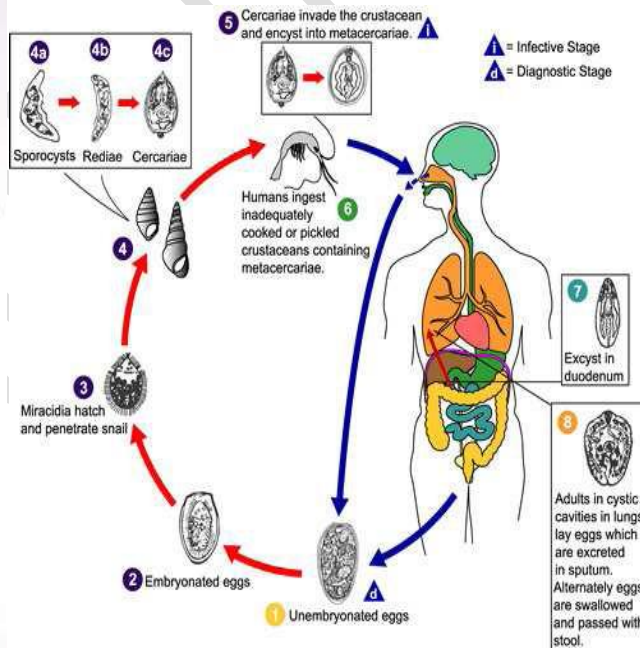




NATIONAL OPEN UNIVERSITY OF NIGERIA
SCHOOL OF HEALTH SCIENCES



COURSE CODE: NSC 106

COURSE TITLE: MEDICAL MICROBIOLOGY AND PARASITOLOGY

COURSE UNITS: 3 (2-0-3)



NATIONAL OPEN UNIVERSITY OF NIGERIA

COURSE GUIDE

NSC106 MEDICAL MICROBIOLOGY AND PARASITOLOGY

COURSE CODE: NSC 106

COURSE TITLE: Medical Microbiology and Parasitology

COURSE UNITS: 3 Credit units (24 hours of instruction online; 12 hours of Discussion forum online/tutorial; 48 hours of laboratory practical)

YEAR: 1

SEMESTER: 2nd Semester

PRE-REQUISITE COURSES: All courses in the BNSC degree programme in the first Semester

CON-CURRENT COURSES: NSc 102, NSc 104, NSc 108

SESSION: 2015/2016

COURSE WEBSITE: www.noun.edu.ng/



COURSE WRITERS

Prof A. Aboderin, Department of Medical Microbiology and Parasitology, College of Health Science, Obafemi Awolowo University, Ile-Ife

Dr O.O. Irinoye, Department of Nursing Science, Obafemi Awolowo University, Ile-ife

Mrs Elizabeth Joseph-Shehu, School of Health Sciences, NOUN, Lagos

COURSE EDITORS: Dr O.O. Irinoye and Mrs. E.M. Joseph-Shehu

COURSE COORDINATOR: Mrs E.M Joseph-Shehu

COURSE FACILITATOR: Professor Dipo Aboderin

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COURSE GUIDE

GENERAL INTRODUCTION

As nurses, you are familiar with the directives of washing hands, cleaning items with antiseptics as regular practices with every action that you take when you relate to clients in clinical settings. Virtually every aspect of nursing practice makes demand for sound foundation in medical microbiology and parasitology. Medical microbiology and parasitology is a compulsory course for Bachelor of Nursing Science students. Microorganisms have their usefulness in various endeavours of human life. They are relevant and have their usefulness in agriculture, medicine (human medicine, animal care and Pharmacy), food and beverages, military to mention a few. However, they are also causes of diseases and destruction and must be appropriately studied and control. The course is covers the structures, the growth and control of these organisms to reduce their negative consequences that compromise life and to help you maximize the benefits for health.

COURSE AIM.

The aim of this course is to update your knowledge and skills in safety practices as you apply the principles of microorganisms control in health care delivery. Your appreciation of the context of infection control should also drive your urge for standard practice and quality assurance for safe health care environment.

COURSE OBJECTIVES

At the completion of this course, you will be able to:

Apply the knowledge, skills and attitude acquired in this course in achieving infection control in clinical practice and in providing safe and quality care while adopting standard measures for infection control in care of patients with different types of diseases.

COURSE IMPLEMENTATION – WORKING THROUGH THIS COURSE

The course will be delivered adopting the blended learning mode, 70% of online but interactive sessions and 30% of face-to-face during laboratory sessions. You are expected to register for this course online before you can have access to all the materials and have access to the class sessions online. You will have the hard and soft copies of course materials, you will also have online interactive sessions, face-to-face sessions with instructors during practical sessions in the laboratory. The interactive online activities will be available to you on the course link on the Website of NOUN. There are activities and assignments online for every unit every week. It is important that you visit the course sites weekly and do all assignments to meet deadlines and to contribute to the topical issues that would be raised for everyone's contribution.

You will be expected to read every module along with all assigned readings to prepare you to have meaningful contributions to all sessions and to complete all activities. It is important that you attempt all the Self Assessment Questions (SAQ) at the end of every unit to help your understanding of the contents and to help you prepare for the in-course tests and the final examination. You will also be expected to keep a portfolio where you keep all your completed assignments.

COURSE MATERIALS

Course Guide

Course Text in Study Units

Textbooks (Hard and electronic)

Book of Laboratory Practical

Assignment File/Portfolio

STUDY UNITS

This course has 7 modules and 22 short units. They are structured as presented

Module 1-	Introduction to Microbiology
Unit 1-	Composition of the Microbial World
Unit 2-	Historical Aspects of Microbiology
Unit 3-	The Relevance and Scope of Microbiology
Unit 4-	Microscopy and Specimen Preparation
Unit 5-	A Brief Survey of Microbes as Friends and Foes
Module 2-	General Characteristics of Microorganisms
Unit 1-	General Characteristics of Bacteria
Unit 2-	General Characteristics of Fungi
Unit 3-	General Characteristics of Viruses
Module 3-	Bacterial Nutrient, Growth and Control
Unit 1-	Bacterial Nutrition and Growth
Unit 2-	Classification and Mode of Action of Antimicrobial Agents
Unit 3-	Sterilization and Disinfection
Module 4-	Evolution of Parasitic Association
Unit 1-	Association in organisms classification of the host organism
Unit 2-	Human helminths infections
Module 5-	Trematodes
Unit 1-	Digenetic Trematodes
Unit 2-	Classification of digenetic trematodes according to their habitat
Module 6-	Cestodes
Unit 1-	Basic body plan of a cestode
Unit 2-	Tapeworms and Examples
Unit 3-	Tapeworms of man and other human's cestode
Module 7 -	Nematodes
Unit 1-	General features and life cycles of nematodes
Unit 2-	Soil transmitted helminths
Unit 3-	Blood and Tissue nematodes
Unit 4-	Air-borne nematode

REFERENCE TEXTBOOKS

1. Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book. Inc.
2. Medigan, M.T. *et al.* (2009). *Brock Biology of Microorganisms*. 12th Edition. Pearson Education Inc.
3. Pelczar, M.J., Chan, E.C.S. & Krieg, R.N. (2001). *Microbiology*. 5th Edition. McGraw-Hill.

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6. Ukoli, F.M.A. (1990). Introduction of Parasitology in Tropical Africa. John Wiley and Sons Ltd., Chichester.
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16. Baumeister, S., Pohlmeyer, K., Kuschfeldt, S., Stoye, M. (1997). Prevalence of *Echinococcus multilocularis* and other metacestodes and cestodes in the muskrat (*Ondatra zibethicus* LINK 1795) in Lower Saxony. *Dtsch Tierarztl Wochenschr.* 104(10):448-52.
17. Khalil, L., A. Jones, and R. A. Bray. eds. 1994. Keys to the Cestode Parasites of Vertebrates. CAB International, Wallingford, U.K. 751 pp.

COURSE REQUIREMENTS AND EXPECTATIONS OF YOU

Attendance of 95% of all interactive sessions, submission of all assignments to meet deadlines; participation in all CMA, attendance of all laboratory sessions with evidence as provided in the log book, submission of reports from all laboratory practical sessions and attendance of the final course examination. You are also expected to:

1. Be versatile in basic computer skills
2. Participate in all laboratory practical up to 90% of the time
3. Submit personal reports from laboratory practical sessions on schedule
4. Log in to the class online discussion board at least once a week and contribute to ongoing discussions.
5. Contribute actively to group seminar presentations.

EQUIPMENT AND SOFTWARE NEEDED TO ACCESS COURSE

You will be expected to have the following tools:

1. A computer (laptop or desktop or a tablet)
2. Internet access, preferably broadband rather than dial-up access
3. MS Office software – Word PROCESSOR, Powerpoint, Spreadsheet
4. Browser – Preferably Internet Explorer, Moxilla Firefox
5. Adobe Acrobat Reader

NUMBER AND PLACES OF MEETING (ONLINE, FACE-TO-FACE, LABORATORY PRACTICALS)

The details of these will be provided to you at the time of commencement of this course

DISCUSSION FORUM

There will be an online discussion forum and topics for discussion will be available for your contributions. It is mandatory that you participate in every discussion every week. Your participation link you, your face, your ideas and views to that of every member of the class and earns you some mark.

COURSE EVALUATION

There are two forms of evaluation of the progress you are making in this course. The first are the series of activities, assignments and end of unit, computer or tutor marked assignments, and laboratory practical sessions and report that constitute the continuous assessment that all carry 30% of the total mark. The second is a written examination with multiple choice, short answers and essay questions that take 70% of the total mark that you will do on completion of the course. Students evaluation: The students will be assessed and evaluated based on the following criteria

- **In-Course Examination:**
In-course examination will come up in the middle of the semester. These would come in form of Computer Marked Assignment. This will be in addition to one compulsory Tutor Marked Assignment (TMA's) and three Computer marked Assignment that comes after the modules.
- **Laboratory practical:** Attendance, record of participation and other assignments will be graded and added to the other scores from other forms of examinations.
- **Final Examination:** The final written examination will come up at the end of the semester comprising essay and objective questions covering all the contents covered in the course. The final examination will amount to 60% of the total grade for the course.

Learner-Facilitator evaluation of the course

This will be done through group review, written assessment of learning (theory and laboratory practical) by you and the facilitators.

GRADING CRITERIA

Grades will be based on the following Percentages

Tutor Marked Individual Assignments	10%	} 30%
Computer marked Assignment	10%	
Group assignment	5%	
Discussion Topic participation	5%	
Laboratory practical	10%	
End of Course examination	70%	

GRADING SCALE

A = 70-100

B = 60 - 69

C = 50 - 59

F = \leq 49

SCHEDULE OF ASSIGNMENTS WITH DATES

Every Unit has activity that must be done by you as spelt out in your course materials. In addition to this, specific assignment will also be provided for each module by the facilitator.

SPECIFIC READING ASSIGNMENTS

To be provided by each module

COURSE OVERVIEW

Medical Microbiology and Parasitology is a course that covers the study of characterisation and classification of micro-organisms, characteristics of bacteria and other micro-organisms like fungi and viruses; medical helminthology; microbial growth, reproduction and control. The course also provide guides and practical experiences to infection control as to assure safety of care providers and recipients of care in clinical sites.

HOW TO GET THE MOST FROM THIS COURSE

1. Read and understand the context of this course by reading through this course guide. paying attention to details. You must know the requirements before you will do well.
2. Develop a study plan for yourself.

3. Follow instructions about registration and master expectations in terms of reading, participation in discussion forum, end of unit and module assignments, laboratory practical and other directives given by the course coordinator, facilitators and tutors.
4. Read your course texts and other reference textbooks.
5. Listen to audio files, watch the video clips and consult websites when given.
6. Participate actively in online discussion forum and make sure you are in touch with your study group and your course coordinator.
7. Submit your assignments as at when due.
8. Work ahead of the interactive sessions.
9. Work through your assignments when returned to you and do not wait until when examination is approaching before resolving any challenge you have with any unit or any topic.
10. Keep in touch with your study centre, the NOUN, School of Health Sciences websites as information will be provided continuously on these sites.
11. Be optimistic about doing well.

COURSE MATERIAL

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MODULE 1- INTRODUCTION TO MICROBIOLOGY

Microorganisms are every where and it can be beneficial and also harmful to human. For instance *escherichial coli* that are found in the lower alimentary canal are harmless at this site (normal body flora) and harmful at another site like vagina (causing infection). This module deals with Composition of the Microbial World, Historical Aspects of Microbiology, The Relevance and Scope of Microbiology, Microscopy and Specimen Preparation and finally a Brief Survey of Microbes as Friends and Foes

Module Objectives

At the end of this module you should be able to:

- i. Describe microorganisms
- ii. Discuss the relevance and scope of microbiology.

CONTENT

Unit 1: Composition of the Microbial World

Unit 2: Historical Aspects of Microbiology

Unit 3: The Relevance and Scope of Microbiology

Unit 4: Microscopy and Specimen Preparation

Unit 5: A Brief Survey of Microbes as Friends and Foes

UNIT ONE- COMPOSITION OF THE MICROBIAL WORLD

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Contents
- 3.1 Microorganisms
- 3.2 Microorganisms are Cells
- 3.3 Classification Systems for Microorganisms
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

Microbiology is the study of microorganisms. These are organisms too small to be seen clearly by the unaided eyes. Microorganisms include bacteria, fungi, algae, protozoa and entities at the borderline of life that are called viruses. The cell is the fundamental unit of life. Most microorganisms are unicellular, in unicellular organisms all the life processes are performed by a

single cell. However, some are multicellular, having more than one cell. This unit examines the definition of microbiology, types of microbial cells, the different groups of microorganisms and the domains in which they are placed and why viruses are not placed in any of the domains.

2.0 Objectives

At the end of this unit, you should be able to:

- i. Define the term microbiology
- ii. List the groups of organisms classified as microorganisms
- iii. Distinguish between prokaryotic and eukaryotic cells
- iv. Explain the distribution of microorganisms into domains
- v. State the characteristics of the microorganisms in each domains
- vi. State the characteristics of viruses

3.0 Main Content

3.1 Microorganisms

Microorganisms are organisms too small to be seen clearly by the unaided eyes. They are very small life forms so small that individual microorganisms cannot be seen without magnification. They include fungi, bacteria, algae, protozoa and viruses. Some microorganisms however, like the eukaryotic microorganisms are visible without magnification. Examples are bread moulds and filamentous algae.

3.2 Microorganisms are Cells

The cell is the fundamental unit of life; a single cell is an entity isolated from other cells. Two fundamental different types of cells exist among microorganisms; which are prokaryotic and eukaryotic.

- **Prokaryotes:** These microbial cells lack membrane-bound nucleus and organelles.
- **Eukaryotes:** Possess a membrane-bound nucleus and organelles.

Differences between Prokaryotic and Eukaryotic Cells

Properties	Prokaryotic cell	Eukaryotic cell
Nuclear membrane	Absent	Present
Chromosome	Single, closed, circular, dsDNA	Multiple linear
Ribosomes	70s(50s + 30s)	80s(60s + 40s)
Mitochondrion	Absent	Present
Examples	Bacteria	Animals, plants, fungi, algae, protozoa

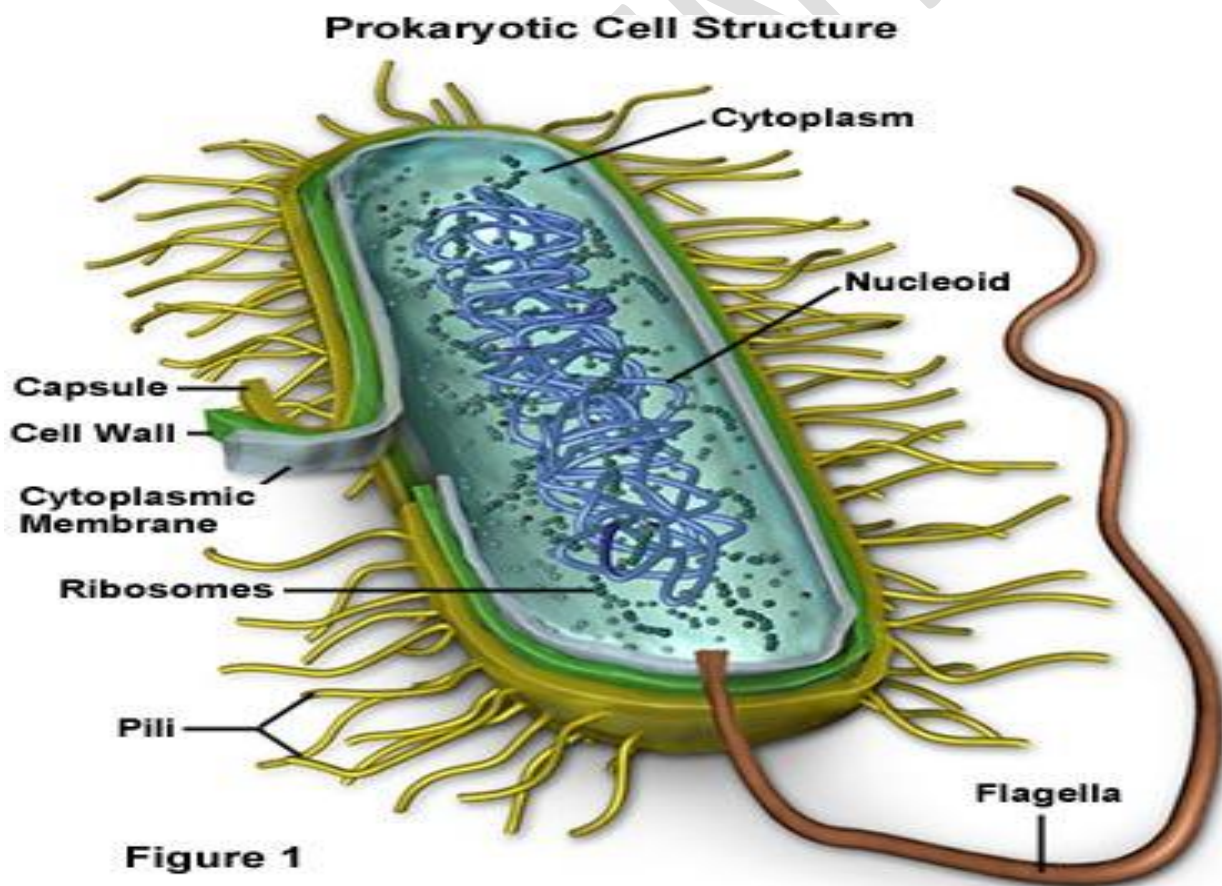


Fig 1.1: Prokaryotic Cell Structure

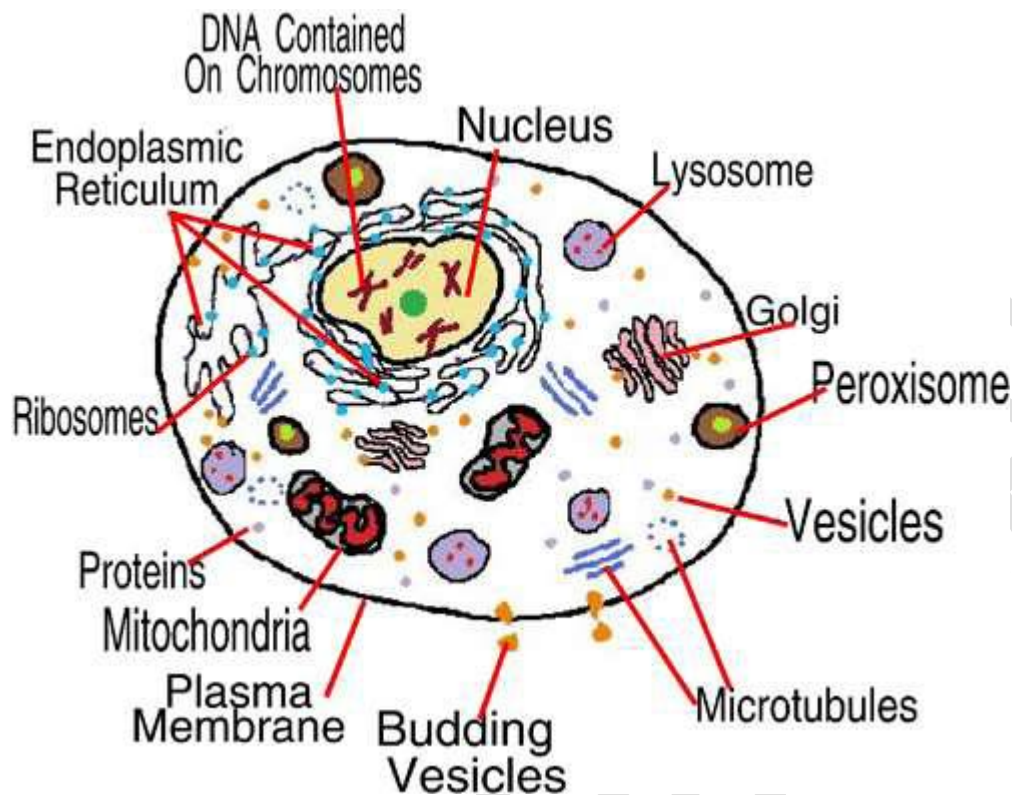


Fig. 1.2: A Eukaryotic Cell

Source: http://www.the-simple-homeschool.com/imagefiles/labeled_cell.gif

3.3 Classification Systems for Microorganisms

The Five Kingdom System of Classification

Based on cell type and mode of nutrition, there was an establishment of the five kingdom system of classifying organisms in which we have:

- i. Monera
- ii. Protista
- iii. Fungi
- iv. Planta
- v. Animalia

Microorganisms except for viruses, which are acellular and have their own classification system, were placed in the first three kingdoms.

The Three Domains System of Classification

Presently, through advances in cell biology, biochemistry and genetics, microorganisms are now placed into three domains, each of which comprises of various kingdoms.

The domains are:

- i. Bacteria (prokaryotic – “true bacteria”)
- ii. Archaea (prokaryotic – “ancient bacteria”)
- iii. Eucarya (eukaryotic)

Domain Bacteria

- i. They are prokaryotic.
- ii. They are single celled organisms.
- iii. They lack membrane bound nucleus and organelles.
- iv. Most have cell wall that contains peptidoglycan.
- v. They are found in the soil, water and air and on other living organisms.
- vi. Some are harmful while others are beneficial to man.

Domain Archaea

- i. They were formerly known as archaeobacteria.
- ii. They are prokaryotic.
- iii. They are single celled organisms.
- iv. They lack membrane bound nucleus and organelles.
- v. They lack peptidoglycan in their cell walls.
- vi. They have unique membrane lipids.
- vii. Some have unusual metabolic characteristics, e.g. methanogens which generate methane gas.
- viii. Many are found in extreme environments.

Domain archaea is distinguished from bacteria based upon

- i. Differences in ribosomal RNA sequences.
- ii. The absence of cell wall peptidoglycan.
- iii. The presence of unique membrane lipids

Domain Eucarya

The major groups of microorganism in this domain are protists and fungi.

Protists

These groups of microorganisms are unicellular algae, protozoa, slime moulds and water moulds.

Algae

- i. They are simple organisms.
- ii. Mostly unicellular.
- iii. They are photosynthetic together with cyanobacteria.
- iv. They produce about 75% of the plant's oxygen.
- v. Commonly found in aquatic environment.
- vi. They are primary producers in food chains in aquatic habitat.

Protozoa

- i. They are unicellular.
- ii. Eukaryotic organisms and animal like.
- iii. They are usually motile.
- iv. Some are free living while some are pathogenic.

Slime Moulds

They are protists which have different forms at different stages of their life cycles. At a stage they are like protozoa and at another stage like fungi.

Water Moulds

These are found on the surface of fresh water and moist soils. They feed on decaying vegetation such as logs and mulch.

Fungi

- i. These are microorganisms that range from unicellular forms like yeasts to moulds and mushrooms which are multicellular with thread like structures called hyphae.
- ii. They absorb nutrients from their environments.
- iii. Many play beneficial roles while others cause diseases in plants, animals and human.

Viruses

- i. They are acellular entities (non cellular).
 - ii. They lack the fundamental structure of living cell but only carry out functions of living organisms when in living cells.
 - iii. They are the smallest of all the microorganisms (10,000 smaller than a typical bacterium).
 - iv. They can only be seen by the electron microscope.
 - v. They cause many diseases of plants, animals and humans.
 - vi. Entities are not placed in any of the domain but are classified on a separate system.
- b. They cause many diseases of plants, animals and humans

4.0 Conclusion**5.0 Summary**

In this unit, you have learnt about the following microorganisms and the classification systems for Microorganisms

6.0 Tutor Marked Assignments

6.1 Activity: Give three examples of diseases caused by each classification of microorganism

6.2 Answer the following questions:

- i. Define the term, microorganism (LO1)
- ii. Compare and contrast prokaryotic and eukaryotic cells (LO3)
- iii. List the three domains under which microorganisms are classified (LO4)
- iv. List three characteristics each of the following domains: a. Bacteria b. Archaea c. Fungi (LO5).
- v. What are the differences between bacteria and archeae? (LO5)
- vi. Why are viruses not placed in any of the domains? (LO6)

7.0 References and other resources

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book. Inc.

Medigan, M.T. *et al.* (2009). *Brock Biology of Microorganisms*. 12th Edition. Pearson Education Inc.

Pelczar, M.J., Chan, E.C.S. & Krieg, R.N. (2001). *Microbiology*. 5th Edition. McGraw-Hill.

Willey, J.M., Sherwood, L.M & Woolverton, C.J. (2008). *Microbiology*. 7th Edition. Boston Bur Bridge, IL: McGraw-Hill Higher Education.

UNIT TWO- HISTORICAL ASPECTS OF MICROBIOLOGY

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
- 3.1 Discovery of Microorganisms
- 3.2 The Spontaneous Generation Conflict
- 3.3 The Recognition of the Role of Microorganisms in Disease
- 3.4 The Development of Microbiology in this Century
- 3.5 Era of Molecular Microbiology
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

The history of microbiology is the story of men and women who developed a technique, a tool or a concept that was generally adopted in the studying of microorganisms. It is also the history of events and metamorphosis of microbiology as a science. In this unit we will be studying the stages in the development of the science of microbiology, some early scientists and their contributions to the field of microbiology.

2.0 Objectives

At the end of this unit, you should be able to:

- i. Explain how microorganisms were discovered
- ii. Discuss the concept of spontaneous generation and the experiments that were performed to disprove the concept
- iii. Discuss Koch's postulate and how they are used to establish a link between a suspected microorganisms and the disease
- iv. Explain development of microbiology in this century
- v. Explain the era of molecular biology.

3.0 Main Content

3.1 Discovery of Microorganisms

The advent of the microscope permitted the studying of microorganisms. The first microscopes were simple ground glass lenses that magnified images of previously unseen microorganisms. Among the first to observe this previously unseen and invisible microbial world were Robert Hooke and Anthony Van Leeuwenhoek.

1. Robert Hooke (1635-1703), an English mathematician and natural historian.

- He coined the term “cells” to describe the “little boxes” he observed in examining cork slices with a compound microscope.
- He was the first to make a known description of microorganisms.
- He made microscopic observation and the earliest description of many fungi.
- Various species of fungi were clearly identified in his drawing and recorded in his book *Micrographia*.

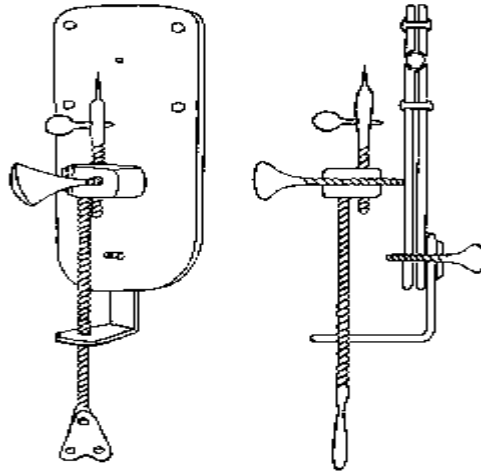


Fig. 2.1: Robert Hooke’s detailed Diagram of Fungi made in 1667

Source: *Microorganisms in our World* by Atlas R. M. (1995)

2. Anthony Van Leeuwenhoek (1632-1723) lived in Delft, Holland.

- He is known as the father of bacteriology and the first person to publish extensive and accurate observations of microorganisms
- He was a draper and an amateur microscope builder.
- He learned lens grinding as a hobby and made over 100 simple microscopes each capable of magnifying an image about 300 times.
- By using simple microscopes, he observed microscopic organisms which he called ‘animalcules’.
- He discovered bacteria in 1676 while studying pepper water infusion and reported his observations in a series of letters to Royal Society of London which published them in 1684 in English translation.
- He made sketches of the different shapes of bacteria.



(a)

(b)

Fig. 2.2: (a) Antony Leeuwenhoek (1632-1723) Holding one of his Microscopes (b) Leeuwenhoek's Microscopes and some of the Sketches of Bacteria from Human Mouth
Source: *Microorganisms in our World* by Atlas R. M. (1995)

After Van Leeuwenhoek's death, the study of microbiology did not develop rapidly because microscopes were rare and interest in microorganisms was not high. Scientists then were debating the theory of spontaneous generation.

3.2 The Spontaneous Generation Conflict

The concept spontaneous generation states that living organisms could develop from non-living matter. The proponents of the concept of spontaneous generation claim that living organisms could develop from non living or decomposing matter.

1. Francesco Redi (1626-1697) challenged this concept by showing that maggots on decaying meat came from fly eggs deposited on the meat, and not from the meat itself.

- He carried out a series of experiments on decaying meat and its ability to produce maggot spontaneously.
- He placed meat in three different containers, one was not covered, and the second was covered with fine gauze to exclude flies.
- Flies laid eggs on the uncovered meat and maggots developed.
- The two other meats did not produce maggots.
- Spontaneously, flies were attracted to the gauze-covered container and laid their eggs on the gauze, these later produced maggots.
- Hence, it become evident that the generation of maggots resulted from the presence of fly eggs and that meat (a non-living matter) did not spontaneously generate maggots as previously believed.

2. Louis Jablot (1670) conducted an experiment in which he divided a hay infusion that had been boiled into two containers: a heated container that was closed to the air and a heated container that was freely open to the air. Only the open vessel developed microorganisms. This further helped to disprove abiogenesis.

3. John Needham (1713-1781) showed that mutton broth boiled in flasks and then sealed could still develop microorganisms, which supported the theory of spontaneous generation.

4. Lazzaro Spallanzani (1729-1799) showed that flasks sealed and then boiled had no growth of microorganisms, and he proposed that air carried germs to the culture medium. He also commented that external air might be needed to support the growth of animals already in the medium. The latter concept was appealing to supporters of spontaneous generation.

5. Louis Pasteur (1822-1895) was a Professor of Chemistry. He devised a series of swan necked flasks known as Pasteur-flasks, filled the flasks with broth and heated the broth to sterilisation. After cooling, the flasks were opened to the air, but bends on the neck of the flasks prevented microorganisms from falling on the broth and contaminating it rather the microorganisms fell on the neck of the bottle. Pasteur proved that no growth occurred because dust and germs were trapped on the wall of the curved necks. If the neck were broken, growth will occur. By these experiments he disproved and defeated the theory of spontaneous generation.

Figure 3 shows the defeat of spontaneous generation.

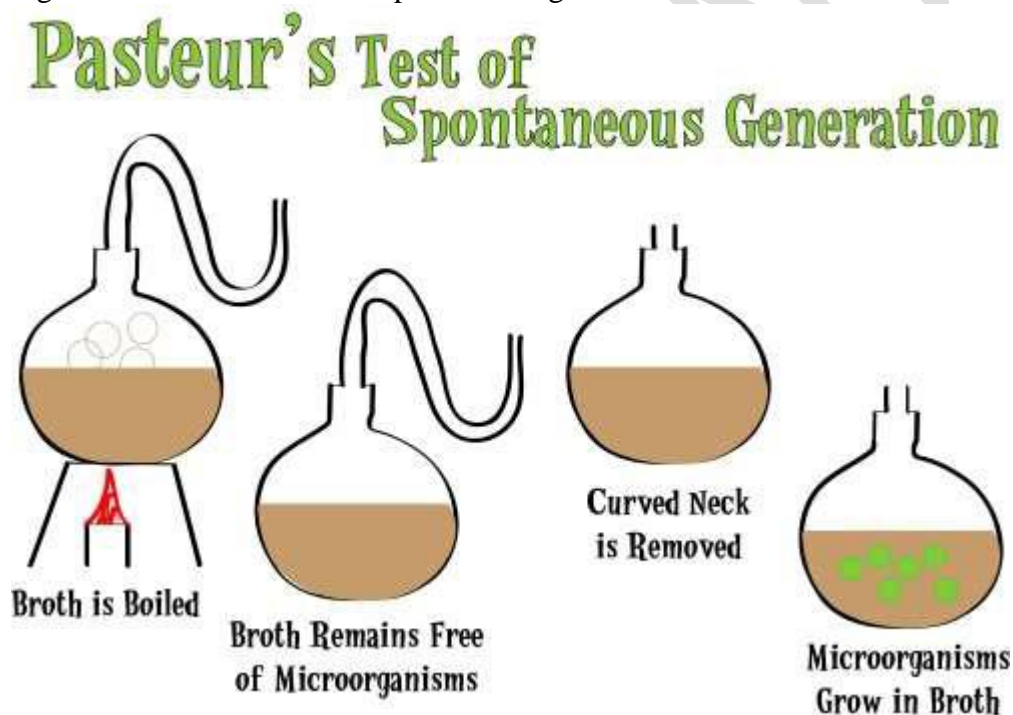


Fig. 2.3: The Defeat of Spontaneous Generation - Pasteur's Experiment with the Swan-Necked Bottles. Source: Amoebamike.wordpress.com

Apart from the defeat of the concept of spontaneous generation:

- Pasteur's work led to an effective sterilization method which involve holding juices and milk at 62.8OC (145OF) for 30 minutes known as Pasteurization.
- He discovered that alcoholic fermentation was catalyzed by Living Yeast Cells.

- He developed vaccines for the diseases anthrax, fowl cholera and rabies between 1880 and 1890.
- As a result of his research on rabies, he became a legend and the French government built the Pasteur Institute in Paris in 1888. It was originally established as a clinical centre for treating rabies, but is now a major biomedical research centre for antiserum and vaccine production.
- He postulated the **Germ Theory of Disease** which states that microorganisms are the cause of infectious diseases.
- Pasteur's work ushered in the Golden Age of Microbiology.

3.3 The Recognition of the Role of Microorganisms in Disease

1. Joseph Lister (1872-1912) developed a system of surgery designed to prevent microorganisms from entering wounds. He implemented the use of sterile surgical instrument, and used carbolic acid (phenol) during surgery and on wound dressings.

2. Robert Koch (1843-1910) was a German physician. He was the first to directly prove the role of microorganisms in causing diseases. He established the relationship between *Bacillus anthracis* and the disease it causes, anthrax. Using mice as experimental animals, he demonstrated that when a small amount of blood from a diseased mouse was injected into a healthy mouse, the healthy mouse quickly developed anthrax.

From this work he developed Koch's postulates which are:

- i. The suspected disease-causing organism should be present in all cases of the disease and absent from healthy animals.
- ii. The suspected organism must be cultivated in a pure culture away from the animal body.
- iii. The isolated organism must cause the disease when inoculated into a healthy susceptible animal.
- iv. The organism must be re-isolated from these experimental animals and culture again in the laboratory after which it should still be the same as the original organism.

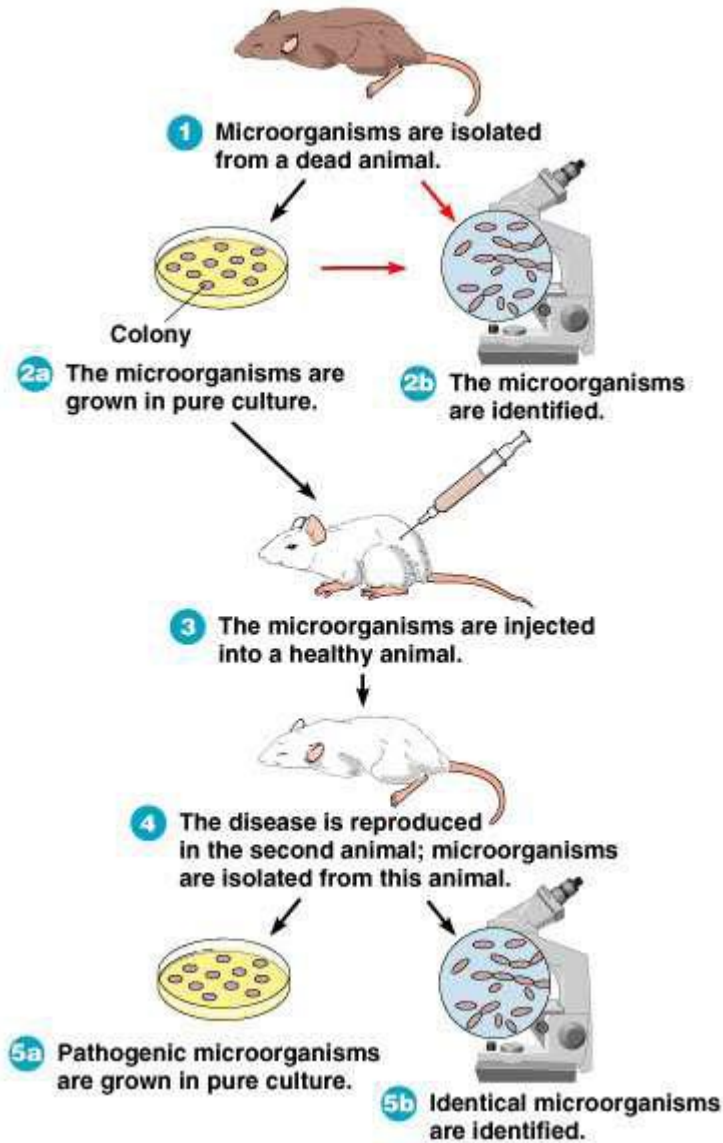


Fig. 2.4: Diagrammatic Illustration of Koch's Postulate

Using these principles, Koch discovered causative organisms of anthrax (1876), tuberculosis (1882) and cholera (1883).

- He was the first to grow bacteria on solid culture media to get pure culture; hence he developed the pure culture concept and developed different solid media.
- Koch's discovery of solid culture media and pure culture concept supplied the most needed tools for the development of microbiology as a field of science.
- For his contribution on tuberculosis, he was awarded the 1905 Nobel Prize for Physiology or Medicine.

Today, "Molecular Koch's postulates" have been established in light of advances in the molecular biology of pathogenic microbes.

3. Edward Jenner (ca. 1798) used a vaccination procedure to protect individuals from smallpox.
4. Emil Von Behring (1854-1917) and Shibasaburo Kitasato (1852-1931) induced the formation of diphtheria tetanus antitoxins in rabbits which were effectively used to treat humans, thus demonstrating humoral immunity.

3.4 The Development of Microbiology in this Century

Microbiology established a closer relationship with other disciplines during the 1940s because of its association with genetics and biochemistry.

1. George W. Beadle and Edward L. Tatum (ca. 1941) studied the relationship between genes and enzymes using the bread mould, *Neurospora*.
2. Salvatore Luria and Max Delbruck (ca. 1943) showed that mutations were spontaneous and not directed by the environment.
3. Oswald T. Avery, Colin M. McLeod, and Maclyn McCarty (1944) provided evidence that deoxyribonucleic acid (DNA) was the genetic material and carried genetic information during transformation.

3.5 Era of Molecular Microbiology

This Began in the 1970s with:

- Advancement in the knowledge of bacterial physiology, biochemistry and genetics.
- Genetic manipulation which involves the transfer of DNA from one organism into another or a bacterium and the proteins encoded by the DNA harvested led to the development of the field of Biotechnology.
- DNA sequencing revealed the phylogenetic (evolutionary) relationships among bacteria which led to revolutionary new concepts in microbial systematics.
- In 1990s, DNA sequencing gave birth to the field of genomics.

4.0 Conclusion

5.0 Summary

In this unit you have been exposed to the following:

- i. The Discovery of Microorganisms
- ii. The Spontaneous Generation Conflict
- iii. The Recognition of the Role of Microorganisms in Disease
- iv. The Development of Microbiology in this Century
- v. Era of Molecular Microbiology

6.0 Tutor Marked Assignments

6.1 Activity: Observe a decay meat under the microscope and discuss your findings in the discussion forum

6.2 Tutor Marked Tests

Answer the following questions:

- i. Write short note on Robert Hooke and Anthony Van Leeuwenhoek contribution to the discovery of microorganisms (LO1)
- ii. State the concept of spontaneous generation (LO2)
- iii. Discuss the experiments that were performed to disprove the concept of spontaneous generation (LO2)
- iv. Explain the steps involved in using Koch's postulate to establish the link between a suspected microorganism and a disease (LO3)
- v. Explain development of microbiology in this century (LO4)
- vi. Explain the era of molecular biology (LO5)

7.0 References and other resources

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book. Inc.

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UNIT THREE- THE RELEVANCE AND SCOPE OF MICROBIOLOGY

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 The Basic Aspects of Microbiology
 - 3.2 The Applied Aspects of Microbiology
 - 3.3 The Future of Microbiology
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
 - 6.1 Activity
 - 6.2 Tutor Marked Tests
- 7.0 References/Further Reading

1.0 Introduction

Modern microbiology is a large discipline with different specialized areas. This is because the entire ecosystem depends on the activities of microorganisms and microorganisms influence human society in countless ways. Microbiology has a great impact on medicine, agriculture, food

science, ecology, genetics, biochemistry and other fields. In this unit, we shall examine the different aspects of microbiology and their relevance to human life.

2.0 Objectives

At the end of this unit, you should be able to:

- i. Explain the two branches of microbiology
- ii. Discuss the different areas of study in basic and applied microbiology.

3.0 Main Content

The main branches of Microbiology are Basic and Applied. Both branches intertwine and are complementary to each other.

3.1 The Basic Aspects of Microbiology

The basic branch of microbiology is concerned with the study of the biology of microorganisms. Fields of study here include:

- i. **Bacteriology:** This is the study of bacteria.
- ii. **Mycology:** The study of fungi such as yeasts, molds, and mushrooms.
- iii. **Algology:** The study of algae.
- iv. **Protozoology:** The study of protozoa; a branch of protozoology called parasitology deals exclusively with the parasite or disease producing protozoa and other parasitic micro and macro organisms.
- v. **Microbial Cytology:** Studies the structures of microbial cells.
- vi. **Microbial Physiology:** Studies of the nutrients that microorganisms require for metabolism and growth and the products that they make from nutrients.
- vii. **Microbial Genetics:** Focuses on the nature of genetic information in microorganisms in microorganisms and how it regulates the development and functions of cells and organisms.
- viii. **Microbial Ecology:** The study of microorganisms in their natural environment. It also studies the global and local contribution to nutrient cycling. In addition, it employs microorganisms in bioremediation to reduce pollution.
- ix. **Microbial Taxonomy:** This is the study of the classification of microorganisms or the grouping of microorganisms.
- x. **Biochemistry:** This deals with the discovery of microbial enzymes and the chemical reactions they carry out.

3.2 The Applied Aspects of Microbiology

The applied aspect of microbiology deal with practical application of microorganisms to solve problems related to diseases, water and waste water treatment, food spoilage and food production. The various fields of study in applied microbiology include:

- i. **Medical Microbiology:** Studies of the causative agents of diseases, diagnostic procedures for identification of the causative agents and preventive measures.
- ii. **Agricultural Microbiology:** This is the study of microbial processes in the soil to promote plant growth. It involves the study of soil microorganisms which has led to the discovery of antibiotics and other important chemicals. It also deals with the methods of combating plant and animal diseases caused by microbes, methods of using microbes to

- increase soil fertility and crop yields. Currently, much work is being done on using bacterial and viral insect pathogens to substitute chemical pesticides.
- iii. **Industrial Microbiology:** This is the large scale growth of microorganisms for the production of medicinal products such as antibiotics and vaccines; fermented beverages; industrial chemicals; production of hormones and proteins by genetically engineered microorganism.
 - iv. **Aquatic and Marine Microbiology:** Aquatic and Marine Microbiology deals with microbial processes in lakes, rivers, and the oceans. It also examines issues that concern water purification, microbiology examination and biological degradation of waste.
 - v. **Public Health Microbiology:** This is closely related to medical microbiology. It deals with the identification and the control of the spread of communicable diseases. It involves monitoring of community food establishments and waste supplies so as to keep them safe and free from infectious agents.
 - vi. **Immunology:** Deals with how the immune system protects the body from pathogens and the response of infectious agents. It also involves practical health problem such as the nature and treatment of allergies auto-immune diseases like rheumatoid arthritis.
 - vii. **Food and Dairy Microbiology:** Deals with the use of microbes to make foods such as cheese, yoghurt, wine and beer. It also deals with the methods of preventing microbial spoilage of food and the transmission of food-borne diseases such as Botulism and Salmonellosis. Microorganisms are also used as single cell protein, which is an important source of protein or nutrients to livestock and humans.
 - viii. **Aeromicrobiology:** Advances thought in the dissemination of diseases in the air, contamination and spoilage.
 - ix. **Exomicrobiology:** Exploration for life in outer space.
 - x. **Geochemical Microbiology:** Coal, mineral and gas formation; prospecting for deposits of coal, oil and gas and recovery of minerals from low-grade ores.

3.3 The Future of Microbiology

There are many promising areas of microbiological research and their potential practical impacts in the future. These areas include combating new and re-emerging human diseases such as HIV/AIDS, SARS, TUBERCULOSIS, POLIOMYELITIS, etc. For this combat to be effective there would be need for the production of new drugs and vaccines. The use of molecular biology and recombinant DNA technology will be applied to give solutions to these problems.

Microorganisms would be needed for environmental bioremediation of pollutants which is on the increase globally. Much work will also be needed to be done on microorganisms living in extreme environments such as to advance the development of new antimicrobial agents, industrial processes and bioremediation. Analyses of genome and its activities will advance the field of bioinformatics and help to investigate biological problems.

4.0 Conclusion

In this unit, we discussed Modern microbiology as a large discipline with many different specialised areas. It is subdivided into two main areas of research (basic and applied). The basic area of research in microbiology deals with the biology of microorganisms and includes fields such as bacteriology, mycology, microbial ecology. The applied aspect of microbiology deals with the practical application of microorganisms to solve various human problems related to

diseases, water and waste treatment, food production and spoilage, etc. The field of microbiology will be faced with many important future challenges such as finding new ways to new and reemerging diseases, reduced environmental pollution and investigating biological problems.

5.0 Summary

In this unit, you have learnt about the following:

- i. The Basic Aspects of Microbiology
- ii. The Applied Aspects of Microbiology
- iii. The Future of Microbiology

6.0 Tutor Marked Assignments

6.1 Activity

Discuss the relevance of microbiology to nursing practice and list five basic areas of research in microbiology and state what each area entails. Share your submission in you group forum.

6.2 Please answer the following questions:

- i. List the fields of microbiology that deal with the following:
 - a. Metabolism (LO2)
 - b. Enzymology (LO2)
 - c. Nucleic Acid and Protein Synthesis (LO2)
 - d. Microorganisms in the Natural Environment (LO2)
 - e. Microbial Classification (LO2)
 - f. Microbial Cell Structure (LO2)
- ii. Explain what the field of medical microbiology entails (LO1)
- iii. State the importance of microbiology in five different fields of human endeavours (LO1)

7.0 References and other resources

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book. Inc.

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UNIT FOUR- MICROSCOPE AND SPECIMEN PREPARATION**CONTENT**

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main Content
- 3.1** The Light Microscope
- 3.2** Electron Microscope
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
- 6.1** Activity
- 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 Introduction

Microbiology is the study of organisms too small to be seen distinctly with the unaided eyes. The nature of this discipline makes the microscope of crucial importance because the study of microorganisms is impossible without the microscope. Microscopes provide magnification which enables us to see microorganisms and study their structures. The magnification attained by microscopes range from x100 to x400,000 in addition there are different types of microscopes and many techniques have been developed by which specimens of microorganisms can be prepared for examination. This unit examines the different types of microscopes, how the microscopes work and how specimens are prepared for examination.

2.0 Objectives

At the end of this unit, you should be able to:

- i. Define the term microscope
- ii. State the two categories of microscope
- iii. Describe the bright field microscope
- iv. Explain the resolving power
- v. Describe methods of preparing and staining specimens
- vi. Describe the scanning electron microscope and the transmission electron microscope.

3.0 Main Content

The Microscope: A microscope is an instrument for producing enlarged images of objects too small to be seen unaided. There are two types of microscopes: Light (optical) and electron depending on the principle on which magnification is done.

3.1 The Light Microscope

This is a type of microscope in which magnification is obtained by a system of optical lenses using light waves. It includes:

- i. Bright Field Microscope
- ii. Dark Field Microscope
- iii. Fluorescence Microscope

iv. Phase Contrast Microscope

Modern microscopes are compound microscopes. That is, the magnified image formed by the objective lens is further enlarged by one or more additional lenses. Most undergraduate students of microbiology perform most of their examinations with the bright field microscope which is the most widely used instrument for routine microscopic work. The other types of microscope are used for special purposes or research investigation.

The Bright Field Microscope

- The ordinary microscope is called a bright field microscope because it forms a dark image against a brighter background.
- The microscope consists of a sturdy metal body or stand made up of a base and an arm to which the remaining parts are attached.
- A light source, either a mirror or an electric illuminator, is located at the base.
- Two focusing knobs, the fine and coarse adjustment knobs are located on the arm and can move either the stage or the nose piece to focus the image.
- The stage is positioned about halfway up the arm and hold microscope slides by slide clips or a mechanical stage clip.
- There is a substage condenser mounted within or beneath the stage which focuses a core of light on the slide.
- The upper part of arm of the microscope holds the body assembly to which a nose piece and one or more eyepieces or ocular lenses are attached.
- Most advanced microscopes have eyepieces for both eyes and are called binocular microscopes.
- The nose piece holds three to five objective lenses of different magnifying power and is easily rotated to position any objective.
- The image you see when viewing a specimen is focused by the objective and ocular lenses working together.
- Light from the specimen which has been illuminated is focused by the objective lens creating an enlarged image within the microscope. The ocular lens further magnifies this primary image.
- The total magnification is calculated by multiplying the objective and eye piece magnification together; e.g. if a 45x objective is used with a 10x eyepiece, the overall magnification of the specimen will be 450x.



