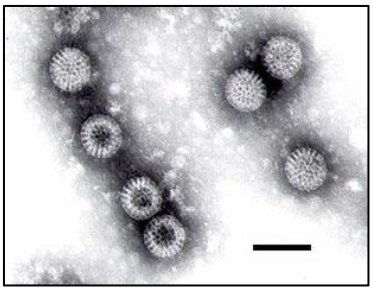

Figure 5.2: Negative-stain transmission electron micrograph of rotavirus particles (http://en.wikipedia.org/wiki/File:Rotavirus.jpg. Original image from http://www.epa.gov/nerlcwww/rota.htm. Image by F.P. Williams)

5.4.1 Classification, structure and replication

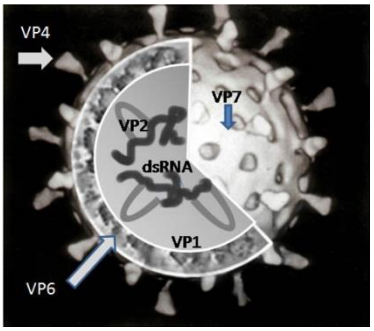

Figure 5.3: Rotavirus structure (http://en.wikipedia.org/wiki/File:Rotavirus_Structure.png)

Rotaviruses are the main cause of diarrhoea in young children and animals. Rotaviruses can be classified into five species (A–E), depending on the antigenic epitopes found on the VP6 structural protein. The viral particle is triple-layered, with channels between the outer and inner capsid and spikes on the surface. (For an illustration, see figure 37-4 in Brooks.) In cross-section under an electron microscope the channels resemble the spokes of a wheel; hence the name rotavirus. The rotavirus genome is made of 11 segments of different sizes, totalling around 18.5 kbp. Each segment codes either a structural protein (VP) or a non-structural protein (NSP).

Group A rotaviruses are the most common human pathogens, and cause over 90% of human rotavirus infections. Within group A there are different serotypes. The G protein (VP7) defines the G serotypes and the P protein (VP4) defines the P serotypes. Group B and C rotaviruses also infect humans.

Rotaviruses enter and replicate within cells of the intestine. To see the rotavirus replication cycle illustrated, see figure 37-3 in Brooks.

5.4.2 Pathogenesis, immunity, clinical features and laboratory diagnosis

The virus is transmitted by the faecal–oral route. When it enters the gut it infects and damages the cells in the villi of the small intestine. The virus replicates in the cytoplasm of enterocytes. These damaged cells then slough off into the intestinal lumen and are eliminated with the stool. In healthy people, infection and viral excretion lasts 2 to 12 days. Diarrhoea occurs because of impaired sodium and glucose absorption, resulting in watery stools. Fever, vomiting and nausea often precede the diarrhoea. Dehydration is the main contributor to mortality.

Laboratory diagnosis is normally through detection of the virus in a stool sample. The presence of the virus is typically demonstrated by enzyme immunoassays (EIAs) or immune electron microscopy (IEM). Other methods, such as PCR for genotyping or ELISA to detect antigens in stool samples, may also be employed.

5.4.3 Activity 5.2

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, discuss the structure of a rotavirus. Include notes on the location of each protein in the final structure (core, inner or outer capsid). It may help to draw a diagram of the virus.
(b) In your own words, discuss the pathogenesis and pathology of rotavirus infections. Refer to the location of infection, the effect on the cells and the resulting effects on the patient (including signs and symptoms).
(c) In your own words, discuss the diagnosis of rotavirus infections.
(d) What is an enzyme immunoassay?

5.4.4 Feedback on activity 5.2

When describing the structure of rotavirus, you may have found the information in figure 37-4 in Brooks helpful.

Enzyme immunoassays are quick, accurate tests that are performed in a laboratory to detect the presence of identifiable molecules. They involve the use of antibodies that specifically bind the molecule of interest. If binding occurs, then it is assumed that the specific molecule is present.

5.4.5 Epidemiology, treatment and control

Rotaviruses are found throughout the world, and studies show that most children have serum antibodies against at least one type of rotavirus by the time they are 3 years old. Worldwide, rotavirus infections may account for up to 50% of acute gastroenteritis hospitalisations of children, and may result in a million deaths annually. Infections in temperate countries exhibit seasonal peaks and troughs, with rotavirus infections being more common in the winter months. In tropical

countries, infections occur throughout the year. The most common age for symptomatic infections is 6 to 24 months. Nosocomial infections are also common.

The treatment is supportive, ensuring rehydration, either orally or intravenously. No effective antiviral agent is known.

As transmission is via the faecal–oral route, sanitation of water is important in controlling infections. Two different vaccines against rotavirus are available, and are given to young infants. They have been shown to be effective in protecting infants from rotavirus infection.

5.4.6 Activity 5.3

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, discuss the epidemiology of rotavirus infections in general. Individuals of what age are typically infected?
(b) In your own words, discuss treatment of patients infected with rotavirus.
(c) Discuss rotavirus control methods (include the role of vaccines, if relevant).

For your own interest:

(d) Conduct your own research to establish which types dominate in your country. Are infections in your country seasonal, or do they occur throughout the year?

5.5 Conclusion

A number of reoviruses infect humans. These include rotavirus, which is an important cause of infant gastroenteritis.

Review the outcomes of this module and think about whether you have achieved them. Can you describe the replication of reoviruses? Do you understand the classification of rotaviruses? Can you discuss the pathology, clinical features, diagnosis epidemiology and treatment of rotavirus?

Learning unit 6

Papillomavirus

6.1 Introduction

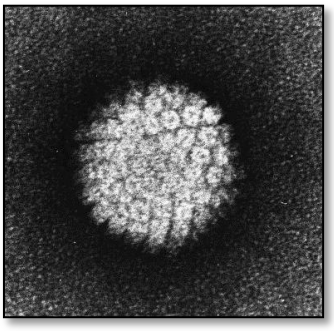

Figure 6.1: Electron micrograph of a negatively stained human papillomavirus (HPV), which occurs in human warts (http://en.wikipedia.org/wiki/File:Papilloma_Virus_%28HPV%29_EM.jpg)

The Papillomaviridae are a large family of viruses that infect many animals and humans.

- Some papilloma viral infections are asymptomatic.
- Others cause small benign tumours, called papillomas or warts.
- Yet other infections cause papillomas that may become cancerous.

They generally infect the skin or mucosal epithelium of the mouth, genitals, anus, or respiratory tract.

It is estimated that worldwide 660 million people have human papillomavirus (HPV) genital infections. HPV causes cervical cancer (99% of cervical cancers can be linked to genital infection with HPV). According to the WHO, there were an estimated 529 000 new cases and 274 00 deaths in 2008 due to cervical cancer.

To complete the study unit, you will need to refer to chapter 43 of Brooks. You will find additional information about papillomaviruses on the following websites: http://pathmicro.med.sc.edu/lecture/retro.htm and http://virology-online.com/viruses/Papillomaviruses.htm.

6.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- identify the structure of papillomaviruses
- describe the replication of papillomaviruses
- identify risk categorisation and discuss the classification of human papillomaviruses
- discuss the clinical features and pathogenesis of HPV infections
- discuss HPV and cancer
- discuss HPV transmission, epidemiology and diagnosis
- explain the role of vaccines in the control of cervical cancer

6.3 Papillomaviridae

Recommended reading: the section on papillomaviruses on pages 602–605 in chapter 43 of Brooks

Papillomaviruses are DNA viruses that infect the squamous epithelia or mucous membranes of a number of mammal, bird, reptile and amphibian species. They are generally highly host specific, and cross-species infections are rare. Warts (also known as papillomas) are common manifestations of papillomavirus infections. Each papillomavirus type is highly tissue specific, and will only infect a particular area of the body.

Papillomas are typically benign, but may become malignant carcinomas. In 1935 Francis Peyton Rous demonstrated that a papillomavirus infection could bring about skin cancer in rabbits. It was the first demonstration that cancer in mammals could be caused by a virus. Numerous elements may contribute to the development of malignancy, including the host's immune system and genetics, and external co-factors such as chemical exposure.

6.3.1 Structure, genome organisation and replication

The viral particles are 55 nm in diameter, and are composed of double-stranded DNA and protein. They lack an envelope, meaning that the capsid of the virus is not covered by a lipid membrane. The icosahedral capsid is made up of 72 capsomeres surrounding the supercoiled double-stranded DNA (dsDNA). The genome is around 8 kbp in size, and contains a non-coding regulatory region, an early region and a late region (refer to figure 48-3 in Brooks).

Papillomaviruses replicate in keratinocytes. The life cycle begins in the basal layer of the epithelium after viruses enter epithelial cells, often through some form of shearing trauma. After infection of a host cell, the six early open reading frames (E1, E2, E4, E5, E6, and E7) are transcribed. At this stage viral DNA can be detected, but complete virions are not present in the basal cells. As the epithelial cells differentiate the viral genome is replicated. In the upper layers of the host epithelium, the late genes L1 and L2 are transcribed. These encode for the structural proteins that encapsulate the viral genomes, and so complete viral particles are found only in terminally differentiated keratinocytes. Once the viral particles have matured, the virions are then sloughed off with the dead host epithelium. Refer to figure 43-9 in Brooks.

The *Nature* article Fehrmann, F. & Laimins, L. (2003) Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. *Oncogene* 22, 5201–5207 is very interesting and discusses the genome, life cycle and epidemiology of HPV. You can access it by clicking on this link: http://www.nature.com/onc/journal/v22/n33/full/1206554a.html.

6.3.2 Activity 6.1

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words define the following terms:
- non-enveloped
- icosahedral capsid
- supercoiled double-stranded DNA

(b) Describe the genome of papillomaviruses. Include notes on the early and late genes and the different products they produce.

(c) Why do you think basal cells are not infectious? In your answer, refer to the location of maturation of the virus.

6.4 Human papillomaviruses (HPV)

Papillomaviruses are not only species specific; they are also often tissue and site specific. Different HPVs can be classified according to genus, species, type, sub-types and variants. Many different types of HPV exist, with almost 200 dissimilar types being classified. They can also be categorised according to risk factor.

The classification of microorganisms is often under revision, with the result that different sources may give conflicting information. In the case of virus taxonomy, the acknowledged authority is the **International Committee on Taxonomy of Viruses (ICTV)**.

For more information, visit the ICTV website at: http://ictvonline.org/index.asp.

For the most recent release year, look up papillomaviruses.

- How many genera are listed?
- How many species of HPV are listed?

Transmission of HPV viruses occurs through close contact. HPV infections can be grouped into

- cutaneous infections and
- mucosal infections.

