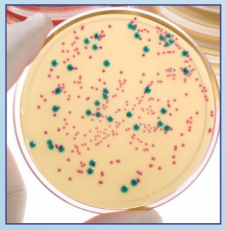
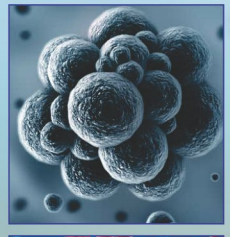
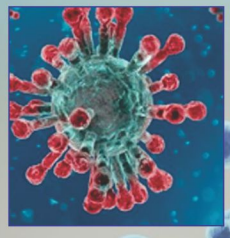
Essentials of Microbiology

An Integrated Clinical Based Approach

Including Parasitology







As per the latest CBME Guidelines |
Competency Based Undergraduate
Curriculum for the Indian Medical Graduate

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Preface

The book is according to the latest competency based curriculum. The key role of Medical Microbiology undergraduate medical curriculum in the MBBS program has become indisputable after the Covid-19 pandemic. Medical Microbiology plays a key role in the diagnosis and management (including prevention) of infectious diseases. However, majority of the medical students fail to grasp that essence, while studying the subject. They struggle with the vast microbiological information available in the microbiology textbooks and are unable to see its relevance, besides finding difficulty in its recapitulation and integration with the medical curriculum. The current book is not a compilation of facts but comprises meaningful integrated clinical data. Such a style also encourages application of microbiological information.

The Competency based curriculum has emphasized a clinical oriented approach, to make the subject relevant to the national health priorities. This book is designed in this direction. In a first level medical microbiology progamme, a strict clinical system wise study of the microbes would not only hinder the understanding and concept building of Medical Microbiology in the students but could imprint erroneous perceptions, as most microbes know no anatomical borders. Competency based medical education curriculum advocates a horizontal and vertical integration of Microbiology with other subjects based on clinical systems. On this ground, microbiology has been a victim in the new CBME. How does one tackle this dilemma? The book has introduced more than 100 integrated clinical cases (including 23 of Parasitic diseases); referenced clinical system wise in exclusive Infectious Diseases section and dealt in the relevant microbiology category. So the book imbibes the spirit and word of the competency based curriculum and yet retain the traditional approach of microbe learning. It has bidirectional linkages, which permit navigation from clinical cases to core microbiology and otherwise. All the main chapters with clinical cases have been provided with linkages for providing comprehensive grasping of topics. Numerous bacterial agents have diverse microscopic, metabolic features, colony characteristics, media requirements, varying laboratory diagnosis profiles, treatment profiles and vaccines. To understand them and to recall them, there is no better way than to tabulate them on a mega scale covering entire topics. This approach has been followed comprehensively for these parameters.

To meet the CBME challenges, more than 15 Integrated clinical based studies in CNS, 24 in CVS (including BSI), 23 in RTI, 28 in GIT (including hepatobiliary), 3 in Kidney, 5 in Genitourinary system, 15 in skin, soft tissue and musculoskeletal system, 3 in Zoonotic and miscellaneous (belonging to 55 Bacterial & Fungal, 25 Viral and 23 Parasitic categories) have been incorporated to make a sound foundation of the Infectious diseases in the budding doctors. They have been worked out in a systematic Q and A based format for clear understanding. An exclusive section on Infection control and Pandemic management has been incorporated. All key chapters in this book start with an opening vignette/clinical case; often with relevant quotations to convey the theme of the topic. Subsequently the topic is worked out systematically in Q-A format. This is done, so that the study becomes relevant & interactive and the relevance of the subject matter becomes clear to the student.

The book has been divided into 8 parts ,which have been categorized into 17 sections for the organization purpose. Parts 1 and 2 deal with General Microbiology and Immunology, respectively. There are more than 27 General Microbiology (including Infection control and Pandemic management) and 15 Immunology based clinical vignettes worked out in a systematic fashion. Both the sections are opening clinical vignette/integrated clinical case based in Q-A format. Part 3 deals with Clinical Infectious syndromes of various anatomical systems (already elaborated). Clinical Infectious syndromes part has been organized anatomical system wise with integrated clinical case based studies in Q-A format including references of clinical cases in appropriate sections/chapters.

Part 4 deals with Bacterial diseases which have been divided into IVA -X sections. In these sections, before the integrated clinical case based studies are depicted, there are chapters devoted to the bacterial and disease characteristics, so that these can be applied and understood in the clinical cases. In each section the first chapter deals with Classification and metabolic and microscopic features of bacteria, second with overview of media requirements, colonial characters and laboratory diagnosis characteristics, third with clinical profile (pathogenicity) and then the integrated clinical case based studies. The laboratory diagnosis and treatment (overview) profiles are

provided towards the end part of each of the sections, just before the Assessment/Examination questions chapter.

Part 5 deals with Viral diseases which has sections from XI to XIII. Section XI deals with General Virology. Sections XII and XIII deals with Infections due to DNA and RNA Viruses, respectively. The latter two sections begin with overview of clinical profile, followed by integrated clinical based studies in Q-A format and end with outline of the laboratory diagnosis.

Part 6 deals with Parasitic diseases. Section XIV deals with diseases caused by Protozoans and Section XV deals with diseases caused by Helminths. In both the sections; Chapter 2 deals with the Morphological profile, Chapter 3 with the Transmission/Life cycle & Host's profile, Chapter 4 with the Clinical profile. In section XV last three chapters deal with the laboratory diagnosis profile, treatment profile and assessment questions respectively; whereas in section XVI these aspects have been dealt in chapters 18, 19 and 21; respectively .

Part 7 deals with Medical Mycology (Fungal diseases). Section XVI has been divided into six chapters for easy understanding of the mycological aspects. In it, chapters 2 to 5 deal with the clinical units of mycoses. Each of this chapter starts with a clinical based case study to highlight the key aspects to be followed by other aspects related to case theme/examination assessment.

Part 8; Section XVII deals with Infection Control and Pandemic management module (Microbiology component). In it, chapters 1 to 7 deal with Infection Control and Chapters 8 to 14 with the Pandemic module (includes two clinical studies on Covid-19). Each chapter starts with opening vignette/integrated clinical case based studies in Q-A format and has relevant quotations.

At places it appears that the information is getting duplicated, this is a deliberate attempt to reinforce some important information to the undergraduates, so that they remember it! The author has seen that many times students are not able to understand and retain the basic information, the material has been so arranged and depicted, that the student overcomes this difficulty and develops confidence. To ensure that the novice student does not get lost in the sea of microbiological information, all chapters have question and answer format (except those tabulating key microbiological information) and all sections are referenced with a list of key examination/ assessment questions (to which references/answers are provided). This approach would be helpful especially to students, who lack command on the English language, but have to clear the examination in the English language.

Bacterial and viral outbreaks that have actually occurred in S.E. Asia are incorporated. Besides the coverage of the microbiological facts, the economic and the social implications involved in these episodes have also been highlighted.

The other key features of book include:

- Molecular biology aspects highlighted.
- Some original classical experiments described to maintain touch with history.
- All biochemical reactions/bacterial/viral vaccines covered in a compact tabular format.
- For examination purpose at places, short notes incorporated separately.
- Space is provided to incorporate new and changed concepts in the book.
- More than 50 quotations to inspire the student.
- Varying font size to grade varying importance of information.
- Footnotes provided for difficult terms.

Studying with understanding may be time consuming initially, but is a sound investment in the student's long medical career. It is with this in mind, the book has been presented. The author is confident that the student will retain more information, score well in the examination, develop a rational approach to the subject, be able to analyze microbiological data in clinical cases rationally in his medical career and continue to learn throughout his medical career.

A textbook should not merely provide information but should make it meaningful and realistic.

"A teacher…who has no living traffic with his knowledge but merely repeats his lesson to his students, can only load their minds, he cannot quicken them…" **—Rabindranath Tagore**

I hope the challenges are met and the subject reclaims its importance both amongst the teachers and students. Any feedback or criticism would be welcome at my email–vsrandh@gmail.com

V.S. Randhawa

List of Contents

Sterilization and Disinfection

4

- *Male sterilization procedure-Vasectomy*
- *Autoclaving surgical items-Sterilization*
- *'Soap, water and common sense are the best disinfectants'* — *William Osler*

Let's study this challenging subject, as three integrated clinical based studies.

Integrated Clinical Case Based Study-1

There is an increase in number of cases of *surgical site infections, who got operated in the surgical OT of a leading hospital. Investigations revealed that there was a lapse in the sterilization technique, used to sterilize the drapes, used in preparing the cases in the OT. Central sterile supplies department (CSSD) is usually involved in supplying sterile material to OTs.

***Are infections in the body following surgery**

What procedure is used to sterilize drapes used in the OT?

A.1 (a) Commonly, autoclave (steam sterilizer) is used to sterilize the drapes in a hospital setting (Fig. 1.4.1a). Such items are also available in packaged form, which have been sterilized by ionizing radiation; as gamma rays.

Define the terms: Sterilization, Disinfection and Asepsis.

A.1 (b)
- Sterilization is a process by which an article is made free of all microorganisms (including viruses and spores)
- Disinfection is a process by which an article is freed of all pathogenic organisms except spores.
- Asepsis is a technique to prevent infection gaining to an uninfected site.

Fig. 1.4.1a: Autoclave

Mention briefly about Antiseptics, sanitation and sanitizer.

A.1 (c)
- Antiseptics are chemicals used on living tissue to prevent infection.
- Sanitation is a process by which microbes are reduced to numbers considered to be 'safe' on inanimate objects. (The process is valid for a short period, as item is not sterilized and microbes can regrow)
- Sanitizer is an agent used during sanitation to reduce number of bacteria to a safe level.

Classify the methods used in sterilization and disinfection.

A.1 (d) Physical methods

- Heat*: (i) Natural-Sunlight
  (ii) Dry Heat (oxidizes molecules)
  (iii) Moist Heat (denatures proteins and disrupts hydrogen bonds)
- Filtration
- Radiation
- Gases (as ethylene oxide and formaldehyde)

Chemical methods:

(i) Alcohols (ii) Aldehydes (iii) Phenol (iv) Halogen (v) Salts (vi) Surface active agents (vii) Dyes

* It is the preferred methods for sterilization, unless the article to be sterilized can get damaged by it.

Mention the broad uses of sterilization and disinfection techniques.

A.1 (e) (i) In surgical and diagnostic procedures (as asepsis)

(ii) In Microbiology for providing sterile media and reagents; besides techniques in processing clinical samples

(iii) In Food and Drug industry for dispensing food and drugs

(iv) In hospital waste management

What type of autoclaves is available to sterilize material of operation theaters?

A.2 They are of three types namely; *Simple, Steam jacketed* (Steam jacket heats side walls of the autoclave independently of the steam, so it facilitates the drying of the load) and *Prevaccum type* (air is removed from the autoclave by vaccum pump, so less time is required for the sterilization).

Commonly the 'Prevaccum' type is used for sterilizing the material used in operation theatre, as faster sterilization of load facilitates the critical working of the OTs.

How can the surgeon ensure that the material that the OT receives is sterile?

A.3 The surgeon/anaesthetist should ensure that the autoclave processing the supplies is having an appropriate indicator control 'test', which it must pass.

Autoclaves use three types of indicators namely:

(a) *Chemical:* Bowie Dick types are frequently used in which appropriate color or design change, indicates that appropriate temperature and conditions were used.

(b) Thermocouple is placed in articles inside the autoclave, with wire outside to the potentiometer to record the temperature.

(c) *Biological:* Ampoules containing spores of *Geobacillus stearothermophilus* are placed in the material to be sterilized. After sterilization, the ampoule is transferred to appropriate medium, incubated at appropriate temperature (55° C) for appropriate time. If growth occurs, it indicates failure of the sterilization process to kill the spores. Spores of this organism require an exposure of 121°C for 12 minutes to be killed.

What is the physical agent used for sterilization in autoclave and hot air oven?

A.4 (a) Moist heat is used in autoclave and dry heat is used in hot air oven

What is the mechanism of action of dry heat?

A.4 (b) Dry heat causes carbonization of the microbial material (destruction of microorganisms)

Describe the methods, which use dry heat for sterilization.

A.4 (c) (i) *Flaming:* It involves passing of an item in naked flame for a few seconds, few times. It is used during handling of glass slides, scalpels and mouth of culture tubes.

(ii) *Red heat:* It involves direct heating of an instrument in direct flame, till it becomes red hot. It is a method to sterilize inoculating wires/loops, tips of forceps etc. The flame of bunsen burner has a temperature of around 1500°C.

(iii) *Incineration:* It uses a high temperature of 800-1000°C for sterilizing and disposing contaminated material by direct burning. Incinerators are frequently installed in hospitals and results in safe destruction of infective material; as dressings, bandages, bedding and other infective material. Air pollution by this system is of concern and polystyrene material should not be fed into this system, as causes extreme pollution (black smoke).

(iv) **Hot air oven:**

Principle: It involves exposure of items at 160°C for 2 hour or 180°C for 30 minutes in an electrically operated oven, which generates radiating dry heat. It may be noted that time required by this technique in comparison to autoclave and boiling (at 100°C) is more, as this type of energy has poor penetration into materials.

Limitations:
- Plasticware can get melted (in heat)
- Water does not mix well with oily substances

Indications: For sterilizing of glassware such as flasks, pipettes, test tubes, glass petri dishes etc.
- Sterilization of surgical instruments*, forceps, scissors, scalpels.
- Sterilization of swabs
- Sterilization of pharmaceutical products; as liquid paraffin, dusting powder, fats etc.

* As those used in eye surgery (as cutting edge does not get dulled and no corrosion occurs)

Fig. 1.4.2: Hot air oven

Culture Media

7

"Our food should be our medicine and our medicine should be our food (man to provide food in the lab for microbes with the same spirit)." — *Hippocrates*

Man must be able to study the role of microbes in disease and other conditions. To achieve this goal, three things must be achieved namely,

***I*-cultivation (growth) of the organisms, for it media required (Chapter 7) *II*-isolation (culture) of the organisms (chapter 8) and finally**

***III*-identification of the organisms (chapter 9).**

What is one attempting to do, while cultivating bacteria?

A.1 Basically, one is attempting to simulate the '*in vivo*' conditions of the organism in the laboratory, so that the organism can easily be cultivated. This actually does not really occur totally in the laboratory but one attempts to approximate the '*in vivo*' conditions of the organism. Since the exact simulation may not many times be possible, so many organisms never get cultivated.

Can the metabolism of an organism be a limit on its cultivation and isolation in the laboratory? Provide a landmark example.

A.2 Yes. Initially it was concluded that *E. coli* was the predominant species of colon, as the cultures were incubated only aerobically. Later it was realized that the colon environment would be anoxic and would have anaerobes. This happened to be true, when the colonic contents were incubated on media, which was heated to expel oxygen and incubated anaerobically. This led to the discovery of Bacteriodes and Peptostreptococci. Bacteriodes spp. happens to be the most predominant organism in the colon,

Are most microorganisms cultivable?

A.3 Surprising it may appear, but most organisms are not cultivable in the laboratory. These are designated as viable but noncultured **(VBNC)** organisms. This fact emphasizes the need of providing appropriate media and environmental conditions in an attempt to cultivate the organisms.

What do you understand by medium (plural-media)?

A.4 They are specific nutrients (liquid or solid form) that can support growth of a group or a subgroup of microorganisms; as bacteria (or fungi)

Broadly what are the physical categories into which the media can be categorized?

A.5 • Liquid media • Solid media • Semisolid

What do you understand by 'culture methods or techniques'?

A.6 These are methods used for growing (cultivating) microbes.

What is the key ingredient added to liquid medium to make it solid? How does solid media helps to obtain pure cultures?

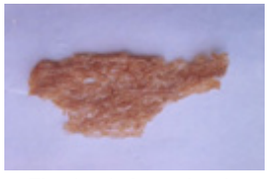

Fig. 1.7.1: Agar shreds

A.7 Agar is added to the liquid medium to make it solid. It is synthesized from agar shreds (Fig. 1.7.1). This agent was suggested to Robert Koch by Angelina Hesse, wife of one of Koch's associates. She used it to harden jelly, while making various recipes in her kitchen.

The inocula (sample) is diluted on the surface of solid media by spreading and thus getting diluted to eventually result in isolated colonies on the surface of solid medium. Previous to agar, gelatin was used to solidify media but it had the disadvantage of liquefying around room temperature.

What are the properties of agar that resulted in it being universally accepted as an ingredient for solid media?

A.8
- It can be sterilized easily by heating and does not get denatured
- It remains stable at high temperature (unlike gelatin, which melts at around room temperature).
- Once melted, it remains liquid until cooled to about 40°C. At a temperature of about 45°C, heat sensitive nutrients and living organisms can be added to the medium without fear of the medium getting solidified (it does not melt below 95°C) but once melted it solidifies around 40°C.
- Very few bacteria can degrade it.

Why does one need to study the nutritional factors that can affect the growth of microorganisms?

A.9 Different organisms have varying nutritional requirements and one needs to cultivate the various microbes in the lab. Broadly the number of nutrient requirement of an organism is determined by the type and number of enzymes it has. Basically, the organisms with many enzymes have simpler nutritional requirements, as they can synthesize most of the substances they require. The organisms with fewer enzymes have complex nutritional requirements, as they are unable to synthesize many substances e.g., lactic acid synthesizing bacteria.

What are the nutritional factors that need to be provided for growth of microorganisms?

A.10 *Water* is the most essential requirement, as it is a primary constituent of the organism accounting for about 80% of its total weight. It is a source of hydrogen and oxygen.

Next in importance is a substance acting; as a *carbon* source. This is used as a source of energy and as carbon containing building block for synthesis of cell components. The source of it varies; depending on whether the bacteria is a autotroph (Lithotroph), which uses inorganic chemicals; as CO_2 or a heterotroph (organotroph), if it uses organic carbon sources. Substances acting as nitrogen source are also important, as these are required in the synthesis of enzymes, proteins and nucleic acid. *Sulfur* requirement in the organism is obtained from inorganic sulphate salts and sulfur containing amino acids. *Phosphorus* requirement of the organism is obtained from inorganic phosphate ions ($PO4^{3-}$). Phosphorus is used in the synthesis of ATP, phospholipids and nucleic acids. The above nutrients are categorized as *macronutrients*, as they are required in relatively large amounts and play a key role in cell structure and metabolism.

The nutrients that are required in minute quantities but essential for the functioning of organism; often as part of key enzymes are called *micronutrients*. All organisms require some sodium and chloride. The variety of trace elements that are often required include iron, zinc, copper and cobalt. Certain organism require some other organic factor; as X and V factors required by *H. influenzae*.

What are the key components of culture media?

A.11
- Water
- Electrolytes (often NaCl etc.)
- Peptone–it is a complex mixture of partially digested proteins. They can be of animal or plant (vegetable) source, obtained by enzyme digestion. The latter are preferred by some groups, as there is no fear of infectious agents such as those, which cause Bovine spongiform encephalopathy.

  The preparation contains proteoses, polypeptides, amino acids, inorganic salts (as phosphates), minerals (as K, Mg) and accessory growth factor (as riboflavin).
- Meat extract–it is commercially available as 'Lab lemco'
- Agar (if medium has to be solid or semisolid)
- Other factors (as blood, yeast extract etc. depending on type of medium).

What are the ways in which media can be classified? Explain how do these classifications help the microbiologist and clinician in achieving their mandate.

A.12 I. On the basis of physical state of the medium. It is categorized as
- Liquid
- Semisolid (floppy) and
- Solid

II. On the presence/absence of molecular oxygen and reducing substances in the media

(i) Aerobic media (ii) Anaerobic media

If from the clinical sample, anaerobic bacteria are expected, then anaerobic media must be inoculated for successful isolation of anaerobes. One has to be very careful in this regard, as even common medium; as blood agar plate, if it not stored in reducing environment, may not support anaerobes. Prolonged exposure of the medium to environmental oxygen may make such medium ineffective for anaerobic work. Ideally PRAS.

III. *Bacterial/fungal*: If the clinical picture suggests a fungal lesion; as dermatophytes or dimorphic fungi, dermatophyte

media/Sabourauds dextrose agar should be used additionally to routine media for fungal isolation.

IV. *Synthetic (defined)/complex/undefined*

Most of the media in usage are in the complex category. This is because often what is present as a component in a accurately weighed ground meat, milk or plants is not exactly known. The composition of the medium also varies with the digestion protocol followed, while preparing it with the various enzymes, e.g., nutrient broth, beef extract. It is difficult to know the exact nutrient requirements of a suspected pathogen in a clinical sample and create such an medium. For this reason, clinical microbiology labs often use this *complex medium* in the primary isolation of an organism.

In the synthetic media (defined), as the name indicates, the composition of the medium is accurately known as only defined and weighed components are added to the medium, e.g., Dubo's medium with Tween 80.

V. *Transport media/plating media*: *Transport media* are those, which are used by the clinician or the patient to transport the specimen to the microbiology laboratory, where the sample processing is to be performed. The composition of these media is to be such that the pathogens present in the sample do not proliferate but remain viable till they reach the laboratory. They usually contain buffers and salts and lack carbon and nitrogen source.

Plating media are those media in which the clinical sample is inoculated in the laboratory. Rarely this medium can also be used to inoculate CSF samples at the bedside and transport to the laboratory.

VI. *Enrichment media/selective media*:

Enrichment media; as the name indicates, enrich the desired organisms, from a mixture or organisms present in the specimen, e.g., tetrathionate broth and selenite broth used in allowing preferential growth of typhoid bacilli. These are liquid media, which allow small number of pathogens to outgrow inhibited (commensal) organisms, before subculture on to culture plates. So the indication of using them, is when you want to select out few pathogenic bacterial present in a mixture of unwanted organisms. This medium should not be confused with the enriched media, which is a similar sounding term. Enrichment media are always liquid media and enhance the isolation of desired organism by apparently shortening the lag phase of the desired organisms. This makes these organisms reach earlier the log phase, thus they become relatively predominant in comparison to other commensal bacteria in the earlier period of the growth curve. So to take advantage of this dynamics, sample after 6-8 hours of inoculation into a enrichment medium, must be subcultured onto a selective medium, or the commensal bacteria would also enter log phase, making lose the advantage that may have occurred.

Selective media, as the name indicates, select out the desired organisms from a mixture of organisms. It is a medium with ingredients to inhibit the growth of certain microbes from a mixture of organisms, while permitting (selecting) the growth of certain others. These media are always solid. In Deoxycholate citrate agar (DCA), sodium deoxycholate inhibits the growth of gram positive cocci and makes the medium selective for gram negative bacilli. In MacConkey agar, sodium taurocholate inhibits the gram positive cocci making the medium selective for gram negative bacilli. More examples see Table 1.7.1.

VII. *Simple (basal)/Enriched media*: The *simple basal media* are, as the name indicates are the simple and routinely employed diagnostic media. It can be both in liquid or solid forms, e.g., nutrient broth, which contains peptone, meat extract (1%), sodium chloride and water. Addition of 2% agar to nutrient broth makes it *nutrient agar*. Sugar media often used in fermentation tests also belong to this category. It contains 1% sugar (glucose/lactose/sucrose/mannitol) in peptone water along with indicator (often, andrades) and sometimes an inverted tube called Durham's tube (to see gas production).

Enriched media as the name indicates are enriched with blood, serum, ascitic fluid or egg. These are obviously expensive than simple media and are used; if fastidious organisms are expected in a clinical sample or fastidious organisms are to be cultivated.

nb: Many media can also be of multiple types eg differential and indicator. e.g., Blood agar, is both an enriched and indicator medium, L.J. medium is both an enriched and selective medium.

VIII. *Other categories*: *Indicator media* (differential) as the name indicates, provide an indication to the type of growth on the medium. E.g., MacConkey's medium can differentiate between lactose fermenters, and non-fermenters. However, it is also a selective medium, as the sodium taurocholate in it inhibits gram positive cocci. Another example of this would be blood agar, where for instance small pin-point β haemolytic colonies would indicate the organism to be β haemolytic Streptococci. However this plate is also an enriched medium. So this classification for media is not absolute and some media can be categorized into numerous categories.

The commonly used media are categorized in table 1.7.1.

Table 1.7.1: Common media and their characteristics

Media for Transporting Samples		Type	Name & Essentials Components	Role of the Components	Functions of Media
		Pike' s medium	• Blood agar • Crystal violet (1 in 1,000,000) • Sodium azide	• Enriched base • *S. pyogenes*, resistant to it • Preservative	Transport of specimen likely to contain *S. pyogenes, H. influenzae, or Pneumococci*
		Buffered glycerol saline	• Glycerol • Saline • Phenol red	• Prevents dessication • Preserves structures • Indicator (if medium turns yellow, it indicates growth of contaminants)	Transport of stool specimens likely to contain organisms, as Shigella
		Stuart's transport medium	• Soft agar (non nutrient) • Charcoal • Sodium thioglycollate • Sodium glycerophosphate • Calcium chloride • Methylene blue	• To provide solidity • Neutralize bacterial inhibitors • Reducing agent • Buffer • Buffer • Indicator	Transport of fastidious organisms including anaerobes (ensures survival but not proliferation of organisms)
		Cary Blair medium	• Sodium thioglycollate (pH 8.4) • Disodium phosphate • NaCl • Alkaline pH	• Provides low oxidation-reduction potential • Buffers medium • Osmotic equilibrium [non-nutritive] • Minimizes bacterial destruction due to acid production	Transport medium for Shigella, Salmonella and Vibrio
		Amies medium	• Mineral solution - sodium chloride, potassium chloride, MgCl2, disodium phosphate etc. • Sodium thioglycolate • Charcoal • Agar (semi-solid media)	• Provides low oxidation-reduction potential • Charcoal neutralizes toxic materials • Minerals help survival of organisms	Transport medium by *Neisseria gonorrhoeae*-throat, vaginal samples Can also be used for Campylobacter
		Venkatraman Ramakrishnan (V-R) medium	• Crude sea salt • Peptone • Water	• High salt increases survival of vibrios	Transport faeces from suspected cholera infection
For Viruses		Viral transport Medium (VTM)	• Hanks balanced Salt solution with Ca & Mg ions • fetal bovine serum • Gentamicin, Amphotericin B (May be available as different formulations according to the manufactures)	• Enhances viral survival & inhibits bacteria and fungi	Transport various swabs for viral detection
For Fungi	*Solid*	Sabouraud dextrose agar; SDA (Fig. 1.7.2)		• Lower pH favour growth of fungi over bacteria • High sugar concentration also favours growth of fungi	Isolation of fungi
		Sabouraud dextrose agar with antibiotics		• Cycloheximide inhibits molds and yeasts • Chloramphenicol inhibits bacterial gowth	Isolation of fungi from contaminated samples
		Dermatophyte test medium agar	• Nutrient base with glucose • Phenol red • Tetracycline, Gentamicin • Cycloheximide	• Indicator • Inhibits Contaminants	Isolation of Dermatophytes
		Brain heart infusion agar (BHIA)	• Calf brain infusion • Beef heart infusion • Salts and buffers		Growth of Dimorphic fungi

Contd.

Contd.

		Cornmeal agar	• Corn meal infusion • Agar	• Stimulate chlamydospore formation	To identify *C. albicans* (formation of chlamydospore, blastoconidia & pseudohyphae, occur in 48 hrs
Aerobic Bacteria					
	Semi-solid	Cragie's tube	• Have 0. 2 - 0. 5% agar		Motility studies
		Oxidation_Fermentation (OF) medium	• Casein enzymic hydrolysate • Carbohydrate • Dipotassium phophate • Bromothymol blue • Agar (low concentration)	• Nutrition. • One to be tested • Buffer • Indicator • Permits motility and diffusion of acidity	Determine oxidative or fermentative metabolism of carbohydrates by GNB
		Fletcher's agar	• Peptone • Beef extract • NaCl • Agar • D.W.	• Leptospires multiply the upper zones of the tubes forming turbidity	Suitable for isolating for leptospires and maintaining them for months
	LIQUID				
	Basal	Peptone water pH 7. 4 (Fig. 1.7.3)	• Peptone - 1% • NaCl - 0. 5%		• Routine culture • As basal medium for carbohydrate Fermentation medium
		Nutrient broth (has variants like Digest broth)	• Peptone water • Meat extract		• Routine culture
	Enriched	Glucose broth	• Nutrient broth • Glucose - 0. 5%	• • • Also acts as a reducing agent	Luxuriant growth of many organism
		Todd Hewitt (meat infusion) broth	• Glucose - 0. 2% • Infusion		Luxuriant growth of organism's; as Streptococci
		Serum peptone broth/Hiss's serum slope	• Serum	• For growth	For carbohydrate fermentation tests with fastidious organism such as Streptococci, *C.diphtheriae*
		Brain heart infusion broth			Recovery of bacteria and fungi
		Mueller Hinton broth (Fig. 1.7.4)			Bacterial susceptibility test medium
		Trypticase soy broth			Cultivation of fastidious organisms; as Brucella
		PPLO broth (medium be free of toxins)	• Bovine heart infusion broth • Horse serum (20%) • Yeast extract (fresh) • Glucose • Phenol red	• For growth • For growth • Indicator	Isolation of mycoplasma
		Middlebrook's 7H10			For isolation of tuberculosis group of organisms
	Enrichment				
		Bile broth	• Peptone, sodium taurocholate		Isolate S.Typhi from Blood culture
		Selenite F broth	• Peptone water • Sodium selenite	• Inhibits most enterobacteriaceae	Enrichment medium for Salmonella & Shigella
		Tetrathionate broth	• Nutrient broth/Peptone base broth • Sodium thiosulphate • Bile salts • Calcium carbonate • Iodine solution	• Inhibits grams positive organism • Neutralizes toxic metabolites • Inhibits enterobacteriaceae	Enrichment medium for Salmonella & Shigella
		Alkaline peptone water (APW)	• High pH	• Optimal for vibrio multiplication	Enrichment medium for Vibrio

Contd.

Contd.

		Gram negative broth	• Peptone, Glucose • Mannitol • Sodium citrate • Sodium deoxycholate • Mineral salts, water	• Less inhibitory than selenite F	Isolation of salmonellae and shigellae
	SOLID				
	Basal (majority have 2% agar as solidifying agent)	Nutrient agar (Fig. 1.7.5)	• Nutrient broth • Agar (2%)		Routine medium
	Enriched				
		Blood agar; BA (Fig. 1.7.6)	• Nutrient agar • 5 -10% sheep / horse / human blood - rarely used if sheep/ horse blood not available		For isolating organisms as Group A streptococci, Haemophiius (fastidious organisms)
		Blood agar with *S. aureus* streak	• *S. aureus* streak	*S. aureus* provides V factor	For isolating Haemophilus organisms
		Blood agar with X and V discs	X disc V disc	• Provides hemin • Provides NAD	For isolating Haemophilus organisms
		Chocolate agar (blood agar slowly heated to 80°C) (Fig. 1.7.7)		Heating blood, releases nutrients	For isolation of fastidious organisms as *H. influenzae*, *N. gonorhoeae* & *N. meningitidis*, *Gardnerella vaginalis*
		Loeffler's serum slope (has no agar)	• Nutrient broth • Serum (Horse/sheep) • Glucose	• Growth within 6-8 hours • Enhances granule formation	For rapid growth of *C. diphtheriae*
		Egg yolk agar	• Proteose peptone • Hemin • Salts • Egg yolk	• Nutrient • Enhance anaerobic growth • Buffer • Lipase (produced by microbes), break down fat into fatty acid, provide iridescence	For Clostridia isolation and other anaerobes
		Dorset's egg	• Nutrient broth • Hen's egg		For isolation of Mycobacteria & other fastidious organisms
		PPLO agar	PPLO broth • Antibiotics as Penicillin, ampicillin, polymyxin B	• Inhibits contaminant bacteria and Fungi	For isolation of Mycobacteria
		Bordet gengou medium	• Blood • Potato • Glycerol It has methicillin (final conc. 2. 5 µg/ml)	Nutrition	For isolation of Bordetella
		Legionella medium	• Mueller Hinton medium supplemented with ferric salts, L-cysteine etc	• Provides reducing condition (L-cysteine)	For cultivation of Legionella spp.
		Francis blood dextrose cystine agar	• Blood, dextrose • Cystine		For isolation of *Francisella tularensis*
		Serum dextrose agar	• 1% glucose • 5% serum	–	*For isolation of Brucella spp.*
		BHI agar; BHIA			Routine medium
	Selective				
		MacConkey	• Peptone • Lactose • Agar • Neutral red • Taurocholate (sodium)	• Indicator substrate • Indicator, dye • Inhibits gram positive cocci	• Routine medium • Differential medium for the demonstration of lactose fermentation by gram negative rods
		Salmonella & Shigella medium	• Bile salts (higher concentration) • Sodium citrate • Ferric citrate	• Inhibits GPCs and coliforms • Blackening occurs due to formation of ferric sulfide	
			• Lactose • Neutral red	• Indicator substrate • Indicator dye	

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		Bile salt agar (alkaline) pH 8.2	• Sodium taurocholate (0. 5%)	Inhibits most gram negative organism	Selective plating media for Vibrios
		Thiosulfate citrate bile salt sucrose agar (TCBS)	• Peptone base agar • Yeast extract • Bile salts • Citrate • Sucrose • Ferric citrate • Sodium thiosulphate • Bromothymol blue • pH-alkaline	• Inhibits GPCs • Inhibits most GNBs • Indicator substrate • Allows H_2S detection • Sulfur source • Indicator • Helps recovery of vibrio	Selective plating media for Vibrios (recovery of vibrios)
		Monsur's gelatin taurocholate trypticase tellurite agar	• Gelatin • Sodium taurocholate • Tellurite	• Vibrios can hydrolyze gelatin & produce halo around colonies • Inhibits gram positive cocci • Reduction of it imparts black color to colonies	For isolation and identification of Vibrio cholerae
		Vibrio media containing 8% NaCl		Halophilic vibrio can tolerate 8% NaCl but not 10%	Differentiation of halophilic vibrios from *V. cholerae*
		Deoxycholate citrate agar (DCA)	• Nutrient agar • Sodium deoxycholate • Lactose • Sodium citrate • Neutral red	• Nutrition • Inhibits gram positive bacteria • Indicator substrate • Inhibits gram-positive bacteria and intestinal commensals • Color indicator	Selective medium for Salmonella & Shigella
		XLD agar (Xylose Lysine deoxycholate) medium	Details beyond undergraduate level		Selective/Indicator medium for Enterobacteriaceae, especially for Salmonella and Shigella
		Wilson&Blair bismuth sulfite medium	• Bismuth ammonium citrate • Sodium sulfite • Salts	Formation of H_2S renders black color to colonies	Selective medium for S. Typhi
		Campy Blood agar	• Brucella agar base with sheep blood with Antibiotics as • Trimethoprim • Cephalothin • Polymyxin B • Vancomycin • Amphotericin B	• Inhibits Proteus spp. (contaminants) • Inhibits gram positive organism • Inhibits most gram negative organisms • Inhibits gram positive organisms • Inhibits yeasts	
		Fildes blood digest agar	• Fildes peptic digest of blood • Nutrient agar • NaCl • NaOH • Chloroform	• Contains abundant of X factor and NAD. "Supports good growth of *H. influenzae* and *Clostridium tetani*, while high haemin inhibits many organisms including throat commensal streptococci	Selective for *H. influenzae*
		Lowenstein Jensen medium (Fig. 1.7.8)	• Eggs, Mineral salts • Asparagine • Glycerol, • Malachite green	• Nutrition, solidifying agent • Inhibits organisms other than mycobacteria and provides contrast to buff colored colonies	Selective for mycobacteria
		Dorset medium	Egg based		For isolation of Mycobacteria
		Middlebrook 7H11	Agar based		For isolation of Mycobacteria
		Bile esculin agar	• Nutrient agar base • Esculin • Ferric citrate react with above	Hydrolysis of esculin by Group D streptococci, provide blackening	Differential isolation & presumptive isolation of enterococci
		Crystal violet blood agar	• Blood agar • Crystal violet	In concentration of 1 in 1,000,000 inhibits *Staphylococcus aureus*	Selective isolation of *Streptococcus pyogenes*

Contd.

Contd.

		PNF media	• Sterile horse blood • Polymyxin B • Neomycin • Fusidic acid • Nutrient agar	Inhibits Staphylococcl and coliform bacteria	*Selective for* β*-hemolytic streptococci*
		Buffered charcoal yeast extract agar (BCYE)	• Agar • Yeast extract • Salts supplements with ketoglutarate, L-cysteine	• To provide pH of 6.85 – 7	Selective for Legionella sps
		PLET medium	• Heart infusion agar • Polymyxin • Lysozyme • EDTA • Thalious acetate	Thalious acetate and polymyxin inhibits most strain of *B. cereus*, *B. subtilis*, other bacillus sp., enterobacteria, pseudomonads	Selective for *B. anthracis*
		Skirrow agar	• Peptone & soy protein base • agar • lysed horse blood • Vancomycin • Polymyxin B • Trimethoprim	• Inhibits gram positive organisms • Inhibits most gram negative organisms	Selective for campylobacter & Helicobacter
		Cycloserine-cefoxitin fructose agar (CCFA)	• Egg yolk base Fructose • Neutral red • Cefoxitin • Cycloserine	• Indicator dye • Inhibits gram negative rods • Inhibits faecal flora	Selective for *Clostridum difficile*
		Cycloserine cefoxitin egg yolk agar (CCEY)	• Egg yolk base • Cefoxitin, cycloserine • Cholic acid sodium salt • Hydroxyphenylacetic acid • Lysed blood		Selective for *Clostriduum difficile*
		Cystine-tellurite blood agar	• Agar base with 5% sheep blood • Potassium tellurite	Reduction of potassium tellurite produces black colonies	Selective isolation of *C. diphtheriae*
		Tinsdale medium	• Peptone • Sodium chloride • Ox serum • L-cysteine • Potassium tellurite • Sodium thiosulphate • NaOH	• Serum enhances corynebacterium growth • Tellurite and thiosulphate inhibits other bacteria • Tellurite also acts as indicator	Selective foe pathogenic corynebacterium
		Thayer Martin agar (variant of choclate agar)	• Blood agar base enriched • with haemoglobin and supplement B • Colistin • Nystatin • Vancomycin • Trimethoprim	• Inhibits gram negative contaminants • Inhibits yeast • Inhibits gram positive organism • Inhibits gram negative contaminants	Selective for *N. meningitidis* and N. gonorrhoeae
		Mannitol salt agar	• Peptone base • Mannitol • Salt concentration of 7.5% • Phenol red	• Acts as indicator substrate • Inhibits growth of most bacteria • Indicator	Selective for *Staphylococcus aureus*
	Miscellaneous	Cystine Lactose electrolyte deficient agar	• Peptone base agar • Lactose • L-cystine • Bromothymol blue	• Inhibits swarming of Proteus • Indicator substrate • Indicator dye	isolation & quantification of bacteria
		Modified Kelley's medium (BSK)			Cultivation of *Borrelia burgdorferi*
		EMJH (Ellinghausen, Mc Cullough, Johnson Harris)			Cultivation of *Leptospira interrogans*
		Castaneda's medium (biphasic medium)	Trypticase soy broth and agar		Cultivation and isolation of Brucella

Contd.