Fig. 4.1: The Bright Field Microscope

Source: http://biology.unm.edu/ccouncil/Biology_203/Images/Microscopes/microscope6.jpeg

The Dark-Field Microscope

- The dark field microscope is used to observe living unstained cells and organisms as a result of change in the way they are illuminated.
- A hollow core of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective only light that has been reflected or refracted by the image forms an image.
- The field surrounding the specimen appears black while the object itself is brightly illuminated.
- The dark field microscope is useful in revealing many internal structures in larger eukaryotic microorganisms.
- It is also used in the examination of unstained microorganisms suspended in fluids, e.g. wet mount and hanging drop preparation.

The Phase-Contrast Microscope

- This type of microscope converts slight differences in refractive index and cell density into easily detected variations in light intensity and is used to view living cells.
- The background formed by the undeviated light is bright while the unstained objects appear dark and well defined.

- This microscope is very useful for studying microbial motility, determining the shape of living cells and detecting some bacterial components such as endospores and inclusion bodies. It is also used in studying eukaryotes.

The Fluorescent Microscope

- This type of microscope exposes a specimen to ultraviolet, violet or blue light and forms an image of the object with resulting fluorescent light.
- The most commonly used fluorescence microscope light is epifluorescence microscope which is also called incident light or reflected light microscope.
- Epifluorescence microscope employs an objective lens that also acts as a condenser. A mercury vapor arc lamp or other source produces an intense beam of light that passes through an exciter filter. The exciter filter transmits on the desired wavelength of excitation light
- The excitation light is directed down the microscope by a speed mirror called the dichromatic mirror.
- This mirror reflects light of shorter wavelength but allows light of longer wavelength to pass through.
- The excitation light continues down through the objective lens to specimen stained with spaced dye molecules called fluorochromes.

Microscope Resolution

Resolution is the ability of a lens to separate or distinguish between small objects that are close together, i.e. the microscope must produce a clear image and not just a magnified one. It is also known as the resolving power. Resolution is described mathematically by an equation in the 1870s by Ernest Abbe, a German physicist. The Abbe equation states that the minimal distance (d) between two objects that reveal them as separate entities depends on the wavelength of light (λ) used to illuminate the specimen and on the numerical aperture of the lens ($n \sin \alpha$) which is the ability of the lens to gather light.

$$d = \frac{0.5 \lambda}{n \sin \alpha}$$

As d becomes smaller, the resolution increases and finer details can be discerned in a specimen; d becomes smaller as the wavelength of light used decreases and as the numerical aperture (NA) increases. Hence, the greatest resolution is obtained using a lens with the largest NA and light with the shortest wavelength.

The relationship between NA and resolution can be expressed as follows:

$$d = \frac{\lambda}{2NA}$$

where d = resolution and λ = wavelength of light. Using the values 1.3 for NA and $0.5 \mu\text{m}$, the wavelength of green light, for λ , resolution can be calculated as

$$d = \frac{0.55}{2 \times 1.30} = 0.21 \mu\text{m}.$$

From these calculations, we may conclude that the smallest details that can be seen by the light microscope are those having dimensions of approximately $0.2 \mu\text{m}$.

Preparation for Light-Microscope Examination

There are two general methods used for preparing specimens for light microscope examination.

- i The organisms are suspended in a liquid (the wet-mount or the hanging drop technique), and
- ii The organism is dried fixed and stained before observing under the microscope.

The wet mount or hanging drop technique

The technique permits examination of organisms in a normal living condition. A wet mount is made by placing a drop of fluid containing the organisms on a glass slide and covering the drop with a cover slip. Petroleum jelly may be used to provide a seal between the slide and covers slip after which the slide is viewed under the microscope.

This method is desirable because:

- i. It prevents distortion of the morphology of spiral bacteria when they are stained and dried.
- ii. It reveals whether organisms are motile or not.
- iii. Some cell inclusion bodies are easily observed.
- iv. Spore formation and germination may also be observed in living cells.

Fixed, Stained Smears of Microorganisms

These are frequently used for the observation of the morphological characteristics of bacteria. The procedure makes the cell more clearly visible, and differences between cells of different species and within the same species can be demonstrated. The essential steps in this procedure are:

- i. Preparation of the film or smear
- ii. Fixation and
- iii. Application of one or more staining solution.

Fixation

Fixation is the process by which the internal and external structures of cells and microorganisms are preserved and fixed in position. It inactivates enzymes that might disrupt cell morphology and tough cell structures so that they do not change during staining and observation. A microorganism usually is killed and attached firmly to the microscope slide during fixation.

There are two fundamentally different types of fixation.

- i. Heat Fixation: Is routinely used to observe prokaryotes. Typically, a film of cells (a smear) is gently heated as a slide is passed through a flame. Heat fixation preserves overall morphology but not structures within cells.
- ii. Chemical Fixation: Is used to protect fine cellular sub-structure and the morphology of larger, more delicate micro organisms. Chemical fixatives penetrate cells and react with cellular components, usually proteins and lipids, to render them inactive, insoluble, and immobile. Common fixative mixtures contain such components as ethanol, acetic acid, mercuric chloride, formaldehyde, and glutaraldehyde.

Staining of Specimens

Although living microorganisms can be directly examined with the light microscope, they often must be fixed and stained to increase visibility, accentuate specific morphological features, and preserve them for future study.

Types of Staining

- **Simple staining:** This is a kind of staining in which a single stain or dye is used. Basic dyes such as crystal violet, methylene blue, and carbolfuchsin are used in simple staining to determine the size, shape and arrangement of prokaryotic acids.
- **Differential staining:** These are staining procedures that make visible the differences between bacterial cells or part of a bacterial cell. It usually involves more than one dye used for staining.
- **Gram staining:** The Gram stain was developed in 1884 by the Danish physician Christian Gram. It is the most widely used differential staining procedure.

The steps involved are as follows:

- i. i The smear is stained with the crystal violet (which is the primary stain).
- ii. ii This followed by treatment with iodine functioning as a mordant.
- iii. iii The smear is decolourised by washing with ethanol or acetone.
- iv. iv The smear is counterstained with a simple dye safranin.

Bacteria stained by the Gram stain method fell into two groups:

- Gram positive bacteria which retain the crystal violet and appear deep violet in colour and Gram negative bacteria which, lose the crystal violet and are counterstained with safranin appear red in colour.
- **Acid fast staining:** This is another differential staining procedure commonly used to identify *Mycobacterium tuberculosis* and *Mycobacterium leprae*, the pathogens responsible for tuberculosis and leprosy respectively.

These bacteria have cell walls with high lipid content in particular, mycolic acid which prevents dye from readily binding to the cells. In the acid fast staining procedure, the red stain and carbol fuchsin is used as primary stain; next acid-alcohol is used as a decolouriser. The acid-alcohol will remove the red stain from bacteria such as *Escherichia coli* which the acid fast mycobacteria will remain red.

3.2 Electron Microscope

This type of microscope uses a beam of electron in place of light waves to produce the image. There are two types:

- Scanning electron microscope
- Transmission electron microscope.

The Transmission Electron Microscope

Electron microscopes use a beam of electrons to illuminate and create magnified images of specimens. Electrons replace light as the illuminating beam. They can be focused, much as light is in a light microscope, but their wavelength is around 0.005mm approximately 1000,000 times shorter than that of visible light. Therefore, electron microscopes have a practical resolution roughly 1,000 times better than the light microscope, with many electron microscopes point closer than 0.5nm can be distinguished, and the useful magnification is well over 100,000x. In transmission electron microscope, the electron beam is transmitted through the specimen.

The Scanning Electron Microscope

The scanning electron microscope produces an image from electron released from atoms on an object's surface. It has been used to examine the surfaces of microorganisms in great detail. Many SEM has a resolution of 7nm or less.

4.0 Conclusion

In this unit, we learnt that Electron microscope uses a beam of electron in place of light waves to produce the image of an object. The ordinary compound microscope is called the bright field microscope because it forms a dark image against a bright background. In the bright field microscope which is a compound the primary image is formed by an objective lens and enlarged by the eye piece or ocular lens to form the final image. We also discussed that Electron microscopes use a beam of electrons to illuminate and create magnified images of specimens.

5.0 Summary

In this unit, you have learnt about the following:

- i. The Light Microscope
- ii. Electron Microscope

6.0 Tutor Marked Assignments

6.1 Activity: Prepare two specimen for light microscope examination

6.2 Answer the following questions

- i. Define microscope (LO1)
- ii. With the aid of a well label diagram describe a bright field microscope (LO3)
- iii. What is microscope resolution? (LO4)
- iv. List the stages involved in preparing a specimen for observation under the light microscope (LO5)
- v. What is the basic difference between a transmission electron microscope and a scanning electron microscope? (LO2)
- vi. Define the resolving power (LO4)
- vii. List 5 parts of a light microscope and state the function of each (LO3)

7.0 References and other resources

Atlas, R.M. (1995). *Microorganisms in our World*. Mosby Year Book. Inc.

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UNIT FIVE- A BRIEF SURVEY OF MICROBES AS FRIENDS AND FOES**CONTENTS**

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main Content
- 3.1** Microorganisms as Friends
- 3.2** Microorganisms as Foes
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
- 6.1** Activity
- 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 Introduction

Microorganisms occur in large numbers of most natural environments and bring about many changes. Some are desirable and others are undesirable. Microorganisms affect the well being of people in many ways. Many are beneficial to man and can be called 'friends' while some are harmful and can be regarded as 'foes' to man. The beneficial impact of microorganisms ranges from the production of goods and pharmaceutical products, to enhancement of soil fertility, environmental cleanup while their harmful effect can be seen in their ability to cause disease in man, animals and plants as well as their usage in biological warfare. However, there are more species of microorganisms that perform friendly and beneficial functions than those that harm other living organisms. This unit gives us a brief survey of microorganisms as friends and foes.

2.0 Objectives

At the end of this unit, you will be able to:

- i. Explain the different ways in which microorganisms can act as friends to man
- ii. Explain ways in which microorganisms can act as foes to man.

3.0 Main Content**3.1 Microorganisms as Friends**

Microorganisms have found application in various aspects of life. They are useful in food industries to produce many food substances, in medicine to produce vaccines and antibiotics, in environmental protection, and in agriculture to optimize yield. Few of these various aspects will be discussed in this material.

Microorganisms and Food Production

- Many microorganisms are used to produce many of the foods and beverages we consume. Microbially-produced food products have properties that are very different from those of the starting materials. Most of these food products are produced by fermentation.

- Fermentation is the chemical transformation of organic compounds carried out by microorganisms and their enzymes. In industrial fermentation, raw materials (substrate) are converted by microorganisms in a controlled favourable environment (created in a fermentor) to form a desired end product substance.
- The accumulation of fermentation products such as ethanol and lactic acid produces characteristic flavours and other desirable properties in food substances.
- Pickles and some sausages are also produced by fermentation processes.
- Microorganisms are used to produce fermented dairy products such as cheese, yoghurt and acidophilus milk.
- They are also used to produce alcoholic beverages such as beer by conversion of sugar to alcohol and carbon dioxide.
- Wine fermented from fruits using yeast strains *Saccharomyces cerevisiae* and bread is also produced by using yeasts.
- Microorganisms can also be used as direct source of food known as single cell protein. Various species of yeasts, algae are grown as single cell protein and use as animal feeds thus helping to meet the world food needs.

Production of Pharmaceuticals

Microorganisms are used to produce different pharmaceuticals such as antibiotics, steroids, vitamins, hormones, etc. Antibiotics are microbially produced substances or substances synthetically derived from natural sources that inhibit or kill microorganisms, Steroids regulate various aspects of human metabolisms and are produced by organisms such *Rhizopus nigricans*. Vaccines are produced using microorganisms with the antigenic properties to elicit a primary immune response; they are used to prevent many once deadly diseases such as polio, small pox, tuberculosis, measles, diphtheria and whooping cough.

Vitamins

Vitamins are essential animal nutritional factors; some vitamins are produced by microbial fermentation, e.g. Vitamin B12 by *Streptomyces*, B12 by *Pseudomonas denitrificans* and *Propionibacterium shermanii*. Riboflavin produced by various species of *Clostridium* and *Ashbya gossypii*. Human insulin and human growth hormone are produced by genetically engineered bacteria.

Production of Organic Acids

Various organic acids are produced by microorganisms examples are:

- i. Gluconic acid: used as a pharmaceutical to supply calcium to the body by several fungi including *Penicillium* and *Aspergillus* species. Citric acid produced by *Aspergillus niger* and used as a food additive especially in the production of soft drinks.
- ii. Lactic acid by different lactic acid bacteria for example, *Lactobacillus delbrueckii*, lactic acid is used in foods as preservatives, in leather production for delimiting hides and in the textile industry for fabric treatment, plastics making in baking powders.

Hygiene

- i. Hygiene is the avoidance of infection and food spoilage by eliminating microorganisms from the surrounding.

- ii. Our knowledge of how disease causing microorganisms spread has permitted us to reduce the incidence of many diseases. Also improved sanitation practices have helped to reduce the incidence of diseases.
- iii. Microorganisms from the surroundings can be totally removed by methods such as sterilization or reduced to acceptable levels using methods such as disinfection and antiseptics. In food preparation, microbes are reduced to acceptable levels using methods such as pasteurization, addition of vinegar. While complete sterility is achieved by autoclaving or irradiation.

Useful in the Study of Science

Microbes are essential tools in biotechnology, biochemistry, genetics molecular biology and genomics. Examples are the yeasts (*Saccharomyces cerevisiae*) and fission yeast (*Shizosaccharomyces pombe*) which are model organisms in science. They can easily be grown rapidly in large quantities and are easily manipulated.

Biotechnology uses genetic engineering which is the artificial manipulation of genes and gene products.

Genes from any source can be manipulated and modified using microorganisms and their enzymes as molecular tools, e.g. human insulin, a hormone which is very low in people with diabetes is produced by genetically engineered bacteria into which human genes have been inserted.

Microorganisms and the Environment

- i. Microorganisms can be used to clean up pollution created by human activities in a process called bioremediation.
- ii. Pollutants such as pesticides, spilled oil solvents which could pose human health hazard are degraded to nontoxic substances by microorganisms.
- iii. Microorganisms are used to degrade wastes and pollutants so as to maintain and restore environmental quality.

3.2 Microorganisms as Foes

Microorganism can act as foes to man and other living organisms by causing diseases and by their usage as biological weapons.

Microorganisms as Disease Agents

Microbial diseases are still the major cause of death in many developing countries. Microorganisms cause different diseases in man such as:

- i. AIDS (Acquired Immune Deficiency Syndrome) caused by the Human Immuno Deficiency Virus (HIV).
- ii. Tuberculosis caused by a bacterium, *Mycobacterium tuberculosis*.
- iii. Cholera caused by a bacteria *Vibrio cholera*.
- iv. Malaria caused by four species of the Protozoa called *Plasmodium* transmitted by the female anopheles mosquito.
- v. Other emerging diseases include: bird flu and swine flu.

Microorganisms as Agents of Warfare and Terrorism

Biological warfare is also known as germ warfare. It is the use of pathogens such as viruses, bacteria, or the toxins produced by them as biological weapons or agents of warfare. A biological weapon may be used to kill, incapacitate or seriously impair a person, group of people or even an entire population. It can be used as a military technique by nations during wars.

There are four kinds of biological warfare agents, bacteria, viruses, fungi and rickettsias. They are living organisms that reproduce with their host victims who then become contagious with a deadly if weakening multiple effects.

Toxins on the other hand do not reproduce in the victims but within a short incubation period (usually with a few hours) kill the victims.

4.0 Conclusion

In this unit, we have discussed Microorganisms in details. Their usefulness as well as their potential disadvantages were also treated.

5.0 Summary

In this unit, you have learnt about the following:

- i. Microorganisms as Friends
- ii. Microorganisms as Foes

6.0 Tutor Marked Assignments

6.1 Activity: Mention at least five microorganisms different from the ones discussed in this text can cause diseases in man

6.2 Answer the following questions

- i. Explain the role of microorganisms in food production (LO1)
- ii. Explain the role of microorganisms Production of Pharmaceuticals (LO1)
- iii. Describe the various organic acids produced by microorganisms (LO1)
- iv. Outline the relevant of microorganism in hygiene (LO1)
- v. Explain the usefulness of microorganisms in the study of science (LO1)
- vi. What are the relevant of microorganisms to the environment (LO1)
- vii. Explain two ways in microorganisms are harmful to man (LO2)

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MODULE TWO- GENERAL CHARACTERISTICS OF MICROORGANISMS

Introduction

As a practice nurse, you are aware of some characteristics features of microorganisms such as reproduction, movement etc. Microorganisms have different characteristics features which will be discussed in this module. The microorganisms that will be discussed are those that have direct link with man. This module deals with general characteristics of bacteria, fungi and virus.

Module objectives

At the end of this module you will be able to describe the general characteristics of microorganisms and their links and usefulness to humans.

Contents

Unit 1: General Characteristics of Bacteria

Unit 2: General Characteristics of Fungi

Unit 3: General Characteristics of Viruses

UNIT 1- GENERAL CHARACTERISTICS OF BACTERIA CONTENTS

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main Content
 - 3.1** General characteristics of bacteria
 - 3.2** Size, Shape and Arrangement of Bacterial Cell
 - 3.3** Bacterial Structures
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
 - 6.1** Activity
 - 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 Introduction

Bacteria are characterised based on the cell shape, size and structure cell arrangement, occurrence of special structures and developmental forms, staining reactions and motility and flagella arrangement. They are also characterised by the cell wall component, Gram stain reaction, cellular respiration and mode of nutrition. This unit examines the general characteristics of bacteria, shapes and forms of bacteria, structures external and internal in bacteria among other things.

2.0 Objectives

At the end of this unit, you should be able to:

- i. describe the general characteristics of basic bacteria
- ii. identify and name the general shapes and forms of bacteria

- iii. describe the external and internal structures of bacteria
- iv. explain the significance of the cell wall structure and composition
- v. explain the modes of nutrition and energy source in bacteria
- vi. explain the modes of cellular respiration in bacteria
- vii. explain the modes of reproduction in bacteria.

3.0 Main Content

3.1 General characteristics of bacteria are:

- i. They are prokaryotic
- ii. They are simplest of all microbial cells
- iii. Bacteria are single celled organisms
- iv. They have distinctive cell wall which contain peptidoglycan
- v. They are measured in unit called micrometer
- vi. Bacteria lack a true nucleus but have a region called the nucleoid region, i.e. DNA is free floating
- vii. They may have additional DNA called a plasmid
- viii. Their reproduction is by binary fission
- ix. They are extremely diverse and numerous in soils and waters.

3.2 Size, Shape and Arrangement of Bacterial Cell

Size

Bacteria are very small, 0.5 to 1.0 μ m in diameter. Because of their small size, they have high surface area/volume ratio which results in a high growth and metabolism rate. No circulatory mechanism is needed for nutrients taken in because the mass of cell substance to be nourished is very close to the surface. Examinations of a microbial cell require the use of a high power microscope usually of about 1,000 diameters.

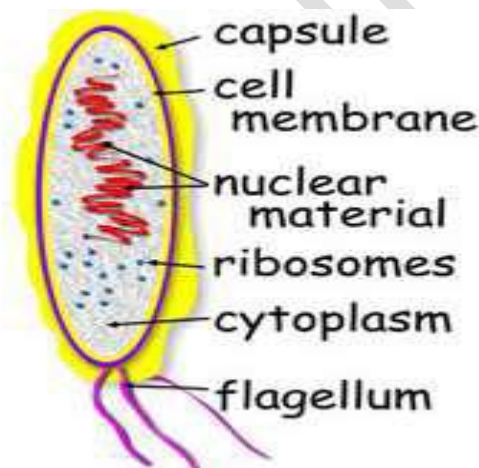


Fig.1.1: A Basic Bacterium Cell (Source: *Microorganisms in our World* by Atlas (1995))

Shape and Arrangement

The shape of a bacterium is governed by its rigid cell wall which gives it a definite shape. Typical shapes of bacteria are:

- Cocci (Singular: Coccus), e.g. *Staphylococcus*
- Bacilli (rods) (Singular: rod, bacillus), e.g. *Bacillus subtilis*
- Vibrios (Singular: *Vibrio*)
- Spirilla (Singular: Sprillum)
- Spirochaetes (Singular: *Spirochaete*), e.g. *Treponema pallidum*

Some species of bacteria are pleomorphic, i.e. they are able to change their forms especially when grown on artificial media.

- i. **Cocci:** They are round, oval or spherical in diameter characteristic arrangement when multiplying is based on arrangement of cells, they are called:
 - Diplococci: cocci in pairs, e.g. meningococci and gonococci.
 - Streptococci cocci in chains.
 - Staphylococci: cocci in irregular clusters (like a bunch of grapes).
 - Tetrads: cocci in a group of four cells.
 - Sarcinae: cocci in regular clusters.
- ii. **Bacilli (Rod):** These are stick like bacteria with rounded, square, tapered or swollen ends. They measure 1-10 μm in length by 0.3-1.0 μm in width.

Bacilli are not arranged in patterns as complex as cocci. Most occur singly. Other arrangements are:

- Diplobacilli: Rods in pairs.
 - Streptobacilli: Rods in chains.
 - Trichomes: Similar to chains but have larger area of contact between adjacent cells.
 - Mass together, e.g. *Mycobacterium leprae*.
 - Palisade arrangement cells are lined side by side like matchsticks and at angles to each other like Chinese letters, e.g. *Corynebacterium diphtheriae*.
- iii. **Vibrios:** These are small slightly curved rods, or comma shaped 3-4 μm in length by 0.5 μm in width. Most are motile with a single flagellum at one end, e.g. *Vibrio cholerae*.
 - iv. **Spirilla:** These are helical bacteria, small, regularly coiled, rigid, organisms measuring 3-4 μm in length. Each coil measures about 1 μm , e.g. *Spirillum minus*.
 - v. **Spirochaetes:** They are helical, (complete twist), flexible, coiled organisms, can twist and contort their shapes. Spirochaetes are divided into three main groups.
 - *Treponemes:* Tiny and delicate with regular tight coils, measuring 6-15 μm by 0.2 μm in width, e.g. *Treponema pallidum* and *Treponema pertenue*.
 - *Borreliae:* Large spirochaetes with irregular open coils 10-20 μm in length by 0.5 μm in width, e.g. *Borella.*, *duttoni* and *Borrelia vinceti*.
 - *Leptospire:* Tiny spirochaetes with many tightly packed coils that are difficult to distinguish; 6-20 μm in length by 0.1 μm in width and have hooked ends, e.g. *Leptospira interrogans*.

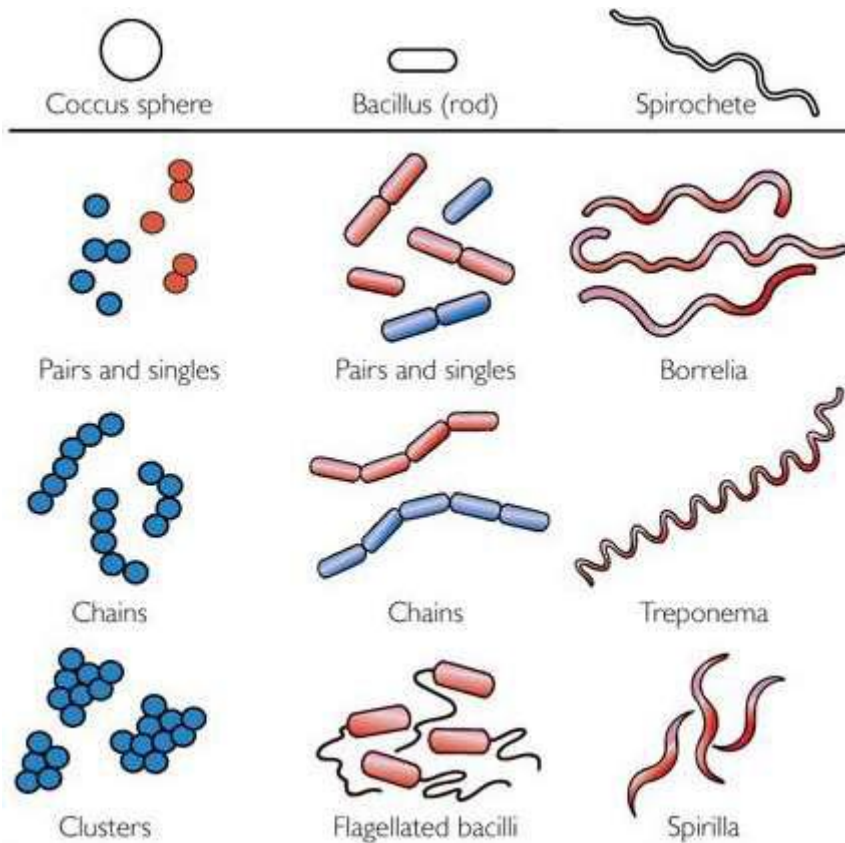


Fig.1. 2: Common Shapes of Bacteria
(Source: *Microorganisms in our World* by Atlas (1995))

In addition to the common bacterial shapes, many others also occur in different shapes, which include:

- pear shaped cells, e.g. *Pasteuri*
- lobed spheres, e.g. *Sulfolobus*
- rods with squared ends, e.g. *Bacillus anthracis*
- disk arranged stacks of coins, e.g. *Caryophanon*
- rods with helically sculptured surfaces, e.g. *Seliberia* and many others.

The shape of a cell affects its survival and activity in the environment.

3.3 Bacterial Structures

Examination of a bacterial cell will reveal several components and structures. Some are external to the cell wall while others are internal to the cell wall.

Structure External to the Cell Wall

1. Flagella (Singular: Flagellum): These are hair like, helical appendages that protrude through the cell wall, $0.01 - 0.02\mu\text{m}$ in diameter and simple in structure. Based on their location on the cell, flagella may be polar or lateral.

- i **Polar:** At one or both ends of bacterium.
- ii **Lateral:** Along the sides of the bacterium.

A flagellum is composed of three parts:

- i A basal body associated with the cytoplasmic, membrane and cell wall.
- ii A short hook and a helical filament which is usually several times as long as the cell.
- iii A flagellum grows at the tip rather than at the base.

Types of Flagella

- **Monotrichous:** A single polar flagellum. Many that appear and function as monopolar or bipolar flagella consist of bundles of 2 to 50 single units (polytrichous). eg *Vibrio cholerae*
- **Lophotrichous:** A cluster of polar flagella or multiple polar flagella eg *Bartonella bacilliformis*
- **Amphitrichous:** Flagella, either single or clusters at both cell poles or single bipolar flagella eg *Spirillum serpens*
- **Peritrichous:** Cell surrounded by lateral flagella or flagella distributed over the entire cell eg *Escherichia coli*

Function of Flagella

Bacteria propel themselves by rotating their helical flagella.

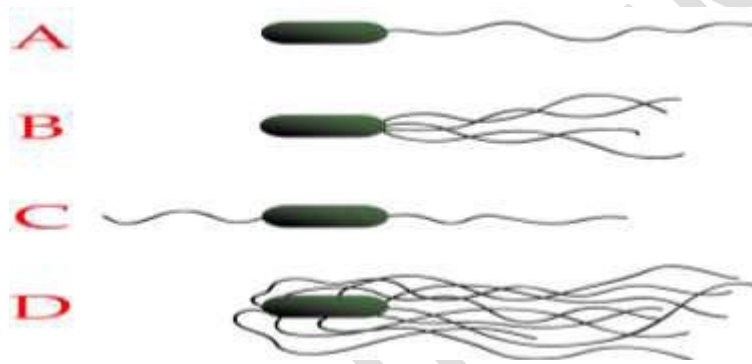


Fig. 1.3: Types of Flagella (Source: bioweb.uwlax.edu)

2. Pili (Singular: Pilus): They are also called fimbriae. They are hollow, non-helical filamentous appendages that are thinner, shorter and more numerous than flagella: long, thin, straight threads 3-25 μ m in diameter and 12 μ m in length. They do not function in motility since they are found on non-motile and motile species. Several functions are associated with different types of pili.

F pilus (Sex pilus) serves as the path of entry of genetic material during bacterial mating. Some play major role in human infection by allowing pathogenic bacteria to attach to the epithelial cells lining the respiratory, intestinal or genitourinary tracts, this prevents the bacteria from being washed away by the flow of mucous or body fluids and permits infections to be established.

3. Capsules: This is a viscous substance forming a covering layer or envelope around the cell wall of some bacteria. Capsules are known to confer resistance to phagocytosis because complement cannot penetrate it, hence protecting bacteria against host defense to invasion. Also protects the bacteria from desiccation. Capsules are usually antigenic for identification. Capsules can be categorised into three based on their visualisation by light microscope using special staining methods.

If the covering layer can be visualised by light microscope using special staining methods, it is termed capsule.

- **Microcapsule:** If the layer is too thin to be seen by light microscope.
- **Slime:** If it is so abundant that many cells are embedded in a common matrix.

Most bacterial capsules consist of polysaccharides which can be homopolysaccharides or heteropolysaccharides.

- **Homopolysaccharides:** Capsule made up of/composed of a single kind of sugar usually synthesized outside the cell by exocellular enzymes, e.g. glucan (a polymer of glucose) from sucrose by *S. mutans*.
- **Heteropolysaccharides:** Composed of several kinds of sugars. A few capsules are polypeptide, e.g. *Bacillus anthracis* has a capsule made up of a polymer of glutamic acid.

Functions

- They may provide protection against temporary drying by binding water molecules.
- They may block attachment of bacteriophages.
- They may be antiphagocytoc, i.e. they may inhibit the engulfment of pathogenic bacteria by white blood cells. Hence contribute to invasive or infective ability (*virulence*).
- Promote attachment of bacteria to surfaces.

Example of capsulated bacteria include; *Neisseria gonorrhoeae*, *Streptococcus pneumonia*, *Hemophilus influenza*.

4. Sheaths: Some bacterial species form chains or trichomes enclosed by a hollow tube called sheaths. These sheaths consist of a heteropolysaccharides containing glucose, glucuronic acid, galactose and fucose.

Functions

- In a few bacteria, they facilitate moderate change of position.
- Sheaths enable individual cells to stay associated in cell colonies.

5. Prosthecae and Stalks

Prosthecae: They are semi rigid extensions of the cell wall and cytoplasmic membrane and have a diameter less than that of the cell. Found in some aerobic bacteria from fresh water and marine environment.

Functions

- Increase surface area of the cell for nutrient absorption.
- Some have adhesive substances that aid attachment to surfaces.

Stalks: They are non-living ribbon -like or tubular appendages excreted by some bacterial cells, e.g. found in *Gallionella* or *lanctomyces*.

Functions

They aid in attachment of the cells to surfaces.

Some bacteria possess oval structures called endospores (these are not appendages) formed within certain bacteria specie that represent a dormant stage in the growth cycle of the organism. They are formed in response to nutritional deprivation within the vegetative bacterial cell. Highly resistant to injurious effects of heat, drying, pressure, and many chemical disinfectants. Seen in

Bacillus and Clostridium spp. And can reverse to vegetative form when environmentally convenient.

The Cell Wall

This provides structural rigidity and forms barrier against the outside environment. Has a high tensile strength conferred on it by a layer composed of a substance called peptidoglycan (aka murein or mucopeptide).

Bacteria are classified as Gram +ve or Gram -ve according to their response to Gram staining procedure i.e based on the propensity of their cell wall to hold fast to the primary dye (crystal violet) or otherwise when exposed to a decolorizing agent such as acetone or 95% alcohol.

It is the site of antigenic determinant of the cell surface. Lipopolysaccharide component of the Gram -ve wall is responsible for non specific endotoxin activity. The cell wall also Shows differences in Gram reaction thereby gives basis for classifying bacteria.

Gram positive cell wall.

About 80nm thick with several layers of peptidoglycan(40-80% dry weight).

Trapped within this peptidoglycan matrix are a variety of proteins, polysaccharides and unique molecules called teichoic acids which stabilize the wall, chelate small ions necessary for cell function and participate in cellular interaction and adherence to mucosal surfaces and are antigenic forming basis for antigenic grouping in some organisms.

The enzymatic biosynthesis of peptidoglycan form target sites for inhibition of cell wall synthesis by specific antibiotics.

Inhibitors of cell wall synthesis

- i. Beta lactams- they possess lactam rings. They are penicillins, cephalosporins, monobactams and carbapenems.
- ii. Vancomycin Glycopeptides
- iii. Teicoplanin Glycopeptides
- iv. Fosfomycin
- v. Bacitracin
- vi. Cycloserine

Beta lactamase inhibitors- clavulanic acid, sulbactam, tazobactam.

Gram negative cell wall

It is thinner but highly complex and multilayered of about 5-10nm thickness

Composed of:

- i. A peptidoglycan layer- relatively thinner than that of Gram +ve wall i.e about 2nm thick (5-10% of dry mass)
- ii. Outer membrane- A bilayered structure, inner and outer leaflets are asymmetrical. Contains numerous proteins, up to 50% by mass. Lipopolysaccharides is attached by a weak cohesive forces (ionic and hydrophobic interactions) to the outer leaflet.
- iii. Lipopolysaccharides (LPS) – attached to outer leaflet of the outer membrane. LPS is responsible for endotoxin activity of GN organisms in GN sepsis.

LPS has three components-

- a) Lipid A- much of endotoxin effect.
- b) Core polysaccharide region.

- c) O-specific (somatic antigen) polysaccharide- for much identification.
1. Lipoprotein – cross-link outer membrane and peptidoglycan layer. Function to stabilize outer membrane and anchor it to the peptidoglycan layer.
 2. Periplasmic space – seen immediately outside the cytoplasmic membrane. Contains peptidoglycan layer and gel-like solution of proteins. Periplasmic proteins include substrates-binding proteins (for sugars, amino acids, vitamins and ions); hydrolytic enzymes; detoxifying enzymes (e.g. β -lactamase and aminoglycoside phosphorylase). Detoxifying enzymes cause antibiotic resistance.

Gram staining

This staining technique makes use of properties in the cell wall of the bacteria I.e. the peptidoglycan content in the cell wall. For gram positive bacteria that has higher peptidoglycan content, they retain the primary stain (crystal violet) despite decolourisation by acetone thereby appearing purple or blue but gram negative will quickly lose the primary stain after brief decolourisation due to reduced content of peptidoglycan in their cell wall thereby taking the colour of the counterstain (neutral red/safranin) and appearing red. Stains in gram staining are- Crystal violet (primary stain), lugol's iodine (mordant), acetone (decolouriser), neutral red/safranin (counterstain).

Structures Internal to the Cell

1. Cytoplasmic Membrane

- This lies immediately beneath the cell wall.
- It is approximately 7.5 μ m (0.0075 μ m) thick and composed primarily of phospholipids (20 to 30 percent) and protein (60 to 70 percent).
- It serves as a barrier to most water soluble molecules.
- It contains various enzymes involved in respiration, and metabolism and in synthesis of capsular and cell wall component.
- Proteins are also synthesized in the cytoplasm.

2. Protoplast

A protoplast is the portion of a bacterial, all made up of the cytoplasmic membrane and the cell material bounded by it.

3. The Cytoplasm

This is the cell material bounded by the cytoplasmic membrane and it may be divided into:

- i The cytoplasmic area, granular in appearance and rich in the macromolecular RNA-protein bodies called Ribosomes on which proteins are synthesised.
- ii The chromatin area rich in DNA and
- iii The fluid portion with dissolved substances.

4. Nuclear Material

Unlike eucaryotic cells bacterial cells do not have a distinct membrane enclosed nucleus but they have an area near the centre of the cell that is regarded as the nuclear structure, the DNA of the cell is confined to this area. The DNA is circular and bears the genes of the cell.

5. Spores and Cysts

Certain bacteria produce spores either within the cells (endospores) or external to the cell (exospores). The spore is metabolically dormant form which under appropriate condition

can germinate to form a vegetative cell. Endospores are extremely resistant to desiccation, staining, disinfecting chemicals, radiation and heat.

Cysts are also dormant, thick walled desiccation resistant forms that can germinate also under favourable conditioning.

Nutrition

The nutrition requirements of bacteria vary widely. Based on their source of energy, they are classified as:

i Phototrophs: These are bacteria that use light energy as their energy sources.

ii Chemotrophs: They obtain their energy by oxidizing inorganic or organic – chemical compounds.

Based on the source of carbon which is the major source of nutrient for all cells bacteria can be classified as:

- Heterotrophs: These are bacteria that derive carbon from preformed organic nutrients such as sugar or carbohydrate.
- Autotrophs: They derive carbon from inorganic sources such as carbon dioxide.

Cellular Respiration

Based on whether they need oxygen to survive or not, bacteria may be:

i **aerobic or strict aerobes**: these require oxygen, e.g. *Bacillus cereus*.

ii **anaerobic bacteria or strict anaerobes**: they cannot tolerate oxygen, e.g. *Clostridium* spp.

iii **facultative anaerobes**: These are generally aerobes but have the capacity to grow in the absence of oxygen, e.g. *Staphylococcus* spp.

Reproduction

Bacteria reproduce mainly by asexual method which most of the time is transverse binary fission. This is a process in which a bacterial cell divides to give two daughter cells after developing a transverse septum (cross wall).

4.0 Conclusion

In this unit, we have learnt that Bacteria are prokaryotic single celled organisms that lack membrane-bound organelles. They are very small, with sizes ranging from 0.5 to 1.0µm in diameter. Also discussed were structures external to bacterial cell wall such as flagella, pili, capsules, sheaths, prosthecae and stalks.

5.0 Summary

In this unit, you have learnt about the following:

- i. General characteristics of bacteria
- ii. Size, Shape and Arrangement of Bacterial Cell
- iii. Bacterial Structures

6.0 Tutor Marked Assignments

6.1.1 Activity: Differentiate the cell walls of Gram positive and Gram negative bacteria. Also mention the stains in gram staining technique

6.2 Answer the following questions:

- i. Describe the general characteristics of basic bacteria (LO1)
- ii. What are the general shapes and forms of bacteria (LO2)
- iii. List four different structures external to the cell wall of bacteria and state one function of each. (LO3)
- iv. Explain the term gram positive and gram negative cell wall (LO4)
- v. Describe the structures internal to bacteria cell (LO3)
- vi. Explain the modes of nutrition and energy source in bacteria (LO5)
- vii. Explain the modes of cellular respiration in bacteria (LO6)
- viii. explain the modes of reproduction in bacteria. (LO7)

7.0 References and other resources

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UNIT 2- GENERAL CHARACTERISTICS OF FUNGI**CONTENTS**

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main Content
- 3.1** Distinguishing Characteristics of Fungi
- 3.2** Structure and Forms of Fungi
- 3.3** Nutrition and Metabolism
- 3.4** Reproduction
- 3.5** Physiology
- 3.6** Importance of Fungi
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
- 6.1** Activity
- 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 INTRODUCTION

Fungi are eukaryotic spore bearing organisms that lack chlorophyll and generally reproduce both sexually and asexually. They are of great practical and scientific importance. One of the reasons for this is that many fungi are of microscopic cellular dimensions. Fungi have a diversity of morphological appearances depending on the species. Fungi comprise the molds, mushrooms and yeasts. Molds are filamentous and multicellular while yeasts are unicellular. They are widely distributed and found wherever moisture is present. They are of great importance to man in both beneficial and harmful ways. This unit examines the general characteristics of fungi, the distribution, morphology, nutrition and reproduction of fungi.

2.0 LEARNING OBJECTIVES

At the end of this unit, you should be able to:

- i. Define a fungus
- ii. State the general characteristics of fungi
- iii. Describe the structure of a yeast
- iv. Describe the structure of a mold
- v. Explain the mode of nutrition in fungi
- vi. Explain the methods of asexual reproduction and sexual reproduction in fungi.

3.0 Main Content**Definition of Fungi**

Fungi are eukaryotic spore bearing organisms that lack chlorophyll and generally reproduce both sexually and asexually.

3.1 Distinguishing Characteristics of Fungi

They are large, diverse and widespread group of organisms, the molds, mushrooms and yeasts.

- i. Fungi are Eucaryotic. They are members of the domain Eucarya.
- ii. They contain a membrane-enclosed nucleus and several other organelles.
- iii. They have no chlorophyll.
- iv. They are chemo organotrophic organisms.
- v. The body of the fungi is called thallus.
- vi. The thallus may consist of a single cell as found in yeasts.
- vii. The thallus may consist of filaments, 5 to 10 μ m across which are commonly branched as found in molds.
- viii. The yeast cell or mold filament is surrounded by a true cell wall (exception is the slime mould which have a thallus consisting of a naked amoeboid mass of protoplasm).
- ix. Some fungi are dimorphic, that is they exist in two forms. Some pathogenic fungi of humans and other animals have a unicellular and yeast like form in their host but when growing saprobically in soil or on a laboratory medium they have a filamentous mold form.
- x. Habitat distribution of fungi is diverse. Some are aquatic, living primarily in fresh water and a few marine fungi are terrestrial. They inhabit soil and dead plant. Some are parasitic, inhabiting and infecting living hosts either plants or animals. Some form beneficial relationships with other organisms as mycorrhizae.
- xi. The study of fungi is known as mycology.

3.2 Structure and Forms of Fungi

The body or vegetative structure of a fungus is called a thallus (plural thalli). It varies in complexity and size ranging from the single cell microscopic yeasts to multicellular moulds and mushrooms. The fungal cell is usually enclosed in a cell wall of chitin.

Yeasts

- They are unicellular fungi that have a single nucleus.
- They are commonly egg-shaped but some are elongated and some spherical. Yeasts have no flagella or other organelles of locomotion.
- They possess most of the other eukaryotic organelles.
- Yeast cells are larger than most bacteria. Yeasts vary considerably in size ranging from 1 to 5 μ m in width and from 5 to 30 μ m or more in length.
- They reproduce asexually by budding and traverse division or sexually through spore formation.

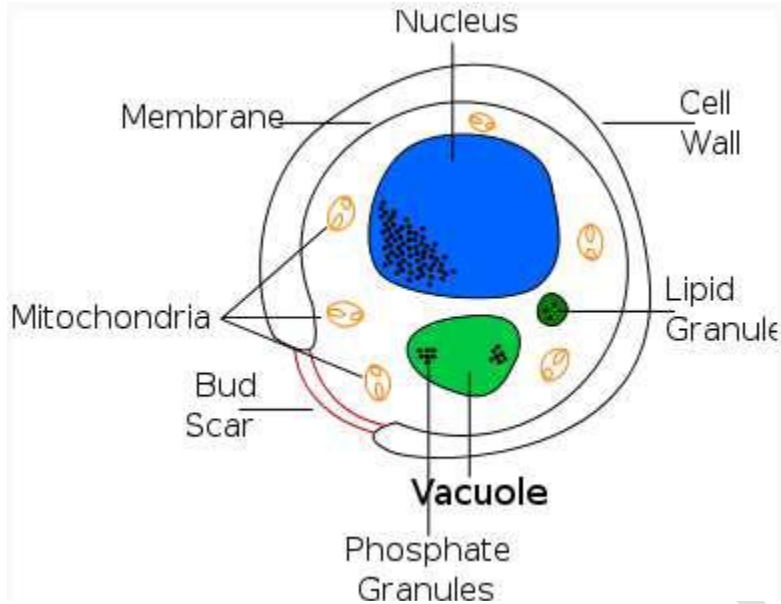


Fig. 2.1: A Diagram of a Yeast Cell

Source: Wikimedia Commons by Frankie Robertson using Inkscape (2009)

Molds

The thallus of a mold consists of long branched threadlike filaments of cells called hyphae. These hyphae form a mycelium which is a tangled mass or tissue like aggregation of hyphae.

Hyphae

- i. Each hypha is about 5 to 10 μ m wide. Hyphae are composed of an outer tube like wall surrounding a cavity the *Lumen* which is filled or lined by protoplasm. Between the protoplasm and the wall is the *plasmalemma*, a double layer membrane which surrounds the protoplasm.
- ii. The hyphal wall consists of microfibrils composed of hemicelluloses or chitin. True cellulose occurs only in the walls of lower fungi.
- iii. Wall matrix material in which the microfibrils are embedded consists of proteins, lipids and other substances. Growth of a hypha is distal near the tip.

The Mycelium

- i. The mycelium is a complex of several filaments called hyphae (singular, hypha). New hyphae generally arise from a germinated spore. The germinated spore puts out a germ tube or tubes which elongate to form hyphae.

These hyphae form a tangled mass or tissue like aggregation.

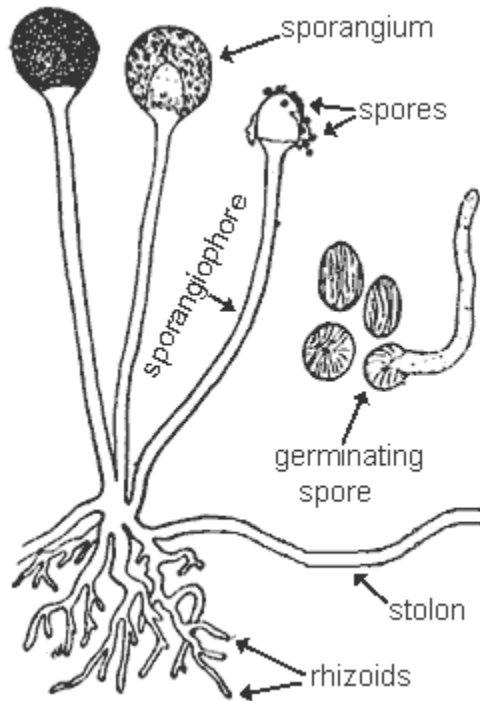


Fig. 2.2: *Rhizopus stolonifer*

Source: Retrieved from The Backyard Nature Website at file:///G:/Bread mold fungus, *Rhizopus stolonifer*.htm.

In some fungi, protoplasm streams through hyphae uninterrupted by cross walls, these hyphae are called coenocytic or aseptate.

The hyphae of others have cross walls called septa (*s. septum*) with either single pore or multiple pores that enables cytoplasmic streaming.

These hyphae are termed septate.

Summarily, hyphae can be said to occur in three forms:

- i. Nonseptate or coenocytic; such hyphae have no septa.
- ii. Septate with uninucleate cells.
- iii. Septate with multinucleate cells. Each cell has more than one nucleus in each compartment.

3.3 Nutrition and Metabolism

Most fungi are saprobes, securing their nutrients from dead organic matters. They release hydrolytic exo-enzymes that digest external substrates and absorb the soluble products.

They are also chemoorganoheterotrophs, i.e. they use organic compounds as a source of carbon, electrons and energy. Fungi are usually aerobic; however, some yeasts are facultatively anaerobic and can obtain their energy by fermentation. Obligately anaerobic fungi are found in the rumen of cattle.

3.4 Reproduction

Reproduction in fungi can either be asexual or sexual.

Asexual Reproduction

Asexual reproduction is a type of reproduction involving only one parent that produces genetically identical offspring by budding or by the division of a single cell or the entire organism into two or more parts. Asexual reproduction, also called somatic or vegetative reproduction is accomplished in several ways and does not involve the fusion/union of nuclei, sex cells or sex organs. It may be accomplished by:

- fission of somatic cells yielding two similar daughter cells
- budding each bud a small outgrowth of the parent cell develops into a new individual
- fragmentation or disjoining of the hyphal cells each fragment becoming a new organism spore formation.

There are several types of asexual spores each with a name.

- Sporangiospores:** These are single-celled spores formed within sacs called sporangia (singular: sporangium) at the end of special hyphae called sporangiospores).
- There are two types of sporangiospores:** Aplanospores which are non-motile and zoospores which are motile. Motility is due to the presence of flagella.
- Conidiospores or conidia (singular, conidium). These are formed at the tip or side of a hypha. Single celled conidia are called microconidia while large multicelled conidia are called macroconidia.
- Oidia (singular oidium) or arthrospores:** These are singlecelled spores formed by disjoining of hyphal cells.
- Chlamydospores:** These are thick walled single celled spores which are highly resistant to adverse conditions. They are found from cells of the vegetative hypha.
- Blastospores:** These are spores formed by budding.

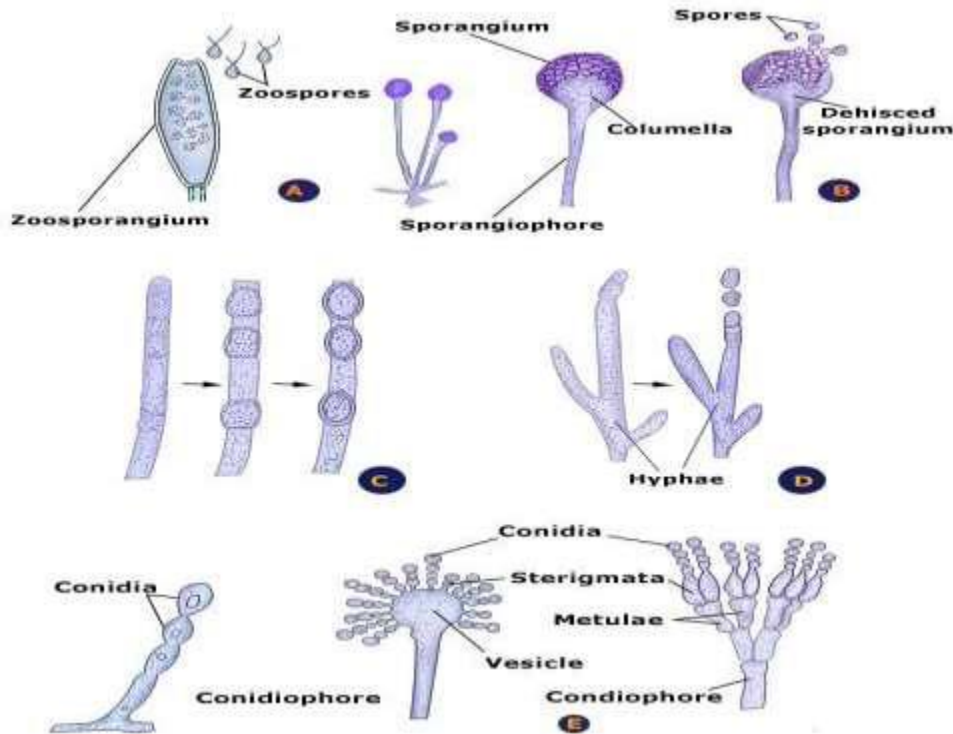


Fig. 2.3: Different types of Asexual Spores

Source: (<http://mb0804mycology.wordpress.com/2008/07/29/reproduction-of-fungi/>)

Sexual Reproduction

Sexual reproduction is a type of reproduction in which two parents give rise to offspring that have unique combinations of genes inherited from the gametes of the two parents.