Different HPV types affecting different sites produce different forms of warts (refer to table 43-7 in Brooks). Cutaneous warts are generally benign. Immunological factors of the host influence the behaviour of the lesions, and nearly all HPV infections are cleared and are undetectable within two to three years.

6.4.1 Activity 6.2

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) Discuss the various forms of warts and lesions that the different types of HPV can cause (refer to table 43-7).
(b) Discuss disease progression of HPV infections (including the role played by host factors).

6.4.2 HPV and cancer

International studies show that papillomaviruses are associated with approximately **10% of all cancers**, and **15% of female cancers**. HPV DNA is present in 99% of invasive cervical cancers. The specific HPV types vary worldwide, with demographic and socioeconomic factors having an influence on distribution. The most common HPV types in cervical cancer are HPV-16 and

HPV-18, and these are involved in 70% of all cervical cancers. Anal cancer is also linked to HPV infection, with 80% of anal cancers associated with genital infection by HPV. The presence of a high-risk HPV type, however, is not in itself a guarantee of malignancy. Oropharyngeal cancers are also associated with HPV infection.

Animal studies on papillomaviruses and human epidemiological studies suggest that in addition, a number of other elements, called **co-factors** (or risk factors), play a role in cancer development. Examples of co-factors are smoking, immunosuppression and radiation exposure.

Some papillomaviruses, particularly those categorised as high-risk, employ a different form of virus–cell interaction: they induce changes in the properties of the host cell. This process is known as **transformation**. The tumour-causing potential of HPV comes from the ability of genes E6 and E7 to cause transformation of host cells, and this appears to be related to the integration of viral DNA into host DNA. In low-grade infections, viral DNA exists in episomal form only, whereas in malignant forms, high-risk HPV DNA is frequently found to be integrated.

Integration frequently disrupts E1 and E2, and since one of the functions of these genes is to down-regulate the expression of E6 and E7, integration allows higher levels of these genes to be expressed. The E6 and E7 proteins inactivate two tumour suppressor proteins, p53 and Rb, and, therefore, increase the chance of malignancy. Refer to the map of HPV in figure 43-8 in Brooks to help you understand the function of the different genes of HPV.

6.4.3 Activity 6.3

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) What is the difference between a virus that has integrated and one that is episomal?
(b) In your own words, discuss the pathogenesis of HPV-related carcinomas.

6.4.4 Feedback on activity 6.3

As HPV is linked to cancer, it is important that you understand what forms of cancer it can cause. The role of HPV in anogenital cancers is described in the section, “Clinical findings and epidemiology” on page 604 of the textbook.

6.4.5 Transmission, epidemiology and diagnosis

Approximately 660 million people worldwide are infected with genital HPV. Transmission of HPV viruses occurs through close contact. Viral particles are emitted from papillomatous lesions of an infected individual and enter microlesions in the new host. Genital wart transmission occurs mainly through sexual contact, but may also take place as a result of vertical transmission. Men are known to be carriers of HPV, and are therefore involved in the transmission of the virus. Most penile HPV infections are asymptomatic. Immunocompromised people have an increased risk of warts or cancer of the cervix, and, therefore, these conditions occur more frequently in people with HIV or AIDS.

Infection with various cutaneous HPVs is common in healthy adults. Most never result in any clinical symptoms, while others may cause common warts in some infected individuals. Infection with cutaneous HPV occurs through direct contact or via fomites, generally during childhood. Trauma, a cut or other wound allows the virus to reach the basal epithelium.

Diagnosis of HPV may involve morphological, molecular or serological methods. Abnormal Pap smear results may indicate testing for infection by high-risk HPV types. If high-risk HPV types are present, the patient would need to be monitored more frequently for cervical cancer development. Molecular methods generally involve the detection of HPV DNA in clinical samples.

6.4.6 Treatment and control

Many people with HPV infections are completely asymptomatic, and most infections resolve themselves. However, genital warts, recurrent laryngeal warts and intra-epithelial neoplasia may require treatment which may involve surgery and/or chemotherapy. Even with drug treatment, persistence and recurrence of the disease is a problem.

Two different HPV vaccines were approved in the USA in 2006 and 2007. These vaccines are for different types of HPV that are associated with cervical cancer. The vaccines contain particles made up of HPV L1 proteins. They are effective in preventing infections by the targeted HPV types, and stop the formation of the genital precancerous lesions. They do not, however, have any effect against established HPV infections. As the vaccines are against HPV that are associated with cervical cancer, they are recommended for adolescent and young adult females before they become sexually active.

6.4.7 Activity 6.4

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) Discuss what steps a person infected with genital HPV should take to prevent spreading the disease.
(b) In your own words, discuss the composition and efficacy of the HPV vaccines.

6.5 Conclusion

Review the outcomes of this module and think about whether you have achieved them.

Can you describe the structure of papillomaviruses and their replication? Do you understand how the virus is transmitted and the clinical features of HPV infection? Do you understand the link between HPV infections and cervical cancer, and the role of vaccines in the control of cervical cancer?

Learning unit 7

Retrovirus

7.1 Introduction

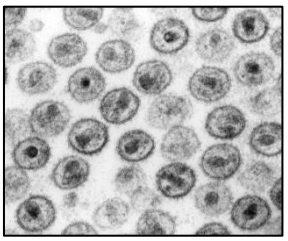

Figure 7.1: Transmission electron micrograph of HIV-1 (http://commons.wikimedia.org/wiki/File:HIV-1_Transmission_electron_micrograph_AIDS02bbb_lores.jpg. The original image is from the Public Health Image Library (PHIL) of the Centers for Disease Control and Prevention, with identification number #948.)

There are a large number of retroviruses. Of these, the best known is human immunodeficiency virus (HIV), the virus that causes AIDS. AIDS has become a worldwide epidemic, with millions of people being infected. There is no known cure, and without treatment most HIV-infected individuals die from opportunistic infections within ten years. Many different treatment options are now available which suppress replication of the virus, thus delaying the onset of AIDS.

In this study unit we will focus on HIV. To complete the study unit, you will need to refer to chapter 44 of Brooks. You will also be able to obtain further information from the internet sources I indicate in the text.

7.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- discuss the origin of HIV
- discuss the classification of HIV
- discuss the genome organisation of HIV
- discuss the replication of HIV
- discuss HIV and AIDS in terms of
    - pathogenesis and pathology
    - diagnosis
    - treatment
    - epidemiology
    - control

7.3 Retroviridae

Recommended reading: the whole of chapter 44 of Brooks

The Retroviridae are a large family of viruses affecting many different species. Retroviruses are all enveloped single-stranded RNA viruses that contain the enzyme reverse transcriptase in their core, enabling them to produce DNA from the viral RNA.

Generally DNA is transcribed into RNA, and the RNA is translated into proteins. Retroviruses are different in that they reverse transcribe their RNA into DNA using the enzyme reverse transcriptase. This process is the reverse of the normal pattern, hence the name retrovirus ("retro" means "backwards"). The new DNA is then inserted into the host genome by the enzyme integrase, and the virus becomes a provirus. The host then transcribes and translates the viral genes as if they were its own, resulting in new copies of the virus.

According to the ICTV classification of the Retroviridae, the family can be divided into two sub-families:

- the Orthoretrovirinae and
- the Spumaretrovirinae.

This study unit will concentrate on human immunodeficiency viruses (HIV-1 and HIV-2), which are part of the genus *Lentivirus*, which falls within the Orthoretrovirinae sub-family.

7.4 Lentiviruses

Lentiviruses are associated with chronic, slowly progressing diseases that are often fatal. They have been isolated from humans, non-human primates and non-primates, although in many of the non-human primates no disease is observed in the host of origin, but is observed in other monkey species after transmission.

- human immunodeficiency virus (HIV),
- simian immunodeficiency virus (SIV),
- feline immunodeficiency virus (FIV), and
- equine infectious anaemia virus (EIAV)

are all examples of lentiviruses.

Human immunodeficiency viruses are closely related to simian immunodeficiency viruses, and HIV is thought to have originated as a result of cross-species transmission through contact with infected primate blood, probably on multiple occasions.

7.4.1 Human immunodeficiency virus

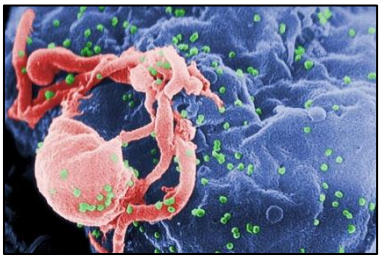

Figure 7.2: Scanning electron micrograph of HIV-1 budding (in green) from cultured lymphocyte. This image has been coloured to highlight the different features. (http://en.wikipedia.org/wiki/File:HIV-budding-Color.jpg. Image credit: CDC/C. Goldsmith)

The human immunodeficiency virus is extremely variable, and mutates readily, so (perhaps more than in the case of any microorganism discussed so far in this module) any printed classification system is likely to be out of date before the publication can be read!

In brief, though, HIV can be divided into two types:

- HIV-1 and
- HIV-2.

HIV-1 is more virulent and infective than HIV-2, and is responsible for the majority of human HIV infections. HIV-1 can be further subdivided into groups, sub-types, sub-subtypes and circulating recombinant forms (CRFs) based on variations in nucleotide sequence. The different strains of HIV-1 can be divided into four groups:

- group M, the major group,
- group O, the outlier group and
- two other groups, N and P

(Older texts will refer to fewer groups.)

Within group M there are at least 11 discrete subtypes or clades, A1, A2, B, C, D, F1, F2, G, H, J and K (for an illustration, see the figure below). This is not a final list, as it is possible that additional sub-types will be found in the future. CRFs form when there is recombination between different sub-types in an individual infected with more than one sub-type. They are each given a number, for example CRF35_AD, which is a recombinant form of sub-types A and D.

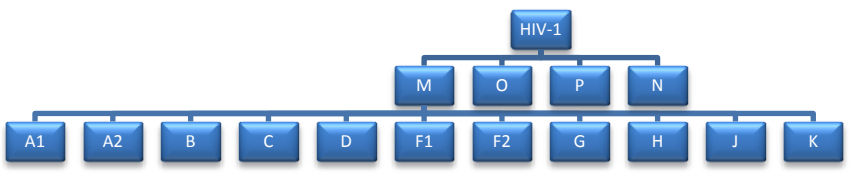

Figure 7.3: Current HIV-1 types and subtypes

The HIV Databases website, http://www.hiv.lanl.gov/content/index, is regularly updated and contains a wealth of other information and tools related to HIV. Another site worth consulting is http://www.avert.org/hiv-types.htm – it is not as up to date, but it is easy to understand.