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For Anaerobic bacteria	*Liquid*	Robertson cooked meat medium (Fig. 1.7.9)	• Meat broth • Solid meat particles • Liquid parraffin	• As nutrient • Lower oxidation reduction potential • Block environmental oxygen	Cultivation of anaerobes support growth of anaerobes, aerobes, microaerophilic & fastidious Organisms
		Thioglycollate broth	• Pancreatic digest of casein • Soy broth & glucose • Thioglycollate • Agar • L cysteine & vitamin	• Lower Eh (reduction potential/ redox) • Act as reducing agent	Cultivation of anaerobes including Actinomycetes
		Anaerobic investigation medium	Peptone, yeast extract, Trypticase, NaCl, Cysteine HCl, Hemin and menadione, are sodium carbonate	–	–
	Solid enriched				
		Blood agar (plain) _PRAS	Pre-reduced anaerobically sterilized medium prepared & packaged in oxygen free environment		Cultivation of anaerobes
		Blood agar (with additives) _PRAS	Yeast extract, haemin, Vitamin K, Neomycin	Support isolation of anaerobes	Cultivation of anaerobes
		Serum/egg yolk agar (6%)	Peptic digest of blood 20% human serum or 5% egg yolk	6% agar Inhibits swarming	To demonstrate Nagler reaction by *C. perfringens*
	Selective				
		Colistin_ Nalidixic acid Blood agar		Antimicrobials make medium selective	Supports selective growth of anaerobic & facultative anaerobes (mostly gram positive, inhibits most gram negative)
		Kanamycin_ Vancomycin Blood agar (*laked* blood agar)	• Kanamycin • Vancomycin • Blood		Selective isolation of anaerobes
		Phenylethyl alcohol sheep blood agar	• Phenylethyl alcohol • Blood		Supports growth of most gram positive & gram negative bacilli
		Bacteriodes bile esculin agar	• Trypticase soy agar base • Hemin • Ferric ammonium citrate • Bile salts, Gentamicin, phenylethyl alcohol	Enriches Gentamicin - inhibits most aerobic gram negative contaminants including Proteus spp.	Supports selective growth of Bacteriodes sps, other bacteria can also grow, supports growth of most gram positive and negative anaerobes (inhibits facultative, gram negative bacilli)
Animate media!	*Tissue culture (cell lines)*	McCoy cells (rendered non-replicating by irradiation or treatment with antimetabolites as cycloheximide)		Organism cannot grow on inanimate media	Isolation of Chlamydia and cultivation of viruses
		HeLa cells (treated with DEAE dextran)			Isolation of Chlamydia
	Chick embryo (6-8 day old)	Yolk sac			For isolation of Chlamydia, Rickettsiae (not suitable for primary isolation) and viruses
		Chorioallantoic membrane			For cultivation of *Borrelia recurrentis*, Leptospira interrogans
	Laboratory animals	Mice (various routes)	Foot pad of mice		Cultivation of *M. leprae*
			Intranasal		For isolation of Chlamydia
			Intracerebral Intraperitoneal		• Isolation of Rickettsia, *Spirillum minus*, • Leptospira interrogans
		Rabbit testes			Cultivation of *T. pallidum, T. pertenue*
		Rabbit kidney (Noguchi's medium)	Rabbit kidney, Ascitic fluid		Cultivation of *Borrelia recurrentis*
		Armadillo (*Dasypus novemcintus*)			Cultivation of *M. leprae*

Monoclonal Antibody

6

Donald Trump the 45th President of USA, downplayed the gravity of Covid-19 pandemic. As this virus spares nobody, even he got infected with SARS-CoV-2 just before the US presidential elections. He was admitted in the state of art hospital. However his having number of co-morbid conditions was a matter of concern. Experts believe that one of the factors in his successful recovery was drug *Casirivimab*. In May 2021, this drug was approved for emergency usage by CDSCO in India during the second Covid-19 wave.

What class of drug does Casirivimab belong to?

A.1 The suffix *'mab'* in the drug indicates it to be a monoclonal antibody and the affixing of *'i'* to mab indicates it to be a chimeric monoclonal antibody.

What is the mechanism by which Casirivimab acts?

A.2 The drug was authorised by FDA for emergency usage in Covid-19 infection. It is believed that the neutralization of the SARS-CoV-2 virus results in early resolution of disease.

Compare and contrast the terms monoclonal antibody and polyclonal antiserum (antibodies) and mention the difficulties in producing monoclonal antibody.

A.3 (a) The term *monoclonal* antibody indicates single clone of antibody from a single B cell lineage. This term contrasts with the term *polyclonal* antiserum (antibody), which means that the serum has antibodies with multiple specificities derived from several clones having different B cell lineages. For medical, diagnostic and therapeutic purposes, monoclonal antibodies are required but these are not often available. The commonly available antiserum has multiple antibodies due to heterogeneous antibody responses. The reason for it is that, when an antigen is introduced into an organism, several clone of B cells proliferate, producing different antibodies, as an antigen has multiple epitopes.

The history of monoclonal antibody dates with the discovery of (tumor) multiple myeloma in mice, which consisted of genetically identical plasma cells and produced pure antibodies of single specificity indefinitely.

Who are the two scientists credited with the discovery of monoclonal antibodies, for which they were awarded the Nobel prize?

A.3 (b) The technological breakthrough to produce monoclonal antibodies was achieved by *G. Kohler* and *C. Milstein* in 1975, using the hybridoma technology for which they were awarded the Nobel prize in physiology/medicine in 1984. Hybridoma technology is based on the principle that when two cell types are mixed in culture in the presence of chemical (as polyethyelene glycol), it is possible to fuse two different cells.

Describe the principle and procedure of synthesis of monoclonal antibodies. Diagramatically illustrate the procedure of synthesis of monoclonal antibodies.

A.4 It is extremely difficult to stimulate a specific clone of lymphocytes in 'vivo' and to collect the resulting antibodies. In lab it, would be possible to isolate a single specific B cell, stimulate it and collect the antibodies, however the antibody production would be short lasting.

Kohler & Milstein developed the ingenious technique (for monoclonal antibody production) in which they used

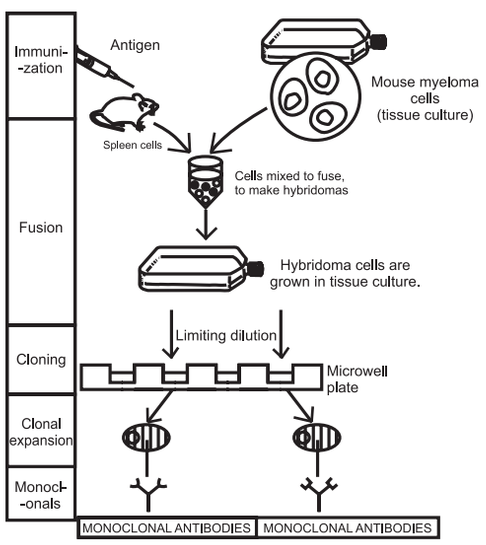
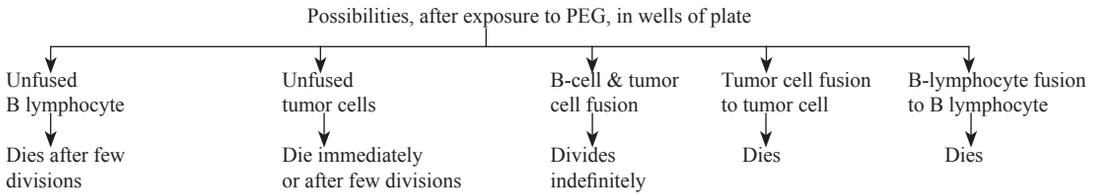


Fig.2.6.1:Schematic representation of technique of monoclonal antibody production

the growth potential of murine myeloma cell and fused with the specific antibody secreting B cells of the spleen (Fig. 2.6.1). The rate of successful hybrid formation is very low, hence in the technique, the need to develop a strategy to select the rare successful fusions. The myeloma cells that are used, lack the capacity to synthesize hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) enzyme (this enzyme enable the cell to synthesize nucleotides, using hypoxanthine as a precursor in the 'salvage pathway'). Such cells cannot survive in a basal medium containing HAT (hypoxanthine, aminopterin and thymidine), as the cells cannot use the 'denovo' pathway for nucleotide synthesis, as the aminopterin binds competitively to the dihydrofolate reductase enzyme to block tetrahydrofolate synthesis (which is essential for purine and pyrimidine synthesis. Thus only those hybrid cells can survive indefinitely that have taken the HGPRT enzyme from the B cells and the growth potential from the tumor cell (depicted in flow diagram below).



Procedure:

1. Antigen of interest injected into experimental animal; usually a mouse following a certain protocol
2. After adequate period, splenic lymphocytes (B) are harvested
3. The spleen lymphocytes are incubated with mouse myeloma cells (HPRT enzyme deficient) grown in culture (tissue culture bottle) with polyethylene glycol, so that fusion of cells can occur.
4. The resulting cells are diluted, such that each microwell has one (fused) cell. The basal culture contains HAT.
5. The microwells are tested for (hybrid) clones (by looking for products)
6. The clone that makes appropriate antibody is selected out, using an appropriate assay. The process uptil this step may take many months to years to standardize.
7. The isolated clone can be grown in large culture vessels to obtain significant amount of antibody. The clone (*hybridoma*) can also be cultivated (propagated) in mice to obtain greater amount of antibodies.

What are the clinical limitations of the monoclonal antibodies raised in mouse?

A.5 (a) Until recently, only mouse monoclonal antibodies were available. These had the limitation in therapeutic use, when used in human of evoking a human antimouse antibody response, that resulted in an (obtained with mouse myeloma cell line) accelerated clearance of the monoclonal antibodies from the blood stream and lowering the effectiveness of the administered antibodies.

How can these limitations be overcome?

A.5 (b) One technique to overcome this limitation is to synthesize human monoclonal antibodies by *genetic engineering techniques* for clinical use. However due to technical problems, generation of hybridomas secreting human antibodies is difficult. To overcome these limitations, genetic engineering including recombinant technology has been employed. This has resulted in the creation of *human-mouse chimeric* monoclonal antibodies, which as the name indicates are chimeras (molecular hybrids of human and mouse antibody). Another variant available in the market for clinical use is called the *humanized monoclonal antibody*, in which the antibody is such engineered, such that all of the antibody is human except for the complementarity determining regions in the variable portion of the light and heavy chain.

In the product Inflixbimab, what does 'imab' indicate?

A.6 It indicates that it is a chimeric monoclonal antibody

Mention the uses of monoclonal antibodies and name the key drugs based on monoclonal antibodies available for clinical usage?

A.7 One of the common applications of monoclonals is in the diagnostics and imaging. This occurs, as monoclonal antibodies are standardized and would give same results, when used anywhere in the world, due to standardized technique in their raising. One of the such kits commercially available in the market, is the pregnancy kit, which can detect the HCG hormone in the serum in just 10 days after conception. Other available kits based on this technology are useful in rapid diagnosis of many infections; as Hepatitis, Herpes and Chlamydia.

Radiolabelled monoclonal antibodies are useful in imaging of some primary and metastatic tumors in patients, which would go undetected by some other lesser sensitive scanning techniques, for instance some monoclonal antibodies labeled with Iodine-131, when introduced into blood, permit earlier detection of spread of breast tumor into regional lymph nodes.

Monoclonal antibodies have great potential in treatment of human disease but are limited by the fact that most monoclonal antibodies are of mouse origin and many humans are hypersensitive to them. Many methods are under development which would use appropriate drug or radioactive substance to be attached to a monoclonal antibody to be delivered exactly at the cells bearing the appropriate antigen. Currently a wide range of drugs are available for use in transplantation, viral chronic diseases and malignancies. Some examples are depicted in table 2.6.1.

Monoclonal antibodies are also used in other diverse applications as measuring blood levels of various drugs, enumerating human lymphocyte subpopulations, matching histocompatibility antigens and detecting specific tumor antigen.

Table 2.6.1: Some monoclonal antibodies in common clinical usage

Monoclonal antibody	Nature of antibody	Target	Treatment for
Muromonab CD3□	• Mouse mAb	• T cells	• Acute rejection of liver and kidney transplants
Infliximab	• Human-mouse chimeric	• TNFα (tumor necrosis factor α)	• Rheumatoid arthritis and Crohn's disease
Palivizumab*	• Humanized mAb	• Respiratory syncytial virus (on F protein)	• RSV infection
RituximabΔ	• Human mouse chimeric	• B cell	• Relapsed or refractory non-hodgkin lymphoma

nb: Abzymes-This term is derived from antibody (ab) and enzymes, indicating monoclonal antibodies to be having enzymatic activity, which can be clinically utilized.

Δ Suffix *'imab'* indicates chimeric antibody.

□ Suffix *'monab'* indicates mouse monoclonal antibody.

* Suffix *'umab'* indicates humanized monoclonal antibody.

Gastrointestinal Infectious Diseases and Hepatobiliary Infections

5

'All diseases begin in the gut' **— Hippocrates**

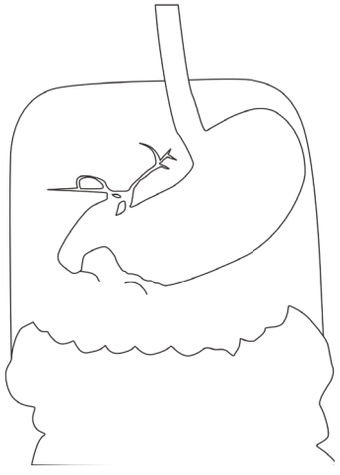
Digestion is quickly shut down during stress…The parasympathetic nervous system, perfect for all the calm, vegetative physiology, normally mediates the action of the digestion. Along comes stress: turn off parasympathetic, and forget about digestion'.

— Robert M. Sapolsky

Let's begin a study of the gastrointestinal and hepatobiliary system infectious diseases with the core aspects of the system to be followed by references of integrated clinical based studies on them. Hoping to keep the microbial stressors in control to have a life full of gastronomic delights.

Classify gastrointestinal infectious diseases / syndromes

A.1 Classification



- Stoma / Oral cavity
  - Stomatitis (Oral thrush, Dental infections, Vincent's angina)
- Oesophagus
  - Oesophagitis (Candidiasis, HHV–5; CMV, Herpetic, Idiopathic)
- Stomach
  - Gastric ulcerative disease (GUD)
- Small to large intestine
  - Gastroenteritis* (Diarrhoea** / Dysentry***/ Food poisoning****)
  - Necrotizing enteritis
  - Pseudomembranous enterocolitis
  - Whipple's disease
- Appendix
  - Appendicitis
- Rectum
  - Proctitis (inflammation of the rectum)
- Peritoneum
  - Peritonitis (Primary / Secondary)

nb: Odynophagia (painful eating); Dysphagia (painful swallowing) entities are not covered.

Gastroenteritis:* It may be defined; as its name indicates, an inflammation of the mucus membrane of the stomach and intestine, often accompanied with alteration in the stool character with clinical symptoms; as abdominal pain.

*Diarrhoea**:* It may be defined as an increase in the frequency, volume (amount) or fluid of the stool in an individual.

WHO defines *acute* diarrhea as one lasting less than 7days and *persistent* as one lasting more than 14 days. Some authorities define chronic diarrhoeae; as one lasting more than thirty days.

*Dysentry***:* It may be defined; as the presence of blood and/or mucus in stool, often accompanied with $^{\Delta}$tenesmus.

ΔFeeling of incomplete evacuation despite empty colon

*Food poisoning****:* It may be defined; as an illness acquired due to ingestion of food containing microbes, microbial toxins or chemicals. It often presents; as acute diarrhoea with or without vomiting.

nb: *Traveller's diarrhoea:* It is an acute diarrhoeal disease observed occasionally in visitors from foreign countries, during their stay in the developing countries.

Classify the etiological agents which cause stomatitis and peritonitis.

A.2 Stomatitis (inflammation of oral cavity)

Bacterial: Spirochaetes, Fusobacterium spp.

Fungal: Candida albicans [oral thrush]

Others (as dissemination from remote sites; as disseminated histoplasmosis)

Viral

Herpes simplex, Coxsackie A,

Measles, HHV-3 (chicken pox)

Enterovirus (as hand and mouth disease)

nb: Vincent's angina (is caused by *Borrelia vincentii* and Fusobacterium]

Peritonitis (inflammation of peritoneum)

Table 3.5.1: Etiological agents in Peritonitis

Primary- in children	**In adults**
S.pneumoniae	*E. coli* (commonest)
S. pyogenes	*S. pneumoniae*
M.tuberculosis	*M. tuberculosis*
Coliforms	*N. gonorrhoeae*
Staphylococci spp.	*C. trachomatis* (In sexually active young women)
Secondary (in sequel to some underlying process in abdomen; as perforated viscus, so etiological agent depends on the pathologic process).	

Classify Gastroenteritis agents

A.3 (a)

Table 3.5.2: Gastroenteritis agents (according to pathogenicity)

Pathogenicity	Etiological	Agents
Noninflammatory Gastroenteritis (diarrhoea)	**Bacterial:** • *Vibrio cholerae* (cholera) • *Escherichia coli:* (Enteropathogenic, Enterotoxigenic, Enteroaggregative) • *C. botulinum* (Botulism) • *Staphylococcus aureus* • *Bacillus cereus* (Food poisoning) • *Aeromonas hydrophila* • *Plesiomonas shigelloides* **Viruses:** • Rotavirus • Norovirus • Adenoviruses-40, 41 • Caliciviruses • Torovirus • Bocavirus • Picobirnaviruses* **Fungi:** • Microsporidia	**Protoxoal** • *Giardia lamblia* • *Cryptosporidium parvum* • *Cyclospora cayetanensis* • *Cystoisospora belli* **Helminthic:** • *Ascaris lumbricoides* • Hookworm • *Strongyloides stercoralis* • *Enterobius vermicularis* • *Trichinella spiralis* • *Taenia saginata solium* • *H. nana* • *F. buski* • *Dipylidium caninum* • *Diphyllobothrium latum*
Inflammatory Gastroenteritis (diarrhoea)	• Non-typhoidal Salmonellae • *Yersinia enterocolitica* • *Listeria monocytogenes* • *Clostridioides difficile* • *Plesiomonas shigelloides* • *Klebsiella oxytoca*	

Invasive Gastroenteritis (dysentery)	• *Yersinia enterocolitica (Yersiniosis)* • *Shigella* species (Shigellosis) • *Campylobacter jejuni* (Campylobacteriosis) • Enterohaemorrhagic *E. coli* • Enteroinvasive *E. coli* • *Vibrio parahaemolyticus* (Vibriosis)	**Parasitic** • *Entamoeba histolytica* • *Balantidium coli* • *Trichuris trichiura* • *Schistosoma mansoni* • *Schistosoma japonicum*

* Have been named so, as these viruses resemble the viruses belonging to family Birnaviridae

nb: HIV enteropathy is an entity in PLHIV, if diarrhoea is not responding to conventional antidiarrhoeal drugs.

How do you differentiate between Enteritis and Colitis?

A.3 (b)

Table 3.5.3: Differences between Enteritis and Colitis

	Enteritis	**Colitis**
Site	Involves S. Intestine	Involves L. Intestine
Volume of stool	Water, large volume	Frequent, small volume (urgent)
Weight loss	Significant	Not associated
Pain Abdomen	Usually absent	Cramping lower abdominal type
Bloating	Usually present	Usually not present

nb: Enteric fever caused by S. Typhi is better classified as a systemic illness, though can be categorized in the Invasive gastroenteritis category.

Classify Food poisoning according to pathogenicity and incubation period.

A.3 (c)

Table 3.5.4(a): Etiological agents for food poisoning (according to pathogenicity)

Infective
• All Salmonellae except Typhi, Paratyphi A & B
• *Clostridium perfringens* type A (In U.K., a common agent, due to meat being a common ingredient in food)
• *Vibro parahaemolyticus* (In Japan, a common agent due to common marine food consumption)
Toxic
• *Staphylococcus aureus* (enterotoxin produces strain belonging to phage group III or phage type 42D)
• *B. cereus*

The etiological agents may also be categorized according to the incubation period [table 3.5.3(b)]

Table 3.5.4(b): Etiological agents of Food poisoning according to incubation period

Incubation period	**Etiological agent**
1 – 6 hours	• *S. aureus*, *Bacillus cereus* (performed toxin)
8 – 16 hours	• *C. perfringens*, *B. cereus* (diarrheagenic toxin), Mycotoxicoses (6 – 24 hours)
> 16 hours	• Bacterial: *E.coli* (ETEC, EHEC), *V. cholerae,* Non-typhoidal salmonellae, Shigella spp., *C. botulinum, C. jejuni, Listeria monocytogenes* • Others: Norovirus, Mycotoxins

Enumerate the samples to be collected in a diarrohea/food poisoning case.

A.4 (a) Samples (in diarrhoeal/food poisoning disease)

1. Stool 2. Rectal swab 3. Vomitus 4. Food sample 5. Serum

Describe the collection procedure of stool and rectal swab. Comment on their transportation.

A.4 (b) Collection of these samples

Stool:

The liquid/non-formed/bloody/pus/mucus part* is preferred over a formed (normal) stool fraction for sampling and collected into a clean, (not necessary to be sterile) leak-proof, wide mouth container. Three samples preferably on consecutive or alternate days can be processed. This approach could be especially useful in detecting parasitic infection, where the ova/cysts are being excreted intermittently in varying quantas.

Rectal swab:

A swab is inserted about 2-5 cms beyond the anal sphincter. It is rotated gently to swab the anal crypts. A properly sampled swab should get stained with faeces. A stool specimen is preferable to a swab specimen, as a tiny amount of sample gets sampled with a swab specimen. The swab may be transported as such or in the transport medium to the laboratory.

nb: Stool specimen from patients admitted in hospital for >3 days is not accepted by some laboratories.

* These fractions represent pathologic part of the stool likely to be in contact with infected intestinal mucosa.

Transportation:

The stool specimen without any holding (transport) medium should be transported to the laboratory within 1 hour at room temperature. In case; a delay is likely, the stool specimen can be kept at 4°C for less than 24 hour.

In case, Cary Blair medium is used for holding, the specimen can be kept at room temperature for ≤24 hours. Buffered glycerol transport medium can also be used for transportation.

References of clinical case based studies on GIT infections / syndromes

Category	Etiological agent/ disease	Section	Chapter	Pages
BACTERIAL	1. *C. botulinum* / Food poisoning	V	14	327-328
	2. *E. coli* / diarrhoea	VI	4	345-348
	3. Shigella / dysentery	VI	5	349-351
	4. Salmonella / Food poisoning	VI	6	352-355
	5. *V. cholerae* / Cholera	VII	5	376-378
	6. *C. jejuni* / Campylobacteriosis	VIII	10,11	398-399
	7. *H. pylori* / Peptic ulcer [G.U.D.]	VIII	12	400-401
VIRAL	1. Adenovirus / Diarrhoea	XII	3	496-498
	2. Rotavirus / Diarrhoea	XII	2A	533-535
PROTOZOAL	1. *E. histolytica / amoebiasis*	XIV	5	634-637
	2. *G. lamblia / Giardiasis*	XIV	7	640-643
	3. *P. falciparum / Pernicious malaria (diarrhoea)*	XIV	11	650-652
	4. *Cryptosporidum spp/Cryptosporidiosis*	XIV	13	655
	5. *B. coli / Balantidiasis*	XIV	14	656
HELMINTHIC	1. *T. solium* / Taeniasis	XV	5	679-680
	2. *D. latum* / Diphyllobothriasis	XV	8	685-686
	3. *F. buski* / Fasciolopsis	XV	10	688
	4. *E. vermicularis* / Enterobiasis	XV	11	689
	5. *A. lumbricoides* / Ascariasis	XV	12	690-694
	6. *S. stercoralis* / Strongyloidiasis	XV	14	698-699

Classify Hepatobiliary Infections / syndromes.

A.5

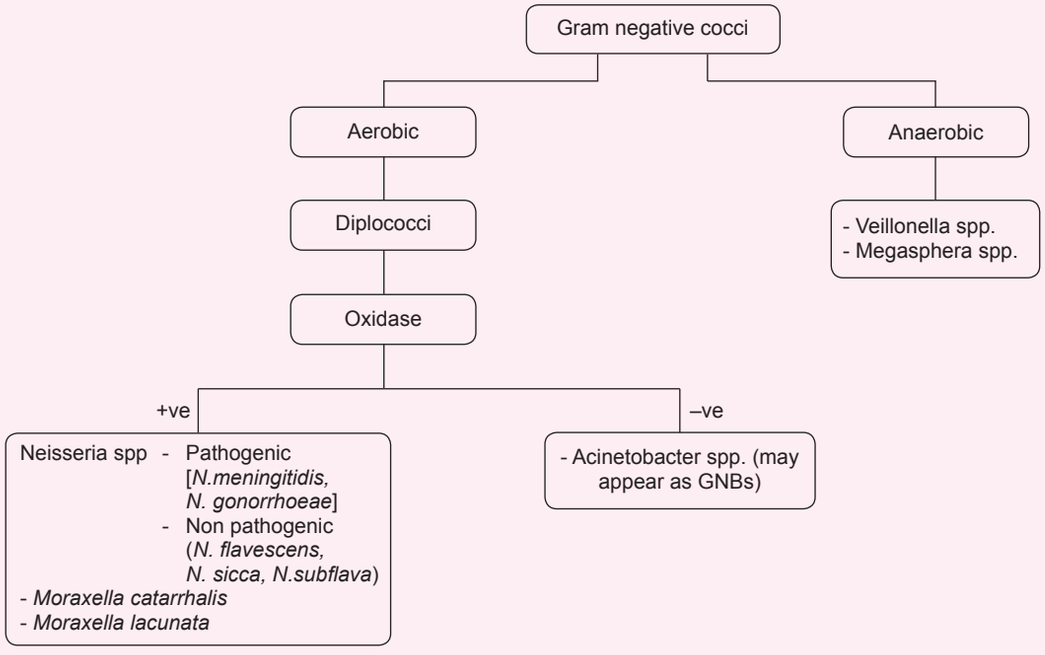
- Liver
  - Hepatitis
  - Abscess
- Bile duct system
  - Cholangitis (is inflammation of the bile duct caused usually by bacteria ascending from junction with duodenum, associated with gallstones.
- Gall – bladder
  - Cholecystitis

Section IVB: Diseases Caused by Gram Negative Cocci

Classification, Metabolic and Microscopic Features of Gram Negative Cocci (GNC)

1

Algorithm for Identification of GNC



Metabolic and Microscopic Features of Gram Negative Cocci

Organisms	Growth Requirements							Cellular Morphology and Staining Characteristics					
	O2 Requ.	Optimal Temp.	CO2 Requ.	Incubation Period Days	Weeks	Months	Shape	Gram	Arrangement	Capsule	Motility	Spore	Special Staining/ microscopy/ Special Features
Neisseria meningitidis	Strictly aerobic	37°C	+	1	–	–	Cocci in pairs	–ve	in pairs Fig. 4b 1.1	+ polysaccharide)	–	–	–
N. gonorrhoeae	Strictly aerobic	35-37°C	+	1-2	–	–	Cocci in pairs	–ve	In pairs Fig. 4b 1.2	+ (polyphosphate)	–	–	–
Acinetobacter spp. [Common spp. are baumani and iwofii]	Strictly aerobic	37°C	–	1	–	–	Cocco-bacili	–ve	In pairs, singly, chains	+	–	–	May appear as diplococci and cause diagnostic confusion
Moraxella calanhalis	Aerobic	37°C	–	1	–	–	diplococci (adjacent sides flattened)	–ve	–	±	–	–	–
Moraxella lacunata	Strictly aerobic	37°C	–	1	–	–	Bacili	–ve	pair, chains, dumps	±	–	–	–
Vellonella spp.	Anaerobic	37°C	–	1	–	–	Cocci	–ve		(–)	–	–	–

± More research is necessary to definetly demonstrate the presence and define its role.

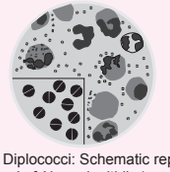


Fig. 4b.1.1: Diplococci: Schematic representation of dipplococci of *N.meningitidis* (gram negative) demonstrating adjacent edges to be flat

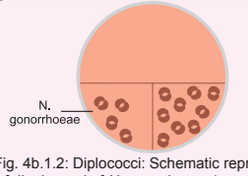


Fig. 4b.1.2: Diplococci: Schematic representation of dipplococci of *N.gonorrhoeae* (gram negative) demonstrating adjacent edges to be concave

An Overview of the Media Requirement, Colonial Characters and Diagnostic Characteristics of Key Gram Negative Cocci

2

	Basal media	Enriched media	Selective/others	Characterization and confirmation of isolate
• N. meningitidis	No growth	- Blood agar: Small (1-2 mm), translucent, haemolytic colonies - Choclate agar & Mueller - Hinton media also used for isolation	- MacConkey: No growth - Thayer Martin medium with antibiotics used (Vancomycin, colistin, trimethoprim and nystatin) (p. 65)	- Microscopic and staining characteristics - Catalase +ve - Oxidase +ve - Sugar fermentations (using serum sugars) - Ferments glucose and maltose with acid only but no gas - Serogrouping (using poly and monovalent sera)
• N. gonorrhoeae	No growth	- B.A.-Small, translucent colonies (five types) known, T1-T5 - Chocolate agar and Mueller Hinton media used	- MacConkey-no growth - Thayer Martin medium with antibiotics used - Mueller Hinton medium (also used)	- Microscopic and staining characteristics - Catalase +ve - Oxidase +ve - Sugar fermentation using serum sugars (Fig. 4b.2.1) - Ferments glucose with acid only but no gas - Aerobe (can grow anaerobically)
• Moraxella catarrhalis	N.A. (+)	+	–	- Microscopic and staining features - Catalase +ve - Oxidase +ve - Doesn't ferment sugar
• Moraxella lacunata	N.A. – NG	- B.A- (+) - Serum agar (+) (pitting colonies)	–	- Microscopic and staining features - Catalase +ve - Oxidase +ve - Doesn't ferment sugars
• Veillonella spp.	Anaerobic microbe	B.A. (+) (details beyond U.G. level)		- Microscopic and staining features - Sugars oxidatively utilized
• *Acinetobacter sps (baumanii & iwofii are two key species)	N.A. (+)	(+)	- MacConkey (+) (pinkish)	- Microscopic and staining features - Obligate aerobe - Oxidase negative - Characterized as glucose oxidizer/Nil fermenter. - Acid produced without gas from glucose (in *A. baumanii)*

nb: (i) NA is Nutrient agar, (ii) NG indicates no growth, (iii) (+) indicates growth, (iv) *also characterized as an gram negative bacilli by some authorities, as has features of both cocci and bacilli, i.e., is a cocco-bacilli.

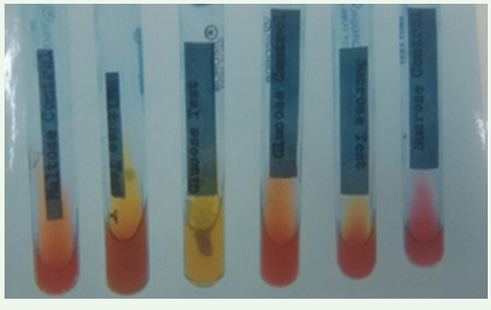


Fig. 4b.2.1: Biochemical reactions of *N.gonorrhoeae* fermenting only glucose with acid production (but no gas production)

Clinical (Pathogenicity) Profile of Infections Caused By Gram Negative Cocci

3

Neisseria meningitidis	Carrier state (localized infection in nasopharynx)
	Meningococcaemia, Meningitis • [Case: pg. 283-284]
	Waterhouse Friderischen syndrome (DIC shock, damage to adrenal gland), chronic meningococcal bacternia
N. gonorrhoeae	Carrier state in women
	In Men
	- Acute gonorrhoea (primarily urethra involved) (Fig. 4b.3.1); Case; p. 285-286
	- Sometimes epididymitis and prostatitis
	- Other lesions less common; as arthritis, meningitis, proctitis etc.
	In women
	- Acute gonorrhoea (primarily cervix involved), sometimes PID.
	- Other lesions less common
	In both sexes
	Pharyngitis, proctitis (gonococcal) and keratitis***
	In children
	- Ophthalmia neonatorum
	- Conjunctivitis
	- Gonorrhoea [Case: pg. 285-287]
Moraxella catarrhalis	- Lower respiratory tract infection (Organism is part of normal flora of upper respiratory tract and genital tract
Moraxella lacunata	- Conjunctivitis (angular and other)
Veillonella spp.	- Bacteremia
Acinetobacter spp.	- Opportunistic and healthcare associated infections; as pneumonia, septicaemia and meningitis and soft tissue infections

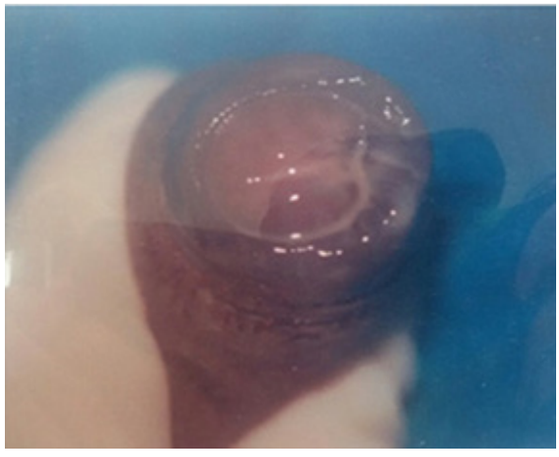


Fig. 4b.3.1: Gonorrhoea: Profuse purulent discharge per urethra

* results because of anal intercourse
** is inflammation of the cornea

Integrated Clinical Based Study of *N. meningitidis*/Meningitis

4

A seven year old girl Sameena, presented to the paediatric emergency with history of fever, headache, stiff neck and double vision. She had all the routine immunizations on schedule. CT scan revealed mild cerebral oedema.

Linkages: Pg. 279-281, 288, 289, 818

What is your differential diagnosis of this case?

A.1 Meningitis, encephalitis, brain abscess, cerebral neoplasm or any space occupying lesion of the brain.

A L.P. is done on the child. The C.S.F. when gram stained revealed presence of polymorphs and numerous gram negative diplococci in pairs (Fig. 4b.4.1).

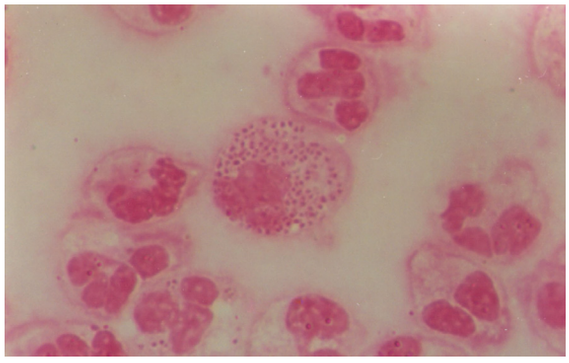


Fig. 4b.4.1: Diplococci: Gram stained smear of cerebrospinal fluid from a case of acute meningitis demonstrating gram negative diplococci in leucocytes

What is the likely pathogen based on these findings?

A.2 (a) *N. meningitidis* (first isolated by Weichselbaum in 1887)

Name some commensal Neisseria of the respiratory tract.

A.2 (b) *N. flavescens*, *N. sicca*

How are they differentiated from N. meningitidis?

A.2 (c) The commensal Neisseria are characterized by their ability to grow on ordinary media (non enriched), producing pigmented colonies and fermenting a number of carbohydrates. (see Pg. 280, Chapter 3)

What is the reservoir of N. meningitidis?

A.3 (a) Man is the only reservoir for meningococcus. This fact is employed in control of meningococcal outbreaks by isolation of the cases.

Are asymptomatic carriers known with N. meningitidis?

A.3 (b) The asymptomatic carrier rate is about 5-10%. This rate often rises before an impending epidemic. Hence, sometimes trends of the nasopharyngeal colonization rate are used for intervention in a community.

Describe the epidemiology of meningitis caused by N. meningitidis.

A.3 (c) Epidemiology:

Agent: *N. meningitidis*

- Agent serogroups* A, B, C, Y, and W-135 responsible for > 90% of meningococcal infections worldwide.
- Group A strains have the potential to cause epidemics (in past; epidemics have occurred at intervals of 8-10 years)
- Group A meningococcus epidemic occurred in Delhi in 1985
- Serogroup B strains have been associated with epidemics in developed countries
- Techniques; as PFGE (classifies bacteria into electrophoretic types-ETs), bacterial genome sequences amplification by PCR and multilocus enzyme electrophoresis help in strain identification.

* Serogroup : Group of strains containing a related structures, which generate a similar antibody response

The organism is unique among the major bacterial agents to cause both epidemic as well as endemic (sporadic) disease.

Reservoir infection: Human nasopharynx is the primary reservoir of human infection (cases and carrier)

Source of infection: Nasopharyngeal and Bronchial secretion (infective).

- Carriers are the most important source of infection.
- In interepidemic periods, approximately 10% of healthy individuals are colonized as carriers of meningococcus. This rate may exceed 80% in close communities during epidemics.

Transmission occurs through inhalation of respiratory droplets from a carrier or a patient (in early stages of disease). It is less often by fomites. The transmission requires close contact and susceptibility (lack of specific antibodies).

Host

- It predominantly causes disease in children and adult of both sexes.
- Attack rate is higher among children than among adults (although one-third to one half of all cases of sporadic meningococcal disease are reported in adults).
- All ages are susceptible, although younger age groups are more susceptible than older age groups, as immunity in them is lower.
- Absence of meningitis in a case of meningococcemia may be a poor prognostic sign, as it could indicate that multiplication has occurred too fast for meningeal seedling to occur or elicit inflammation in CSF.

Environmental factors: Outbreaks occur more frequently in the dry and cold months of the year.

Outbreaks (epidemics) occur commonly among the poorest groups, where crowding and lack of sanitation are common. The places of outbreaks include schools (day care centers), prisons and army camps. During epidemics, both chemoprophylaxis; which gives short term protection and vaccination for long term protection is indicated, (if a case occurs in a family setting). Recently; epidemics have been reported from Australia, China, Netherland and Africa.

How many serogroups are known for this pathogen?

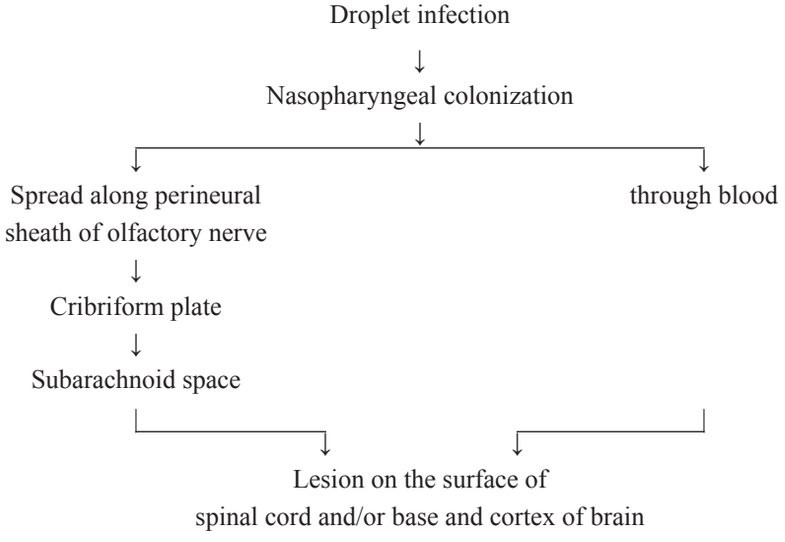
A.4 It is divided into 13 serogroups. Some serogroups are associated more frequently with causation of epidemics. Typing of isolate helps in characterizing the outbreaks, which occur differently in the various parts of the world. The vaccine is constituted by those serogroups that are prevalent in a geographical region. Serogroup B is poorly immunogenic, hence not included in common polyvalent vaccine.

Why does an outbreak with N. meningitidis cause so much fear?

A.5 (a) Because meningococcal meningitis is associated with severe morbidity and high mortality, sometimes (upto 70% mortality has been reported in some outbreaks).

Outline the pathogenesis of meningitis caused by N. meningitidis.

A.5 (b)



What measures are taken to control a meningococcal meningitis outbreak? Does immunization and chemoprophylaxis have any role in the control of an outbreak?

A.6 Control measures; include isolation of cases, chemoprophylaxis and immunization of contacts. The latter two have a role; unlike in some disease outbreaks, as cholera, where their role is minimal.

What pharmacokinetic aspect of antimicrobial is to be considered, when administering drugs for treatment of meningitis?

A.7 The antimicrobial should be able to cross the blood-brain barrier (meninges) to reach the brain

What are the current challenges in the study of N. meningitidis?

A.8

- To understand, why certain populations are susceptible to *N. meningitidis* meningitis.
- To understand the sporadic (endemic) nature of the disease, which sometimes occurs with this agent.
- To understand the mechanism, by which some persons become carrier with this pathogen and the mechanism of the eradication of the organism.
- To make group C vaccine more immunogenic in children under 2 years of age (not clear, why it is less immunogenic).
- Development of group B polysaccharide vaccine (not clear, why this group is less immunogenic).