It is carried out by fusion of the compatible nuclei of two parent cells. The process of sexual reproduction begins with the joining of two cells and fusion of their protoplast (plasmogamy) thus enabling the two haploid nuclei of two mating types to fuse together (karyogamy) to form a diploid nucleus. This is followed by meiosis, which again reduces the number of chromosomes to the haploid number.

The sex organs of fungi if present are called gametangia. They may form differentiated sex cells called gametes or may contain instead one or more gamete nuclei. If the male and female gametangia are morphologically different, the male gametangium is called the antheridium (plural antheridia) and the female gametangium is called the Oogonium (Oogonia).

Methods of sexual reproduction include:

- i Gametic copulation: This is the fusion of naked gametes, one or both of which are motile.
- ii Gamete-gametangial copulation: Two gametangia came into contact but do not fuse; the male nucleus migrate through a pore or fertilization to be into the female gametangium.
- iii Gametangial copulation: Two gametangia or their protoplast fuse and give rise to a zygote that develops into a resting spore.
- iv Somatic copulation: Fusion of somatic or vegetative cells.
- v Spermatization: Union of a special male structure called a spermatium (plural spermatia) with a female receptive structure.

The spermatum empties its content into the female during plasmogamy.

Sexual spores are produced by the fusion of two nuclei. Examples are:

- i **Ascospores:** These are single-celled spores produced in a sac called an ascus. There are usually eight ascospores in each ascus.
- ii **Basidiospore:** These are single celled spores borne on a club shaped structure called a basidium.
- iii **Zygosporos:** These are large thick walled spores formed when the tips of two sexually compatible hyphae or gametangia fuse together.
- iv **Oospores:** These are formed with a special female structure, the oogonium. Fertilization of the eggs or oospheres by the male gametes formed in an antheridium give rise to oospores.

3.5 Physiology

- Fungi are better able to withstand certain extreme environments than other microorganisms. They can tolerate more acidic conditions than other microbes. Some types of yeasts are facultative; they can grow under both aerobic and anaerobic conditions. Molds and many types of yeast are usually aerobic microorganisms.
- Fungi grow over a wide range of temperature. The optimum temperature for most saprobic species is 22 to 30°C, while pathogenic fungi have a higher temperature optimum of 30 to 37°C.
- Some fungi will grow at or near 0°C and thus can cause spoilage of meat and/or vegetables in cold storage.

3.6 Importance of Fungi

- About 90,000 fungal species have been described according to literature. However, some estimates suggest that 1.5 million species may exist. Fungi are important to humans in both beneficial and harmful ways.
- Beneficially, fungi act as decomposers. They degrade complex organic materials in the environment and release simple organic and inorganic molecules like carbon, nitrogen, phosphorus needed by other living organisms.
- Moulds and yeasts are used in many industrial processes involving fermentation to produce beer, wine and bread, cheese, soy-sauce, organic acids and many antibiotics.
- They are important research tools in the study of fundamental processes such as cytology, genetics, biochemistry and microbiology.
- On the other hand, fungi cause many diseases of plants, animals and humans. About 20 new human fungal pathogens are documented each year.

4.0 Conclusion

In this unit, we have discussed about general characteristics of fungi and their nature as being eukaryotic spore bearing organisms that lack chlorophyll and reproduce both asexually and sexually. Also discussed was its grouping into molds or yeasts based on the development of the thallus.

5.0 Summary

In this unit, you have learnt about the following:

- i. Distinguishing Characteristics of Fungi
- ii. Structure and Forms of Fungi
- iii. Nutrition and Metabolism
- iv. Reproduction
- v. Physiology
- vi. Importance of Fungi

6.0 Tutor Marked Assignments

6.1 Activity: Give five examples of infection caused by fungi

6.2 Answer the following questions:

- i. Describe the structure of a mold. LO4
- ii. Describe each of the following types of asexual fungal spores:
 - a. Sporangiospore
 - b. Conidiospore and
 - c. Blastospore. (LO6)
- iii. Describe the structure of yeast? (LO3)
- iv. Describe the structure of mold (LO4)
- v. Enumerate general characteristics of fungi (LO2)
- vi. Describe sexual reproduction as it occurs in fungi (LO6)
- vii. Describe the mode of nutrition in fungi (LO5)

7.0 References and other resources

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book.Inc.

Medigan, M.T. *et al.* (2009). *Brock Biology of Microorganisms*. (12th ed.). Pearson Education Inc.

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UNIT 3- GENERAL CHARACTERISTICS OF VIRUSES**CONTENTS**

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main Content
- 3.1** General Characteristics of Viruses
- 3.2** Virion Size
- 3.3** The Structure of Viruses
- 3.4** Viral Genomes
- 3.5** Virus Reproduction
- 3.6** The Cultivation of Viruses
- 3.7** Virus Purification and Assay
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
- 6.1** Activity
- 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 INTRODUCTION

Viruses are acellular entities. They are genetic elements that cannot replicate independently of a living cell called the host cell. Viruses have extracellular forms which enable them to exist outside the host for long periods. But to multiply, they have to enter a cell in which they can replicate causing infection. Viruses are the most numerous microorganisms on earth and infect all types of cellular organisms. The study of viruses is known as virology. This unit examines the general characteristics of viruses, their structures, genomes, symmetry, replication in hosts and purification.

2.0 Objectives

At the end of this unit, you should be able to:

- i. Define the term virus
- ii. State the general characteristics of virus
- iii. Describe the structure of a typical virus particle
- iv. Explain virus genome
- v. Explain the process of viral replication in susceptible host
- vi. State various methods of cutting viruses
- vii. State the various methods of virus purification.

3.0 Main Content

Definition

Viruses are simple acellular entities that can only reproduce within living cells.

3.1 General Characteristics of Viruses

- i. They are the smallest microorganisms. They range in size from 10 to 400 μm in diameter and can only be viewed under an electron microscope.
- ii. They are acellular, i.e. not cellular and non living.
- iii. They only reproduce when present within living cells.
- iv. They are infectious agents.
- v. A complex virus particle or virion consists of one or more molecules of DNA or RNA enclosed in a coat of protein.
- vi. Viruses can exist in two phases: extracellular and intracellular.
- vii. The extracellular phase known as virion possesses few if any enzymes and cannot reproduce independent of living cells. It is metabolically inert and does not carry out respiration.
- viii. In the intracellular phase, viruses exist primarily as replicating nucleic acids in the host cells that induce host metabolism to synthesise virion components which are later released.

Viruses differ from living cells in three ways:

- i. They have simple acellular organisation.
- ii. The presence of either DNA or RNA but not both in almost all virions.
- iii. They do not have the ability to reproduce independent of cells and carry out cell division as procaryotes and eukaryotes do.

3.2 Virion Size

Virions range in size from about 10 to 400 μm in diameter. The smallest viruses are a little larger than ribosomes whereas the pox viruses which include vaccinia are about the same size as the smallest bacteria and can be seen in the light microscope. Most viruses however, are too small to be visible in the light microscope and must be viewed with scanning and transmission electron microscope.

3.3 The Structure of Viruses

A virus is made up of a central genetic nucleic acid molecule surrounded by a protein coat called a capsid. The combination of both is called the nucleocapsid. The capsid surrounds and protects the viral nucleic acid.

The capsid also gives the virus a characteristic shape and help to establish the specificity of the virus for a particular host cells. Capsids are large macromolecular structures that self assemble from many copies of one or a few types of proteins. The protein used to build the capsids is called protomers. The simplest virus is a naked virus (nucleocapsid) consisting of a geometric capsid assembled around a nucleic acid. On the other hand, we can have a virus made up of a nucleocapsid surrounded by a flexible membrane called an envelope. This type of virus is called an envelope virus.

The various morphology types of viruses results from the combination of a particular type of capsid symmetry with the presence or absence of an envelope which is a lipid layer external to the nucleocapsid.

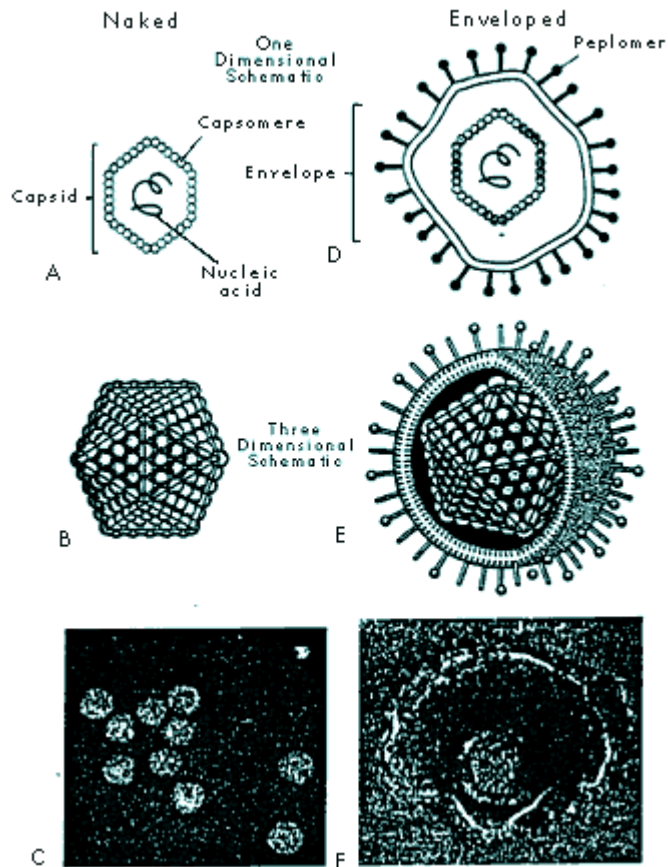


Fig. 3. 1: Generalised Structures of Viruses Source: triroc.com

3.4 Viral Genomes

All cells contain double stranded DNA genomes. By contrast, viruses have either DNA or RNA genomes (one group of viruses does use both DNA and RNA as their genetic material but at different stages of the replication cycle). Hence, we have RNA viruses or DNA viruses.

Virus genomes can be classified based on whether the nucleic acid in the virion is DNA or RNA and further subdivided to whether the nucleic acid is single or double stranded. Linear or circular, some viral genomes are circular but most are linear. We can have single stranded DNA, double stranded DNA, single stranded RNA and double stranded RNA. All four types are found in animal viruses. Most plant viruses have single stranded RNA genomes and most bacteria viruses contain double stranded RNA.

3.5 Virus Reproduction

Viruses need a host cell in which to reproduce; hence the first step in the life cycle of a virus is attached to a host. This is followed by entry of either the nucleocapsid or the viral nucleic acid into the host. If the nucleocapsid enters uncoating of the genome usually occurs before further steps can occur.

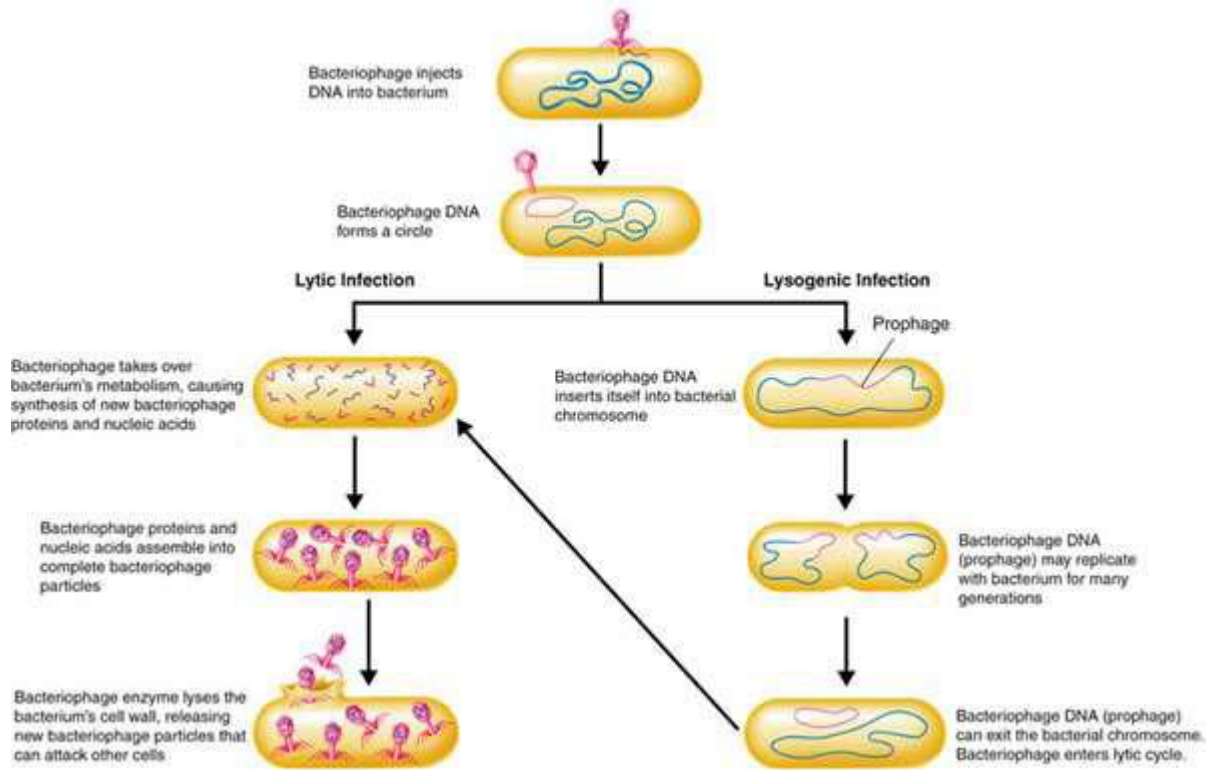


Fig. 3.2: Generalised Illustration of Virus Reproduction (Source: goldiesroom.org)

Once free in the cytoplasm, genes encoded by the viral genome are expressed, i.e. the viral genes are transcribed and translated. This allows the virus to control the host cell's biosynthetic machinery so that new virions can be made.

The viral genome is then replicated and viral proteins are synthesised. New virions are constructed by self assembly of coat proteins with the nucleic acids and finally, the matured virions are released from the host.

Summarily, the steps involved in viral replication or reproduction are:

- attachment of the virion to a susceptible host
- penetration or entry of the virion or its nucleic acid into the host
- synthesis of virus nucleic acid and protein by cell metabolism as directed by the virus
- assembly of capsids and packaging of viral genomes into new virions
- release of mature virions from the cell.

However, there is great variation in the details of virus reproduction for individual virus species.

3.6 The Cultivation of Viruses

Because viruses are unable to reproduce independent of living cells, they cannot be cultured in the same way as prokaryotic and eukaryotic microorganisms. Animal viruses are cultivated by inoculating suitable host animals or embryonated egg – fertilised chicken eggs incubated about 6 to 8 days after laying. More recently, animal viruses have been grown in tissue (cell) culture on monolayers of animal cells.

3.7 Virus Purification and Assay

Viral purification and Assays are necessary so as to accurately study virus structure, reproduction and other aspects of their biology.

Virus Purification

This involves getting or isolating the viral particle in its pure state, purification makes use of several virus properties. Four of the most widely used methods to isolate and purify viruses are:

- differential and density gradient centrifugation. This is often used in the initial purification steps to separate virus particles from host cells.
- precipitation of viruses particles.
- denaturation of contaminants.
- enzymatic digestion of host cells constituents.

Virus Assays

The quantity of viruses in a sample can be determined either directly by counting particle numbers using the electron microscope or indirectly by measurement of an observable effect of the virus using techniques such as the hemagglutination assay.

4.0 Conclusion

In this unit, we have discussed the general characteristics of viruses and established the fact that Viruses are simple acellular entities that can only reproduce within living cells. A virus is made up of a central genetic nucleic acid molecule which could be DNA or RNA surrounded by a protein called capsid.

5.0 Summary

In this unit, you have learnt about the following:

- i. General Characteristics of Viruses
- ii. Virion Size
- iii. The Structure of Viruses
- iv. Viral Genomes
- v. Virus Reproduction
- vi. The Cultivation of Viruses
- vii. Virus Purification and Assay

6.0 Tutor Marked Assignments

6.1 Activity: Discuss three diseases common in your hospital that are caused by virus

6.2 Answer the following questions:

- i. mention three ways viruses differ from living cells (LO2)
- ii. enumerate the characteristics of virus (LO2)
- iii. write briefly on virus genome (LO4)
- iv. describe the structure of a typical virus particle (LO3)
- v. explain how viruses are cultivated in different hosts (LO6)

- vi. explain four major approaches by which viruses may be purified (LO7).
- vii. define the following terms:
 - a. virus (LO1)
 - b. nucleocapsid (LO2)
- viii. Explain the processes involved in viral replication or virus reproduction (LO5)

7.0 References and other resources

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book.Inc.

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<http://www.goldiesroom.org>

<http://www.triroc.com>

Module 3- Bacterial Nutrient, Growth and Control

Introduction

Infection control is a core duty of all hospital workers that are directly involved in the care of patients. Nurses are faced with infection and infection control challenges in their daily practice. This module will deal with bacterial nutrition, growth and control.

Module Objectives

At the end of this module, you should be able to discuss Bacterial Nutrient, its Growth and how it can be Controlled.

CONTENTS

Unit 1: Bacterial Nutrition and Growth

Unit 2: Classification and Mode of Action of Antimicrobial Agents

Unit 3: Sterilization and Disinfection

UNIT 1- Bacterial Nutrition and Growth

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main Content
- 3.1** Common Nutrients Requirement
- 3.2** Nutritional Types of Microorganisms
- 3.3** Growth factors
- 3.4** Nutrient Uptake
- 3.5** Culture Media
- 3.6** Bacterial Growth
- 3.7** Environmental Factors Influencing Growth
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
- 6.1** Activity
- 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 Introduction

Microbial cells are structurally complex and carry out numerous functions. Nutrients are required as materials that are used in biosynthesis and to make energy available. The growth of microorganisms depends upon an adequate supply of nutrient, pH, oxygen and temperature. They require the elements present in their chemical composition. Nutrients must provide these elements in a metabolically accessible form.

2.0 Objectives

At the end of this unit, you should be able to:

- i. Itemize nutrient requirements of microorganisms
- ii. Describe the nutritional types of microorganisms
- iii. State the requirements for carbon, hydrogen, oxygen and electrons
- iv. Identify microbial growth factors
- v. Describe nutrient uptake mechanisms in bacteria
- vi. Describe culture media
- vii. Explain the different phases of bacterial growth
- viii. Explain factors that influences bacterial growth

3.0 Main Content

3.1 Common Nutrient Requirements

The nutrients may be in form of:

- i. Macronutrients or macroelements
- ii. Micronutrients or trace elements

Macronutrients

These include Calcium, Oxygen, Hydrogen, Nitrogen, Sulphur, Phosphorus, Potassium, Calcium, Magnesium and Iron (C, O, H, N, S, P, K, Ca, Mg, and Fe).

They constitute over 95% of cell dry weight and are needed in relatively large quantities.

C, O, H, N, S, and P are components of carbohydrates, lipids, proteins, and nucleic acids while the remaining four elements (K, Ca, Mg, F) exist in the cell as cations and play a variety of roles.

Micronutrients

These include Manganese, Zinc, Cobalt, Molybdenum, Nickel and Copper (Mn, Zn, Co, Mo, Ni, and Cu). They are used in very small amounts. In nature, they are ubiquitous and probably do not usually limit growth.

Requirements for Carbon, Hydrogen, Oxygen and Electrons

- All organisms require a source of carbon, hydrogen, oxygen, and electrons.
- Carbon is needed for the skeleton of all the organic molecules from which organisms are built.
- Hydrogen and oxygen are also important elements in organic molecules.
- The movement of electrons through the electron transport chain and during oxidation-reduction reactions provide energy for cellular work.

3.2 Nutritional Types of Microorganisms

Microorganisms can be grouped into three sources based on their nutritional needs for growth.

i. Carbon sources:

- **Autotrophs:** use CO₂ as their primary source of carbon; they must obtain hydrogen and electrons from other sources.

- **Heterotrophs:** use organic molecules as their source of carbon. These molecules often supply hydrogen, oxygen, and electrons as well. Some heterotrophs also derive their energy from their organic carbon source.
- ii. **Energy sources:**
 - **Phototrophs:** use light energy.
 - **Chemotrophs:** obtain energy from oxidation of chemical compounds.
- iii. **Electron sources:**
 - Lithotrophs: Electrons are extracted from reduced inorganic substances
 - Organotrophs: Electrons are extracted from reduced organic compounds

3.3 Growth Factors

These are organic factors that are essential cell components or precursors of such components but cannot be synthesised by the organism.

The **three major classes** are:

- i. **Amino acids**
- ii. **Purines and Pyrimidines**
- iii. **Vitamins**

Vitamins are small organic molecules that usually are components of enzyme cofactors (riboflavin, folic acid etc).

Practical applications: microbes needing a growth factor can be used in bioassays that detect and quantify the growth factor; those that do not need a growth factor can sometimes be used to produce the growth factor in industrial settings.

3.4 Nutrient Uptake

Microorganisms make use of several different transport mechanisms:

- i. Facilitated diffusion
- ii. Active transport
- iii. Group translocation

Although some nutrients can enter cells by **passive diffusion**, a membrane carrier protein is usually required.

Facilitated diffusion - the transport protein simply carries a molecule across the membrane in the direction of decreasing concentration, and no metabolic energy is required.

Active transport systems use metabolic energy and membrane carrier proteins to concentrate substances actively by transporting them across a gradient. ATP is used as energy source by ABC transporters. Gradients of protons and potassium ions also drive solute uptake across membranes.

Bacteria also transport organic molecules while modifying them, a process known as **group translocation** e.g. many sugars are transported and phosphorylated simultaneously.

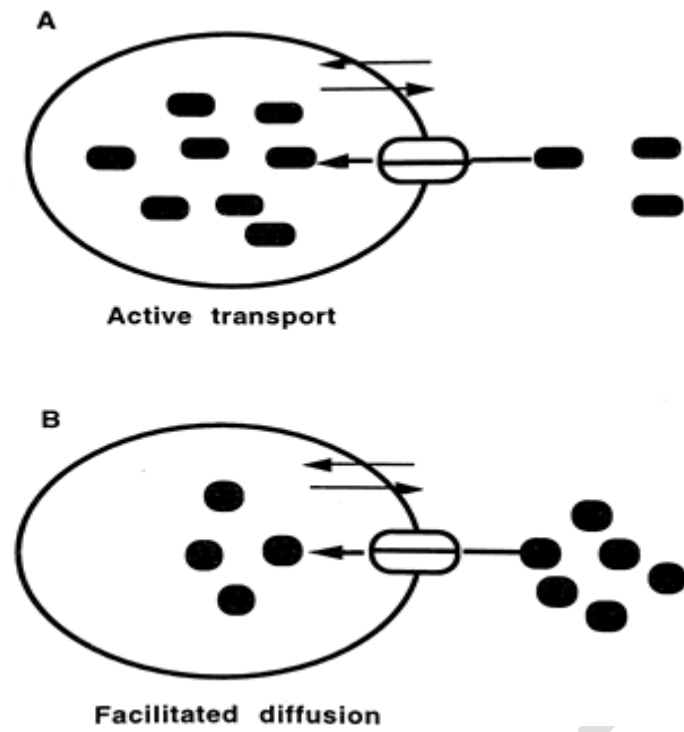


Fig. 1.1: a. Active transport b. Facillitated diffusion (source: Flylib.com)

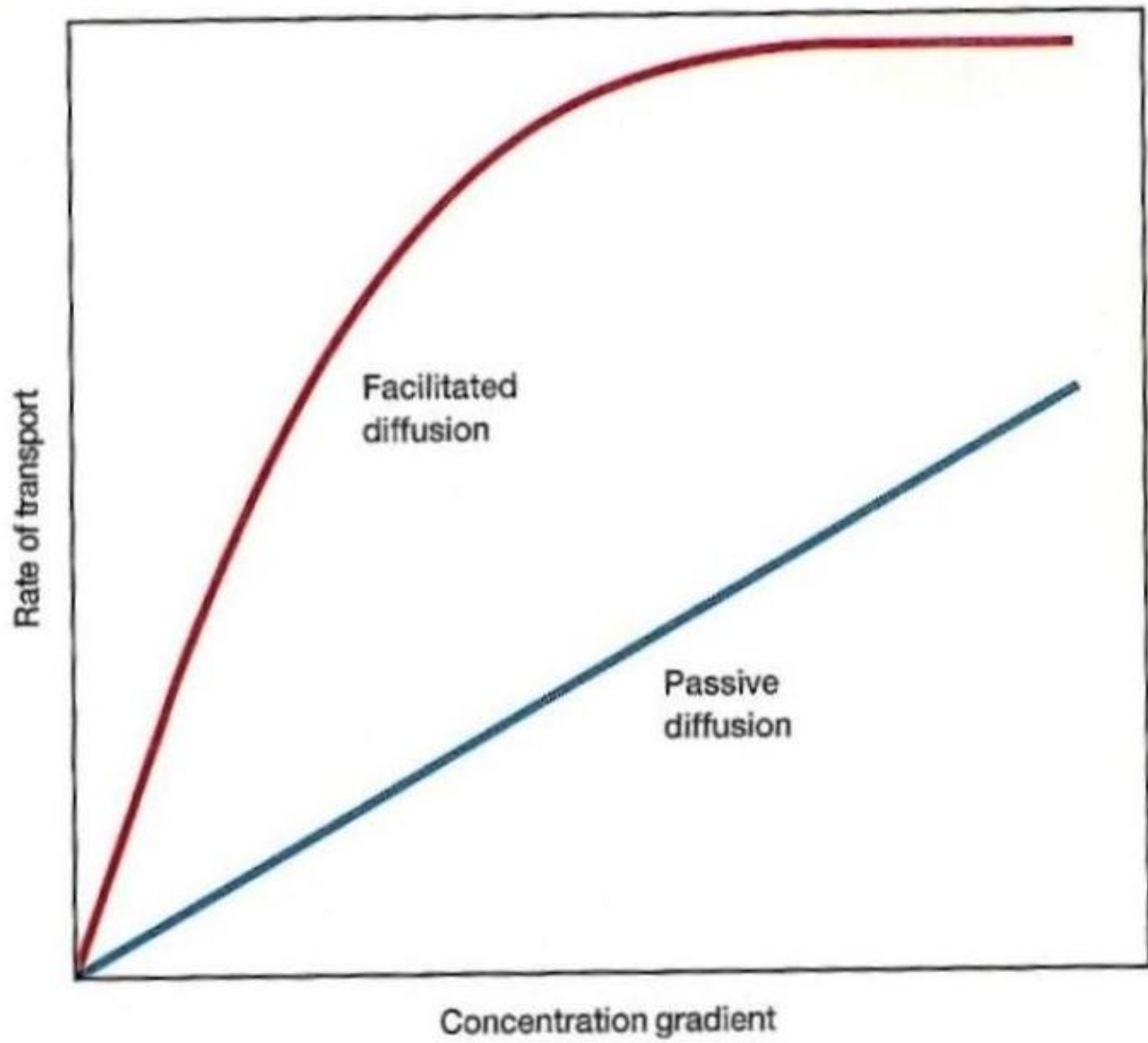


Fig 1.2: Movement of nutrients across the cell membrane (source: learning.uonbi.ac.ke)

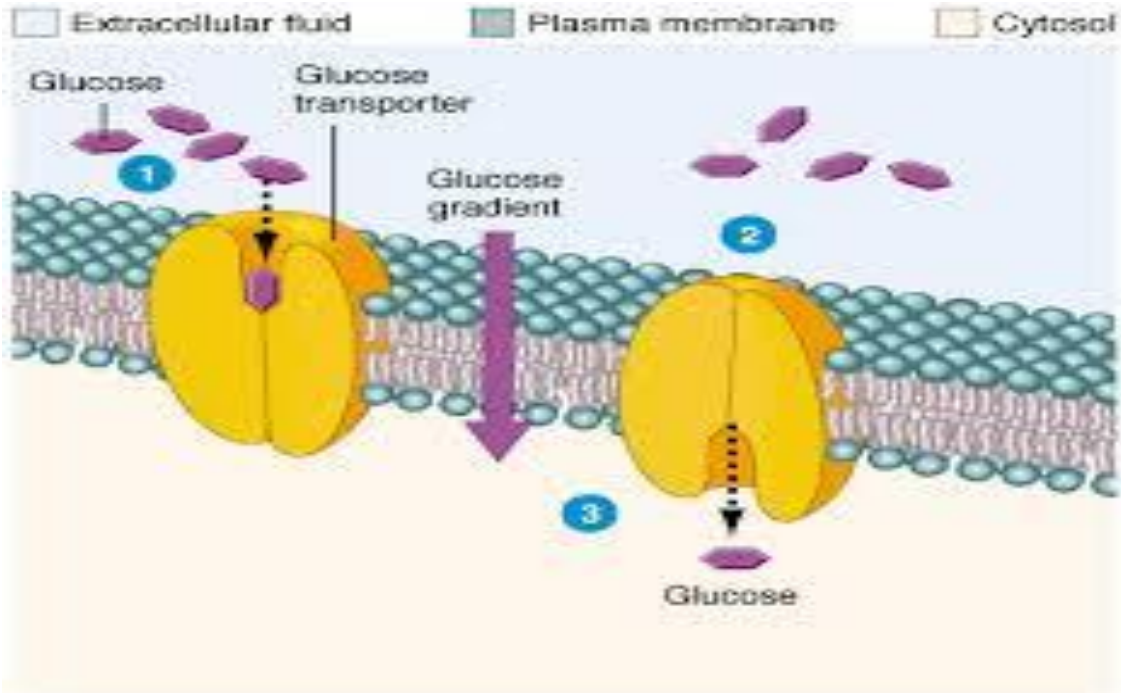


Fig 1.3: Facilitated diffusion: Carrier-mediated uptake of glucose into the cell (Source: classroom.sdmesa.edu)

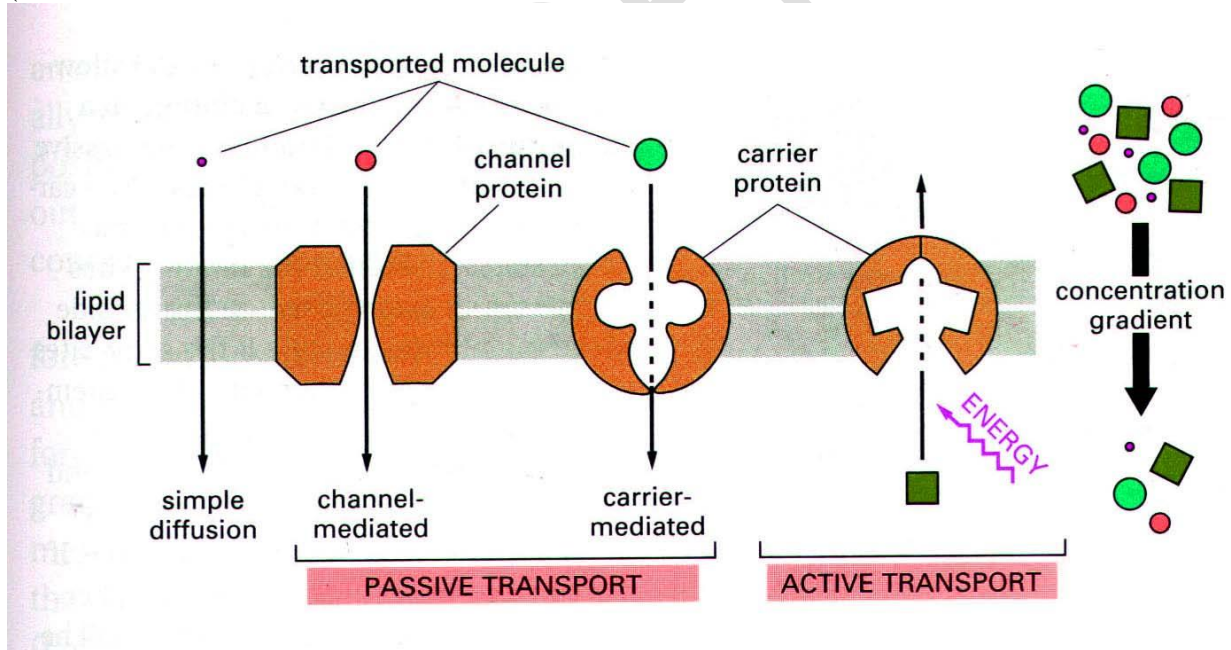


Fig 1.4: Transportation of nutrient across the cell membrane (Source: 12knights.pbworks.com)

3.5 Culture Media

Culture media are solid or liquid preparation used to grow, transport, and store organisms. An effective medium must contain all the nutrients the microorganism requires for growth.

They are classified on the basis of several parameters:

- i. Chemical constituents from which they are made

- ii. Physical nature
- iii. Function

Types of Media

Physical Nature	Chemical Composition	Functional Type
Liquid	Defined (synthetic)	Supportive (general purpose)
Semisolid	Complex	Enriched
Solid		Selective
		Differential

Culture media can be constructed completely from chemically defined components (defined or synthetic media) or constituents like peptones and yeast extract whose precise composition is unknown (complex media).

Culture media can be solidified by the addition of agar, a complex polysaccharide from red algae.

Classification

Enriched media are supportive media that contain additional nutrients needed by fastidious microbes.

Selective media contain components that select for the growth of some microbes.

Differential media contain certain components that allow microbes to be differentiated from each other, usually based on some metabolic capability.

TABLE 6.5		Culture Media
Type	Purpose	
Chemically defined	Growth of chemoautotrophs and photoautotrophs, and microbiological assays.	
Complex	Growth of most chemoheterotrophic organisms.	
Reducing	Growth of obligate anaerobes.	
Selective	Suppression of unwanted microbes; encouraging desired microbes.	
Differential	Differentiation of colonies of desired microbes from others.	
Enrichment	Similar to selective media but designed to increase numbers of desired microbes to detectable levels.	

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Cultivation of microorganisms

This involves:

Isolation

Identification

Preservation

3.6 Bacterial Growth

Growth is an orderly increase of all the components of an organism and not merely of some of its constituents.

Growth occurs in various nutrient-containing preparations - culture media.

The population of cells is referred to as a culture.

Growth occurs first by increasing the amount of cellular organelles and then later through binary fission, in which a parent cell divides to form a progeny of two cells.

The time required for a single cell or population of cells to double is called the generation or doubling time.

A population of bacterial cells goes through a number of phases from the time it is introduced into the medium until it ceases growth typified by the growth curve:

A “typical” Bacterial Growth Curve

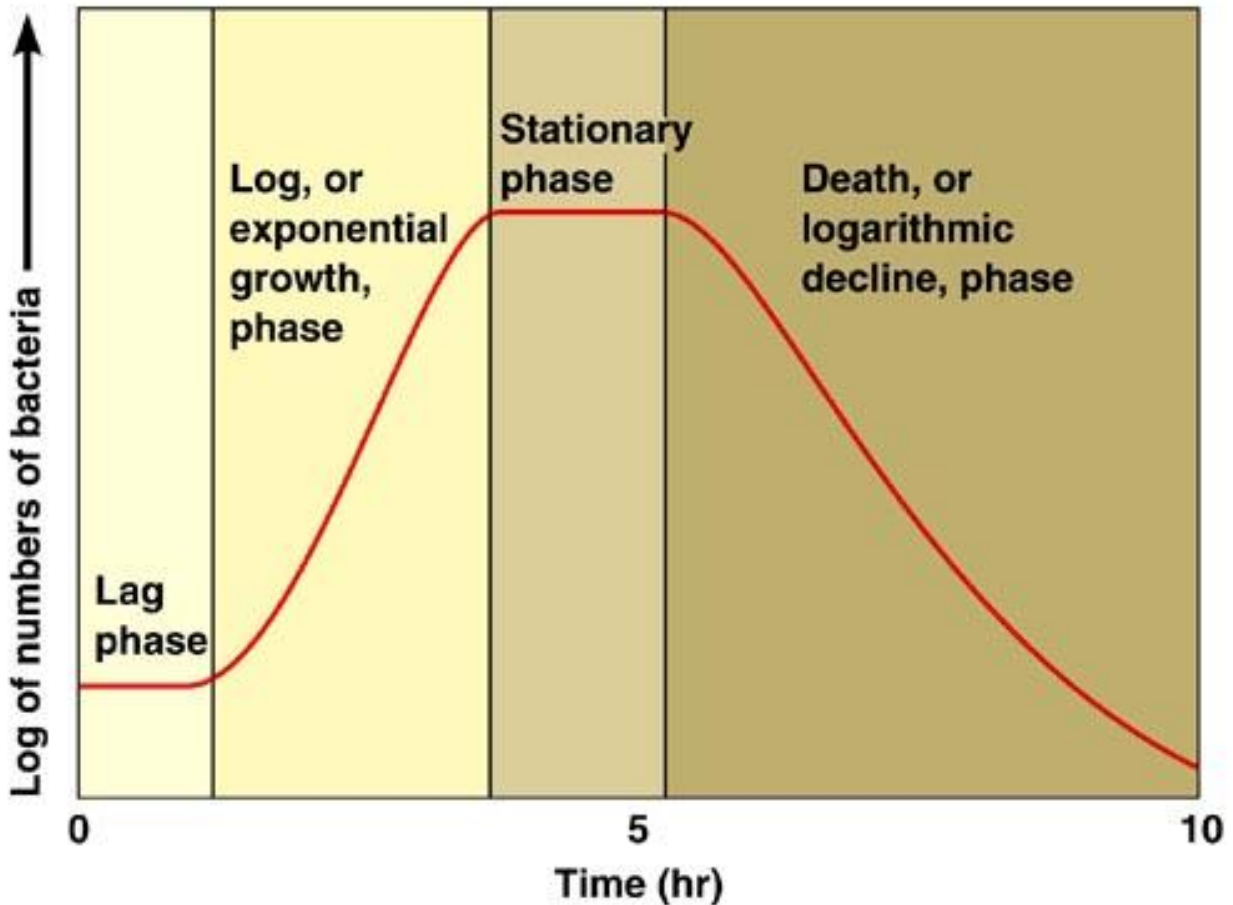


Fig 1.6:

Lag phase -During this time, the organism adapts itself to the new environment with cell numbers remaining constant. There is considerable increase in RNA and total protein content of each cell but the DNA content remains approximately the same. The length of this phase usually depends on the physiological condition and size of the inoculum used.

Logarithmic or exponential phase -In this phase the organisms are growing at the maximum rate achievable in the medium employed, the cells dividing at minimum generation period with cell concentration increasing exponentially. The cells in this phase are at the peak of their

metabolic activity hence they are most frequently used in experimental studies. The duration of this phase depends on whether or not the organisms in question are fast or slow growing.

Stationary phase-After a phase of active growth, there follows a phase when once again there is little or no increase in the number of organisms so that the number of organisms remains constant. One explanation for this is probably that there is exhaustion of essential nutrients and energy sources resulting from the activities of the exponential growth phase. Other reasons might be the accumulation of toxic metabolic wastes in the culture medium.

Death or decline phase -This is essentially the reverse of the exponential growth phase with the cells dying in a geometric progression fashion. The total cell counts may remain constant initially but the total number of viable cells continues to decline. This pattern is ascribable to the increase in toxic metabolites within the medium as well as the release of lytic enzymes by the dying cells.

The type of growth described above is known as batch culture. However, it is possible to use an open system in which there is a continuous supply of fresh nutrients into the culture medium and a continuous removal of grown bacteria by means of a constant level device. This type of continuous culture system is achieved by a **chemostat** or a **turbidostat**.

3.7 Environmental Factors Influencing Growth

These factors include:

- i. pH: (**Negative logarithm of Hydrogen ion concentration**) - most bacteria grow best between pH 6 and 8. Some however are sensitive to acid but tolerant of alkali e.g. *Vibrio cholerae*
- ii. **Temperature**- each bacterium multiplies best within a restricted temperature range. Psychrophiles - grow below 20⁰C, usually quite well below 0⁰C. For example, soil and water bacteria. They cause spoilage of refrigerated and frozen food. Mesophiles – most cause disease in humans. T°: 30°-37°C. Thermophiles – are incapable of growth at the normal body temperature. They are not involved in infectious disease of humans. T° is 45 – 70°C. They are cause of spoilage in under-processed canned foods, since many form spores of exceptionally high heat-resistance.
- iii. **Osmotic pressure**- as a result of the presence of semi-permeable cytoplasmic membrane, bacterial resembles other cells in being subject to osmotic pressure. Sudden exposure of bacteria to solution of high salt concentration causes loss of water from the cells, and shrinkage of protoplast (plasmolysis). Plasmolysis prevents growth.
- iv. Oxidation-reduction (Redox) potential
- v. Carbon dioxide
- vi. Moisture and dessication
- vii. Light and other radiations

Gaseous nutrients- it is necessary to provide oxygen for a strict aerobe and to remove it completely from the environment of strict anaerobe.

Descriptive Term	Definition	Representative Microorganisms
pH		
Acidophile	Growth optimum between pH 0 and 5.5	
Neutrophile	Growth optimum between pH 5.5 and 8.0	<i>Escherichia</i> ,
Alkalophile	Growth optimum between pH 8.0 and 11.5	
Temperature		
Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	<i>Bacillus psychrophilus</i>
Psychrotroph	Can grow at 0-7°C; has an optimum between 20 and 30°C and a maximum around 35°C	<i>Listeria monocytogenes</i> , <i>Pseudomonas fluorescens</i>
Mesophile	Has growth optimum around 20-45°C	<i>Escherichia coli</i> , <i>Neisseria gonorrhoeae</i> , <i>Trichomonas vaginalis</i>
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	<i>Geobacillus stearothermophilus</i> , <i>Thermus aquaticus</i>
Hyperthermophile	Has an optimum between 80 and about 113°C	
Oxygen Concentration		
Obligate aerobe	Completely dependent on atmospheric O ₂ for growth	<i>Micrococcus luteus</i> , <i>Pseudomonas</i> , <i>Mycobacterium</i> ; Most protists and fungi
Facultative anaerobe	Does not require O ₂ for growth, but grows better in its presence	<i>Escherichia</i> , <i>Enterococcus</i>
Aerotolerant anaerobe	Grows equally well in presence or absence of O ₂	<i>Streptococcus pyogenes</i>
Obligate anaerobe	Does not tolerate O ₂ and dies in its presence	<i>Clostridium</i> , <i>Bacteroides</i>
Microaerophile	Requires O ₂ levels below 2-10% for growth and is damaged by atmospheric O ₂ levels (20%)	<i>Campylobacter</i> , <i>Treponema pallidum</i>

4.0 Conclusion

In this unit, we have learnt about how bacteria grow and their nutrition. Also discussed were factors such as carbondioxide, light, moisture and dedication and how they affect bacteria growth.

5.0 Summary

In this unit, you have learnt about the following:

- i. Common Nutrients Requirement
- ii. Nutritional Types of Microorganisms
- iii. Growth factors
- iv. Nutrient Uptake
- v. Culture Media
- vi. Bacterial Growth
- vii. Environmental Factors Influencing Growth

6.0 Tutor Marked Assignments

6.1 Activity: Discuss how environmental factors like carbondioxide, moisture and desiccation, light and other radiations affect the growth of bacterial.

6.2 Answer the following questions:

- i. what are the nutrients requirements of microorganisms (LO1)
- ii. explain the nutritional types of microorganisms (LO2)
- iii. outline the requirements for Carbon, Hydrogen, Oxygen and Electrons (LO3)
- iv. what are microbial growth factors (Lo4)
- v. explain the following terms:
 - a. Facilitated diffusion
 - b. Active transport
 - c. Group translocation (LO5)
- vi. classify culture media and mentioning their uses (LO6)
- vii. Describe a “typical” Bacterial Growth Curve (LO7)
- viii. The period between inoculation of bacteria in a culture medium and beginning of multiplication is known as:
 - (a) lag phase
 - (b) log phase
 - (c) stationary phase
 - (d) decline phase (LO7)
- ix. When a substance is added to a solid medium which inhibits the growth of unwanted bacteria but permits the growth of wanted bacteria, it is known as:
 - (a) selective medium
 - (b) enrichment medium
 - (c) enriched medium
 - (d) differential medium (LO6)

7.0 References and other resources

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UNIT 2- CLASSIFICATION AND MODE OF ACTION OF ANTIMICROBIAL

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
- 3.1 Classification of antimicrobials (majorly antibacterial);
- 3.2. Bacteriostatic
- 3.3 Bacteriocidal
- 3.4 Sites of action
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

A chemotherapeutic drug is a chemical compound that is used in the treatment of disease. The compound may come from natural sources or may have been synthesized by a chemist in the laboratory. An antibiotic is an antimicrobial agent that is derived from a microorganism while antimicrobial agent is a drug that acts primarily against infectious organisms.

Sir Flemming discovered Penicillin in 1928. Antibiotics were originally natural, produced by other organisms, but most are now semi-synthetic –modified from the original compounds (eg beta-lactams), a few are completely synthetic (eg quinolones, oxazolidinones, sulfanamides) while drugs like the aminoglycosides are still produced from living organisms .

2.0 Learning Objectives

At the end of this unit, you should be able to:

- i. Explain the classification of antimicrobials
- ii. Classify antibiotics according to site of action.
- iii. Explain mode of action of each group with specific examples.

3.0 Main Content

3.1 Classification of antimicrobials (majorly antibacterial);

- i. Bacteriostatic or Bacteriocidal

- ii. Site of Action
- iii. Chemical structure
- iv. Range of Activity

3.2 Bacteriostatic

Inhibit growth of the microorganism at normal concentrations. Duration of therapy must be sufficient to allow cellular and humoral defense mechanisms to eradicate the bacteria. Final elimination is dependent on host immune system. Examples are: Tetracyclines, Erythromycin, Sulphonamides, Chloramphenicol.

3.3 Bacteriocidal

Kill the microorganism. Bacteriocidal antibiotics should be used to treat infections of the endocardium or the meninges. Host defenses are relatively ineffective in these sites. Dangers imposed by such infections require prompt eradication of the organisms. E.g., Aminoglycosides, Fluoroquinolones, Penicillins, Cephalosporins.

3.4 Sites of action

There are five major modes of action:

- interference with cell wall synthesis,
- inhibition of protein synthesis,
- interference with nucleic acid synthesis, and
- Inhibition of a metabolic pathway.
- Disruption of bacterial membrane structure.

Interference With Cell Wall Synthesis-

- β -lactam agents inhibit synthesis of the bacterial cell wall by interfering with the enzymes (PBPs) required for the synthesis of the peptidoglycan layer. Eg: penicillins, cephalosporins, carbapenems, monobactams.
- Glycopeptide also interfere with cell wall synthesis, but by binding to the terminal D-alanine residues of the nascent peptidoglycan chain, thereby preventing the cross-linking steps required for stable cell wall synthesis. Eg vancomycin, teicoplanin.

β -lactams

The 1st antibiotic discovered was a β -lactam, i.e., penicillin in 1928 by Alexander Flemming. The work of Florey, Chain and associates in 1941 made possible the commercial production of penicillin G. All β -lactam antibiotics have a β -lactam nucleus in their molecular structure. Penicillins and derivatives, cephalosporins, carbapenems, monobactams and β -lactam inhibitors. The basic structure consists of a thiazolidine ring – the β -lactam ring – and a side chain. The β -lactam ring is essential for antibacterial activity. The side chain determines in large part the antibacterial spectrum and pharmacologic properties.

Examples of β -lactams

- a. Penicillins- penicillin G, penicillin V
- Penicillinase – resistant penicillin
 - Methicillin, nafcillin

- Isoxazolyl Penicillins - cloxacillin, Flucloxacillin, Oxacillin
- Aminopenicillins-
 - Ampicillin, Amoxicillin, Bacampicillin,
- Antipseudomonal (ureidopenicillins)-
 - Azlocillin, Carbenicillin, ticarcillin, Mezlocillin, Piperacillin
- b. Carboxypenicillins-
 - Carbenicillin, ticarcillin

c. **Cephalosporins**- discovered as naturally occurring substances from the mould *Cephalosporium* Cephalosporin C, obtained from the cultures of *Cephalosporium acremonium* and is the foundation on which current cephalosporin antimicrobials are constructed.

The β -lactam ring is fused to a six-membered dihydrothiazine ring (yielding the cephem nucleus). Contrast to penicillins in which the comparable unit is a five-membered thiazolidine ring.

Generations of cephalosporins- Based on spectrum of activity and timing of the agent's introduction.

- 1st generation: Relatively narrow spectrum of activity focused primarily on the gram-positive cocci. Eg - cephalothin , cephradine
- 2nd generation: Variable activity against gram-positive cocci but have increased activity against gram-negative bacteria. Eg - cefuroxime, cefoxitin
- 3rd generation: Very marked activity against the gram-negative bacteria; some of them have limited activity against gram-positive cocci, particularly MSSA. Eg - Cefotaxime (claforan), Ceftriaxone (rocephin), Ceftazidime (fortum), Cefoperazone.
- 4th generation. Good true broad-spectrum activity against both Gram-negatives and Gram-positives. Eg Cefepime
- 5th generation - MRSA-active cephalosporins and currently includes ceftaroline and ceftobiprole.

Cephamycins- Closely related to cephalosporins. They contain oxygen in place of sulfur in the dihydrothiazine ring, rendering them more stable to beta-lactamase hydrolysis. The cephamycins are noted for their additional activity against gram-negative anaerobic bacteria, such as *Bacteroides* spp. They are grouped together as Second generation cephalosporins. Eg cefoxitin, cefotetan, cefmetazole.