Although the image below is not recent, it shows how the main infective sub-types vary in their distribution around the world.

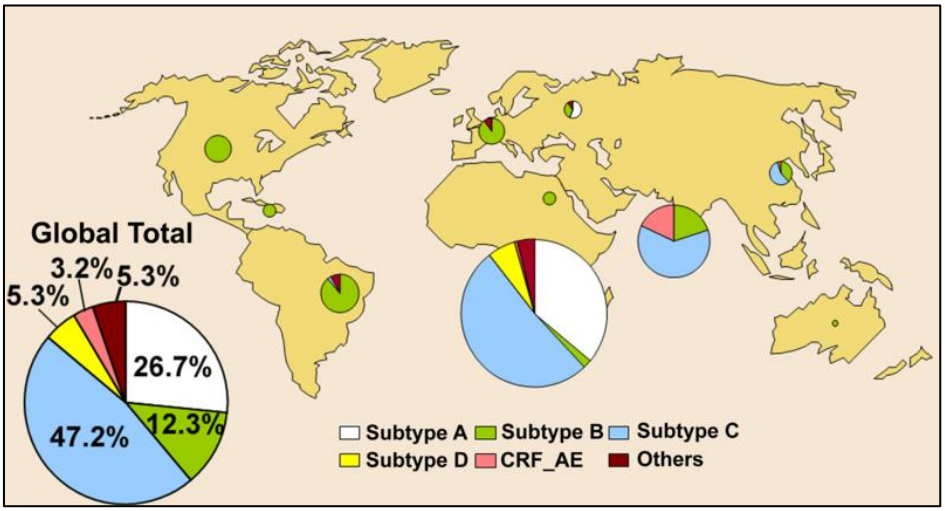

Figure 7.4: Map showing HIV-1 sub-type prevalence in 2000 (http://en.wikipedia.org/wiki/File:HIV-1_subtype_prevalence_in_2002.png)

7.4.2 Activity 7.1

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, discuss the various categories into which HIV can be divided.
(b) Discuss the geographical distribution of the major HIV types and sub-types. Refer to recent texts that you find on the internet or in the library to obtain up-to-date information.
(c) Which sub-type is most common in South Africa?

7.4.2.1 Structure and genome organisation

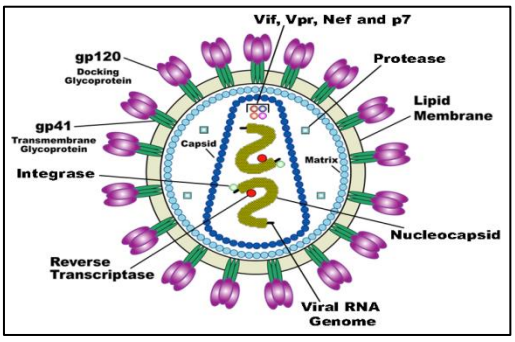

Figure 7.5: Diagram of the HI virus (http://en.wikipedia.org/wiki/File:HIV_Virion-en.png)

The HIV genome is 9–10 kbp in size, and consists of two linear molecules of single-stranded, positive-sense RNA. These RNA molecules are enclosed in a core made up of p24 capsule proteins. This core is surrounded by a matrix of p17 proteins. The enzymes reverse transcriptase, proteases, and integrase are also present within the viral particle. This is then enclosed in an outer envelope consisting of lipid and viral proteins. To see this illustrated, refer to the figure above and figure 44-2 in Brooks. The protein Env is embedded in the viral envelope and allows the virus to attach to and fuse with the target cell. Env is made up of a cap (comprising three glycoprotein, gp 120, molecules) and a stem (containing three gp 41 molecules). Although the env proteins have been considered as targets for vaccines, the different HIV strains often show considerable variation in these proteins.

The HIV genome encodes for nine genes (*gag, pol, env, tat, rev, nef, vif, vpr, vpu*), encoding for 19 different proteins. *Gag*, *pol* and *env* are common to all retroviruses. *Pol* codes for four proteins vital in replication, including reverse transcriptase and protease; *gag* encodes for a number of structural proteins; and *env* encodes for gp160, which is cleaved into Gp120 and gp41 that embed in the viral envelope. In HIV, *tat* codes for a protein involved in the transcriptional activation ("transactivation") of other genes. Transactivation is particularly efficient in HIV, which may account in part for the virulence of HIV infections.

7.4.2.2 Replication

Retroviruses get their name from their ability to reverse transcribe DNA from RNA. This process is catalysed by an RNA-dependent DNA polymerase called reverse transcriptase, and occurs in the cytoplasm. Once synthesised, the DNA enters the host cell nucleus, where it is integrated into the host DNA. Figure 43-3 in Brooks provides an overview of the replication cycle of the retrovirus HTLV. HIV has a very similar replication cycle, which you can see illustrated in figure 7.6 below.

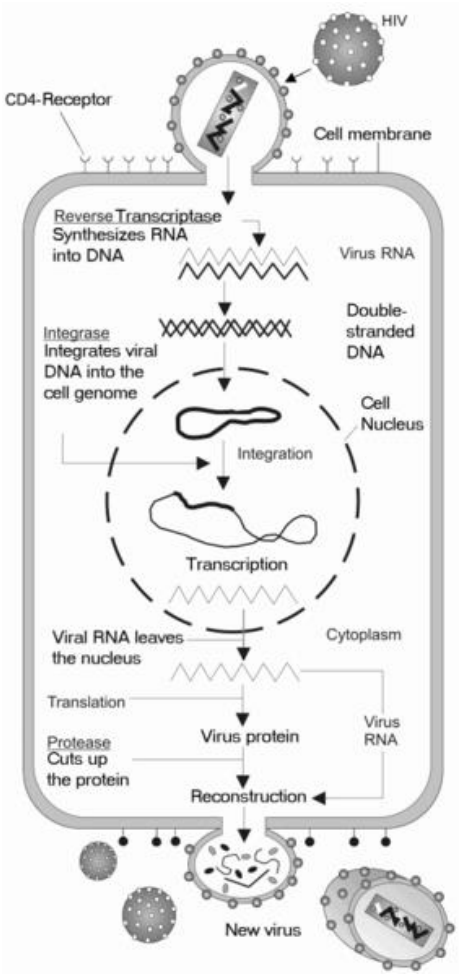

Figure 7.6: HIV replication (http://commons.wikimedia.org/wiki/File:HIV_gross_cycle_only.png)

The following YouTube video shows the HIV replication cycle:
https://www.youtube.com/watch?v=HhhRQ4t95OI

7.4.3 Activity 7.2

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, discuss the genome of HIV and the various gene products.
(b) In your own words, discuss the replication of HIV. Include notes on

- attachment to the cell: receptors and co-receptors
- cell entrance
- DNA production
- integration
- transcription
- virion production

7.5 HIV and AIDS

The following websites have further information on the topics covered in this study section:

- http://www.unaids.org/en/
- http://aidsinfo.nih.gov/
- http://www.iavi.org/Pages/home.aspx

7.5.1 Pathogenesis and pathology

Left untreated, a typical HIV infection in adults has a duration of around ten years (refer to figure 44-4 in Brooks), and goes through these stages:

- primary infection
- spread of virus to lymphoid organs
- asymptomatic phase (clinical latency – length varies; may be as much as ten years)
- increased viral load
- clinical disease
- death

During this time, a range of diseases may be observed. Without treatment, death usually occurs within two years of the onset of clinical symptoms.

Counts of a patient's CD4+ cells are useful in assessing disease progression. Once the CD4+ count declines to around 200/µl, the patient is diagnosed as having AIDS (acquired immune deficiency syndrome). As the immune system becomes overwhelmed, disease may manifest in one or more organ complexes, such as the cardiovascular, pulmonary, gastrointestinal or nervous systems, and skin, liver and kidneys. The patient is also more susceptible to cancers and to opportunistic infections.

7.5.2 Activity 7.3

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, discuss the progress of HIV infections with reference to

- viral load (RNA and viral proteins)
- the role of CD4 T cells and memory cells
- the role of monocytes and macrophages
- the role of the lymphoid organs

(b) Co-infections with other pathogens are common in people who are HIV positive. Discuss some common co-infections.

7.5.3 Clinical symptoms and diagnosis

The symptoms of HIV are nonspecific and consist of fatigue, headache, nausea, night sweats and rash. Some otherwise asymptomatic patients may show persistent generalised lymphadenopathy.

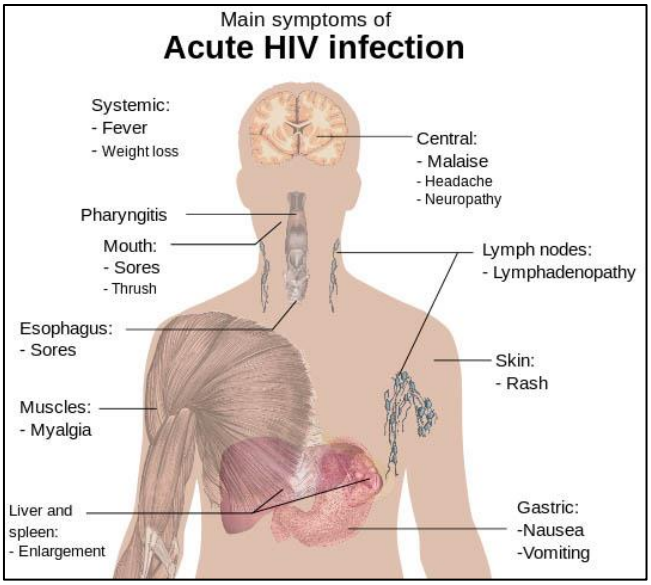

Figure 7.7: Main symptoms of acute HIV infection (http://simple.wikipedia.org/wiki/File:Symptoms_of_acute_HIV_infection.svg)

Laboratory diagnosis

Infection by HIV can be diagnosed in an individual by

- isolating viral particles from a clinical sample
- demonstrating the presence of antiviral antibodies
- detection of viral nucleic acid or antigens

The diagnostic methods entail a number of techniques, including

- reverse transcriptase polymerase chain reaction (PT-PCR)
- serum tests for antibody or antigen
- EIA/ELISA
- virus isolation and culture

7.5.4 Activity 7.4

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) Discuss some examples of the complications arising from HIV infections. Include information on each of the following areas:

- neurological
- liver
- skin
- gastric
- cancer
- opportunistic infections

(b) In your own words, discuss the diagnosis of HIV by viral isolation, serology or the detection of nucleic acid or antigens. Compare the available diagnostic tools, and discuss the advantages and disadvantages of the different techniques.