Integrated Clinical Based Study of *N. gonorrhoeae*/Gonorrhoea

5

A 20 year college going male student Shahid, reported to the medical OPD with complaints of burning micturition, dysuria and profuse seropurulent urethral discharge (Fig. 4b.3.1, p. 281). A history of having sexual relation with three female partners in the past three months was elicited. A gram stain of the urethral discharge revealed PMNs with intracellular diplococci, Fig. 4b.1.2. (p. 279). Urine culture did not reveal any pathogens.

Linkages: Pg. 279-281, 285, 288, 289

What is your provisional diagnosis in the above case?

A.1 **(a)** Urethritis (acute) most likely due to *N. gonorrhoeae.* This organism was first described by Neisser in 1879; in exudates.

How is urethral specimen collected?

A.1 **(b)** A fine, flexible swab is inserted about 4 cm into the urethra, rotated twice and removed. If fresh exudate is available, then the surface exudate is discarded and the freshly expressed one can be utilized for processing. The patient should not have urinated, at least one hour before collection of this sample.

What is the sensitivity of gram stain in diagnosing gonococcol urethritis in males?

A.1 **(c)** Approximately 95% in males.

What is the reliability of this technique in diagnosing gonococcal cervicitis in females?

A.1 **(d)** The reliability of gram stain in detecting the infection in females is low, as it is difficult to distinguish commensal Neisseria in genital flora from pathogenic *N. gonorrhoeae*. The positivity of Gram stain of cervical specimen for this pathogen is only about 50 to 60%, making it an unreliable tool. In females, smears should be prepared from urethral discharge and/or cervical swab.

What are the non gonococcal (NGU) causes of urethritis?

A.1 **(e)** It includes C.*trachomatis, Ureaplasma urealyticum, Mycoplama genitalium, Trichomonas vaginalis*, Human Herpes viruses and Adenoviruses

What microbiological test can be done to isolate N.gonorrhoeae from clinical specimen suggestive of gonorrhoea?

A.2 **(a)** Conventionally, the urethral exudate can be cultured onto Chocolate agar/Thayer Martin medium for extended period (of at least 36-48 hrs) in environment of 5% CO_2 for isolation of *N. gonorrhoeae*.

What rapid diagnostic techniques can be employed on a clinical sample with suspected N. gonorrhoeae infection?

A.2 **(b)** The gonococcal antigen in clinical specimens, as urethral discharge and endocervical discharge can be detected utilizing assays, utilizing direct fluorescent antibody or enzyme linked antibody principles.

Molecular amplification techniques (NAATs) based on PCR, LCR and TMA (transcription based amplification) technology for detection of this pathogen are also commercially available.

Is it unusual for the urine culture of this case not to reveal any pathogen in this case?

A.2 **(c)** In urine, this pathogen can be demonstrated, using antigen detection or genome amplification detection techniques. However a conventional culture using blood agar medium incubated for only 24 hrs in an environment without increased CO_2 concentration is unlikely to lead to isolation of this pathogen.

What is the reservoir of N. gonorrhoeae?

A.3 **(a)** The reservoir of this pathogen is infected men and women, who are cases and carriers. In men, the commonest site is urethra and in the women; endocervix. Women play a bigger role in the transmission of this infection, as they are often asymptomatically infected, so go undetected. Infection of rectal and pharyngeal regions has also been seen especially in individuals, who practice uncommon sexual practices.

Is it possible for an woman infected with N. gonorrhoeae to be asymptomatic?

A.3 **(b)** Yes.

What is the mode of infection for gonorrhoea? Mention its epidemiology.

A.3 **(c)** It is primarily venereal. This may be contrasted with Ophalmia neonatorum, which is a non-venereal gonococcal infection.

Agent: *N. gonorrhoeae* (types T1-T4)

Source:

- It is an exclusively human pathogen found only in infected cases (not as part of normal human flora).
- Gonorrhoea is a veneral disease involving inflammatory discharge from the urethra or vagina.
- One infection, doesn't confer immunity and repeated infections are possible.
- One can get this infection (and other STDs) by having vaginal, anal or oral sex with someone, who has gonorrhoea. So to avoid this and other STDs, one has to restrict these three categories of sexual activities with infected partners.
- Higher proportion of females, then males are asymptomatic carriers of this pathogen. They act as reservoir of this infection and are important in transmission not only of this but other STDs. Contact tracing and empirical treatment of sex partners of index case is important.

Environmental factors:

- Fomites don't play any role in the transmission of the disease, as the organism is fragile.
- Numerous social factors as prostitution, broken homes and sexual disharmony are involved in the spread of this infection.

What complications can occur in a woman, who has been infected with N. gonorrhoeae, but has remained asymptomatic for years?

A.3 **(d)** Pelvic inflammatory disease; which may result in infertility and ectopic pregnancy. Disseminated gonococcal infection may also occur.

Should asymptomatic women infected with N. gonorrhoeae be treated? Explain.

A.4 **(a)** Asymptomatic women must be treated, so that complications; as PID can be prevented from occurring and the sexual contacts of woman do not contract this infection.

What does PPNG represent? What are some factors that promoted the development of PPNG?

A.4 **(b)** PPNG represents: penicillinase producing *N. gonorrhoeae,* i.e., implying that the isolate (strain) would be resistant to penicillins in varying degrees. This was reported in 1970s and was mediated by plasmids. Currently drug resistance due to chromosomal mutation is also reported. Quinolone resistance in this pathogen is also reported.

Some of the factors that led to the development include:

(i) Practise of chemoprophylaxis, especially among prostitutes

(ii) Availability of the drugs without prescription (i.e., over the counter availability)

(iii) Inadequate course of antibiotics (i.e., use of suboptimal doses)

(iv) Laboratory manipulation of gonorrhoea strains, as their cultivation in media containing; vancomycin.

(v) Change in the cultural practices; as homosexualty, making gonoccus getting exposed to bile acids and fatty acids in male rectum; favouring the development of resistance to these and other agents.

What virulent factors are responsible for the pathogenicity of N. gonorroheae?

A.5 **(a)** *N. gonorrhoeae* has the ability of producing intense inflammatory response, which manifests; as uretheral exudates in males and PID in females. Two important virulent factors are pili; which helps in attachment to cells and lipo-oligosaccharide (LOS); which stimulates inflammatory reaction in the infection site.

Describe the pathogenesis of gonorrhoea.

A.5 **(b)** The infection gets initiated by sexual contact. The adhesion to mucosal cells is initiated by piliated strains (i.e., strains having pili). The penetrating microvilli from the host cells, aid the phagocytosis of gonococcus penetrating through cell to reach intercellular spaces and then to submucosal tissue. The inflammatory response is generated, caused by release of cytokines. The damage to the genital structures is responsible for the pathogenicity.

What is the probability of acquiring this infection from a surface; as an dry western toilet seat?

A.5 **(c)** It is very low, as the organism is a fragile one. It is susceptible dehydration and common disinfectants.

What are the other infections, the individual mentioned in this case is prone to?

A.6 Since this case is involved in increased sexual activity with multiple sexual partners, he is prone to STDs; as AIDS, syphilis and chlamydial infections.

The case should be screened for other associated STDs and managed according to coinfections, he may be having. He should be counselled and all his sexual contacts traced, screened and treated, if required

What is the reason for non availability of vaccine against this organism?

A.7 Gonococci undergoes frequent rearrangement of the pilin genes making it difficult to produce a protective vaccine. Its pathogenicity is not toxin based, unlike tetanus against which protective immune response can be easily generated.

Aspect related to case theme/examination assessment

Describe Non gonococcal urethritis.

A.8 NGU is an entity of chronic urethritis, which should be differentiated from urethritis caused by gonococcus. This differentiation is important as the management (including treatment) strategy for urethritis, varies, according to disease category.

- **Etiological agents:**
  Bacterial: Chlamydia trachomatis, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*
  Viral: Human Herpes virus 5 (CMV),
  Fungal: Candida albicans,
  Protozoal: Trichomonas vaginalis
- **Pathogenicity:** The onset usually occurs more than one week after contact. The uretheral discharge is mucopurulent in nature in contrast to gonococcal urethritis, where it is purulent in nature.
- **Diagnosis:** It depends on the incriminated etiological agent.
- **Treatment:** For *Chlamydia trachomatis* and *Ureaplasma urealyticum*, tetracycline is effective. For *Trichomonas vaginalis* and *Candida albicans*, Metronidazole and antifungal; as Clotrimazole are effective, respectively.

Laboratory Diagnosis and Treatment (Overview) 6

An Overview of the Comparative Approach in Laboratory Diagnosis of Key Gram Negative Cocci (Aerobic) Organisms

Organism/ Disease	Specimen	Stain enhanced microscopy	Detection of Microbial antigen/metabolite/ genome	Serological Tests	Culture of Organism In Media / Characterization of Isolate	Differential Diagnosis	Antimicrobial Susceptibility Tests
Neisseria meningitidis	- Cerebrospinal fluid - Nasopharyngeal* swab (for carriers) - Blood - Serum - Petechiae - Synovial fluid	CSF is divided into three parts: - First part is centrifuged and deposit, used for gram staining. (Gram –ve diplococci) - Second part is used for culture purpose - Third part is inoculated into glucose both and kept as stock	- Latex agglutination coagglutination, counterimmuno-electrophoresis and enzyme linked immunoassay techniques, available to detect microbial antigen in sample (rapid diagnostic test) - Limulus amoebocyte assay can be done to detect endotoxin in C.S.F./PCR available for meningococcal DNA in CSF	- may attempt specific antibody demonstration by haemagglutination and fixation techniques, when culture is negative	- Basal media: NG Enriched - Chocolate agar(+) - Mueller Hintor agar(+) MacConkey: NG For characterization and confirmation of isolate See p. 280	- *N.gonorrhoeae* - Commensal Neisseria as : *N flavescens* : *N.sicca* - *N.catarrhalis*	- Special protocol to be followed
** role only in epidemiology/research, as isolation from this site gives no information on the existence of systemic disease.*							
Neisseria gonorrhoeae	- exudate : Urethral meatus : Cervical os (in female) : Pharynx (esp those who pratice oral sex) - Conjunctival secretion (in new-born) - Blood (if suspect bacteremia) - Synorial fluid, cerebrospinal fluid in disseminated cases - For specimen transport, charcoal coated swab in stuart's medium or Amie's medium with charcoal	- Smear examination is unreliable; as commensal flora can resemble gonococci - Sensitive and specific fluorescent antibody test available	- ELISA can detect gonococcal antigen in sample/DNA probe available for uretheral discharge Because of legal implications, reliance is on culture or two NAAT (targetting two different nucleic acid sequences) - For nucleic acid probe test, target is gonococcal 16S ribosomal RNA	• Specific antibodes (IgG and IgA) appear against pilli, outer membrane protein and lipopolysaccharide in serum and genital fluid. These can be detected by ELISA and other techniques, but not of much diagnostic significance	- Basal media: NG - Blood agar (scanty growth) - chocolate agar (good growth) - Thayer Martin with antibiotics for sample from infected site(+) - For characterization and confirmation of isolate, see p. 280	- N.meningitidis - Commensal Neisseria	- Special protocol, to be followed - β lactamase producting strains should be detected
nb: urethral swab (discharge) is not a sample in females, as urethritis is not common in them.							

An Overview of the antimicrobial Options for Infections caused by key Gram Negative Cocci (Key)

	Cell Wall Inhibitors	Cell-Membrane Inhibitors	Amino Acid Synthesis Inhibitors	Nucleic Acid Synthesis Inhibitors	Others
• Neisseria meningitidis	• PnG (DOC)* • Cephalosporins		Chloramphenicol (in penicillin susceptible cases)	Fluoroquinolones	
• Neisseria meningitidis** • *Carriers*, for eradication of organism			Rifampicin (given at end of therapy to eradicate organism)****		
• *N.gonorrhoeae* (most penicillinase producing)	• [Ceftriaxone or cefixime	+	Azithromycin or doxycycline (DOC)]	• Ciprofloxacin*** • Ofloxacin • Gatifloxacin	
• *Moraxella catarrhalis* (previously Branhamella catarhalis)	• Cephalosporins as cefuroxime (DOC)		• Doxycyline	• Fluoroquinolones • TMP-SMZ	
• Moraxella lacunata	PnG				
• Acinetobacter spp.	• Carbapenems (DOC) • Piperacillin tazobactam • Ceftazidime	• Polymyxin B	• Doxycycline • Minocycline • Aminoglycosides often combined with imipenem/ ceftazadime for serious infections.	Fluroquinolones TM-SMZ	

- Treatment should be administered based on antimicrobial susceptibility testing, however the choices mentioned are general indications.
- DOC is Drug of first choice
- TMP-SMZ is Trimethoprim-Sulfamethoxazole

* In tentative cases, give third generation cephalosporins; as meningitis caused by *S. pneumoniae* and *H. influenzae* may not respond.

** Chemoprophylaxis is indicated; as house-hold contacts of cases, as they do get infected (nasopharyngeal colonization).

*** In early 2000s, ciprofloxacin resistance started getting reported in *N. gonorrhoeae*. Around 2007, CDC stopped recommending fluoroquinolones; as empiric treatment for gonorrhoea. Cefexime or ceftriaxone, then became the recommendations.

**** This drugs is not used in treatment.

Assessment/Examination Questions

1.	Outline the morphology and cultural characteristics *of N. meningitidis*.	p. 279-280
2.	What is the reservoir of *N. meningitidis*? Are asymptomatic carriers known to occur with this pathogen? Describe the epidemiology of *N. meningitidis* infections.	A 3a,b,c., p. 282-283
3.	Describe the pathogenesis of meningitis caused by *N. meningitidis.*	A 5b., p. 283
4.	Describe the laboratory diagnosis of meningococcal meningitis.	p. 288, p. 279, 280
5.	Describe the antigenic structure of *N. meningitidis* and describe the meningococcal vaccine.	A 3c., p. 282, p. 818
6.	Outline the morphology and cultural characteristics *of N. gonorrhoeae.*	p. 279-280
7.	What is the reservoir of this pathogen? Describe the epidemiology of gonorrhoea.	A 3 a-d., p. 285-286
8.	What virulent factors are responsible for the pathogenicity of this pathogen? Describe the pathogenesis of gonorrhoea.	A 5a, b., p. 286
9.	What does PPNG stand for? What are some of the factors that promoted the development of this entity?	A 4b. p. 286
10.	Describe the laboratory diagnosis of gonorrhoea.	p. 288, 279, 280
11.	Should asymptomatic women infected with this pathogen be treated? Explain.	A 4a., p. 286
12.	Enumerate the differences between *N. meningitidis* and *N. gonorrhoeae.*	p. 279, 280
13.	Describe non gonococcal urethritis.	A 8., p. 286-287
14.	Describe *Moraxella catarrahalis.*	p. 280-281
15.	Name a gram negative bacilli that may appear as gram negative cocci.	p. 279
16.	Describe Acinetobacter spp.	p. 279-281

For Single Response Assessment/Examination Questions, See Appendix 5, pg. 844

The genome of all DNA viruses consist of single, double stranded molecule; except in parvovirus, where it is single stranded. The smallest virus contains about 4000 nucleotides (4 kilobases), whereas the largest DNA virus contains about 200000 (200 kb) base pairs. The DNA can be linear or circular.

In contrast to DNA viruses, most RNA viruses have single-stranded genome except Reoviruses, which exists as double stranded-molecule. For most viruses, RNA exists as a single molecule. For some, it exists as segmented form; for example in Arenaviridae; as 2 segments, in Bunyaviridae; as 3 segments, in orthomyxoviridae; as 8 segments and in Reoviridae; as 10-12 segments. In retroviridae, segment is single, but two copies exist. Generally RNA viral genomes are smaller than DNA viral genomes (not gauzed by virus particle size), as depend on host enzymes for replication. More errors occur during RNA replication than DNA, as RNA polymerase lacks efficient proof reading.

nb: One 1kb pair contains enough genetic information to code for about one average size protein.

All viral RNAs are linear, none exists as a covalently closed circle. However, the ssRNA of arenaviruses and bunyaviruses have sticky ends which make these molecules exist as circles. In cases of segmented RNA genomes of orthomyxoviruses and retroviruses, one may consider most of the segments to be individual genes.

The single stranded RNA viruses can also be categorized according to its polarity (or sense). If the single stranded RNA is capable of acting as a mRNA (i.e., can be directly translated by host ribosomes), it is called positive sense. If the virus has a nucleotide sequence complementary to that of the mRNA (i.e., can not be directly translated by host ribosome), it is said to have a negative polarity (minus sense). Such viruses must carry a RNA-dependent RNA polymerase in the virion, so that the mRNA can be transcribed. In one genus of bunyavirus, one of the RNA segments is ambisense (i.e., part has positive polarity and part has negative polarity or positive and negative regions attached end to end).

Enzymes

Many viruses don't have any enzymes; as part of their structure and composition, as they are dependent even for enzyme synthesis on their host. However some viruses as negative stranded RNA viruses, whose initiation of viral replication cycle cannot occur, need enzymes as RNA dependent RNA polymerase to make the negative stranded RNA virus into a positive stranded virus.

Enumerate 'Virus like' agents.

A.6 (b) Viroids and Prions (see chapter 18, section XIII, p. 599). Initially it was believed that viruses were the smallest particles to be agents of infectious disease. However this concept changed with the discovery of 'virus like' agents.

What are viroids?

A.6 (c) They are single stranded circular RNA molecules without capsid. They are mostly plant pathogens.

Classify viruses of medical importance on the basis of their structural details.

A.7 (a)

Table 11.1.4: Classification of viruses of medical importance on structural basis (Physical and biochemical characteristics)

Nucleic acid core	Capsid symmetry	Virion: enveloped or naked	Ether sensitivity	Virus particle size (nm)[1]	Physical type of nucleic acid[2]	Virus family
DNA	Icosahedral	Naked	Resistant	18-26	ss	Parvoviridae
				45	ds, circular	Polymaviridae
				55	ds, circular	Papillomaviridae
				70-90	ds	Adenoviridae
		Enveloped	Sensitive/	40-48	ds, circular	Hepadnaviridae
			Resistant	150-200	ds	Herpesviridae
	Complex	Complex coat	Resistant	230x400	ds	Poxviridae
RNA	Icosahedral	Naked	Resistant	28-30	ss/plus sense	Picornaviridae
				28-30	ss/plus sense	Astroviridae
				27-40	ss/plus sense	Caliciviridae
				60-80	ds, segmented	Reoviridae
		Enveloped	Sensitive	50-70	ss/plus sense	Togaviridae
	Unknown or complex	Enveloped	Sensitive	40-60	ss/plus sense	Flaviviridae
				50-30	ss/minus sense	Arenaviridae
				80-110	ss diploid/plus sense	Retroviridae
	Helical	Enveloped	Sensitive	120-160	ss/plus sense	Coronaviridae
				80-120	ss (segmented)/minus sense	Orthomyxoviridae

				80-120	ss (segmented)/minus sense	Bunyaviridae
				80-125	ss/minus sense	Bornaviridae
				75x180	ss/minus sense	Rhabdoviridae
				150x300	ss/minus sense	Paramyxoviridae
				80x1000	ss/minus sense	Filoviridae

[1]Diameter, or diameter x length

[2]ss= single stranded, ds = double stranded

Classify diseases caused by DNA viruses.

A.7 (b) (i) **Table 11.1.5:** Common DNA Viruses And Diseases Caused In Man (details see Section 12)

Family/Shape (symmetry)	Genus/Virus	Disease
• Parvoviridae/Icosahedral	• Parvovirus B 19 Erythrovirus • Dependovirus (adeno-associated Virus)	Fifth disease
• Papillomaviridae/Icosahedral	• Papillomavirus/Human Papilloma Virus	• Warts, Cancer (cervical & penile)
• Polymaviridae/Icosahedral	• BK/JC Polyoma virus, Simian virus 40	• See pg. 490-492, Section 12
• Adenoviridae/Icosahedral	• Mastadenovirus/Human adenoviruses (>51 serotypes)	Associated with many syndromes (pg. 496-498)
• Herpesviridae/Icosahedral, (enveloped)	• Simplexvirus/HHV 1 & 2 • Varicellovirus/HHV-3 (Varicella zoster virus) • Cytomegalovirus/HHV-5 • Roselovirus/HHV-6 • Lymphocryptovirus/HHV-4 (Epstein- Barr virus)	• Local & genital herpes • Chickenpox & Herpes zoster • CMV infection • Roseola subitum/roseola infantum • Infectious mononucleosis
• Poxviridae/Brick/ Complex (enveloped)	• Orthopoxvirus/Variola virus, Vaccinia virus/Cowpox virus/ Monkey pox virus • Parapoxvirus/Orf virus, Milker's node virus • Molluscipoxvirus/Molluscum contagiosum virus Yatapoxvirus/Yabapox virus, Tanapox virus	• Smallpox, Vaccinia Milker's node, Orf Molluscum contagiosum Yabapox, Tanapox
• Hepadnaviridae/ Icosahedral (tubular forms also exist)	Orthohepadnavirus/Hepatitis B virus	Hepatitis B Infection

nb: Icosadeltahedron represents a complex icosahedron

Classify diseases caused by RNA viruses.

A.7 (b) (ii) **Table 11.1.6:** Common RNA Viruses And Diseases Caused In Man (details see Section 13)

Family/Shape	Genus/Species	Disease
• Reoviridae/Doubled shelled, Icosahedral	• Orthovirus/Human Reovirus (reo, refers to respiratory, enteric orphan) • Rotavirus/Rotaviruses • Orbivirus/Kemerovo Virus • Coltivirus/Colarado tick fever virus	• Gastroenteritis and other
• Orthomyxoviridae/Spherical	• Influenzavirus A/Influenza A virus • Influenzavirus B/Influenza B virus • Influenzavirus C/Influenza C virus	• Influenza
• Paramyxoviridae/Spherical	• Respirovirus/Human parainfluenza viruses (types 1 & 3), Sendai virus • Rubulavirus/Human parainfluenza viruses (type 2), Mumps virus • Morbillivirus/Measles virus, Rinderpest virus • Pneumovirus/Human respiratory syncytial virus, • Human metapneumovirus • Henipavirus/Hendra virus, Nipah virus, Sendai virus • Avulavirus/Newcastle disease virus	• Respiratory Infections • Mumps • Measles • Respiratory syncytial disease • Respiratory tract infection
• Rhabdoviridae/Bullet	• Vesiculovirus/Vesicular Stomatitis virus • Lysaavirus/Rabies virus	• Rabies

• Filoviridae/Filamentous & pleomorphic	• Filovirus/Ebola virus, Marburg virus, Reston Virus	• Ebola and other
• Bunyaviridae/Spherical	• Bunyavirus/California encephalitis virus, Oropouche • Phelbovirus/Rift valley fever virus, Sandfly fever virus • Nairovirus/Crimean-Congo haemorrhagic virus (CCHF) • Hantavirus/Hantaan virus, Sin Nombre virus, Puumala, Ganjam virus	• Arboviral infections; as Sandfly fever, Rift valley fever, CCHF and others
• Arenaviridae/Spherical [*arena* = "sand"]	Arenavirus/Lymphochoriomeningitis virus, Lassa virus, Machupo virus, Junin virus, Sabia virus & others	Lymphocytic choriomeningitis, Lassa fever and others
• Caliciviridae/Icosahedral	Calicivirus/Human Calciviruses, Hepatitis E virus, Norwalk virus	Diarrhoeal disease, Jaundice
• Picornaviridae/Icosahedral	See A1c, e, p. 563-564	A1c,e, Chapter 9, p. 563-568
• Coronaviridae/Petal shaped spikes project (peplomer) project from surface	Coronavirus/Human Coronaviruses [SARS - CoV, MERS - CoV and SARS - CoV-2]	Respiratory infection
• Flaviviridae/spherical	• Flavivirus (Flavi=yellow)/Yellow fever virus, Dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus • Hepacivirus/Hepatitis C virus & others	• Yellow fever, Dengue, Encephalitis • Hepatitis C infection
• Togaviridae/Icosahedral (Toga, Greek for "Mantle")	• Alphavirus/Chickengunya virus, Sindbis virus, Eastern, Western and Venezuelan equine encephalitis viruses (EEE., WEE and VEE) • Rubivirus	• Chickengunya, • Encephalitis Rubella
• Retroviridae (positive strand/positive sense)/spherical	• Delta retrovirus/Human T lymphotropic virus 1, Human T lymphotropic virus 2 • Lentivirus/HIV- 1,HIV- 2	• Adult T cell leukemia/Lymphoma (Associated with HTLV-1) • AIDS

Provide a detailed account of viral replication.

A.8 It is important to understand this, as it helps to appreciate the pathogenesis of viral diseases, find the role of viruses in cancer, understand antiviral chemotherapy and devise newer strategies for antiviral drugs and vaccines. It must be appreciated that while discussing viral multiplication, the term *replication* is preferred over *reproduction*. This is so, as in viral multiplication, classical asexual and sexual processes are not utilized.

These are number of steps involved in the virus replication, but the overall production of viruses can be studied in the one-step growth curve. It is a representation of the overall change with time, in the amount of infectious virus in a single cell that has been infected by a single virus particle. The curve begins with the eclipse period to be followed by the exponential growth period and the plateau phase. The eclipse period represents the time elapsed from initial viral entry, disassembly of the parental virus to the assembly of the first progeny virion. This period for most viruses of human viruses varies from one to twenty hours. The exponential growth period is characterized by exponential increase in the number of progeny virus produced within an infected cell. The maximum yield per cell is characteristic for each virus cell system and the yield can vary from 100 to several thousands virions per cell.

The viral replication can be divided into 6 phases, though there may be overlapping in some phases (Fig. 11.1.3).

1. **Adsorption/Attachment:** A pre-requisite for this step is a collision between the *virion and the host cell.

   This is the first step in the infection of host cell. Ideally, to prevent the viral infections, the antiviral drugs and vaccines, should be targeting this step. During this step, viral attachment protein (VAP); as the surface capsid/ envelope attach to receptors on host cell (Table 11.1.7). Damage to VAPs can inactivate the virus. Production of antibodies against these structures can also prevent viral infection.

   This step involves the interaction of specific viral structure; as glycoprotein spikes in rabies virus or gp 120 in HIV, with receptor (specific) on target cells of the host. If the receptors are lacking from a target cell, then the natural viral infection is not possible. Lack of acetylcholine receptors in the rodents, explains their resistance to the rabies disease. If this phase of adsorption is bypassed and the nucleic acid of rabies virus is introduced directly into the rodent cells, they become susceptible to this disease.

   nb: *Virion –A mature, extracellular particle that is virulent (which can establish infection in a host) is called virion.

Section XIII: Infection Due to RNA Viruses

Overview of Clinical Profile (Pathogenicity) of RNA Viral Infections

Virus	*Disease*
• Rotavirus	• Acute diarrhoea (commonest viral agent for this disease, for children under 5 years) Details Chapter 2a, pg. 533-534
• *Influenza virus* (H1N1)	• Influenza (details A2b,c, pg. 536)
• *'Swine' flu* (H1N1)	• Respiratory infection of pigs, can spread to human and result in outbreaks See Chapter 3, pg. 542-545
• *Influenza virus* (H5N1, 'Avian' flu)	• Respiratory infection of birds; as fowl, can spread to man, outbreak in India occurred in 2008 See Chapter 4, pg. 544-545
• *Parainfluenza viruses*	Respiratory infections (See A11, pg. 547-548, Case 5)
• *Newcastle disease virus* • (Ranikhet virus)	Conjunctivitis (in individuals exposed to infected birds; as poultry workers)
• *Respiratory syncytial virus*	Respiratory syncytial virus infection See Chapter 5, pg. 546-547
• *Measles virus*	Measles • See Chapter 6 A1, 2, p. 549, SSPE is a complication
• *Mumps virus*	• Mumps • Parotid gland enlargement in 95% cases (non suppurative parotitis) • Complications; as meningitis, meningoencephalitis, pancreatitis, orchitis and others
• *Human Metapneumovirus*	Respiratory tract infection in children and adults (RSV-like illness)
• *Rabies virus*	Rabies • See Chapter 7, pg. 557, A5
• *Rabies related viruses* as *Mokola virus, Duvenhage virus*	Human infection resembing rabies See Chapter 7, pg. 559 A.10
• *Filoviruses; as Marburg*, Ebola**	Haemorrhagic fever (feared for the high mortality rates)
• *Hepatitis A virus*	Infectious Hepatitis • Details see Chapter 8, pg. 561, A5
• *Enteroviruses*	See A.1(e) Chapter 9, pg. 564
• *Poliovirus*	Poliomyelitis • Details see Chapter 9, pg. 563-565., A.4,5
• *Coxsackieviruses*	See A.5(b), p. 565 and See A.14 (ii), pg. 567
• *Echoviruses* (Enteric cytopathogenic human orphan)	• Mostly cause asymptomatic infection • Some serotypes associated with aseptic meninigitis (common cause), pericarditis, myocarditis, infantile diarrhoea and encephalitis
• *Enterovirus type 70*	Acute haemorrhagic conjunctivitis
• *Rhinoviruses*	Common cold
• *Hepatitis E virus*	Hepatitis • See Chapter 11, pg. 571-572
• Caliciviruses; as *Norovirus Sapovirus Astrovirus*	Gastroenteritis

Contd.

* Named after the location from, where they were originally isolated

Contd.

• *Rubella virus*	• Rubella (German measles); *germanus*⌂ = "similar" • Congenital rubella syndrome. see Chapter 11, pg. 571., A1d) • Postnatal rubella, see A5; pg. 572
• *Dengue virus (4 serotypes)*	Dengue ('break bone' fever) • Details see Chapter 12, pg. 574., A5c,d
• *Japanese encephalitis virus*	• Encephalitis See Chapter 13, pg. 575., A3a)c) and chapter 2, section 3, p. 195
• *Hepatitis C virus*	• Hepatitis • Less severe disease than Hepatitis B and more than half of the cases develop chronic hepatitis. Cases can develop cirrhosis or hepatocelluar carcinoma. (details Chapter 14, pg. 580-581, A.5(b)
Coronaviruses	
• *MERS-CoV*	Middle east respiratory syndrome (upper and lower respiratory tract infection)
• *SARS-CoV*	• Severe acute respiratory syndrome (SARS). Outbreak started in 2002 in South China to involve several countries; including India
• *SARS-CoV-2*	Covid-19 (details see A2b, p. 804-805)
• *Eastern equine encephalitis virus*	Encephalitis • see Chapter 2, pg. 194 (Section 3)
• *Western equine encephalitis virus*	Encephalitis • see Chapter 2, pg. 194 (Section 3)
• *Chikungunya virus*	• Chickungunya (severe joint pain,fever, lyphadenopathy, conjunctivitis and rash), details see Chapter 13, A.7(iii), pg. 577-578
• *Phelbovirus*	Rift valley fever, Sandfly fever
• *Nairovirus*	Crimean-Congo haemorrhagic fever
• *Hantavirus*	Hantaan and others
• *Bunyavirus*	Chittor, California encephalitis
• *Human Immunodeficiency virus type 1 (HIV -1 virus)*	• Acquired immunodeficiency syndrome (AIDS) Details see Chapter 15, A.3(d), A.7, A.9(c), pg. 588, 590
• *Human Immunodeficiency virus type 2*	Acquired immunodeficiency syndrome (often milder type)
• *Human T cell lymphotropic virus type 1 (HTLV-I)*	Adult T cell leukemia and Spastic tropical paraparesis
• *Human T cell lymphotropic virus type II (HTLV-II)*	Role not clear
• ∆*Arenaviruses as:* *Lymphocytic Choriomeningitis virus,* *Lassa virus* *Junin virus* *Machupo virus*	 Occasionally influenza like illness and aseptic meningitis (Lymphocytic choriomeningitis) Lassa fever (haemorrhagic fever) Argentine haemorrhagic fever Bolivian hoaemorrhagic fever
• *Hepatitis D virus*	Hepatitis • Details see Chapter 16, pg. 597-598, A1,4
• *Prions**	Transmissible degenerative (spongiform) encephalopathies Details see Chapter 17, pg. 599-601, A2a.

* Is not a RNA virus, but proteinaceous particle.

∆ These viruses are likely to have jumped from rodents to man, as a result of increased agricultural ventures that brought infected rodents in contact with man

⌂ Termed so, as first described by German and entity believed to be a variant of measles.

Integrated Clinical Case Based Study of Influenza Virus/'Avian Flu'

3

In January 2008 in West Bengal (India), an outbreak occurred in poultry (hen and ducks), which resulted in over 1 lakh fowl deaths. To control the outbreak, over 10 lakh hens and ducks were culled (killed), by over 900 teams, that were sent by the Government of India in the same month. The livelihood of thousands of families got affected, who depended on 'backyard' poultry. Prices of chicken came crashing down to that below of vegetables and people stopped having their favorite chicken dish (including eggs) in restaurants and at home.

In January 2021, an outbreak of Avian Influenza was again reported affecting twelve states. It affected the poultry, crow, migratory and wild birds.

Linkages: Pg. 459, 460, 531 and 602

What was the virus that was implicated in the above outbreak?

A.1 (a) It was an avian subtype of Influenza virus.

How is it designated?

A.1 (b) It is designated as H5N1, which is a subtype of influenza A virus. 'H' stands for haemagglutinin and 'N' stands for neuraminidase. The numbers represent the subtypes for the two surface proteins.

Where was the diagnosis of the 'avian' influenza outbreak confirmed?

A.1 (c) The specimens from the infected birds were sent to high security animal disease laboratories (HSADL) in Bhopal and National Institutes of Virology in Pune, where laboratory confirmation of this disease occurred.

Why do only few labs perform such confirmatory tests?

A.1 (d) The laboratory should have a high biosecurity level (BSL); as exists in the above two labs, so that the laboratory personnel don't get infected with exotic viral agents and the local environment also doesn't get contaminated with it. The (HSADL) lab at Bhopal has BSL 4 facility.

What is a common differential diagnosis of this disease?

A.1 (e) Ranikhet disease

Why was there so much concern about the 'avian' influenza outbreak?

A.2 It was due to the following reasons:

(i) Economic loss to people

(ii) Ecological imbalance with loss of so many birds

(iii) Spread of the disease to neighbouring states and countries and possible emergence of a pandemic (if reassortment of genes between epidemic human strain and lethal avian strain occured)

(iv) Spread to human from infected birds i.e., animal to human transmission documented

(v) Possibility of high case fatality rate in man, as man may not have protective (antibodies) immunity against this virus.

(vi) Possible spread of infection from human to human, if the virus undergoes reassortment of genes and/or has mutation.

(vii) No human vaccine is in common usage.

What was the reason for culling of these birds?

A.3 (a) The infected birds represented the animal reservoir for this virus.* Culling of the birds would reduce the risk of spread of the virus to other birds and humans. This would also prevent the emergence of a new virus by reassortment and/or mutation.

*culling means a deliberate killing of animal

What technique was employed to cull the infected birds?

A.3 (b) The neck of the bird was pulled and then twisted to silently kill the bird. The process resulted in a deliberate dislocation of the cervical vertebral column of the animal; resulting in the destruction of its spinal cord.

What was the advantages of this technique?

A.3 (c) The advantage of this technique was that no blood spill occurred; which minimized the risk of viral contamination.

What were the culling teams equipped with?

A.4 Each member of the culling team was equipped with a personnel protective equipment kit, which consisted of N95 mask (filters particles larger than 0.3 micrometer with efficiency of 95%), gloves and oseltamivir (tamiflu) tablets.

How were the culled birds disposed?

A.5 (a) The culled birds were put in a pit, which was at least nine feet deep. It was then covered with lime and sodium hypochlorite, which was then covered with a thick layer of earth.

What was the importance of following this technique?

A.5 (b) The importance of this technique was that the virions of the killed birds are destroyed and their spread was minimized.

How were the local people motivated to hand over their live birds; which were the source of livelihood, to the government personnel for culling?

A.6 (a) In camps set up near the villages, the local people were encashed of the compensation slips, which were given in exchange for the hen. For a hen producing egg, the compensation slip was Indian Rupees 40.

What advise was given to poultry workers on sick farms?

A.6 (b) They were advised to wear gown, face-mask and goggles for protection. The poultry workers could wear shoe covers, so that shoes did not carry the virus.

How can such 'outbreak' scenario be prevented in the future?

A.7 (a) The susceptible birds can be vaccinated against this virus. This wouldn't result in the elimination of this virus from the birds but would prevent new birds from this disease, by the induced protective antibodies. There has to be an effective surveillance that should result in improved communication network; between the villages, the veterinary hospitals and laboratories, so that an outbreak can be nipped at the early stage, before it becomes a big problem. This approach has been followed in China, Hongkong and other places.

What is the difference between quarantine and isolation?

A.7 (b) Quarantine is enforced to separate exposed individuals from healthy individuals whereas isolation is enforced to separate diseased (sick) individuals from healthy individuals.

What is the I.P. of 'avian' influenza?

A.8 1-5 days

How do you define a probable human case of 'avian' influenza (H5N1) infection?

A.9 A possible case of influenza has limited laboratory evidence of influenza A/H5 infection (positive laboratory confirmation of influenza A infection but insufficient evidence of H5N1) or no evidence of another cause of disease.

What advice would you give to international traveler visiting a place having an 'avian' influenza outbreak?

A.10 The traveller should avoid poultry farms and contact with animals in live food market; including surfaces that are contaminated with faeces of poultry or other animals. The advice is also not eat half-boiled egg and undercooked chicken.