Other β -lactams;

- **Monobactams** : narrow-spectrum antibiotics .Active only against aerobic, gram-negative bacteria.eg Aztreonam
- **Carbapenems**: They are derivatives of thienamycin, a compound produced by *streptomyces cattleya*. They diffuse easily in bacteria and are considered as broad-spectrum antibiotics active against virtually all groups of organisms with few exceptions (such as *Stenotrophomonas maltophilia*). Eg Meropenem , Imipenem and Ertapenem

β -lactamase Inhibitors (suicide inhibitors)- Do not contain the β -lactam ring

- Clavulanate, Sulbactam.Tazobactam . They can be combined with other β -lactams eg amoxicillin to enhance antimicrobial spectrum. They do not affect the pharmacokinetics nor does it increase side -effects. It Increases resistance to β - lactamases. Possess

negligible antimicrobial activity. May be reversible or irreversible. All used in clinical practice are irreversible.

- Amoxicillin-clavulanate (Augmentin)
- Ampicillin-sulbactam (Unasyn)
- Ticarcillin-clavulanate (Timentin)
- Piperacillin-tazobactam (Zosyn)

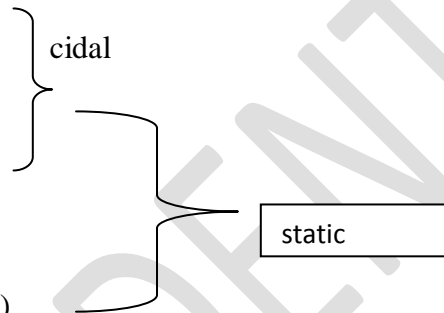
Other Inhibitors of cell wall synthesis asides β – lactams.

- **Glycopeptides** - Vancomycin, Teicoplanin, Ramoplanin, Decaplanin
- Bacitracin
- Cycloserine
 - Fosfomycin

Inhibition of Protein Synthesis.

Antibiotic Classes

- Aminoglycosides
- Streptogramin
- Glycylcyclines
- Tetracyclines
- Chloramphenicol
- Macrolides
- Lincosamides
- Fusidic acid
- Oxazolidones (Linezolid)



Antimicrobials that Attack the 30s Ribosomal Subunit Blocking Protein Synthesis

Aminoglycosides - The antibiotics inhibit bacterial protein synthesis by irreversibly binding to 30S ribosomal proteins. Originally they were isolated from *Streptomyces* species. Gentamicin was isolated from *Micromonospora* species. Amikacin is a synthetic derivative of kanamycin. Broad spectrum. Act in synergy with other agents. Streptomycin, neomycin, kanamycin, tobramycin.

Tetracyclines -Tetracycline, Oxytetracycline, Doxycycline and Minocycline. Oral absorption – poor with food, milk, orange juice, antacids iron containing tonics. Mode of action is by reversible binding to the 30S ribosome and inhibition of binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome.

Spectrum of activity – Broad spectrum; Useful against intracellular bacteria. Resistance is Common with Adverse effects including Destruction of normal intestinal flora resulting in increased secondary infections and staining of the structure of bone and teeth.

Glycylcycline- Tigecycline. which is a Synthetic analogue of Tetracycline .Broad spectrum . Useful against strains resistant to tetracyclines and other antibiotics. Clinical use: skin and soft tissue, intra-abdominal infections.

Spectinomycin- reversibly interferes with m-RNA interaction with the 30S ribosome. It is structurally similar to aminoglycosides but does not cause misreading of mRNA . Used in the treatment of penicillin-resistant *Neisseria gonorrhoeae*.

Oxazolidinones - Linezolid . Attach to 30s ribosomes. Affect translation by inhibiting the formation of N-formylmethionyl-tRNA . Activity mainly against Gram positive organisms. Antimicrobials that Attack the 50s Ribosomal SubUnit- Blocking Protein Synthesis.

Chloramphenicol, lincomycin, clindamycin - bind to the 50S ribosome and inhibit peptidyl transferase activity. Chloramphenicol is broad spectrum while Lincomycin and clindamycin are narrow spectrum. Resistance is however common

Chloramphenicol can be toxic (bone marrow suppression) but it is used in the treatment of bacterial meningitis. Lincomycin and clindamycin predispose to Pseudomembranous colitis.

Macrolides- Erythromycin, Azithromycin, roxythromycin, clarithromycin . Inhibit translocation in protein synthesis. Good coverage against Gram-positive bacteria, *Mycoplasma*, *Legionella* .

Streptogramins- (Bacteriocidal) Eg: Quinupristin/dalfopristin. Synergistic activity when used together causing irreversible binding to different sites of the 50S ribosome. Good against Gram positive organisms with little resistance developed.

Inhibitors of Nucleic Acid Synthesis

Inhibitors of DNA replication Quinolones (fluoroquinolones). A family of synthetic antimicrobial agents. The first quinolone, nalidixic acid was identified among by-products of chloroquine synthesis in 1962. NA has bactericidal activity against Gram-negatives.

2nd generation quinolones has a fluoride atom at position 6 of quinolone molecule with enhanced biological activity. Fluoroquinolones discovered in the 1980s e.g. ciprofloxacin, ofloxacin, perfloxacin, and norfloxacin.

3rd generation – (fluoro) quinolones e.g. levofloxacin with activity against both Gram-positives and Gram-negatives

The quinolones selectively interfere with bacterial DNA replication by inhibiting two enzymes involved in DNA synthesis: the type II topoisomerase known as DNA gyrase, and DNA topoisomerase IV. They generally have broad spectrum activity.

Inhibitors of RNA polymerase -Rifamycins . Bind A-dependent RNA polymerase and block initiation of synthesis of mRNA. Wide spectrum of action but is used most commonly in the treatment of tuberculosis. Since resistance is common, rifampin is usually used in combination therapy.

Nitroimidazoles -Interact with DNA leading to breaks in the DNA – Metronidazole, Tinidazole . Active against anaerobes and some protozoa.

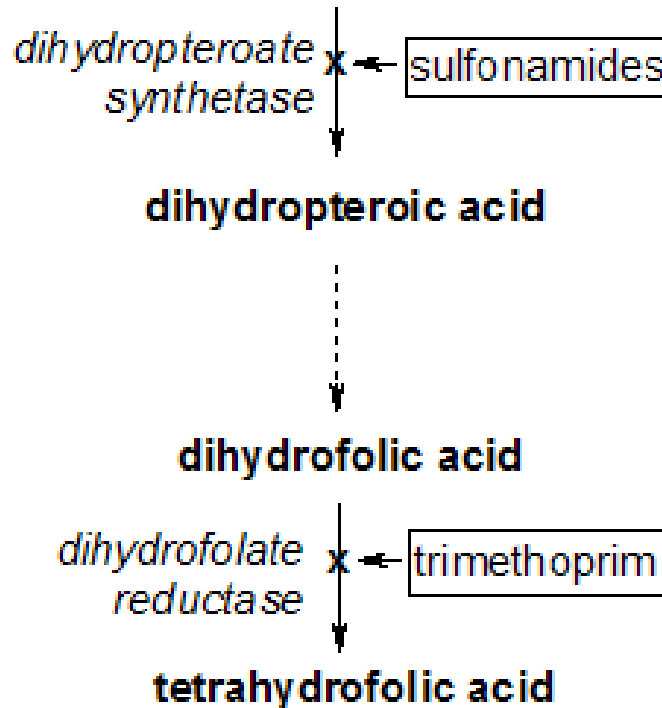
Inhibition of a Metabolic Pathway.

Inhibitors of Folic Acid Synthesis

The selectivity of these antimicrobials is a consequence of the fact that bacteria cannot use pre-formed folic acid and must synthesize their folic acid. In contrast, mammalian cells use folic acid obtained from food.

Trimethoprim- Available since 1962. The last truly new antibacterial agent introduced into clinical practice. All late developed agents are variations of older antibiotics. It is completely synthetic.

dihydropteroate diphosphate + p-aminobenzoic acid (PABA)



Trimethoprim, methotrexate, pyrimethamine (bacteriostatic) - bind to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid. Broad Spectrum and used primarily in urinary tract infections and in *Nocardia* infections. Resistance however is Common.

Sulfonamides, sulfones (bacteriostatic) - analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteroic acid.

Combination therapy – Trimethoprim used in combination with the sulfonamides. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains

Inhibition of Cell Membrane Function

Antibacterial - Polymyxins ;Very toxic (Nephrotoxic), used mainly topically. Gram negative organisms except Proteus. Polymyxin E– only one used parenterally (colistin). Polymyxin B-topical on skin binds to the lipid A portion of lipopolysaccharide and also to phospholipids. However, it binds preferentially to lipid A. This disrupts the outer membrane of Gram negative bacteria. Since the cell membrane is not exposed in Gram positive bacteria polymyxin has little activity against them. It is toxic to human cells, since it can also lyse eukaryotic membranes; hence has limited clinical use.

Antifungal drugs- Polyenes –Nystatin and Amphotericin B . Bind to fungal ergosterol. It cross reacts with human cholesterol.

Antibacterial – The cyclic lipopeptide, daptomycin inserts its lipid tail into the bacterial cell.

4.0 Conclusion

In this unit, we have learnt about different classes of antimicrobial and their mode of actions.

5.0 Summary

In this unit, we have learnt about the following:

- i. Classification of antimicrobials (majorly antibacterial);
- ii. Bacteriostatic
- iii. Bacteriocidal
- iv. Sites of action

6.0 Tutor Marked Assignments

6.1 Activity:

What are the groups of Antibiotics commonly used in your hospital and what inform the choice of these groups of drugs?

6.2 Answer the following questions:

- i. Describe the mode of action of penicillins (LO1)
- ii. List the generations of cephalosporin and two examples each (LO1)
- iii. Explain how antimicrobials inhibit protein synthesis (LO2)
- iv. List antimicrobials that affect the nucleic acid of organisms (LO2)
- v. Write short note on the following
 - a. Bacteriostatic
 - b. Bacteriocidal (LO1)
- vi. Describe antimicrobials that attack the 30S ribosomal subunit blocking protein synthesis (LO3)

7.0 References and other resources

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UNIT 3- STERILIZATION AND DISINFECTION

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content

- 3.1 Basis of Infection Control Practices
- 3.2 Principles of Infection Control
- 3.3 Definition of Terms
- 3.4 Rationale for Choice of Procedure
- 3.5 Sterilization
- 3.6 Disinfection
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

In healthcare settings, various surgical and medical procedures are usually performed. These procedures involve contact by medical devices or surgical instruments with patients' sterile tissues or mucous membrane. A major risk of all such procedures is the introduction of pathogens that can lead to infection.

Failure to properly disinfect or sterilize equipment carries not only risk associated with breach of host barriers but also risk for person-to-person transmission (e.g. of hepatitis B virus) and transmission of environmental pathogens (e.g. *Pseudomonas aeruginosa*).

2.0 Objectives

At the end of the lecture, you should be able to:

- i. State the basis of infection control practices.
- ii. List the principles of infection control
- iii. Differentiate between sterilization and disinfection
- iv. Itemise various methods available for sterilization and disinfection, including the newer Disinfectants
- v. Explain a disinfection policy

3.0 Main Content

3.1 Basis of Infection Control Practices

Involves use of practices and procedures that prevent or reduce the likelihood of infections being transmitted from a source (e.g., person, contaminated body fluids, equipment, and environment) to a susceptible individual.

3.2 Principles of Infection Control

These include:

1. Handwashing
2. Protective Clothing
3. Cleaning Disinfection and Sterilization
4. Management of Linen
5. Management of Waste
6. Management of Blood spillage

7. Management of Inoculation and Contamination incidents
8. Specimen handling and transportation

3.3 Definition of Terms

Cleaning- The physical removal of organic material or soil from objects. It involves use of water with or without detergents. It removes; not to kill microbes.

Sterilization- The total elimination of all forms of microbial life including spores.

Disinfection- The elimination of vegetative organisms without elimination of spores.

3.4 Rationale for Choice of Procedure

This entails categorizing medical devices, equipment and surgical materials on the basis of risk of causing infection, into:

- i. Critical items
- ii. Semi-critical items
- iii. Non-critical items

Critical items

These are instruments or objects that will be introduced directly into the blood stream or into other normally sterile areas. These include surgical instruments, implants, blood compartment of a haemodialyzer, cardiac catheters. **The minimum standard required is Sterilization.**

Semi-critical items

These items come in contact with intact mucosal surfaces but do not ordinarily penetrate body surfaces. They have intermediate risk of causing infection. These include non-invasive flexible and rigid endoscopes, endotracheal tubes, cystoscopes, anaesthesia breathing circuits.

Sterilization is preferred but is not absolutely essential. A **high-level disinfection procedure** that can be expected to destroy vegetative microbes, most fungal spores, tubercle bacilli and small non-lipid viruses can be **recommended.**

Non-critical items

These items do not ordinarily touch the patient or touch only intact skin and have a low risk of transmitting infection. These items include crutches, blood pressure cuffs, stethoscopes, tourniquets etc. **Cleaning with water and detergent may be adequate though a low-level disinfectant may be preferred in all cases.**

3.5 Sterilization

There are various methods available for sterilization. For example, use of:

1. Moist heat under pressure (autoclaving)
2. Dry heat
3. Ethylene oxide gas
4. Vapor phase Hydrogen peroxide
5. Ionizing radiation

Moist Heat under Pressure (Autoclaving):

- This involves autoclaving at 121°C for 15mins /flash sterilisation 270°C for 3mins (not for implants).
- It is very reliable and efficient.
- It can be used to sterilize dressings, instruments, glasswares.
- It is not suitable for powders, some plastics, anhydrous oils (i.e., heat & moisture sensitive)



Fig. 3.1 **An Autoclave**

Dry Heat

- Sterilization using dry heat at 160°C for 2hrs. Hot air oven is used.
- This method is used for heat stable materials, glassware, oils, powders.



Fig. 3.2 Flaming



Ethylene Oxide Gas

- This is effective at low temperature.
- It has good penetrating power, and compatible with most medical materials.
- However, it is expensive, toxic and inflammable.

Vapor phase Hydrogen Peroxide

- Sterilization occurs at low temp. It is safe, no toxic residuals; simple to operate, install and monitor.

- Sterilization takes place in small chamber. It requires synthetic packaging.
- Devices with long or narrow lumens can not be processed.

Ionising Radiation

This sterilization method is used for packaging materials, mainly industrial. For example, use of cobalt 60 gamma rays or electron accelerators.

Central Sterile Services Department (CSSD)

- Cleaning, Disinfection and Sterilization of patients' supplies should be performed in the CSSD.
- It should be divided into several areas separated by physical barriers.
- Temperature should be between 18°C – 22°C.
- Relative humidity should be between 35% -70%.
- Airflow should be directed from clean to relatively soiled areas.

Rules of sterilization

- All items should be thoroughly cleaned before Sterilization
- Presoaking in disinfectants is ineffective and should be discouraged
- Where possible precleaning should be automated
- All items should be double wrapped
- Wrapping should be compatible with the sterilization process, inexpensive, impervious to bacteria (140-thread-count muslin, kraft paper etc.), durable, flexible, free of pinholes

Monitoring the Sterilization Process

Physical monitoring: Monitoring temperature, pressure, time.

Chemical monitoring: Involving colour or physical change indicators that monitor exposure to sterilizing agents/conditions.

Biological monitoring: This is the most important monitoring method. It is done weekly.

Bacillus stearothermophilus is the biological indicator used for sterilizers and *Bacillus subtilis* var *niger* or var. *globigii* for Ethylene oxide sterilizers

3.6 Disinfection

Methods of Disinfection

Broadly divided into:

- Boiling** - Occurs at 100°C. It is not sporicidal.
- Chemical Disinfection**- Disinfectants are of different types. Their actions are dependent on many factors.

Classification of chemical disinfectants

Based on their microbicidal activity into:

- High level disinfectants
- Intermediate level disinfectants
- Low level disinfectants

Low level Disinfectants: They can kill only vegetative bacteria and enveloped viruses but not the tubercle bacilli, spores or small and non-lipid (non-enveloped) viruses; though they may kill fungi after prolonged contact.

Intermediate level disinfectants: They kill vegetative bacteria, tubercle bacilli fungi and enveloped viruses. They have no effect on spores and non-enveloped (non-lipid) viruses at normal contact times. They may exhibit limited virucidal (against non-enveloped) activity on prolonged contact. Examples include Chlorine compounds, Alcohols.

High level disinfectants: They kill everything except spores. At extended contact times they are capable of actual sterilization. Examples include 2% gluteraldehyde, Heat, Chlorine dioxide, Peracetic acid.

Factors affecting disinfectants

These include:

- i. Type (Chemical component)
- ii. Concentration
- iii. pH of the medium
- iv. Temperature
- v. Volume
- vi. Contact time
- vii. Length of storage
- viii. Nature and amount of contamination
- ix. Presence of inactivating substances
- x. Surface to be disinfected
- xi. Prior cleaning

IT IS IMPOTRANT TO ALWAYS USE DISINFECTANTS ACCORDING TO THE MANUFACTURER'S INSTRUCTION

Types of disinfectants

- i. **Aldehydes** – e.g., Gluteraldehyde/formaldehyde
- ii. **Halogens** – e.g., Hypochlorite/chlorine
- iii. **Alcohols** – e.g., Isopropyl/ethyl/methyl
- iv. **Chlorhexidine** – e.g., Hibiscrub (chl + 4% detergent), Hibisol (chl + alcohol + glycerine), Chl. Ointment
- v. **Iodophors/iodine** – e.g., Povidone iodine
- vi. **Phenolics** – e.g., Phenol, Chloroxynelol, Hexachlorophen. They are environmental & laboratory disinfectants
- vii. **Quanterary Ammonium Compounds-** e.g., Cetricimide

Newer disinfectants

Peracetic Acid – NuCidex

They are:

- Strong oxidising agent
- Rapid bactericidal agent

High level disinfectant

Peroxygen based compounds-Virkon

They are:

- Broad spectrum
- Good environmental disinfectant (may damage equipments)
- For semi-critical items

Superoxidised Water

They:

- Are environmentally friendly
- Have broad spectrum of action
- May be useful for semi-critical items

Rules for use of disinfectants

- i. Follow manufacturers' instructions
- ii. Check Expiry date of solution
- iii. Ensure optimum dilution
- iv. Always wash and clean articles before disinfection
- v. **DO NOT REFILL DISINFECTANT CONTAINERS BETWEEN EACH USE –**
 - a. **TOPPING UP IS NOT ALLOWED**
- vi. Disinfectants should be supplied ready for use from the pharmacy
- vii. Return empties to pharmacy – do not discard or use for other purposes
- viii. Do not use to sterilize instruments or equipment (unless specified in the disinfection policy)
- ix. Open containers are a serious NO! NO! in any hospital environment
- x. Where disinfectants are indicated for use on surfaces, **WIPE – DO NOT BATHE**

Disinfection policy

- i. List purposes for which disinfectants are used
- ii. Identify unnecessary or dangerous practices
- iii. Select effective disinfectants for remaining indications
- iv. Ensure optimum dilution
- v. Arrange distribution and collection of empties/expired
- vi. Ensure proper labeling
- vii. **MUST BE WRITTEN AND PROMINENTLY DISPLAYED**
- viii. **MUST BE REVIEWED EVERY TWO YEARS**

The role of the pharmacy

- i. Ensure cleanliness of containers
- ii. Ensure proper dilution
- iii. Ensure correct labeling
- iv. Ensure proper distribution and collection

Some myths about disinfection in operating theatres

- i. Transfer areas where patients are transferred from ward trolleys to clean OR trolleys
- ii. Routine culturing of OR personnel

- iii. Routine culturing of the environment
- iv. Ultraviolet rays for disinfecting theatres
- v. Disinfecting theatre floors
- vi. Patient antiseptic baths or showers before surgery

4.0 Conclusion

Discussed in this unit, were the what sterilization and disinfection are, why they need to be carried out and how they can be performed.

5.0 SUMMARY

In this unit, you have learnt about the following:

- i. Basis of Infection Control Practices
- ii. Principles of Infection Control
- iii. Definition of Terms
- iv. Rationale for Choice of Procedure
- v. Sterilization
- vi. Disinfection

6.0 Tutor Marked Assignments

6.1 Activity: List the disinfectants you usually used and write out their solutions.

6.2 Answer the following questions:

- i. What are the basis of infection control practices (LO1)?
- ii. List the principles of infection control (LO2)
- iii. Differentiate between sterilization and disinfection (LO3)
- iv. Enumerate the various methods for sterilization and disinfection (LO4)
- v. Outline the newer disinfectants (LO4)
- vi. Explain a disinfection policy (LO5)
- vii. The process of total elimination of all forms of microbial life, including spores is:
 - (a) sterilization
 - (b) disinfection
 - (c) antisepsis
 - (d) cleaning
- viii. Heating in a hot air oven at 160⁰C for one hour is used for sterilization of:
 - (a) glass syringes
 - (b) oils and jellies
 - (c) powders
 - (d) all of the above

7.0 References and other resources

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CONFIDENTIAL

MODULE 4- EVOLUTION OF PARASITIC ASSOCIATION

Introduction

Organisms are associated to each other for different reasons like nutrition, protection etc. this module will describe the association of organism, parasitic helminth and different types of host. The module has two units.

Module Objectives

At the end of this module you should be able to

- i. Describe the various association that exist between organisms
- ii. Describe human helminthes

Contents

Unit 1: Association in Organisms and Classification of Host Organisms

Unit 2: Human helminths infections

Unit 1- ASSOCIATION IN ORGANISMS AND CLASSIFICATION OF HOST ORGANISMS

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main contents
- 3.1 Types of Association
- 3.2 Classification of the parasitic organisms
- 3.3 Types of host
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

Organisms frequently associate together, often closely. There are a number of "motives" for these associations, including protection, nutrition, and as an aid to the dispersion (both geographically and temporally) of the organism. There are four main ways animals of different species may be associated to one another; Symbiosis, Mutualism, Commensalism and Parasitism. These classifications however, on closer inspection, may become blurred, one type taking on the aspects of another, for example over time as the relationship evolves. However, as a general guide these terms are still very useful.

2.0 Objectives:

At the end of the study, you should be able to

- i. Explain the various types of association existing between organisms
- ii. Give examples of various types of association

- iii. Describe the concept of parasitism and types of parasites
- iv. Explain the different types of parasites' host

3.0 Main Contents

3.1 Types of Association

Symbiosis

Here both organisms are dependent on each other. Examples being the association of flagellate protozoa in the gut of termites, where termites are dependent on the protozoa for breaking down their food stuffs, and the protozoa are dependent on the termites as host organisms. Another good example here which is often cited is the association between clown fish and anemones in tropical reefs; where the fish is dependent on anemone for protection and food while the anemone does not appear to gain anything from the association, except possibly cleaning. However, it has been observed that in some cases, in the absence of the fish partner the anemones tend to disappear from their reef home, indicating a true symbiotic rather than a mutualistic or commensal relationship.

Another well known example is found with the lichens, symbiotic association composed of fungi and algae. These associations may become very close, and it is thought that the eukaryotes as a group evolved as a result of such an association.

Intracellular organelles such as the mitochondria and chloroplasts appear to have their origin as intracellular symbiotes of early eukaryotes, (some extremely primitive eukaryotes, such as the intestinal parasite *Giardia lamblia*, lack these organelles). Other forms of symbiosis may be much less close, for example an organism that uses another organism purely as a means of dispersal. For example bacterial or fungal spores on the legs of flies, or coelentrates and barnacles on the carapaces of marine crustaceans. This particular form of symbiosis is sometimes called Phoresis.

Mutualism

Here the associates may or may not be dependent on each other for their existence, but both benefit when they are associated. A good example of this occurs with the association of sea anemones on the backs of crabs. Both gain from the association (the anemone providing some food for the crab, which in turn gives extra motility to the anemone), but both can survive on their own.

Another less well known example is found between certain species of ants and the caterpillars of some of the Lycaenidae butterflies (particularly the 'Blues'), where the caterpillar is protected by the ants within their nests, in return for which the caterpillar secretes a honeydew which the ants collect. In this case from the point of view of the ant, it benefits from the association, but does not appear to need it, (i.e. the association is facultative, or opportunistic). However, from the point of view of the caterpillar, this association is required for its survival (i.e. the association is obligatory). This illustrates that these definitions may become blurred, and, over time, one form of association may evolve into another.

Commensalism

Neither organism is dependent on the other for its existence, but in this case only one of the partners benefits from the association, the other being unaffected. An example of this, found in humans, are the non-pathogenic obligate commensal protozoa such as the amoebae *Entamoeba*

gingivalis, commonly found in the mouth, feeding on bacteria, dead epithelial cells and food particles. Purely commensal relationships tend to be rather rare, as on a closer inspection elements of mutualism or parasitism may become apparent.

Parasitism

Here one of the associates lives either partly or wholly at the expense of the other associate, the other partner (the host organism) not gaining anything from the association. This association may give rise to extreme pathology in the host, or the parasitism may be generally not very pathogenic. Parasitism is carried out by many organisms, the main groups including viruses, bacteria, protozoa (these usually being endoparasitic), and various metazoan groups (multicellular eukaryotic animals), these being mostly groups of helminths (often endoparasitic), and arthropods (usually ectoparasitic), as well as some higher organisms, such as ectoparasitic lampreys and hagfish. Generally however, for partly historical reasons, the term parasitology generally only refers to the study of infection with eukaryotic protozoan, and invertebrate metazoan parasites, not bacteria, viruses or the higher chordate parasites, even though these are parasites in the true sense.

3.2 Classification of the parasitic organism

Organisms in these associations may either be on the outer surface of the host organism, (in which case the prefix **Ecto-** is used), or inside the host organism, (in which case the prefix **Endo-** is used). These prefixes may be used with any of the animal associations listed above. For example the flagellate protozoa in the termite guts are **Endosymbionts**, while the anemone can act as an **Ectocommensal** with the crab. Parasites may act as both **ecto-** and **endoparasites**. Parasites may also be classified according to the closeness of the relationship. For example **Facultative Parasites** (such as many bacteria) are those where the parasitic lifestyle is only taken up opportunistically, whereas **Obligate Parasites** (such as all viruses, and most of the helminth parasites described below) are those in which the organism must parasitise another organism. These parasites may often cause diseases, in which case they are referred to as **Pathogenic Parasites**.

In a somewhat wider interpretation of the term parasitism, some organisms exhibit parasitic behaviour only early in their life cycle, these being referred to as **Brood Parasites**. Examples of these include caterpillars of the Large Blue butterfly, which chemically mimic other caterpillars with mutualistic associations with ants (see above), but both fail to produce honeydew as compensation and consume ant grubs, and may in fact destroy the nest, (thereby acting as a pathogenic parasite for the ants). In this case, the parasitic lifestyle probably evolved from the mutualistic lifestyle of the other, related butterflies, again illustrating how one form of association may change into another. Another well known example of a brood parasite is a bird, the cuckoo.

Some parasites establish themselves in hosts in which they do not ordinarily live. These are called the **Incidental Parasites**. A **temporary parasite** is free-living during part of its existence and seeks its host intermittently to obtain its nourishment whereas **Permanent Parasite** remains on or in its host's body from early life until maturity, sometimes for its entire life. Parasites that have passed through the alimentary tract without infecting the host are called **Coprozoic or Spurious Parasite**.

Parasites often lack the necessary organs for assimilation of raw materials and depend upon the host for predigested food. An adequate supply of moisture is assured inside the host, but during the free-living existence of the parasite, inadequate moisture may either prove fatal or prevent the larval development.

Temperature is likewise important. Each species has an optimal temperature range for its existence and development. Both high and low temperatures are detrimental and even lethal.

3.3 Types of Host

Parasitic helminths may have either simple or complicated lifecycles. The terms used to describe the hosts harbouring different stages in these lifecycles are however the same. The degree of damage done to the hosts is however varied. For example in definitive host, the greatest harm is seen being the one the adult stage of the parasite is found. Sometimes, a host might assume dual functions, and therefore could be difficult to classify strictly into one type. Human host during infection by malarial parasite is one of such. Human could be classified as the definitive host being the one in which greatest harm is seen. Also, intermediate host because human harbours the asexual stages of the parasite (merozoites and trophozoites). A clear understanding of the relationship between host and parasite and function of host in survival and transmission of parasite is therefore necessary for a better classification.

Definitive Host

The adult parasites are found in the **definitive host**. This is where the parasite's sexual cycle usually takes place, with either cross or self fertilisation with hermaphroditic parasites, or sexual reproduction if the parasites have separate sexes, followed by production of eggs, or more rarely with viviparous helminths, larvae. The greatest harm is usually seen in this host.

Intermediate Host

In many cases the parasites larvae are found in different hosts, these are called the **Intermediate Hosts**. Parasitic helminth larvae may have one, two or more intermediate hosts in their lifecycles, or they may have no intermediate hosts. Often asexual stages of reproduction occur in these intermediate hosts, (for example with Platyhelminth parasites). Note that when describing hosts of parasitic protozoa, these terms are slightly different owing to the asexual characteristics of many of these organisms. With parasitic protozoa the vertebrate host is generally referred to as the definitive host, whilst the invertebrate is the intermediate host. Some parasitic nematodes (e.g. *Strongyloides stercoralis*) are Facultative parasites, having completely free living lifecycles in addition to parasitic ones. The two terms definitive and intermediate host are the most important in Parasitology when referring to the type of host. A **vector** however, should not be mistaken for intermediate host. A **vector** actively transmits infection to a host without necessarily harbouring the asexual stage of the parasite e.g. the vector of African trypanosomiasis *Glossina* spp. These groups of vectors pick up parasite (the infective stage) from the reservoir hosts during blood meal and transmit it to a susceptible definitive host. However, some vectors can still serve as intermediate hosts harbouring the asexual or the larval forms of the parasite e.g. certain *Anopheles* mosquitoes that harbour the microfilariae of filarial worms.

Accidental Host

Accidental hosts are those in which the parasites do not normally develop (for example to lack of exposure to infective forms of the parasite), but when occasionally chance infections occur, the parasite is able to complete its lifecycle.

Hosts where the parasite can complete its lifecycle are called **Permissive hosts**, and include true definitive and intermediate hosts as well as many accidental hosts. Examples here include such parasites as *Fasciola hepatica*, where the normal definitive hosts are ruminants, but humans and other animals may also be infected and viable adult parasites develop. Another example is human infection with the nematode *Angiostrongylus cantonensis* in the far East.

In comparison another form of the accidental host is the **Non-Permissive host** where the parasite, although it may develop to some extent, reaches effectively a dead end, the parasite not being able to complete its lifecycle and eventually dying within the host. These forms of infection often occur where the parasite has intermediate hosts which may be accidentally ingested by animals other than the true definitive host. For example, with various marine ascarids of the family Anisakidae such as *Anisakis* sp., which give rise to the condition of 'Anisakiasis' on ingestion of raw infected fish.

Paratenic Host

Paratenic hosts may also be included in parasitic helminth lifecycles. In these forms of infection the parasites undergo an arrested development on infection, larval forms accumulating in these hosts until they have a chance of infecting the definitive host (e.g. in the Pseudophyllidean tapeworms). These hosts are therefore not essential to completion of the parasites lifecycle. This is in contrast to the case with true intermediate hosts whose ingestion is essential to the lifecycle, for example *Echinococcus* sp.

Reservoir Host

These are accidental hosts and hosts of parasites which have zoonotic patterns of infection (i.e. normally infect a wide range of hosts), may act as **Reservoir Hosts** for the parasite. These are also a form of permissive hosts as fully viable infections develop, and a more accurate term would be alternative definitive hosts (though this is not in fact used). The term reservoir host is usually only used when describing the epidemiology of human infections. An example of parasites with zoonotic infections is *Schistosoma japonicum*. This parasite, as well as infected man, can also infect other mammals as definitive hosts, including rodents, cats, dogs, domesticated ruminants such as water buffalo and a wide range of other mammals. In Human African Trypanosomiasis (HAT), the reservoir host are cattle which serve as **sources of active infection** to man. The presence of these **Zoonoses** has implications for the control of the parasite in the field.

4.0 Conclusion

In this unit, we learnt that organisms interact with each other at different degrees. While some are solely dependent on each other, however, some are opportunistic and can adopt different means for their survival. Various types of association, classification of the parasitic organisms and different types of hosts were also discussed.

5.0 Summary

In this unit, you have learnt about the following:

- i. Types of Association
- ii. Classification of the parasitic organisms
- iii. Types of host

6.0 Tutor Marked Assignments

6.1 Activity: What is the type of association that exist between salmonella typhi and its host?

6.2 Answer the following questions:

- i. write short note on the following: Symbiosis, Mutualism, and Commensalism (LO1)
- ii. what is/are the differences between symbiosis and mutualisms (LO1)
- iii. give one example in each case of (a) Commensalism, (b) symbiosis (c) mutualism (LO2)
- iv. describe the different types of Parasites with example (LO3)
- v. explain the various types of host (LO4)

7.0 References and other resources

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book.Inc.

Medigan, M.T. *et al.* (2009). *Brock Biology of Microorganisms*. (12th ed.). Pearson Education Inc.

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UNIT 2- Human Helminth Infections

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Contents
- 3.1 Nematode infections
- 3.2 Digenean trematode infections
- 3.3 Cestode (tapeworm) infections
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

This unit aims to introduce the student to the diversity of helminth infections in man, and even more importantly, to the numbers of individuals that harbour these infections in all regions of the world. There are three major groups of helminthes containing members that have man as their

main hosts, these being the **Digenean Flukes**, the **Tapeworms (Cestodes)**, and the **Roundworms (Nematodes)**.

2.0 Learning Objectives

At the end of this unit, you should be able to:

- i. Describe various helminthes parasites
- ii. Explain examples of various helminthes parasites

3.0 Main Contents

3.1 Nematode Infections

- i. ***Enterobius vermicularis*** - Pinworm, Threadworm.

E. vermicularis infection, an extremely common nematode infection, particularly in temperate areas such as Western Europe and North America, (being relatively rare in the tropics) and particularly in children. It has been estimated that the annual incidence of infection is over 200 million, this probably being a conservative figure. Samples of caucasian children in the U.S.A. and Canada have shown incidence of infection of from 30% to 80%, with similar levels in Europe.

- ii. ***Ascaris lumbricoides*** - The Large Human Roundworm.

Again the incidence rates for this parasite are very high with > 1500 million cases of infection annually, of which about 210 million cases are symptomatic.

- iii. ***Trichuris trichiuria*** - The Large Human Roundworm.

The incidence rates for this parasite are also very high, with estimates of about 1300 million cases of infection annually, of which >133 million cases are symptomatic.

- iv. **The Hookworms.**

These are represented by two parasites, *Necator americanus* in the tropics and sub tropics worldwide and the S. E. states of the U.S.A., and *Ancylostoma duodenale*, again with a worldwide distribution in the tropics and sub tropics as well as the Mediterranean region. There are > 1200 million cases of hookworm infection annually, of which about 100 million cases are symptomatic.

- iv. **Lymphatic filariasis** - Elephantiasis

This disease is caused principally by two parasites, *Wuchereria bancrofti* with an annual rate of infection of about 106 million cases, and *Brugia malayi* with an annual rate of infection of about 12.5 million. The total number of people infected with other types of lymphatic filarial worms is much smaller, at about 1.5 million cases. These lymphatic filarial worms, along with the related filarial parasite *Onchocerca volvulus*, are unusual among the nematodes in that they develop with, and are transmitted by insect vector intermediate hosts.

- v. ***Onchocerca volvulus*** - River Blindness

The incidence rates for this parasite are not as high as some of the previously described parasites, with an annual rate of infection of about 18 million, however, due to the extreme pathology

associated with this parasite, often with all adult members of affected villages losing their sight, along with severe skin conditions, the infection is significant.

vi. *Dracunculus medinensis* - Guinea Worm

The incidence rates for this parasite are much lower, with an estimated annual rate of infection of about 100 000. This is much lower than in the recent past, when up to 50 million people were infected. This reduction in incidence illustrates how successful helminth control programmes can be effective in reducing the disease caused by these organisms.

Other important nematode infections include; *Trichinella spiralis*, *Strongyloides stercoralis*, and a number of more **rare infections**. Nematodes that normally infect other animals may still cause disease in man. These include *Toxocara canis* and a number of nematodes causing *anisakiasis*.

3.2 Digenean Trematode Infections

i. *Schistosomiasis* - Bilharzia.

Schistosomiasis is caused by *Schistosoma mansoni*, *S. haematobium*, *S. intercalatum*, *S. japonicum* and *S. mekongi*. This disease is the most important human helminthiasis in terms of morbidity and mortality. The numbers of people infected are lower than those of many of the nematode infections, with an estimated annual incidence of infection of > 200 million cases. In terms of active disease however, the parasite is much more important, with an estimated annual mortality rate of about 1 million deaths directly due to infection with these parasites.

ii. *Opisthorchis sinensis* - The Chinese Liver Fluke

This is also a very important trematode infection, with an estimated annual incidence of infection of about 20 - 30 million cases, mostly in the Far East, in Japan, China, Taiwan and South East Asia.

iii. *Paragonimus* spp. - The Lung Fluke

This fluke causes a pulmonary disease, the adult parasites living in the lungs of their definitive hosts (e.g. man). There are a number of different species of this parasite; the most well documented being *P. westermani* in the Far East. It may however, be locally very common, with up to 40 to 50% of the population infected.

There are a number of other digenean trematode infections. These include various **Echinostome** infections as well as a number of other flukes. In addition, there are a number of these parasites that usually infect domesticated animals, but also cause well known human infections as well. These include *Fasciola hepatica* and *Dicrocoelium dendriticum*.

3.3 Cestode (Tapeworm) Infections

i. *Taenia saginata* - The Beef Tapeworm

This only causes very limited pathology in man, but the annual incidence of infection is high, at an estimated 50 million cases.

ii. *Taenia solium* - The Pork Tapeworm

This has a similar estimated annual incidence of infection of about 50 million cases. However, in this case the consequences may be more severe, due to the added risk of contracting infection with the larval metacestode, (cysticercosis).

This may have extreme consequences in terms of the pathology associated with infection, with an estimated annual mortality rate of about 50,000 deaths. For the cestodes, these annual incidence rates are based on detection of infection with the adult parasite. This is achieved by examination of faeces, urine or sputum for **parasite eggs**. Diagnosis of infection with larval metacestode parasites, such as *Echinococcus* sp. is very difficult, due to the lack of non invasive diagnostic techniques. It is in consequence very difficult to estimate annual rates of infection, even though these metacestodes may be very important pathogens.

4.0 Conclusion

In this unit, we have discussed that Parasitic helminths can be grouped into nematodes, cestodes and trematodes. The nematodes are the most diversified groups. Parasitic helminths infect a wide range of hosts, ranging between man, domestic animals and wild animals. Morbidity is often high leading to death of several thousands of people

5.0 Summary

In this unit, you have learnt about the following:

- i. Nematode infections
- ii. Digenean trematode infections
- iii. Cestode (tapeworm) infections

6.0 Tutor Marked Assignments

6.1 Activity

6.2 Answer the following questions:

- i. Write short note on Nematode Infections, Digenean Trematode Infections and Cestode (Tapeworm) Infections (LO1)
- ii. Describe the following Lymphatic filariasis, *Enterobius vermicularis*, *Dracunculus medinensis*, Schistosomiasis – Bilharzia, and *Taenia solium* (LO2)
- iii. Mention the 3 types of fluke with 2 examples of each species (LO2)

7.0 References and other resources

- Brown, H.W. and Neva, F.A. (1983). Basic Clinical Parasitology (5th edition). pp1-17
- Ukoli, F.M.A. (1990). Introduction of Parasitology in Tropical Africa. John Wiley and Sons Ltd., Chichester.
- Hunter, G.W., Swartzwelder, J.C., Clyde, D.F (1976). A Manual of Tropical Medicine. 5th Ed. WB Saunders, Philadelphia.

MODULE FIVE- TREMATODES

Introduction

Module Objectives

At the end of this module, you should be able to:

Contents

Unit 1: Digenetic Trematodes

Unit 2: Classification of digenetic trematodes according to their habitat

Unit 1- Digenetic Trematodes

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main contents
- 3.1 The Adult Digenean Fluke
- 3.2 The Basic Lifecycle of the Major Groups of the Digeneans
- 3.3 The Digenean Trematode Egg
- 3.4 The Larval Digeneans
- 3.5 Features of digenetic trematodes
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
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1.0 Introduction

The phylum Platyhelminthes comprises six classes which include the free living forms and those that are of zoological, medical and economic importance. The medically important groups are the trematodes and cestodes. Trematode also called flukes cause various clinical infections in humans which occur worldwide. The parasites are so named because of their conspicuous suckers, the organs of attachment (*trematos* means "pierced with holes"). All the flukes that cause infections in humans belong to the group of digenetic trematodes.

The class Trematoda comprises 3 subclasses

Subclass 1 – Aspidogastrea: They have large ventral adhesive organ subdivided by longitudinal and transverse septa into sucking discs. They are parasites of turtles, fishes and molluscs

Subclass 2 – Didymozoida: These are tissue-dwelling parasites of fish. They are greatly elongated, dioecious, with sexual dimorphism. No complete life cycle is known

Subclass 3 – Digenea: This contains parasites of medical and economic importance to man and therefore will be dealt with more extensively.

2.0 Objectives

At the end of this unit, you should be able to

- i. Identify the striking features of digenetic trematodes
- ii. Describe the morphology of the egg, larva, and adult stage of various digenetic trematodes
- iii. Explain the general transmission patterns of some trematodes

3.0 Main Contents

3.1 The Adult Digenean Fluke

The basic body form of the adult trematode takes a number of different forms, some of which are illustrated below;

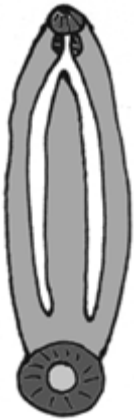


Fig 1.1 Amphistomee: These have large fleshy bodies, with a prominent sucker at the posterior of the body (e.g. *Gastrodiscoides hominis*)



Fig 1.2: Distome: These are the most common type, with the mouth surrounded by the oral sucker and a ventral sucker, present anywhere on the ventral surface except the extreme posterior (e.g. *Fasciola hepatica*)



Fig 1.3: Echinostome: Similar to the distomes, except that the oral sucker is surrounded by a prominent collar, equipped with spines (e.g. *Echinostoma* sp.)

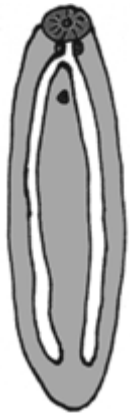


Fig 1.4: Monostome: In these there is either only one sucker present (usually only the oral sucker), or there are two suckers, but one very reduced, or in some cases no suckers (e.g. *Notocotylus attenuatus*)

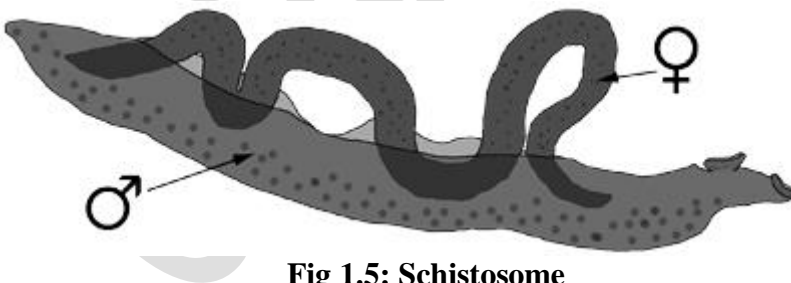


Fig 1.5: Schistosome

Elongate trematodes, with separate sexes, the male generally larger, holding the female within a groove formed by a folding of the male body (the gynaecophoric canal). Found within the circulatory system. (e.g. *Schistosoma mansoni*)

There are other forms as well, for example the 'Holostome' type, where the body of the trematode is divided into two distinct regions, the anterior of which may hold an additional adhesive organ, (e.g. *Diplostomum* sp.), and the 'Gasterostome', where the gut is a very simple, sac like, structure, attached to a mouth situated near the centre of the body (reminiscent of the arrangement of some of the free living platyhelminthes).

3.2 The Basic Lifecycle of the Major Groups of the Digeneans

Most digeneans are hermaphroditic (the major exception being the schistosomes, and one other group). In the majority of these parasites self fertilization may occur, but cross fertilization between different individuals is more generally the rule. The sperm enter the female system, either via the Laurers canal or more commonly through the common genital atrium, which opens into the uterus.

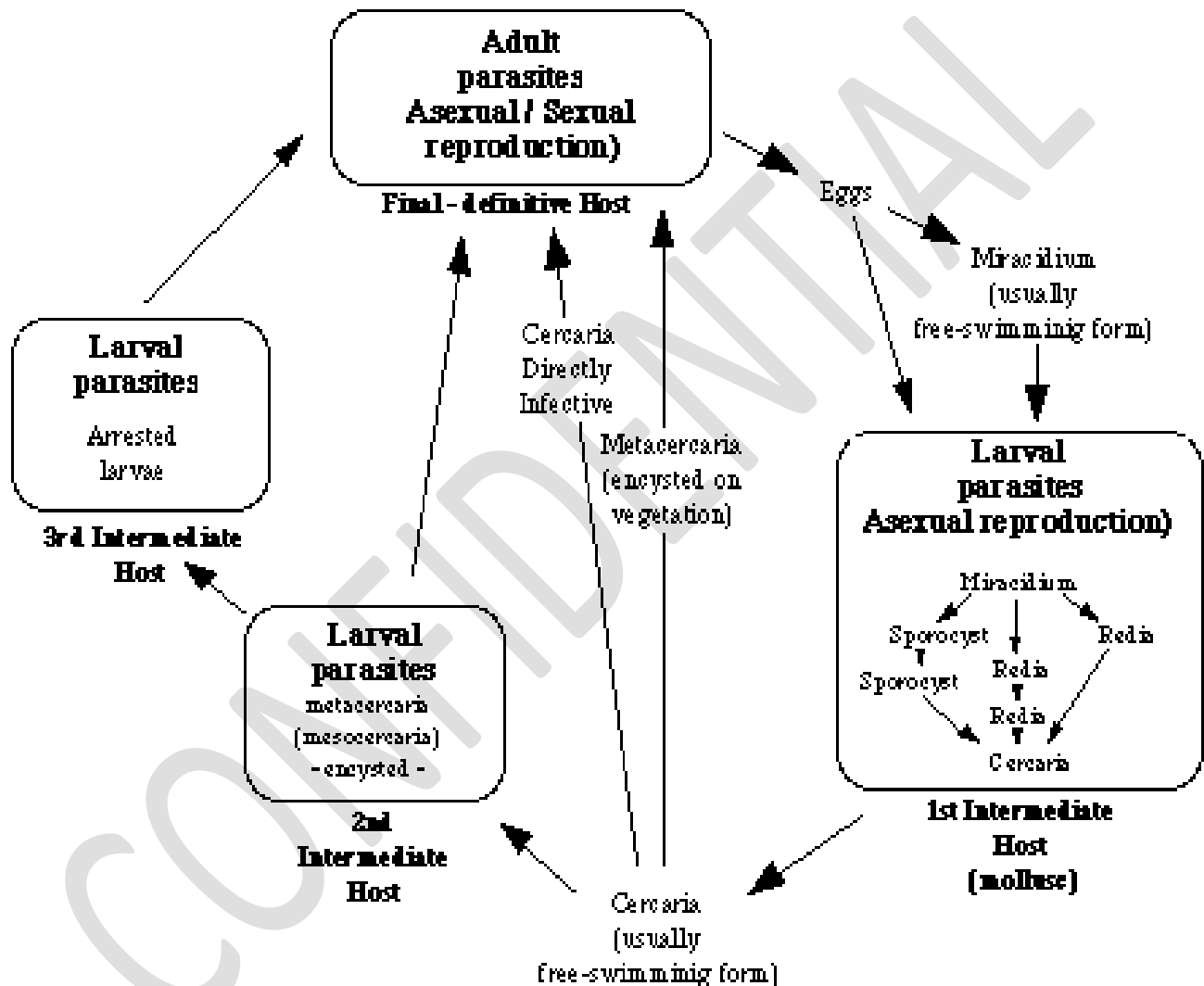


Fig 1.6: The generalised life cycle of digenetic trematodes (imgarcade.com)

3.3 The Digenean Trematode Egg

The formation of the digenean egg follows that described for the platyhelminthes as a group. Briefly, as the egg enters the öotype of the fluke it becomes surrounded by a predetermined number of vitelline cells, the number of which will be specific for different parasites, which form the food reserve of the egg. These vitelline cells produce globules of a mixture of proteins and

phenols, which are extruded to the outer surface of the developing egg. Here the phenols oxidise to form quinone, which then coalesces with the protein, reacting to form sclerotin, a hard inert yellowish substance, making up the egg shell. As the eggs of different species may vary in thickness, their colours may vary from yellow, to a dark brown. The digenean egg is usually operculate, in common with other platyhelminthes. Exceptions to this may occur however, the most important being with the schistosomes. Here the eggs are non-operculate, and are ornamented with spines, the appearance of which are characteristic for different species of schistosome.



Fig 1.7: operculate egg

The eggs hatch of operculate eggs involves the release of the opercular cap. This takes place under a variety of conditions, modified according to the particular species of trematode. For example some trematode lifecycles involve the ingestion of the egg before hatching (e.g. *Dicrocoelium dendriticum*, the lancet fluke), whilst others such as those of *Fasciola hepatica*, (the liver fluke), hatch in water. For the eggs that hatch in the external environment, a number of factors may be important, for example light, temperature and changes in osmotic pressure. Again the exact details of these environmental requirements will be optimised for the particular conditions which will maximise the chances of completion of the parasite lifecycle. In all cases the egg hatches to release the miracidium.

3.4 The Larval Digeneans

The miracidium

The miracidium is the name of the ciliated larval stage that is hatched from the digenean egg. In comparison with the other larval platyhelminthes, it is very similar to the larvae of the monogeneans, (the oncomiracidium) and the larval cestodarian, or lycophore. In most cases the miracidium is usually a free swimming stage, that seeks out the primary, and in some cases only, intermediate hosts of these parasites. In all cases these primary, or 1st intermediate hosts are molluscs. In the few examples where the miracidium is not a free swimming stage the eggs are ingested, as with the lancet fluke *Dicrocoelium dendriticum*. Here the eggs hatch in the intestine of the mollusc liberating the miracidium, from where it immediately penetrates the intestinal wall to invade the molluscan tissues. In the free swimming miracidia the larval parasite exhibits distinct behavioural responses that enable it to enter the environment of or detect the presence of its hosts. These behavioural responses have principally been studied in the case of the schistosome miracidium. Morphologically, the surface of the miracidium is covered with a series of ciliated plates, which may be clearly seen using electron microscopy after the removal of cilia. These ciliated epidermal plates (in some species the cilia being replaced by spines) are discontinuous, not being in contact with each other but being separated by extensions of the underlying subepidermal layer. The whole structure is being illustrated below.