(c) What is seroconversion, and how does it affect the accuracy of antibody tests?

7.5.5 Epidemiology

AIDS was first recognised in the 1980s. Since then it has become a worldwide epidemic, with an estimated 35 million people worldwide infected with HIV at the end of 2012. The WHO reported in January 2012 that over 25 million people worldwide had died as a result of AIDS.

The prevalence of HIV infection varies from country to country, with the highest rates in sub-Saharan Africa (http://www.unaids.org/globalreport/HIV_prevalence_map.htm). The 2010 UNAIDS report estimated that 5 600 000 South Africans were living with HIV.

7.5.6 Treatment and control

Once HIV proviral DNA becomes incorporated into host DNA, the infection is permanent. The search for a vaccine that will prevent infection is ongoing, but for already infected patients, current treatment goals are to minimise obvious disease, prolong life and reduce the spread of infection to others.

Since HIV is highly mutable, any single drug tends to quickly become ineffective. To counter this, current best practice is to combine several drugs (often referred to as HAART, which stands for highly active antiretroviral therapy), with each drug of the combination targeting different aspects of the virus or its replication cycle. With combined treatment, extended inhibition of viral replication can occur, making HIV a chronic treatable, but not curable, disease. Treatment must be for life. Some drugs have serious side effects, and the development of drug resistance may limit therapy options.

Classes of antiretroviral drugs include

- nucleoside reverse transcriptase inhibitors
- non-nucleoside reverse transcriptase inhibitors
- protease inhibitors
- fusion inhibitors
- integrase inhibitors

The development of a vaccine against HIV that can protect individuals who do not have HIV from contracting the virus offers the best hope for managing the spread of the disease. HIV is highly mutagenic, which makes vaccine development difficult.

The most effective way to prevent HIV infection is to reduce any potential exposure to the virus. The main routes of transmission are through

- exposure to infectious blood,
- sexual contact and
- mother-to-infant transmission.

As HIV can be transmitted in blood and other bodily fluids such as semen, the handling of these fluids needs to be controlled. Blood and organ donors also need to be carefully screened. Drug treatment of HIV-positive pregnant women can greatly reduce the chance of vertical transmission of the virus during pregnancy and childbirth.

7.5.7 Activity 7.5

Do the following activity and add it to your portfolio.

(a) In your own words, discuss the different routes of HIV transmission.
(b) Discuss global trends in HIV epidemiology, including

- sub-types
- modes of transmission
- treatment programmes

(c) Describe the classes of antiretroviral drugs and how each acts against HIV.
(d) Discuss the difficulties inherent in finding a preventive vaccine against HIV, and current advances in this field.
(e) Discuss steps that can be taken to prevent the spread of infection from HIV-positive individuals.

7.5.8 Paediatric infection

Risk of transmission of HIV infection from an infected woman to her child during pregnancy, childbirth or postnatally (e.g. via breast milk) may be as high as 40% in untreated cases, but with appropriate antiretroviral treatment this risk may be reduced to just 2%.

Infected neonates are more susceptible to the effects of HIV because their immune systems are not fully developed at the time of infection. This, paediatric disease progression may be significantly different from that of adults.

7.6 Conclusion

HIV/AIDS is a global problem that affects millions of people worldwide. Extensive research is being conducted to find measures to prevent, treat or cure HIV/AIDS. You need to be able to describe the classification, genome organisation and replication of HIV along with the pathology and clinical symptoms that occur with infection by HIV. The diagnosis, epidemiology, prevention, treatment and control of HIV infection are also important aspects of the disease that you need to be familiar with.

Learning unit 8

Prion diseases

8.1 Introduction

Transmissible spongiform encephalopathies (TSEs) are degenerative diseases of the central nervous system and brain. The most accepted theory is that these illnesses are transmitted by **prions**, which are essentially **infectious misfolded proteins**. In this study unit I will tell you more about the transmissible spongiform encephalopathies, and specifically human prion diseases.

To complete the study unit, you will need to refer to chapter 42 of Brooks. You will find additional information on this topic at http://www.microbiologybytes.com/virology/Prions.html.

8.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- explain what scrapie is
- discuss bovine spongiform encephalopathy (BSE)
- describe human TSE diseases by elaborating on
    - how they are acquired
    - the various forms of CJD
    - other forms of TSE
- analyse the health risks associated with prions

8.3 Transmissible spongiform encephalopathies

Recommended reading: the section on slow virus infections and prion diseases on pages 585–588 in chapter 42 of Brooks et al (2012)

The diseases covered in this study unit are unique in that the etiologic agents are entirely unlike any other disease-causing agent you have studied so far in this module. These agents have been given the name "proteinaceous infectious particle", or prion. They are believed to consist only of **protein**, and lack any form of nucleic acid. They are transmissible, and cause numerous **holes** to form within the tissues of the central nervous system, causing the affected tissues to develop a sponge-like (“**spongiform**”) appearance. They elicit no response from the host immune system.

Prions are believed to function by causing certain proteins to misfold into non-functional forms, with the prion protein acting as a template guiding the misfolding process. The newly misfolded proteins are now prions themselves, and can affect the folding of correctly folded proteins of the same type. These proteins gradually accumulate in the body, especially in nerve cells, which subsequently die.

Prions are of considerable concern because

- the diseases they cause are fatal
- they are resistant to many of the forms of disinfection that kill bacteria, viruses, fungi and spores

The neurological diseases produced by prions may have incubation periods of years before the symptoms are clinically manifested.

8.4 Animal spongiform encephalopathies

8.4.1 Scrapie

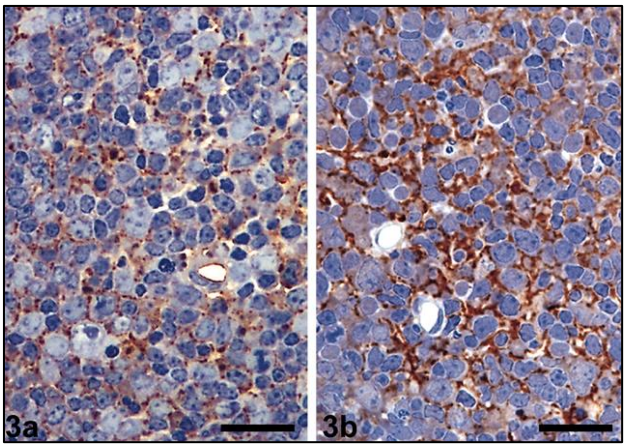

Figure 8.1: Lymph nodes from (a) healthy and (b) infected sheep – colouring with antibodies shows scrapie prions in the intracellular tissue of the infected sheep (http://en.wikipedia.org/wiki/File:Scrapie_lymph_node_immunoglobulin_labeling.png)

Scrapie is a neurological disease that affects sheep and goats, and does not appear to be transmissible to humans. Different breeds of sheep have different susceptibility to the disease, ranging from 0% to 80% in dissimilar breeds. The name comes from the fact that the disease causes itching, and so the sheep rub against rocks or trees, scraping the fleece off their bodies.

Although the disease was observed in sheep as early as the 18th century, it was only in the early 20th century that it was proven to be transmissible. As the disease progresses, amyloid plaques form in the central nervous system of the infected animals. The incubation period is generally two to five years. No treatment is available, and so the most common way to contain outbreaks is to quarantine and destroy the infected animals.

The following pdf file has more information on scrapie:
http://www.cfsph.iastate.edu/Factsheets/pdfs/scrapie.pdf

8.4.2 Activity 8.1

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

In your own words, describe the transmission and pathogenesis of scrapie.

8.4.3 Bovine spongiform encephalopathy and other animal prion diseases

Bovine spongiform encephalopathy (BSE, or “mad cow disease”) was first identified in the 1980s in the United Kingdom. For some time before this, protein in the form of sheep and cattle bone meal

and meat had been fed to cattle as a supplement, without ill-effect. However, in the mid-1980s there was a change in the way that the offal was processed, and the unforeseen outcome was that contaminants were not destroyed, and cattle became infected. Further recycling of these contaminated animals may have amplified the effect.

The source of the original infection is a matter of debate: one possibility is that a strain of scrapie was involved, while other research suggests that BSE may have developed from a sporadic form of cattle prion disease called bovine amyloidotic spongiform encephalopathy (BASE).

If you are interested, you will find more information on BASE and BSE in the following article: http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.0030031#ppat-0030031-b016.

Of greater concern to medical microbiologists is a human disease believed to have arisen from BSE, namely **variant Creutzfeldt Jakob disease (vCJD)**. The new vCJD and BSE are thought to be caused by the same agent, and it is thought that vCJD arose from infection of humans with BSE. The disease is assumed to have been transmitted to human beings through food contaminated with BSE. I will say more about vCJD in the next section.

8.5 Human prion diseases

The first form of human transmissible spongiform encephalopathy (TSE) was described by Hans Gerhard Creutzfeldt and Alfons Maria Jakob in the early 1920s, and named after these two German neurologists. At that time the cause was unknown. Since then several other forms of TSE have been detected in humans, including kuru, Gerstmann Sträussler Scheinker syndrome (GSS), and variant Creutzfeldt Jakob disease (vCJD). These diseases can be grouped according to the manner in which they are acquired. They can be either

- idiopathic (spontaneously occurring),
- familial, or
- acquired.

8.5.1 Activity 8.2

Do the following activity and add it to your portfolio.

(a) Describe the different modes of transmission of idiopathic, familial and acquired prion diseases.

To complete the following question you will need to conduct your own research by consulting sources other than the textbook. You may obtain the information you need from the internet or the Unisa library.

(b) Human TSE, especially CJD and vCJD, are widely covered in the literature. Make notes on each of the forms of human TSE (kuru, GSS, CJD and vCJD). In each case provide a description of the disease, how it is acquired, age of onset, and the symptoms and prognosis.

Discussion forum question

This is a discussion activity that you should answer in the Discussions tool on the module web site. If you do not have internet access, you can write your response and add it to your portfolio.

Given the development of BSE and the subsequent development of vCJD, what is your opinion regarding the European Commission's proposal to relax rules on feeding meat to animals, as discussed in the following article? http://www.independent.co.uk/environment/nature/meat-back-on-menu-for-animal-feed-20-years-after-bse-crisis-2072188.html

Go to the discussion topic entitled: “Bovine spongiform encephalopathy”, and post your opinion about the content of the article.

Please also read the other students’ postings and respond to at least one other posting. Mention anything you found particularly interesting about that posting.