Laboratory Diagnosis and Treatment (Overview)

18

Virus/ Syndrome/ Approach	Specimens	Direct Demonstration Of Viral Antigen/ Genome/Particle In Clinical Specimen/ Animal Inoculation/Chicken Egg Inoculation	Viral cell line	Growth/ Confirmation	Serological Tests	
					Type	Interpretation
• **Rotavirus**	• Faeces • Serum	• Detected (antigen) by latex agglutination test, RPHA, ELISA/ Polyacrylamide gel electrophoresis analysis of viral RNA permits, discrimination between strains • During acute stage about 10^{11} virus particles per ml are present in faeces, can be detected by E/M I.E.M • Genotyping of RNA by PCR	• Primary monkey kidney cell lines as MA	• CPE is not characteristic	• Serum antibody can be measured by EIA, RIA, HI or CF	• Not refined to the extent to be used in routine diagnosis
• **Influenza viruses A, B, C**	• Nasal / throat washing • Swab of nasopharynx/throat • Sputum • Lung (at autopsy) is collected using suitable buffered salt solution. Specimen should be processed immediately. If short delay is expected, store at 4°C. If longer delay is expected store at 70°C. The specimen is treated with antibiotics to destroy bacteria	*Direct fluorescent test* can demonstrate antigen in epithelial cells within 2 hours. In nasal wash specimen,both IFA and EIA are rapid techniques • Chick embryo can be inoculated by intramniotic or intraallantoic routes (this technique was standard about 3 decades back, nowadays chick embryo essentially used for vaccine production, though in some cases, it may have better sensitivity) • After 2-3 days of incubation viral haemagglutination can be demonstrated in the fluid • Further typing and subtyping of influenza isolates • RT-PCR technique (rapid, sensitive and specific)	• Primary monkey kidney • Human embryo kidney cells • MDCK (Madin- Darby canine kidney continuous cell line) Note: culture should have trypsin to split neuraminidase	• Growth of virus at 33°C to 35°C in cultured cells is recognized by haemoadsorption of guinea pig red blood cells, after few days (3-7) • Isolate is then identified by haemagglutination inhibition • Some strains of influenza A can agglutinate only guinea pig RBC's, while influenza B can agglutinate both guinea pig & fowl RBC's. Influenza C can agglutinate only fowl RBC at 4°C. Fresh isolates of influenza fail to produce CPE in monkey kidney cell culture.	• Complement fixation, haemagglutination inhibition and Neutralization test can be used Novel subtypes arising by antigenic shift may have novel neuraminidase as well as new haemagglutinin, so the isolate should be tested to detect major & minor changes, arising by antigenic 'shift' or drift, using reference antisera against purified HA and NA.	• Four fold or higher rise of antibody titre between acute and convalescent phase sera has to be demonstrated (use in epidemiological studies) The problem of 'original sin' complicates the identification of strain-specific antibodies (where the key antibodies elicited by the current strain of virus are against the first strain of influenza virus, one experienced years earlier and represent anamnestic response)
• **Parainfluenza viruses 1-4**	• Mouth washing • Throat swab (esp. posterior pharynx) • Exfoliated cells, aspirated from respiratory tract • Respiratory mucus (must be adequately solubilized for elaborating antigen for ELISA)	• Immunofluorescent staining of exfoliated cells from respiratory tract • Detection of antigen in mucus by ELISA or RIA • NAATs are preferred method of diagnosis	• Primary monkey kidney or human kidney	• Inoculated cells incubated at 33-36°C (Roller apparatus gives better result) • Little CPE, except by Parainfluenza type 2, which induce syncytium formation • Viral growth is detected by haemadsorption of guinea pig RBC's (4 and 25°C) • Differentiation from other haemadsorbing respiratory viruses is done by fluorescent antibody staining or by haemagglutination inhibition, using virus from cell culture supernatant	• ELISA, CFT test (in paired sera)	• Demonstrate rise in titre

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• **Measles (Rubeola virus)/** Most cases are diagnosed clinically, laboratory diagnosis may be necessary in cases of atypical measles	• Respiratory secretion • Serum • Urine • Conjunctival swab	• Viral antigen can be demonstrated in epithelial cells, secretions, conjunctiva and urine, by using fluorescent staining, monoclonal antibody • Giemsa staining of sample will show Cowdry type A inclusion body and giant cells (Warthin-Finkeldey cells)	• Monkey/human kidney cell line	• Multinucleate giant cells produced in 7-10 days (both intranuclear and intracytoplasmic inclusion bodies) • Can confirm with fluorescent monoclonal antibody or haemadsorption with chick RBC	• CFT, HI, NT, IgM capture ELISA	• Rising titre or measles-specific IgM antibody is significant
• **SSPE** (Also see A1.d, p. 549 and A.3c, p. 550)	• Serum • CSF • Brain biopsy	• *Fluorescent antibody test* on neural tissue	• Monkey kidney or other susceptible cell (isolation from a brain of patient is difficult)	• Co-cultivation technique used - layer affected brain cells onto the monkey kidney cell line	• CFT in serum; especially CSF	• High titre of antibodies especially in CSF, suggest that these antibodies are produced in brain and have not crossed blood-brain barrier
• **Mumps/** Atypical cases and presentation; as meningo-encephalitis require laboratory help (Also see A9. p. 551)	• Saliva (or swab from orifice of Stensen's duct) • Throat secretion • Urine • CSF	• *Immunofluorescence test* can demonstrate viral antigen	• Monkey kidney or HEp2 line	• becomes +ve in 3-5 days, identified by cell line haemadsorption	• Traditionally CFT (using soluble(s) and viral (v) antigens –IgM ELISA HI (Used to monitor immune status in vaccination studies	• Detection of mumps specific IgM in serum drawn early in infection suggests recent infection
• **Respiratory syncytial virus/** Typically patient is 1-3 month infant with respiratory symptoms, who may progress quickly to cyanosis	• Nasal secretion, Pharyngeal secretion (nasopharyngeal aspirate). Virus is extremely fragile, so sample should be added without delay and freezing (some even recommend bed side inoculation)	• *Immunofluorescence* test with conjugated monoclonal antibodies can give result in less than 1 hour (Direct & Indirect)	• Hela, HEp2, monkey cell cultures	• Growth occurs in 5-15 days with development of giant cells and syncytia. • Monoclonal antibodies can detect CPE earlier • Absence of haemadsorption distinguishes RSV from all other paramyxoviruses • Immunofluorescence test/RT-PCR can also identify definitively	• CFT • Neutralization test	• Test not very useful, as babies have poor immune response
• **Rabies/** *In Animals* - Commonly required to know, if the animal known to have inflicted the bite is rabid *In Man* - Antemortem Postmortem	• Animal sacrificed and hip-pocampus, brain stem or cerebellum region can be processed • If can examine within 2 days, can refrigerate (If longer delay, can transport on dry ice or in 50% glycerol saline) • Corneal smear • Corneal biopsy • Saliva • Skin biopsy from (a) nape of neck (b) bite site • CSF _Serum (to assess response to vaccination) • Salivary glands • Hippocampus • Brain stem • Cerebellum	E/M can demonstrate viral particle/ • In skin biopsy, corneal smear or saliva can demonstrate genomic RNA or viral mRNA by PCR or dot- blot hybridization assay (with 32P labelled nucleic acid probes) • Giemsa's stain or Sellers technique can demonstrate inclusion body (Negri body may be absent in 20% of patients)/ Mouse pathogenicity test- intracerebral inoculation of sample into suckling mice, observe for 28 days	• Vero monkey kidney • BHK (Baby hamster kidney) • Mouse neuro-blastoma	• No CPE occurs • After 18-24 hrs of inoculation, presence in cell line can be demonstrated by fluorescent antibody test	• In serum and CSF, specific antibodies can be demonstrated by ELISA	• These occur usually late in disease, not relevant. Vaccination can cause confusion (vaccinated individuals have low titres of < 1:64 of neutralizing antibodies)
• **Marburg & Ebola/** Filoviruses are classified as Biosafety Level 4 pathogens, so utmost care in isolation. Prompt notification of public health authorities is mandatory for suspected cases, before any diagnostic attempts are made	• Serum • Blood • Autopsy specimens e.g. from liver Samples must be refrigerated & packed according to IATA regulation to world's few containment facilities	• Virions can be demonstrated in blood, liver, lungs by E/M & staining fluorescent antibody (following precautionary irradiation)	• Vero cell cultures Virus culture must be attempted only in maximum security laboratories	-	• Antibody by immunofluorescence & RIA	• Antibody are demons-trable 7-10 days, after infection (is an exotic antigen)

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• **Lymphocytic choriomeningitis virus**/ Diagnosis is suggested by history of rodent contact No person to person transmission of infection has been documented	• Serum • Blood • C.S.F.	• Intracerebral inoculation of Blood/CSF to weaning (1 month old mice or young guinea pig)	-	-	• Rising titre of serum antibodies by indirect IF, C.F.T, Nt tests	
• **Calciviruses**	• Faeces • Serum	• Viral antigen detected by ELISA/ by Immunoelectron microscopy viral particle can be demonstrated		-	• IgG and IgM specific antibodies by ELISA	
• **Hepatitis E**/ Serologic & viral antigen demonstration approach is resorted to (previously exclusion approach)	• Faeces • Serum	• Viral antigen in stool/ • RT. PCR can demonstrate viral genome/ Viral concentration in stool is usually low & is present only during first week, after onset of jaundice *Immune electron microscopy* can demonstrate aggregated Calicivirus like particles using Monoclonal antibodies	• Virus not cultivable	-	IgG - anti HEV, IgM - anti HEV IgG and IgM antibodies by ELISA and western blot assay	
• **Rubella (German measles)**/ Indications for diagnosis 1. A woman considering vaccination wants to know her immune status 2. An unimmunized woman in 1st trimester of pregnancy develops rash, want to know, if she hascontracted disease & should have an abortion 3. A baby is born with signs suggestive of rubella syndrome (in latter two conditions culture can have role)	• Throat swab • Serum • Cord blood • Amniotic fluid in pregnant woman by amniocentesis *Infants with CRS* • Throat swab • Urine in new born • C.S.F • Leucocytes	• Molecular typing (can identify virus genotype in throat swab)	• Vero • Rabbit kidney (RK- 13, SIRC)	• CPE is inconspicuous • Infection detected by interference technique in which inoculated monkey kidney cells are challenged by Coxsackie A • Culture not used routinely, as is expensive, tedious & may require as long as 2 weeks for demonstrable effect	• Traditionally haemagglutination inhibition (H.I.) was the test & gold standard • IgG & IgM specific antibodies by ELISA	• Presence indicate immunity (as single serotype) For confirming primary rubella, 4 fold rise to be demonstrated between acute & convalescent sera Becomes +ve 1 week after appearance of rash & can remain positive for few months
• **HIV I & II (AIDS)**/diagnosis essentially serologic initial screening tests ELISA/ rapid ICT based (Immunochromatographic based tests common) *Confirmation* of result done by repeating the serological test of different type Bacterial, Viral, Parasitic, Fungal infection & malignancies may need to be identified Other non-specific indicators are: • Lymphopenia: • Lowering of CD4 : • CD8 ratio (even reversal): • Hypergammaglobulinemia: • Deficient C.M.I • Diagnosis in new born (p.592)	• Serum • Blood • Other specimens according to opportunistic infections • Dried blood spots for infants (6 weeks to 6 months)	1. *p24 levels* (can be detected in serum by ELISA in 30% patients during 'window' pe- riod i.e., have role before specific antibodies are detectable. Levels can also help in monitoring ART. 2. P.C.R role in: (a) Early infection (before antibodies appear) (b) Retesting viral load, so monitoring treatment (by RT-PCR) (c) In diagnosing babies borne toinfected mothers (d) In diagnosing double infection (e) Detecting sequence variabilities of HIV genome (by gene sequencing)	• Culture a research tool Lymphocyte culture co-cultivation done: Patient's peripheral lymphocytes inoculated in presence of mitogens; as PHA & interleukin-2 (T cell growth factor), can be isolated from all the stages & numerous specimens; as bone-marrow, lymph node etc.	Growth identified by: (i) RT activity (ii) E/M to detect viral particle (iii) p24 in supernatant (iv) Indirect immunofluoresence, using specific monoclonal antibodies to detect HIV proteins	• HIV1 & 2 detected with separate tests (combined kits also available) Negative when patient in 'window periods' *Screening tests:* ELISA : *Rapid test:* (i) Latex agglutination test (ii) Dot blot (ICT based test): *Confirmatory tests* repeat test of different type, Western blot (antibodies to specific viral proteins detected) Appropriate HIV testing strategy to be applied Details: p. 593	-Highly sensitive & specific Require expensive equipment & about 4 hrs. - Time required is less than 30 mins. Easy interpretation More expensive -Antibodies to: core protein: reverse transcriptase: surface antigen, detected (sometimes test is ambiguous, then designated as indeterminate & then repeated after 6 months)

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• **Poliomyelitis**	• Faeces • Throat swab • C.S.F.(Difficult to isolate from it) • Serum Laboratory diagnosis is important, unless epidemiologic support exists It is important to differentiate wild virulent strain from attenuated vaccine strain	• Nucleic acid hybridization to differentiate from vaccine strain (as nucleic acid sequences of both strains are known) • Direct electron microscopy/Immune electron microscopy can demonstrate viral particle/ • Intraspinal inoculation of monkey was done to differentiate wild strain from vaccine strains (develop typical sign & symptoms)	• Any human/simian cell line	- C.P.E develops within a few days, early changes include cell retraction, increased refractility, cytoplasmic granularity & nuclear pyknosis - Identification of serotype is made with antisera - Neutralization tests with pooled and antisera of 3 types	• Neutralizing antibody • C.F.T	• Paired sera are required for interpretation
• **Coxsackie** group A-23 serotype group B - 6 serotype	• Faeces (isolation from it should be interpreted cautiously, as asymptomatic shedding can persist) • Throat swab • C.S.F.	Intracranial inoculation into suckling mice Pathological changes in suckling mice *Group A*: - flaccid paralysis; - Generalized myositis; - Death within a week *Group B*: - spastic paralysis; - focal myositis; - localized lesions within liver, pancreas & other organs	• Human diploid embyonic lung fibroblast, human rhabdomyosarcoma cell line	• C.P.E resemble those of polioviruses but develop slowly • Identification involve, several pools of reference sera	• Not much role	
• **HAV**	• Serum/Blood • Faeces	• IEM (virion in faecal extract)/RT-PCR for viral RNA	• Can cultivate on human cell line (research)	-	• IgM HAV antibody demonstration (mainstay)	-
• **HCV**	• Serum • Liver biopsy	• HCV core antigen assay/RT-PCR for HCV RNA (can confirm, quantity and genotype)	-	-	• ELISA (for screening) • RIBA for confirmation	-
• **Coronavirus SARS-CoV-2 (COVID-19)**	• Nasopharyngeal/nasal/ swabs & aspirate • Throat swab • Oropharyngeal swab • Lower respiratory tract specimens (if patient undergoing invasive procedure) • Serum	• Detection of viral RNA by NAATs is the recommended test. • Rapid antigen testing - lateral flow immunochromatographic assay based on presence of a colloid gold conjugate pad and a membrane strip pre-coated with SARS-CoV-2 antibodies, less recommended due to high false negative rate.	Not yet cultivated	-	• **Point-of-care** (POC) **tests** generally are lateral flow devices that detect IgG and IgM, or total antibody in serum, plasma, whole blood, and/or saliva. • Laboratory tests use ELISA (enzyme-linked immunosorbent assay) or CLIA (chemiluminescent immunoassay) methods	• Play an important role in understanding the viral epidemiology in the general population and identifying groups at higher risk for infection. Serological studies can determine different aspects of immune response and functionality of antibodies against SARS-Cov-2.

Assessment/Examination Questions

19

Chapter 2a

1.	Classify viruses which cause gastroenteritis. To which family does Human Rotavirus belong? How has the name of the family been derived?	A3a., p. 215, A4a, b., p. 533
2.	Describe the morphology of the Rotavirus. Describe the epidemiology of rotavirus diarrohea.	A 4c., p. 533, A 5a., p. 534
3.	Describe the pathogenesis of rotavirus diarrohea.	A 6., p. 534
4.	What approach is commonly made to demonstrate this etiological agent? Describe the laboratory diagnosis of rotavirus diarrohea.	A 3b-e., p. 533, p. 602

Chapter 2b

1.	Mention key historical features in relation to Influenza. Compare and contrast the features of the Orthomyxoviruses and Paramyxoviruses.	A 4c., p. 537, A 4b., p. 537
2.	Illustrate the structure of the Influenza virus (in a figure). Briefly describe the structure of Influenza virus and tabulate the differences between haemagglutinin and neuraminidase.	A 5b., p. 538-539
3.	Describe the types and subtypes of Influenza virus.	A 4d., p. 537-538
4.	What are the key virulent factors of Influenza virus? Describe the pathogenesis of Influenza.	A 4a., p. 537, A 3b., p. 536-537
5.	Describe the epidemiology of Influenza. Describe the emergence of antigenic subtypes of Influenza A in the last century.	A 6b, c., p. 539-540
6.	Explain the emergence of antigenic drift and shift in a community using a hypothetical viral structure. Tabulate the differences between antigenic drift and shift.	A 6d., p. 540, A 6a., p. 539
7.	Describe the laboratory diagnosis of Influenza.	Pg. 602, P. 536
8.	Describe vaccines used for prevention of Influenza.	A 8a-c., p. 541, pg. 823-824
9.	Enumerate key preventive strategies with reference to Influenza. What is the basis of the hypothesis that has led to the recommendation of keeping pigs, birds and human separately as for as possible?	A 9a,b., p. 541

Chapter 3

1.	Describe ‘avian’ (Bird flu) Influenza.	P. 542-543
2.	During the recent outbreak of ‘avian influenza’, why was there so much scare?	A 2., p. 542
3.	Why were the birds ‘culled’ (killed) during the outbreak?	A 3a., p. 542
4.	How can such ‘outbreak’ scenario be prevented in the future?	A 7a., p. 543

Chapter 4

1.	Describe ‘Swine flu’.	P. 544, 545
2.	What is ‘swine flu’ in pigs? Does ‘swine Influenza virus’, normally cause human infection?	A 1, A2., p. 544
3.	What was the likely origin of the ‘swine influenza strain’ that was involved in the outbreak in 2009?	A 3., p. 544
4.	Why was there a scare during the ‘swine flu’ outbreak progression?	A4., p. 544
5.	How is a confirmed case of ‘swine flu’ infection defined?	A 5., p. 544
6.	Is it safe to eat pork that is likely to be infected with this virus?	A 10., p. 545

Chapter 5

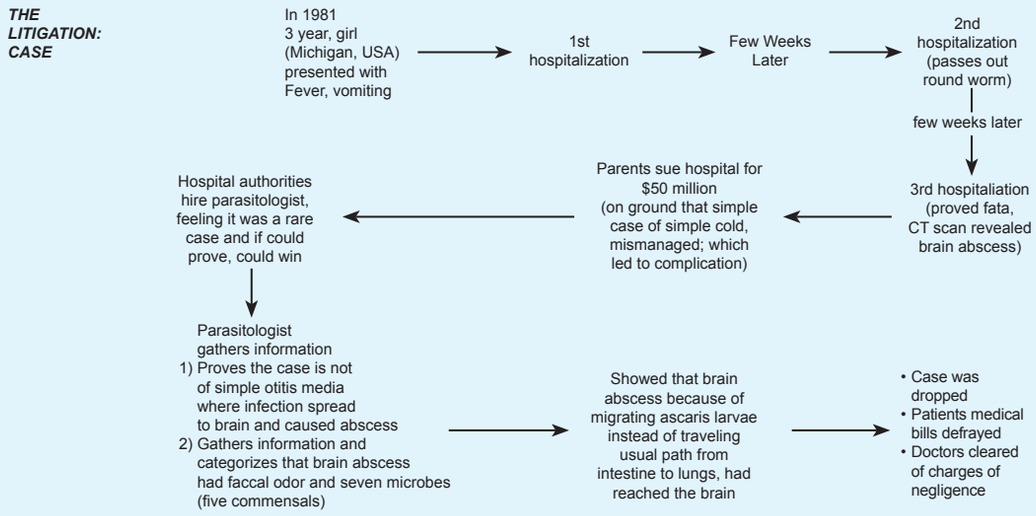
1.	Classify Paramyxoviruses. Describe morphology of Paramyxoviruses.	A 3c., p. 546, A3b., p. 546 and p. 459 (A7a)
2.	Write briefly on Parainfluenza viruses.	A 3c., p. 546

Section XIV: Diseases Caused by Protozoans (Protozoology)

Introduction to Parasitology

1

"We humans are the greatest of earth's parasites" ***—Martin H. Fisher***



Adapted from August 1991 issue of Medical Economics

What is the 'take home' message of the 'Litigation case'?

A.1 (i) There has to be a heightened awareness of parasitic diseases, as they constitute as a differential diagnosis to innumerable infectious as well as non-infectious diseases.

For a clinican to be able to consider them in the differential diagnosis, the intricate life cycles and pathogenicity of various parasites must be understandable to them. Students often ponder over the reason to learn the details of complex life cycles of parasites, of the day of migration etc.

(ii) Parasitic disease can occur even in areas, where the disease are not expected to be present because of the heightened levels of travel and increased populations having immunocompromised status.

What is the approximate global estimate of common parasitic infections?

A.2

- Amoebiasis - 10% of world's polulation
- Giardiasis - 200 million cases
- Trichomoniasis - 200 million cases
- Malaria - 300 to 400 million
- Toxoplasmosis - 1/3 of world's population
- Hoskworm infection - 576 million cases
- Ascariasis - 807 million cases

What is the definition of Parasite?

A.3 **(a)** It is defined; as an organism, which lives in or another organism (host) and derives its nutrition from it.

Medical Parasitology deals with the study of parasites, which infect and produce disease in man.

Do Bacteria, viruses and fungi also don't depend on another organism for nutrition? If so, why are they not categorized as parasites?

A.3 (b) Bacteria, viruses and fungi also do depend on another organism for nutrition.

By definition; they could also get categorized as parasites, but conventionally only protozoans and helminthes are categorized as Parasites.

What are the common parasitic problems in India?

A4. Table 14.1.1: Parasitic problems of India

DISEASE (PARASITE)	SUB – GROUP	GROUP
• Amoebiasis (*E. histolytica*)	• Sarodina (Presence of Pseudopodia)	Protozoa
• Giardiasis (*G. lamblia*) • Trichomoniasis (*T. vaginalis*) • Leishmaniasis (*L. donovani* complex)	• Mastigophora (Presence of flagella)	
• Malaria (Plasmodium spp.) • Cryptoporidiosis (Crypstosporidium spp.) • Toxoplasmosis (*T. gondii*)	• Apicomplexa/ Sporozoan(Presence of microtubule complex)	
• Balantidiasis (*Neobalantidium coli*)	• Ciliophora (Presence of cilia)	
• Taeniasis (Taenia spp.)-Fig.14.1.1 • Cysticercosis (*T. solium*) • Hydatid cyst (Echinococcous spp.)	• Cestodes	Helminths
• Fasciolopsis reported from limited regions (*F. buski*) • Heterophyiasis reports from limited regions (*H. Heterophyes*) • Paragonimiasis Reports from N.E. India (*P. westermani*)	• Trematodes	
• Hookworm infestation –Fig.14.1.2(*A. lumbricoides*, *N. americanus*) • Strongylodiasis (*S. stercoralis*) • Ascariasis Fig.14.1.3(*A. lumbricoides*) • Filariasis –Fig.14.1.4(*W. bancrofti, B. malayi*) • Trichinellosis (*T. spiralis*)	• Nematodes	

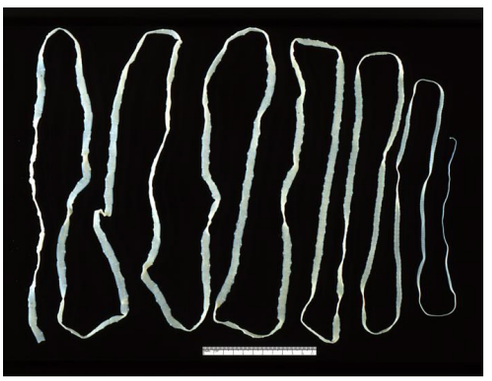


Fig:14.1.1: Photograph depicts adult *Taenia saginata* approximately 4mts. long.

Courtesy: CDC

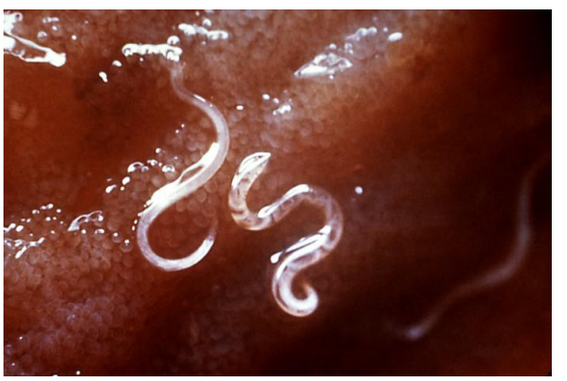


Fig:14.1.2: Photograph depicts presence of hookworms, atop the intestinal villi.

Courtesy: CDC

Compare and contrast Ectoparasitc and Endoparasite.

A.5 (a) Ectoparasite lives on the outside surface of the body of host e.g.: *Sarcoptes scabei*; which causes scabies.

Endoparasite lives inside the body of the host e.g., Leishmania in the reticuloendothelial system.

Fig.14.1.3: The Laboratory technician holds a mass of round worms passed by a child in kenya.
Courtesy: CDC

Fig.14.1.4: Man with highly enlarged scrotum (pendulous nature) due to filariasis
Courtesy: Dr. William R. Smart/CDC

Compare and contrast obligate and facultative parasite.

A.5 (b) Obligate parasite is one which cannot exist without it's host e.g., Malarial parasite (Plasmodium spp.). This can be contrasted with a Facultative parasite, which can exist with or without it's host, depending on the condition as *Naegleria fowleri*.

Compare and contrast Accidental and Aberrant parasite.

A.5 (c) Accidental parasite is one which infects an unusual host e.g., Dog tapeworm (*E.granulosus*) infects man accidentally. Aberrant parasite is one which infects a host, where it cannot live or develop further e.g., ascarid roundworm of dog (*Toxocara canis*) which infects man

Parasites can cause a spectrum of illness in host, which can be categorized as (i) Asymptomatic carrier state, (ii) varying grades of morbidity and (iii) Lastly mortality in the host.Which of the three states would be desirable from the parasite's and host`s perspective? Justify.

A.6 The asymptomatic carrier state would be desirable to both to the host and parasite, as the goal of the parasite is to proliferate maximally and the host should not be adversely affected. This is only possible in this state.

If the parasite cause some degree of morbidity in the host, the host immune response would be elicited, which would inhibit the proliferation ability of the parasite. If the parasite kills the host, obviously the parasite has to then find a new host.

Elucidate the concept of definitive and Intermediate host.

A.7 (a) Some Protozoans and many helminths require more than one host to complete the life cycle. In such a scenario; definitive host is one, which harbors the adult or sexual stage of the parasite. Intermediate host is one in which the larval stage or the asexual form exists.

The hosts can be vertebrate or invertebrate. The commonest examples of invertebrate host are arthropod vectors; as mosquito and sand fly.

To which host category, does man belong to in most of the parasitic infections?

A.7 (b) In majority of the parasitic infections, man is a definitive host.

Enumerate the parasites in which man is an intermediate host?

A.8 (i) Plasmodium spp., (ii) *Toxoplasma gondii*, (iii) *Taenia solium* (occasionally), (iv) *Echinococcus granulosus*, (v) *E. multilocularis*, (vi) *E. vogeli*, (vii) *Trichinella spiralis*, (viii) *Spirometra mansoni* (a cestode), (ix) *Taenia multiceps*.

How do you classify common parasites existing (in India) on the basis of sign/symptoms/syndrome and organ system involvement?

A.9 **Table 14.1.2:** Parasitic classification according to system

Central Nervous System

Sign/Symptoms/Syndrome	Parasite
Altered consciousness, seizure, coma	*P. falicparum*
Pyogenic meningitis	Naegleria spp.
Seizure, space occupying lesion	Toxoplasma spp., *T. solium*, Acanthamoeba spp, *Balamuthia mandrillaris*.

Eye

Sign/Symptoms/Syndrome	Parasite
Visual loss	Toxoplasma spp.
Retinal mass	Toxocara spp.
Painful corneal ulcer	Acanthamoeba spp.

Lungs

Sign/Symptoms/Syndrome	Parasite
Pulmonary abscess	Paragonimus spp.
Loeffler's Syndrome (Cough, transient infiltrates, eosinophilia)	Migrating larvae of Ascaris, Hookworm and Strongyloides.
Pulmonary oedema and adult respiratory distress syndrome (ARDS)	*P. falciparum*

Blood

Sign/Symptoms/Syndrome	Parasite
Red blood cells	Plasmodium spp., Babesia spp.
White blood cells	Leishmania spp., Toxoplasma spp.
Whole blood/plasma	Trypanosomes, Microfilaria

Gastrointestinal Tract

Sign/Symptoms/Syndrome	Parasite
Hepatomegaly	*E. histolytica*, Echinococcus spp.
Hepatosplenomegaly	*L. donovani* complex, Malaria (multiple episodes)
Chronic splenomegaly	Leishmania, Malaria and Schistosoma
Cholangitis	Cryptosporidum spp., *Clonorchis sinensis*
Dysentry (bloody diarrhea)	*E. histolytica, B. coli*
Diarrhea (Watery)	*G. lamblia*, Cryptospordium spp, *Isospora belli*, *D. latum, F. buski*
Passage of big worm	Ascaris
Passage of small segments	*T. solium/ saginata*

Genitourinary System

Sign/Symptoms/Syndrome	Parasite
Discharge with itching	*Trichomonas vaginalis*
Haematuria	Schistosoma haematobium

Muscular

Sign/Symptoms/Syndrome	Parasite
Myalgia, Myositis	Trichinella spp.

Skin

Sign/Symptoms/Syndrome	Parasite
Ulcer (painless)	Leishmania
Rash	Hookworm, Strongyloides, Toxocara, Onchocerca

Is there any parasitic disease which has been eradicated from India?

A.10 Dracunculiasis (guinea worm disease) has been eradicated from India and it has been declared as a guinea worm free country by WHO in 2000.A unique form of the worm removal was practiced for it(Fig: 14.1.5)

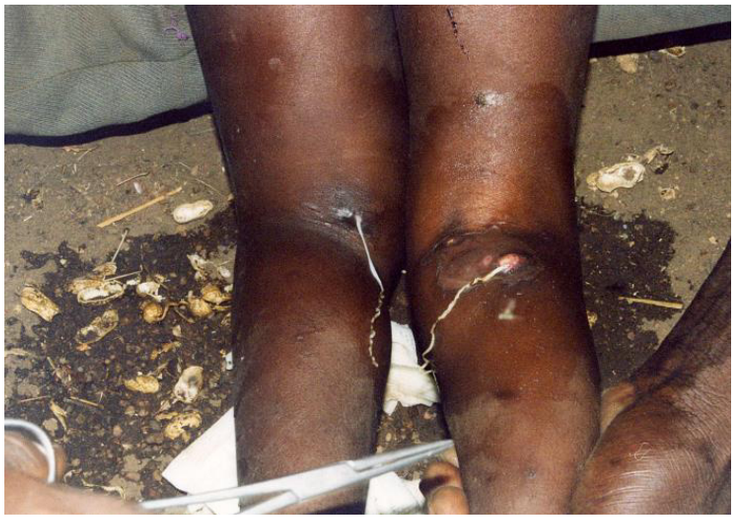


Fig. 14.1.5: Image depicts emergence of two female Guinea worms. These worms were pulled by a health care worker. (The worm can be pulled out only a few centimeters each day taking many weeks for the process to complete)

Courtesy: The Carter Center/CDC

nb: – For list of parasites associated with immunocompromised individuals as AIDS, see pg. 590.

– For list of zoonotic parasitic diseases see pg. 241.

– *S. haematobium* and *C. sinensis* are associated with bladder carcinoma and cholangiocarcinoma;respectively.

Introduction to Protozoology and an Overview of the Morphological Profile of Key Protozoans

2

'Perhaps the ghastliest disease endemic to mosquitia is Mucocutaneous Leishmaniasis, sometimes called *white leprosy*'.
—*Douglas Preston*

Compare and contrast the general characteristics of protozoal and helminthic infections.

A.1

Table 14.2.1: General characteristics of Parasites

	Protozoal	***Helminthic***
• Structure	Unicellular (microscopic)	Multicellular (macroscopic)
• Site of residence	Intracellular or extracellular	Extracellular (as size is large)
• Replication type	Mostly asexual, without passing to exterior	Replicate sexually, may pass outside human host
• Replication site	Essentially replicate within human host and sometimes in arthropod vectors also	Replicate in man and intermediate host (one or multiple)
• Number of animal hosts (to complete life cycle)	One (except Toxoplasma)	Multiple (except in Strongyloides)
• Number of exposures	Single exposure can result in severe (overwhelming) infection (so traveler's can have such presentation)	Multiple exposures required for severe infection (so travellers with few exposure unlikely to have such presentation
• Risk of disseminated infection in immunocompromised cases	Exists (Cryptosporidium, Leishmania, *Trypanosoma cruzi* and Toxoplasma)	Only; Strongyloides
• Availability of vaccine	None (except one Pre-erythrocytic falci-parum malaria vaccine in phase IV trial)	None

How can Protozoa be classified?

A.2 In the traditional 1980s classification,protozoa are classified on the basis of the locomotion method. There are four basic groups

Table 14.2.2: Basic Protozoal groups

	Group	**Criteria**	**Example**
1	Sarcodina	Presence of Pseudopodia	• *E.histolytica* • Free living amoebae
2	Mastigophora	Presence of flagella	Intestinal : *G.lamblia* Oval/vaginal : *Trichomonas vaginalis* Blood : Leishmania, Trypanosomes
3	Apicomplexa (also referred as Sporozoan)	Apical microtubule complex presence and involves alternation of sexual and asexual phase and involving two hosts	Blood : Plasmodium Tissue : Toxoplasma Intestine : Cryptosporidium, Cystoisospora, Cyclospora, Sarcocystis
4	Cilophora	Presence of cilia	Balantidium

The current 2000s classification is based on ribonucleic acid and protein sequences of the protozoans. This classification has permitted to arrange these parasites within categories on evolutionary distances (Table 14.2.3).

Table 14.2.3: Molecular classification (2000) of Protozoa infecting humans

Kingdom	Subkingdom	Phylum	Class	Order	Genus
Protozoa	Archezoa	Metamonada	Trepomonadea Retortamonadea Trichomonadea	Diplomonadida Retortamonadida Trichomonadida	Giardia, Chilomastix Trichomonas
	Neozoa	Amoebozoa (amoebae)	Archamoebea Amoebaea	Entamoebidae Acanthopodida	Entamoeba, Endolimax, Iodamoeba Acanthamoeba, Balamuthia
		Percolozoa Euglenozoa (blood and tissue flagellates)	Heterolobosea Kinetoplastidea	Schizopyrendia Trypanosomatida	Naegleria Leishmania, Trypanosoma
		Apicomplexa	Coccidea	Eimerida	Eimeria, Toxoplasma, Cryptosporidium, Cyclospora, Cystoisospora, Sarcocystis
		Ciliophora	Litostomatea	Haemosporida Piroplasmida Vestibuliferida	Plasmodium Babesia Balantidium
Fungi		Microspora	Microsporea	Microsporida	Enterocytozoon, Encephalitozoon, Microsporidium,
Chromista	Chromobiota	Bigyra	Blastocystea		Blastocystis

Adapted from: Topley and Wilson's Microbiology and Microbial Infections, 10th edition .Wiley:2009

Enumerate some non-pathogenic protozoans.

A.3 *Entamoeba gingivalis* (in mouth), *Trichomonas tenax* (teeth and gums), *Trichomonas hominis* (ileocaecal region), and *Trypanosoma rangeli* (blood). This relationship is actually commensalism (from latin; meaning "eating at the same table"), in which one organism is benefiting from the association and other is not harmed by it.

Overview of the Morphological profile of key Protozoans infecting man

A.4 **Table 14.2.4:** Morphological profile of Protozoans

PARASITE	MORPHOLOGICAL	FORMS
Entamoeba histolytica	• Trophozoite: 20-30μm, single nucleus with central karyosome, actively motile, characteristic cytoplasmic inclusions are RBCs and leucocytes (Fig.14.2.1)	• Cyst: 10-20μm, 1 to 4 nuclei with central karyosome, Non motile (Fig.14.2.1 and 14.2.2)
Naegleria fowleri	• Trophozoite: 10-20μm, motile, with characteristic bulbous pseudopodia, single nucleus with central karyosome (Fig. 14.2.3)	• Cyst: 7-10μm, uninucleate, cyst wall present (stage not found in man/tissue) (Fig. 14.2.3)
Acanthamoeba spp	• Trophozoite: 15-45μm, characteristic fine tapering pseudopodia, uninucleate (stage seldom seen in man) (Fig. 14.2.4	• Cyst: 20μm (approximately), Double walled, outer wall wrinkled. (Fig.14.2.4and 14.2.5)
Balamuthia mandrillaris	• Trophozoite: Form similar to Acanthamoeba spp. (Fig. 14.2.6)	• Cyst: Form similar to Acanthamoeba spp. (Fig.14.2.6)

Contd.

Contd.

Giardia lamblia	• Trophozoite: 14μm (long) / 7μm (wide), pear shaped, bilaterally symmetrical, central axostyle, binucleate& Four pairs of flagella (Fig. 14.2.7 to 14.2.10b)	• Cyst: 12/7μm, oval shape, central axostyle, four nuclei, No flagella (Fig.14.2.10a,14.2.10b,14.2.11)
Trichomonas vaginalis	• Trophozoite: 10-30μm long, pear shaped, characteristic twitching motility, central axostyle, 4 anterior flagella and a posterior trailing flagellum (Fig. 14.2.12)	• Cyst stage: Does not exist
Leishmania donovani	• Amastigote form: [Leishman Donovan (LD) body] 3-5μm, rount to oval, non-motile, central roundish nucleus, kinetoplast exists at right angle to nucleus, axoneme arises from blepharoplast (represents intracellular portion of flagellum), No external flagellum (Fig.14.2.13,14.2.14,14.2.15)	• Promastigote form: 15-20μm (long), 1-2μm (wide), spindle shaped, motile, central nucleus, single flagellum, kinetoplast exists transversely to nucleus, axoneme arises from blepharoplast. (Fig. 14.2.16)
Trypansoma brucei complex	• Trypomastigote (long slender form): 15-30μm long, 1.5-4μm wide, spindle shaped, blunt posterior end, pointed anterior end, motile, kinetoplast is at posterior end, flagellum arises at posterior end and moves as long undulating membrane. (Infective form in man) (Fig. 14.2.17)	• Epimastigote: Resembles trypomastigole except that the kinetoplast is close to the nucleus and lies anteriorly. (Infective form in Testse fly)
Trypanosoma cruzi	• Trypomastigote form (appears in blood of cases) and Amastigote form appear in tissues; as muscle and heart of cases. (Fig.14.2.18)	• Promastigote and Epimastigote forms appears in Reduvid bug.

Plasmodium vivax (Fig: 14.2.19, 14.2.20, 14.2.21, 14.2.22)	• Ring form: Single, 1.5-2μm, single chromatin dot on ring, cytoplasm opposite chromatin is thicker	• Trophozoite form: Large, irregular, amoeboid, vacuole present	• Schizont form: Large, 9-10μm in diameter (almost fills RBC), Golden brown Pigment, 12-24 merozoites	• Gametocyte form: Large, rounded, male gametocyte smaller than female
Plasmodium falciparum (Fig.14.2.19, 14.2.23, 14.2.24)	• Ring form: Multiple rings, size of each 1-1.5μm, multiple chromatin dots on ring, cytoplasm of ring is fine and regular. Accocele present (parasitic chromatin and part of cytoplasm remain attached to outside of edge of RBC)	• Trophozoite form: Compact form, pigments gather into single mass	• Schizont form: Small, 4.5-5μm diameter, fills two thirds of RBC (normal size), Dark brown 'Rigment, 18-24, merozoes pigment	• Gametocyte form: Sickle/banana shaped, female one is longer and more slender than male gametocyte

Toxoplasma gondii	• Trophozoite (Tachyzoite) form: 7μm/3μm (breadth), crescentic / oval, gets stained with Giemsa stain (invades all mammalian cells except non nucleated RBCs, during acute stage infection) (Fig.14.2.25,14.2.27)	• Tissue cyst: 20-100μm in diameter, variable size, contain cyst wall, gets stained with periodic acid Schiff (PAS), contains bradyzoites similar to tachyzoites, but smaller (Stage is infectious to animals that ingest them) (Fig. 4.2.26)	• Oocyst: 10-12μm diameter, oval, has thick wall, has two sporocysts, each has four aporozoites. (stage is formed in cat by sexual reproduction)

Contd.

Contd.