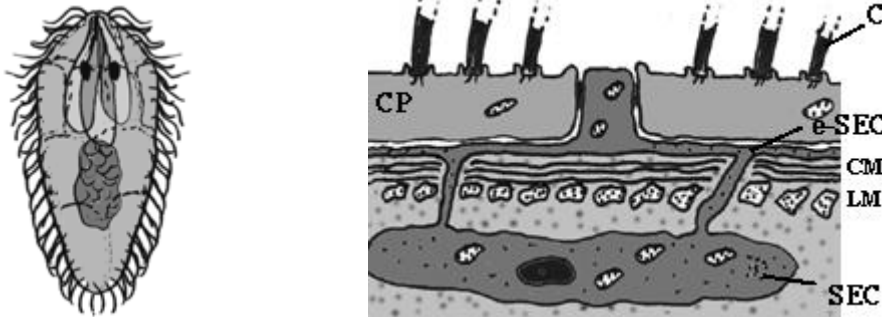


Fig 1.8

CP = Ciliated Plate

C = Cilia

SEC = Sub-epithelial cell

e-SEC = extension of subepithelial cell

CM = Circular Muscle

LM = Longitudinal Muscle

The plates themselves show a definite arrangement, being placed in four to five transverse rows, the exact arrangement of which may vary between different trematodes. Beneath the plates are layers of muscle fibres. At the anterior end of the larvae is a non-ciliated conical projection, the terebratorium, (or anterior papillae), bearing apertures of the apical and penetration glands. These themselves are found at the anterior end of the body. Miracidia possess a number of sensory organs, the most important of which are the dorsally situated eye spots, beneath which is found the cerebral mass. Other sensory organs are situated within folds of the terebratorium. Below all of the structures is found the miracidium's large rounded germinal cells, which are often grouped in clusters called germ balls.

Finally the miracidia possess a protonephridial excretory system, basically similar to that found in the adult parasites. On examination of eggs containing mature miracidia, it is clearly seen that flame cell activity is the first sign of the initiation of hatching of the egg. On invasion of the molluscan tissue the miracidium sheds its ciliated plates, in almost all cases rapidly transforming into an endoparasitic form, the sporocyst, although in a few unusual groups the miracidium may contain a fully developed redia.

The sporocyst

The sporocyst develops within the molluscan host as a hollow fluid filled germinal sac, into which protrude germinal masses. At the conical anterior of the sporocyst body a birth pore is located, from which subsequent generations of larvae emerge. The germinal masses develop internally into either daughter sporocysts, which are essentially the same as their parent sporocysts, or into a second larval stage, the redia.

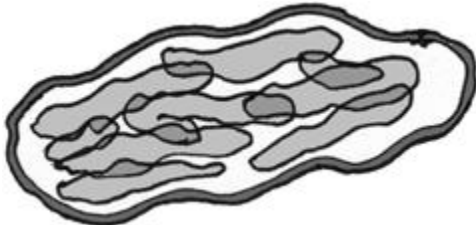


Fig 1.9: The sporocyst

The redia

The redia are the second larval form to develop within the molluscan host (but may be absent in some groups, such as the schistosomes). They are similar to sporocysts, containing germinal masses within a fluid filled sac, which may develop into either second generation daughter redia, or more commonly into the final larval stage within the mollusc, the cercaria.



Fig 1.10

They differ from the sporocysts however, in that they are a much more active form, and importantly they possess simple gut. The tissue they feed on is predominantly molluscan in origin, but the redia of some groups (e.g. those of the echinostomes) may actively seek out the developmental stages of other trematodes (e.g. schistosome sporocysts) within the same intermediate.

The gut itself consists of a mouth, opening into a large muscular pharynx, which in turn opens into a simple rhabdocoel like intestine. Externally, behind the mouth many redia have a ridge-like collar, below which the birth canal opens and from which either cercariae or daughter redia emerge. Further along the body are lobelike extensions of the body, which are thought to aid the movement of the parasite within its host's tissues.

An interesting exception to the general rule that cercaria are produced by the redia is found in a few trematodes where the redia produce progenetic metacercaria, fully capable of producing viable eggs. In these few very unusual cases, the trematode may only have a single molluscan host, although the metacercaria may still be capable of developing in a second host as well. Exceptions such as these, and those described above involving miracidia containing fully developed redia is evidence of the evolutionary past of these organisms. It has been noted that the redia bears some resemblance to some of the more advanced turbellarians, and as described above, this stage is a very active form of the parasite, fully capable of actively ingesting host material, and in some cases even predation of competing parasites within their hosts. It has been postulated that the group as a whole emerged from an ancestral parasitic turbellarian, with a single molluscan host, after the development of internal division and asexual reproduction, later developing specialised forms to exploit the varying environments that these organisms have to cope with.

The cercaria

Some of the types of Cercariae

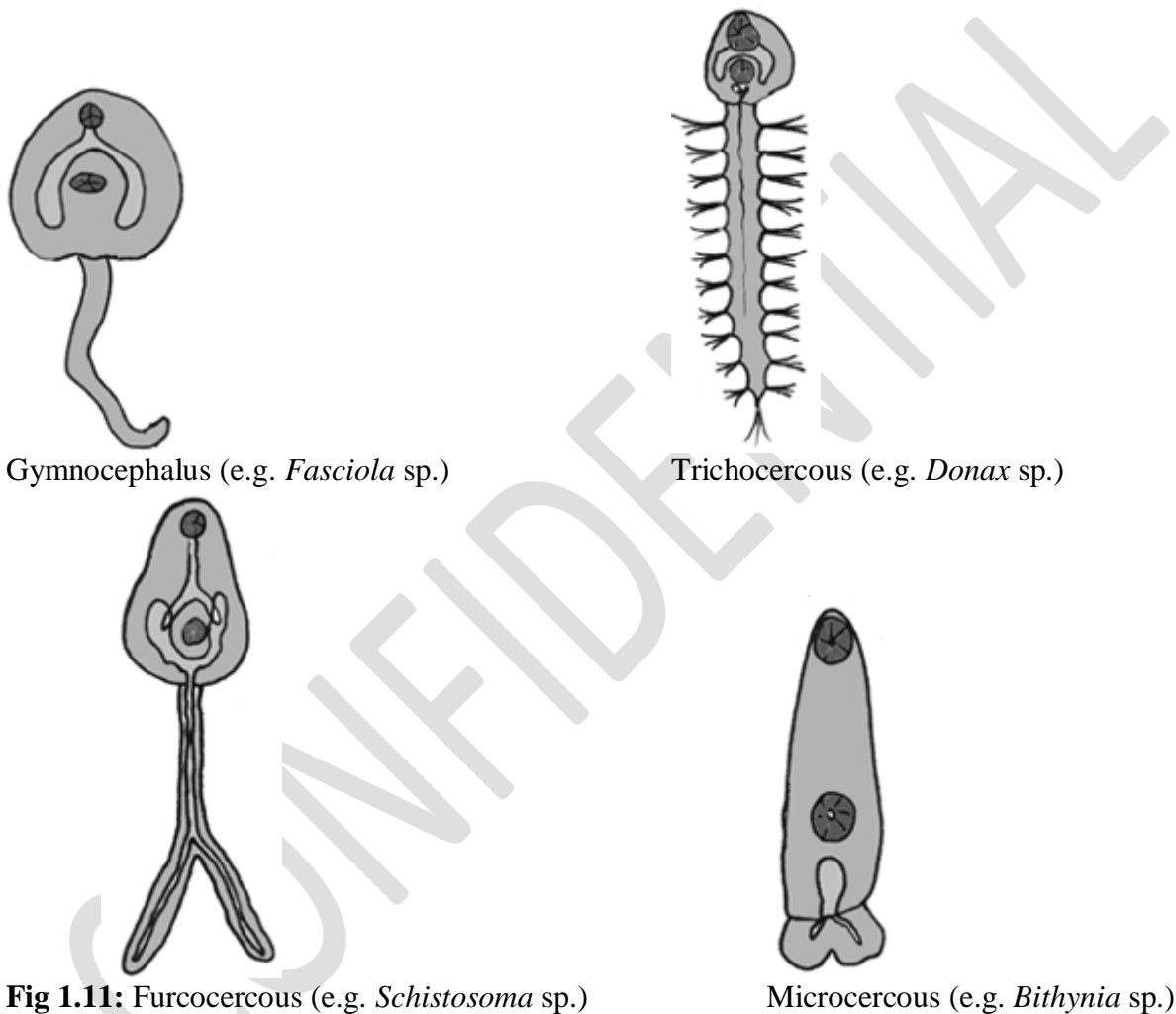


Fig 1.11: Furcocercous (e.g. *Schistosoma* sp.)

Microcercous (e.g. *Bithynia* sp.)

In almost all species of trematode, it is the cercarial stage that emerges from the mollusc, and is the infective form for the vertebrate host, although there may be exceptions to this general rule. For example in some cases a sporocyst, modified to have a thickened internal wall resistant to the environment, emerges, to be ingested by a second intermediate host, (e.g. as is the case in the trematode *Dicrocoeloides petiolatum*). Other exceptions, involving redia producing progenetic metacercaria, have already been described above.

The trematode cercaria exhibits considerable variations in structure, which is very important taxonomically, and reflects in many cases adaptations to the specific lifecycle of the parasite involved. Because of this great diversity of form, a system of cercarial classification has evolved,

based on the gross morphology of these larval forms. Firstly cercariae may be divided into three major groups;

- i. **Monostome Cercariae** - These lack a ventral sucker, and have simple tails. These forms develop within rediae
- ii. **Amphistome Cercariae** - In these the large ventral sucker is situated at the base of a slender unbranched tail. These forms develop within rediae.
- iii. **Distome Cercariae** - This is the commonest cercarial form, with the ventral sucker lying some distance from the posterior end, in roughly the anterior third of the body.

These distome cercariae may themselves be divided into a large number of subgroups, based on other morphological features, particularly the form that the cercarial tail takes. Some of these forms are described below;

a) Leptocercous Cercariae - These cercariae have straight slender tails, which are much narrower than the cercarial body. This form is further subdivided into;

- i) *Gymnocephalous Cercariae* - In these, the suckers are equal in size. This is a common form, represented within such species as *Fasciola hepatica*, and develop within rediae
- ii) *Xiphidiocercariae* - These are similar to the gymnocephalous forms, but in these the oral sucker is equipped with a stylet, used in penetration of their next hosts, and they generally develop within sporocysts
- iii) *Echinostome Cercariae* - In these there is a ring of spines at the anterior end of the larvae, as in adult forms of these parasites. These are found within trematodes of the genus *Echinostoma*, and develop within rediae.

b) Trichocercous Cercariae - These forms have long tails, equipped with rings of fine bristles. They are usually found in marine trematodes.

c) Cystocercous Cercariae - In these the end of the tail is highly enlarged, with a cavity into which the larval body may be retracted. These usually develop within sporocysts.

d) Microcercous Cercariae - Cercaria with vestigial tails, and which may develop within both rediae and sporocysts.

e) Cercariaea Cercariae - Cercaria with no tails, where the cercaria is not a free swimming form, and may develop within both rediae and sporocysts.

f) Furcocercous Cercariae - In these the tails are forked at the end. The cercaria of the most important group of trematodes, the schistosomes, have cercariae of this form. This form develops within sporocysts.

Otherwise, both externally and internally the structure of the body of the cercaria resembles that of the adult trematode into which they grow. For example, the ring of spines found at the anterior end of echinostome cercariae is also present in the adult flukes.

The outer surface of the cercaria is a tegument, which may however differ from that found in the adult form in a number of ways. For example in the schistosomes the tegument is covered with a trilaminar plasma membrane, (as opposed to the two bi-lipid membranes found in the adult), on

the outer surface of which there is a glycocalyx, (absent in the adult). However, many other features of this tegument appear similar to that of the adult, the differences almost certainly being adaptations due to the differing environments that these two lifecycle stages experience. For example, spines found on the surface of both forms of tegument, and the overall structures of a syncytium connected to subtegumental cells are the same. Within the cercarial body a number of different types of gland cells may be found, including cystogenous gland cells, used by the larvae to secrete a cyst wall during the formation of the metacercarial stage, and penetration gland cells, used by the cercaria to penetrate its next host, either a second intermediate host, or in some groups the definitive host, (such as the schistosomes), where the cercaria is the final larval stage. The cercariae released from their molluscan intermediate host are usually a free swimming form. These must then locate either their next, and usually final intermediate host, their definitive host which they actively penetrate (e.g. in members of the family Schistosomatidae), or locate a suitable solid substrate to encyst upon, or be ingested by their definitive host (members of the family Azygiidae).

To locate these various targets the cercariae are equipped with a variety of sensory organs. These commonly include two or more eye spots, as well as touch receptors, and allow specialised cercarial behaviour, designed to bring the cercariae into an environment giving the maximum probability of infecting their next hosts. For example, the cercariae of the schistosomes exhibit negative phototrophy (swimming to the surface of the water), and positive thigmotrophy and thigmotrophy, being attracted to warm objects moving in the water. As well as these behavioural responses within the free swimming cercariae, the parasite exhibits definite circadian rhythms in terms of shedding from the molluscan host, again being shed at times optimal for bringing them into contact with their next host. For example, the schistosome cercariae are generally shed during daylight, in the morning, whilst those of other species emerge only at night.

In a few groups, such as *Alaria* spp. However, the parasite employs three intermediate hosts. In these cases the cercaria penetrates the second intermediate host to form a resting stage, the mesocercaria described below. In these cases this second intermediate host is in turn ingested by a third intermediate host, where it encysts to form a metacercaria.

The mesocercaria

The mesocercaria is essentially a resting stage within the parasitic life cycle, employing a second intermediate host in a parasite lifecycle utilising four hosts.

The mesocercaria is a definite prolonged stage in the adult generation of strigeate trematodes, which closely resembles the cercarial body, from which it develops in the second intermediate host, and which does not possess metacercarial features; it develops in turn into the metacercaria in another host.

In parasites having this larval stage the mesocercaria are capable of infecting and surviving within a very wide range of paratenic hosts which may ingest the second intermediate host, thus in effect increasing the number of hosts which the parasite may use in its lifecycle. For example, amphibians infected with mesocercaria of *Alaria* may themselves infect a wide variety of other amphibians, reptiles, birds and mammals if they are ingested by these animals.

The metacercaria

This is a much more common "resting" larval stage of the trematode parasitic lifecycle, formed either in a final intermediate host (when a mesocercaria, or more commonly a cercaria enters its body), or on a solid substrate in the external environment. The final intermediate host may be a fish (e.g. *Opisthorchis sinensis*), an arthropod (e.g. *Dicrocoelium dendriticum*, employing an ant second intermediate host, and *Paragonimus westermani* employing a crustacean), or another mollusc, as with some of the echinostomes. As stated above, some trematodes however do not have second intermediate hosts, but either encyst as metacercariae on solid substrate's, such as aquatic vegetation or on shells of aquatic organisms, which will in turn be ingested by the parasites definitive host, or in some groups such as the schistosomes, as already described, the cercariae directly penetrate the skin of, and infect, the parasites definitive host. Although generally the metacercariae are inactive encysted forms, the metacercaria of some species do remain free and active. In most other metacercariae however, encystment does occur. The structure of the cyst wall itself varies considerably, though generally it is a complex mixture of tanned proteins, lipids and polysaccharides. Within the cyst wall the morphology of the larva usually closely resembles that of the cercarial body, although as described above, in some groups sexual maturation may occur either fully or partially. To continue further the metacercaria must be ingested, either along with the body of the intermediate host it inhabits by a carnivorous definitive host, or along with the vegetation it has encysted on by a herbivorous or omnivorous host.

The Larval Digeneans - the Juvenile Adult Stages

On ingestion the metacercaria (or cercaria) must transform into the adult form. The precise details of this process will vary considerably, depending on how the definitive host was infected. For example, in some species the adult flukes are found within the alimentary tract. In these cases the metacercarial cyst wall is broken down to release what is essentially a young fluke, which only has to migrate a short distance to reach their preferred site within the hosts body. In other groups however the adult forms are located in other sites within the body. In these cases the liberated young fluke must penetrate the gut wall, or in the case of the schistosomes penetrate the hosts skin. Then they must undergo a migration through the hosts body. This is usually via the circulatory system, but again the precise details of the migratory path will vary considerably.

3.5 Features of digenetic trematodes

- i. Digenetic trematodes are unsegmented leaf-shaped worms that are flattened dorsoventrally.
- ii. They bear 2 suckers, one surrounding the mouth (oral sucker) and another on the ventral surface of the body (ventral sucker). These serve as the organs of attachment.
- iii. The sexes of the parasites are not separate (monoecious). An exception is schistosomes, which are diecious (unisexual).
- iv. The alimentary canal is incomplete, and no anus is present.
- v. The excretory system is bilaterally symmetrical. It consists of flame cells and collecting tubes. These flame cells provide the basis for the identification of the species.
- vi. The reproductive system consists of male and female reproductive organs and is incomplete in each fluke.

- vii. The flukes are oviparous. They lay operculated eggs. An exception is schistosome eggs, which are not operculated.
- viii. All have complicated life cycles, with alternating asexual and sexual developments in different hosts.

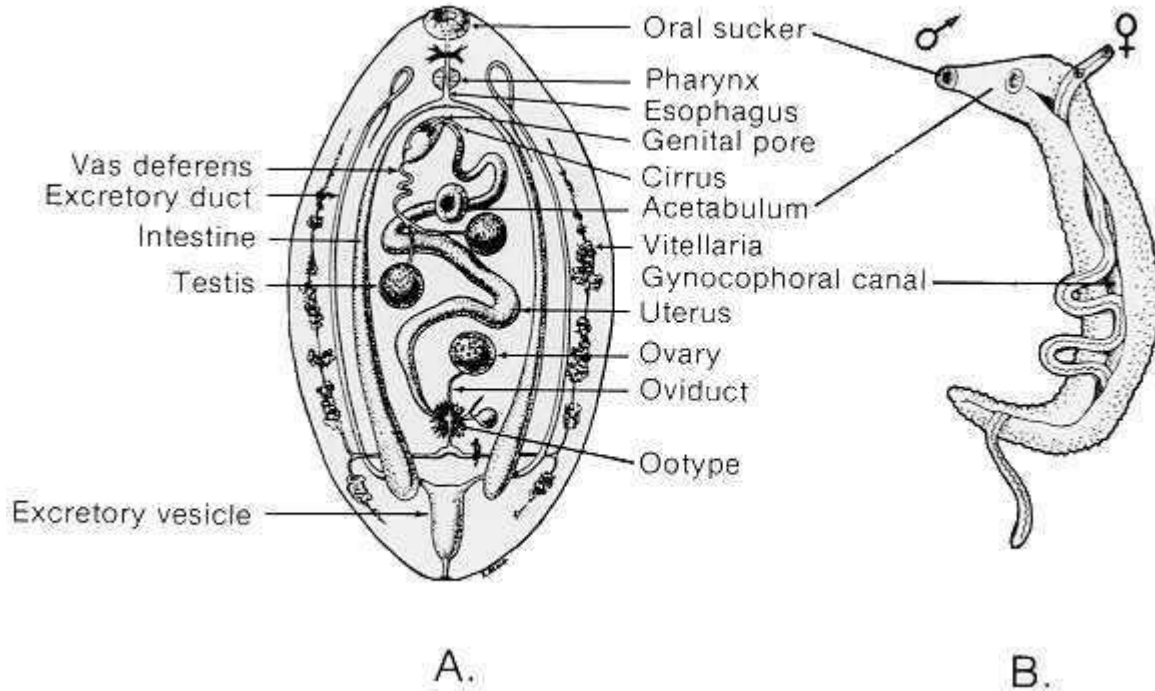


Fig 1.12 Structure of digenetic flukes. (A) Hermaphroditic fluke. (B) Bisexual fluke.

4.0 Conclusion

In this unit, we have learnt that digeneans are medically important trematodes. The digenea are unsegmented leaf-shaped worms that are flattened dorsoventrally with two suckers (the oral sucker and ventral sucker). The digeneans have heteroxenous life cycles having one or more intermediate hosts. The adult worms lay eggs within the definitive host which hatch miracidia in water medium. Miracidia develop within the snail intermediate hosts of particular species. The life cycles continue following a specific pattern depending on the parasites' species giving rise to other larval stages like sporocysts, rediae, cercariae and metacercariae

5.0 Summary

In this unit, you have learnt about the following:

- i. The Adult Digenean Fluke
- ii. The Basic Lifecycle of the Major Groups of the Digeneans
- iii. The Digenean Trematode Egg
- iv. The Larval Digeneans
- v. Features of digenetic trematodes

6.0 Tutor Marked Assignments

6.1 Activity: Observe the larval digeneans under a microscope and report your findings in the log book.

6.2 Answer the following questions:

- i. What are the striking features of digenetic trematodes (LO1)?
- ii. With a well labeled diagram describe the generalised life cycle of digenetic trematodes (LO2)
- iii. Write short note on the following:
 - a. The miracidium
 - b. The sporocyst
 - c. The redia
 - d. The cercaria
 - e. The mesocercaria
 - f. The metacercaria
 - g. The juvenile adult stages (LO2)
- iv. outline the features of digenetic trematodes (LO3)

7.0 References and other resources

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UNIT 2- CLASSIFICATION OF DIGENETIC TREMATODES ACCORDING TO THEIR HABITAT

CONTENTS

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main content
- 3.1** Blood flukes (*Schistosoma* species)
- 3.2** Lung flukes (*Paragonimus* species)
- 3.3** Liver fluke (*Fasciola hepatica* and *F. gigantica*)
- 3.4** Intestinal flukes (*F. buski*, *H. heterophyes*, *M. yokogawai*)
- 3.5** Pancreatic flukes (*Eurytrema pancreaticum*, *E. coelomaticum*, *E. ovis*)
- 4.0** Conclusion
- 5.0** Summary

- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

The digeneans are a group of specialised endoparasitic platyhelminthes. A common feature is that all have complex lifecycles, involving one or more intermediate hosts, the first of which is always a mollusc, which is usually aquatic. As adults they are found in most vertebrates groups, including fish, amphibians, reptiles, birds, and mammals, acting as definitive hosts, where they may be highly pathogenic. They may be located in most of the internal organs of these definitive hosts, including the lungs, bladder and blood stream, although the majority are found in the gastrointestinal tract, or closely associated organs such as the bile duct and liver. They exhibit a flattened leaf-like body, structurally similar to many of the free living turbellarians. The digeneans are classified below based on their locations in the definitive hosts.

- i. **Blood flukes** - *Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum*
- ii. **Liver flukes** - *Fasciola hepatica*, *F. gigantica*, *C. sinensis*, *Opisthorchis felineus*, *O. viverrini*, *Dicrocoelium dendriticum*, and *D. hospes*
- iii. **Pancreatic flukes** - *Eurytrema pacreaticum*, *E. coelomaticum*, and *E. ovis*
- iv. **Lung flukes** - *Paragonimus westermani*, *P. mexicana*, and *P. skrjabini*
- v. **Intestinal flukes** - *Fasciolopsis buski*, *Metagonimus yokogawai*, *Echinostoma ilocanum*, *Watsonius watsoni*, *Heterophyes heterophyes*, and *Gastrodiscoides hominis*

2.0 Objectives

At the end of this unit, you should be able to;

- i. Explain the transmission cycles of digenetic trematodes
- ii. Describe types of digeneans with examples
- iii. Identify the factors responsible for transmission of digenetic trematodes
- iv. Identify the control measure to be taken to prevent transmission of digenetic trematodes
- v. Identify each parasite using the diagnostic features of the eggs
- vi. Explain the pathology caused by parasite

3.0 Main Content

3.1 Blood flukes (*Schistosoma* species)

Schistosomiasis, or bilharzia, is a tropical parasitic disease caused by blooddwelling fluke worms of the genus *Schistosoma*. Over 200 million people are infected in at least 75 countries with 600 million or more people at risk of infection. The main schistosomes that infect human beings include *S. haematobium* (transmitted by *Bulinus* snails and causing urinary schistosomiasis in Africa and the Arabian peninsula), *S. mansoni* (transmitted by *Biomphalaria* snails and causing intestinal and hepatic schistosomiasis in Africa, the Arabian peninsula, and South America), and *S. japonicum* (transmitted by the amphibious snail *Oncomelania* and causing intestinal and hepatosplenic schistosomiasis in China, the Philippines, and Indonesia).

S. intercalatum and *S. mekongi* are only of local importance. *S. japonicum* is a zoonotic parasite that infects a wide range of animals, including cattle, dogs, pigs, and rodents. *S. mansoni* also infects rodents and primates, but human beings are the main host. A dozen other schistosome species are animal parasites, some of which occasionally infect humans. Unlike other trematodes, schistosomes have separate sexes, but males and females are found together. The male is short and stout and holds the relatively long female worm in its gynaecophoric canal, a groove like structure. With *S. haematobium*, both male and female live together in the veins that drain the urinary bladder, pelvis, and ureter, whereas *S. japonicum* and *S. mansoni* live in the inferior and superior mesenteric veins, respectively. Hence, these flukes are known as blood flukes. These species are distinguished from the other *Schistosoma* species based on the morphology of their eggs and their adult and cercarial forms. *S. haematobium* eggs have a terminal spine, whereas *S. mansoni* and *S. japonicum* eggs have lateral spines and central spines, respectively.

Morphology

The adult males measure up to 15 millimetres in length and females up to 10 mm. The schistosomes remain in copula throughout their life span, the uxorious male surrounding the female with his gynaecophoric canal. The male is actually flat but the sides roll up forming the groove. The cuticle of the male is covered with minute papillae. The female only possess these at the anterior and posterior end as the middle section being covered by the male body. Oral and ventral suckers are present, with the ventral one being larger serving to hold the worms in place, preventing them from being carried away by the circulatory current.

The ova of *S. mansoni* are 114-175 μm long by 45-68 μm wide. They are light yellowish brown, elongate and possess a lateral spine. The shell is acid fast when stained with modified Ziehl-Neelsen Stain.

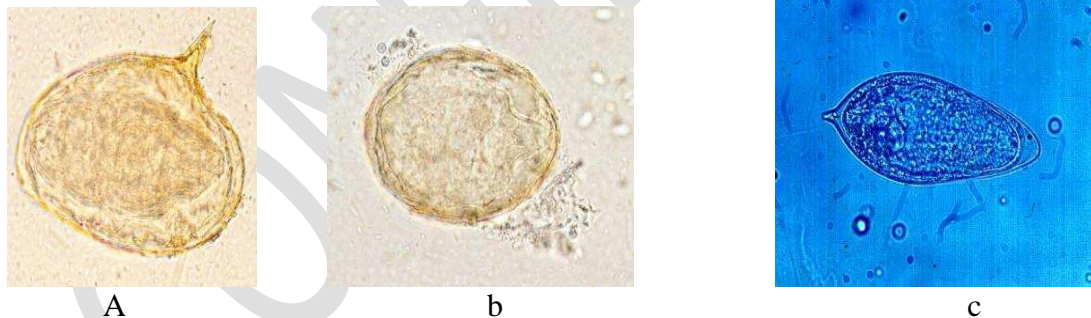


Fig 2.1: Saline smear of (a) *S. mansoni* (b) *S. japonicum* (c) *S. haematobium* ova showing their spine position; a good distinguishing feature when identifying Schistosome ova

Comparative Features of Major Human *Schistosoma* Species

Stages	<i>S. haematobium</i>	<i>S. mansoni</i>	<i>S. japonicum</i>
Adult			
Body surface of Male	Finely tuberculate	Grossly tuberculate	Nontuberculate(smooth)
Testes	4-6, in a cluster	6-9, in a cluster	7, in a linear series
Position of ovary	Posterior to middle of body	Anterior to middle of body	Posterior to middle of body
Number of eggs in uterus	20-30	1-4	50-300
Egg Size and shape	110-170 µm long 40-70 µm wide Terminal spine	114-175 µm long 45-68 µm wide Lateral spine	70-100 µm long 50-65 µm wide Central spine
Cercaria Cephalic glands	2 pairs, oxyphilic	2 pairs, basophilic 4	pairs, oxyphilic

Life Cycles and Transmission of Schistosomes

Once the eggs are laid by the adult female worms, the majority of them first pass through the veins of the blood vessel in which the worm is living, and then into the lumen of the intestine and are passed in the faeces (*S. mansoni* and *S. japonicum*) or into the lumen of the bladder, and are then passed in the urine (*S. haematobium*). Those eggs that reach fresh water hatch, releasing a miracidium which, to develop further must infect a specific snail species within 24 hours. The eggs of each species are markedly different but each produce virtually identical miracidium. A single miracidium can multiply in the snail to produce nearly 100,000 cercariae.

Asexual multiplication takes place in the snail, and results in the release of cercariae (minute in size with forked tails, 200µm long) into the water about 3 – 6 weeks later. Cercariae actively swim around and when they have located, or come into contact with a definitive host, they actively penetrate the skin. They can stay active looking for a host for 24-48 hours after which if they don't find a host they will die.

The head of the cercariae migrates to the liver and develops into either adult male or female worms (flukes), where they pair up and then migrate to their region of the venous blood system (species specific sites). The females leave the males and moves to smaller venules closer to the lumen of the intestine or bladder to lay her eggs (about 6 weeks after infection). The majority of adult worms live from 2-4 years, but some can live considerably longer.

SCHISTOSOMA MANSONI, HEMATOBIMUM AND JAPONICUM

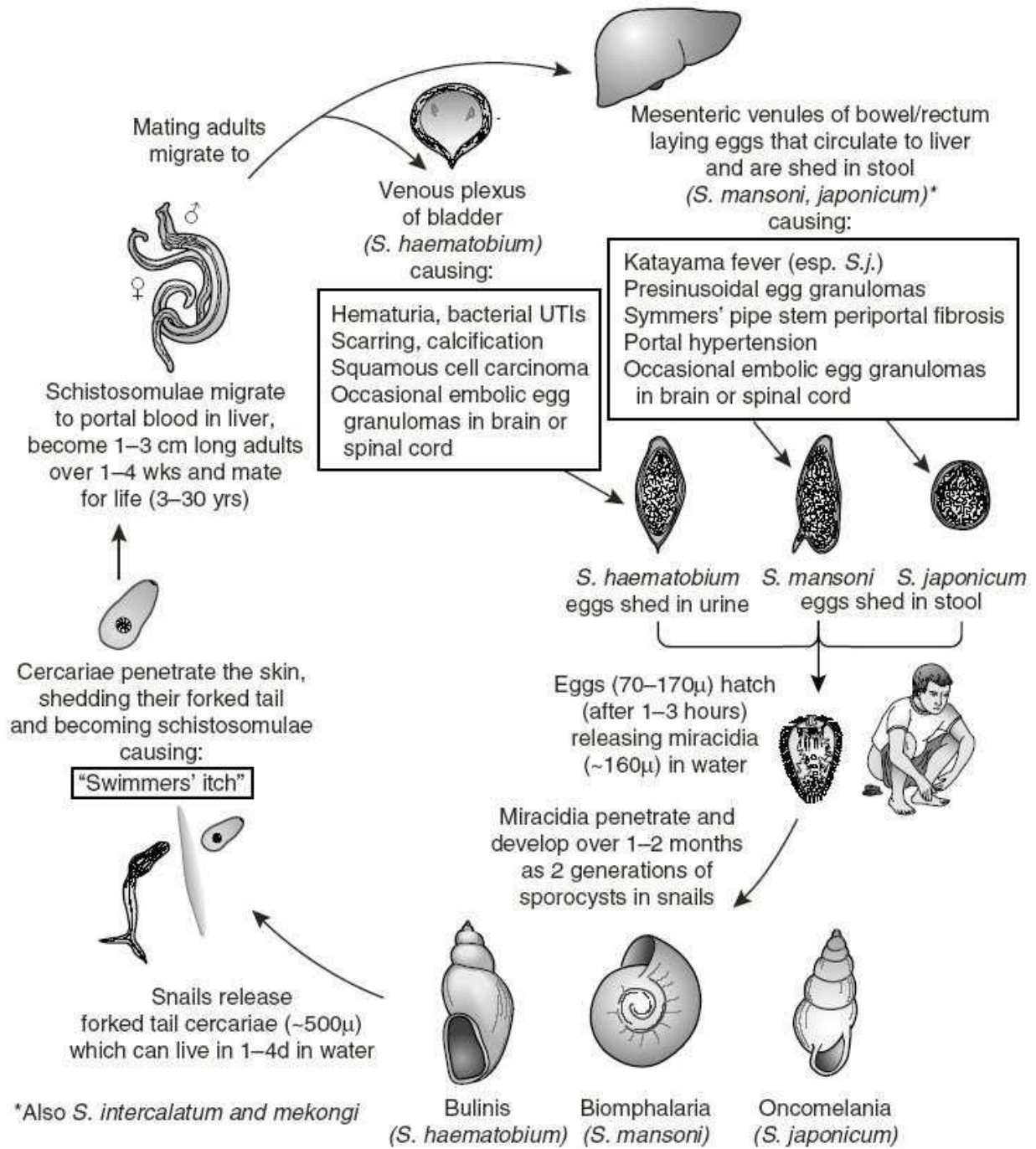


Fig 2.2: Life Cycle of *Schistosoma* spp

Vectors and Geographical Areas Associated With Certain Trematode Types

Snail Host	Geographical Area	Trematode
<i>Biomphalaria glabrata</i>	Brazil	<i>S. mansoni</i>
<i>Biomphalaria pfeifferi</i>	Nigeria	<i>S. mansoni</i>
<i>Bulinus globosus</i>	Nigeria	<i>S. haematobium</i>
<i>Bulinus truncates</i>	Iran	<i>S. haematobium</i>
<i>Oncomelania hupensis nosophora</i>	Japan	<i>S. japonicum</i>
<i>Thiara granifera</i>	China	<i>Paragonimus westermani</i>
<i>Semisulcospira libertine</i>	China	<i>Paragonimus westermani</i>
<i>Pirenella conica</i>	Egypt	<i>Heterophyes heterophyes</i>
<i>Lymnaea truncatula</i>	England	<i>Fasciola hepatica</i>
<i>Lymnaea natalensis</i>	Nigeria	<i>Fasciola gigantica</i>

Pathology and clinical symptoms

- **Acute manifestations**

i. Cercarial dermatitis, also known as swimmer's itch, is an allergic reaction caused by the penetration of cercariae in persons who have been exposed to cercariae in salt water or fresh water. Cercarial dermatitis manifests as petechial haemorrhages with oedema and pruritus, followed by maculopapular rash, which may become vesicular. The process is usually related to avian schistosomal species of the genera *Trichobilharzia*, *Gigantobilharzia*, and *Orientobilharzia*, which do not develop further in humans.

ii. Katayama syndrome corresponds to maturation of the fluke and the beginning of oviposition. This syndrome is caused by high worm load and egg antigen stimuli that result from immune complex formation and leads to a serum sickness –like illness. This is the most severe form and is most common in persons with *S. mansoni* and *S. japonicum* infections. Symptoms include high fever, chills, headache, hepatosplenomegaly, lymphadenopathy, eosinophilia, and dysentery. A history of travel in an endemic area provides a clue to the diagnosis.

- **Chronic manifestations**

i. Symptoms depend on the *Schistosoma* species that causes the infection, the duration and severity of the infection, and the immune response of the host to the egg antigens.

ii. Terminal haematuria, dysuria, and frequent urination are the main clinical symptoms of urinary schistosomiasis.

iii. The earliest bladder sign is pseudotubercle, but, in long-standing infection, radiography reveals nests of calcified ova (sandy patches) surrounded by fibrous tissue in the submucosa.

iv. Dysentery, diarrhoea, weakness, and abdominal pain are the major symptoms of intestinal schistosomiasis.

v. A reaction to schistosomal eggs in the liver causes a periportal fibrotic reaction termed Symmers clay pipestem fibrosis.

vi. Haemoptysis, palpitation, and dyspnea upon exertion are the symptoms of schistosomal cor pulmonale that develops as a complication of hepatic schistosomiasis.

vii. Headache, seizures (both generalized and focal), myeloradiculopathy with lower limb and back pain, paresthesia, and urinary bladder dysfunction are the noted symptoms of CNS schistosomiasis due to *S.japonicum* infection.

Diagnosis

Intestinal schistosomes

- Laboratory confirmation of *S. mansoni* and *S. japonicum* infection can be made by finding the eggs in the faeces. When eggs cannot be found in the faeces, a rectal biopsy can be examined.
- Serological tests are of value in the diagnosis of schistosomiasis when eggs cannot be found. An enzyme linked immunosorbent assay (ELISA) using soluble egg antigen, is employed at HTD.

Urinary schistosome

The definitive diagnosis of urinary schistosomiasis is made by finding the characteristic ova of *S. haematobium* in urine. Terminal urine should be collected as the terminal drops contain a large proportion of the eggs. The urine can then be centrifuged and the deposit examined microscopically for ova. Eggs can sometimes be found in seminal fluid in males.

- A bladder biopsy is seldom necessary to make the diagnosis. A rectal snip may show the presence of ova as they sometimes pass into the rectal mucosa.
- Serological tests can be of value when eggs cannot be found in clinical samples. An enzyme linked immunosorbent assay using soluble egg antigen to detect antischistosome antibody is most sensitive.

Note: There is a marked periodicity associated with the time when most eggs are passed out. Higher numbers of eggs are encountered in urine specimens passed between 10 am and 2pm, presumably as a result of changes in the host's metabolic and physical activities.

Epidemiology of Schistosomiasis

The following factors are of epidemiological importance in the transmission of schistosomiasis:

- The presence of water bodies such as rivers, streams, lakes, dams suitable for the breeding of the snail intermediate hosts
- Presence of appropriate snail hosts necessary for the developments of the asexual stages and transmission of the infective stage to the human definitive host
- Contamination of natural water bodies with infected human urine and faeces
- Human water contact activities including swimming, laundry and fetching
- Factors that promote intramolluscan development of parasite and subsequent transmission to man
- Socio-economic status of the people such as good sanitary system and water supply

Control

- Reduction of human-water contact
- Improved sanitation by proper waste disposal
- Attacking the adult forms of parasite through chemotherapy to reduce the worm burden or egg production
- Eradication or reduction of snail population through the use of molluscicides
- Development of vaccine to induce immunity

- Modification of the ecology of the snail habitat
- Biological control through the introduction of competitors snails into the snail habitat
- Education

3.2 Lung flukes (*Paragonimus* species)

The genus *Paragonimus* contains more than 30 species that have been reported to cause infections in animals and humans. Among these, approximately 10 species have been reported to cause infection in humans, of which *P. westermani* is the most important. *P. westermani*, also known as the Oriental lung fluke is most common in China, Korea, Thailand, Philippines, and Laos. Isolated endemic foci have also been reported from the states of Manipur, Nagaland, and Arunachal Pradesh in India. A low prevalence has been reported from African countries of Cameroon and Nigeria, where infections with *Paragonimus africanus* and *Paragonimus uterobilateralis* were reported. Humans are infected by eating raw or partially cooked crab or crayfish or crabs soaked in wine as a food delicacy or by drinking juice from raw crabs or crayfish as a part of a food habit.

It inhabits parenchyma of the lung close to bronchioles in humans, foxes, wolves, and various feline hosts (e.g, lions, leopards, tigers, cats). *Paragonimus* species belong to the family 'Troglotrematidae' and they possess the following striking features:

- The adult worm is reddish brown fluke.
- The body of adult worm is thick, fleshy, and ovoid in shape
- The tegument is spiny or scaly
- Have weak suckers
- Testes lie side by side
- Uterus is short with a few tight uterine coils forming a 'rosette'
- Extensive vitellaria in lateral fields
- Cercariae are microcercous xiphidiocercariae
- The eggs are ovoid, brownish yellow, thick shelled and operculated.

Life cycle and Transmission

The infection is typically transmitted via ingestion of metacercariae contained in raw freshwater crabs or crayfish. Additionally, consumption of the raw meat of paratenic hosts (e.g, omnivorous mammals) may also contribute to human infection. Freshwater snails and crabs are first and second intermediate hosts of *Paragonimus* species, respectively. In the duodenum, the cyst wall is dissolved, and the metacercariae are released. The metacercariae migrate by penetrating through the intestinal wall, peritoneal cavity, and, finally, through the abdominal wall and diaphragm into the lungs. There, the immature worms finally settle close to the bronchi, grow, and develop to become sexually mature hermaphrodite worms.

Adult worms begin to lay the eggs, which are unembryonated and are passed out in the sputum. However, if they are swallowed, they are excreted in the feces. The eggs develop further in the water. In each egg, a ciliated miracidium develops during a period of 2-3 weeks. The miracidium escapes from the egg and penetrates a suitable species of snail (first intermediate host), in which it goes through a generation of sporocysts and 2 generations of rediae to form the cercariae. The cercariae come out of the snail, invade a freshwater crustacean (crayfish or crab), and encyst to form metacercariae. When ingested, these cause the infection, and the cycle is repeated.

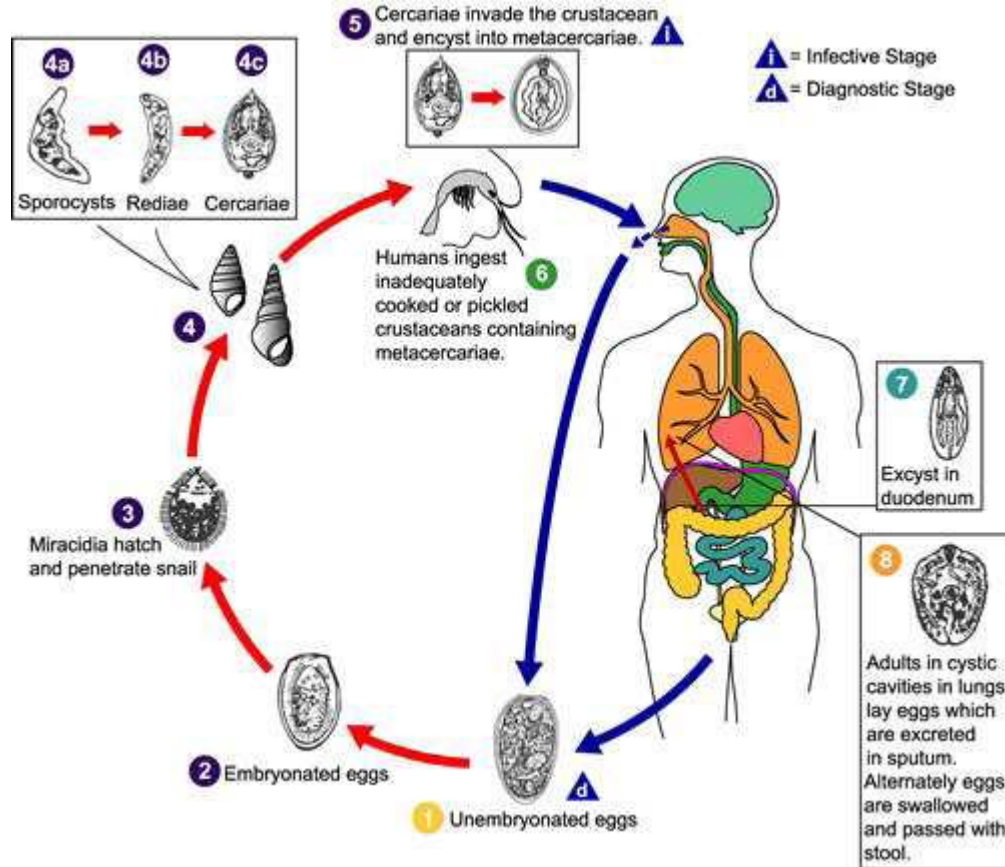


Fig 2.3: Life cycle of *Paragonimus* spp

Pathology and clinical symptoms

- i. **Acute manifestations:** Acute pulmonary infection is characterized by low-grade fever, cough, night sweats, chest pain, and blood-stained rusty-brown sputum.
- ii. **Chronic manifestations:** Lung abscess or pleural effusion develops in individuals with chronic infections. Fever, haemoptysis, pleurisy pain, dyspnea and recurrent attacks of bacterial pneumonia are the common symptoms. The condition mimics pulmonary tuberculosis.
- iii. Fever, headache, nausea, vomiting, visual disturbances, motor weakness, and localized or generalized paralysis are the symptoms of cerebral paragonimiasis.

Diagnosis

Diagnosis is based on finding the characteristic eggs in brown sputum. The eggs can also be found in the faeces due to swallowing sputum. A chest x-ray may show cystic shadows and calcification. Serological tests, in particular, the ELISA method, are useful diagnostic tests.



Fig 2.4: Saline smear of *Paragonimus westermanni* egg. The egg shells are thick and operculated

Epidemiology

The epidemiology of the disease (Paragonimiasis) depends on one of the following

- Presence of appropriate snail, crab and mammalian reservoir hosts in the area
- Pollution of snail habitats with sputum and faeces of man as well as natural mammalian reservoir hosts infected with the parasite
- Consumption of metacercariae through eating of raw or undercooked crabs or through contamination of the fingers and cooking utensils with metacercariae while cleaning

Control

- Proper cooking of crabs before consumptions
- Proper waste disposal

3.3 Liver fluke (*Fasciola hepatica* and *F. gigantica*)

Fascioliasis is a zoonotic disease caused by infection with *F. hepatica*. It is a major disease of livestock that is associated with important economic losses due to mortality; liver condemnation; reduced production of meat, milk, and wool; and expenditures for anthelmintics. The disease has a cosmopolitan distribution, with cases reported from Scandinavia to New Zealand and southern Argentina to Mexico. Also of importance is the West Africa species of *Fasciola* (*F. gigantica*).

The two share similar morphology, life cycle and pathogenicity. They belong to the family 'Fasciolidae' having the following major features;

- They are large with flattened leaf-like forms
- They have ramifying and complicated digestive and reproductive systems
- Most members of the family inhabit the liver and the bile duct. However, *Fasciolopsis buski* inhabits the intestine
- Cercariae are gymnocephalous
- Metacercariae encyst on vegetation thus establishing a two-host cycle

Morphology of the Adult

- They are leaf-like with oral cone and shoulder at anterior end
- The intestinal caeca, testes and ovary are branched
- Tight and relatively short uterus is opposite to the ovary at the anterior end
- Vitellaria are extensive and are laterally distributed

Distinctions between *F. hepatica* and *F. gigantica*

- *F. gigantica* is larger (75 by 12mm) while is smaller (30 by 13mm)
- *F. gigantica* is oblong with prolonged posterior end while *F. hepatica* is more or less triangular in shape
- The eggs of *F. gigantica* is also larger

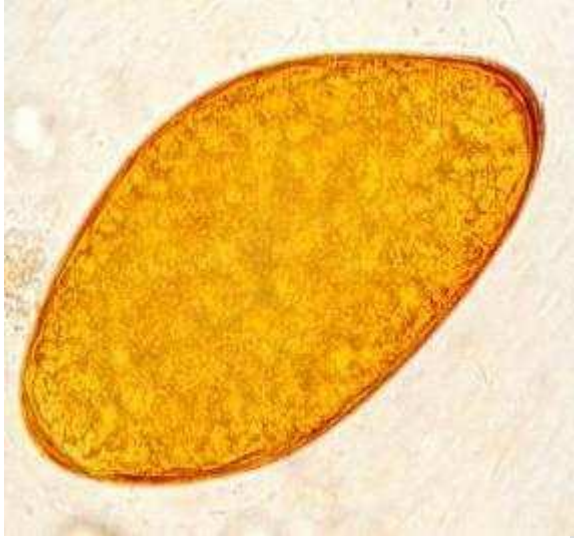


Fig 2.5: Egg of *Fasciola*; ova of *Fasciola* are ovoid in shape, quinine colour and often showing an inconspicuous operculum. *Fasciola hepatica* ova measure 130 - 150 μ m by 63 - 90 μ m. There is much cross-over in ova size between all of the *Fasciola* species.

Life cycle and Transmission

Opercular eggs are passed out from the faeces of the infected animal (cattle or sheep). The eggs embryonate in the presence of light as stimulus and hatch into miracidia which locate an appropriate snail intermediate host by a chemical response called **chemotaxis**. The snail host of *F. hepatica* is *Lymnaea truncantula* while that of *F. gigantica* is *L. natalensis*. The intramolluscan development of miracidium produces sporocysts which in turn develop rediae. The mother rediae produce second generation of rediae (daughter rediae) which later give rise to gynocephalous cercariae. This crawls out of the snail, locates submerged plant, loses its tail and encysts into metacercariae. Infection occurs when sheep and cattle ingest plant with metacercariae during grazing. Metacercariae excyst in the duodenum and the emerging young adult punctures its way into the body cavity and wanders around until it locates the liver capsule. It burrows into the tissues, feeding on the cells until it gets to the bile duct where it eventually attains maturity.

Pathology

- Pathology depends on the intensity of infection and duration of the disease.
- Fluke causes biliary obstruction. Because of pressure, toxic metabolic products, and feeding habits, the worms provoke inflammatory, adenomatous, and fibrotic changes of the biliary tract.
- Parenchymal atrophy and periportal cirrhosis develop.
- Severe headache, chills, fever, urticaria, a stabbing substernal pain, and right upper quadrant pains that radiate to the back and shoulders may be the first evidence of infection.

- As infection progresses, an enlarged tender liver, jaundice, digestive disturbance, diarrhoea and anaemia develops.

Laboratory diagnosis

- Definitive diagnosis is made by observing the ova in faeces.
- Where identification cannot be made from the size of the ova, clinical information and the source of infection may help to provide a diagnosis. This includes an enlarged tender liver and a febrile eosinophilic syndrome
- Positive complement-fixation test and intracutaneous reactions with *Fasciola* antigens are used when direct faecal examination fails to reveal the eggs.

Epidemiology and Control

Fascioliasis is prevalent in areas where cattle or sheep graze and in areas where appropriate lymnaeid snail hosts flourish. Therefore control measures involves

- Treatment of animals to improve general condition and reduce egg output
- Breaking of transmission cycle by eradicating the snail hosts. However, this is difficult to achieve on the field
- Infection in humans can be prevented by eliminating raw water cress and other uncooked green vegetable from the diets.
- A safe water supply is also necessary

3.4 Intestinal flukes (*Fasciolopsis buski*, *Heterophyes heterophyes*, *Metagonimus yokogawai*)

F. buski is the most common intestinal nematode that causes infections in humans. It is widely distributed in Asia and the Indian subcontinent, especially in areas where humans raise pigs and consume freshwater plants. The trematodes *H. heterophyes* and *M. yokogawai* are less-common causes of human infection.