8.5.2 Feedback on activity 8.2

If you did not manage to find information about human prion diseases, you may find the following web pages helpful:

http://www.cdc.gov/ncidod/dvrd/prions/
http://www.prion.ucl.ac.uk/welcome/
http://www.hopkinsmedicine.org/healthlibrary/conditions/nervous_system_disorders/prion_diseases_134,56/

8.6 Conclusion

The prion diseases have a unique aetiology in that they are caused by an infectious protein agent. Scrapie, BSE and TSE were discussed as examples of prion disease that infect sheep, cows and humans respectively. As there are many health risks associated with prion diseases and they are all fatal once acquired, extensive research is being conducted on prions.

Recently scientists have discovered prions that infect yeasts and fungi. Hopefully understanding of prions in simple organisms will increase our knowledge of prion diseases and ultimately aid in the development of a way to treat transmissible spongiform encephalopathies. If you are interested in further information, refer to http://genesdev.cshlp.org/content/18/5/470.long, which is an in-depth paper on prions that affect mammals and yeasts.

Learning unit 9

Fungal diseases

9.1 Introduction

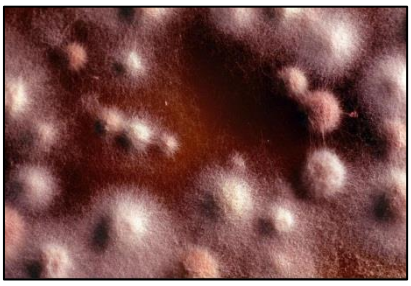

Figure 9.1: Cultured athlete's foot fungus (http://en.wikipedia.org/wiki/File:Athlete%27s_Foot_Fungus_microscope.jpg. Image credit: Ecorahul)

It is reported in the *Dictionary of the Fungi* (Kirk, P.M., Cannon, P.F., Minter, D.W. and Stalpers, J.A. (2008) *Dictionary of the Fungi* (10th edition). CABI) that just over 97 000 species of fungi have been described, although it has been estimated that the actual number of species may exceed 5 million. Despite these large numbers, only a relatively small number of fungi (fewer than 500) are of medical importance. Of these, just 50 species cause more than 90% of fungal infections in humans and animals.

In this study unit we will look at fungi and fungal diseases in general, and then we will focus on *Candida* spp. as an example of a pathogenic fungus. To complete the study unit, you will need to refer to chapter 45 of Brooks. You will find further information in the internet sources that I indicate in the text.

American Journal of Botany 98(3):426–438

9.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- describe the forms that fungi take
- explain the difference between the various types of mycoses
- discuss the epidemiology of the different forms
- differentiate between obligate and opportunistic pathogens
- discuss the diagnosis and treatment of fungal infections
- identify *Candida* spp. and the infections caused by this genus

9.3 *Fungi and fungal diseases*

Recommended reading: the sections dealing with the general properties and the classification of fungi, superficial mycoses, cutaneous mycoses and subcutaneous mycoses on pages 625–635 in chapter 45 of Brooks

Fungi are chemotrophs, and make use of saprophytic (involving the decomposition of organic substrates) or parasitic modes of nutrition. They secrete enzymes that break down a range of complex organic substrates into nutrients, which they then absorb. Generally fungi are beneficial to humans and are essential in recycling organic matter in the environment. Humans have used fungi for thousands of years in the production of food (e.g. bread and cheese) and beverages (e.g. beer). Some fungi have been exploited and used in the production of antibiotics and other drugs. As pathogens they have a significant influence on the agricultural industry, where they result in huge crop losses every year.

Fungal species are spread over four phyla: the

- Chytridiomycota,
- Zygomycota,
- Ascomycota and
- Basiodiomycota.

The study of these organisms is known as **mycology**, and infections caused by fungi are called **mycoses**.

Fungi grow in two main forms: as either

- unicellular yeasts or
- multicellular moulds.

Some are dimorphic, as they are able to form filamentous hyphae at environmental temperatures and assume the yeast form in a host.

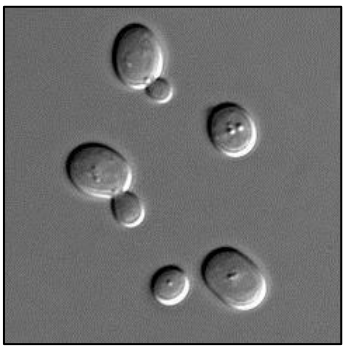

Figure 9.2: *Saccharomyces cerevisiae* yeast cells (http://en.wikipedia.org/wiki/File:S_cerevisiae_under_DIC_microscopy.jpg)

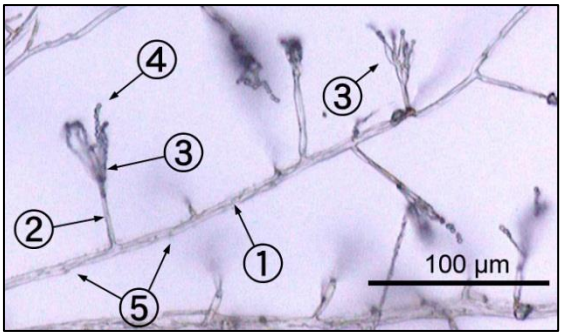

Figure 9.3: An environmental isolate of *Penicillium* growing as a multicellular mould: 1. hypha, 2. conidiophore, 3. phialide, 4. conidia, 5. septa (http://en.wikipedia.org/wiki/File:Penicillium_labeled_cropped.jpg)

Unlike all the other disease-causing agents we have discussed so far in this module, fungi are **eukaryotic** organisms, with at least one membrane-bound nucleus (often they are multinucleate). They also contain **membrane-bound organelles**, for example mitochondria, secretory organelles, and membrane systems such as endoplasmic reticulum. Similar to plant cells, they possess a thick cell wall, which in fungi takes the form of polysaccharide chains (mostly glucan and mannan) as well as chitin and glycoproteins. Plant cell walls, in contrast, consist mostly of cellulose.

9.3.1 Activity 9.1

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

In your own words, discuss the general characteristics of fungi, and describe the forms that they take.

9.4 Incidence and types of infection

Infections by pathogenic fungi can be classified as

- superficial mycoses
- cutaneous mycoses
- subcutaneous mycoses
- systemic mycoses
- opportunistic mycoses

A particular fungal species may be responsible for more than one form of disease. Furthermore, a specific disease may be caused by more than one species of fungus.

9.4.1 Activity 9.2

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) What are systemic pathogens and opportunistic pathogens?
(b) Provide one example each of a fungus that causes superficial, cutaneous and subcutaneous infection respectively. Make sure you understand the difference between superficial, cutaneous and subcutaneous mycoses.

9.5 Diagnosis and treatment

Recommended reading: pages 654–660 in chapter 45 of Brooks

Diagnosis of fungal disease requires a combination of clinical observations and laboratory techniques. In the laboratory, specimens are generally studied by microscopic examination or culturing.

Most mycoses are **difficult to treat**, as they are eukaryotes, and many agents that are toxic to fungi are also toxic to mammals. In this way they are unlike bacteria, which have very different gene products and biochemical pathways that can be targeted without resulting in toxicity to the host.

There are, however, a number of drugs that target fungi. They are imperfect in that they often have severe side effects, a narrow antifungal spectrum (in other words, they act on only a few species) or poor penetration of human tissues. The development of drug resistance is also a problem.

For a list of common antifungal drugs and their mechanism of action, see table 45-5 in Brooks.

9.5.1 Activity 9.3

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

Briefly describe the mechanisms of action and side effects of the anti-fungal agents amphotericin B, flucytosine and azoles.

9.6 Candida spp.

Recommended reading: the sections dealing with opportunistic mycoses and candidiasis on pages 646–649 in chapter 45 of Brooks

To give you a better understanding of fungi and fungal diseases, we will deal with a specific genus, *Candida*, in more detail in this section. We will not be covering other fungal diseases in detail in this module.

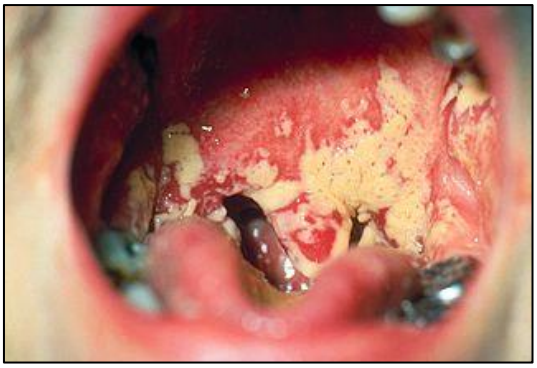

Figure 9.4: Oral thrush caused by *Candida albicans* (http://it.wikipedia.org/wiki/File:Oral_thrush_Aphthae_Candida_albicans._PHIL_1217_lores.jpg)

There are over 150 described species of *Candida*, several of which form part of the normal skin, gastrointestinal and mucous membrane flora. Under certain circumstances, the *Candida* population may increase, resulting in a range of opportunistic infections. Nine *Candida* species are frequently pathogenic, and together they are the most common cause of fungal infections.

Some important *Candida* species are

- *C. albicans*
- *C. glabrata*
- *C. krusei*
- *C. parapsilosis*
- *C. tropicalis*
- *C. guilliermondii*
- C. dubliniensis

Most *Candida* species grow as yeasts and can form pseudohyphae. *C. albicans* is dimorphic, and can also form true hyphae.

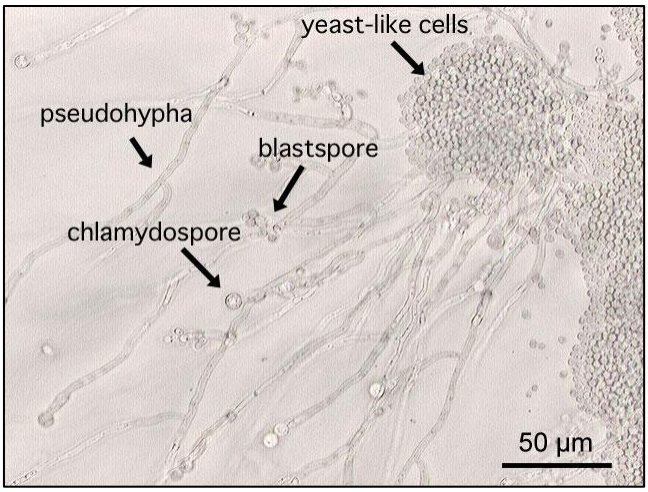

Figure 9.5: *Candida albicans*, grown on cornmeal agar (http://en.wikipedia.org/wiki/File:C_albicans_en.jpg. Image credit: Y Tambe)

On agar, Candida species form soft, cream-coloured colonies, which you can see in the image below.