Cryptosporidium parvum	• Oocyst: 2-6μm in diameter, spherical, colorless, cyst wall present, contain 4 crescentic shaped sporozoites (anterior end pointed), • Acid fast, does not stain with iodine. (Fig. 14.2.28,14.13.1)
Cystoisospora (Isospora) *belli*	• Oocyst: 20-33μm x 10-15μm, ellipsoidal, two layered smooth cyst wall, contains two sporocysts, each with four sporozoites, Acid fast. (Fig. 14.2.28)
Cyclospora cayetanensis	• Oocyst: 8-10μm in diameter, spherical, contains two sporcyst, each with two sporozoites, • Acid fast (variable) (Fig. 14.2.28)

Balantidium coli	• Tropohzoite: 60-70 x 40x70μm (breadth), Oval, ciliated, anterior end narrow and posterior broad, one large kidney shaped macronucleus and one small micronucleus in the concavity of macronucleus. (Fig. 14.14.1)	• Cyst: 50-60 μm in diameter, spherical, double layered wall, macronucleus, micronucleus and vacuoles present. (Fig. 14.14.1)

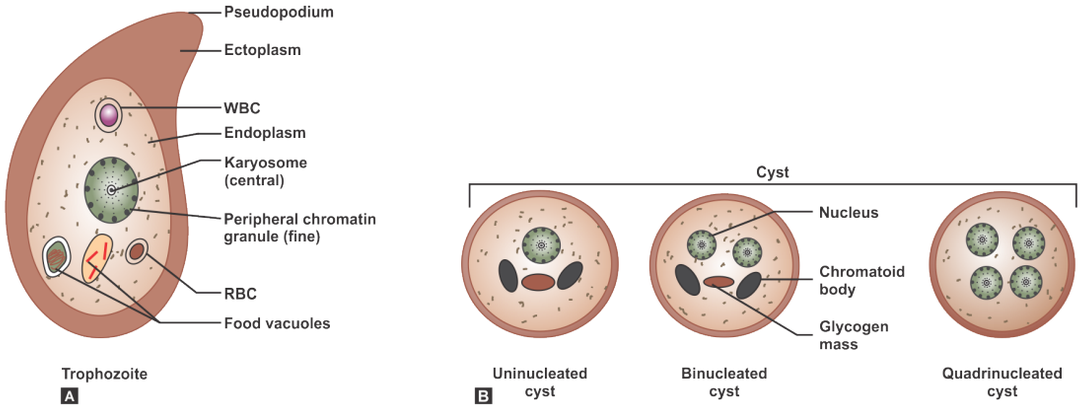


Fig.14.2.1: Schematic representation of A. Trophozoite B. Cysts of *E. histolytica*

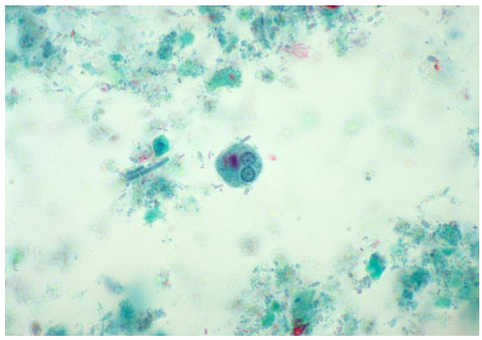


Fig.14.2.2: Photomicrograph depicting binucleated cyst of *E.histolytica*, with two chromatoid bodies (Trichrome stained).
Courtesy: Dr. Mae Melvin/CDC

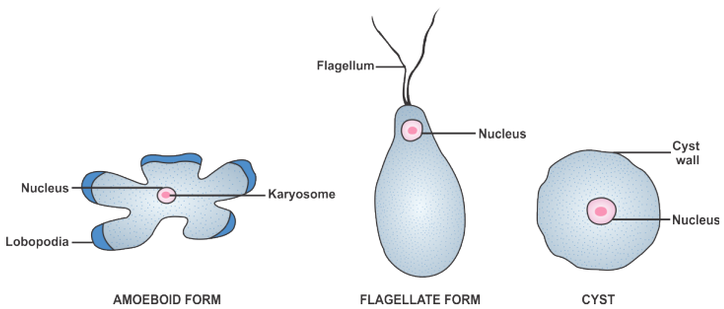


Fig.14.2.3: Schematic representation of morphological forms of *Naegleria fowleri*

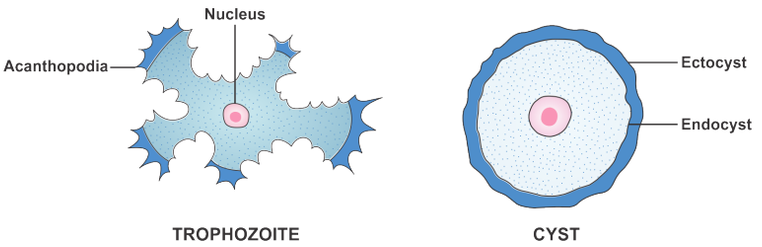


Fig.14.2.4: Schematic representation of Trophozoite and Cyst forms of Acanthamoeba species

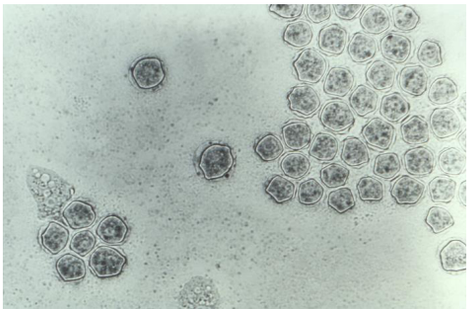


Fig.14.2.5: TEM image of Acanthamoeba spp. organisms in cystic stage, obtained from a keratitis case (specimen cultured on fibroblast tissue culture agar plate).

Courtesy: .Dr. John Hierbolzer / CDC

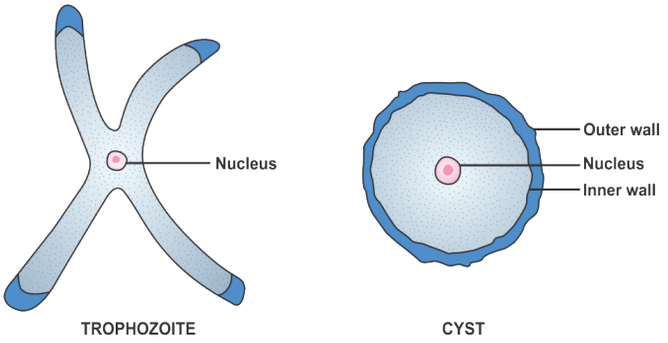


Fig.14.2.6: Schematic representation of morphological forms of *Balamuthia mandrillaris*

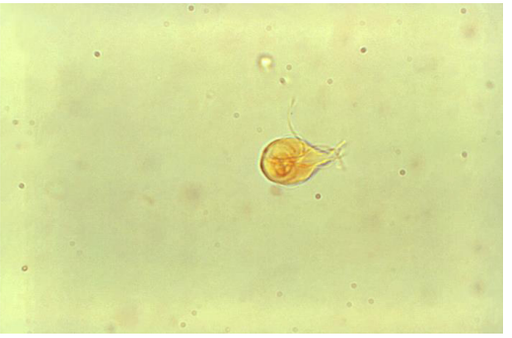


Fig.14.2.7: Photomicrograph of trophozoite of *G.lamblia* (Iodine staining)

Courtesy: Dr. Mae Melvin / CDC

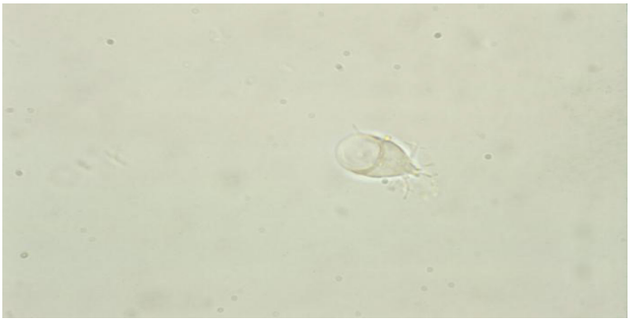


Fig.14.2.8: Photomicrograph of trophozoite of *G.lamblia* trophozoite with its sucking disc visible

Courtesy: Dr. Mae Melvin / CDC

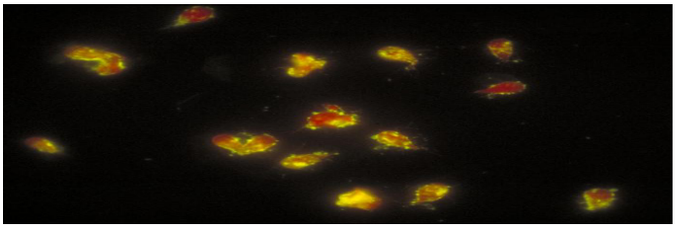


Fig.14.2.9: Photomicrograph depicting a positive IFA test for G.lamblia.

Courtesy: Dr. G.S. Vivesvara / CDC

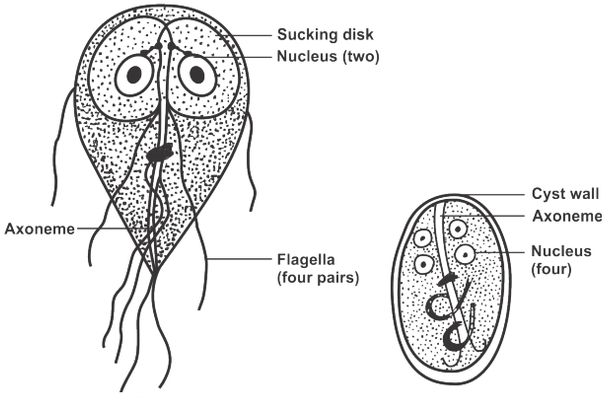


Fig.14.2.10a: Schematic representation of Trophozoite and Cyst forms of *G. lamblia*

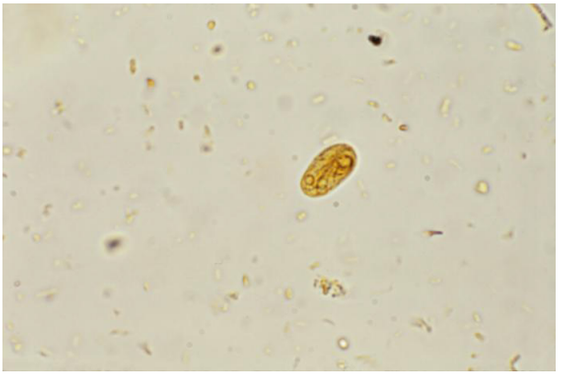


Fig.14.2.10b: Photomicrograph of cyst of *G.lamblia* cyst.

Courtesy: Dr. Mae Melvin / CDC

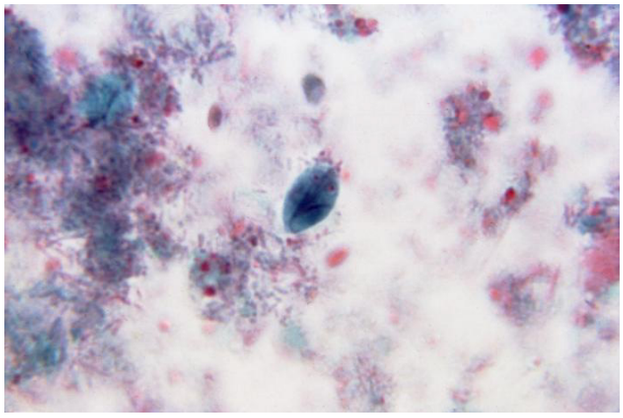


Fig.14.2.11: Photomicrograph of cyst of *G.lamblia* (Trichrome staining)

Courtesy: Dr. Mae Melvin / CDC

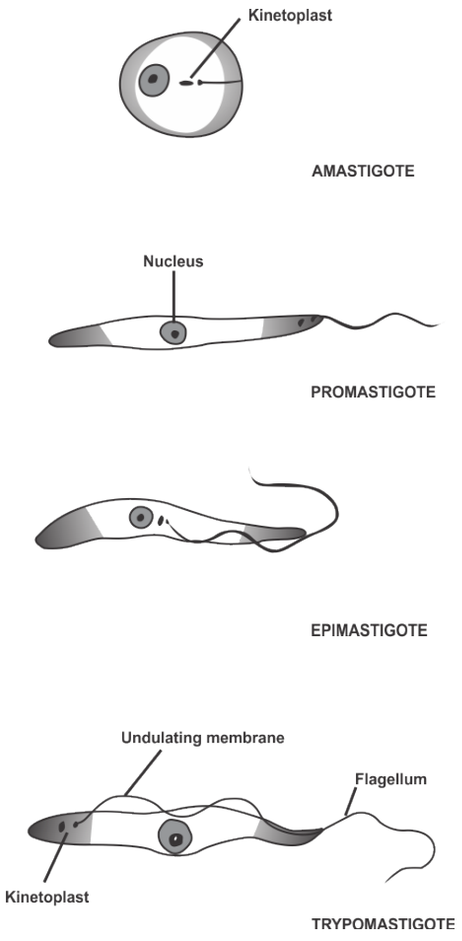


Fig.14.2.13: Morphological forms of human flagellates

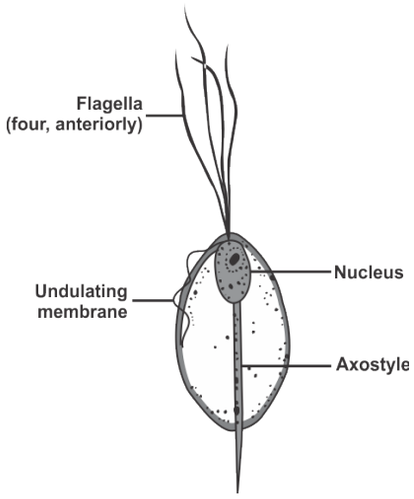


Fig.14.2.12: Schematic representation of *T. vaginalis* (has only trophozoite stage)

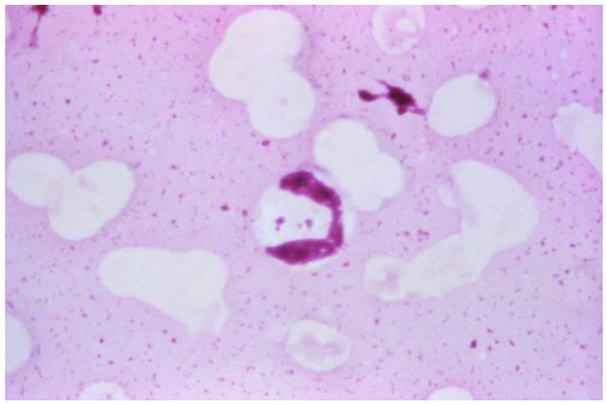


Fig. 14.2.14: Photomicrograph of bone marrow specimen, depicting *L. donovani* amastigotes within a WBC.

Courtesy: Dr. L.L.A. Moore, Jr. / CDC

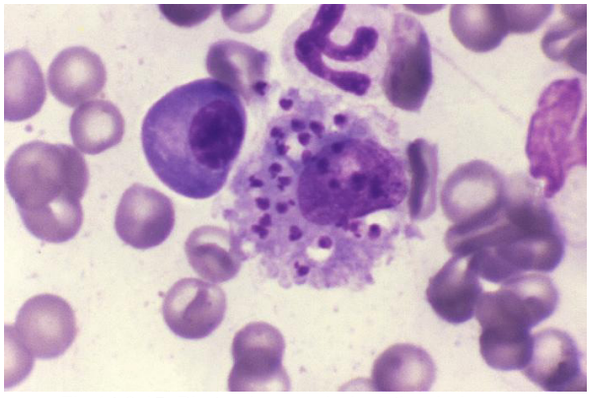


Fig.14.2.15: Photomicrograph depicting *L.donovani* amastigotes within one of bone marrow histiocytes.

Courtesy: Dr. Francis W. Chandler / CDC

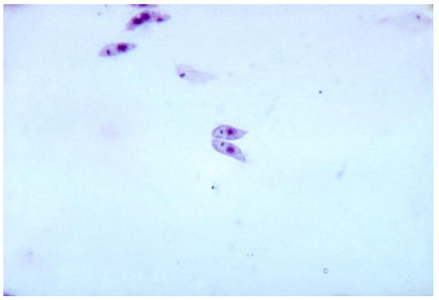


Fig.14.2.16: Photomicrograph depicting promastigotes of *L.donovani* inside midput of sandfly.

Courtesy:CDC

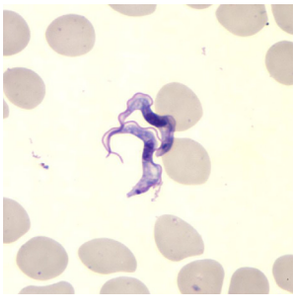


Fig.14.2.17: Giemsa stained blood smear revealing two trypomastigotes of *T. brucei*

Courtesy: Blaine Mathson / CDC

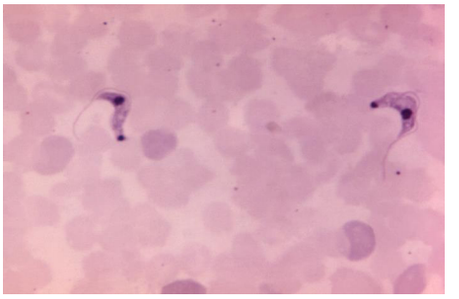


Fig.14.2.18: Photomicrograph of blood smear specimen revealing trypomastigotes of *T. cruzi*.

Courtesy: Dr. Myron G. Schultz / CDC

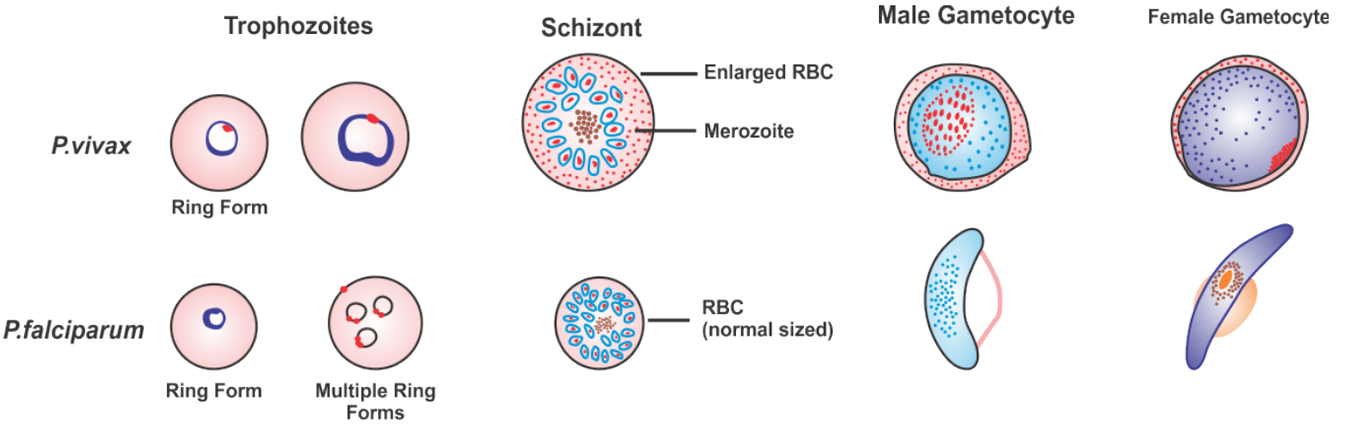


Fig.14.2.19: Schematic representation of morphological forms of *Plasmodium vivax* and *P. falciparum*

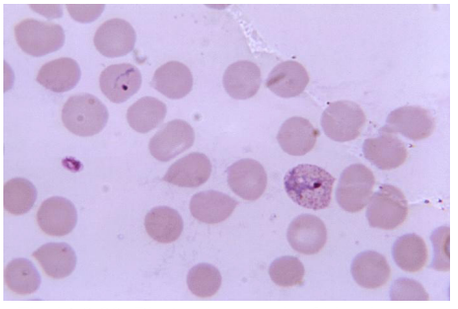


Fig.14.2.20: Photomicrograph of blood smear revealing ring form of *P.vivax* trophozoite form (upper left) and a growing amoeboid trophozoite displaying schufner's dots (right).

Courtesy: Dr. Mae Melvin / CDC

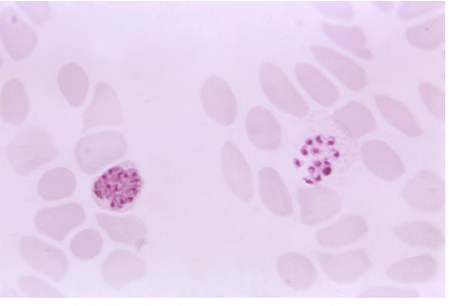


Fig.14.2.21: Photomicrograph of thin blood smear depicting two schizonts of *P.vivax* (immature form on the left and mature form on the right)

Courtesy: Dr. Mae Melvin / CDC

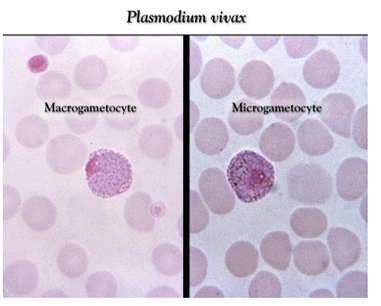


Fig.14.2.22: Photomicrographs compares microgametocyte of and microgametocyte of *P.vivax*

Courtesy: Steven Glenn / CDC

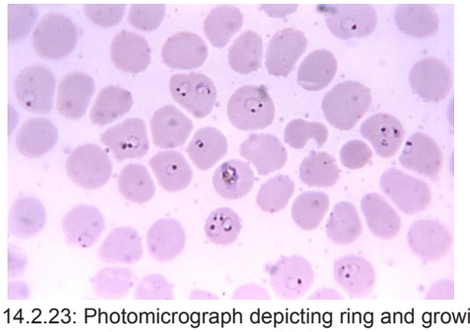


Fig.14.2.23: Photomicrograph depicting ring and growing band form trophozoite of *P.falciparum*.

Courtesy: Dr. Mae Melvin / CDC

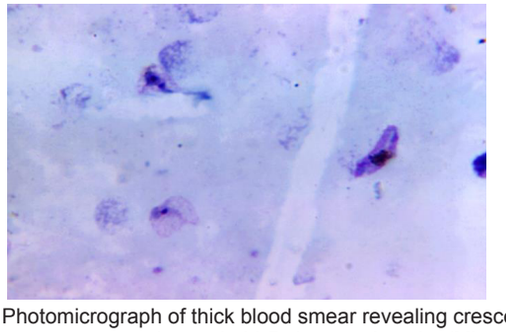


Fig.14.2.24: Photomicrograph of thick blood smear revealing crescent shaped *P.falciparum* gametocyte and amoeboid *P.vivax* trophozoite (Mixed infection)

Courtesy: Dr. Mae Melvin / CDC

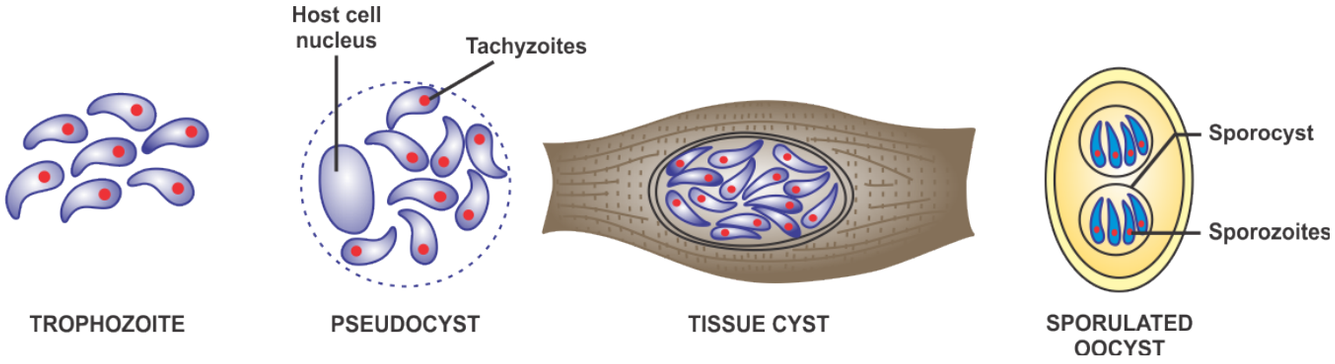


Fig.14.2.25: Schematic representation of morphological forms of *Toxoplasma gondii*

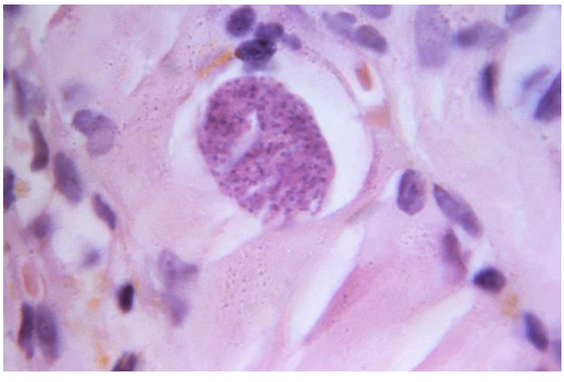


Fig.14.2.26: Photomicrograph of HAE stained human muscle tissue specimen reveals *T.gondii* tissue cyst, containing developing bradyzoites.

Courtesy: Dr. Martin Hicklin / CDC

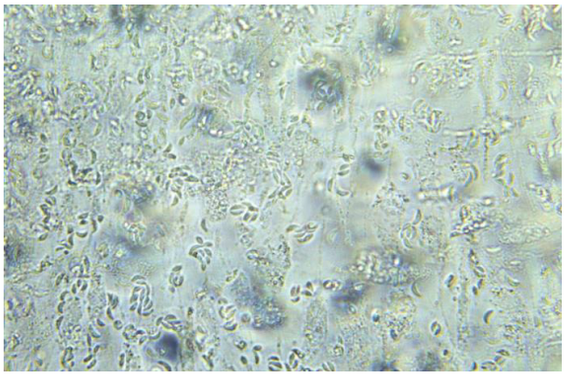


Fig.14.2.27: Photomicrograph depicts numerous crescent shaped parasites of *T.gondii* amongst the cultured fibroblasts.

Courtesy: Dr. Morris T. Suggs , Jr ; / CDC

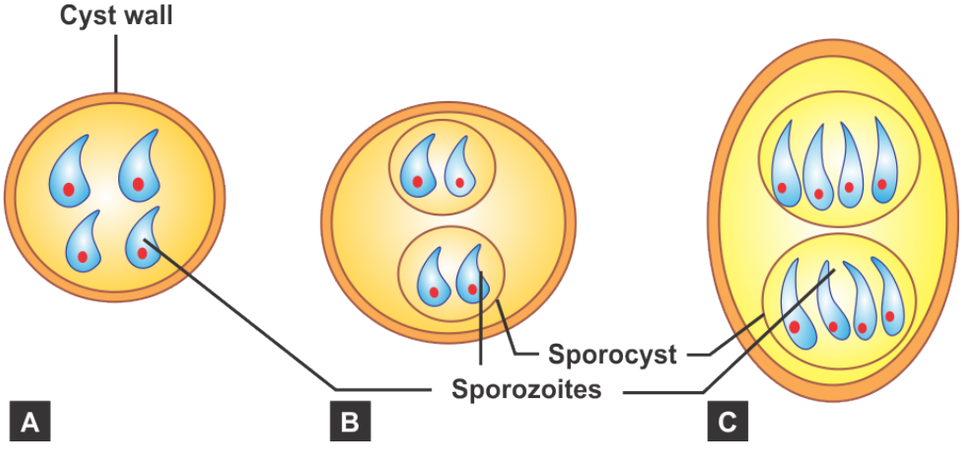


Fig.14.2.28: Schematic reprersentation of Sporulated Oocyst of A. Cryptosporidium spp., *B. Cyclospora cayatensis*,*C. Cystoisospora belli*

An Overview of the Transmission/Life Cycle and Host's Profile of Protozoan Parasites

3

Differentiate between Reservoir and paratenic host.

A.1 *Reservoir host* is one, which harbors the parasite and serves as an important source of infection to other susceptible hosts e.g., dog (canines) for hydatid disease. *Paratenic host* is one in which the parasite survives, but cannot develop further and serves as a carrier host.

Differentiate between Simple (Direct) and Complex (Indirect) Life cycle.

A.2 In simple (Direct) life cycle, only one host is required to complete the life cycle of parasite, whereas in complex (indirect) life cycle two or more hosts are required to complete the life cycle of parasite.

Tabulate the transmission profile of protozoan parasites.

A.3

Table 14.3.1: Transmission profile of Protozoan parasites

Parasite/Disease	*Mode of transmission (including infective form)*	*Location of Parasite in man*	*Definitive host*	*Intermediate host*
• *E.histolytica*/ Amoebiasis	- Ingestion of cysts/ - Faecal-oral (commonest)/ - Sexual contact (in homosexual males; orogenital/ anogenital contact (Fig. 14.5.2)	Large intestine / Liver / others less common	Man	-
• *Naegleria fowleri* / Primary meningocencephalitiis	- Inhalation of warm water containing amoeboid form trophozoite/ Nasal route (Fig. 14.3.1)	CNS	Man	-
• Acanthamoeba spp. / Primary meningoencephalitis	- Inhalation of aerosol/ dust containing trophozoite / Cyst from soil / fresh water - Direct contact with cornea / Nasal route, Eye. (Fig.14.3.2)	CNS / Sinuses / Lung	Man	
• *Balamuthia mandrillaris* / Granulomatous amebic encephalitis	- Similar (Fig. 14.3.3)	Not Known	Man	-
• *Giardia lamblia* / Giardiasis	- Ingestion of cyst (from water & food / Faecal – oral AND Sexual route (homosexuals) (Fig. 14.7.2	Smal intestine / common bile duct / gallbladder	Man	-
• *Trichomonas vaginalis* / Trichomoniasis	- Trophozoles / Sexual route (rarely non-sexual) (Fig. 14.8.2)	Vagina / urethra / Prostrate / Seminal vesicle	Man	-
• *L.donovani* complex / Visceral leishmaniasis (Kala azar)	- Promastigole form / Bite of infected female sand fly. (Phelbotomus) (Fig. 14.9.2)	Liver / spleen/ L.N. / Bone (R.E. System)	Man / other animals	Sand fly (*Phelbotomus argentipes*)
• *L. brazilensis* complex / Mucocutaneous leishmaniasis	- Promastigole form / Bite of infected sand fly	Skin / mucous membrane	Man / other animals	Sand fly (Lutzomyia)
• *L. tropica* / oriental sore • (Cutaneous leishmaniasis)	- Promastigote form/ Bite of infected sand fly	Skin (Intracellular)	Man / other animals	Sand fly (Phelbotomus)
• *T.brucei* / Africian trypanosomiasis	- Trypomastigole / Bite of infected Tsetse fly. (Fig. 14.3.6)	Blood lymphatics / CNS	Man	Tsetse fly (Glossina) (Fig.14.3.4)

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• *T.cruzi* / Chaga's disease	- Trypomastigote / Faeces of infected Reduvid bug. (Fig. 14.3.7)	Blood / R.E. System / Heart / brain	Man / other animals	Reduvid bug (Triatoma) (Fig.14.3.5)
• Plasmodium spp.	- Sparozoite / Bite of infected female anopheles mosquito / AND - Blood transfusion / congenital (in it trophozoite form)	RBCs / R.E. system	Anopheles mosquito (Fig.14.3.8)	Man
• *T.gondii* / Toxoplasmosis	- Bradyzoites (Ingestion of infected meat, having tissue cyst) / - Sporulated oocyst in cat's faces (Ingestion of contaminated food, water)/ - Tachyzoites (by blood transfusion, transplantation, congenital). (Fig. 14.12.2, 14.12.3)	R.E. system (cell) / Other organs as eye, brain	Cat	Man / other animals
• *C. parvum* / Cryptospordiosis	- Ingestion of Oocyst (ingesting contaminated food/water) Faecal – oral (Fig. 14.3.10)	Intestinal epithelium / (intracellular) Biliary and respiratory tract (in immunodeficient individuals)	Man / other animals	-
• *Cystoisospora belli* / Cystoisosporiasis	- Sporulated oocyst (ingestion of contaminated food / water) Faecal – oral. (Fig. 14.3.9)	Small intestinal epithelium (intracellular)	Man	-
• *Cyclospora cayantensis* / Cyclosporiasis	- Sporulated Oocyst (Ingestion of contaminated food / water) Faecal – oral	Small intestine (Intracellular) / Biliary tract (in immunocompromised individual)	Man	-
• *Palantidium coli*/ Balantidiasis	- Cyst (Ingestion of contaminated food / water) Faecal – oral. (Fig. 14.14.1)	Large intestine	Pig / Man (accidental host)	-

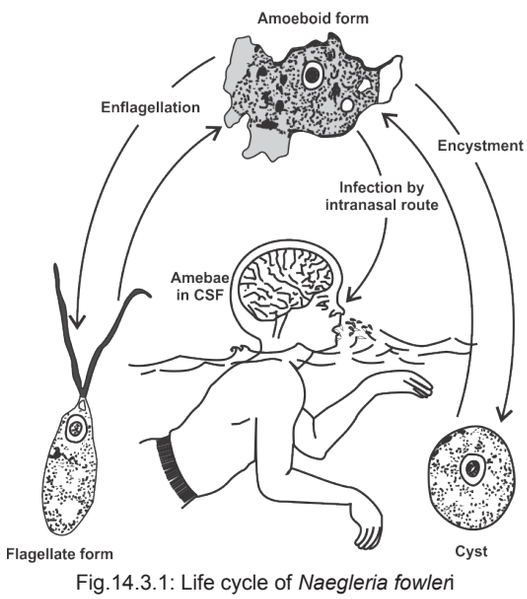

Fig.14.3.1: Life cycle of *Naegleria fowleri*

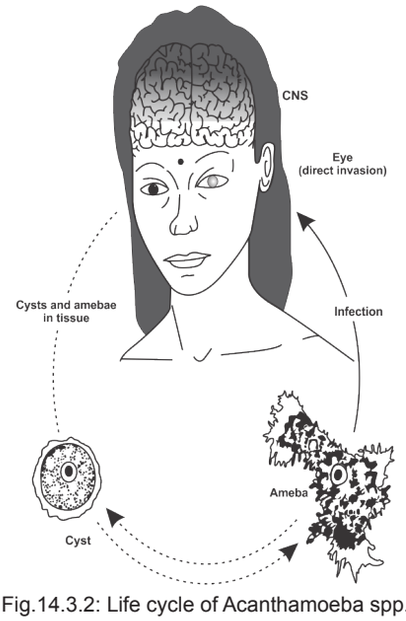

Fig.14.3.2: Life cycle of Acanthamoeba spp.

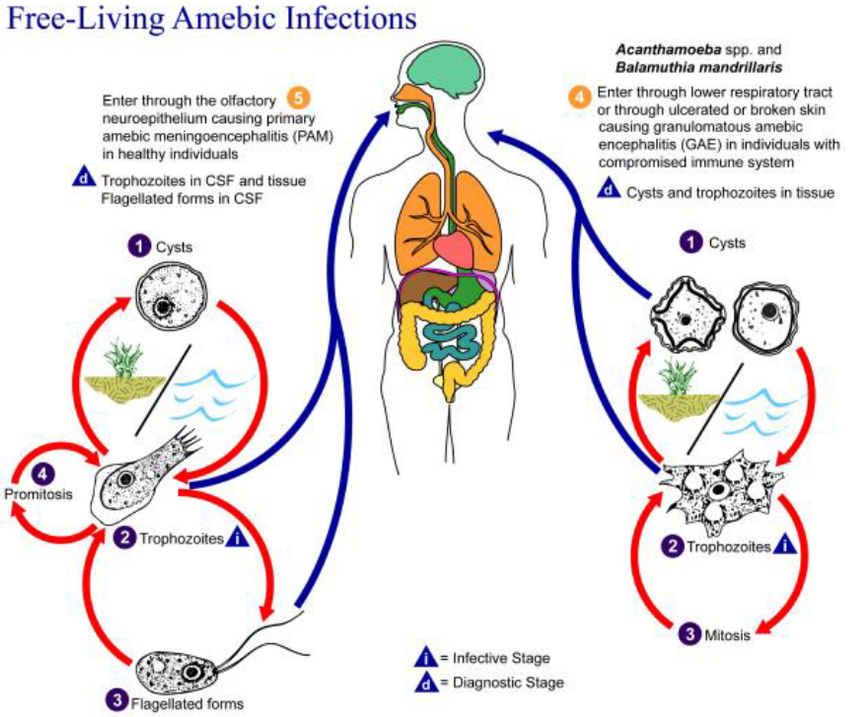

Fig.14.3.3: Schematic representation of life cycle of different species of free-living amebic infections

Courtesy: Dr. Alexander J. da Silva; Melanie Moser, / CDC

Fig. 14.3.4 Image of Tsetse fly (Genus Glossina), depicting the forward pointing proboscis for extracting blood meal (process also result in parasite transmission)

Courtesy: Kay DeWitt / CDC

Fig.14.3.5 Dorsal view of Reduvid bug(kissing bug,Triatoma), Which transmits Chaga's disease

Courtesy: Kay DeWitt / CDC

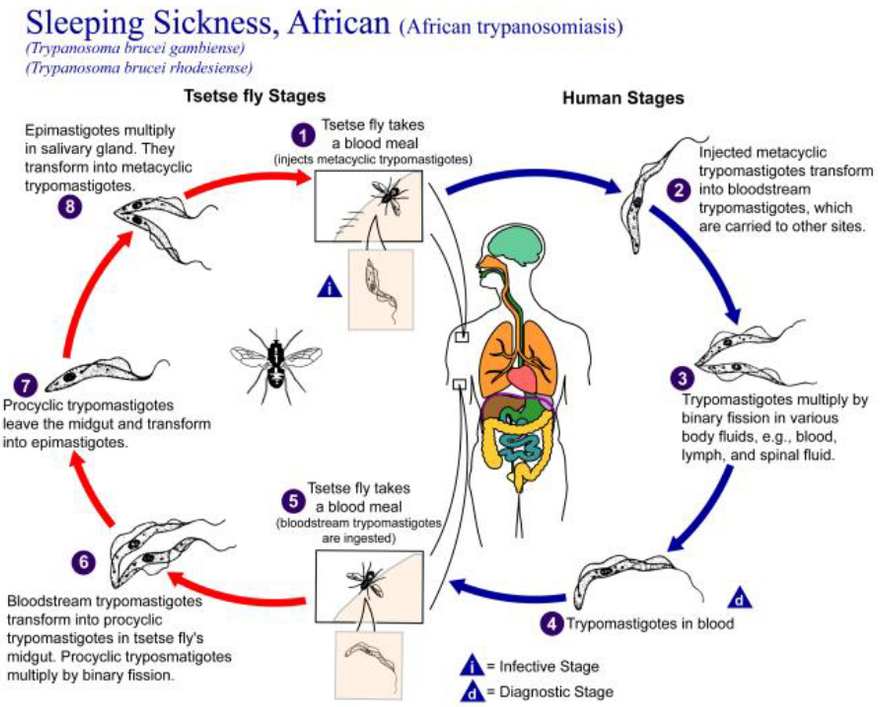

Fig. 14.3.6 .Schematic representation of life cycle of T.*brucei*

Courtesy: Dr. Alexander J. da Silva M. Moser / CDC

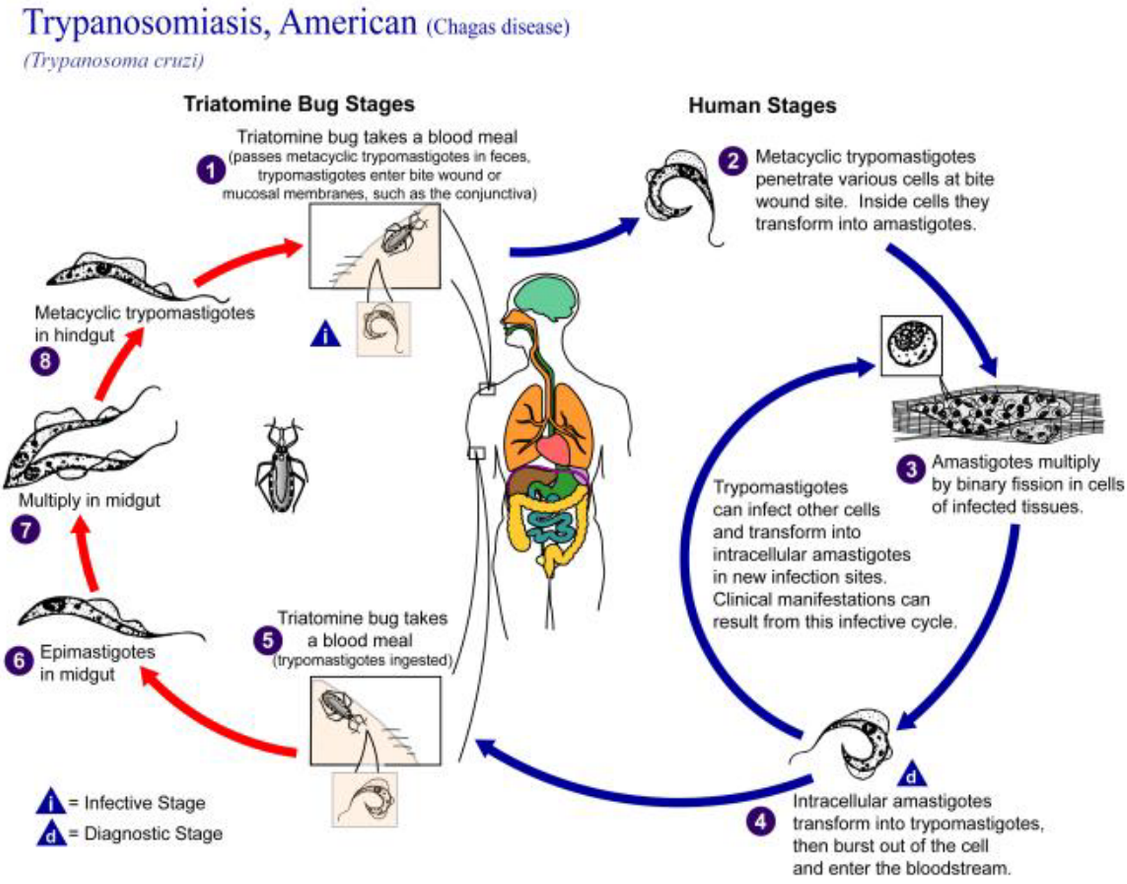

Fig.14.3.7: Schematic representation of life cycle of *T.cruzi*

Courtesy: Dr. Alexander J. da Silva/M. Moser / CDC

Fig.14.3.8.Image depicting lateral view of female feeding anopheles mosquito. Abdomen is red colored & filled with blood

Courtesy: James Gathony / CDC

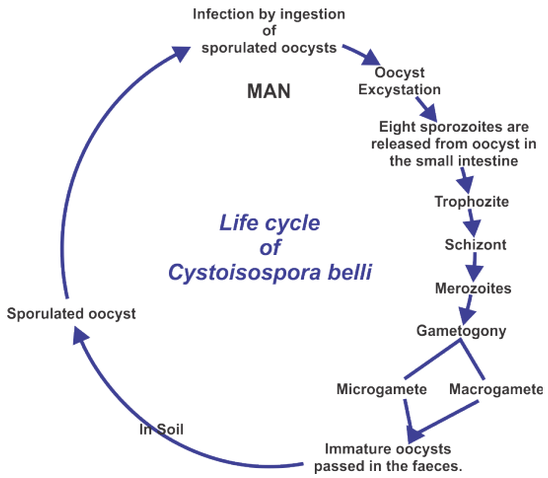

Fig.14.3.9: Life cycle of *Cystoisospora belli*

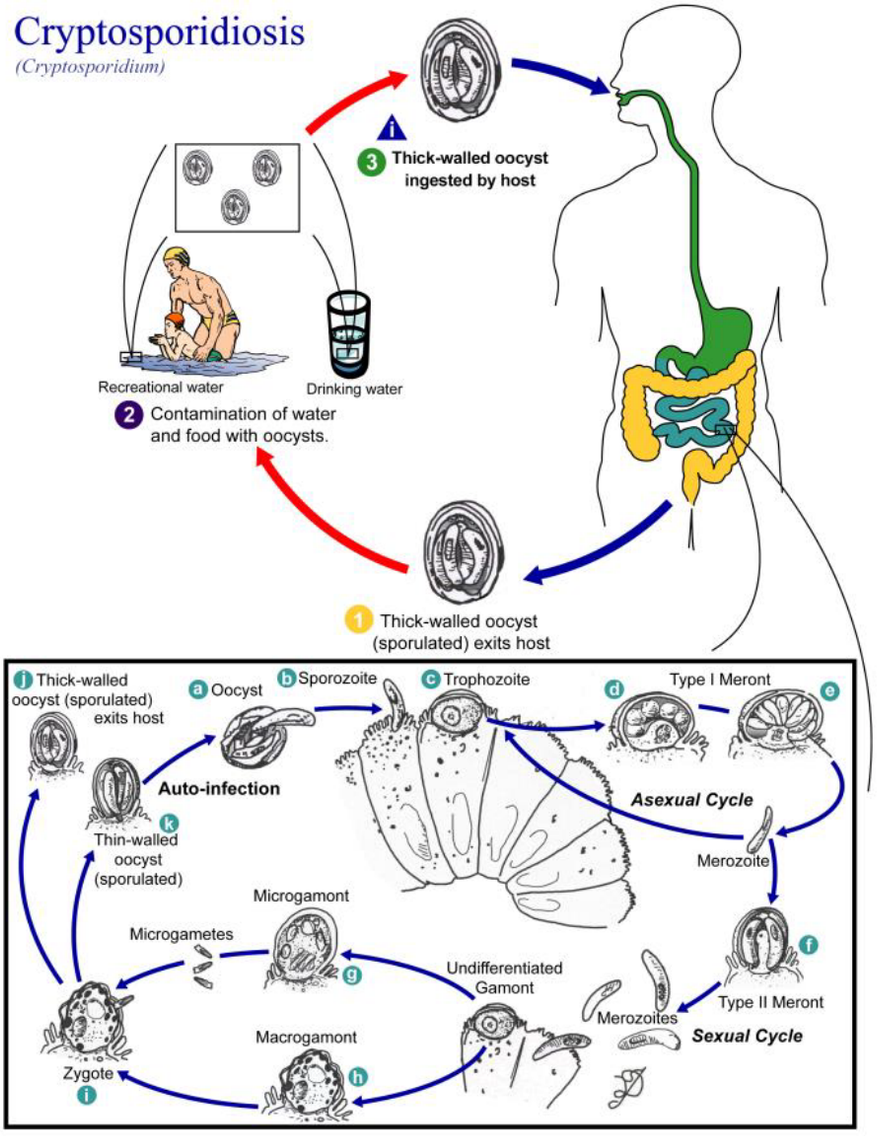

Fig.14.3.10: Life cycle of Cryptosporidium spp.