Life cycle

F. buski, known as the giant intestinal fluke, is found in the duodenum and jejunum of pigs and humans and is the largest intestinal fluke to parasitize humans. Humans are infected by eating freshwater aquatic plants such as water caltrops, water chestnuts, and water bamboo, which can harbour the metacercariae. In the intestine, the metacercariae excyst, attach to the duodenum or jejunum, develop, and grow into adult worms. They lay unembryonated eggs, which are excreted in the faeces.

In water, inside the egg, a ciliated miracidium develops, comes out, and penetrates a suitable snail host. Inside the snail, after several stages of asexual multiplication, large numbers of cercariae are produced. The latter emerge from the snail and encyst on the surface of aquatic plants to metacercariae. Ingestion of these plants causes infection in humans, and the cycle is repeated.

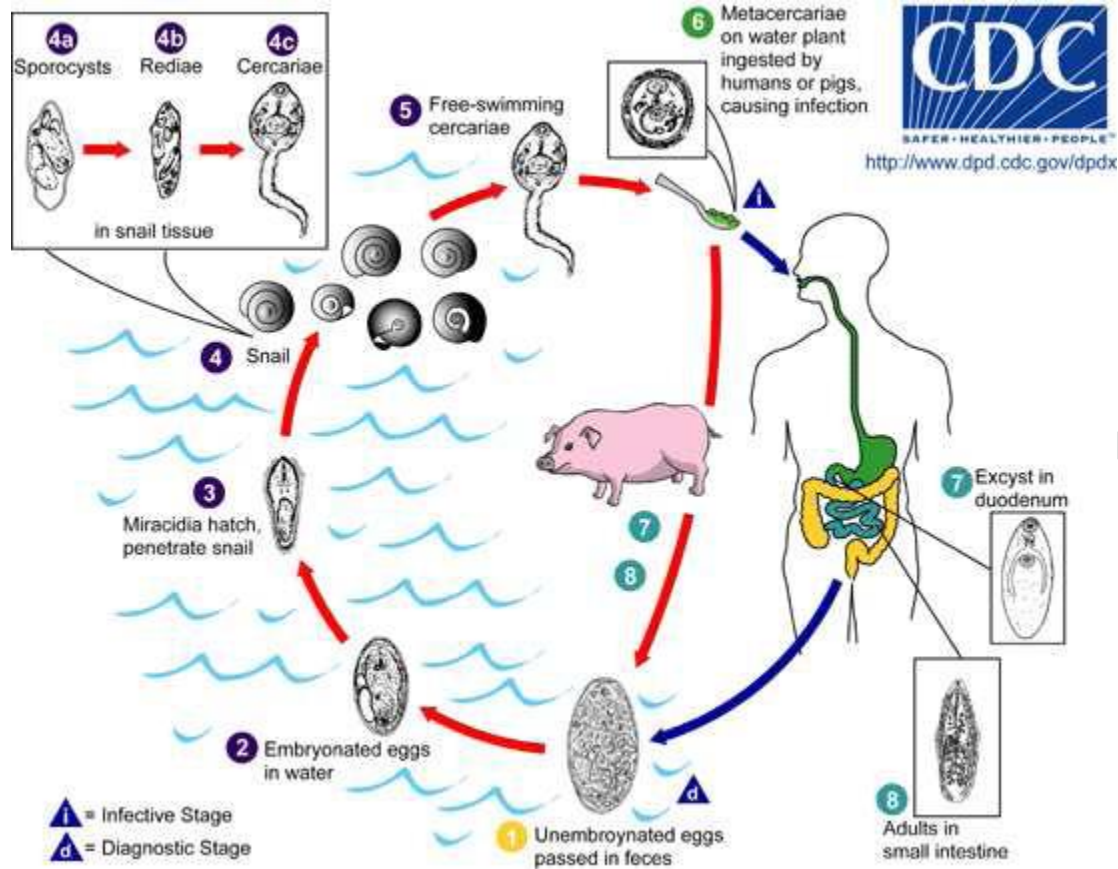


Fig 2.6: Life cycle of *F. buski*

Source: <http://www.dpd.cdc.gov/dpdx>

Morphology

Eggs of *Fasciolopsis buski* are broadly ellipsoidal, operculated and measure 130-150 μm long by 60-90 μm wide. The eggs are unembryonated when passed in feces. The eggs of *F. buski* can be difficult to distinguish from *Fasciola hepatica*, although the abopercular end of the latter often has a roughened or irregular area.

The adults of *F. buski* measure 20-75 mm long and have poorly-developed oral and ventral suckers. Adults reside in the intestine of the mammalian host.



Fig 2.7: The egg and the adult forms of *F. buski*

Clinical Features

Most infections are light and asymptomatic. In heavier infections, symptoms include diarrhoea, abdominal pain, fever, ascites, anasarca and intestinal obstruction.

Laboratory Diagnosis

Microscopic identification of eggs, or more rarely of the adult flukes, in the stool or vomitus is the basis of specific diagnosis. The eggs are indistinguishable from those of *Fasciola hepatica*.

3.5 Pancreatic flukes (*Eurytrema pancreaticum*, *E. coelomaticum*, *E. ovis*)

These are widely distributed in China, Korea, Japan, Hong Kong, South America, etc. *E. pancreaticum* is a common parasite of pancreatic (or rarely bile) ducts of herbivorous mammals, i.e., cattle, sheeps, goats, monkeys, and camels.

Life cycle

The adult flukes live in the pancreatic passages of the herbivores. Eggs are passed in the faeces and ingested by land snail, which is the first intermediate host (snail). The cercariae develop into infective metacercariae only if ingested by grasshoppers, the second intermediate host. The life cycle is completed when the infected insects are eaten by grazing herbivores. The metacercariae excyst and migrate to the pancreatic passage, where they develop into adults. Humans become infected when they accidentally swallow infected grasshoppers.

Morphology

The parasite (10~18 x 5~9 mm in size) is broad, flat, and oval to fusiform. The suckers are large, the oral sucker is larger than the ventral sucker. The eggs (50~80 x 35~40 μm) are embryonated in the uterus.



Fig 2.8: *E. pancreaticum* : from pancreas of cattle, Acetocarmine stain, X40

Pathology and clinical symptoms

Eurytrema is usually characterised by mild symptoms. Heavy infections, however, may be marked by gastrointestinal disturbances, including abdominal distress, flatulence, vomiting, diarrhoea or constipation, jaundice, an enlarged liver, and systemic symptoms. Eosinophilia is rare.

Diagnosis

Diagnosis is made by finding the characteristic eggs in faeces. Spurious infection must be ruled out by repeated examination. Eggs of the *Dicrocoelium dendriticum* and *E. pancreatum* are almost indistinguishable. Definitive diagnosis can be made by recovery of adult flukes at surgery or autopsy.

Prevention

Human infections are generally accidental.

4.0 Conclusion

5.0 Summary

Digenetic trematodes are the medically important groups of trematodes that inhabit different tissues and organs of their hosts. Hence, they are named according to their various locations in the parasitised hosts. Diagnosis is dependent on the route through which parasites' eggs are voided out of the host. Therefore, the faecal, urine and sputum samples are examined microscopically to identify the characteristic eggs. The pathological effects vary from mild to severe due to the parasite burden in the host. Proper waste disposal, and proper cooking of crabs, fishes and land snails which act as intermediate host are some of the control measures.

6.0 Tutor Marked Assignments

6.1 Activity: Examine the eggs of the various digenea trematode

6.2 Answer the following questions:

- i. Describe Blood flukes (*Schistosoma* species) (LO2)
- ii. What are the main schistosomes that infect human beings? (LO2)
- iii. Tabulate the comparative features of major human *Schistosoma* species (LO2)
- iv. With a well labeled diagram describe the life cycles and transmission of schistosomes (LO1)
- v. Explain the Pathology and clinical symptoms of blood flukes (LO6)
- vi. Describe the diagnosis of blood flukes (LO5)
- vii. Enumerate the epidemiology of schistosomiasis (LO2)
- viii. How can schistosomiasis be controlled? (LO4)
- ix. Highlight the differences in the mode of transmission cycles of *S.haematobium* and *H. heterophyes* (LO3)
- x. What are the morphological differences in the species of schistosomes? (LO5)

7.0 References and other resources

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MODULE SIX- CESTODES**Introduction**

The cestodes consist of two separate subclasses, the Cestodarians, parasites of fish and other cold blooded vertebrates. These are non-segmented parasites, with only a single set of sexual organs. In contrast, the more well known members of the Subclass Eucestoda are parasites of both warm and cold blooded vertebrates, including mammals such as man. The module has three units and deals with basic body plan of a cestode, tapeworms and examples and tapeworms of man and other human's cestodes

Module Objective

At the end of this module you should be able to:

- i. Describe tapeworms of man and other human cestodes

Contents

Unit 1: Basic body plan of a cestode

Unit 2: Tapeworms and Examples

Unit 3: Tapeworms of man and other human's cestodes

UNIT 1- BASIC BODY PLAN OF A CESTODE**CONTENT**

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main contents
 - 3.1** The adult parasite
 - 3.2** The cestode integument
 - 3.3** Larval metacercaria
 - 3.4** Metacestodes
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
 - 6.1** Activity
 - 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 Introduction

Cestodes resemble a colony of individual animals in that their bodies are divided into a series of segments (the proglotids), each with their own complete set of internal organs. There may be many hundreds of these proglotids, resulting in the complete parasite having a long, ribbon like body. The appearance of this long body is the origin for the common name for these parasites, the tapeworms. The common names of these parasites are often derived from their intermediate hosts, ingestion of which results in their infection, e.g. the Fish, Beef and Pork Tapeworms. Alternatively they may be named after the definitive host that the adult parasites are normally

found in. For example the Rat tapeworm *H. diminuta* and the Dog Tapeworm *Dipylidium caninum*. The study of the morphology of the cestode body may be divided into two distinct areas.

Firstly, the morphology of the adult cestode (the tapeworm) and secondly the morphology of the cestode larvae, or Metacestode.

2.0 Learning Objectives

At the end of this unit, you should be able to

- i. Identify the striking features of cestodes
- ii. Describe the egg, larva and adult stage morphology of common cestodes

3.0 Main Content

3.1 The Adult Parasite

The body of the adult tapeworm may be divided into three regions.

The Scolex

This is the "head" and attachment organ of the parasite. There are four main types of scolex, by which the tapeworm may be taxonomically classified.

i. No special attachment organs

The scolices of some tapeworms of the order Caryophyllidea (parasites of freshwater fish) have no special attachment organs. (NB. Some authors do not recognise this taxonomic order, placing these parasites within the Pseudophyllidea).

ii. **A Bothria** - This is composed of a pair of shallow, elongated, weakly muscular grooves. Tapeworms of the order Pseudophyllidea are equipped with bothria on their scolices.

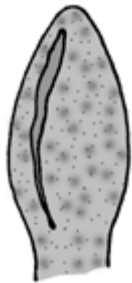


Fig 1.1: The bothria of Pseudophyllidea

iii. **A Bothridia** - These are broad, leaflike muscular structure, exhibiting a large degree of variation. Some bothridia are sessile, some are stalked, whilst others are hooked with accessory suckers. Tapeworms of the order Tetraphyllidea and others are equipped with bothridia.

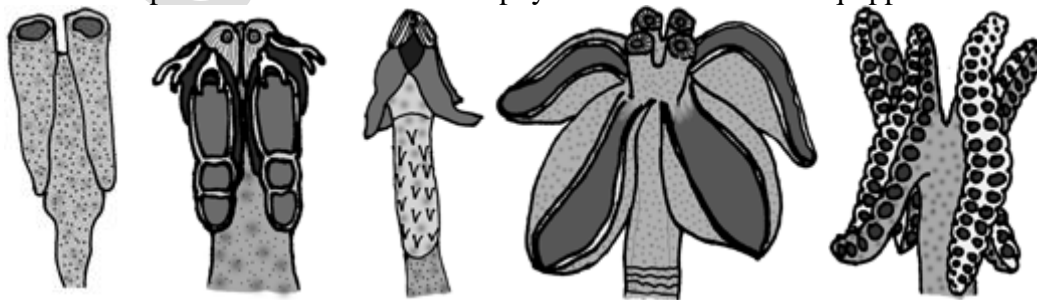


Fig 1.2

iv. Acetabulate Suckers - Tapeworms of the order Cyclophyllidea are equipped with four acetabulate suckers. Parasites in this order may also have additional features at the apex of the scolex such as;

- Glandular areas
- Protrusible suckers
- Suckers armed with hooks
- Hooks (e.g. *Taenia*)
- A rostellum, an eversible muscular proboscide, often covered with hooks (e.g. *Hymenolepis*, *Echinococcus*, *Dipylidium*)
- A Myzorhynchus (a protrusible muscular mass).

The Neck

This is the area of proliferation of the parasite, from which the proglottids of the strobila grow.

The Strobila

This is composed of a series of proglottids. Each proglottid contains a complete set of male and female reproductive organs, although these organs usually mature at different rates. Usually the male organs develop before the female organs, and degenerate before the female organs mature. The large, gravid proglottids at the posterior end of the tapeworm are full of developing, or in the extreme terminal proglottids, mature eggs.

3.2 The Cestode Tegument

The related cestodarians that also belong within the cestodes, have a tegument that appears to be intermediate with that of the eucestodes and monogeneans. This is another piece of evolutionary evidence that indicates a monogenean origin for the tapeworms. In this case the surface of the cestodarian tegument is covered with numerous microvilli, similar in form to the eucestode microtriche (see below), but lacking the electron dense cap seen in these parasites.

The cestode tegument is a syncytial layer, showing many features typical of that found in other parasitic platyhelminthes.

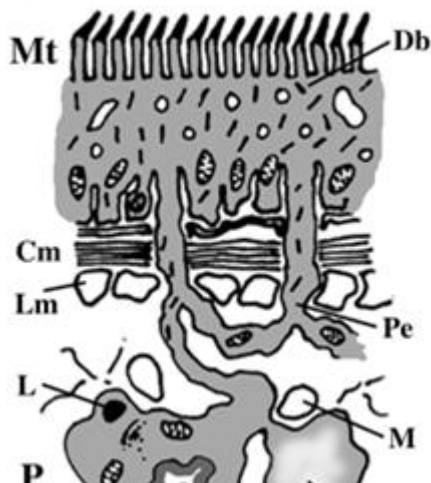


Fig 1.3: Diagram showing eucestode tegument

There are however, a number of distinguishing features present in these parasites. On the very outer surface of the tegument a surface glycocalyx is seen to cover the outer plasma membrane. Below this glycocalyx, a characteristic feature of the eucestode tegument is the presence of numerous microtriches (**Mt**), long spine like processes that are in fact a highly modified form of microvilli. Each microtrich has a hard, pointed, electron dense cap which is separated from the rest of the microtrich by a crescent shaped membranous cap. The microtriches are thought to serve two functions. Firstly, the tapeworms do not possess a gut and must absorb all of their nutrients across the surface tegument. The microtriches greatly increase the surface area of the parasite, and can be seen as an adaptation to maximise the amounts of nutrients available to the parasite. This is supported by the finding of microtubules in the shaft of the microtriches. Secondly the spine like character of the microtriches probably help the parasite maintain its position in the gut. This can be more clearly seen by comparing the microtriches found in different regions of the parasite's body. It has been noted in many species that the microtriches found covering the scolex, the attachment organ of the parasite, were much longer than those covering the strobila, and in some species show special adaptations. For example the microtriches covering the strobila of *E. granulosus* have been found to show curved hooks or sometimes even barbs. Below the layer of microtriches the main syncytial layer of the tegument is found. This has been seen to contain numerous vesicles and membrane bound, electron dense rod-like structures, referred to as disc-shaped bodies (**Db**). Finally numerous mitochondria, mainly in the distal region of the tegument, may be seen. These are unusual in that they do not have many cristae, reflecting the anaerobic metabolism of the organism. The tegumental nuclei are however not located in this outer layer, but are found within subtegumental cell bodies (**StC**), located beneath the circular (**Cm**) and longitudinal muscle (**Lm**) layers, embedded within the parenchymal tissues (**P**) and mesenchymal musculature (**M**). These subtegumental cell bodies also contain other cellular elements such as golgi apparatus and lipid inclusion bodies (**L**) which are connected to the outer syncytium and areas of glycogen storage (**Gs**) by long protoplasmic extensions (**Pe**). The location of these important cellular elements away from the outer surface of the parasite, exposed to immunological attack by the parasites host, is an important adaptation to a parasitic lifecycle adopted by all of the parasitic platyhelminthes. The parenchymal tissues are similar to those of the trematodes and fill the spaces between the parasites internal organs (all cestodes and other platyhelminthes being acoelomate organisms). These tissues are a syncytial network formed by anastomosis of mesenchymal cells, with spaces filled with carbohydrate rich parenchymal fluid.

3.3 Larval Metacercaria The Larval Cestodes

i. The Cestodarians

The cestodarians larvae, or **lycophore** are free swimming, being covered in cilia. They have a set of ten hooks at the extreme anterior of the body, thus differing from the larval eucestodes, which are equipped with 3 pairs of hooks. Anteriorly they are armed with penetration glands. The bodily form of these larvae bears a marked resemblance to the larvae of the trematodes, such as the miracidium in the digeneans, and the larval monogenean, the oncomiracidium.

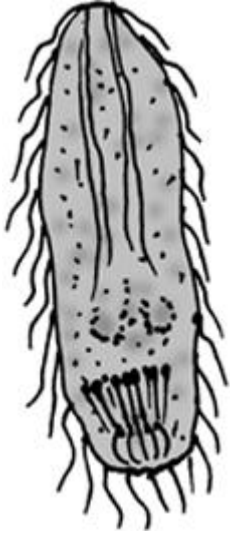


Fig 1.4: A lycopore

ii. The Eucestodes (Tapeworms)

The eggs of Pseudophyllidean and Cyclophyllidean cestodes differ considerably. The egg of the pseudophyllidean tapeworm closely resembles that of the trematodes, having a thin shell wall, and an operculum, which on hatching opens to release the free swimming larvae. This illustrates the close relationship between the two major groups of platyhelminth parasites. In contrast, the egg of the cyclophyllideans tapeworms is very different, having a very thick, resistant egg shell, with no operculum.



Fig 1.5: The pseudophyllidean and cyclophyllidean ova

The larvae emerging from these eggs also differ. The pseudophyllidean egg hatches to release a free swimming larvae called a coracidium. This has an outer layer of ciliated epidermal cells with which it swims through the water before being ingested by the parasites first intermediate host. This is often a copepod. Inside the copepod the ciliated epidermis is shed, to release a larvae that initially resembles that of the newly hatched cyclophyllideans. This has 6 hooks, arranged in pairs, and is a common feature throughout the eucestodes. On the basis of the presence of these hooks, present in both the eucestodes and cestodarians, many authors believe that the cestodes originally evolved from an ancestor common to the extant monogeneans.



Fig 1.6: Coracidium larva

The larval cyclophyllidean, as with the pseudophyllidean, is equipped with 3 pairs of hooks. Both groups use these hooks to penetrate the gut wall of its intermediate host after being ingested, before developing into the other larval forms described below in more detail.

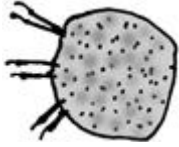


Fig 1.7: Larva of cyclophyllidean

3.4 Metacestodes

A number of different larval forms of cestodes (metacestodes) are seen, these include the following;

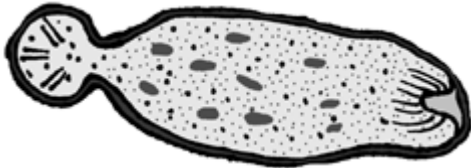


Fig 1.8: A Proceroid of **Pseudophyllidean** (e.g *D. latum*)

A larval form of **Pseudophyllidean** cestodes, (e.g. *D. latum*, *Ligula intestinalis*). Here two forms of the proceroid are shown. Firstly an immature proceroid, and secondly a mature infective proceroid. In the lifecycle of these parasites there are two intermediate hosts. The proceroid being found in the first of these (usually a small crustacean e.g. *Cyclops*). In appearance these larvae have solid bodies with the remains of the embryonic hooks from the onchosphere larvae at the posterior of the parasite.

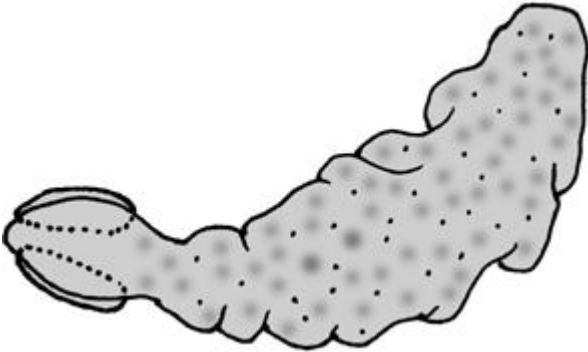


Fig 1.9: A Plerocercoid of **Pseudophyllidean** (e.g *D. latum*)

A larval form of **Pseudophyllidean** and other Cestodes, (e.g. *D. latum*, *Ligula intestinalis*). In the lifecycle of these parasites there are two intermediate hosts (see the cestode life cycle page). The plerocercoid being found in the second of these (usually a fish or amphibian). In appearance, these are elongated larvae with solid bodies which are much larger than the preceding proceroid larvae. In these stages the embryonic hooks are absent. The plerocercoids of some Pseudophyllideans already show the start of the development of the sexual organs (e.g. *Schistocephalus solidus*, *Ligula intestinalis*), whilst those of *Schistocephalus solidus* are also already divided into proglottids).



Fig 1.10: A larval form of **Cyclophyllidean** Cestodes, (e.g. *Hymenolepis* sp.)

A larval form of **Cyclophyllidean** Cestodes, (e.g. *Hymenolepis* sp.). This larval form is usually found in species where the intermediate host is an invertebrate, usually an insect.

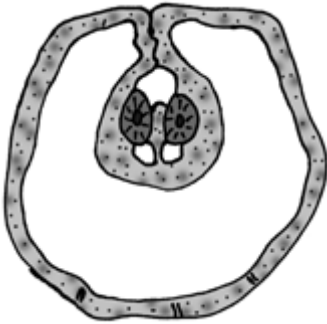


Fig 1.11: A larval form of **Cyclophyllidean** Cestodes, (e.g. *Taenia solium*)

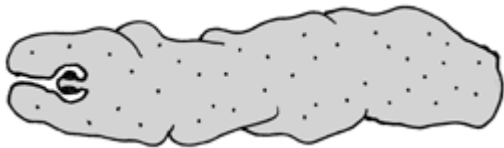


Fig 1.12: A larval form of **Cyclophyllidean** Cestodes, (e.g. *Mesocystoides* sp.).



Fig 1.13: A larval form of **Cyclophyllidean** Cestodes, (e.g. *Taenia taeniaeformis*).

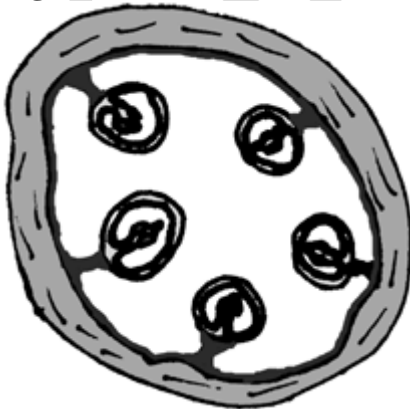


Fig 1.14 A larval form of **Cyclophyllidean** Cestodes, (e.g. *Taenia multiceps*).



Fig 1.15: A larval form of **Cyclophyllidean** Cestodes, (e.g. *Echinococcus granulosus*). - Hydatid cyst

4.0 Conclusion

5.0 Summary

The body plan of adult cestode is divided into scolex, neck and strobila. The scolices of the order Caryophyllidea (parasites of freshwater fish) have no special attachment organs while the Pseudophyllidea have weakly muscular grooves which are armed with bothria. The Cyclophyllidea have four acetabulate suckers. In addition to these are glandular areas, protrusible suckers and rostellum depending on the species of the cyclophyllidean. The strobila is made up of proglottids containing the male and the female reproductive organs. The larvae of cestodes vary with species with some being ciliated and as such are free swimming. Some however, have operculum with thin shell wall. Others have thick shell wall with 6 hooks.

6.0 Tutor Marked Assignments

6.1 Activity:

6.2 Answer the following questions:

- i. Describe the four main types of scolex (LO1)
- ii. Differentiate between the larvae of pseudophyllidean and cyclophyllidean (LO2)
- iii. Give a concise description of the various larval forms of cestodes (LO2)

7.0 References and other resources

Ukoli, F.M.A. (1990). Introduction of Parasitology in Tropical Africa. John Wiley and Sons Ltd., Chichester.

UNIT 2- TAPEWORMS AND EXAMPLES**CONTENT**

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main contents
- 3.1** *Diphyllobothrium latum* (the broad fish tapeworm)
- 3.2** *Dipylidium caninum* (the dog tapeworm)
- 3.3** Tapeworm of the Genus *Hymenolepis*
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
- 6.1** Activity
- 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 Introduction

Cestodes or tapeworms are the most specialised of the Platyhelminthe parasites. All cestodes have at least one, and sometimes more than one, secondary or intermediate host as well as their primary host. While the intermediate hosts are often invertebrates of some sort, the primary host is normally a vertebrate. However, in some cases both hosts are vertebrates, as in the common Beef Tapeworm (*Taenia saginatus*), and in a few species there may be only a single host. A number of tapeworms include mankind in their life cycles but infection is not normally a serious health problem and can be cured. There are more than 1,000 species of tapeworms known to science, and nearly every species of vertebrate is liable to infection from at least one species of tapeworm.

2.0 Objectives

At the end of this unit, you should be able to:

- i. Describe the morphology and life cycle of a named cestode.
- ii. Explain the epidemiology and control of these parasites

3.0 Main Content**3.1 *Diphyllobothrium latum* (the Broad Fish Tapeworm)**

Diphyllobothrium latum is the fish tapeworm of man. It has a fairly cosmopolitan distribution, but is particularly common in the Baltic region, Russia and the Great Lakes region of the U.S.A.

Morphology of the adult Tapeworm

The adult parasites are typically between 2 and 12 m in length by up to 2 cm in width, but may grow even longer in some cases. The anterior organ of attachment is a bothria, a pair of shallow, elongated muscular grooves, typical of tapeworms of the order Pseudophyllidea. The body is divided into proglottids, as is the case of all pseudophyllidean tapeworms. These proglottids are

broader than they are long, except at the terminal end, where they are approximately square in shape. Internally the proglottids are typical of pseudophyllidean tapeworms, with numerous testes and vitellaria arranged on the lateral margins of the proglottid, with a central bilobed ovary. An important difference between this parasite and the other tapeworms of man is that the uterus open to the exterior (cyclophyllidean tapeworms have closed uteruses). Eggs are therefore actively deposited by the parasite, in contrast to the disintegration of the proglottids seen in the other human tapeworms.

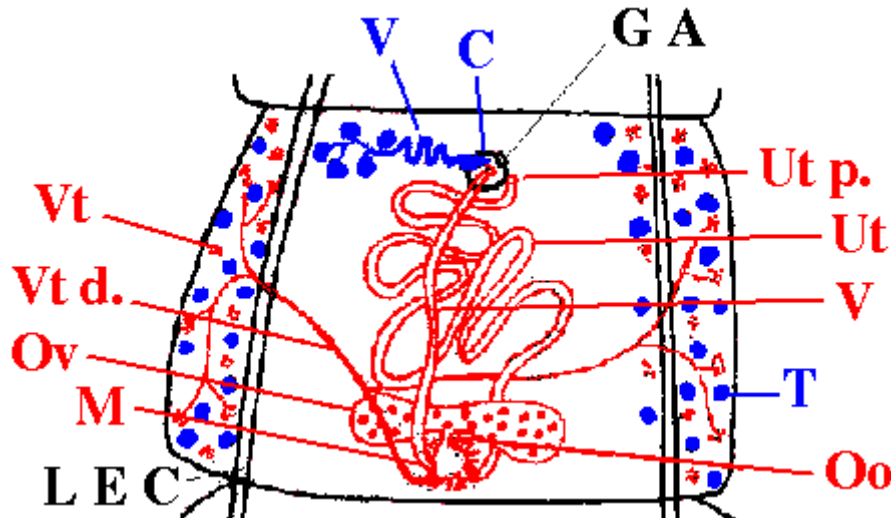


Fig 2.1: The Female Reproductive System The Male Reproductive System of *D. latum*

Key - Ov - Ovary (bilobed in *D. latum.*); Oo - Ootype (where the egg is formed); Ut - Uterus (In the pseudophyllideans this opens to the outside, via the uterine pore); Ut p. - Uterine pore (not present in the cyclophyllideans); V - Vagina (a long straight tube); Vt - Vitelline glands (secreting substances that make up the egg yolk and shell); Vt d. - The Vitelline duct (connecting the vitelline gland, which are diffuse and are situated laterally in *D. latum*); M - The Mehlis gland (A cluster of unicellular shell glands, absent in some species) T - Testes (dorso-lateral in *D. latum*); V - Vas deferens; C - Cirrus (a protrusible muscular organ, opening anterior to the vagina in a common genital atrium); GA - Genital Atrium (a cup shaped sinus, where the cirrus and vagina have common openings); L E C - The Lateral Excretory Canal

Life cycle and Transmission

Diphyllobothrium latum has a typical Pseudophyllidean tapeworm lifecycle. In addition to the adult parasites in the definitive host, (i.e. man), there are two intermediate hosts containing larval stages. Eggs are passed from man in the faeces and hatch in water to release a small motile embryonic parasite, the **Coracidium**. This is internally similar to the hexacanth larvae of the Cyclophyllidean tapeworms, being equipped with 6 hooks, but this hexacanth larva is covered in a ciliated embryophore. The coracidium is a free swimming stage, but cannot survive long. For further development, it must be ingested by the first intermediate host, a copepod. On ingestion the embryonic larvae penetrates the arthropods gut wall, entering the haemocoel to develop into the first larval stage, the proceroid, measuring 50µm in length. This larva, (as well as the next larval stage, the plerocercoid described above) is very different from the cyclophyllidean parasite larvae in that they have elongated and solid bodies. In addition, the proceroid bears the embryonic hooklets on a posterior bulb like rounded growth, the cercomer. To continue the

lifecycle, the copepod must be ingested by the next intermediate host, a fish. The proceroid penetrates the gut wall of the fish, and develops into the next larval stage, the plerocercoid (**sparganum**), measuring 4 - 5 mm in length, in the viscera or musculature of the fish. These plerocercoids have again elongated solid bodied parasites, but differ from the proceroids in the absence of the cercomer and hooklets, and at the anterior end having a developed attachment organ, the bothridium, similar to the adult parasite. A number of different species of fish may act as intermediate hosts for the plerocercoids of *D. latum*, but the highest densities of plerocercoids are found in carnivorous fish such as the pike. These high parasite loads are because, in addition to infection by ingestion of the copepod plus proceroid, if another infected fish is eaten the plerocercoids within the body tissues of this predated fish are released in the intestine of the carnivorous fish. These then migrate through the intestinal wall, to invade the new host, which is then acting as a Paratenic host for these secondary plerocercoids. The plerocercoids are, in addition, very long lived, and may achieve very high parasite densities. Man is infected by ingestion of raw or undercooked fish, the plerocercoids emerging in the intestine to grow into the adult parasite. In addition to man a number of other fish eating mammals may also be infected, including cats, dogs, pigs, bears. Therefore *D. latum* in addition to being a parasite of man, also causes zoonotic infection. In man multiple infections may occur, sometimes of very high numbers (up to 143 worms have been reported from a single individual). In these cases the parasites length is considerably reduced.

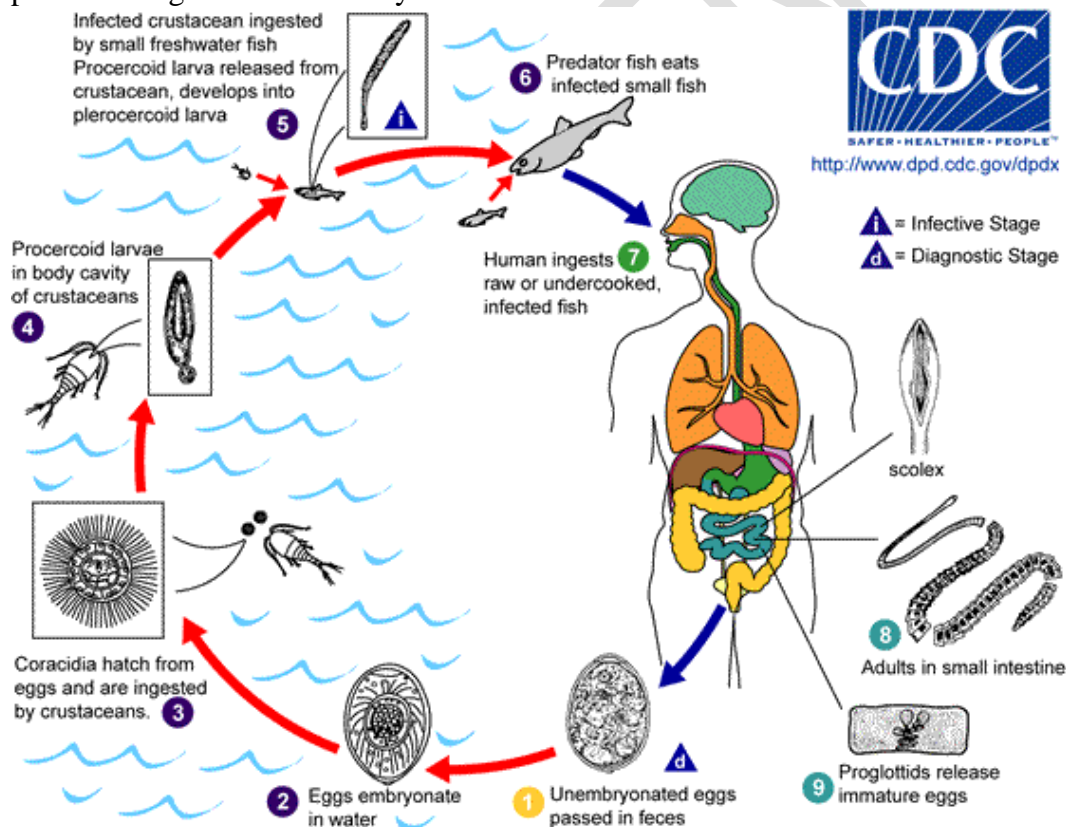


Fig 2.2: Life cycle of *D. latum* (source: <http://www.dpd.cdc.gov/dpdx>)

Pathology of Infection

Infection, as is often the case with adult tapeworms, presents a variable range of pathology, but again is not commonly the cause of serious disease in man. Symptoms, when they occur, include a variety of non-specific abdominal signs, including abdominal pain and loss of weight, and are often very similar to the symptoms displayed during infection with adult *Taenia*. However, *D. latum* differs from *Taenia* in absorbing much more vitamin B12, (between ten and fifty times more) than other tapeworms. Infection may therefore result in a macrocytic hypochromic anaemia in some cases, vitamin B12 having an important role in formation of blood cells. This feature of the disease is much more common in the Baltic region, particularly in Finland. This tapeworm derived anaemia may be due to host derived genetic factors. It is also more commonly seen when the tapeworm is situated higher in the intestine.

Epidemiology and Control

Infection occurs by consuming raw or undercooked fish harbouring sparganum. Therefore to avoid infection in man, fish should be properly cooked, killing the infective plerocercoids.

3.2 *Dipylidium caninum* - (the Dog Tapeworm)

This Tapeworm is primarily a parasite of the Dog and the Cat. However man, and in particular children, may also be infected.

Lifecycle

Similar to *Taenia saginata*, the proglottids of this tapeworm are actively motile, and are able to crawl out of the anus of the definitive host as well as being passed in the faeces. The eggs of this species of tapeworm are contained in egg-capsules, each containing up to twenty eggs. These eggs are ingested by the parasite's intermediate host, in this case an invertebrate arthropod such as fleas (only the larval flea can be infected) or the dog louse *Trichodectes canis*. The onchosphere larvae is released in the arthropods gut and penetrates through the gut wall, developing into a cysticercoid, similar to the hymenolepid larval tapeworms. Infection of the Definitive host, whether dog, cat or man, occurs on ingestion of the larval parasite, either when the intermediate host is ingested, or ingestion of the crushed bodies of these hosts. For example, if the dog licks the face of the child just after it has bitten a flea or louse. On ingestion the cysticercoid larvae develops into the adult parasite in the small intestine in about twenty days.

Morphology

Larvae - The larvae are roughly pear-shaped, and follow the typical cysticercoids body pattern.

Adults - These are relatively short tapeworms, measuring between 15 and 17cm in length and consisting of up to 170 proglottids. These are elongated in form, the gravid proglottids, measuring approximately 12 x 3mm and packed full of eggcapsules, having the appearance of grains of rice. The scolex by which the parasite attaches to the wall of the small intestine has four large acetabulate suckers, a retractile rostellum and six rows of 30 to 150 rose-thorn shaped hooks. The eggs which are typical Cyclophyllidean tapeworm eggs, are round in shape and measure up to 60µm, and are held within egg-capsules.

Pathology of Infection.

The infection appears to be asymptomatic and generally non-pathogenic, although there may be some degree of mild pruritis, or itching, around the perineum due to the presence of emerging proglottids.

3.3 Tapeworms of the Genus *Hymenolepis*

There are a number of species in this genus, two of which are common parasites of man.

H. nana - The Dwarf Tapeworm

This tapeworm is relatively small, growing up to 4cm in length, the size of the parasite being inversely proportional to the number of worms present in the infection. Infections, which are more commonly seen in children in warmer climates, are characterised by the presence of numerous parasites (both cysticercoid larvae and adults) in the small intestine. Infection is by ingestion of soil contaminated with faeces containing eggs and may give rise to abdominal discomfort.

H. diminuta - The Rat Tapeworm

This tapeworm is much longer than *H. nana*, growing up to 60cm or more in length. This is primarily a parasite of the rat, humans only being infected by accidental ingestion of the insect intermediate host. This species is of more importance as a research model for the study of the biochemistry, physiology, chemotherapy and immunology of tapeworm infections. In addition there are a number of species found in animals, including;

H. carioca - A common non-pathogenic parasite of fowl in the USA.

H. microstoma - A parasite of rodents.

H. lanceolata - A pathogenic parasite of ducks, geese and other anseriform domestic fowl.

H. coronula - A parasite of anseriform domestic fowl.

H. cantaniana - A parasite of chickens and other galliform domestic fowl.

Morphology

Apart from their relative sizes, these two parasites of man are very similar, *H. nana* being up to 4cm in size, the strobila consisting of up to 200 proglottids, whilst *H. diminuta* grows up to 60cm or more in length and the strobila consists of up to 1000 proglottids. These proglottids are trapezoidal in shape, and are approximately four times as wide as they are long. Each proglottid contains three round testes, a bi-lobed ovary, a compact vitelline gland and a large uterus opening to a lateral genital pore (as does the cirrus). The scolex in both parasites have four suckers and a retractile rostellum which in *H. nana* is equipped with 20 -30 hooks (the rostellum is unarmed in *H. diminuta*). Finally the eggs of the two species both have the characteristic thickened walls of all cestode eggs, but may easily be differentiated. Those of the yellowish brown *H. diminuta* eggs are much rounder than colourless *H. nana* eggs and are larger with 60 - 80µm in diameter.

In *H. nana* the eggs are oval in shape, measuring ~ 40 by 50µm and contains an oncosphere equipped with 3 pairs of embryonic hooks (i.e. a "hexacanth" larvae) and long wavy filaments (absent in *H. diminuta*) which lie in the space between the larvae and the egg shell wall.

The two species infecting man have rather different lifecycles which will be considered separately here.

H. nana

This parasite has rats and mice as well as man as the definitive host, and differs from *H. diminuta* and almost all other tapeworm in that an intermediate host is not required, although fleas and beetles may be used. The embryonated eggs are passed in the faeces where they contaminate soil. If the eggs are ingested by the definitive host the oncosphere is activated and breaks out of the egg and penetrates the gut villus. Here it develops as a cysticoid larvae in about 4 days before rupturing into the gut lumen. Once ruptured, the scolex attaches to the gut mucosa and the parasite develops into the adult tapeworm after about 15 to 20 days. If the insect intermediate hosts are utilised the lifecycle is similar to that of *H. diminuta* below. In heavy infections eggs liberated by adult worms in the intestine may hatch here rather than passing out of the body, to give autoinfection.

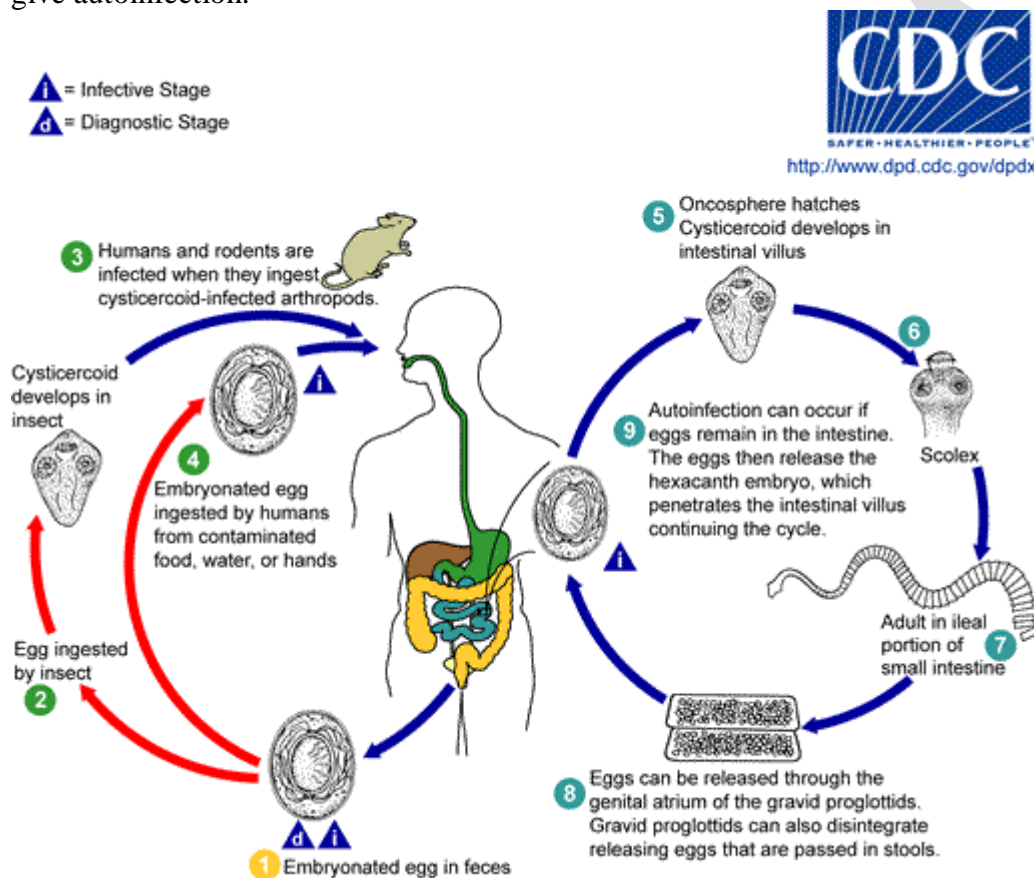


Fig 2.3: Life cycle of *H. nana* (source: <http://www.dpd.cdc.gov/dpdx>)

H. diminuta

This parasite as in most tapeworms does require an intermediate host. Embryonated eggs pass out of the body of the definitive host in the faeces and are ingested by the insect intermediate hosts. Many insects may act as intermediate hosts for this parasite, the most common being fleas and beetles such as the flour beetle. When ingested by the intermediate host the oncosphere larvae become activated, break out of the egg shell and penetrate into the insect's body cavity where they develop into a cysticercoid larvae. For completion of the lifecycle, the infected intermediate host must be eaten by the definitive host. On ingestion, the cysticercoid larva becomes activated, the scolex becomes attached to the gut mucosal wall, and the parasite

develops into the adult tapeworm. An interesting feature of *Hymenolepis* tapeworms is that they undergo a diurnal migration within the gut, which is associated with the feeding patterns of the host. From about 4pm to 4am few parasites are seen in the lower part of the small intestine, whilst from about 4am to 4pm many parasites are seen in the upper part of the small intestine. This was first observed in *H. diminuta* and subsequently in other species, and is indicative of a nocturnal feeding pattern by the parasite.

Pathology of Infection.

These parasites are not very pathogenic, usually with asymptomatic infections. In man infected with *H. nana* there may be a slight irritation of the gut mucosa and slight abdominal pain, and with very heavy infections (>2000 worms) there may also be some diarrhoea. In the bird species there may be enteritis and intestinal obstruction with some species.

4.0 Conclusion

5.0 Summary

D. latum 'a pseudophyllidean' infects fish which in turn infects man when fed on raw or undercooked fish. Rats and mice as well as man are the definitive hosts of *H. nana*, and therefore differs from *H. diminuta* and almost all other tapeworm in that an intermediate host is not required, although fleas and beetles may be used. *Dipylidium caninum* are dog tapeworms that can as well infect human especially children.

6.0 Tutor Marked Assignments

6.1 Activity: Conduct a physical examination of different types of tapeworm and report your findings in the log book.

6.2 Answer the following questions:

- i. What are the striking features of cestodes ?(LO1)
- ii. Explain the transmission cycle of *D. latum* (LO2)
- iii. What are morphological differences between the mature proglottids of *Dipylidium caninum* and *Hymenolepis* spp? (LO2)
- iv. Describe the life cycle of *D. latum* (LO2)

7.0 References and other resources

Bonsdorff (1977). Diphyllbothriasis in man, New York, Academic Press, Inc.

UNIT THREE- TAPEWORMS OF MAN AND OTHER HUMAN'S CESTODES

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main contents
- 3.1 *Taenia* spp
- 3.2 Other *Taenia* cestodes
- 3.3 *Echinococcus* spp
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

Two species from the genus *Taenia* are common parasites of man, these being *Taenia solium* (the Pork tapeworm) and *Taenia saginata* (the Beef tapeworm). *Taenia saginata* has a cosmopolitan distribution, with estimates of approximately 50 million cases of infection world-wide annually. As with *T. saginata* and *T. solium* this parasite has a cosmopolitan distribution, with estimates of approximately 50 million cases of infection world-wide annually. However the incidence of infection may vary considerably, and may be influenced by a number of factors such as religious inhibitions on eating pork, as in many Islamic countries, or in other countries by high degrees of sanitation, limiting exposure of the intermediate hosts to human faeces. This parasite has pigs as the main intermediate host, but man may also act as an intermediate host for this parasite as well as being infected with the adult tapeworms. This aspect of the parasites lifecycle has important implications for the pathology associated with infection with this parasite. *Echinococcus granulosus* is one of the three species of *Echinococcus* that is generally accepted as parasites of man. It is the causative agent of Hydatid disease in man and many other mammals. It occurs in Europe and Arctic region of North America.

2.0 Objectives

- i. Discuss the epidemiology and control of human tapeworms
- ii. Describe the morphology of *Taenia* spp
- iii. Explain the pathology infection of *Taenia* spp

3.0 Main Content

3.1 *Taenia* spp

Life cycle of *Taenia* spp

This parasite has cattle or related animals as its main intermediate hosts, although other animals such as camels, llamas and some antelopes may also occasionally be infected. The larval form in these animals is a cysticercus in the muscles and heart. These are infected by ingestion of the

eggs of the tapeworm, shed from the faeces of the carnivorous definitive host, in this case man. Once ingested the eggs hatch to release the hexacanth larvae, which migrate through the intestinal wall to reach the blood or lymphatic systems, from where it is carried to the tissues, particularly the heart and other muscles to develop into the cysticercus. Man is infected by ingestion of undercooked or raw meat, the bladder wall of the cysticercus being digested in the intestine to release the scolex of the parasite. This attaches to the intestinal wall and grows into the mature adult tapeworm.

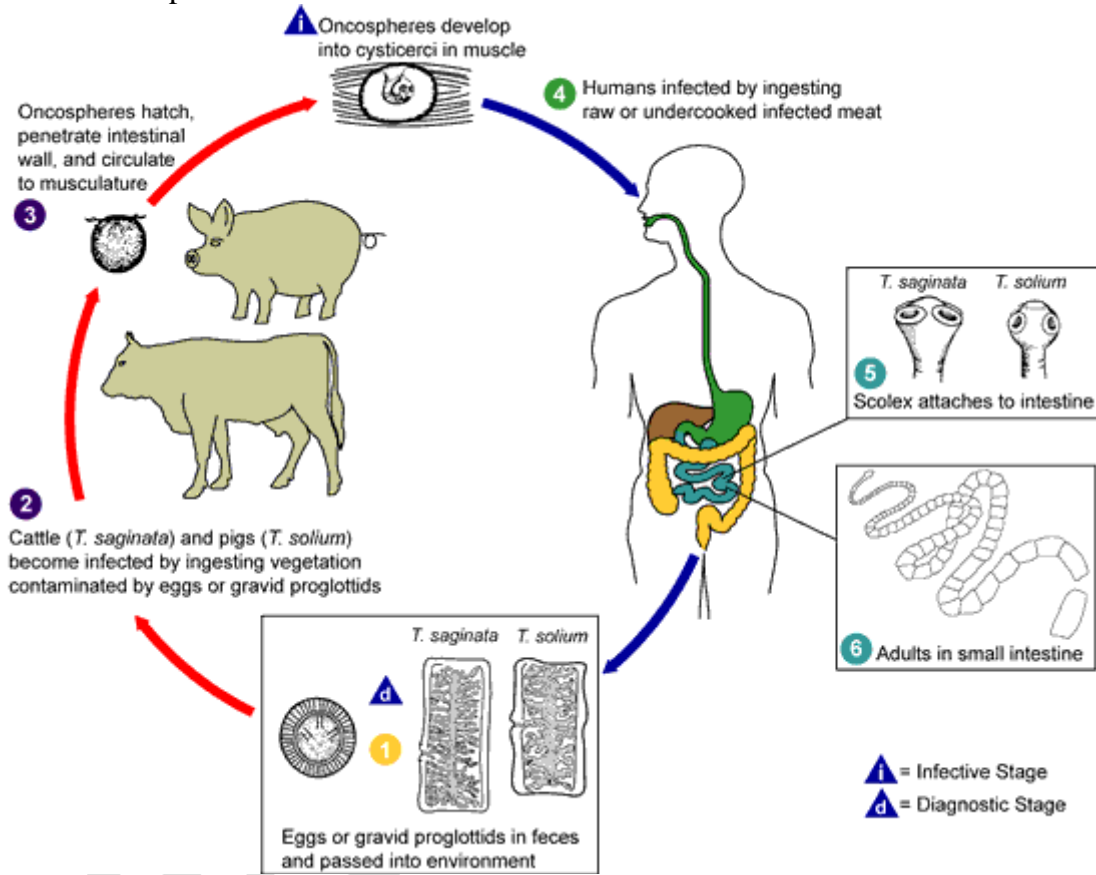


Fig 3.1: Life cycle of *T. saginata* and *T. solium*

Morphology

Taenia saginata

Larvae - These cysticerci are approximately 7.5-10mm wide by 4-6mm in length.