Figure 9.6: This is an image of a SABHI agar plate culture of the fungus *Candida albicans* grown at 20 °C. (http://en.wikipedia.org/wiki/File:Candida_albicans_PHIL_3192_lores.jpg)

9.6.1 Superficial *Candida* infections

Superficial cutaneous or mucosal candidiasis can occur when there is damage to the skin or epithelium and the numbers of *Candida* increase. Some examples of superficial *Candida* infections are

- thrush
- gastrointestinal candidiasis
- vulvovaginitis

A range of risk factors contribute to the development of superficial mycoses, including diabetes, reduced immunity, treatment with antibiotics or corticosteroids, and trauma.

9.6.2 Systemic *Candida* infections

Systemic candidiasis occurs when *Candida* enter the blood stream. Candidaemia is often nosocomial in origin, and can be acquired from surgery or catheters, for instance. In healthy individuals the infection is usually short-lived, and the yeast is eliminated by the immune system. In immunocompromised individuals more serious illness may result, including:

- kidney infections
- bone and joint infections
- skin lesions
- central nervous system (CNS) candidiasis
- cardiac candidiasis
- disseminated candidiasis

9.6.3 Activity 9.4

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, discuss the clinical features of superficial *Candida* infections. (Include any associated risk factors.)
(b) Explain how microscopic examination, culturing and serology are used in the diagnosis of *Candida* infections.
(c) Discuss the treatment and control of *Candida* infections.

9.7 Conclusion

Review the outcomes of this module and think about whether you have achieved them.

Can you describe the general structure of fungi and the difference between the various types of mycoses? Do you understand how fungal infections are diagnosed and treated, with *Candida* as an example?

Learning unit 10

Medical parasitology

10.1 Introduction

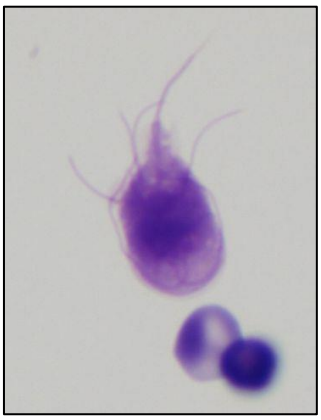

Figure 10.1: *Giardia lamblia* from small bowel biopsy of a patient infected with *Giardia* (http://en.wikipedia.org/wiki/File:Giardia_lamblia_cytology_closeup.jpg. Image credit: Jerad M Gardner, MD)

A **parasite** is an organism that lives in or on another organism of a different species, from which it obtains nutrients. **Medical parasitology is the study of parasites that infect humans, and the diseases they cause.** The diagnosis, treatment, prevention and control of parasites are all important aspects of medical parasitology. Parasites may be **endoparasites**, which live within a host, or **ectoparasites**, which live on the surface of the host. This study unit will focus on selected endoparasites of humans. Parasites of humans include

- *Plasmodium* spp., which cause malaria,
- *Giardia* spp., which cause giardiasis,
- *Schistosoma* spp., which cause bilharzia and
- *Taenia* spp. (tapeworm), which infect the intestines.

For a comprehensive list of parasites of humans refer to the CDC web page, http://www.dpd.cdc.gov/dpdx/az.html.

To complete the study unit, you will need to refer to chapter 46 of Brooks. You will also find more information on this topic at: http://pathmicro.med.sc.edu/book/parasit-sta.htm and http://www.dpd.cdc.gov/DPDx/.

10.2 Learning outcomes

Upon completion of this learning unit you should be able to:

- define various terms which relate to parasitology
- discuss the characteristics of the four groups of parasitic protozoa
- explain what parasitic helminths are

- discuss Plasmodium, Giardia, Trichomonas and Schistosoma in terms of
    - life cycle
    - morphology
    - pathogenesis and pathology
    - epidemiology
    - diagnosis
    - treatment
    - control

10.3 Parasites of humans

Recommended reading: pages 665–670 in chapter 46 of Brooks

The endoparasites of humans can be broadly divided into two groups:

- parasitic protozoa and
- parasitic helminths.

This categorisation is purely for convenience, and is not systematically correct. The "protozoa" in particular do not form a valid taxonomic group, with the organisms falling into a number of different phyla (which are also under constant revision).

There are numerous routes by which a parasite can be transmitted.

- They can be transmitted sexually.
- They can be food-borne.
- They can be water-borne.
- They can be transmitted through direct and indirect contact.
- They can be transmitted by means of vectors.

10.4 Parasitic protozoa

The parasitic protozoa can be divided into four groups based on their modes of locomotion and reproduction. So, we have the

1. flagellates, which have at least one flagellum
2. ciliates, which have rows or sections of cilia, and two nuclei
3. amoebae, which move using pseudopodia
4. sporozoa, which have complicated life cycles with alternating sexual and asexual reproductive phases

Figure 10.2: False colour SEM of promastigote form of *Leishmania mexicana* (http://en.wikipedia.org/wiki/File:LeishmaniaMexicana_Promastigote_SEM.jpg. Image credit: Zephyris)

10.4.1 Activity 10.1

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) Distinguish between the terms in each of the following pairs:

- parasite and host
- endoparasite and ectoparasite
- pathogenic and commensal parasite

(b) Refer to table 46-1 in Brooks. Make notes on the different sites of infection and the mechanism of infection of the various parasites of humans.

(c) Give at least one example of a human parasite from each group of parasitic protozoa, except the ciliates, and in two sentences describe the disease that each causes. Remember: there are four groups of parasitic protozoa, so because you are not including the ciliates, you will be providing three examples.

10.4.2 Feedback on activity 10.1

When examining table 46-1 you should have noticed that parasites infect a number of different sites within the body, and that parasitic infections can be acquired by various means.

There are four groups of parasitic protozoa: the flagellates, ciliates, amoebae and sporozoa. *Balantidium coli* is the only ciliate parasite of humans, and is very rare, so we will not be discussing it. There are a number of representatives of human parasites from the other groups of protozoa. If you page through chapter 46 of your textbook you will see illustrations of *Giardia lamblia* (intestinal flagellate) on page 669, *Entamoeba histolytica* (intestinal and tissue amoeba) on page 670, and *Cyclospora* (intestinal sporozoa) on page 673. You should not have limited yourself to the examples I have given, as there are many other examples in the textbook.

10.5 Parasitic helminths

Parasitic helminths are worms that parasitise humans. They fall into two phyla:

- nematodes (roundworms) and
- platyhelminthes (flatworms).

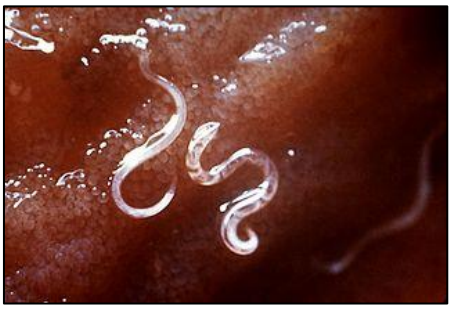

Figure 10.3: *Ancylostoma caninum*, a type of hookworm, attached to the intestinal mucosa. (http://en.wikipedia.org/wiki/File:Hookworms.JPG. Original image from the Public Health Image Library (PHIL) of the Centers for Disease Control and Prevention, with identification number #5205)

Nematodes are a very diverse group of worms that inhabit a broad range of environments. They are long and tapered at both ends, and in cross-section they are round. There are many different species that parasitise humans, such as hookworms, pinworms and whipworms. These worms typically live in the intestines of humans, and are generally acquired through ingestion of the egg or larval stage.

Figure 10.4: *Enterobius vermicularis*, a pinworm parasite (http://en.wikipedia.org/wiki/File:Threadworm.jpg)

Platyhelminthes are flatworms. They are dorsoventrally flattened in cross-section. The pathologically relevant flatworms belong to two classes:

- the cestodes (tapeworms) and
- trematodes (flukes).

Cestodes are flat, and are made up of a ribbon-like chain of segments. The beef tapeworm, *Taenia saginata*, and the pork tapeworm, *Taenia solium*, infect millions of people worldwide. *Taenia saginata* can grow up to 20 m in length. Infection generally occurs when the cestode larvae are ingested; these then mature into adult worms in the intestine of the host. The symptoms of infection vary greatly between species.

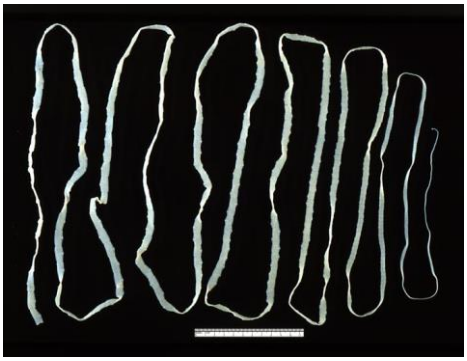

Figure 10.5: An adult *Taenia saginata* tapeworm
(http://phil.cdc.gov/PHIL_Images/20031208/87d4bff74e41427cb278526bd9cbe76a/5260_lores.jpg)

Schistosomiasis (also known as bilharzia) is an example of a parasitic disease caused by the trematode *Schistosoma.* We will discuss schistosomiasis later in the study unit.

10.5.1 Activity 10.2

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) Give at least one example each of a nematode, a trematodes and a cestode that parasitise humans, and briefly discuss the disease that each causes. (Table 46-7 may help.)
(b) In your own words, describe what the terms “reservoir” and “vector” mean with regard to parasites.

10.6 Examples of human parasites

In this section we will focus on a few relevant parasites:

- *Plasmodium* (a sporozoan transmitted by an insect vector)
- *Giardia* (a flagellate normally transmitted through water)
- *Trichomonas* (a sexually transmitted flagellate)
- *Schistosoma* (a water-borne trematode)

10.7 *Plasmodium* spp.

Recommended reading: pages 677–681 in chapter 46 of Brooks

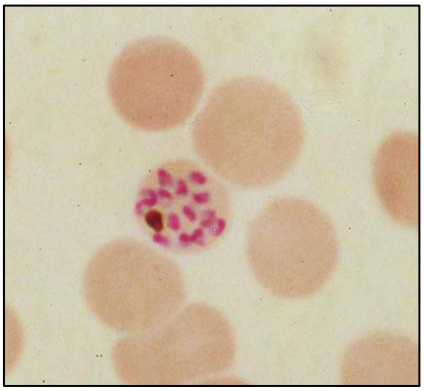

Figure 10.6: Human red blood cell infected by the malaria parasite *Plasmodium falciparum* (http://en.wikipedia.org/wiki/File:P.falciparum_schizont.jpg)

Figure 10.7: Mosquito (http://commons.wikimedia.org/wiki/File:Anopheles_minimus.jpg)

Malaria is a mosquito-borne disease of humans caused by *Plasmodium*. Of the parasitic diseases, it is the most significant killer. According to the WHO, there were about 207 million cases of malaria in 2012, and an estimated 627 000 deaths. Most of the fatalities were children under 5 years old.