An Overview of Clinical Profile (Pathogenicity) of Infections Caused by Protozoan Parasites

4

Table 14.4.1: Clinical profile of Protozoal Infection

Organism (Disease)	Clinical features (major clinical syndrome)
Entamoeba histolytica (amoebiasis)	• *Amoebic dysentery:* colitis like picture (complications include perforation, peritonitis and amoeboma formation), (Fig.14.4.1), amebic liver abscess (commonest extraintestinal presentation) nb: *E.dispar* is responsible for many cases of asymptomatic carriage, previously attributed to *E.histolytica*
Naegleria fowleri	• Primary meningoencephalitis (features include nausea, vomiting, frontal headache and nuchal rigidity. Later convulsions and coma can follow (in young population often involved in water sports)
Acanthamoeba spp.	• Granulomatous amoebic encephalitis (Fig.14.4.2), keratitis, sinusitis and bone infections. • (involves older population, often immunocompromised; with no history of swimming)
Balamuthia mandrillaris	• Granulumatous amoebic encephalitis
Giardia lamblia (Giardiasis)	• Asymptomatic giardiasis (mostly) • Acute giardiasis (non inflammatory diarrhoea) • Chronic giardiasis. Person can present with recurrent diarrhoea and often associated with flatulence, distension and epigastric abdominal cramps (which are characteristic). Various grades and types of malabsorption occur. nb: It is important to recognize the different types, as the clinican can confuse them with other clinical entities. One individual can move from one type (phase) to another and in one family, individuals can be in different phases.
Trichomonas vaginalis (Trichomoniasis) Fig.14.4.3	• A venereal infection. *In females*; classical presentation is vaginal discharge; which may be copious, frothy and malodorous. May be associated with itching in vulvar region and thigh. Estimated that half of infected individuals may be asymptomatic. *In males*; may cause persistent urethritis and prostatitis, usually asymptomatic.
Leishmania donovani complex	• Visceral leishmaniasis (Kala azar meaning 'black fever'). Splenomegaly is most characteristic feature. Other features are hepatomegally, fever, anemia and weight loss. Skin in light-skinned individuals acquire a greyish pigmentation, responsible for the disease acquiring the name of Kala azar. Post-Kala-azar leishmaniasis (PKDL) is a non-ulcerative lesion of the skin, which follows 1–2 years after treatment.
Leishmania brazilensis complex	• Mucocutaneous leishmaniasis (Espundia). Characterized by ulcers of skin, oral and nasal mucosa (nose and lips are destroyed creating a ghastly site)
Leishmania tropica	• Oriental sore (Delhi boil). Skin ulcer (single or multiple); which may self heal.
Trypanosoma brucei complex (T. b. brucei, T.b. gambiense, T.b. rhodesiense)	• African trypanosomiasis (*Sleeping Sickness*). Trypanosomal chancre, fever, headache and Winterbottom's sign (enlargement of posterior cervical lymph nodes). In later stage, characteristic progressive daytime somnolence, responsible for the name of sleeping sickness. Terminally; meningoencephalitis.
Trypanosoma cruzi	• American trypanosomiasis (*Chagas disease*). Chagoma (inflammatory lesion at site of deposition of bug's faeces), Romana's sign –Fig.14.4.4 (conjunctivitis in eye, when bug enters through conjunctiva), Fever, hepatosplenomegaly and acute myocarditis (Fig.14.4.5). In chronic Chaga's disease; megaoesophagus (dilated oesophagus) and megacolon (dilated colon) may occur.
Plasmodium vivax (Malaria; benign tertian malaria)	• (i) Febrile paroxysm, (ii) Anemia, and (iii) Splenomegaly. • The fever recurs every third day (tertian fever). Each paroxysm comprises of cold, hot and sweating stages. However classical picture not seen often, as erythrocytic cycles are not entirely synchronous.
Plasmodium falciparum (Malaria, malignant tertian malaria)	• Presentation is like of *P.vivax*. However associated with significant morbidity and mortality. The complications include Black water fever and pernicious malaria; which may present as cerebral malaria, algid malaria or septicemic malaria.

Contd.

Contd.

Toxoplasma gondii (Toxoplasmosis)	• Congenital: If infection is acquired in first trimester, may result in still birth or abortion. If acquired later can result in neurological damage • Chorioretinitis is the commonest delayed complication of congenital toxoplasmosis; manifesting in the second or third decade of life. • Postnatally acquired in normal individuals: Mostly asymptomatic. Commonest presentation is lymphadenopathy, cervical group often involved. Other presentations rarely occur. • In immunocompromised: There can be widespread dissemination of infection manifesting as necrotizing pneumonitis, myocarditis (Fig.14.4.6) and encephalopathy. • In AIDS cases, encephalitis is a common presentation.
Cryptosporidium parvum (Cryptosporidiosis)	• In immunocompetent: Self limiting non-inflammatory diarrhea.(lack of flatulence may differentiate, it from Giardia infection) • In immunocompromised: Chronic severe diarrhoea can occur. • Extraintestinal presentation; as cholangitis etc., can occur in AIDS cases.
Cystoisospora belli: (Isospora) – Cystiosoporiasis	• Diarrhoea, abdominal pain, anorexia and weight loss. • A severe infection occurs in immunocompromised individuals.
Cyclospora cayatensis (Cyclosporiasis)	• Diarrhoea, anorexia, abdominal pain and weight loss.
Sarocystis spp.	• Myositis, fever.
Balantidium coli (Balantidiasis)	• Majority of cases remain asymptomatic. In acute cases; colitis and diarrhoea are present.
Blastocystis spp. (hominis)	• Blastocystosis, presents as diarrhoea.

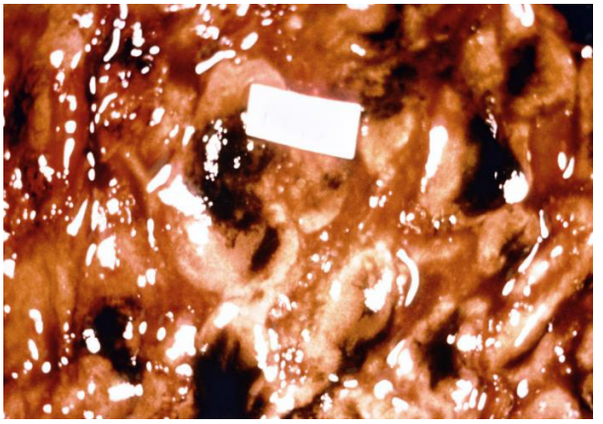

Fig.14.4.1 Multiple mucosal lesions in the intestine; indicative of amebiasis

Courtesy: Dr.Healy/CDC

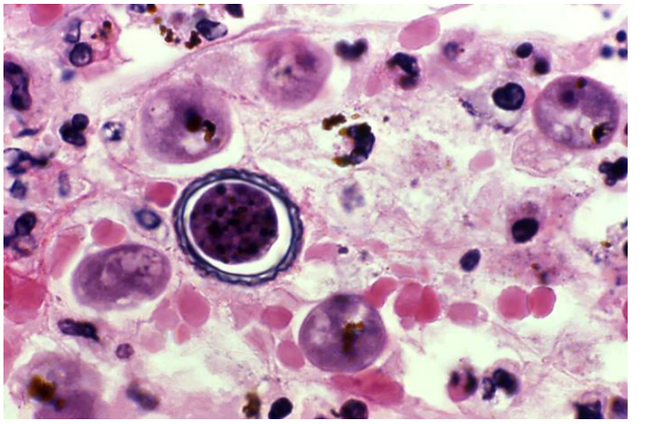

Fig.14.4.2.Photomicrograph depicting brain tissue with centrally located Acanthamoeba cyst.

Courtesy: Dr. George Healy/CDC

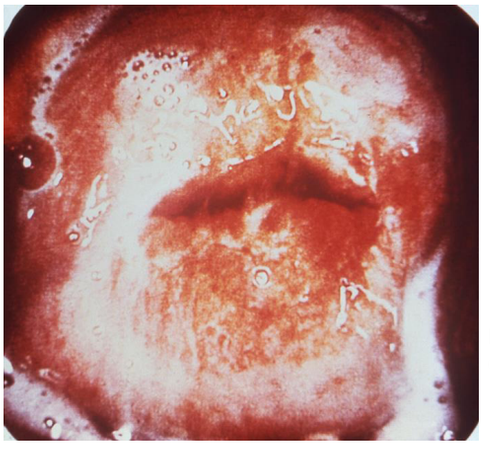

Fig.14.4.3 Colposcopic (Intravaginal) view of cervix revealing purulent exudate caused by *T.vaginalis*

Courtesy: Jim Pledger / CDC

Fig.14.4.4 Image of a child suffering from Chaga's disease has swollen night eye (Romana's sign +ve)

Courtesy: Dr. Mae Melvin / CDC

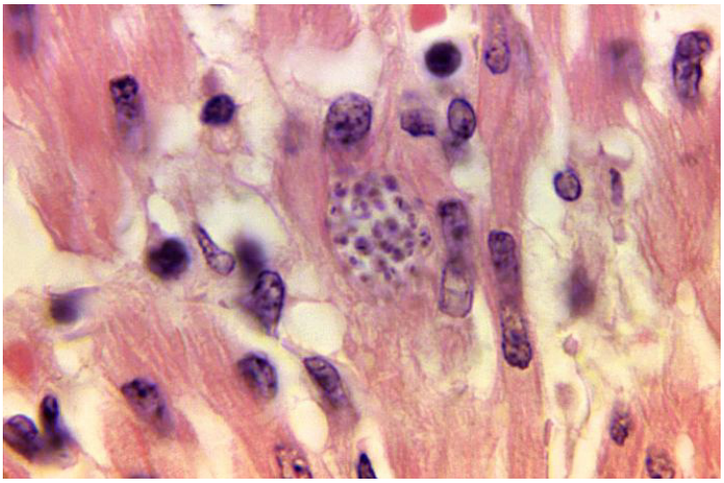

Fig.14.4.5 Photomicrograph of heart tissue sample revealing cyst containing *T.cruzi* bradyzoites.

Courtesy: Dr. L.L. Moore, Jr / CDC

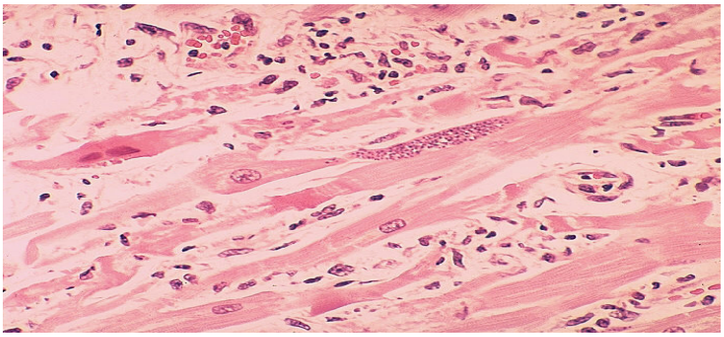

Fig.14.4.6 Photomicrogrpah reveals cyst containing numerous *T.gondi*i bradyzoites within a myocyte of cardiac tissue sample

Courtesy: Dr. Edwin P. Ewing, Jr; / CDC

An Integrated Clinical Case Based Study on *E. histolytica*/Amoebiasis

5

Rahul, a forty five year male presented with passing of 6-8 motions per day. It was copious in amount and red tinged. Tenesmus was present. The case was able to perform his daily duties. There was no history of any outbreak in the locality.

Linkages: Pg. 616, 619, 621, 626, 631, 657, 660

What is your clinical diagnosis?

A.1 (a) Dysentry (Amoebic or Bacillary).Tenesmus (feeling of incomplete evacuation) indicates ulceration in the rectal area.

A microscopic examination of the faeces was performed. It revealed many motile trophozoites about 15 μm in size and few cysts (approximately 10μm) (Fig. 14.5.1)

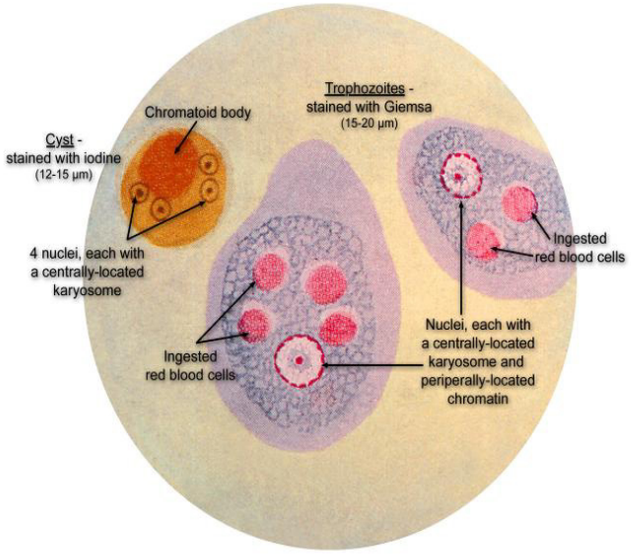

Fig.14.5.1: Schematic representation of findings of cyst of *E.histolytica* depicting four nuclei with centrally located karyosome. The trophozoites depict ingested RBCs, single nucleus with centrally located karyosome and peripherally located chromatin.

Courtesy: CDC

How do you clinically differentiate amoebic from bacillary dysentery?

A.1 (a)

Table 15.4.1: Clinical differences between amoebic and bacillary dysentry

	Amoebic dysentery	Bacillary dysentery
• Age	Uncommon in children	Common in children
• Incubation period	Fortinight to month	One week or less
• Onset	Insidious	Acute
• Symptoms	Categorized as 'Walking dysentery'	'lying down dysentry'

What is your parasitological differential diagnosis?

A.2 (a) The protozoa on the basis of morphological parameters is most likely *E. histolytica*. However only on morphological basis, it is very difficult to differentiate *E. histolytica* from *E. dispar*, *E.moshkovskii* and *E.bangladeshi.*

What are free living amoebae?

A.2 (b) As the name indicates, these are amoebae which are freely living in the natural environment (as soil and water). These amoebae are aerobic; as they possess mitochondria, unlike the human intestinal ones. They can cause opportunistic infections of the CNS (including eye). The important genera associated with human disease include *Naegleria fowleri*, Acanthamoeba spp., *Balamuthia mandrillaris* and Sappina spp.

What is the biological significance of the trophozoite and cyst form?

A.2 (c) The trophozoite a fragile state is the metabolically active stage, which causes pathology at the host level. However when it has to transmit to infect another host, it has to traverse the unfavourable environment. To enable this, this forms secretes a cyst wall and transforms to cyst; process known as encystation (in conrast to excystation; where cyst converts to trophozoite). This form is metabolically inactive.

What instructions need to be given to the case for collection of stool?

A.2 **(d)** Providing of an appropriate stool sample to the laboratory by the patient is of paramount importance. The principle is that the stool sample representative of the intestinal pathology should come to the laboratory. Liquid/semi formed (with pus and blood) have priority over formed part of the stool for processing. The patient should send a minimum of three specimens over a 7-10 days period. The stool can be collected in a clean, wide mouthed container; so that the appropriate sample can be sent. The time of collection should be prior to administration of antidiarrhoeal drugs and before a barium meal etc. The stool should not be allowed to mix with urine (as it has acidic pH, which can destroy trophozoites) and toilet water.

What are the modes of transmission of amoebiasis? Outline the life cycle of amoebiasis.

A.3 For modes of transmission, see pg. 626, table 14.3.1

For life-cycle, see table 14.5.2

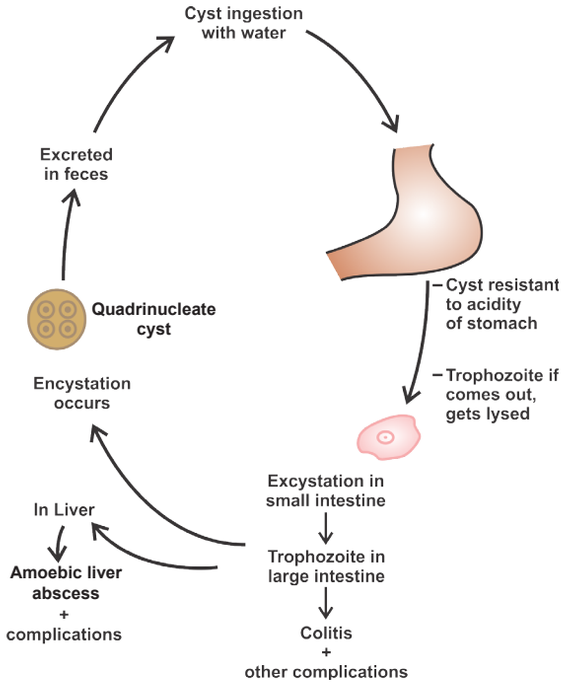

Fig.14.5.2: Life cycle of Entamoeba histolytica

Enumerate the differences between E.histolytica and E.coli (cyst and trophozoite stages). Why is it important to differentiate them?

A.4 It is important to differentiate *E. histolytica* from *E. coli*, as the former is a pathogen and the latter is a commensal.

Table 14.3.2: Differences between *E. histolytica* and *E. coli*

	E. histolytica	*Entamoeba coli*
Trophozoite • Size • Cycloplasm	 • 20-30μm • Relatively 'clean'	 • 20-40μm • 'messy' with numerous vacuoles and ingested debris(Fig.14.5.3)
• Cytoplasmic inclusions	• RBC & leucocyte(Fig.14.5.4) • Never bacteria	• Bacteria & Other material • Never RBC
• Nucleus	• Central Karyosome • Compact karyosome • Delicate nuclear membrane lined with fine chromatin granules	• Eccentric karyosome • More diffuse karysome • Thick nuclear membrane lined with coarse chromatin granules
Cyst • Size • Nuclei • Chromatoid body (collection of ribosomes)	 • 6-15μm (diameter • 1-4 • Rounded	 • 15-20 μm (diameter) • 1-8 • Filamentous (Fig.14.5.5)

Many times the fragile trophozoites get destroyed before the technologist examines the faecal preparation due to delay in examination, In such a scenario, is it possible to culture (cultivate) this parasite? Describe its role in diagnosis.

A.5 Yes, it is possible to culture the trophozoite (not cyst) stage of *E. histolytica*. Culture (Cultivation) of *E. histolytica* is not required for routine diagnosis of amoebiasis. However to study the metabolic properties of the parasite, to produce antigen (for serological tests) and to screen drugs, culture may be performed. Currently axenic culture media; as Trypticase panmode serum (TPS) and TYS (Trypticase Yeast Serum-iron) medium are utilized. Axenic culture is defined as a pure culture without any other biological associate. Polyaxenic culture medium is defined; as one in which the medium has more than one microbe eg. Robinson medium.

nb: Polyvinyl alcohol can be used as a preservative in stool, if trophozoites are to be examined later on.

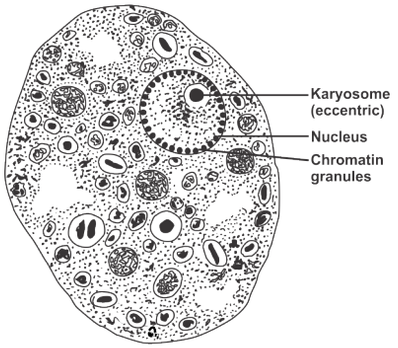

Fig.14.5.3: Schematic representation of the trophozoite of *Entamoeba coli* emphasizing nuclear details

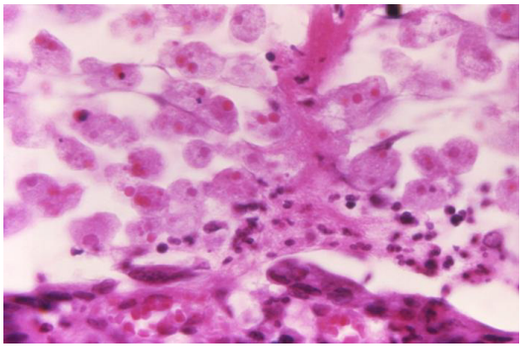

Fig.14.5.4: Photomicrograph of intestinal mucosa revealing numerous *E.histolytica* trophozoites with numerous ingested RBCs, process known as erythrophagocytosis. (500x)

Courtesy: CDC

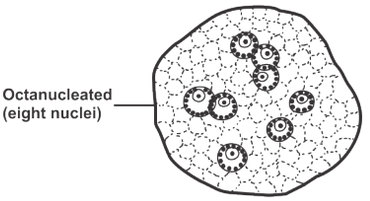

Fig.14.5.5: Schematic representation of Cyst of *E coli*

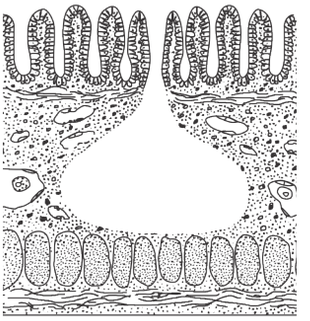

Fig.14.5.6: Diagrammatic representation of Flask shaped ulcer in Intestinal amebiasis

Enumerate the virulence factors of E.histolytica involved in causing tissue damage. Describe the pathogenesis of intestinal amoebiasis. Briefly comment on the pathologic findings in intestinal amoebiasis.

A.6 The key virulence factors are

(i) A 260 kDa N-acetyl – D – galactosamine inhibitable lectin.

(ii) An amoebapore (which can insert in the host cell membrane and produce channels or pores)

(iii) A 56 kDa cysteine protease (which can degrade protein structures).

Three steps are involved in the amebic disease. First is the adherence of the trophozoite to colonic mucosa via the 260 kDa lectin. This step is inhibitable by D-galactose. Second step is the cytolysis of the host cell. This step requires presence of calcium, pore forming enzyme, phospholipase A activity, intact acid vesicle system and intact microfilament function. The third and last step is the phagocytosis of the dead human cell.

The amebic ulcer most commonly develops in the caecum; to be followed by ascending colon, sigmoid colon, appendix and terminal ileum. Classically it is flask shaped i.e. when viewed from the mucosal surface is a small defect, but if probed inside, is a much bigger defect (Fig. 14.5.6). Microscopically the central part of the ulcer has a picture of tissue destruction with cells in various stages of degeneration. In the edge of the ulcer, you can demonstrate the trophozoites. There is a minimal acute inflammatory response and in contrast to bacillary dysentery, as mucosa between ulcers is normal.

Which form of E.histolytica can be demonstrated in the ulcer?

A.7 The trophozite stage can be demonstrated in the ulcer, as it is the active parasitic stage.

From which site in the ulcer, the biopsy should be taken, why?

A.8 The basic principle in diagnostic pathology is to take a sample from the active site i.e. the junction of the normal and pathologic tissue. In this condition such a site would be the edge of the ulcer. Taking a sample from any other site can result in a false negative result.

Amebic granulomas in large bowel; simulate carcinoma in barium enema study. Discuss the significance of this finding?

A.9 A variation in the pathology of intestinal amoebiasis (in the colon) is the amoeboma formation (instead of classical ulcer formation). As the name of entity indicates; it is a tumor like growth due to amoeba. However it is a pseudotumoral lesion with findings of inflammation. This is usually a single lesion, found in the caecum, rectosigmoid region and hepatic/splenic angles of colon. This lesion can be confused with a malignancy and ulcerative colitis (Inflammatory bowel disease). Amoebic granulomas of large intestine simulate malignancy in barium enema study. In Western countries, where tropical diseases are not common, this lesion at times has been mistakenly diagnosed; as a colonic malignancy, resulting in radical surgical procedures; as colectomy. Differentiation of amoeboma from IBD is possible, if trophozoites can be demonstrated in the lesion. It is important to correctly diagnose IBD, as treatment of choice in it is immunosuppressive therapy and if such therapy given in amebic colitis, can result in fatal dissemination of amoebae.

Does serology (antibody detection) has any role in the diagnosis of intestinal amoebiasis?

A.10 Serrological diagnosis based on demonstration of specific antiamebic antibodies has usually minimal role in diagnosis of intestinal amoebiasis, as these arise only late in infection and then persist for long periods.

Mention the role of antigen detection and molecular diagnosis in the diagnosis of intestinal amoebiasis.

A.11 Amebic antigen demonstration in stool of intestinal amoebiasis has a role in the diagnosis. Their presence as of Gal/GalNAc lectin indicates recent and active disease. The sensitivity of the technique is however low and technique often employed for its detection is ELISA.

Molecular biology techniques for detection of amoebic genome in the stool specimen are emerging as gold standard for amoebic dysentry diagnosis. However, utility in developing countries is limited, where clinicians give a *combo* antimicrobial trial for diagnosing and treatment. Details see table 14.15.1, page 657.

How do you treat the case in discussion?

A.12 See page 660, table 14.16.1

Which stage of E.histolytica is responsible for spread of amoebiasis in the community? Mention the preventive aspects.

A.13 As mentioned previously, it is the cyst stage, which is responsible for spread of amoebiasis in the community.

As far as the prevention is concerned, it is difficult to implement and improve the environmental sanitation. Health education is required as diseases spread primarily by faeco-oral route in our set up. Emphasis should be given to early detection and treatment of cases.

An Integrated Clinical Case Based Study on *E. histolytica*/Amebic Liver Abscess

6

Mukesh, a 30 years man from Aligarh, UP presented with 2 week history of pain in right flank and anorexia of 5 days duration. He gave history of loose motions of 10 days duration about 4 months back. His physical examination revealed enlarged liver, which was about 2 cm below right costal margin.

TLC was 21, 490/cu mm with 70% of these being polymorphs. Other lab findings were within normal limits. CECT examination of abdomen revealed a solitary cystic lesion in liver(Fig.14.6.1 and 14.6.2).

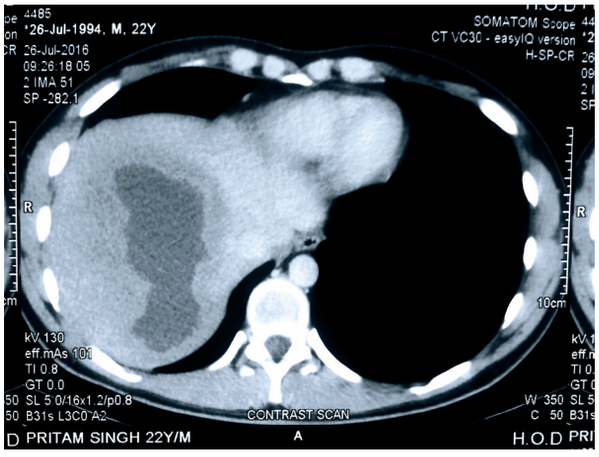

Fig.14.6.1" CECT abdomen depicting single large hepatic abscess.

Courtesy: Dr. Nikhil Talwar, RMLH, New Delhi

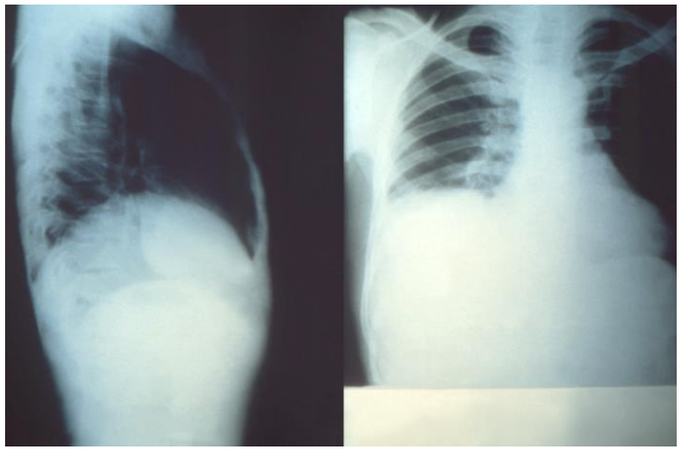

Fig.14.6.2 Hepatomegaly in amoebiasis case, Right lateral and AP views depict 'dome' of enlarged liver encroaching the thoracic cavity.

Courtesy: Dr. Trenton K.Ruebush, III; /CDC

The case has been contributed by Dr Anusha Rathi, Post graduate, LHMC, New Delhi

Linkages: Pg. 619, 626, 631, 657, 660

What is your provisional clinical diagnosis?

A.1 A young male from an endemic area for amoebiasis, with a past history of suspected amoebic colitis and with a solitiary hepatic lesion would suggest a diagnosis of amoebic liver abscess.

What microbiological test performed on a specimen obtained non-invasively can help to support the clinical diagnosis?

A.2 The serum of the case can be assayed for the titre of antiamoebic antibodies. Presence of significantly raised specific IgM antiamoebic serum antibodies (level of significant titre of antibodies can vary from place to place) can support the provisional clinical diagnosis.

Describe the key charateristics of amoebic liver abscess.

A.3 **(a)**

- Number: Mostly single lesion.
- Site: mostly posterior portion of right lobe (as it receives most of the blood from right colon through portal vein)
- Gross: The pus in this entity has been described as 'anchovy sauce' appearance. It consists of liquefied necrotic liver, (which signifies tissue destruction). The 'pus' is not suppurative in nature but mixture of necrosed tissue and blood. It is thick ,odorless and resembles 'chocolate syrup'.

– Microscopic features: it consists of degenerated liver cells, few RBCs and occasional leucocytes. The 'pus' is bacteriologically sterile. It usually contains no trophozoites, as these get localized in the cyst wall.

Why is aspiration of the amebic abscess usually not resorted to as a diagnostic or therapeutic modality?

A.3 **(b)** One, amoebic antigen can also be detected in serum of cases having active infection by ELLSA technique. Two, majority of the amoebic liver abscess cases respond favourably to antiamebic drugs and do not require surgical intervention as aspiration. Thirdly, investigations, as amebic serology (including antigen detection) and radiological investigations; as ultrasonography can diagnose correctly a majority of infected case.

If it is decided to aspirate the abscess for diagnostic purpose, which part of the abscess is likely to yield the amoebic antigen and/or the trophozoite?

A.4 The last part (or last few drops) of the aspirate is likely to be positive for amoebic antigen and / or trophozoites, as that part it is contact with the active lesion at the outer edge of the abscess. The initial and the middle parts of the drained abscess represent the necrosed tissue material.

What are the indications of aspirating a 'suspected' amoebic abscess?

A.5 Routinely it has no role in the management, however it can be considered in the following cases:

- Lack of improvement in 72 hrs. of medical management (using antiamoebic drugs).
- Markedly tender or painful liver (may indicate a bacterial infection component).
- Left lobe abscess (if ruptures can cause complications, as lies close to critical anatomical structures).
- Abscess greater than 10 cm.
- Seronegative abscess (for amoebic infection).
- Markedly elevated diaphragm and difficulty in breathing.

What are the clinical indicators that suggest a liver abscess to be having an amoebic etiology?

A.6

- Male sex.
- Insidious onset.
- History of amoebic dysentery (present in about 30% cases).
- Right pleuritic pain.
- Single hepatic lesion.

Which stage of the amoeba is responsible for causation of the liver abscess?

A.7 Trophozoite stage.

Which species of Entamoeba has been recently discovered that is indistinguishable morphologically from E. histolytica and which may explain conflicting data about the clinical profile of E. histolytica infections?

A.8 *E. dispar* is a non pathogenic amoeba unlike *E. histolytica*. It resembles morphologically *E. histolytica* and can be distinguished from it by biochemical and molecular studies. Many studies, which concluded predominant asymptomatic clinical picture of *E. histolytica* infection in past, may have actually been *E. dispar* infections.

How should this case be managed?

A.9 This case has a moderately sized abscess, which would respond very well to conventional antiamoebic drugs; as tinidazole. Amoebic abscess is an exception to the dictum, that the management of abscess must require an surgical intervention, as incision. The indications of aspirating a suspected amoebic abscess are given in A5. Open surgical drainage is very rarely indicated. It may be considered if (i) there is poor yield on needle aspiration, despite repeated attempts. (ii) very large abscess and (iii) if complicated amoebic liver abscess occurs.

An Integrated Clinical Case Based Study on *G. lamblia*/Giardiasis

7

Janak, a five year old male presented to the paediatric OPD with picture of significant weight loss. Past history of frequent diarrhoea was elicited. Three stool examinations were ordered at a gap of few days, but no abnormal finding was found; except that the stool was greasy and foul smelling. The mother on further questioning informed that the stool used to frequently float in the Western toilet.

Linkages: Pg. 616, 620, 621-623, 626, 631, 657, 660

What is your presumptive diagnosis?

A.1 The boy appears to be a case of chronic diarrhoea. The etiological agents involved in the case could be *G. lamblia* or Cryptosporidium spp. The finding of floating and greasy stools indicate that the boy is having fat malasorption (steatorrhoea). In such a scenario, the etiological agent is likely to be *G. lamblia*.

How do you explain the absence of any parasitological finding in this case?

A.2 The parasitological findings is stool are usually found in acute condition. Chronically infected cases are unlikely to have findings in stool. In giardiasis, it is well known for a case to shift from asymptomatic carrier state to acute giardiasis and chronic giardiasis or in the reverse.

The case under discussion appears to be in the chronic stage i.e., having chronic giardiasis.

What would have been the parasitological finding (in the stool) in the past in the child, when he had episodes of diarrhoea?

A.3 In case the boy was having severe acute infections, trophozoites could be demonstrated in the stool specimen. Usually the trophozoites are not demonstrable in the stool specimen, as these get lysed while exiting from the large intestine outside. In most cases, only giardia cyst gets demonstrable in the stool specimen.

If a case with provisional diagnosis of acute giardiasis presents to you and stool examination reveals no finding, how can you proceed to clinch a diagnosis of giardiasis?

A.4 The sample of choice for diagnosing a case of giardiasis is duodenal aspirate, as the protozoan resides in that part. However, obtaining a duodenal aspirate requires an invasive procedure. Conventionally during upper G.I. endoscopy, duodenal secretions can be aspirated and processed. To circumvent this problem, an *'Entero-test'* has been devised.

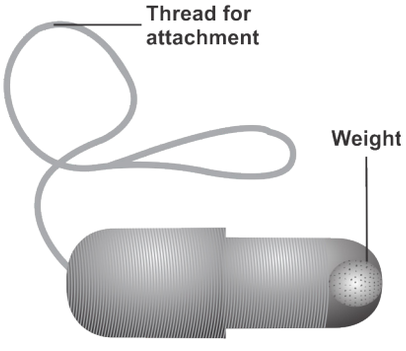

Fig.14.7.1 Entero-test

- Principle: *'Entero-test'* is also designated as a 'string test'. In this test, the patient is instructed to swallow an entero-test capsule (Fig. 14.7.1). One end of the capsule has a thread (string), which is tied to the neck of the patient. The gelatin capsule, once in the stomach, gets dissolved and releases the spool of nylon string attached to a weight. The weight guides the string to reach the duodenum.
- Procedure: After 4 hours, the thread is withdrawn and samples collected from the end to the thread.
- Quality control: to assess if the weight actually reached the duodenum, the color of the thread should be examined for presence of yellow-greenish color. If it is present, it would indicate contact of the thread with bile (present in duodenum).
- Other indications: This technique can be used to assess other upper small intestinal parasites, as Strongyloides and Cryptosporidium also.

What are the forms in which G. lamblia exists? How does this disease transmit?