Adults - The adult tapeworms have an average length of about 5 meters, consisting of approximately 1000 proglottids, but may grow up to 17 metres in length occasionally, and are therefore longer than the adult forms of *Taenia solium*. The mature proglottids have approximately double the number of testes that *T. saginata* has and are larger. The gravid proglottids are also larger, measuring approximately 20mm long by 6mm wide with a uterus with more lateral branches than *T. solium*. These gravid proglottids when detached from the strobila may be very active, not only crawling away from the faeces when passed, but often actively emerging from the anus to deposit eggs from the ruptured uterus around the perianal region. The scolex in this tapeworm may also be differentiated from *T. solium* as it is slightly

larger, at approximately 2mm in diameter and is unarmed, without any hooks, although the 4 acetabular suckers are still present.

Taenia solium

Larvae - These small cysticerci (referred to as *Cysticercus cellulosae*) are approximately 6-18mm wide by 4 - 6mm in length when found in the muscles or subcutaneous tissues (the normal sites for the larva of this parasite). The cysticerci may however be found in other tissues such as those of the central nervous system where they may grow much larger, up to several centimetres in diameter.

Adults - The adult tapeworms have an average length of about 3 meters, but may grow up to 8 metres in length occasionally, and follow the typical morphology of cestode tapeworms. The strobila consists of between 800 and 1000 proglottids. The mature proglottids having trilobed ovaries with a small central lobe in addition to the two lateral lobes and only approximately half the number of testes that *T. saginata* has. The gravid proglottids, measuring approximately 12mm long by 6mm wide, have a uterus with between 8 to 12 lateral branches, less than *T. saginata*. The scolex in this tapeworm may also be differentiated from *T. saginata* as it is equipped with a low rostellum with a double crown of approximately 30 hooks.

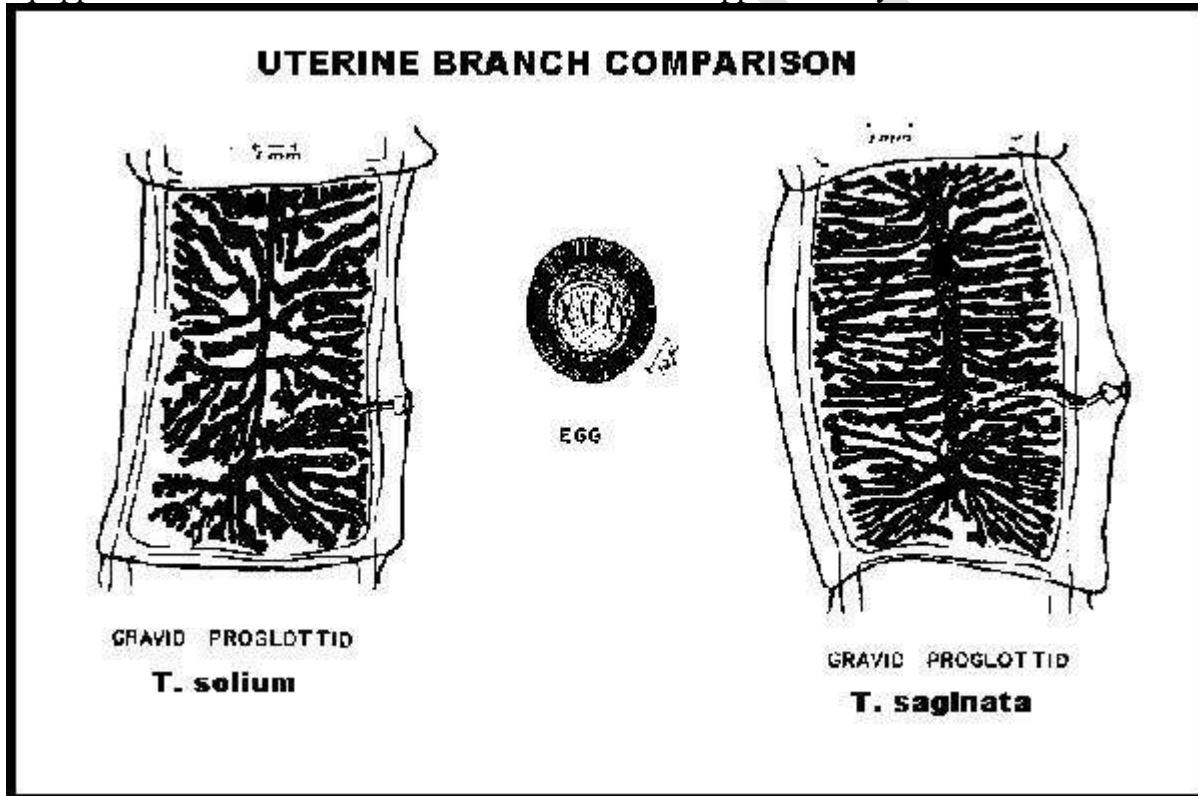


Fig 3.2: showing the distinctions between the proglottid of *T. solium* and *T. saginata*

Major Differences between *T. saginata* and *T. solium*

Features	<i>T. saginata</i>	<i>T. solium</i>
Size	3-7m(sometimes Upto 25m) long	2-3m(sometimes up to 10m) long
No. of proglottids	1000-2000 (sheds 3-10 daily)	800-1000 (sheds 8-10 daily)
No. of eggs per proglottid	100000	40000
Scolex	Cuboidal, up to 2.0mm In diameter	Spheroid, about 1.0mm in diameter
Rostellum	Absent	Present, armed with two circlets of 22-32 hooks
No. of testes	800-1200	300-500
Shape of ovary	Bilobed	Trilobed
Gravid uterus	15-25 lateral branches on each side	7-13 lateral branches on each side
Vaginal sphincter	Present	Absent
Gravid proglottid	When detached, active and creep out through anus and crawl about individually	When detached, passive

Pathology of Infection.***T. saginata***

Larvae - Unlike *T. solium*, *T. saginata* does not utilise man as an intermediate host, and therefore pathology due to the larval form is not a feature in human disease. In cattle the cysticercus, referred to as *Cysticercus bovis* (named before the parasite life cycle had been determined, and the connection between the two forms had been established) is completely asymptomatic.

Adults - The pathology of infection with adult *T. saginata* is highly variable. Often infections are completely asymptomatic, but in other cases some degree of pathology may be seen, most seriously intestinal blockage. In some cases vitamin deficiency may be the result of excessive absorption of nutrients by the parasite, although this aspect of tapeworm pathology is more a feature of infection with the fish tapeworm *D. latum*. In addition infection may be accompanied by a broad range of non-specific symptoms, including more commonly, (if seen at all), abdominal pain, digestive disturbances, excessive appetite *or* loss of appetite, weakness and weight loss.

T. solium

Larvae - Infection with the larval form of *T. solium* *Cysticercus cellulosae*, (called "Cysticercosis") may have severe consequences, the annual world-wide mortality due to cysticercosis having been estimated at approximately 50 000 cases. In man the cysticerci mainly develop in the subcutaneous tissues, but infections in both the Central Nervous System (C.N.S.) and ocular tissues are also very common. Infection of the C.N.S. may cause severe pain, paralysis, optical and/or psychic disturbances and epileptic convulsions, mainly due to mechanical pressure as the larvae develop. Later there may be loss of consciousness and even death. Infections involving the eye may give rise to discomfort, and can cause

detachment of the retina.

Adults - Usually only a single adult specimen is present, which may cause a slight degree of mucosal inflammation. The actual effects on the host may vary considerably, often there are few symptoms, but in some cases a variety of nonspecific symptoms such as constipation, epigastric pain and diarrhoea, are present. Very rarely there may be perforation of the intestinal wall, with subsequent peritonitis. However, more seriously, as detailed above, the presence of adult worms carries the risk of autoinfection due to reverse-peristalsis resulting in cysticercosis, it being estimated that approximately 25% of cases of *Cysticercus cellulosae* infections in man being acquired by this route.

Diagnosis of Infection by *Taenia* spp

- Demonstration of scolex and proglottids in the faeces. However, scolex are rarely excreted in faeces
- The eggs of *T. saginata* and *T. solium* are similar. However, most laboratory diagnosis is through the observation of *Taenia* spp eggs in faecal sample.
- Examination of gravid uterus shows 15-25 lateral branches in *T. Saginata* and 7-13 lateral branches (counted from the main stem) in *T. Solium* when short chains of 5-8 proglottids passed out in faeces are pressed through glass slides.
- The scolex of *T. saginata* is easily distinguished from that of *T. solium* in that it has only 4 suckers but no hooks.
- Radiological examination of the intestinal tract may reveal tapeworm infection.

Epidemiology and control

The prevalence of *Taenia* infection is on the increase due to the following factors;

- i. Intensification of animal production
- ii. Development of meat industries in several developing countries
- iii. Consumption of undercooked beef and pork by tourist visiting highly endemic areas
- iv. Consumption of semi-cooked meat in manufactured food products like hamburgers, etc.
- v. Accelerating urbanisation with decreased efficiency of sewage systems
- vi. Sewage farming

In view of the above listed epidemiological factors that favour transmission, the following measures can be taken to reduce prevalence;

1. Proper meat inspection services before usage in meat industries. Diseased meat should be condemned and destroyed
2. Lightly infected beef with cysticerci can be rendered safe for consumption by freezing at -10oC for at least 10 days
3. Cooking of meat well before eating
4. High standards of sanitation will reduce transmission
5. Immunization against bivariate cysticercosis.

3.2 Other *Taenia* cestodes

Infection by Adult tapeworms

Taenia taeniformis - This parasite has a cosmopolitan distribution, the adult parasites are normally found in cats and related carnivores, but it has been reported from an Argentinean

child. The adult tapeworms are about 60cm long, and are unusual in that they lack a neck. The scolex is large and equipped with two rows of hooks, whilst the posterior gravid proglottids have a characteristic bell shape. The larvae, which is found in wild rodents, is a strobilocercus, a development of a cysticercus where the scolex has evaginated, but is still attached to the bladder of the cysticercus by a short segmented strobila.

Taenia bremneri (Syn. *T. confusa*) - reported from man in Africa, Japan and the United State of America. This parasite may be a synonym of *T. saginata*.

Taenia africanus - reported a few times in East Africa. This tapeworm has broad segments and an unarmed scolex with a small apical sucker.

Infection by Larvae (Metacestode Infections)

Taenia multiceps - The adult tapeworms of this species are found in dogs and related canids. The larva is a fluid containing cyst 5cm or more in diameter, containing several hundred protoscolices, and is called a coenurus. It is normally found in the brain or spinal cord of sheep and goats where it is an important pathogen. In these animals, it causes a condition known as 'gid' or 'staggers' as the coenurus develops along with an associated destruction of nervous tissue. The larval form may rarely infect man, where it causes a condition called coenurus cerebralis, on accidental ingestion of tapeworm eggs from the faeces of dogs.

Taenia serialis - A similar parasite to *T. multiceps*, the coenurus larvae, measuring 4cm in diameter or larger, is usually found in the subcutaneous and intramuscular tissues of lagomorphs. The adult tapeworms are found in dogs and foxes with a cosmopolitan distribution. They measure about 70 cm in length and have a scolex with two rows of about 30 hooks. The larvae have been reported very rarely in man.

Taenia glomerulatus - The larvae normally infect rodents, but the coenurus larvae have also been reported as rarely infecting man in Africa.

3.3 Echinococcus spp

Three species of *Echinococcus* have been generally accepted as parasites of man

Echinococcus granulosus

The causative agent of Hydatid disease in man and many other mammals. The dog acts as the definitive host for this species. A number of sub-species of this parasite have also been described, the most universally accepted being *E. g. granulosus* (thought to be the original species found in Europe, although now more widespread) and *E. g. canadensis* (the indigenous species of the Arctic region of North America, for more details see below). In addition there is considerable strain variation within this parasite, with differing preferences for intermediate hosts. For example in Ireland a strain exists whose larvae only infect the horse, man being resistant to infection.

Life cycle

Dogs and other canids are parasitised by the adult tapeworm. When shed by the tapeworm, the gravid proglottids disintegrate in the dogs intestine, and eggs which are passed in the faeces, are highly resistant, being able to survive freezing and drying on the ground for up to a year. Many mammals apart from man may act as intermediate hosts, in particular sheep and horses. The

situation is highly complex as at least 9 sub-species have been identified, all with different host specificity;

E.g. granulosus - Adult form in most canids apart from the red fox, hydatids in sheep, pigs, cattle, man and many wild ruminants.

E.g. equinus - Adults in canids, hydatids in horses and other Equidae, but probably not man.

E.g. canadensis - Adults in canids, hydatids in caribou, reindeer and man.

E.g. borealis - Adults in canids, hydatids in many cervids and man.

In addition, in parts of Kenya there is a strain or sub-species that is particularly adapted to transmission between man and domesticated dogs.

The egg enters the host by ingestion, either from contaminated grass (as is the case in infections of herbivorous ruminants), or in the case of man, by contamination, (for example by the dog licking face after it has been cleaning itself) or other examples of bad hygiene, followed by transfer to the mouth. The egg then hatches in the intestine, penetrates the gut wall, and travels via the lymphatic or blood system throughout the body, from where they lodge within the body tissues. The cysts may develop anywhere within its intermediate hosts body, but as the circulatory blood stream passes from the mesenteric blood vessels to the liver, it is in the liver that the majority of the cysts (in about 65% of cases) are found. Next in frequency of infection are the lungs (about 20%), brain (1%), peritoneal cavity (8%), kidneys (3%) and bone marrow or other organs. Development of the cysts to produce infective protoscolices takes approximately 1 to 2 years. On the death of the intermediate host, either directly by predation on the part of the dog, or by the scavenging of the dead cadaver, (the protoscolices are also highly resistant, being able to survive in carrion for several weeks), the cyst is ingested along with the offal. The cyst wall is then digested, liberating the protoscolices which quickly evaginate, penetrating deeply into the crypts of Lieberkuhn, and developing to adult worms in approximately 7 to 9 weeks. Due to the presence of many protoscolices in each hydatid cyst, dogs may be infected with many *E. granulosus*

(tapeworms).

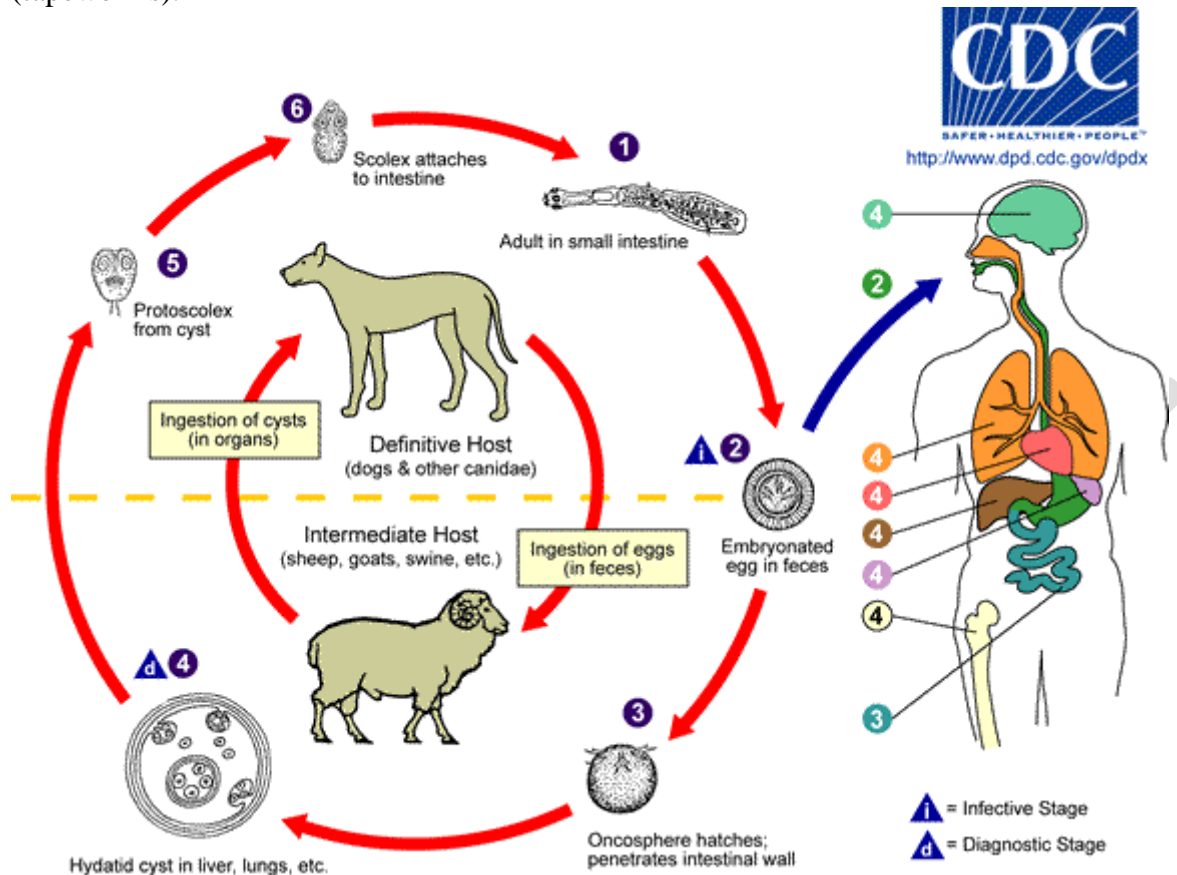


fig 3.3: Life cycle of *E. granulosus* (source: <http://www.dpd.cdc.gov/dpdx>)

Morphology

Larvae - These Metacestodes (called 'Hydatids') are large, roughly spherical, fluid filled hollow bladders, containing numerous protoscolices (forming the so-called hydatid sand), brood capsules, and daughter cysts which are identical in form to their parent cyst. The cyst wall itself consists of an outer laminated hyaline wall, supporting the whole cyst. Beneath this there is a nucleated germinal layer, studded with developing brood capsules, which may eventually break off to float freely in the fluid filled cyst. The protoscolices are formed within the brood capsules, which may rupture to give the free protoscolices in the hydatid fluid.

They vary considerably in size depending on where in the body they form, which may be almost any organ of the body. Those found in the liver (the most common organ affected) may be approximately 20cm in diameter, but those found in the peritoneal cavity may sometimes be very much larger, containing several litres of fluid. For example one case has been reported of a cyst 50cm in diameter, containing 16 litre of fluid.

Adults - The adult parasites in the dog represent one of the smallest of the tapeworms. They measure between 3 and 9mm in length, and usually consist of only 3 proglottids, an immature, a mature, and a gravid proglottid. The scolex is globular in shape, and has a prominent rostellum, armed with a double row of between 30 and 36 hooks. The eggs are very similar to those of the genus *Taenia*, and measure between 30 and 40µm in diameter

Pathology of Infection.

Larvae - In domesticated animals clinical signs appear to be uncommon, whilst in man they will vary in their seriousness depending on where in the body the hydatid develops, and how large it grows. Sometimes, the infection is asymptomatic, the only evidence of infection being the presence of calcified cysts on autopsy after death due to an unrelated cause. The major pathology is due to the size of the cyst, giving rise to pressure related injury. A complication may arise if the cyst is ruptured, possibly due to blows to the body, muscular strain, or during operations. In this case the contents of the hydatid is released into the body's circulatory system, and the liberated protoscolices may give rise to numerous secondary cysts throughout the body. In addition the hydatid cyst fluid is highly allergenic and cyst rupture may result in anaphylactic shock and rapid death.

Adults - The adult tapeworm is usually non-pathogenic to its canine hosts, although sometimes in very heavy infections there may be some inflammation of the intestinal wall.

Echinococcus multilocularis

It is the causative agent of highly pathogenic Alveolar Hydatid disease in man and other mammals. The fox is the most important definitive host, although dogs, and occasionally cats, may also be infected with the adult parasite. Again there appears to be a number of sub-species of this organism, *E. m. multilocularis* in Europe and *E. m. sibiricencis* in North America.

This is very similar to that of *E. granulosus*, but with more adaptations for colder climates. For example the eggs are highly resistant to cold temperatures, being able to survive at -20° for more than 2 weeks. In addition the pre patent period in the definitive host is much shorter, usually between 4 to 5 weeks.

Morphology

Larvae - The larval *E. multilocularis* is very different from that of *E. granulosus*. In this case the 'cyst' grows invasively by external budding, forming a diffuse growth through the infected organ, replacing that organ's tissues. The growth itself, (it cannot truly be called a cyst as there is no real cyst wall), is composed of numerous cavities containing a gelatinous matrix within which protoscolices and numerous brood capsules are produced, and which in its behaviour, most closely resembles a malignant neoplasm. In contrast to *E. granulosus* this growth is also very rapid, infective protoscolices being present after only 2 to 3 months, as compared to the 1 to 2 years in the related metacestode.

Adults - The adult parasite is very similar to *E. granulosus*, being slightly smaller, with a maximum length of approximately 4mm, and consisting of 4 to 5 proglottids.

Pathology of Infection

Larvae - The multilocular cyst is highly pathogenic due to its fast growth rate and invasive nature, in extreme cases completely replacing liver tissue. As the cyst lacks the tough laminated layer seen in *E. granulosus*, and by its nature grows by budding, metastases of growth may also be seen, colonising other body organs. Due to this aspect of the parasite, it may also be transferred by transplantation. This parasite must be considered one of the most pathogenic of the parasitic helminths.

Adults - As with *E. granulosus* the adult tapeworm is usually non-pathogenic to its canine hosts.

4.0 Conclusion

In this unit, we have discussed about tape worms and their effect on humans, how they occur in humans.

5.0 Summary

In this unit, you have learnt about the two major tape worms infecting man; *Taenia saginata* (beef tape worms) and *T. solium* (pork tape worm). Infections by these tapeworms often occur following the consumption of raw or undercooked beef and pork. Others *Taenia* spp which have man as accidental host are *T. taeniformis*, *T. bremneri*, *T. multiceps*, *T. serialis* and *T. glomerulatus*.

Dog and other canids are the definitive hosts of *Echinococcus granulosus* with the hydatid cyst of the parasite causing the pathological effects seen in man. Proper cooking of beef and pork could prevent infection due to *T. saginata* and *T. solium* while good sanitary condition can as well prevent infection by *E. granulosus*.

6.0 Tutor Marked Assignments

6.1 Activity: Conduct a physical examination of the various types of taenia spp and report your findings in the log book.

6.2 Answer the following questions:

- i. Briefly discuss the epidemiology and control of human tapeworms (LO1)
- ii. Highlight the pathology control measures of *Echinococcus granulosus* (LO3)
- iii. Describe the morphology of describe the morphology of *Taenia saginata*, *Taenia solium*, *T. solium* (LO2)

7.0 References and other resources

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MODULE SEVEN- NEMATODES**Introduction**

Nematode infections in humans include ascariasis, trichuriasis, hookworm, enterobiasis, strongyloidiasis, filariasis, and trichinosis, among others. The phylum Nematoda, also known as the roundworms, is the second largest phylum in the animal kingdom, encompassing up to 500,000 species. The module will describe the general features and life cycles of nematodes, soil transmitted helminthes, blood and tissue nematodes and air-borne nematodes

Module Objective

At the end of this module, you should be able to discuss the various types of nematodes in relation to epidemiology, pathology and control

Contents

Unit 1: General features and life cycles of nematodes

Unit 2: Soil transmitted helminths

Unit 3: Blood and Tissue nematodes

Unit 4: Air-borne nematodes

UNIT 1- GENERAL FEATURES AND LIFE CYCLES OF NEMATODES**CONTENT**

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main contents
- 3.1** General features
- 3.2** The Basic Life cycle of the Major Groups of Nematodes
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
- 6.1** Activity
- 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 Introduction

Members of Nematoda are elongated, with bilaterally symmetric bodies that contain an intestinal system and a large body cavity. Many roundworm species are free living in nature. Recent data have demonstrated that approximately 60 species of roundworms parasitize humans. Intestinal roundworm infections constitute the largest group of helminthic diseases in humans. According to a 2005 report by the World Health Organization (WHO), approximately 0.807-1.221 billion

humans have ascariasis, 604-795 million have trichuriasis, and 576-740 million have hookworm infections worldwide.

2.0 Objectives

At the end of this unit, you should be able to:

- i. List the various examples of nematodes with their common names
- ii. Describe the general morphological features of nematodes
- iii. Explain the life cycles of the major groups of nematodes

3.0 Main Contents

3.1 General features

Nematodes are cylindrical rather than flattened; hence the common name roundworm. The body wall is composed of an outer cuticle that has a noncellular, chemically complex structure, a thin hypodermis, and musculature. The cuticle in some species has longitudinal ridges called alae. The bursa, a flap-like extension of the cuticle on the posterior end of some species of male nematodes, is used to grasp the female during copulation.

The cellular hypodermis bulges into the body cavity or pseudocoelom to form four longitudinal cords; a dorsal, a ventral, and two lateral cords which may be seen on the surface as lateral lines. Nuclei of the hypodermis are located in the region of the cords. The somatic musculature lying beneath the hypodermis is a single layer of smooth muscle cells. When viewed in cross-section, this layer can be seen to be separated into four zones by the hypodermal cords. The musculature is innervated by extensions of muscle cells to nerve trunks running anteriorly and posteriorly from ganglion cells that ring the midportion of the esophagus.

The space between the muscle layer and viscera is the pseudocoelom, which lacks a mesothelium lining. This cavity contains fluid and two to six fixed cells (celomocytes) which are usually associated with the longitudinal cords. The function of these cells is unknown.

The **alimentary canal** of roundworms is complete, with both mouth and anus. The mouth is surrounded by lips bearing sensory papillae (bristles). The oesophagus, a conspicuous feature of nematodes, is a muscular structure that pumps food into the intestine; it differs in shape in different species.

The intestine is a tubular structure composed of a single layer of columnar cells possessing prominent microvilli on their luminal surface.

The excretory system of some nematodes consists of an excretory gland and a pore located ventrally in the mid-esophageal region. In other nematodes this structure is drawn into extensions that give rise to the more complex tubular excretory system, which is usually H-shaped, with two anterior limbs and two posterior limbs located in the lateral cords. The gland cells and tubes are thought to serve as absorptive bodies, collecting wastes from the pseudocoelom, and to function in osmoregulation.

Nematodes are usually bisexual. Males are usually smaller than females, have a curved posterior end, and possess (in some species) copulatory structures, such as spicules (usually two), a bursa, or both. The **males** have one or (in a few cases) two testes, which lie at the free end of a convoluted or recurved tube leading into a seminal vesicle and eventually into the cloaca.

The **female** system is tubular also, and usually is made up of reflexed ovaries. Each ovary is continuous, with an oviduct and tubular uterus. The uteri join to form the vagina, which in turn opens to the exterior through the vulva.

Copulation between a female and a male nematode is necessary for fertilization except in the genus *Strongyloides*, in which parthenogenetic development occurs (i.e., the development of an unfertilized egg into a new individual). Some evidence indicates that sex attractants (pheromones) play a role in heterosexual mating. During copulation, sperm is transferred into the vulva of the female. The sperm enters the ovum and a fertilization membrane is secreted by the zygote. This membrane gradually thickens to form the chitinous shell. A second membrane, below the shell, makes the egg impervious to essentially all substances except carbon dioxide and oxygen. In some species, a third proteinaceous membrane is secreted as the egg passes down the uterus by the uterine wall and is deposited outside the shell. Most nematodes that are parasitic in humans lay eggs that, when voided, contain either an uncleaved zygote, a group of blastomeres, or a completely formed larva. Some nematodes, such as the filariae and *Trichinella spiralis*, produce larvae that are deposited in host.

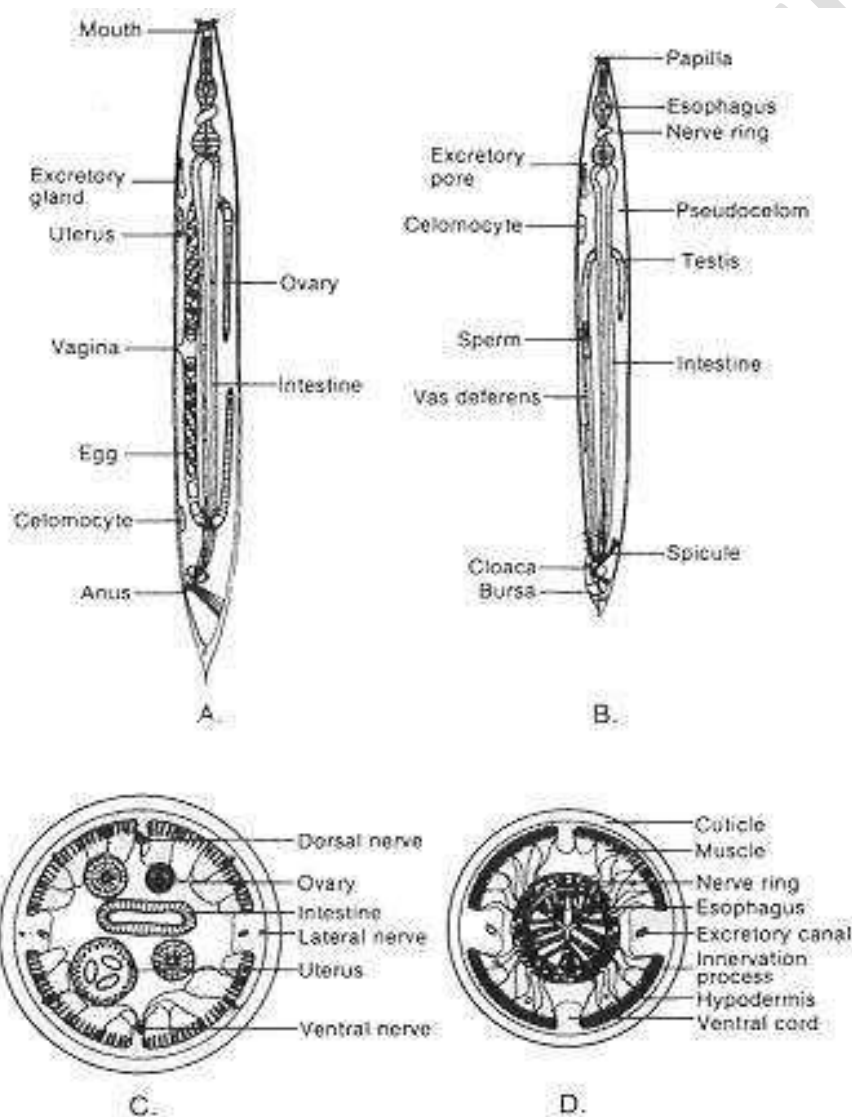


Fig 1.1: Structure of nematodes. (A) Female. (B) Male. Transverse sections through the mid region of the female worm (C) and through the esophageal region (D).

3.2 The basic life cycle of the major groups of nematodes

The life cycles of the parasitic species vary considerably, as would be expected from such a large and diverse group. There are however a number of common features. Firstly, the parasite undergoes a series of moults through larval stages (designated **L1** to the adult **L5** form). Secondly, in most (but not all) nematodes it is the **L3** larvae that is the infective form, important exceptions to this being the Ascarids, such as *Ascaris lumbricoides* and the pinworms, where it is either the **L1** larvae, or eggs containing **L1** or **L2** larvae that are infective. Thirdly the **L3** form onwards in all species undergoes a migration within the body of the definitive host as it matures into the adult parasite, usually via the bloodstream or lymphatic system to the heart, lungs, trachea, and then to the intestine. Finally, in most cases the parasite leaves the definitive host as thin walled eggs in the faeces, important exceptions being the viviparous filarial worms (where **L1** larvae infect intermediate hosts, usually in the blood meals of biting arthropods), *Strongyloides stercoralis*, (where the **L1** larvae are found in the faeces), and the viviparous *Trichinella spiralis*, where the larvae do not leave the body as such, but develop to the **L3** stage which then encysts in the muscles, infection being by ingestion of undercooked contaminated meat. Infection of the definitive host may be by a variety of routes, such as the oral route, where eggs are accidentally ingested, also many filarial worms are infective via the bite of flies, as previously described, and the **L3** larvae of many nematodes such as the hookworms and other related nematodes are directly invasive.

In terms of complexity, the simplest life cycles are those of the pinworms, where adults living in the colon mate and lay eggs which pass out in the faeces, infection being either by the oral route with eggs, or perianally, where eggs hatch around the anus and **L1** larvae migrate back through the anus.

The most diverse is probably that of *S. stercoralis*, where there are a number of alternative lifecycles which it may undergo, either as a completely free living soil nematode, or as the standard infective **L3** larvae with tissue migration to the intestine, or even occasionally full completion of the life cycle within the intestine, and finally in immunocompromised hosts a life-threatening disseminated infection can occur, with parasites found throughout the body.

4.0 Conclusion

5.0 Summary

Nematodes are roundworms with pseudocoelom (lacking a mesothelium lining). The alimentary canal is complete having mouth and anus. The intestine is a tubular structure composed of a single layer of columnar cells possessing prominent microvilli on their luminal surface. The excretory system of some nematodes consists of an excretory gland and a pore with complex tubular excretory system, which is usually H-shaped. Nematodes are usually bisexual. Males are usually smaller than females. Copulation between a female and a male nematode is necessary for fertilization except in the genus *Strongyloides*, in which parthenogenetic development occurs (i.e., the development of an unfertilized egg into a new individual). Parasite undergoes a series of moults through larval stages with **L3** larva mostly being the infective stage. Infection of the definitive host may be by accidental ingestion of eggs, bite of flies and skin penetration by the infective **L3** larval form as in the case of hookworms.

6.0 Tutor Marked Assignments

6.1 Activity: Conduct a physical examination of a mature roundworm and report your findings in the log book.

6.2 Answer the following questions:

- i. Mention examples of nematodes with their common names (LO1)
- ii. With the aid of well labeled diagrams describe the general features of roundworms (LO2)
- iii. Highlight the basic life cycles of nematode (LO3)

7.0 References and other resources

Ukoli, F.M.A. (1990). Introduction of Parasitology in Tropical Africa. John Wiley and Sons Ltd., Chichester.

UNIT TWO- SOIL TRANSMITTED HELMINTHS

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main contents
- 3.1 *Ascaris lumbricoides* (large roundworm of man)
- 3.2 The human hookworms
- 3.3 *Trichuris trichiura* (whipworm)
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
- 6.1 Activity
- 6.2 Tutor Marked Tests
- 7.0 References and other resources

1.0 Introduction

Soil-transmitted helminth (STH) infection is highly endemic in tropical and subtropical areas of sub-Saharan Africa, Asia and Latin America, where up to 2 billion people have active infections. STH infection has remained largely neglected by the global health community because the people most affected are among the most impoverished and because the infection causes chronic ill health with insidious clinical presentations, rather than severe acute illness or high mortality. However, it is now recognized that STH infection causes significant morbidity worldwide with 39 million disability adjusted life years (DALYs) lost each year - more than those lost to malaria (36 million yearly) and approaching those lost to tuberculosis (47 million yearly). Hookworm infection alone causes the loss of 22 million DALYs.

2.0 Objectives

At the end of this unit, you should be able to

- i. Give examples of soil transmitted helminthes (STHs)
- ii. Describe their life cycles with emphasis on the route of infection
- iii. Describe the diagnostic features of the parasites

3.0 Main Contents

3.1 *Ascaris lumbricoides* (large roundworm of man)

Infection with this roundworm is extremely common, with estimates of the annual incidence of infection being greater than 1500 million cases, or around one quarter of the worlds population. In addition to the species in man, *Ascaris lumbricoides*, a morphologically indistinguishable species *Ascaris suum* is found in the pig. Other related genera include *Parascaris* in equines, and *Toxascaris* in a variety of domesticated animals.

Morphology

The adult *Ascaris lumbricoides* are large white, or pinkish-white, cylindrical roundworms, slightly narrower at the head. The more slender males measure between 10 to 30cm long and have a curved tail with two spicules, but no copulatory bursa. The females are very similar, being slightly larger at between 20 to 35cm long, a vulva approximately a third of the length of the body down from the head, and have a blunt tail. They are both characterised by having a smooth, finely striated, cuticle, and a mouth, which is characteristic of all of the Ascarids (e.g. *Toxocara*), having three lips each equipped with small papillae. Internally they follow the generalised body plan of all nematodes, and have a cylindrical oesophagus opening into a flattened ribbon like intestine.

The eggs consist of a thick transparent inner shell which is covered in a thick, warty, albuminous coat.



Fig 2.1: Eggs unfertilized (left) fertilized (right) of *A. lumbricoides*.

Life cycle

These parasites have a direct life cycle, with no intermediate hosts. The adult parasite lives in the lumen of the small intestine of man, usually only feeding on the semi-digested contents of the gut, although there is some evidence that they can bite the intestinal mucous membrane and feed on blood and tissue fluids. The female parasite is highly prolific, laying an estimated 2 million eggs daily. In the intestine, these only contain an unembryonated mass of cells, differentiation occurring outside the host. This requires a temperature less than 30°C, moisture and oxygen, before the development of the young L1 larvae after approximately 14 days. Eggs containing the

L2 larvae take another week to develop, before they are infective to man, and may remain viable in the soil for many years if conditions are optimal. Infection occurs on ingestion of raw food, such as fruit or vegetables, that is contaminated with these infective eggs. The eggs then hatch in the small intestine, to release the L2 rhabditiform larvae (measuring approximately 250 by 15µm in size). These do not simply grow into the adult forms in the intestine, but must then undergo a migration through the body of their host. These L2 larvae penetrate the intestinal wall, entering the portal blood stream, and then migrate to the liver, then heart, then after between 1 to 7 days, the lungs. Here they moult twice on the way to form the L4 larvae, (measuring approximately 1.5mm long), then burrow out of the blood vessels, entering the bronchioles. From here they migrate up through the air passages of the lungs, to the trachea. They then enter the throat and are swallowed, finally ending up in the small intestine where they mature and mate, to complete their life cycle.

Pathology of Infection.

The majority of infections (~85%) *appear* to be asymptomatic, in that there is no gross pathology seen. However the presence of these parasites appears to be associated with the same general failure to thrive in their hosts seen with many of these intestinal nematodes. In terms of more easily identified pathology, this may be divided into three areas;

Pathology Associated with the Ingestion and Migration of Larvae

Severe symptoms of *Ascaris* infection may be associated with the migrating larvae, particularly in the lungs. If large numbers of these larvae are migrating through the lungs simultaneously this may give rise to a severe haemorrhagic pneumonia. More commonly, as is the case with most infections, the haemorrhages are smaller in scale, but still may lead to breathing difficulties, pneumonia and/or fever. A complication here is that many of the parasites proteins are highly allergenic. Due to this the presence of the migrating larvae in the lungs is often associated with allergic hypersensitivity reactions such as asthmatic attacks, pulmonary infiltration and urticaria and oedema of the lips.

Pathology Associated with Adult Parasites in the Intestine

The most common symptoms of infection are due to the adult parasite, and consist of rather generalised digestive disorders, such as a vague abdominal discomfort, nausea, colic (e.t.c.). These symptoms are dependent to some extent on the parasite's burden of the host, which in severe cases may consist of many hundreds or even thousands of parasites, although these are extreme cases. In the case of these heavy infections the presence of many of these large parasites may contribute to malnutrition in the host, especially if the hosts (often children) are undernourished. A more serious, and potentially fatal, condition may arise in these more heavy infections, where the mass of worms may block the intestine and need to be surgically removed. This may also occur sometimes on treatment for other intestinal nematodes such as hookworms, where the curative drug dose for these parasites irritates the ascarids.

Pathology due to "Wandering" Adults outside of the Intestine

Adult parasites often leave the small intestine to enter other organs, (sometimes in response to anti-helminthic drugs used to treat other intestinal nematode infections), where they may cause various types of pathology, sometimes with severe consequences. For example adult *Ascaris*

worms may migrate to the bile duct, which may then become blocked causing jaundice and a general interference in fat metabolism. Adult parasites may also migrate to the appendix, or through the intestinal wall, both conditions which may cause a fatal peritonitis as they may well carry intestinal bacteria to these sites. They may, alarmingly, sometimes migrate forward through the intestinal tract, to be either vomited up or emerging through the nose. More seriously, if they enter the trachea they may cause suffocation.

Diagnosis

Definitive diagnosis is by demonstration of the characteristic eggs in faecal samples or by identifying adult worms passed out spontaneously by the host.

Epidemiology and Control

Infection occurs through ingestion of parasites' eggs in food. The eggs are highly resistant to adverse environmental conditions. This with other factors highlighted below are often associated with transmission of infection;

- Lack or inadequate waste disposal facilities
- Improper washing of hands before eating
- Improper washing of fruits and vegetables before consumptions
- Unkept rooms and dwelling places that harbour mechanical carriers of parasites, etc.

Provision of good waste disposal system and good personal hygiene will help to control infections.

3.2 The human hookworms

The hookworms belong to the Order Strongylida, a very large order, and of great interest as it contains many important pathogens of man and domesticated animals. This order is further subdivided into three Superfamilies, the Strongyloidea (the hookworms in man), and two related groups, the Superfamily 'Trichostrongyloidea', intestinal nematodes which are of veterinary importance in many domesticated animals (e.g. *Haemonchus contortus* in cattle and *Nippostrongylus brasiliensis* in rodents) and members of the Superfamily 'Metastrongyloidea' (the lungworms, in domesticated animals).

In man there are two species capable of causing intestinal infections, *Ancylostoma duodenale* native to parts of Southern Europe, North Africa and Northern Asia parts of Western South America, and *Necator americanus* in Central and Southern Africa, Southern Asia, Australia and the Pacific Islands. These are very important human pathogens. It has been estimated that there are 1200 million cases of hookworm infection in man annually, of which about 100 million of which are symptomatic infections with accompanying anaemia. In addition, the larvae of several species of hookworms infecting domesticated animals may penetrate human skin, causing pathology even though they do not develop to the adult parasites in man.

Morphology

The adult parasites are small cylindrical worms, 0.5-1.5mm long (*Ancylostoma duodenale* being slightly larger than *Necator americanus*). The posterior end of the male worm is equipped with a characteristic copulatory bursa, used to hold the female nematode in place during mating. The females themselves have a vulva situated near the center of the body, slightly anterior in *Necator* and slightly posterior in *Ancylostoma*. The anterior end of the parasites are formed into a

Buccal capsule, absent in members of the other Strongylida superfamilies, by which the different genera and species within the group may be differentiated. For example members of the genus *Necator* have capsules equipped with cutting plates on the ventral margins, and within the capsule itself small dorsal teeth. In contrast members of the genus *Ancylostoma* have pairs of teeth on the ventral margin of the capsule. The number of teeth will vary between different species of *Ancylostoma*, but is usually between one and four pairs.



Fig 2.2: Scanning electron micrograph of the mouth capsule of *Ancylostoma duodenale* (left), note the presence of four "teeth," two on each side and *Necator americanus* (right)



Fig 2.3: Left picture: Copulatory bursa and spines of *N. americanus* (a side view); Right picture: copulatory bursa of *A. duodenale* (a top view)

The eggs are bluntly rounded, thin shelled, and are almost indistinguishable between the different species, measuring approximately 60 by 40 μm , the eggs of *Ancylostoma* being slightly larger than those of *Necator*.

The Morphological Differences between Two species of Hookworms

Features	<i>A. duodenale</i>	<i>N. americanus</i>
Size	Larger	Smaller
Shape	Single curve, looks like C	Double curves, looks like S
Mouth	2 pairs of ventral teeth	1 pair of ventral cutting plates
Copulatory bursa	Circle in shape	Oval in shape
Copulatory spicule	1 pair with separate endings	1 pair of which unite to form a terminal hooklet
Caudal spine	Present	No
Vulva position	Post-equatorial	Pre-equatorial

Life cycle

The life cycles of all the hookworms are very similar. The eggs are passed in the faeces, once exposed to air they mature rapidly if conditions are right, with both moisture and warmth essential for development. When matured, they hatch to liberate a rhabditiform (i.e. having an oesophagus where a thick anterior region is connected via a neckline region with a posterior bulb) L1 larvae after a few days.

These larval nematodes feed on bacteria and organic material in the soil, where they live and grow for about two days before undergoing the first moult. After about five days more growth they moult again, to produce a much more slender L3 larvae. The L3 larvae has a much shorter oesophagus, is a non-feeding form, and is the infective form of the parasite. Infection takes place by penetration of the skin, for example when walking with bare feet over contaminated damp soil, followed by entry into the circulatory system. Here they are carried to the heart, and then lungs. Once in the lungs, they are too large to pass through the capillary bed there. Instead they become trapped, and burrow through the capillary epithelium, entering the air spaces. They then migrate up through bronchi and trachae, and are then swallowed. Once swallowed, they pass into the intestine and bury themselves between the intestinal villi. Here they moult to form the L4 larvae, equipped with a buccal capsule allowing adherence to the gut wall. After about thirteen days post-infection they moult for the final time, producing immature adult worms. These mature over three to four weeks (i.e. five to six weeks after infection), then mate and commence egg laying to complete the life cycle. These parasites show a very high fecundity, female *Necator americanus* producing up to 10,000 eggs daily, while female *Ancylostoma duodenale* produces up to 20,000 eggs daily.

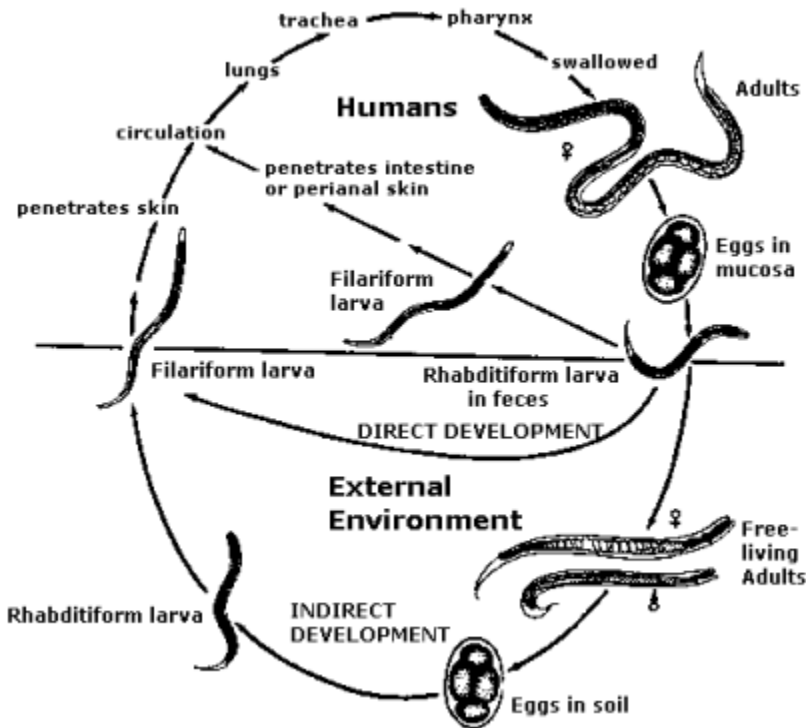


Fig 2.4: Life cycle of Human Hookworms (source: rrh.org.au)

Pathology of Infection.

The Pathology associated with hookworm infections may be divided roughly into two areas. Firstly the pathology associated with the presence of the adult parasite in the intestine, and secondly the pathology associated with the penetration of, and migration of the larval worms within the skin. The adult hookworms attach themselves to the intestinal wall using their buccal capsules. Their preferred site of infestation is in the upper layer of the small intestine, but in very heavy infections (where many thousands of worms may be present) the parasites may spread down as far as the lower ileum. Once attached to the intestinal wall, the hookworm mouthparts penetrate blood vessels, and the parasites obtain nutrition by sucking blood.



Fig 2.5: Adults in intestinal mucosa

A single *Necator americanus* will take approximately 30 μl of blood daily, while the larger *Ancylostoma duodenale* will take up to 260 μl . The gross pathology of the disease is very dependent on the intensity of infection. Light infections appear asymptomatic, but in heavy infections, the continuous loss of blood leads to a chronic anaemia, with down to 2gm of haemoglobin per 100ml of blood in extreme cases. Experiments carried out in the 1930's showed that in dogs infected with 500 *Ancylostoma caninum* a similar species to the human parasite, nearly a pint of blood a day was lost. This leads to permanent loss of iron and many blood proteins as well as blood cells. This in turn has consequences for further production of erythrocytes, which have been shown to contain less haemoglobin, as well as being reduced in size and smaller in numbers. This form of anaemia may be directly fatal, but more often, it induces more non-specific symptoms, the most noticeable being the severe retardation in growth and development, both physical and mental, in infected children, and a general weakness and lassitude, often wrongly interpreted as "laziness".

Diagnosis

Identify characteristic eggs in faecal samples. Note the eggs of *N. americanus* and *A. duodenale* are morphologically identical.



Fig 2.6: Egg of Hookworm

Epidemiology and Control

The factors of epidemiological importance include;

- Poor sanitation through contamination of soil through direct defaecation on the ground.
- Skin exposure to infections e.g. by walking about bare-footed
- Favourable environmental conditions that enhance eggs and larval development.
- Loose, humus soil with reasonable drainage and aeration
- Even distribution of rainfall throughout the year.