Infection of humans occurs when an infected *Anopheles* mosquito bites a person and the sporozoites of *Plasmodium* enter the blood stream. The symptoms of malaria are generally fever, headache, nausea and vomiting.

There are four main species of *Plasmodium* which affect humans:

- Plasmodium falciparum
- Plasmodium vivax
- Plasmodium malariae
- Plasmodium ovale

Plasmodium is a sporozoan. It has a complex life cycle incorporating both **sexual** and **asexual** phases that take place in separate hosts.

- The asexual phase takes place in **humans**, and can be further divided into an
  - exo-erythrocytic cycle (in the liver) and an
  - erythrocytic cycle (in the blood).
- The sexual phase (also called the sporogonic cycle) takes place in **female *Anopheles* mosquitoes**.

Refer to the life cycle diagram below.

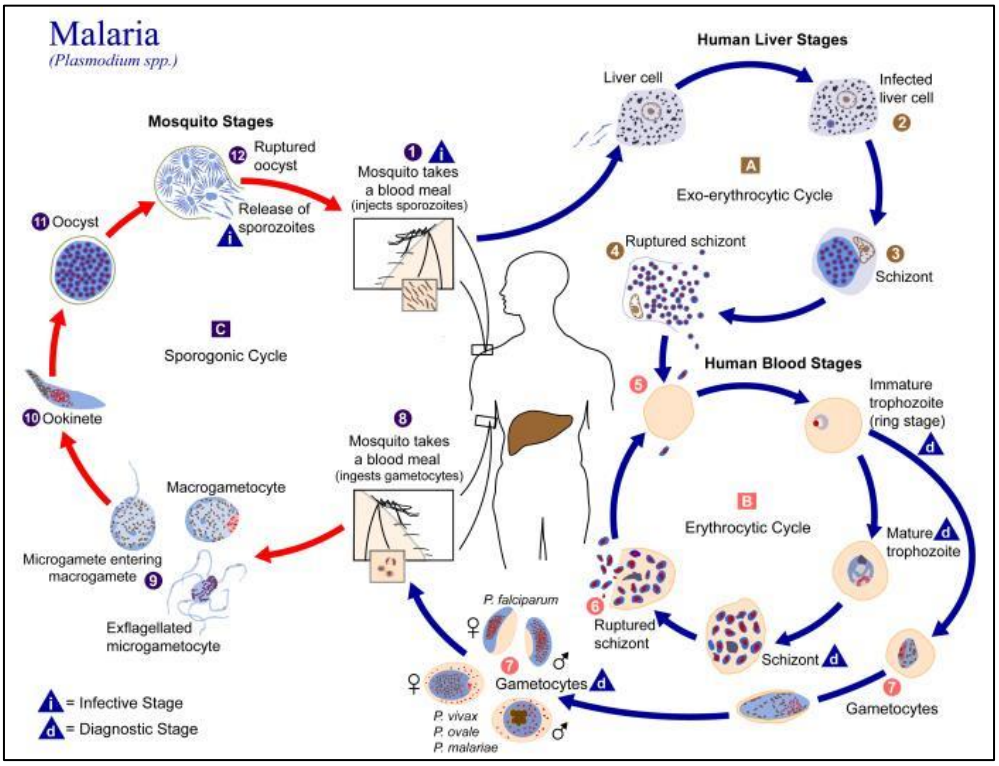

Figure 10.8: This is an illustration of the life cycle of the parasites of the genus Plasmodium that are causal agents of malaria.
(http://commons.wikimedia.org/wiki/File:Plasmodium_lifecycle_PHIL_3405_lores.jpg)

10.7.1 Pathogenesis and pathology

Pathology varies from species to species, and the malaria differs in incubation time, symptoms, severity and response to chemotherapy.

The symptoms of malaria (and the differences between the forms) are linked to the differing life cycles of the distinctive species. Some people also have genetic traits that make them less susceptible to certain malarial forms.

The incubation period for malaria is between 9 and 30 days. *P. falciparum* attacks **both** young **and** old red blood cells, and so the parasite levels may be very high and the **symptoms severe**. Conversely, the other *Plasmodium* species attack **either** young **or** old blood cells (but not both),

resulting in **milder symptoms**. Therefore *P. falciparum* infections are more dangerous and are more likely to be fatal.

The disease is found in the tropical and subtropical regions of sub-Saharan Africa, Asia, and the Americas, as this is the region in which the mosquito is able to live. The different species of *Plasmodium* have slightly different distributions.

10.7.2 Laboratory diagnosis

Various tests are available for diagnosing malaria, but the most reliable method is to study a Romanowsky-stained droplet of blood under a microscope. Under these conditions each *Plasmodium* species has a number of distinguishing characteristics. Table 46-5 and figure 46-10 in the textbook show the characteristic features of the different species of *Plasmodium* when seen under the microscope.

10.7.3 Treatment and prophylaxis

Several different prophylactic drugs are available to prevent malaria. The choice of drug needs to take into account the traveller's destination and other factors. For example, certain malaria drugs have adverse effects at pressure, so should not be taken by travellers intending to scuba dive.

Malaria infection can be treated with a number of drugs, although resistance to antimalarial medicines is a problem.

Mosquito-control measures, for example, using mosquito nets, insect repellents, insecticides and draining any standing water, can all help reduce disease transmission.

10.7.4 Activity 10.3

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

(a) In your own words, explain the steps in the general life cycle of *Plasmodium*.
(b) At what points in the life cycle of *Plasmodium* are sporozoites, trophozoites, and gametocytes present?
(c) In your own words, discuss the pathogenesis of malaria infections.
(d) Describe the treatment and prophylaxis options available, as well as some of the factors that may make a specific drug unsuitable for use.
(e) Discuss possible measures to reduce the risk of infection, both on a personal level and at community level.

10.8 *Giardia lamblia* (also called G. *intestinalis*)

Recommended reading: pages 669–670 in chapter 46 of Brooks

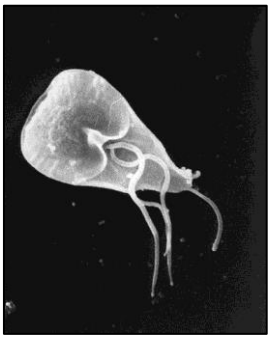

Figure 10.9: Scanning electron micrograph (SEM) of a flagellated *Giardia lamblia* protozoan parasite (http://phil.cdc.gov/PHIL_Images/8698/8698_lores.jpg or http://en.wikipedia.org/wiki/File:Giardia_lamblia_SEM_8698_lores.jpg)

Giardia lamblia is a flagellate which lives in the human small intestine and causes **giardiasis**. An infected person may either be asymptomatic, or develop severe diarrhoea, cramps, nausea and dehydration. *G. lamblia* has a simple life cycle with only two stages: **trophozoites** and **cysts** (refer to the figure below). Cysts are resistant, and can survive for months in cold water. Ingestion of even a small number of cysts can lead to infection.

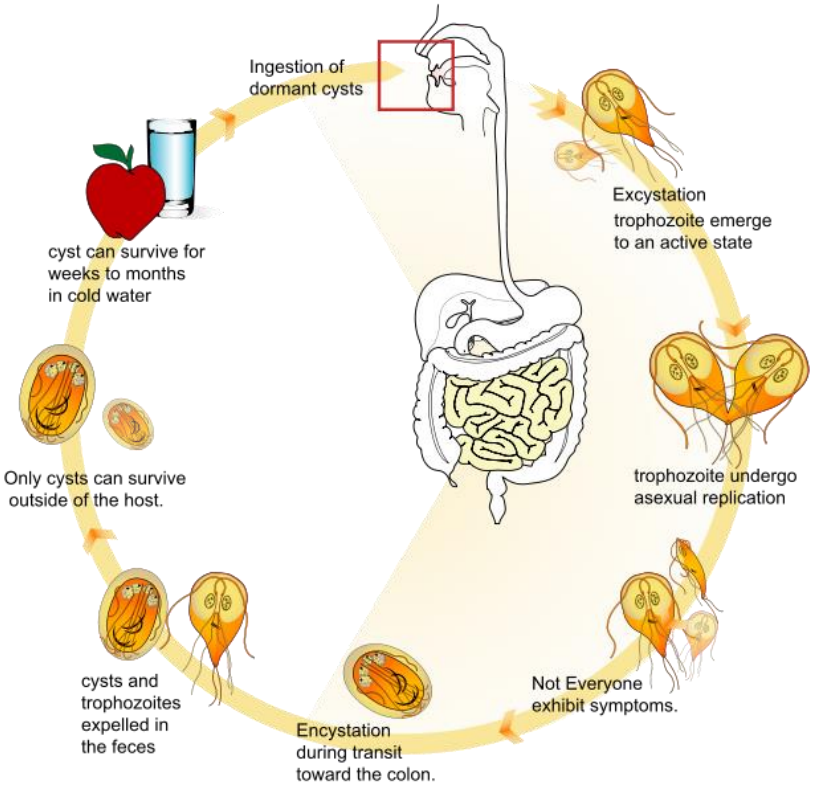

Figure 10.10: Life cycle of the parasite *Giardia lamblia* (http://en.wikipedia.org/wiki/File:Giardia_life_cycle_en.svg)

10.8.1 Activity 10.4

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

Discuss *Giardia* under the following headings:

- Symptoms of disease
- Life cycle
- Epidemiology
- Pathology

10.9 *Trichomonas vaginalis*

Recommended reading: pages 673–635 in chapter 46 of Brooks

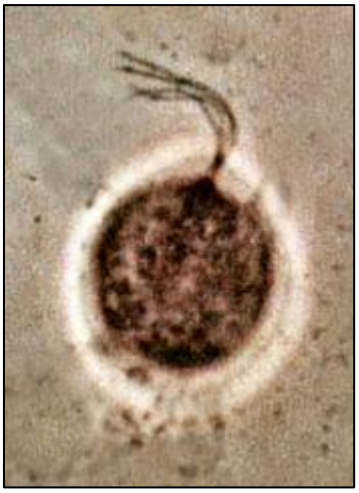

Figure 10.11: *Trichomonas vaginalis* photographed by phase contrast microscopy (http://species.wikimedia.org/wiki/File:Trichomonas_vaginalis_phase_contrast_microscopy.jpg. Image by Dr Graham Beards)

Trichomonas vaginalis is a flagellate which lives primarily in the vagina and causes **genital infections**. It is transmitted sexually, and most infections are mild or asymptomatic. In females symptoms may include vaginal discharge, local tenderness and burning. About 10% of males will have a thin white urethral discharge. Although it is predominantly sexually transmitted, contaminated towels and other articles may result in infection. Babies may also be infected during birth. Diagnosis is generally by microscopic examination of discharge, urine or a tissue scraping. Treatment of both partners is typically with the drug metronidazole.