A.5 See table 14.2.4, pg 620 and table 14.3.1 at pg 626

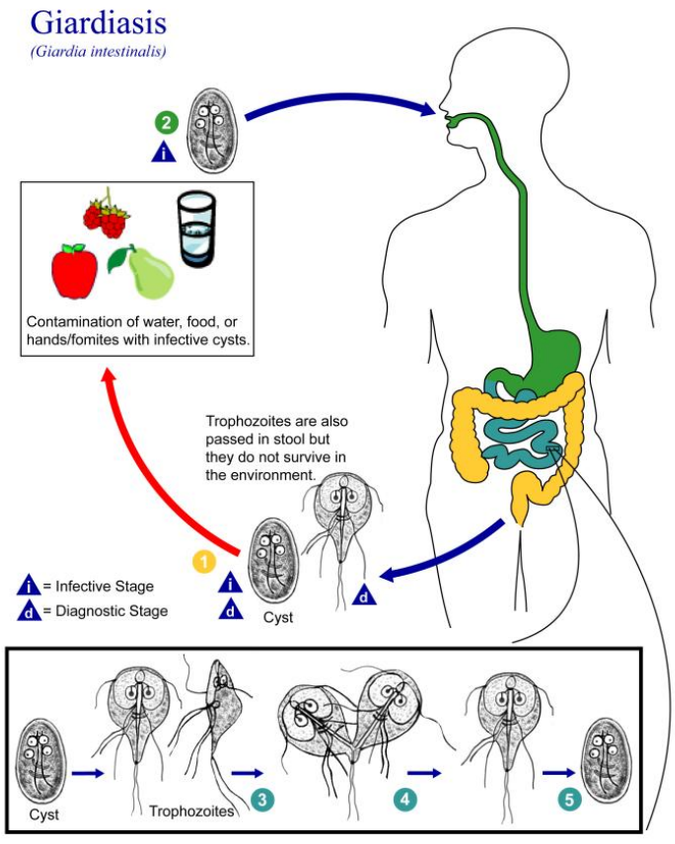

Fig.14.7.2: Life cycle of G.lamblia

Outline the life cycle of G. lamblia.

A.6 The infection starts with ingestion of cyst (Fig.14.7.2). In stomach, the acidity probably acts as a signal for the excystation to start (conversion of cyst into trophozoite). In duodenum, the excystation is complete and each cyst divides into two trophozoites. The trophozoites attach to the intestinal mucosa by the sucker on the ventral surface. Once established, giardia multiplies by binary fission. This goes on till the environmental conditions are favorable. When conditions become unfavourable, encystations starts (conversion of trophozoitc stage to cyst stage). This process begins in the jejunum. The cyst, which is a stable and resistant form, passes out with the faeces to the environment outside.

Comment on the pathogenicity of giardiasis. Make a brief mention on the immunological aspect.

A.7 Many pathogenetic mechanisms have been postulated to explain the pathogenicity of giardiasis. Varying degree of villous atrophy is seen in these cases. The atrophy has been related to decreased surface area for the absorptive purpose. Some believe that trophozoites release substances that are related to bile salt conjugation, related to fat malabsorption. Prostaglandins release has also been implicated in the pathogenicity.

Protective immunity appears to play a great role, as high frequency of giardiasis is seen in patients having hypogammaglobulinemia and rarity of disease in adults. Humoral (including secretory I_gA) and cell mediated immunity both play a part in providing protection against giardiasis.

How do you proceed to make a laboratory diagnosis of giardiasis?

A.8 The outline is given in table 14.15.1, pg 657. One thing needs to be re-emphasized is that the specimen of choice for giardiasis is duodenal aspirate, however, often reliance is placed on stool specimen for obvious reasons. Secondly, many cases who are in chronic giardiasis stage often get missed out in diagnosis.

How can giardiasis be prevented?

A.9 The priority has to be placed on treatment of asymptomatic carrier and improvement in environmental sanitation to minimize faecal oral spread.

An Integrated Clinical Case Based Study on *T. vaginalis*/Trichomoniasis

8

Rahilya Devi, a 26 year woman presented to the Gynaecology OPD with complaints of vaginal discharge, which was copious in amount, frothy and malodorous.

Linkages: Pg. 616, 620, 623, 626, 631, 657, 660

What is the presumptive clinical diagnosis?

A.1 Trichomoniasis

The doctor requisitions for examination of wet mount of the freshly passed vaginal fluid.

What finding in the microscopy would confirm the diagnosis of Trichomoniasis?

A.2 Demonstration of pear shaped trophozoites (7-30 µm in length) with characteristic twitching motility.

This parasite is unique in only having a trophozoite stage. A smear prepared from the secretion, trophozoite can be stained with Giemsa, Leishman or Papanicolaou stain.

The trophozoite has five flagellae, four anteriorly and one posteriorly. The latter forms the undulating membrane, which reaches uptil the middle of the trophozoite.

Name two other Trichomonas found in man.

A.3 *Pentatrichomonas hominis* (found in large intestine) and *Trichomonas tenax* (found in mouth)

Outline the life cycle of T. vaginalis

A.4 See figure 14.8.2

What is the status of the wet mount and staining test?

A.5 The test can also be used to examine prostratic /urethral secretion and urinary sediment.

The test can be used as a primary diagnostic tool. However, it lacks sensitivity (i.e., can miss many cases) although specificity is up to 100%.

What is considered as a gold standard test for diagnosing Trichomoniasis?

A.6 Culture of the clinical specimen (mostly vaginal fluid). This test has a higher sensitivity in many studies reaching 90% with specificity of 100%. However, the test has the limitation that it is demanding, requires 3-4 days for cultivation and if specimen contains non-viable organisms; the result would be false negative.

Two commonly used media are TYM (Trypticase Yeast Maltose media) and CPLM (Cysteine Peptone Liver Maltose media).

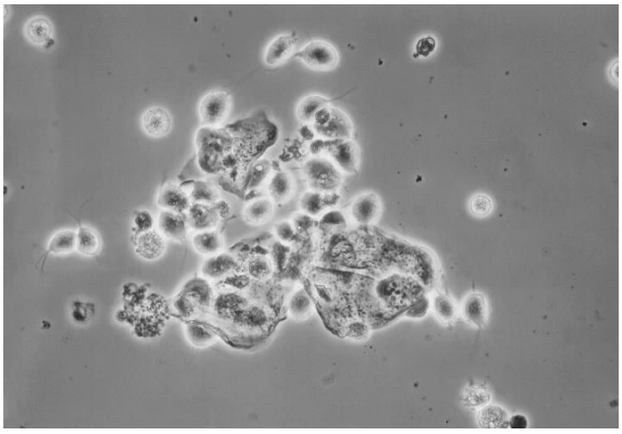

Fig.14.8.1 Photomicrograph of wet-mounted vaginal discharge specimen revealing numerous *T.vaginal*s parasites.

Courtesy: Joe Miller / CDC

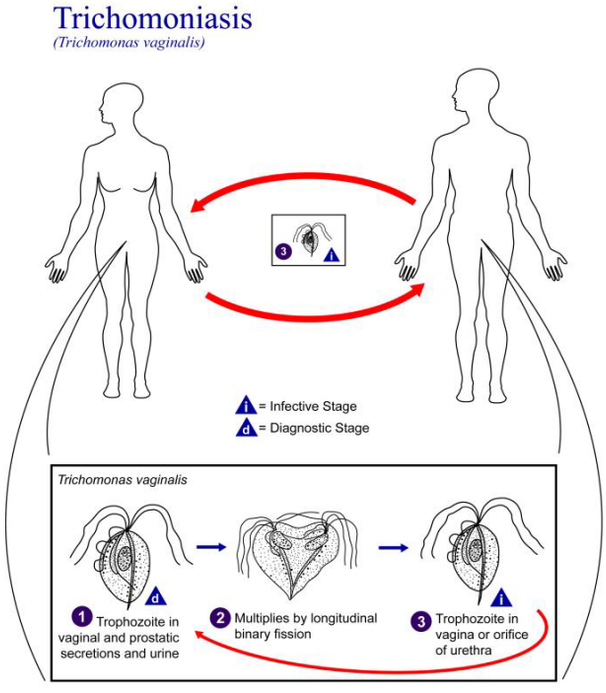

Fig.14.8.2: Life cycle of *T.vaginalis*

Does an antigen detection test exist for Trichomoniasis?

A.7 Yes. An Immunochromatographic test (ICT) is available, which uses Trichomonas protein as antigen. This test has a higher sensitivity than direct microscopy but as expected lower than culture.

Does serology (antibody detection) has any role in diagnosis of Trichomoniasis?

A.8 No (as tests can't differentiate between past and current infections).

Mention about the molecular biology based methods in diagnosis of Trichomoniasis.

A.9 PCR and Transcription Mediated Amplification (TMA) based tests are available. They have been found to have a higher sensitivity than culture and likely to replace culture as a gold standard test in future.

Highlight the clinical profile of Trichomoniasis in males.

A.10 In males, the infection is mostly asymptomatic. Occasionally it may present as urethral discharge (urethritis) and may also cause epididymitis and prostatitis. The asymptomatically infected individuals must be treated to prevent the spread of infection.

Mention briefly about the epidemiology of Trichomoniasis.

A.11 The disease has a worldwide prevalence. It is a venereal infection with prevalence correlating with number of sexual contacts. The incidence in virgins in zero and in prostitutes upto 70%.

Describe the pathogenicity of Trichomoniasis.

A.12 The disease spreads via sexual contact. It is estimated that half of the infected population is asymptomatic, hence the importance of them in transmission is of vital importance.

The trophozoites are the infective form and divide by binary fission (cyst form does not exist).

'Strawberry cervix' is a characteristic clinical sign that can be elicited during colposcopy (increased vascularity, oedema and inflammation in cervical epithelium; Fig. 14.4.3).

What clinical entities is T. vaginalis associated with pregnancy?

A.13 Pre-term labor, premature rupture of of membranes and low-birth weight babies.

What is the treatment, the woman in this case should receive?

A.14 The treatment of choice is single oral dose of metronidazole or tinidazole. Consumption of alcohol within 24 hours of this treatment can result in undesirable side effects.

Can an asymptomatic male transmit infection to this woman?

A.15 Yes. The woman has high risk of getting reinfected within 3 months of treatment. To ensure that she does not get reinfected, she must ensure that all her partners are treated. The sexual activity should be avoided till the symptoms persist and condoms may be used during sexual activity.

An Integrated Clinical Case Based Study on *L. donovani*/Visceral Leishmaniasis

9

Shanti Devi, a thirty four years female from Darbhanga (Bihar) presented with fever of 4 weeks duration, progressive weakness and abdominal discomfort. Physical examination revealed, enlarged spleen 5 cms below left costal margin and associated hepatomegaly. Laboratory investigations revealed Hb level of 7.5 gm/dl.

Linkages: Pg. 616, 620, 623, 624, 626, 631, 658, 660

What is your provisional diagnosis? Justify it.

A.1 Visceral Leishmaniasis (Kala Azar). This is a likely diagnosis, as the patient belongs to an endemic area and has suggestive clinical symptoms and signs. Spleen in a Kala azar case enlarges downward and over a course of many years can reach the pelvis.

Bone marrow aspiration was done in this case. The findings are depicted in figure 14.9.1.

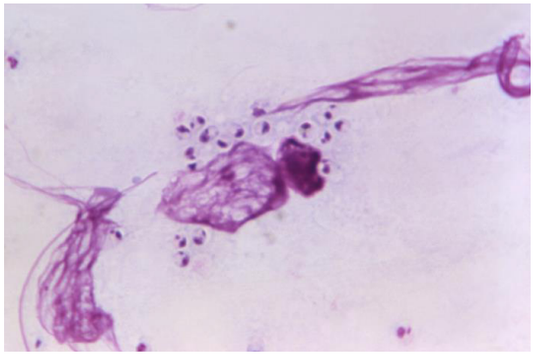

Fig.14.9.1 Photomicrograph of splenic smear, depicting L. donovani amastigotes (1125X)

Courtesy: Dr. Green / CDC

What is your diagnosis?

A.2 (a) The case is a confirmed Visceral Leishmaniasis (VL case, as the findings in this specimen of LD bodies are considered to be gold standard for diagnosis of VL.

What non-invasive test could have helped you to support the clinical diagnosis?

A.2 (b) Demonstrating specific anti-Leishmania serum antibodies against recombinant Kinesin antigen (rK39) using ICT format (test developed and commercialized by AIIMS, New Delhi).

Can L. donovani be cultured? Describe the method.

A.3 (a) Yes; *L. donovani* can be cultured

- Medium: NNN medium (Novy-MacNeal-Nicolle), biphasic medium, composed of salt agar (two parts) and defibrinated rabbit blood (one part).
- Principle: Amastigote form in clinical sample is transformed to promastigote form in the medium.
- Samples (inoculable): Aspirates, tissues etc.
- Procedure: Sample is inoculated into water of condensation and incubated at 22 – 28°C for 3 – 4 weeks.
- Observation: At weekly intervals, water of condensation is examined for presence of promastigotes (can be stained with Giemsa stain)
- Limitation: Technique may miss 25% of cases.

Describe the other diagnostic modalities in diagnosis of visceral leishmaniasis.

A.3 (b) See table 14.15.1, Pg. 658

Mention the modes of transmission of visceral leishmaniasis. Briefly mention the epidemiology of Indian leishmaniasis.

A.4 See table 14.3.1, pg. 626 (for primary mode of transmission).

Rarely transmission can occur by blood transfusion, transplantation, congenital (to foetus 'in utero'), Contact (by laboratory cultures on skin) and by coitus.

Epidemiological characteristics of Indian Leishmaniasis (i) Man is only reservoir of infection (contrast it with domestic dog being a reservoir in Eurasia & Latin America), (ii) Disease is not zoonotic, occurs by bite of sandfly, (iii) Person to

person transmissions seen. In other forms, infection acquired, when enter animal zones, (iv) Disease affects chiefly adults.

Tabulate the features of morphological forms of Leishmania.

A.5 See table 14.2.4, page 620

Outline the life cycle of L. donovani (a haemoflagellate).

A.6 See table 14.3.1, page 626, and fig. 14.9.2.

Briefly mention about the host immune response and pathogenicity in visceral leishmaniasis.

A.7 In Visceral Leishmaniasis; there is proliferation of R.E. cells present especially in the liver and spleen, which leads to massive splenomegaly and also moderate hepatomegaly. These hypertrophied organs may return to normal after effective treatment. The involvement of bone marrow in the disease is responsible for anemia and leucopenia picture. There is a significant increase of gamma – globulins (especially IgG antibodies) because of polyclonal B cell activation. This leads to reversal of normal albumin to globulin ratio.

L. donovani amastigotes are also killed by the alternate pathway of serum complement.

The parasitized macrophages are responsible for the spread of disease throughout the body. These can be induced to be killed in macrophages by the cytokines released from senitized T cells. This is essentially seen in individuals who have a primarily T_{H1} (T-helper –1 response) in which increased production of interferon γ (1FN-γ) and IL–2 activate the macrophage to kill the amastigotes. In contrast, the T_{H2} response primarily causes polyclonal B cell activation leading to hypergammaglobulinemia.

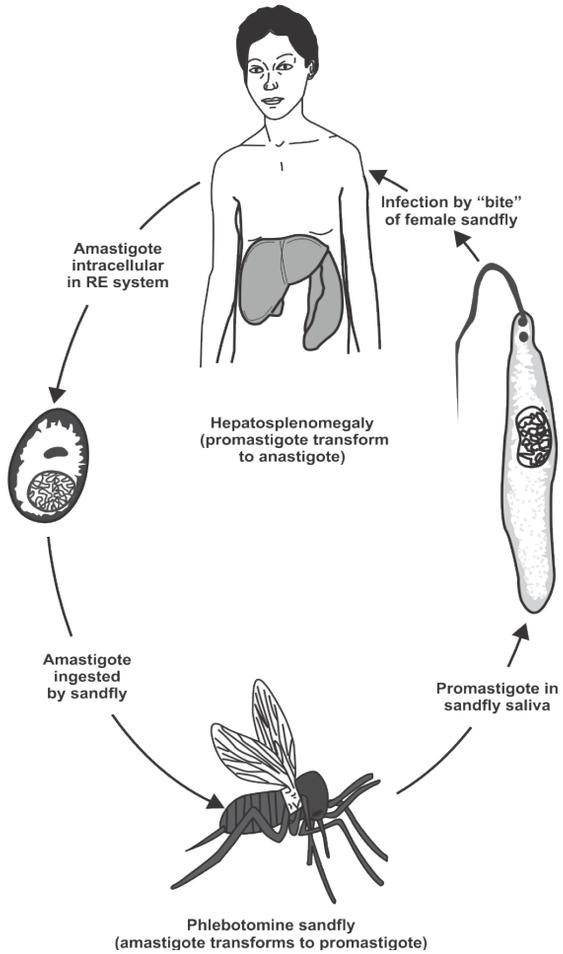

Fig.14.9.2 Life cycle of L. donovani

Briefly describe post-kala azar dermal leishmaniasis (PKDL).

A.8 As the name indicates it is a skin lesion which occurs after recovery from Kala azar (VL).

- Incidence: In 5 – 10% cases of VL.
- Follows: After few years of treatment of VL case.
- Distribution: India and African countries.
- Aggravating factor: Exposure to sunlight.
- Presentation: Starts as an erythematous butterfly rash on nose, cheeks and chin. Then can spread to arms and trunks and assume the form of nodules.
- Diagnosis: Demonstrate amastiogte forms in the skin/nodular lesions.
- Treatment: Higher doses of pentavalent antimony compounds.

How do you treat the case under discussion?

A.9 See table 14.16.1, pg. 660

Mention the preventive aspect in leishmaniasis.

A.10 Several vaccine trials are ongoing, but none is available.

National vector – borne disease control program (NVBDCP) targets control of six vector borne diseases including kala azar.

Protecting from bites of sandflies using insect repellents and bed nets. As the sandfly can not fly but only jump 30 – 35", sleeping at heights greater than it, can also be protective.

Early treatment of all cases including PKDL, to minimize the parasite reservoir.

An Integrated Clinical Case Based Study on *P. vivax*/Malaria

10

A 10 year boy from rural Delhi presented with high grade, intermittent fever of 10 days duration and petechial rash of three days duration. His physical examination revealed enlarged spleen, which was 2 cm below costal margin.

Linkages: Pg. 616, 620, 624, 627, 631, 658, 661

What is your differential diagnosis?

A.1 Dengue haemorrhagic fever, Malaria, Meningococcemia and Bacterial sepsis

What relevant investigations need to be performed in this case?

A.2 Blood culture including CRP and procalcitonin levels
Hemogram including examination for malarial parasite
Detection of Dengue NS1 antigen
Plasmodial LDH test (rapid dipstick test)

Further investigation revealed the following results

- **No bacterial blood pathogen isolated after 24hrs of aerobic incubation**
- **Hb 9.5 gm/dl, TLC 7500/cu.mm, DLC – polymorphs-52, lymphocytes-36, monocytes-5, eosinophils-7, platelet count- 43000/cu.mm.**
- **Thin blood smear did not reveal presence of any malarial parasite**
- **Serum was non reactive for NS1 antigen**
- **pLDH test was reactive (positive) for *P. vivax***

What is your diagnosis based on these findings?

A.3 (a) *P. vivax* infection with thrombocytopenia.

Enumerate the plasmodial species infecting man?

A.3 (b) *P. vivax*, *P. falciparum*, *P. ovale* and *P. malariae*

Which is the fifth species recently discovered that is able to infect man?

A.3 (c) *P. knowlesi* (primarily causes malaria in monkeys)

Why did the peripheral blood smear examination did not reveal any malarial parasite finding?

A.4 (a) Thin blood smear can miss out light parasitemia, as the sensitivity of the thin blood smear is <0.05%.

Outline the life cycle of P. vivax.

A.4 (b) See Figures 14.10.1 and 14.10.2

What is the need of qualitatively characterizing and quantitating the malarial parasitemia before initiating therapy?

A.5 It helps the clinician to characterize the type of infection and monitor the response to treatment ruling out any malarial drug resistance.

What are the common tests that can be done to characterize and quantitate the parasitemia?

A.6 Thick blood smear examination using giemsa stain (quantitating with reference to the number of WBCs cells counted) and Quantitative buffy coat examination using acridine orange (fluorescent dye)

Is it possible for this case to have a 'mixed malarial infection'?

A.7 (a) Yes. It is important to rule out a coinfection with *P. falciparum* infection, as it has an associated high morbidity and mortality.

Comment upon the periodicity patterns of fever in various Plasmodium spp. infections.

A.7 (b) *P. vivax, P. falciparum* and *P. ovale* cause fever classically at intervals of 48 hours i.e., tertian malaria; which recurs every 3rd day. *P. malariae* causes quartan malaria, which occurs at intervals of 72 hours and recurs every fourth day. *P. knowlesi* causes quotidian malaria, which occurs every 24 hours. This pattern is related to the time it takes to complete erythrocytic schizogony. However, this pattern is only rarely seen, as different lots of RBCs get infected at different times and mature (and burst) at various times.

Comment upon Tropical splenomegaly.

A.7 (c) As the name indicates it is a chronic complication of malaria, where there is massive spleen enlargement in cases, who reside in the tropics (countries in Asia and Africa including India). There is an abnormal immune response in these cases, which results in the enlarged spleen. The case often has associated hepatomegaly and elevated IgM levels due to polyclonal B cell activation. These individuals are prone to increased respiratory and skin infections, so consider antimalarial chemoprophylaxis in them.

Populations with which blood group characteristic are protected against P. vivax infection and why?

A.8 Merozoite attachment to RBCs is mediated by Duffy blood group antigen (alleles Fy^a and Fy^b) receptor (specific erythrocyte surface receptor). The absence of this antigen confers protection against merozoite invasion. Most West Africans are duffy antigen negative, hence are resistant to *P. vivax* malaria.

When is an individual categorized as a malaria Carrier?

A.9 (a) A person is considered to be a malaria carrier, when the case has ≥12 gametocytes/cu.mm of blood and the female gametocytes are in excess of the male gametocytes.

Describe 'Transfusion malaria'.

A.9 (b) This is seen in drug abusers or those who have received blood transfusion or organ transplantion. The infective form in this can be merozoites (released) or trophozoite / schizont stages in RBC development but not gametocytes. The incubation period in this is short, as there is no pre-erythrocytic stage or exo-erythrocytic stage. In treatment one should realize that there is no need of radical therapy with primaquine, as development in hepatic cells i.e., pre-erythrocytes stage is bypassed.

What is intrinsic incubation period in malaria?

A.10 Intrinsic incubation period in malaria refers to the time taken (12-30 days) for the appearance of clinical sign and symptoms of malaria after infection (i.e., bite of infected mosquito). This depends on the various periods (days) in the body; as depicted below:

Table 14.10.1: Various periods in Malarial infection

	P.vivax	*P. falciparum*	*P. ovale*	*P. malariae*
• Pre-erythrocytic schizogony	8	6	9	15
• Erythrocytic schizogony	2	2	2	2
• Asexual forms in blood (appear on)	12	9	0	0
• Exocrythrocytic schizogony	+	–	+	–
• Recrudescene	–	+	–	+
• Gametocytes (appear on)	16	21	0	0

How long a mosquito must live to become infective, after taking an infective blood meal?

A.11 This depends on the Extrinsic incubation period (9–28 days), which is the time taken for the mosquitoes to become infected after taking an infected blood meal. It implies that mosquito must live to 10th day after biting to be able to transmit malaria.

Mention the advantages and disadvantages of various techniques used to diagnose malaria.

A.12

Table 14.10.2: Profile of tests in diagnosis of Malaria

	Advantage	**Disadvantage**
Thick blood smear	• Sensitive • Picks up 0.001% parasitemia • Inexpensive	• Experience required • Underestimates true count • Artefacts

Contd.

Contd.

Thin blood smear	• Rapid • Species specific • Inexpensive	• Insensitive • Picks up 0.05% parasitemia
PfHRP2 Dipstick/Card	• Rapid • Sensitive as thick smear	• Detects only *P. falciparum* • Positive weeks after infection • Non quantitative
Plasmodium LDH Dipstick/Card	• Sensitive as thick smear • Differentiates falciparum from other three species	• May miss low level parasitemia • Non quantitative
Culture (RPMI 1640 medium, RBCs, serum)	• Can detect latent infections • Can differentiate vivax and falciparum, if only ring stage seen • Evaluate corrective action of drugs • Study drug resistance	• Never resorted in actual practice
Quantitative Buffy coat examination (Fluorescent stain by acridine orange)	• Rapid • Quantitative	• Expensive • Technical demanding
Molecular methods	• Rapid • Can differentiate species	• Expensive • Technically demanding • Sensitivity less than PBS (peripheral blood smear)
Newer techniques	• FACS • Automated system as MALDI TOF, Flow cytometry.	

Briefly explain the concept of malarial culture.

A.13 The malarial culture technique is actually a misnomer. It should be designated as a concentration technique, as only the erythrocytic cycle of malaria is amplified. It was introduced by Trager and Jensen in 1976 and uses RPMI 1640 medium (Roswell Park Memorial Institute) along with RBCs for culture. This technique is never resorted to in actual practice. But however, can be used in research institute to detect latent infection, mixed infection and evaluate role of various drugs including drug resistance studies.

What is the principle of parasite lactate dehydrogenate test? Tabulate its advantages and disadvantages.

A.14 The malarial parasite lactate dehydrogenase (pLDH) test is based on detecting specific LDH enzyme in the sample. Two formats of kits are available, pan LDH can detect all species of Plasmodium, whereas Pf LDH can only detect *P. falciparum*. These kits are basically of rapid diagnostic category and use the ICT (Immunochromatographic Technique) principle on nitrocellulose membrane.

Table 14.10.3: Profile of pLDH test for malaria

Advantages	Disadvantages
• Rapid result	• Expensive
• Skilled microscopist not required	• Low sensitivity
• Can monitor response to treatment (as enzyme is produced by viable parasites)	• False positives may occur
• Parasitemia can be graded	

What is the drug of choice for treating malaria caused by P.vivax.

A.15 Chloroquine remains the drug of choice, however for malaria caused by *P.falciparum*, artemisinin based combination therapies (ACT) are the first line of treatment

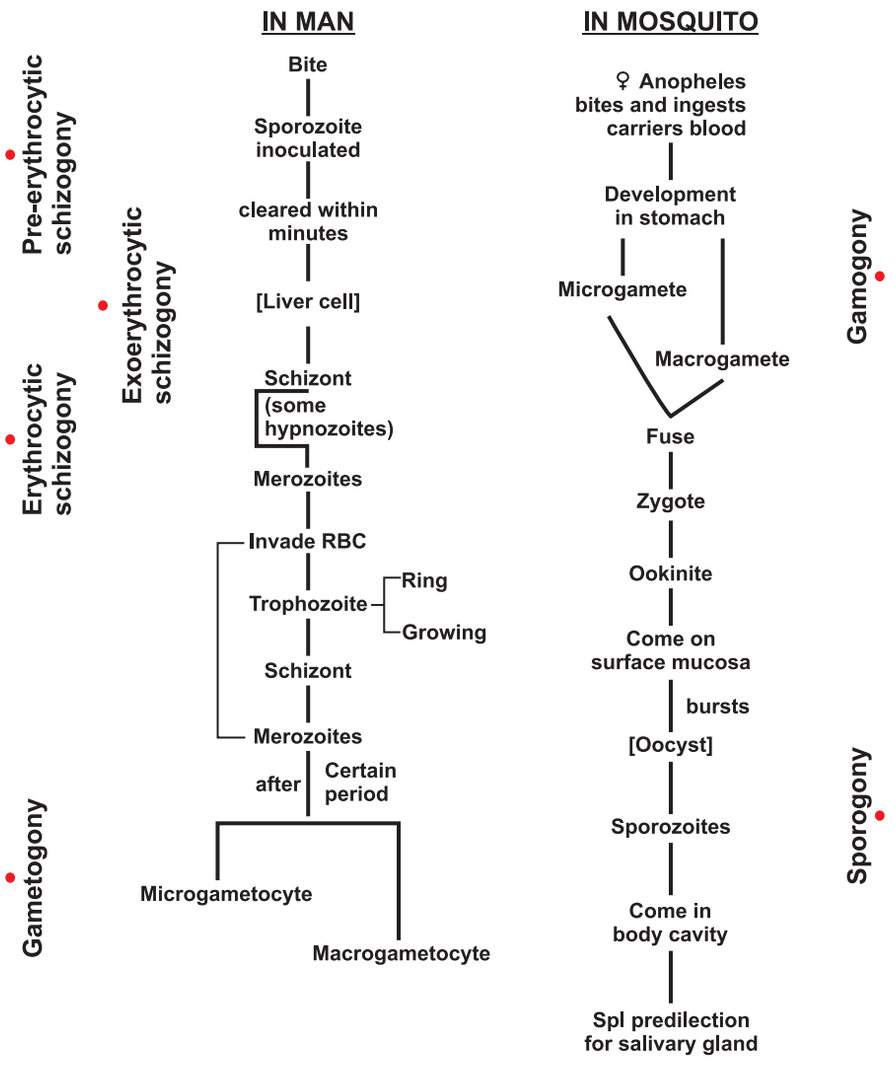

Fig.14.10.1: Life cycle of Plasmodium spp.

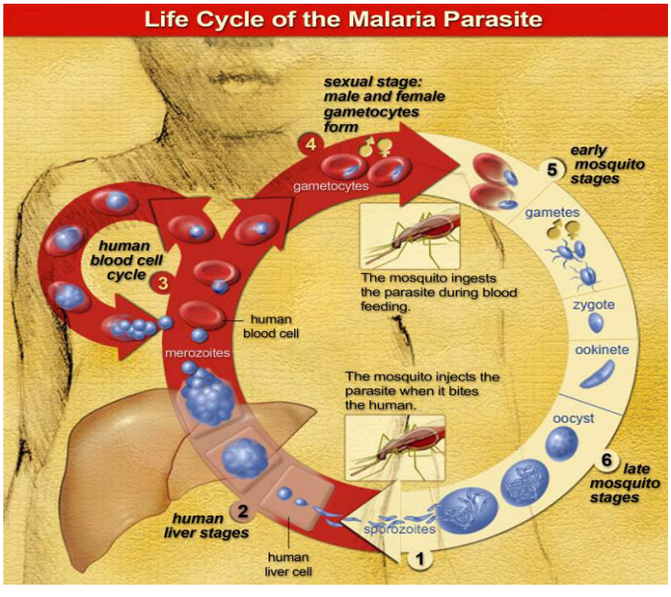

Fig.14.10.2 Diagramatic representation of life cycle of Malarial parasite

Courtesy: NIAID – National Institute Of Allergy & Infection Disease / CDC

An Integrated Clinical Case Based Study on *P. falciparum*/Pernicious Malaria

11

Sahil, a thirty five year old male presented to medical emergency with generalized convulsions and high fever. A battery of tests were performed including brain imaging, but these were inconclusive. An opinion of an Infectious disease consultant was taken. He advised a thick smear examination of blood for malarial parasite, as the thin smear had not revealed any finding. His hunch was correct, as some RBCs with multiple rings were detected in thick smear examination (Fig. 14.11.1).

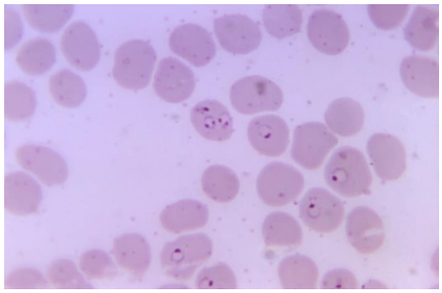

Fig.14.11.1 Photomicroraph of human blood smear depicting numerous ring forms of *P.falciparum* RBC in center contains three parasites two of which have two chromatin dots

Courtesy: Dr. Mae Merlin / CDC

Linkages: Pg. 616, 620, 624, 627, 631, 658, 661

What is the diagnosis of this case?

A.1 **(a)** Pernicious malaria (Cerebral type). It is a life threatening complication in an acute falciparum malaria (malignant tertian malaria) case, which if not treated can be fatal. It can be grouped broadly three categorise namely;

1. Cerebral malaria: The case can have convulsions, abnormal behavior and other neurologic abnormalities.
2. Algid malaria: The case has cold and clammy skin with vascular collapse, which can lead to peripheral circulatory failure.
3. Septicaemic malaria: It is a very broad category in which person has high fever with involvement of several organs.The individual can have acute renal failure, pulmonary oedema, respiratory distress, severe jaundice, bleeding disorders and many other presentations.

The case developed diarrhoea later on.

What may be the mechanism for developing it?

A.1 **(b)** The intestinal mucosal capillaries can get plugged with parasitized RBCs,this may be the mechanism for initiation. It may be noted that diarrhea in Kala azar cases can be due to infiltration of submucosa with Leishmania containing macrophages ,which can lead to mucosal ulceration and diarrhea.

When should malaria be suspected?

A.2 It should be suspected in any illness of unknown aetiology, even if the case has no fever. In non endemic areas, it should also be considered especially, if there is a history of travel to malarious zone and/or history of blood transfusion. This consideration is of utmost importance, as in the acute stage, this stage has been mistaken for TB, Cholecytitis, Yellow fever, appendicitis, Influenza and many other conditions.

What are the reasons the falciparum malaria causing greater morbidity and mortality than vivax malaria?

A.3 (i) Increased cytoadherence of falciparum infected RBCs to endothelial cells in vascular endothelium of various deep organs. This can lead to blockage of vessels of organs, as kidney and brain, leading to infarction of tissues supplied by these vessels. These infected RBCs develop knobs, which act as adhesion protein. *P. falciparum* erythrocyte membrane protein 1 (PFEMP1) is believed to play a key role as a virulence character.

(ii) *P. falciparum* can invade all ages of RBCs. In contrast *P. vivax* can invade only the reticulocytes. Thus *P. falciparum* can invade greater number of RBCs.

(iii) RBCs infected by *P. falciparum* are more rigid than RBCs infected by other plasmodial species, which leads to greater destruction in spleen, thus greater degree of anaemia.

How do you differentiate P. vivax infected RBC from one infected by P. falciparum?

A.4 The changes can be studied in two categories, namely changes in RBC and characteristic of parasite findings in the RBC. The changes in RBC size, shape and other characteristics are depicted below:

	P. falciparum	*P. vivax*
• RBC Size	Not enlarged	Enlarged
• RBC Shape	Round, sometimes crenated	Round or oval, frequently bizarre
• RBC Color	Normal, but may become darker; may have a purple rim	Normal to pale
• Stipling	Maurer's spots, appear as large red spots, loops and clefts; up to 20 or fewer.	Schuffner's dots, appear as small red dots, numerous.
• Pigment	Black or dark brown; in asexual forms as one or two masses; in gametocytes as about 12 rods	Seen as a haze of fine golden brown granules scattered through the cytoplasm

For parasitic findings, see table 14.2.4, Pg. 620

Why does late trophozoite and schizont stages of P. falciparum not get seen in peripheral blood smear of infected cases?

A.5 This development occurs in the capillaries of internal organs, hence these stages are not seen in the peripheral blood smear. However, if the individual is having heavy *falciparum* infection, then these stages do get seen in the peripheral blood smear.

Which drugs can be used to treat 'falciparum' malaria?

A.6 (a) See table 14.16.1, Pg. 661

Who got the noble prize for discovery of a drug, which contributed significantly to the treatment of malaria?

A.6 (b) Ms. Tu Youyou, a Chinese chemist discovered Artemisinin and got the noble prize in physiology or medicine in 2015. This drug is used extensively in the treatment of *falciparum* malaria.

What is a significant challenge in the management of falciparum malaria?

A.7 (a) *P. falciparum* has developed drug resistance to almost all drugs used against it.

What does drug resistance in malaria convey?

A.7 (b) It indicates that the parasite can survive and/or multiply, even after administration of an appropriate drug (and dose) to the patient.

What is the magnitude of P.falciparum chloroquine resistance in India?

A.7 (c) It is atleast 25%

What is the basis of artemisinin-based combination therapies (ACT) in malaria caused by P.falciparum?

A.7 (d) High magnitude of chloroquine resistance in P.falciparum. Artemisinin monotherapy should be avoided as it leads to resistance to this drug. Artemisinin based combination therapies should only be used.

How can drug resistance in a case under treatment be monitored?

A.7 (c) Thick smears of the patient can be prepared at regular intervals. The parasite count is quantitated to monitor, if the parasite clearance occurs and to see, if any recrudescence has occurred. Also see A.7g

Why does drug resistance occur in P. falciparum?

A.7 (d) These occur due to mutations in genes involved in metabolism of antimalarial drugs, as genes encoding transporter proteins.

What are the factors that contribute to emergence of antimalarial drug resistance?

A.7 (e) (i) poor compliance of drug (inadequate and irregular usage), (ii) Mutation in parasite (iii) host immunity, (iv) usage of drugs with longer half life, (v) Great reproductive potential of this parasite.

What strategies can be used to combat antimalarial drug resistances?

A.7 (f) Combination of antimalarial drugs are to be used (see table 14.16.1, Pg. 661). This strategy has also been used in the treatment of TB and AIDS cases. Successful antimalarial vaccines can also combat this problem.

Enumerate the indices for categorizing antimalarial drug resistance?

A.7 (g) WHO indices for assessing the degree of drug resistance (in intense transmission areas)

1. Early clinical failure:
   (a) Development of danger signs/severe malaria on day 1, day 2 or day 3 in the presence of parasitemia.
   (b) Parasitemia on day 2 is higher than day 0.
   (c) Parasitemia on day 3 ≥ 25% of count on day 0.
2. Late Clinical failure:
   Development of severe malaria from day 4 to day 14 along with parasitemia in a patient, where features of early clinical failure were absent.
3. Late parasitological failure:
   Presence of parasitemia on day 14 and fever < 37.5°C without previously meeting any of the criteria of early treatment failure or late clinical failure.

Are there individuals, who have presence of live malarial parasites in blood, but have no symptoms (Hint: premunition / Infection Immunity)

A.8 Yes. This phenomenon is seen in endemic areas; as in many regions of Africa. This phenomenon can be explained by the concept of Premunition. It implies that continuous infection by this parasite during the childhood leads to immunity against malaria, when they reach adulthood. However; if the individual moves to a non-endemic country, then the person becomes susceptible to malaria, as then frequent infection episodes are not experienced by the individual. This type of transmission is categorized as *stable*. This is in contrast to *unstable* transmission, which occurs in areas with low transmission. In these areas, complete protective immunity is not acquired and symptomatic disease may occur at all ages.

Are antimalarial vaccines available for human usage?

A.9 (a) A 'falicparum' circumsporozoite protein based pre-erythrocytic RTS, S/ASO1 vaccine has entered the phase IV trial in Africa. 'R' stands for central repeat region of circumsporozoite protein; 'T' for the T-cell epitopes of the circumsporozoite protein (CSP); 'S' for hepatitis B surface antigen and ASOI is a chemical adjuvant.

What are the broad approaches in the malaria vaccine development?

A.9 (b) (i) Pre-erythrocytic vaccines (block the entry of sporozoites in hepatic cells); (ii) Blood stage vaccines and (iii) Anti-gamete vaccines (block the development of parasite in the mosquito, if the mosquito has the anti-gamete antibodies).

Name an intraerythrocytic protozoan parasite of animals that rarely infects man. Mention its diagnostic criteria.