Control is by improvement in the standard of sanitation, raising the nutritional status of the population especially in relation to iron content, and mass treatment with suitable worm expeller (vermifuge)

3.3 *Trichuris trichiura* (human whipworm)

The first written record of *Trichuris trichiura* was made by Morgani, an Italian scientist, who identified the presence of the parasite in a case of worms residing in the colon in 1740. Exact Morphological description and figures were first recorded in 1761 by Roedere, a German physicist. Soon after morphology and visual representation of the worms, *Trichuris trichiura* was given taxonomy (during the 18th century).

This is the third most common round worm of humans. It is distributed worldwide, with infections more frequent in areas with tropical weather and poor sanitation practices, and among children. It is estimated that 800 million people are infected worldwide. The southern United States is endemic for trichuriasis.

Morphology

Adult worms are usually 3–5 cm long, with females being larger than males as is typical of nematodes. The thin, clear majority of the body (the anterior, whip-like end) is the oesophagus, and it is the end that the worm threads into the mucosa of the colon. The widened, pinkish gray region of the body is the posterior, and it is the end that contains the parasite's intestines and reproductive organs. *Trichuris trichiura* has characteristic football-shaped eggs, which are about 50-54µm long and contain polar plugs (also known as refractile prominences) at each end.

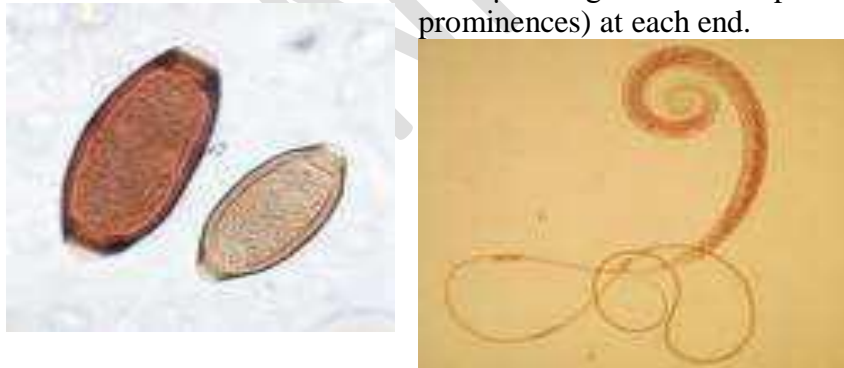


Fig 2.7: Egg and adult of *Trichuris trichiura*

Life cycle and transmission

Humans can become infected with the parasite due to ingestion of infective eggs by mouth contact with hands or food contaminated with egg-carrying soil. However, there have also been

rare reported cases of transmission of *Trichuris trichiura* by sexual contact. Some major outbreaks have been traced to contaminated vegetables (due to presumed soil contamination). Unembryonated eggs (unsegmented) are passed in the faeces of a previous host to the soil. In the soil, these eggs develop into a 2-cell stage (segmented egg) and then into an advanced cleavage stage. Once at this stage, the eggs embryonate and then become infective, a process that occurs in about 15 to 30 days). Next, the infective eggs are ingested by way of soil-contaminated hands or food and hatch inside the small intestine, releasing larvae into the gastrointestinal tract. These larvae burrow into a villus and develop into adults (over 2–3 days). They then migrate into the cecum and ascending colon where they thread their anterior portion (whip-like end) into the tissue mucosa and reside permanently for their year-long life span. About 60 to 70 days after infection, female adults begin to release unembryonated eggs (oviposit) into the cecum at a rate of 3,000 to 20,000 eggs per day, linking the life cycle to the start.

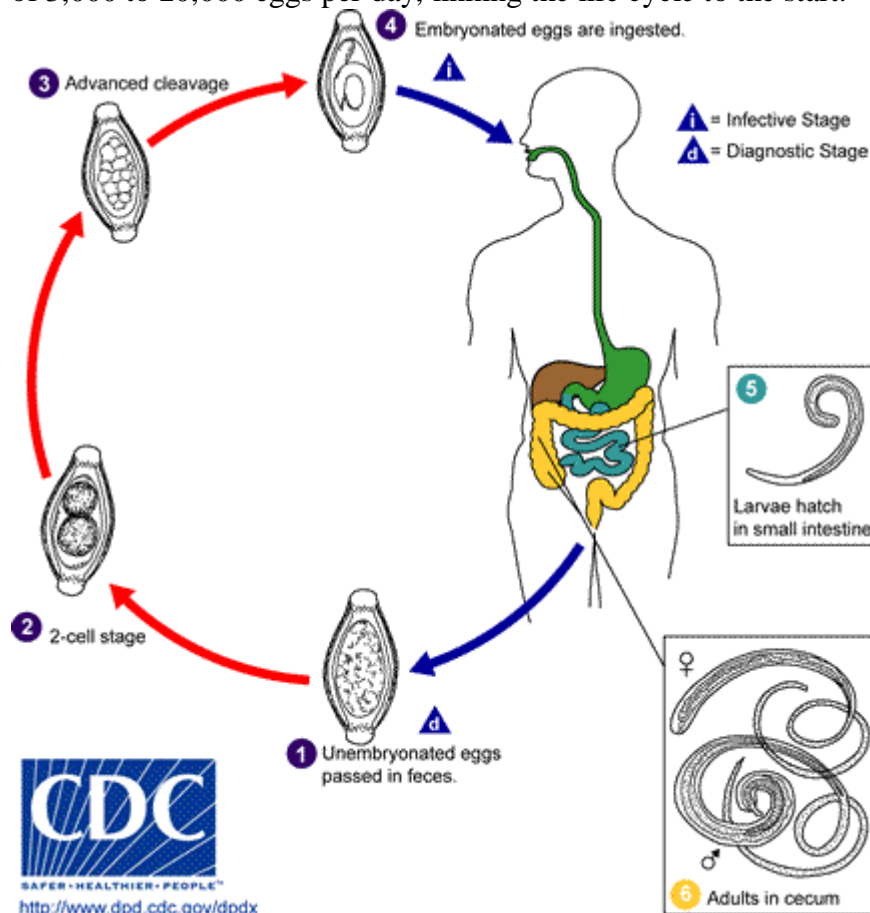


Fig 2.8: Life cycle of *Trichuris trichiura*

Signs and symptoms

Light infestations are frequently asymptomatic (have no symptoms). Heavier infestations, especially in small children, can present gastrointestinal problems including abdominal pain and distention, bloody or mucous-filled diarrhoea, and tenesmus (feeling of incomplete defecation, generally accompanied by involuntary straining). While damage may be done to the GI tissue and appendicitis may be brought on (by damage and oedema of the adjacent lumen) if there are large numbers of worms or larvae present, it has been suggested that the embedding of the

worms into the ileo-cecal region may also make the host susceptible to bacterial infection. Severe infection may also present with rectal prolapse, although this is typically seen only in heavy infections of small children. High numbers of embedded worms in the rectum cause oedema, which causes the rectal prolapse. The prolapsed, inflamed and oedematous rectal tissue may even show visible worms.

Growth retardation, weight loss, nutritional deficiencies, and anaemia (due to long-standing blood loss) are also characteristic of infection, and these symptoms are more prevalent and severe in children.

Diagnosis

A stool ova and parasites examination reveals the presence of typical whipworm eggs. Typically, the Kato-Katz thick-smear technique is used for the identification of the *Trichuris trichiura* eggs in the stool sample. Although colonoscopy is not typically used for diagnosis, but there have been reported cases in which colonoscopy has revealed adult worms. Colonoscopy can directly diagnose trichuriasis by identification of the threadlike form of worms with an attenuated, whip-like end. Colonoscopy has been shown to be a useful diagnostic tool, especially in patients infected by only a few male worms and with no eggs presenting in the stool sample.

Epidemiology

Trichuris trichiura is the third most common nematode (roundworm) of humans. Infection of trichuris trichiura is most frequent in areas with tropical weather and poor sanitation practices. Trichuriasis occurs frequently in areas in which human feces is used as fertilizer or where defecation onto soil takes place. Trichuriasis infection prevalence is 50 to 80 percent in some regions of Asia (noted especially in China and Korea) and also occurs in rural areas of the southeastern United States. Infection is most prevalent among children, and in North America, infection occurs frequently in immigrants from tropical or sub-tropical regions. It is estimated that 600-800 million people are infected worldwide with 3.2 billion individuals at risk.

Control and Prevention

Improved facilities for faeces disposal have decreased the incidence of whipworm. Handwashing before food handling, and avoiding ingestion of soil by thorough washing of food that may have been contaminated with egg-containing soil are other preventive measures. Mass Drug Administration (preventative chemotherapy) has had a positive effect on the disease burden of trichuriasis in East and West Africa, especially among children, who are at highest risk for infection. Improvement of Sewage and Sanitation systems, as well as improved facilities for faeces disposal have helped to limit defecation onto soil and contain potentially infectious faeces from bodily contact. A study in a Brazil Urban Centre demonstrated a significant reduction in prevalence and incidence of geohelminth infection, including trichuriasis, following implementation of a city-wide sanitation programme. A 33% reduction in prevalence of trichuriasis and a 26% reduction in incidence of trichuriasis was found in the study performed on 890 children ages 7–14 years old within 24 different sentinel areas chosen to represent the varied environmental conditions throughout the city of Salvador, Bahia, Brazil. Control of Soil Fertilizers has helped eliminate the potential for contact with human faecal matter in fertilizer in soil.

4.0 Conclusion

In this unit, we have discussed in details what soil transmitted helminthes are with various examples. Also discussed were their life cycles and their diagnostic features.

5.0 Summary

STH infection is caused by four major nematode species: *Ancylostoma duodenale* and *Necator americanus* (hookworms), *Ascaris lumbricoides* (roundworm) and *Trichuris trichiura* (whipworm). Infection is prevalent in areas with overpopulation and inadequate sanitation in tropical and sub-tropical countries, where the climate supports the survival of the parasite eggs or larvae in the warm and moist soil. After infective larvae enter the human body they develop into adult worms and parasitize the gastrointestinal tract, sometimes for years. Some species of worms can produce up to 200,000 eggs per day. Eggs are excreted in the faeces and remain viable in the soil for several weeks or years depending on the species. It is common for a single individual, especially a child, to be infected with all three types of worms. Although STH infection rarely causes fatality, chronic infection with high worm burden can lead to serious health consequences. Infection is typically most intense and debilitating in school-age children, resulting in malnutrition, physical and intellectual growth retardation, and cognitive and educational deficits. *A. lumbricoides* may cause intestinal obstructions that require surgery, and *T. trichiura* may cause chronic colitis. Hookworm infection causes iron-deficiency anaemia because the worms feed on the intestinal wall causing tissue damage and blood loss. Hookworm infection is a leading cause of morbidity in children and pregnant women, and can have adverse results for the mother, the foetus and the neonate.

6.0 Tutor Marked Assignments

6.1 Activity: Conduct a physical examination of *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Trichuris trichiura* under the microscope and report your findings in the log book.

6.2 Answer the following questions:

- i. State the morphological features of *Ascaris lumbricoides* and *Trichuris trichiura* (LO1)
- ii. What strategies you will implore for the control of soil transmitted nematodes? (LO2)
- iii. Describe the life cycles of hookworm with emphasis on the route of infection (LO2)

7.0 References and other resources

- Bethony, J., S. Brooker, M. Albonico, S. M. Geiger, A. Loukas, D. Diemert, and P. J. Hotez. (2006). Soil transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* 367:1521.
- WHO. (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee. WHO Tech Rep Ser No. 912:1.

UNIT THREE- BLOOD AND TISSUE NEMATODES**CONTENT**

- 1.0** Introduction
- 2.0** Objectives
- 3.0** Main contents
- 3.1** Filarial worms
- 3.2** *Trichinella spiralis*
- 4.0** Conclusion
- 5.0** Summary
- 6.0** Tutor Marked Assignments
- 6.1** Activity
- 6.2** Tutor Marked Tests
- 7.0** References and other resources

1.0 Introduction

Filariasis is caused by nematodes (roundworms) that inhabit the lymphatics and subcutaneous tissues. Eight main species infect humans. Three of these are responsible for most of the morbidity due to filariasis: *Wuchereria bancrofti* and *Brugia malayi* cause lymphatic filariasis, and *Onchocerca volvulus* causes onchocerciasis (river blindness). The other five species are *Loa loa*, *Mansonella perstans*, *M. streptocerca*, *M. ozzardi*, and *Brugia timori*. (The last species also cause lymphatic filariasis).

Among the agents of lymphatic filariasis, *Wuchereria bancrofti* is encountered in tropical areas worldwide; *Brugia malayi* is limited to Asia; and *Brugia timori* is restricted to some islands of Indonesia. The agent of river blindness, *Onchocerca volvulus*, occurs mainly in Africa, with additional foci in Latin America and the Middle East. Among the other species, *Loa loa* and *Mansonella streptocerca* are found in Africa; *Mansonella perstans* occurs in both Africa and South America; and *Mansonella ozzardi* occurs only in the American continent.

Another tissue invading parasite is *Trichinella spiralis* whose larval form is found in the muscular tissue of the host animal. *Trichinella spiralis* is in fact a complex of three closely related worm species. They are morphologically identical, but differ in their host specificity and their biochemical characteristics. *T. spiralis spiralis* occurs in moderate regions and infects mainly pigs. *T. spiralis native* occurs in the polar regions (polar bear, walrus). These parasites are resistant to freezing which is important for meat storage. *T. spiralis nelsoni* occurs in Africa and southern Europe with a reservoir in wild carnivores and wild pigs. *T. britovi* and *T. pseudospiralis* rarely cause infections. *T. pseudospiralis* can also infect some birds as well as mammals, unlike the other *Trichinella* species.

2.0 Objectives

At the end of this unit, you should be able to

- i. Explain examples of blood and tissue invading parasites
- ii. Describe their life cycles and clinical features associated with their infections.
- iii. Describe the methods of diagnosis of their infections

3.0 Main Contents

3.1 Filarial worms

Wuchereria bancrofti

Different species of the following genera of mosquitoes are vectors of *W.bancrofti* filariasis depending on geographical distribution. Among them are:

Culex (*C. annulirostris*, *C. bitaeniorhynchus*, *C. quinquefasciatus*, and *C. pipiens*); *Anopheles* (*A. arabinensis*, *A. bancroftii*, *A. farauti*, *A. funestus*, *A. gambiae*, *A. koliensis*, *A. melas*, *A. merus*, *A. punctulatus* and *A. wellcomei*); *Aedes* (*A. aegypti*, *A. aquasalis*, *A. bellator*, *A. cooki*, *A. darlingi*, *A. kochi*, *A. polynesiensis*, *A. pseudoscutellaris*, *A. rotumae*, *A. scapularis*, and *A. vigilax*); *Mansonia* (*M. pseudotitillans*, *M. uniformis*); *Coquillettidia* (*C. juxtamansonia*). During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound. They develop in adults that commonly reside in the lymphatics. The female worms measure 80 to 100 mm in length and 0.24 to 0.30 mm in diameter, while the males measure about 40 mm by .1 mm. Adults produce microfilariae measuring 244 to 296 μm by 7.5 to 10 μm , which are sheathed and have nocturnal periodicity, except the South Pacific microfilariae which have the absence of marked periodicity. The microfilariae migrate into the lymph and blood channels moving actively through lymph and blood. A mosquito ingests the microfilariae during a blood meal. After ingestion, the microfilariae lose their sheaths and some of them work their way through the wall of the proventriculus and cardiac portion of the mosquito's midgut and reach the thoracic muscles. There the microfilariae develop into first-stage larvae and subsequently into third-stage infective larvae. The third-stage infective larvae migrate through the haemocoel to the mosquito's proboscis and can infect another human when the mosquito takes a blood meal.

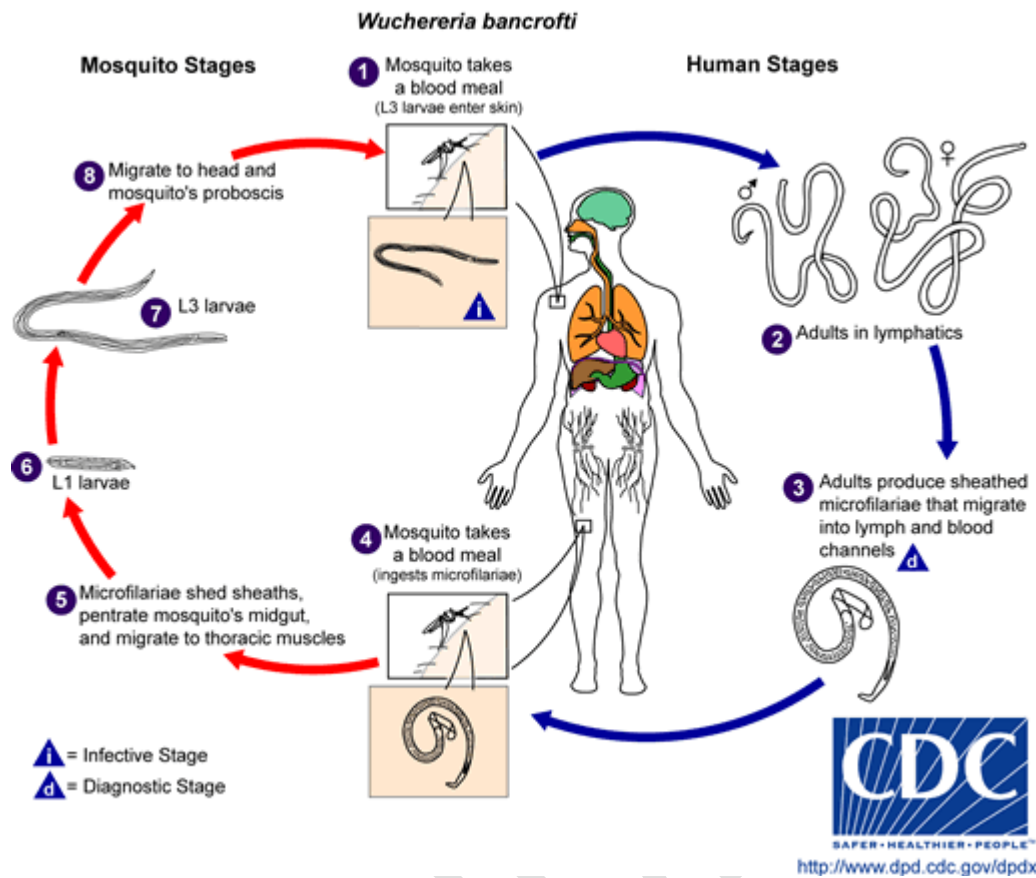


Fig 3.1 Life cycle of *W. bancrofti*



Fig 3.2: Microfilaria of *W. bancrofti*

Onchocerca volvulus

During a blood meal, an infected blackfly (genus *Simulium*) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound. In subcutaneous

tissues the larvae develop into adult filariae, which commonly reside in nodules in subcutaneous connective tissues. Adults can live in the nodules for approximately 15 years. Some nodules may contain numerous male and female worms. Females measure 33 to 50 cm in length and 270 to 400 μm in diameter, while males measure 19 to 42 mm by 130 to 210 μm . In the subcutaneous nodules, the female worms are capable of producing microfilariae for approximately 9 years. The microfilariae, measuring 220 to 360 μm by 5 to 9 μm and unsheathed, have a life span that may reach 2 years. They are occasionally found in peripheral blood, urine, and sputum but are typically found in the skin and in the lymphatics of connective tissues. A blackfly ingests the microfilariae during a blood meal. After ingestion, the microfilariae migrate from the blackfly's midgut through the haemocoel to the thoracic muscles. There the microfilariae develop into first-stage larvae and subsequently into third-stage infective larvae. The third-stage infective larvae migrate to the blackfly's proboscis and can infect another human when the fly takes a blood meal.

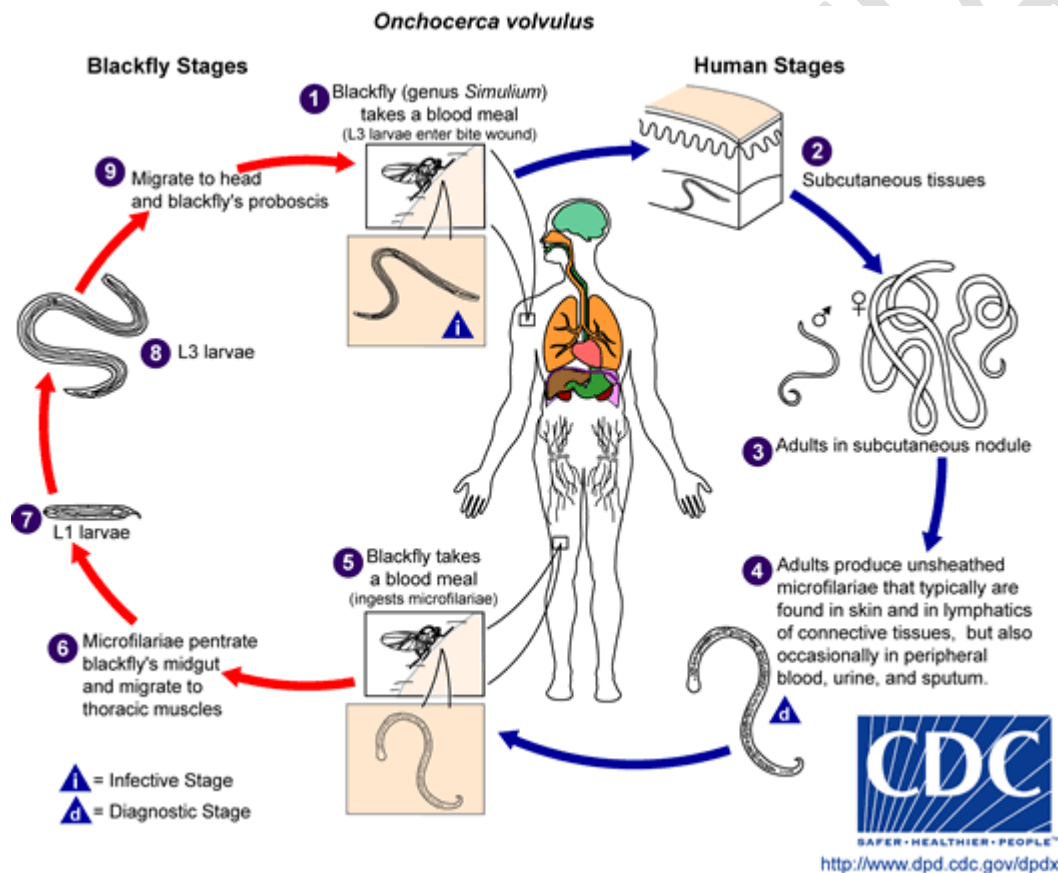


Fig 3.3: Life cycle of *O. Volvulus*

Loa loa

The vector for *Loa loa* filariasis are flies from two species of the genus *Chrysops*, *C. silacea* and *C. dimidiata*. During a blood meal, an infected fly (genus *Chrysops*, day-biting flies) introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound. The larvae develop into adults that commonly reside in subcutaneous tissue. The female worms measure 40 to 70 mm in length and 0.5 mm in diameter, while the males measure 30 to 34 mm in length and 0.35 to 0.43 mm in diameter. Adults produce microfilariae measuring 250

to 300 μm by 6 to 8 μm , which are sheathed and have diurnal periodicity. Microfilariae have been recovered from the spinal fluids, urine, and sputum. During the day they are found in peripheral blood, but during the noncirculation phase, they are found in the lungs. The fly ingests microfilariae during a blood meal. After ingestion, the microfilariae lose their sheaths and migrate from the fly's midgut through the haemocoel to the thoracic muscles of the arthropod. There the microfilariae develop into first-stage larvae and subsequently into third-stage infective larvae. The third-stage infective larvae migrate to the fly's proboscis and can infect another human when the fly takes a blood meal.

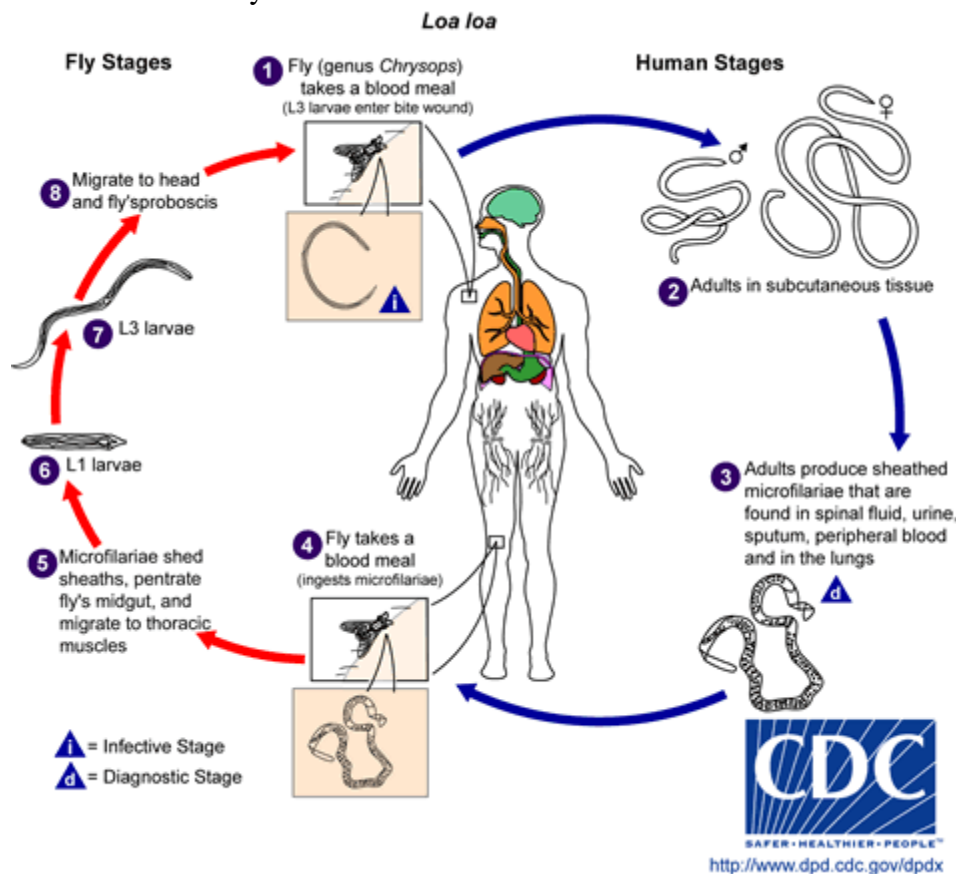


Fig 3.4 Life cycle *Loa loa*

Brugia malayi

The typical vector for *Brugia malayi* filariasis is mosquito species from the genera *Mansonia* and *Aedes*. During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound. They develop into adults that commonly reside in the lymphatics. The adult worms resemble those of *Wuchereria bancrofti* but are smaller. Female worms measure 43 to 55 mm in length by 130 to 170 μm in width, and males measure 13 to 23 mm in length by 70 to 80 μm in width. Adults produce microfilariae, measuring 177 to 230 μm in length and 5 to 7 μm in width, which are sheathed and have nocturnal periodicity. The microfilariae migrate into the lymph and enter the blood stream reaching the peripheral blood. A mosquito ingests the microfilariae during a blood meal. After ingestion, the microfilariae lose their sheaths and work their way through the wall of the proventriculus and cardiac portion of the midgut to reach the thoracic muscles. There the

microfilariae develop into first-stage larvae and subsequently into third-stage larvae. The third-stage larvae migrate through the haemocoel to the mosquito's proboscis and can infect another human when the mosquito takes a blood meal.

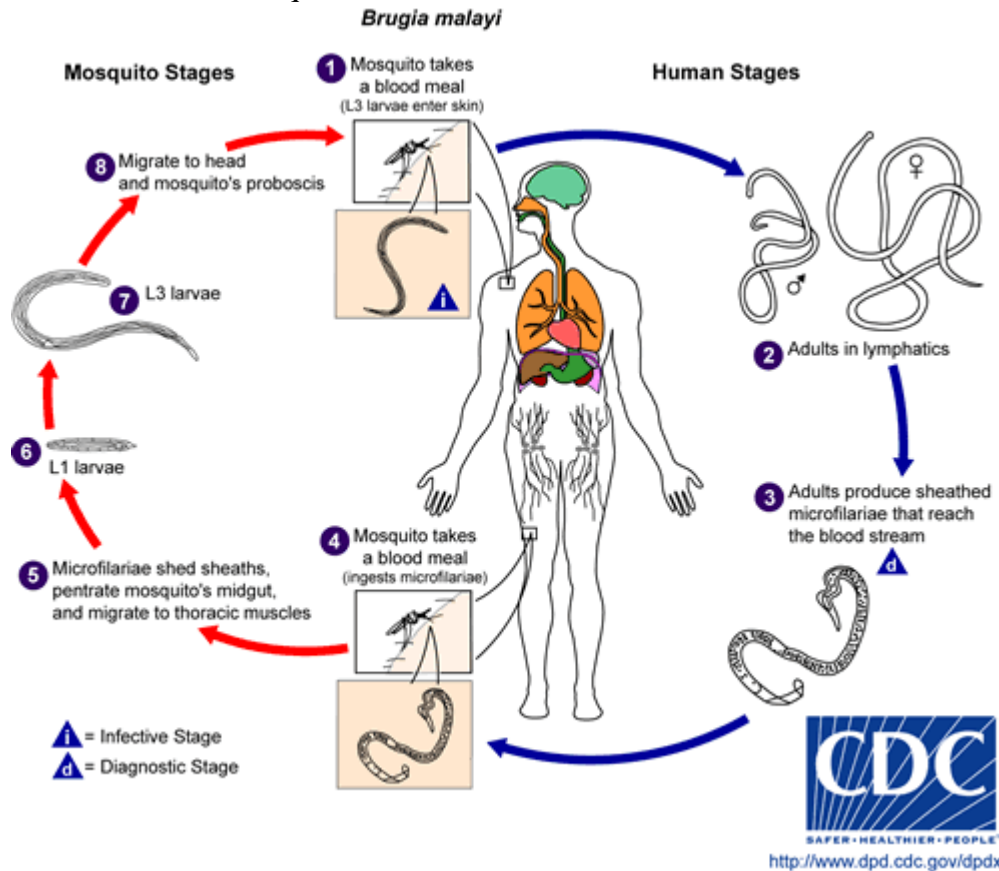


Fig 3.5: Life cycle of *Brugia malayi*

Clinical Features and Pathology

Lymphatic filariasis most often consists of asymptomatic microfilaremia. Some patients develop lymphatic dysfunction causing lymphedema and elephantiasis (frequently in the lower extremities) and, with *Wuchereria bancrofti*, hydrocele and scrotal elephantiasis. Episodes of febrile lymphangitis and lymphadenitis may occur. Persons who have newly arrived in disease-endemic areas can develop afebrile episodes of lymphangitis and lymphadenitis. An additional manifestation of filarial infection, mostly in Asia, is pulmonary tropical eosinophilia syndrome, with nocturnal cough and wheezing, fever, and eosinophilia. Onchocerciasis can cause pruritus, dermatitis, onchocercomata (subcutaneous nodules), and lymphadenopathies. The most serious manifestation consists of ocular lesions that can progress to blindness. Loiasis (*Loa loa*) is often asymptomatic. Episodic angioedema (Calabar swellings) and sub-conjunctival migration of an adult worm can occur. Infections by *Mansonella perstans*, which is often asymptomatic, can be associated with angioedema, pruritus, fever, headaches, arthralgias, and neurologic manifestations. *Mansonella streptocerca* can cause skin manifestations including pruritus, papular eruptions and pigmentation changes. Eosinophilia is often prominent in filarial infections. *Mansonella ozzardi* can cause symptoms that include arthralgias, headaches, fever, pulmonary symptoms, adenopathy, hepatomegaly, and pruritus.



Fig 3.6: Elephantiasis caused by infection by *W. bancrofti*

Treatment and control

Ivermectin is effective in killing the larvae, but does not affect the adult worm. Preventive measures include vector control, treatment of infected individuals and avoidance of black fly.

Laboratory Diagnosis of filarial worms

Identification of microfilariae by microscopic examination is the most practical diagnostic procedure.

Microscopy

Examination of blood samples will allow identification of microfilariae of *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, *Loa loa*, *Mansonella perstans*, and *M. ozzardi*. It is important to time the blood collection with the known periodicity of the microfilariae. The blood sample can be a thick smear, stained with Giemsa or haematoxylin and eosin. For increased sensitivity, concentration techniques can be used. These include centrifugation of the blood sample lysed in 2% formalin (Knott's technique), or filtration through a Nucleopore® membrane. Examination of skin snips will identify microfilariae of *Onchocerca volvulus* and *Mansonella streptocerca*. Skin snips can be obtained using a corneal-scleral punch, or more simply a scalpel and needle. The sample must be allowed to incubate for 30 minutes to 2 hours in saline or culture medium, and then examined for microfilariae that would have migrated from the tissue to the liquid phase of the specimen.

Preparing Blood Smears for Microscopy Examination

If one uses venous blood, blood smears should be prepared as soon as possible after collection (delay can result in changes in parasite morphology and staining characteristics).

Thick smears

Thick smears consist of a thick layer of dehemoglobinized (lysed) red blood cells (RBCs). The blood elements (including parasites, if any) are more concentrated (app. 30×) than in an equal area of a thin smear. Thus, thick smears allow a more efficient detection of parasites (increased sensitivity). However, they do not permit an optimal review of parasite morphology. For

example, they are often not adequate for species identification of filaria parasites: if the thick smear is positive for filaria parasites, the thin smear should be used for species identification.

How to Prepare a thick smears

- i. Place a small drop of blood in the centre of the pre-cleaned, labeled slide.
- ii. Using the corner of another slide or an applicator stick, spread the drop in a circular pattern until it is the size of a dime (1.5 cm²).
- iii. A thick smear of proper density is one which, if placed (wet) over newsprint, allows you to barely read the words.
- iv. Lay the slides flat and allow the smears to dry thoroughly (protect from dust and insects!). Insufficiently dried smears (and/or smears that are too thick) can detach from the slides during staining. The risk is increased in smears made with anticoagulated blood. At room temperature, drying can take several hours; 30 minutes is the minimum; in the latter case, handle the smear very delicately during staining. You can accelerate the drying by using a fan or hair dryer (uses cool setting). Protect thick smears from hot environments to prevent heat-fixing the smear.
- v. Do not fix thick smears with methanol or heat. If there will be a delay in staining smears, dip the thick smear briefly in water to haemolyse the RBCs.

Thin smears

Thin smears consist of blood spread in a layer such that the thickness decreases progressively toward the feathered edge. In the feathered edge, the cells should be in a monolayer, not touching one another.

How to prepare thin smears

- i. Place a small drop of blood on the pre-cleaned, labeled slide, near its frosted end.
- ii. Bring another slide at a 30-45° angle up to the drop, allowing the drop to spread along the contact line of the 2 slides.
- iii. Quickly push the upper (spreader) slide toward the unfrosted end of the lower slide.
- iv. Make sure that the smears have a good feathered edge. This is achieved by using the correct amount of blood and spreading technique.
- v. Allow the thin smears to dry. (They dry much faster than the thick smears, and are less subject to detachment because they will be fixed.)
- vi. Fix the smears by dipping them in absolute methanol.

Special Procedures for Detecting Microfilariae

Blood microfilariae:

A. Capillary (fingerstick) blood

Since microfilariae concentrate in the peripheral capillaries, thick and thin smears prepared from fingerstick blood are recommended.

B. Anticoagulated (EDTA) venous blood (1 ml) should be concentrated by one of the following methods:

1. Centrifugation (Knott's technique)

- a. Prepare 2% formaldehyde (2 ml of 37% formaldehyde + 98 ml H₂O).

b. Mix 9 ml of this 2% formaldehyde with 1 ml of patient's venous blood. Centrifuge at $500 \times g$ for 10 minutes; discard supernatant. Sediment is composed of WBCs and microfilariae (if present).

c. Examine as temporary wet mounts.

d. Prepare thick and thin smears; allow to dry; dip in absolute methanol before Giemsa staining to enhance staining of microfilariae.

2. Filtration

a. Place Millipore® or Nucleopore® membrane filter (5 μm pore) in filter holder with syringe attachment.

b. Mix 1 ml of venous blood (in EDTA) with 10 ml of 10% Teepol® 610 (Shell Co.); allow to stand for several minutes to allow lysis; transfer to a 10 ml Luer-Loc® syringe; attach the filter apparatus.

c. Force the solution through the 5 μm pore filter, followed by several syringes of water to wash out the remaining blood, then 1 or 2 syringes full of air to clear excess fluid.

d. Prepare a temporary wet mount by removing the filter and placing it on a glass slide, adding a drop of stain or dye and a coverslip.

e. For permanent preparations, pass 2 to 3 ml of methanol through the filter while it is still in the holder; remove filter and dry it on a glass slide; then stain it with Giemsa stain, horizontally (so that the filter does not wash off the slide); coverslip filter before examining.

Diagnostic findings

- **Antigen detection** using an immunoassay for circulating filarial antigens constitutes a useful diagnostic approach, because microfilaremia can be low and variable. A rapid-format immunochromatographic test, applicable to *Wuchereria bancrofti* antigens, has been evaluated in the field. However, antibody detection is of limited value. Substantial antigenic cross reactivity exists between filaria and other helminths, and a positive serologic test does not distinguish between past and current infection.
- **Molecular diagnosis** using polymerase chain reaction is available for *W. bancrofti* and *B. malayi*.
- **Identification of adult worms** is possible from tissue samples collected during nodulectomies (onchocerciasis), or during subcutaneous biopsies or worm removal from the eye (loiasis).

3.2 *Trichinella spiralis*

Historical aspect

In 1835, a man died of tuberculosis in St Bartholomew's Hospital, London. Dr Paget, a first-year student, carried out the autopsy and observed fine hard white inclusions in the muscles. Similar inclusions had been observed by doctors from time to time in the past, but were attributed to commonplace muscle calcification, which quickly blunted the dissecting scalpel. Dr Paget inspected the lesions with a hand lens and quickly recognised their worm-like structure. The name "*Trichina spiralis*" was suggested. This name *Trichina* had already been given to a certain fly, however, and the name was later changed to "*Trichinella*". The discovery of the parasite was published by the famous biologist and palaeontologist Richard Owen, at that time assistant conservator of the museum of the Royal College of Surgeons. In 1859 Rudolph Virchow carried out transmission experiments in which infected human muscle was fed to a healthy dog. After only 3 to 4 days adult *Trichinella* worms were found in the dog's duodenum and jejunum.

Life cycle

More than 100 species of mammals are susceptible to the infection. Infections with *Trichinella spiralis* affect chiefly carnivores and omnivores, although infection of horses has also been described. People become infected with this nematode by eating raw or insufficiently cooked infected meat, often pork or wild boar. The larvae of *Trichinella spiralis* which are in the meat develop in a few days into adult worms (2-4 mm) in the wall of the small intestine. There they lay larvae (100 m m). These spread via the bloodstream to various muscles, including the heart, where they undergo encapsulation [*Trichinella pseudospiralis* does not form a capsule]. The larvae cannot continue to survive in the heart. The larvae are localised within the cells of the muscles, which is unique for a worm. After penetrating the muscle cell, a larva excretes a number of signal molecules and proteins, which convert the cell to what is called a nurse cell. In the cell the behaviour of the worm is rather similar to that of a virus. Many of its proteins are glycosylated and often carry an unusual sugar (tyvelose). These proteins are excreted from a special organ in the larva (the stichosome). Various muscle proteins such as actin and myosin change or disappear, nuclear division is stimulated and mitochondria are damaged. Local angiogenesis is stimulated by excretion of a blood vessel growth factor and new blood vessels, originating from nearby venules, develop and form a network around the infected cell. The metabolism of the nurse cell and the parasite is essentially anaerobic. After 1 to 4 months the adult worms die. The larvae in the muscles sometimes survive for years and can remain viable for a long time even in rotting flesh. *Trichinella* is unique among worms in that all development stages take place in the same host. There is never a free stage outside the mammalian body.

Symptoms

Infection may be asymptomatic. In typical cases there is diarrhoea, abdominal pain, vomiting and fever a few days after eating infected meat. After 10 days the fever increases, the patient is very ill and debilitated, there are muscle pains and a typical peri-orbital oedema (differential diagnosis acute trypanosomiasis and nephrosis). This oedema is caused by invasion of the small muscles around the eye. There may be signs of myocarditis, encephalitis, urticaria and asthma. There is often very significant eosinophilia. The myositis causes an increase in the muscle enzymes (creatine phosphokinase, CK). After a few months the symptoms are reduced or disappear. Mild infections are self-limiting.

Diagnosis

Not many nematodes are found in muscle tissue. Occasionally a migrating third stage larva of *Ancylostoma*, *Toxocara* or *Gnathostoma* may be found (visceral larva migrans). *Dracuncula medinensis* may also be found in muscle tissue. Another, less common nematode which may be found here is *Haycocknema perplexum* (Tasmania).

Prevention

- Meat should be well boiled or roasted thoroughly.
- Importance of meat inspection. The diaphragm of a slaughtered animal is inspected (the piece of muscle is flattened between two glass slides and examined using transillumination). This technique (trichinoscopy) is not so good for *Trichinella pseudospiralis* because it is not surrounded by a capsule and is easily missed.
- Pig food (which may include infected rats) should be boiled for 30 minutes.

- To store pork for 10 days at -25° C is generally impractical in developing countries. In the West meat is sometimes irradiated with high doses of gamma rays, which will kill any larvae. *Trichinella spiralis nativa* is cold-hardy.

4.0 Conclusion

Blood and tissues are important ingredients for human survival, however despite their importance, several factors exist that can hinder or reduce their continuous existence. Therefore, this unit discussed various Parasites affecting human blood and tissue. Also discussed were their life cycles and clinical features associated with their infections.

5.0 Summary

Filariasis is caused by nematodes that inhabit the lymphatics and subcutaneous tissues. Eight main species infect humans of which three of these are responsible for most of the morbidity due to filariasis: *Wuchereria bancrofti* and *Brugia malayi* cause lymphatic filariasis, and *Onchocerca volvulus* causes onchocerciasis (river blindness) Infective larvae are transmitted by infected biting arthropods during a blood meal. The larvae migrate to the appropriate site of the host's body, where they develop into microfilariae-producing adults. The adults dwell in various human tissues where they can live for several years. The agents of lymphatic filariasis reside in lymphatic vessels and lymph nodes; *Onchocerca volvulus* in nodules in subcutaneous tissues; *Loa loa* in subcutaneous tissues, where it migrates actively; *Brugia malayi* in lymphatics, as with *Wuchereria bancrofti*; *Mansonella streptocerca* in the dermis and subcutaneous tissue; *Mansonella ozzardi* apparently in the subcutaneous tissues; and *M. perstans* in body cavities and the surrounding tissues. The female worms produce microfilariae which circulate in the blood, except for those of *Onchocerca volvulus* and *Mansonella streptocerca*, which are found in the skin, and *O. volvulus* which invade the eye. The microfilariae infect biting arthropods mosquitoes for the agents of lymphatic filariasis; blackflies (*Simulium*) for *Onchocerca volvulus*; midges for *Mansonella perstans* and *M. streptocerca*; and both midges and blackflies for *Mansonella ozzardi*; and deerflies (*Chrysops*) for *Loa loa*). Inside the arthropod, the microfilariae develop in 1 to 2 weeks into infective filariform (third-stage) larvae. During a subsequent blood meal by the insect, the larvae infect the vertebrate host. They migrate to the appropriate site of the host's body, where they develop into adults, a slow process than can require up to 18 months in the case of *Onchocerca*.

Infections with *Trichinella spiralis* affect chiefly carnivores and omnivores. People become infected by eating raw or insufficiently cooked infected meat, often pork or wild boar. The larvae of *Trichinella spiralis* which are in the meat develop in a few days into adult worms (2-4 mm) in the wall of the small intestine. There they lay larvae (100 mm). These spread via the bloodstream to various muscles, including the heart, where they undergo encapsulation. Vector control in case of filariasis and proper cooking of pork (*Trichinella spiralis*) are the control measures.

6.0 Tutor Marked Assignments

6.1 **Activity:** Prepare thick and thin smears for two patients each.

6.2 Answer the following questions:

- i. Write short note on *Wuchereria bancrofti*, *Onchocerca volvulus*, *Loa loa* (LO1)
- ii. Describe the life cycles and clinical features associated with *Wuchereria bancrofti*, *Onchocerca volvulus*, *Loa loa* infections (LO2)
- iii. Describe the laboratory diagnosis and treatment of filarial worms (LO3)

7.0 References and other resources

Duke (1972). Onchocerciasis. *Br. Med. Bull.*, 28: 66-71

Neva and Ottessen (1978). Tropical (filarial) eosinophilia. *N. Engl. J. Med.*, 298: 1129-1131

UNIT 4- AIR-BORNE NEMATODE

CONTENT

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main contents
- 3.1 *Enterobius vermicularis* (the human pin-worm)
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor Marked Assignments
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1.0 Introduction

The human pinworm *Enterobius vermicularis* is an ubiquitous parasite of man. It is estimated that over 200 million people are infected annually. It is more common in the temperate regions of Western Europe and North America, (it is being relatively rare in the tropics) and is found particularly in children. Samples of Caucasian children in the U.S.A. and Canada have shown incidences of infection of between 30% to 80%, with similar levels in Europe, and although these regions are the parasites strongholds, it may be found throughout the world, again often with high degrees of incidence. For example in parts of South America the incidence in school children may be as high as 60%. Interestingly non-Caucasians appear to be relatively resistant to infection with this nematode. As a species, and contrary to popular belief, *E. vermicularis* is entirely restricted to man, other animals harbouring related but distinct species that are non-

infective to humans, but their fur may be contaminated by eggs from the human species if stroked by someone with eggs on their hands. In man anywhere where there are large numbers of children gathered together, (such as nurseries, play groups, orphanages etc.), especially if conditions are insanitary, are ready sources of infection, as one child may rapidly transmit the parasite to his or her fellows.

2.0 Learning Objectives

At the end of this unit, you should be able to:

- i. Describe the morphology of *Enterobius vermicularis*
- ii. Describe the life cycle of *Enterobius vermicularis*
- iii. Examine the pathology, diagnosis and control of *Enterobius vermicularis*

3.0 Main Contents

3.1 *Enterobius vermicularis* (the human pin-worm)

Morphology

These creamy white coloured nematodes are relatively small, with the female measuring only approximately 10mm by 0.4mm wide. The females have a cuticular expansion at their anterior ends, with a long pointed tail. The male parasites, which are much less numerous than the females, are much smaller, measuring only up to 5mm long, and have a curved tail, with a small bursa like expansion, and a single spicule. The head has a mouth with three small lips.



Fig 4.1: Adult Pinworm

Life cycle

The adult parasites live predominantly in the caecum. The male and females mate, and the uteri of the females become filled with eggs. The gravid females (each containing up to 15000 eggs) then migrate down the digestive tract to the anus. From here they make regular nocturnal migrations out of the anus, to the perianal region, where air contact stimulates them to lay their eggs, before retreating back into the rectum. Eventually the female die, their bodies disintegrating to release many remaining eggs. These eggs, which are clear and measure about 55 by 30µm, then, mature to the infectious stage (containing an L1 larvae) over 4 to 6 weeks. To infect the host, typically these eggs must then be ingested. The ingested eggs hatch in the duodenum. The eggs themselves are sticky, and have a characteristic shape, shared with all members of the group Oxyuridea, with an asymmetrical form, flattened on one side, (see below);



Fig 4.2: The ova of *Enterobius vermicularis*

The larvae then undergo a series of moults, as they migrate down the digestive tract. The adult worms then mature in the caecum, before copulating to complete the cycle (typically 6 weeks). Occasionally the eggs hatch in the perianal region itself, the resulting L1 larvae being fully infective, crawling back through the anus, then migrating up the intestine to the caecum (retroinfection).

Pathology of Infection.

The majority of infections with this nematode are asymptomatic, although in some cases the emerging females and the sticky masses of eggs that they lay may cause irritation of the perianal region, which in some cases may be severe. As the females emerge at night this may give rise to sleep disturbances, and scratching of the affected perianal area transfers eggs to the fingers and under the finger nails. This in turn aids the transmission of the eggs, both back to the original host (autoinfection), and to other hosts.

Diagnosis

Because eggs are rarely passed out with faeces, examination of faecal samples may not reveal them. This may account for negative results of enterobiasis in many of the surveys for helminth infections involving faecal samples in tropical Africa. The most reliable diagnosis is by the cellophane tape swab. This involves the attachment of a piece of cellophane to the perianal region overnight. This is then examined for eggs under the microscope. Alternatively, the anus and perianal area can be examined under bright light at night, at which time adult worms can be seen glittering in the light.

Epidemiology and Control

The eggs of the parasite are air-borne, caught in clothing, household linen, curtain, carpets, etc. As such, infection is common in dry season than rainy season in the tropics. Maintenance of high standards of personal and domestic hygiene is therefore imperative for control and prevention.

4.0 Conclusion

In this unit, the morphology of *Enterobius vermicularis* and their life cycle have been discussed. Also discussed were the pathology, diagnosis and control of *Enterobius vermicularis*

5.0 Summary

Enterobius vermicularis is an air-borne parasitic infection common mostly in the temperate regions of the world. Adult female worms lay eggs in the perianal regions and infection occurs through direct ingestion of eggs containing the L1 larvae. Infection is usually asymptomatic but

sometimes the sticky eggs could cause irritation of the perianal giving rise to scratching and sleep disturbance. Maintenance of high standards of personal and domestic hygiene is imperative for control and prevention.

6.0 Tutor Marked Assignments

6.1 Activity: Conduct a physical examination of an adult pinworm under the microscope and report your findings in the log book.

6.2 Answer the following questions:

- i. With a well label diagram describe the morphology of *Enterobius vermicularis* (LO1)
- ii. Describe the life cycle of *Enterobius vermicularis* (LO2)
- iii. Explain the pathology, diagnosis and control of *Enterobius vermicularis* (LO3)

7.0 References other resources

Brown, H.W. and Neva, F.A. (1983). Basic clinical parasitology (5th edition). pp. 128-132