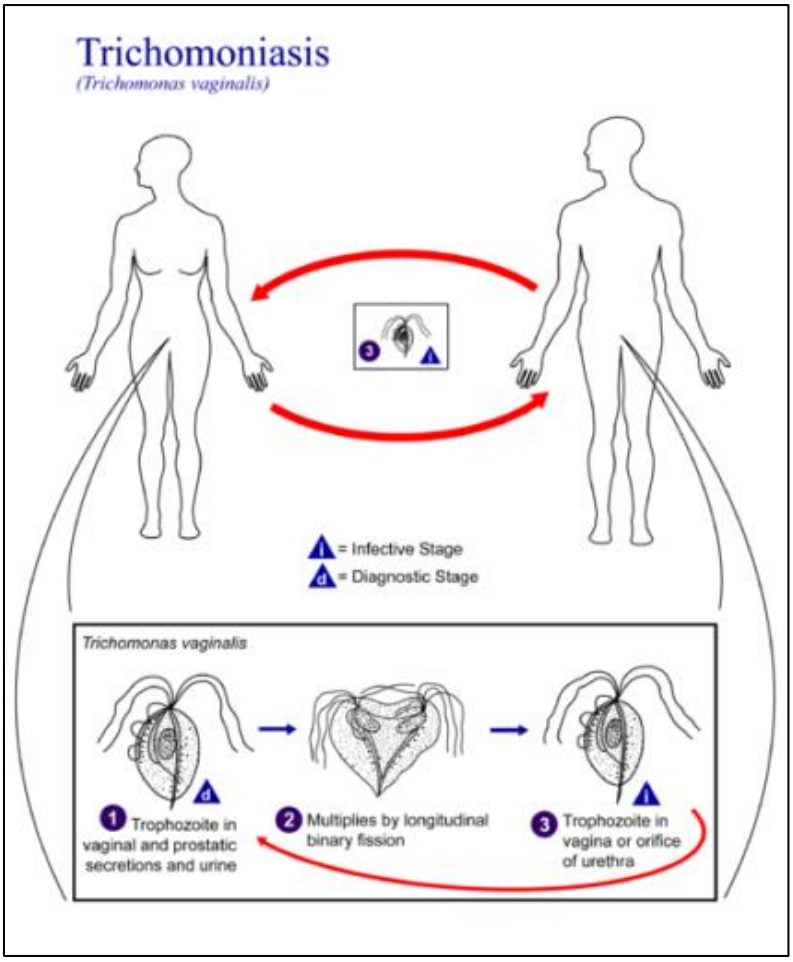

Figure 10.12: Trichomoniasis transmission (http://commons.wikimedia.org/wiki/File:Trichomoniasis_01.png. Image Alexander J. da Silva/CDC)

10.9.1 Activity 10.5

Do the following activity and add it to your portfolio.

Remember, this could serve as part of your summary to use in preparing for the exam!

Discuss *Trichomonas* under the following headings:

- Morphology
- Epidemiology
- Symptoms and pathology
- Prevention

10.10 *Schistosoma* spp.

Recommended reading: pages 696–697 in chapter 46 of Brooks

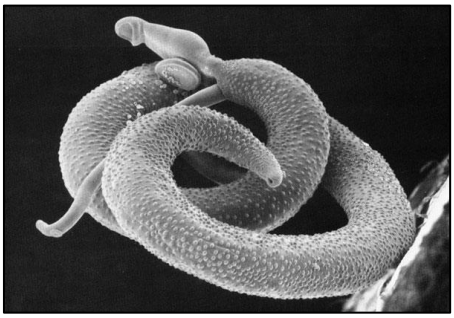

Figure 10.13: Scanning electron micrograph of a pair of *Schistosoma mansoni* (http://commons.wikimedia.org/wiki/File:Schistosoma_mansoni2.jpg)

Schistosoma are parasitic flatworms, commonly known as blood-flukes. They are responsible for the infection **schistosomiasis** in humans. Schistosomiasis is thought to be the second most important parasitic disease of humans after malaria. There are five general species of *Schistosoma* which infect humans. Three of them, S*. mansoni*, *S. haematobium* and *S. japonicum*, are the main etiologic agents of schistosomiasis (also known as **bilharzia**) worldwide. The remaining two, *S. mekongi* and *S. intercalatum*, are more restricted geographically.

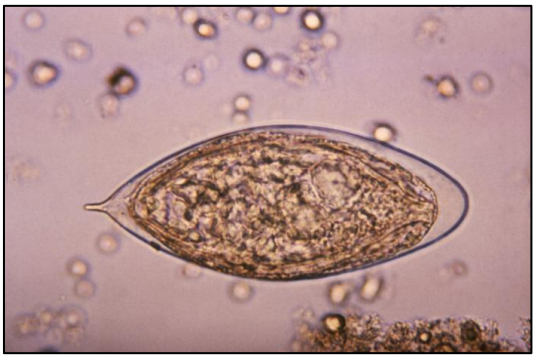

Figure 10.14: This micrograph depicts the egg of a *Schistosoma haematobium* trematode parasite. (http://phil.cdc.gov/PHIL_Images/20031013/b47fc1793d7443d7a5cdbfbc73d95e53/4843_lores.jpg)

The life cycle of *Schistosoma* is complex, consisting of several stages and requiring two main hosts. **Snails** are generally the **intermediate** host, with **humans and other animals** being the **final** host. As animals may also be infected, they act as natural reservoirs for the disease. Refer to the life cycle below. These organisms are dioecious (have separate sexes), with the male being larger than the female. Adult *Schistosoma* worms live together in breeding pairs, with the female worm tucked into a special groove in the male worm.

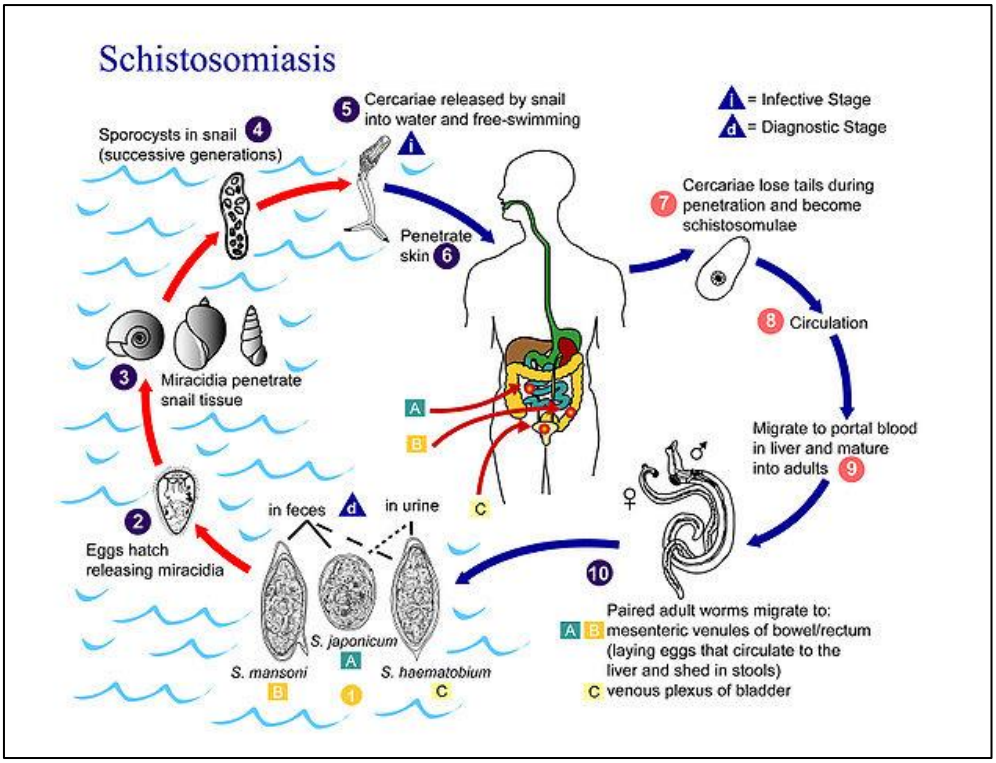

Figure 10.15: Life cycle of *Schistosoma* (http://commons.wikimedia.org/wiki/File:Schistosomiasis_Life_Cycle.jpeg)

Infection is through active penetration of the skin by the cercariae stage of the parasite. The cercariae then migrate through the body to the liver, where they mature into adults.

Schistosomiasis is typically diagnosed through a stool exam for ova and parasites, and treatment is generally with the drug praziquantel.

10.10.1 Activity 10.6

Do the following activity and add it to your portfolio.

Discuss *Schistosoma* under the following headings:

- Morphology and life cycle
- Symptoms and pathology
- Diagnosis

For additional information, read the following article by the WHO: http://www.who.int/mediacentre/factsheets/fs115/en/

10.11 Conclusion

Review the outcomes of this module and think about whether you have achieved them.

Can you discuss the characteristics of the four groups of parasitic protozoa and what helminths are? Can you discuss the life cycle, morphology, pathogenesis, diagnosis and treatment of *Plasmodium*, *Giardia*, *Trichomonas* and *Schistosoma*?

Forum 1: Student Lounge

Use this forum to discuss general matters amongst yourselves

Discussion 1: Introduce yourself

Use this space to get to know your classmates.

In about 250 words, tell us about your current work situation and professional background, and something about yourself as a person.

Discussion 2: Fellow student contact details

Use this space to share your contact details with your classmates and to form study groups.

Forum 2: Learning unit content

Use this forum to discuss any work related to the learning units, possibly some concept you are trying to understand..

Forum 3: Bovine spongiform encephalopathy (course discussion topic)

Use this forum to discuss the question asked in activity 8.2

Announcement 1

Welcome and getting started

Dear Student

Welcome to Medical Microbiology. You should have received a “Getting Started” letter in the mail, explaining what is expected of you as an online student.

Please go to the **Discussion Forums** link on the left-hand side of your screen and access “Forum 1: Student Café”. In Discussion 1 we would like you to participate in your first online activity, during which you introduce yourself to your fellow students. Please participate in this discussion during February / July.

We are looking forward to meeting you online!

Your lecturers