A.10 Babesia species. The blood smear picture is characteristic (Fig: 14.11.2 and 14.11.3)

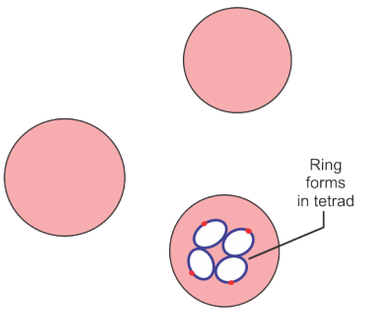

Fig.14.11.2 Diagrammatic representation of ring stage of Babesia spp. (Maltese cross form)

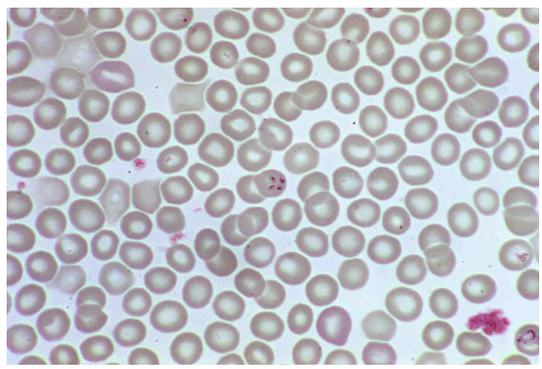

Fig.14.11.3 Photomicrograph of blood smear reveals presence of tetrad trophozoite of Babesia spp.
Courtesy: Dr. Mae Merlin / CDC

An Integrated Clinical Case Based Study on *T. gondii*/Toxoplasmosis

12

Sahil a 40 year old HIV positive male has a CD4 count of 80/μl. He is a vegetarian. He presented with headache, agitation and slurred speech. He was afebrile. CSF obtained by lumbar puncture revealed increased lymphocyte count and normal glucose and protein levels. CSF was negative for cryptococcal antigen. Brain imaging revealed multiple ring-enhancing images.

Linkages: Pg. 616, 620, 625, 627, 632, 659, 661

What is the differential diagnosis of this case?

A.1 The noninfectious causes could include malignancy of brain, as lymphoma. The infectious causes include multiple brain abscesses (from infective endocarditis lesion), CNS tuberculomas, cryptococcomas and lesions by *T. gondii* (as ring-enhancing lesions).

A brain biopsy is obtained, which was stained with Giemsa stain, it revealed numerous crescent shaped parasites (Fig.14.12.1)

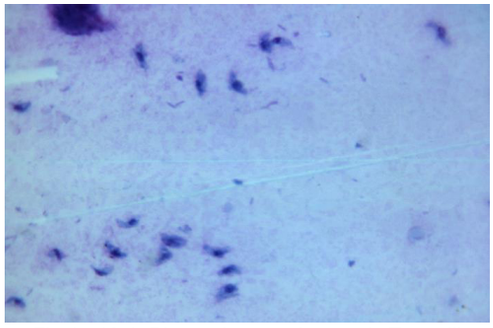

Fig.14.12.1: Photomicrograph reveals T.gondii tachyzoites, as well as a numerous cyst, the upper left corner, containing developing T.gondii bradyzoites.

Courtesy: Dr. Mae Melvin / CDC

What is the diagnosis?

A.2 Acute toxoplasma encephalitis.

Comment on the type of infection in the case.

A.3 Basically the infection type can be of two types; namely an acute infection or a reactivation of the latent infection. This infection is likely to be the latter one, as in an AIDS case, there is classically a reactivation of the latent parasitic forms.

How could this case have acquired the infection?

A.4 As the case is a vegetarian, he is unlikely to have consumed the bradyzoites present in a cyst of a meat preparation. He is likely to have consumed the infective oocyst (containing trophozoites) in the environment with food or drinks.

Outline the life cycle of T. gondii.

A.5 See Fig. 14.12.2 and Table 14.3.1, Pg. 627

Comment upon the IgG avidity test and its role in diagnosis of this toxoplasmosis case.

A.6 All serological tests based on detecting specific antibodies are indirect evidence of infection; especially IgG antibody demonstration. In this case serological tests have no role, as the radiological picture is suggestive and microscopically the parasite has been demonstrated in the affected tissue.

IgG avidity test has the ability to detecting recent infection better that the IgG or IgM based tests. Low IgG avidity, a value of <0.3 (in some formats) indicate recent infection, whereas values more than 0.3 indicate past infection.

Comment on the Sabin-Feldman dye test.

A.7

- *Status:* Considered as a gold standard in the antibody (serological) detection test category.
- Requires a lot of expertise as culture of tachyzoites, hence performed only in reference laboratories.
- *Principle:* Complement mediated neutralizing antigen-antibody test. If the serum (of patient) contains the specific antibody, more than 50 percent of toxoplasma; do not accept the stain and cytoplasm remains colorless.
- *Procedure:* Dilutions of patient's serum prepared. Toxoplasma suspension and accessory factor added to all tubes. A drop of methylene blue added to all. Incubated at 37°C for one hour.
- *Interpretation:* A titer of 128 is taken as diagnostic of active toxoplasmosis. The titer is interpreted as the highest dilution of serum in which 50 percent or more of the organism have unstained cytoplasm.
- *Limitation:* Some other parasites as *T. vaginalis* also give positive test.

How should this case be managed?

A.8 (a) Trimethoprim–Sulfamethoxazole is the drug of choice.

Primary prophylaxis for Toxoplasma encephalitis should continue till CD4 + T cell count remains below 200/µl.

Antiretroviral therapy is to continue life long.

What is the prognosis of this case?

A.8 (b) The case should be carefully monitored. The case can develop other CNS complications, as convulsions and other disabilities.

A.9 The compliance of ART treatment should be high.With good care, the case can lead a satisfactory life.

Outline the preventive strategies for Toxoplasmosis.

A.10
- Special care should be taken for pregnant women and immunologically compromised cases.
- Chemoprophylaxis with Trimethoprim – Sulfamethoxazole should be given to cases, when CD4 + T cell count falls below 200/µl.
- Contact with cat's faeces should be avoided (so that oocyst does not get ingested).
- Meat should be completely cooked to destroy the infective cysts in it.
- If feasible, transplant from seropositive donors should be avoided in immunosuppressed cases.

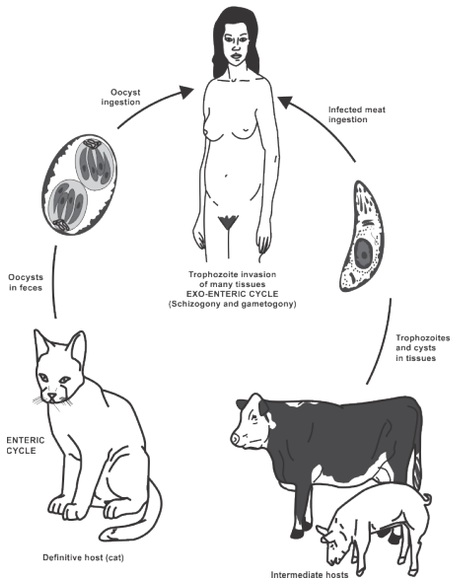

Fig.14.12.2: Transmission in *Toxoplasma gondii*

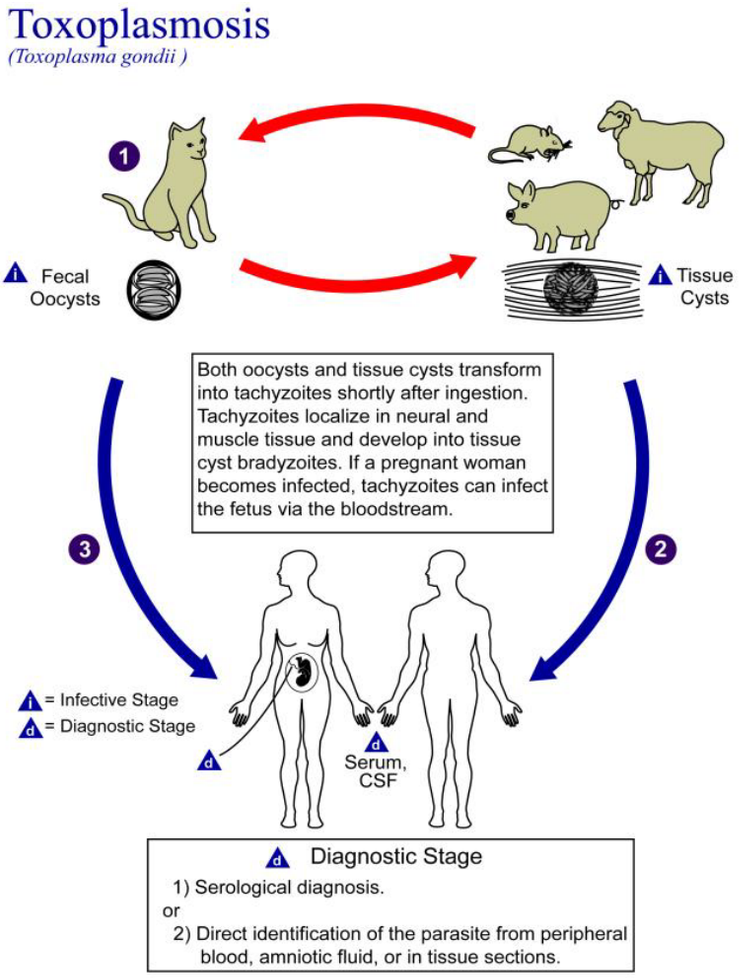

Fig.14.12.3: Life cycle of T.gondii

Courtesy: /CDC

An Integrated Clinical Case Based Study on *Cryptosporidium Spp.*/Cryptosporidiosis

13

Shahnawaz, a seventy old male having multiple myeloma, was on chemotherapy including dexamethasone for many months. He presented with watery diarrohea of three weeks duration, which was not responding to conventional antiamebic drugs comprising tinidazole. Stool examination did not reveal any parasitic finding. Culture for bacterial enteric pathogens did not reveal any significant finding.

Linkages: Pg. 616, 621, 625, 627, 630, 632, 659, 661

What approach should be followed to manage this case?

A.1 This case is likely to be immunocompromised due to the disease and the drugs being administered. In such a scenario, parasitic infections, which are associated with immunocompromised individuals should be considered. In the above scenario, cryptosporidiosis infection should be considered.

The stool specimen was stained with modified acid fast staining technique and findings are depicted in Fig. 14.13.1

What is your diagnosis?

A.2 This case was administered Nitazoxanide and responded well, further supporting a diagnosis of cryptosporidiosis in the given case.

Why was cryptosporidium finding getting missed in this case, despite the case having cryptosporidiosis?

A.3 For demonstrating cryptosporidium oocyst in the stool specimen, special acid fast staining procedure needs to be performed. Such procedure was not performed, as the microbiologist was not provided with the clinical scenario.

Depicit the life cycle of Cryptosporidium spp.

A.4 See Fig, 14.3.10, pg. 630

Mention preventive strategies for cryptosporidiosis.

A.5 As Cryptosporidium spp. can also infect animals, the exposure of infective oocysts in human/animal faeces needs to be minimized.

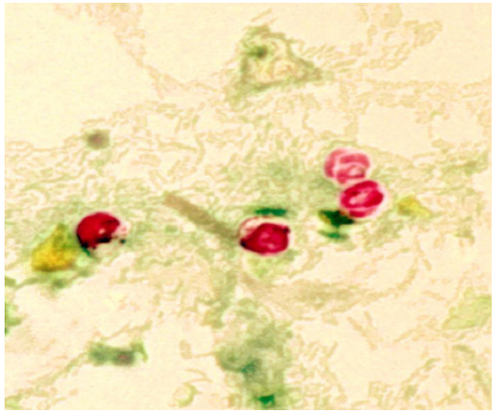

Fig.14.13.1: Morphologic details of Cryptosporidium parvum oocysts, using modified acid-fast method.

Courtesy: CDC / DPDx

What is the take home message of this case?

A.6 Any case of diarrhoea of prolonged nature in immunocompromised individual, consider the diagnosis of Cryptosporidium spp. The clinician should requisition the modified acid fasting staining to be performed on the stool specimen.

An Integrated Clinical Case Based Study on *B. coli*/Balantidiasis

14

Anjali, a 37 year old female was hospitalized on account of fever, anorexia abdominal pain episodes of loose stools with blood for 10 days. Examination of the stool revealed large motile trophozoites (approximately 60 μm length) (Fig.: 14.14.1).

Linkages: Pg. 616, 621, 627, 632, 659, 661

What is your diagnosis?

A.1 Dysentery caused by *Balantidium coli*.

With which entity, this parasitic infection could have been misdiagnosed?

A.2 Amebic dysentery could have caused a similar picture, but stool would reveal small sized trophozite (10 μm size). See Fig. 14.14.1.

Describe the life cycle of B.coli.

A.3 The infection occurs by ingestion of cysts (Table 14.3.1, pg. 627). The cysts excyst (i.e., convert to trophozoites) in small intestine. In large intestine (colon), some of them convert to cysts and pass out in the faeces.

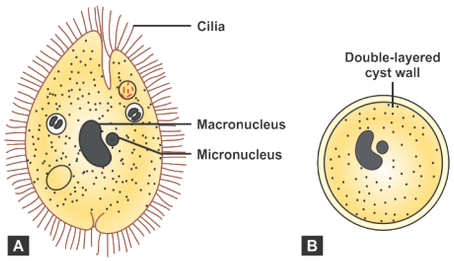

Fig.14.14.1Schematic representation of Morphological stages of Trophozoite (A) and Cyst(B) of *Balantidium coli*

If a microbiological diagnosis was not possible in this case, what would have the likely clinical scenario?

A.4 The case would have been presumptively diagnosed as amebic dysentery and got treated with metronidazole and would have responded well. For balantidiasis, tetracycline is the drug of choice.

How do you prevent Balantidiasis?

A.5 Pigs should be hygienically reared and food/water contamination should be prevented with pig and human faeces.

What is the take home message of this case?

A.6 *B.coli* is the largest protozoan and only ciliate to cause human infection. It may be considered in differential diagnosis of an amebic dysentery case.

An Overview of the Approach to Laboratory Diagnosis of Protozoans

15

Table 14.15.1: Overview of approach to laboratory diagnosis of Protozoal diseases

PARASITE / SYNDROME	SAMPLE	DIRECT DEMONSTRATION OF ANTIGEN/NUCLEIC ACID IN SAMPLE	PARASITE DEMONSTRATION	SEROLOGY	CULTURE	OTHERS (AS RADIOLOGIC)
E.histolytica / Amebic dysentery	1. Stool preferably three samples at gap of few days (can perform concentration technique as F.E. technique) 2. Material, as pus can be aspirated during procedures as sigmoidoscopy 3. Serum (for detecting specific serum antibodies) 4. Colonic mucosal biopsy (rarely) for demonstrating trophozoites	• EIA test for demonstrating Entamoeba specific antigen in stool (*coproantigen*) • A monocolonal EIA kit can detect *E.histolytica* Gal/ GalNAC lectin in stool. • Real time PCR to detect specific rRNA gene in stool. • Multiplex PCR based kit (Gastrointestinal panel in *BioFire filmarray*)	• Saline mount can detect both trophozoites and cysts. • Iodine mount can only detect cysts (as trophozoites get destroyed) • Size estimation can be done in smears stained with H&E, using micrometry. • Morphologically *E.histolytica* can not be differentiated from *E.dispar* or *E.bangladeshi* • Specific antibody based stains useful	• Demonstration of specific serum antibodies not useful in diagnosis of intestinal amoebiasis	• Can cultivate trophozoile stage in axenic media, a research technique.	• Colonoscopy (detect flask shaped ulcers)
E.histolytica / Amebic liver abscess	1. Aspirated abscess 2. Serum	• Antigen and nucleic acid based tests can be used.	• Trophozoite can rarely be demonstrated in abscess	• Specific antibodies demonstrated in serum, useful in extraintestinal amoebiasis cases	• Not much role	• CT Scan / Ultrasound of upper abdomen very useful.
Naegleria spp./ primary amebic meningoecephalitis	• CSF • Brain biopsy	• Tests available to detect specific antigen in CSF • PCR based tests available to detect specific genes	• Typical amoeboid motile trophozoites demonstrable in CSF (Fig. 14.2.3)	• Not available	• Trophozoites can be cultivated on agar culture plate with lawn culture of bacteria	• CT/MRI of brain can provide indication • CSF picture mimicks of bacterial meningitis
Acanthamoeba spp./ primary amebic meningoecephalitis	• CSF • Brain biopsy • Corneal scraping • Others as skin; contact lenses	• PCR based tests available to detect specific genes in various samples	• Characteristic trophozoites &cysts both can be demonstrable in various samples (IFAT based tests useful)	• Not available	• Above technique available	• CT / MRI scan reveals space occupying lesions
Balamuthia mandrillaris/ Granulomatous amebic encephalitis	• CSF • Brain biopsy	-	• Trophozoite & Cysts can be demonstrated (structure resembles Acanthamoeba)	-	• Trophozoites can be cultivated on tissue culture lines as Vero cell line	• CT / MRI scan of brain reveal space occupying lesion.
G.lamblia/ Giardiasis	• Stool specimen (three) • Duodenal aspirate (as parasite inhabits duodenum) • Serum	• EIA test can detect giardia antigen in stool • Multiplex PCR based kit test can detect nucleic acid of giardia cysts (G.I. panel of *BioFire Film* Array test)	• Saline mount can detect both giardia trophozoites and cyst • Iodine mount can only demonstrate the cyst • In duodenal aspirate (by entero test), only trophozoites get demonstrated • In stool, cysts usually get demonstrated however in acute infections, trophozoites also get demonstrated	• EIA & IFA tests can detect specific antibodies in serum	• Duodenal aspirate can be cultivated in axenic media as Diamonds and T.P.S. to cultivate trophozoite stage	• G.I. radiology as barium meal may be helpful in 20% cases. Thickening of mucosal fold & gut spasticity may be seen.

Contd.

Contd.

T. vaginalis / Trichomoniasis	• Vaginal secretion • Prostatic secretion • Urethral secretion • Urine sediment	• Latex agglutination, ICT and EIA techniques can detect specific antigen in samples • PCR and transcription mediated amplification techniques can detect specific nucleic acid in various samples See A7 and A9; p. 643	• Wet mount and staining is a primary diagnostic tool, characteristic twitching motility, technique lacks sensitivity See A5; p. 642	-	• Culture remains gold standard test, TYM medium used (Trypticase yeast extract maltose), culture may take 3-4 days, test would be negative, if non-viable organism inoculated	• Whiff test : Positive on addition of drop of 10% KOH, fishy odour enhanced (as amines produced)
L. donovani complex / Visceral Leishmaniasis (Kala azar)	• Blood • Bone marrow aspirate • Splenic aspirate • Liver biopsy • Lymph node aspirate • Other samples as indicated, if co-infected with AIDS • Serum	• LAT test available to detect specific antigen in urine • PCR based tests available which target Kinetoplast DNA of Leishmania	• Gold standard is demonstration of amastigole in macrophages of tissues smears (Leishman Donovan bodies; LD body) Fig. 14.9.1	• Several tests available to detect specific IgG antibodies in serum as direct agglutination test, EIA and ICT test based on rK39 antigen (recombinant)	• N.N.N. medium available for culture, but is a research tool, inoculated medium incubated at 22-24°C for 3 weeks (amastigotes transform to promastogotes)	• Leishmanin test (Montenegro test) • Delayed hypersensitivity skin test • Napier's aldehyde test (drop of 40% formalin added to 1ml of serum, jellification, a positive test indicates increased serum globulins
L.brazilensis complex / Mucocutaneous Leishmaniasis	• Skin biopsy • Mucosal biopsy • Serum	• PCR based test available	• Gold standard is demonstration of LD bodies in smears (Gicmsa / Leishman staining)	• Tests available as above	• Culture is feasible	-
L. tropica complex / Oriental sove (Cutaneous Leishmaniasis)	• Punch biopsies (Skin)	-	• Demonstration of LD bodies	-	• Culture is feasible	• Positive Leishmanin skin test (in diffuse skin disease, test may be negative)
T. brucei complex / African trypanosomiasis	• Chancre fluid, blood, L.N. aspirate, bone marrow aspirates CSF, serum	• LAT and EIA techniques available to detect antigen in serum and CSF • PCR & other molecular based tests available	• Wet preparation or smear preparation can demonstrate the trypomastigote forms (Fig. 14.2.13, p. 623)	• EIA and card agglutimation based formats available • Persistent high levels of IgM may exist	• Research purpose	-
T. cruzi / Chagas' disease	• Blood • CSF • Chagoma fluid • L. N. aspirate • Tissue specimens • Heart tissue (in autopsy) • Serum	• EIA technique can detect antigen in serum and urine of chronic Chaga's disease • PCR based molecular techniques available	• Demonstration of trypomastigotes in peripheral blood is a key test	• EIA and various other formats available to detect specific IgG antibodies	• NNN medium available, a research tool	• Delayed hypersensitivity skin test • Xenodiagnosis (allowing non-infected Reduvid bugs to feed on patient and examining the faeces of bug after few weeks for parasite)
P. vivax / Benign tertian malaria Consider malaria diagnosis in any illness of unknown etiology, disease often mistaken as T.B, Cholecystitis etc.	• Blood, serum	• Pan malarial pLDH (parasite Lactate dehydrogenase) antigen can be detected using rapid diagnostic test technology • In quantitative buffy technique, nucleus of parasite fluoresces bright green, when stained with acridine orange & examined under U.V. light. • PCR based techniques are available to qualtitatively and quantitatively characterize parasite (including speciation)	• Smear examination to detect the asexual forms of parasite is the gold standard for diagnosis (gametocytes also demonstrable) • Thin and Thick smears prepared • Giemsa / Leishman / J. S. B. stains utilized • Quality control of staining procedure essential • Thick smear can detect light parasitemia, utilized in mass screening and used in drug resistance studies (must scan atleast about 100 fields)	• Various tests available are of epidemiological value only	• RPMI 1640 medium can be used to cultivate (concentrate) erythrocytic schizogony stage, is a Research tool	• Automated systems as Flow cytometry can detect malarial parasite • Techniques available to detect drug resistance in malarial parasite
P. falciparum / Malignant tertian malaria	• Blood serum	• *P. falciparum* specific pLDH antigen detection • Pf. HRP-II antigen detection (Histidine rich protein)	• Smear examination is gold standard test See A4, p. 650	• Epidemiological role	• Research tool	• Drug resistance is common in this parasite, so needs to be assessed

Contd.

Contd.

T.gondii / toxoplasmosis (postnatally acquired)	• Blood • Body fluids • Biopsy from L.N., spleen and brain • Aspirates from L. N. and bone marrow • CSF • Bronchoaveolar lavage (in cases co-infected with AIDS)	• Antigen detection techniques less often used as lack sensitivity • PCR based techniques available to detect specific genes from various samples especially in settings of encephalitis infection	• Specimens stained with Giemsa, PAS, and silver stains • Trophozoites (tachyzoites) can be demonstrated in smears prepared from blood, body fluids and tissues, presence indicates acute infection. • Bradyzoites (PAS positive) can be demonstrated in tissue cysts, their presence does not differentiate acute from chronic infection	• Most common method for diagnosis of toxoplasmosis • Test based on IgG/IgM detection (Interpretation is key) • Sabin-Feldman dye test (considered to be gold standard) • IgG avidity test	• Can be cultivated by animal isoculation or tissue culture • Mice inoculated by intraperitoneal route, in positive case, peritoned fluid and splenic aspirate often show tachyzoites (trophozoites) after 7-10 days of inoculation	• CT/MRI show characteristic ring enhancing lesions in Toxoplasma encephalitis cases • Histopathologic findings as in L.N. biopsy can be characteristic
Congenital toxoplasmosis	• Amniotic fluid (in antenatal diagnosis) • Placenta • Cord	• PCR based techniques have role in this setting	• Trophozoite (tachyzoite) can be demonstrated	• IgG and IgM tests estimating titers • Dye test	• Can be attempted (above)	• Ultrasonography can be performed at 20 weeks of gestation and repeated subsequently.
C.parvum / Cryptosporidiosis	• Stool (three samples) • Serum **In HIV co-infected cases rarely** • Sputum • Jejunal aspirate etc	• EIA and ICT based test available to demonstrate specific antiges • PCR based tests (including multiplex version) available	• Demonstration of the cryptosporidial oocyst in the stool, using modified acid fast staining technique is the classical test (Auramine – Rhodamine staining)	• Only epidemiological role	• Only research tool	• Flow cytometry can detect oocysts • In H & E stained intestinal biopsy, oocysts can be demonstrated
Cystiosospora (Isospora) *belli* / Cystoisosporiasis	• Stool	• PCR based techniques available to demonstrate specific genes of the parasite	• Demonstration of acid – fast oocysts in the stool is the classical test	-	-	-
Cyclospora cayetanensis / Cyclosporiasis	• Stool	• PCR based tests available	• Demonstration of acid fast oocyst in the stool is the classical test	-	-	-
Balantidium coli / Balantidiasis	• Stool (three samples) • Intestinal biopsy • Ulcer scraping	-	• Demonstration of the trophozoites in the stool specimen is the classical test	-	-	• Histopathological examination of intestine can reveal various parasitic forms
Sarcocystis spp	• Stool • Muscle biopsy	-	• Characteristic sporocyst can be demonstrated in stool specimen • Sarcocysti can be demonstrated in muscle.	-	-	*-

Overview of Drugs Used Against Protozoal Infection

16

Table 14.16.1: Drugs used against Protozoal infections

Protozoa	Anti-parasitic agent	Class to which it belongs	Comments
E.histolytica	Tinidazole Metronidazole*, Iodoquinol (anti-luminal agent) Paromomycin	- Nitroimidazole - Nitroimidazole - Aminoglycoside	• Very effective for acute amoebiasis • Useful in prevention of disease transmission
Naegleria fowleri	Amphotericin B	-	Scope of improvement for treatment
Acanthamoeba spp.	Combination of drugs recommended; as pentamidine, Trimethoprim–suflamethoxazole ± Flucytosine	-	Scope of improvement for treatment
Balamuthia mandrillaris	Multiple multidrug therapy regimen considered	-	Scope of improvement for treatment
G. lamblia	• Tinidazole • Metronidazole • Nitazoxanide • Furazolidone • Paromomycin	- Nitroimidazole - Nitroimidazole - Oxazolidinone - Aminoglycoside	• Considered to be D.O.C. • Very effective • Alternative drug • Given in children • Given in pregnancy
T. vaginalis	• Tinidazole • Metronidazole** (single dose) • Treatment Kit 2 (used in syndromal approach)	-	• D.O.C. • Resistance to both drugs reported
L. donovani	• Liposomal amphotericin B • Sodium stibogluconate • Meglumine antimoniate • Miltefosine • Amphotericin B deoxycholate • Paromomycin Combination of above also used	- Antimonial compound - Antimonial compound - Aminoglycoside	• Drug of choice • Resistance reported
T. brucei	• Pentamidine isethionate • Suramin • Eflornithine • Melarosprol		Alternative drug "
T. cruzi	• Benznidazole • Nifurtimox		• Drug of choice

Contd.

Contd.

Plasmodium spp.	• Chloroquine	- 4-Aminoquinoline	• Acts against asexual RBC stages, so it useful in clinical cure and suppressive prophylactic (destroy merozoites released from liver)
	• Quinine		• Used in severe cases • Gametocoidal except *P.falciparun*
	• Artemisinin (Fig.14.16.1) and derivatives (Artemether artesunate)		• ACT-AL and ACT-SP used in treatment of falciparum malaria
	• Primaquine	- 8-Aminoquinolines	• Used in treatment of vivax and falciparum malaria, causes radical cure (as eradicates hepatic forms also)
	• Proguanil	- Folate antagonist	• Causal prophylactic (prevent maturation of sporozoites to schizonts in liver cells), not used alone for treatment
	• Pyrimenthamine	- Folate antagonist	• Suppressive prophylactic
Toxoplasma gondii	• No drug required in immunocompetent individuals		
	• Trimethoprim – sulfamethoxazole	- Folate antagonist	• Drug of choice
	• Dapsone – pyrimethamine		
	• Atovaquone ± pyrimethamine		
	• Spiramycin	- Macrolide	
		Prophylaxis if CD4 + T lymphocyte is less than 200/μL	
Cryptosporidium parvum	• No drug (mild cases as self limiting)		
	• Nitazoxanide		• DOC
	• Paromomycin	- Aminoglycoside	
Cystoisospora belli	• Trimethoprim – sulfamethoxazole		• DOC
	• Ciprofloxacin	- Fluoroquinolone	• Alternative drug
Cyclospora cayetanensis	• Trimethoprim – sulfamethoxazole		• DOC
	• Ciprofloxacin		• Alternative drug
Balantidium coli	• Tetracycline		• DOC
	• Metronidazole		• Alternative drug
Microsporidia	• Albendazole		

*Drug resistance reported against this agent especially in cases of amebic liver abscess

**in refractory cases, therapy prolonged to many weeks as if case occurs in person having co-infection with HIV

nb – ACT – AL is artemisinin combination therapy – artemether – Lumenfantrine

ACT – SP is artemisinin combination therapy – artesunate / sulfadoxine – pyrimethamine

Assessment/Examination Questions and Reference/Answer

17

Chapter – 1

1.	What is the 'take home' message of the 'Litigation case'?	A1; p. 613
2.	Define (i) Parasite (ii) Medical Parasitology	A3a; p. 613
3.	Differentiate between ectoparasite and endoparasite.	A5a; p. 614
4.	Define obligate, Facultative, Accidental andAberrant parasite.	A5b,c; p. 615
5.	Differentiate between Definitive and Intermediate host.	A7a; p. 615
6.	Enumerate the parasites in which man is an Intermediate host.	A8; p. 615
7.	Enumerate the parasites, which involve the various systems of man. Mention also their clinical component.	A9; p. 616-617
8.	Which parasitic disease has been eradicated from India?	A10; p. 617

Chapter – 2

1.	Compare the characteristics of protozoal and helminthic infections.	A1; p. 618
2.	Classify the protozoal parasites on the basis of their locomotion method.	Table 14.2.2; p. 618
3.	Write briefly on the molecular classification (2000) of protozoans infecting man.	Table 14.2.3; p. 619
4.	Enumerate some nonpathogenic protozoans.	A3; p. 619

Chapter – 3

1.	Differentiate between Reservoir and Paratenic host.	A1; p. 626
2.	Differentiate between indirect and complex life cycle.	A2; p. 626
3.	Depict the life cycles of following protozoans (a) *N.fowleri*, (b) Acanthamoeba spp., (c) Free living amoebae (d) *T.brucei*, (e) *T.cruzi*, (f) *Cystoisospora belli*, (g) Cryptosporidium spp.	Pgs. 627-630
4.	Enumerate the zoonotic parasitic diseases.	Table 3.11.1; p. 241

Chapter – 4

1.	Write briefly on Free-living amoebae (Acanthamoeba, Naegleria and others).	Table 14.4.1; p. 631, A4; p. 619, A3; p. 626
2.	Write briefly on sleeping sickness.	Table 14.4.1; p. 631, A4; p. 620, A3; p. 626
3.	Describe Chagas disease.	Table 14.4.1; p. 631, A4; p. 620, A3; p. 626

Chapter – 5

1.	Clinical scenario and analysis.	P. 634-637
2.	Describe the laboratory diagnosis and life cycle of *E. histolytica* (amoebiasis)	A3; p. 635, 657, A4 & A5 (p. 635), A10 & 11 (p. 637)
3.	Tabulate the differences between amoebic dysentery and bacillary dysentery.	A1a; p. 634
4.	Tabulate the differences between *E. histolytica* and *E. coli*.	A4; p. 635

Chapter – 6

1.	Write briefly on Amoebic liver abscess.	P. 638-639

Chapter – 7

1. Clinical scenario and analysis — P. 640-641
2. Describe life cycle of *G. lamblia* and laboratory diagnosis of giardiasis. — A5 and 6; p. 640-641, A8; p. 641
3. Write briefly on *G. lamblia* / Giardiasis — P. 640-641

Chapter – 8

1. Clinical scenario and analysis. — P. 642-643
2. Describe life cycle of *T. vaginalis* and laboratory diagnosis of Trichomoniasis. — A4; p. 642
3. Write briefly on *T. vaginalis* / Trichomoniasis. — P. 658, A5-A9; p. 642-643

Chapter – 9

1. Clinical scenario and analysis. — P. 644-645
2. Enumerate the various haemoflagellates. Describe the life cycle of *L. donovani* and laboratory diagnosis of Visceral Leishmaniasis (Kala azar). — Table 14.2.2; p. 618, A4-A6; p. 644-645
3. Write briefly on L.D. body. — P. 620, A2a; p. 645, Fig. 14.9.1
4. Write briefly on Post Kala-azar dermal leishmaniasis (PKDL). — A8; p. 645

Chapter – 10

1. Clinical scenario and analysis — P. 646-649
2. Describe life cycle of *P. vivax* and laboratory diagnosis of benign tertian malaria. — A4b; p. 646, p. 658, A5-A7; p. 647, A12-A14; p. 648-649

Chapter – 11

1. Clinical scenario and analysis — P. 650-652
2. Describe life cycle of *P. falciparum* and laboratory diagnosis of malignant malaria. — A10; p. 647, Figs. 14.10.2 and 14.10.2; p. 649
3. Tabulate the differences between *P. vivax* and *P. falciparum.* — A4; p. 650-651, Fig. 14.2.4; p. 620
4. Write briefly on cerebral malaria. — A1a; p. 650 and p. 650-652
5. Write briefly on Pernicious malaria / Black water fever. — P. 650-652
6. Write briefly on vaccine approaches for malaria. — A9; p. 652

Chapter – 12

1. Clinical scenario and analysis. — P. 654-655
2. Describe life cycle of *T. gondii* and laboratory diagnosis of Toxoplasmosis. — A5; p. 653, p. 659, A6-A7; p. 653
3. Write short note on Sabin – Feldman dye test. — A7; p. 653
4. Write short note of congenital Toxoplasmosis. — P. 632, p. 659, A10; p. 654

Chapter – 13

1. Clinical scenario and analysis — P. 655
2. Describe the life cycle of *C. parvum* and laboratory diagnosis of Cryptosporidiosis. — A4; p. 655, 659
3. Write short note on Cryptosporidiosis. — P. 655 and linkages

Chapter – 14

1. Clinical scenario and analysis. — P. 656
2. Write briefly on Balantidiasis. — P. 656 and linkages

For Single Response Assessment/Examination Questions, See Appendix 5, pg. 852

Integrated Clinical Based Study on SARS-CoV-2 Case 1

10

During May 2020, a young male was bought to the medical emergency with history of having suddenly collapsed in the public place in 2020 during the 2020 Coronavirus pandemic. He was unconscious & breathless, with heart rate of 120/minute, respiratory rate of 36/ min, BP- 150/75. On examination, he had peripheral cyanosis and bilateral basal crepts. The medical officer on duty wanted to rule out a diagnosis of COVID- 19.

The case has been contributed by Dr Garima Gautam, Jr. Consultant in LHMC, New Delhi

Linkages: Pg. 459, 632, 605, 804-808 [Chapter 11]

What rapid point of care test may be requisitioned so that the report for COVID- 19 becomes available within an hour?

A.1 An antigen detection point of care test may be requisitioned. Most of the currently available and authorized antigen-testing kits target the 'spike protein' that studs the surface or the nucleocapsid of the of SARS-CoV-2 by chromatographic immunoassays.

The test turns out to be negative. The physician still keeps a tentative diagnosis of COVID- 19. Performance of rRT-PCR test for COVID-19 takes around 7-8 hours.

His oxygen level by fingertip measurement using pulse oximeter was SpO_2- 68%. What treatment can be life saving for the case, if it is a case of coronavirus disease?

A.2 (a) Oxygen should be administered by high level nasal cannula and methylprednisolone should be given, dose depending on disease severity.

How does administration of corticosteroids become lifesaving in a moderate to severe case of COVID 19?

A.2 (b) SARS-CoV-2 gains entry by binding of its spike protein to the ACE-2 receptor in the host respiratory epithelial cells. The pathogenesis of COVID-19 (moderate to severe case) is related to the cytokine storm. As the name of the phenomenon indicates, there is excessive release of proinflammatory cytokines such as IL-6, IL-8, IL-10, TNF and many others by the immune cells. This 'cytokine storm' acts as a chemoattractant for neutrophils, CD4+ T cells and CD8+ cytotoxic T cells, which then begin to get sequestered in the lung tissue. These cells are responsible for the subsequent inflammation and lung injury. This leads to pulmonary microvascular thrombosis and haemorrhage. Pulmonary oedema and infiltrates develop in the lung, which is described as 'ground glass' appearance on chest X-ray. These changes can be halted by the corticosteroids, if administered early in the disease.

What is the timeline of COVID- 19 disease?

A.2 (c) Day 0: Infection

Day 1- 5: Asymptomatic case

Day 0-5: Onset of symptoms

Day 0- 7: Window period (only PCR test positive)

Day 7 onwards: IgM positive

Day 1-28: SARS- CoV- 2 RNA and antigens positive

Day 14: IgG positive

Day 14- 21: Decline phase (still infective)

Day 21- 28: Convalescence phase (possibly still infective)

Report of rRT-PCR for COVID-19 turns out to be positive. Due to worsening condition, the patient was intubated. His ABG showed respiratory alkalosis with PaO_2/FiO_2 of 136 (moderate ARDS). Other parameters were, CRP- >120mg/L, LDH- 600 units/L, Troponin I- <2.5ng/L, D-dimer- 1100ng/L and serum ferritin- 560ng/ml. Chest radiograph depicted patchy opacities and bilateral alveolar infiltrates. ECG showed normal sinus rhythm. Blood and sputum culture showed no growth.

What is the role of testing of parameters such as CRP, LDH, D-dimer, troponin and serum ferritin in this case?

A.3 (a) These are prognostic markers of COVID-19 disease. High values of these parameters are risk factors for more severe disease, thromboembolic complications, myocardial damage, and/or worse prognosis.

What can be the cause of death in this case?

A.3 (b) The cause of death in this case could be hypoxemic respiratory failure ,which can lead to acute respiratory distress.

Describe the rRT-PCR test used for COVID- 19.

A.4 (a) rRT-PCR stands for real time reverse transcriptase polymerase chain reaction.

- Status- Considered to be a gold standard test for diagnosis of COVID-19.
- Set-up required- Biosafety level- 2 facility (BSL-2).
- Specimens of choice- Nasopharyngeal swab, Throat/ nasal swabs collected and immersed in viral transport medium (VTM), after collection.
- Specimen collection and transport- The person who collects the sample must don appropriate PPE kit including N95 respirator mask to prevent getting infected. The specimen must be transported in triple layer packing to prevent environmental contamination with the virus. The cold chain should also be maintained during transport to the lab to prevent lysis of the virus.
- Principle of the test- The viral RNA in the sample is converted into complementary DNA by the reverse transcriptase enzyme. The converted DNA is subsequently amplified by real time PCR technology.
- Sequence of testing- In the screening assay, genus specific gene is targeted such as spike protein (S)/ envelope protein (E)/ membrane protein (M)/nucleocapsid protein (N). If the test turns out to be positive, then species specific gene are targeted such as ORF1a/b gene or RNA dependent RNA polymerase (RdRp) gene for confirmation of COVID- 19.
- Interpretation- The PCR test (NAAT, nucleic acid amplification test) usually becomes positive after 5 days of infection and starts becoming negative after third week. The report is expressed as Ct value, which stands for threshold cycle. It is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e., intersection between the amplification curve and the threshold line). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. These values are relative. Values between 12-36 are considered positive, values between 36-40 as inconclusive/marginal and those greater than 40 are considered negative (Fig 17.10.1).

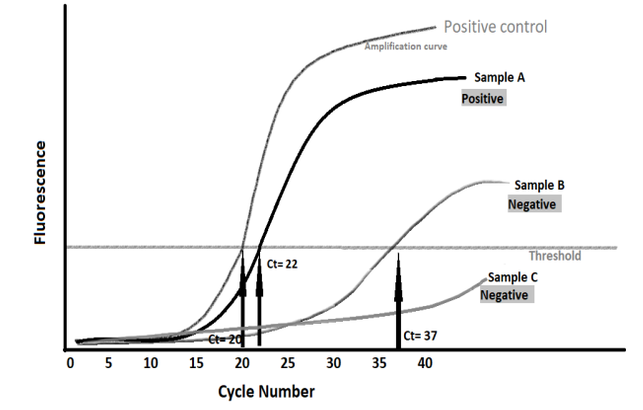

Fig. 17.10.1: Interpretation of rRT-PCR test

What are the other alternative nucleic acid platforms available for testing COVID- 19?

A.4 (b) Two commonly used alternatives are Cartridge based nucleic acid amplification (CBNAAT) and *TrueNAT*. The former system was initially used in the diagnosis of Tuberculosis and has the advantage of providing results within an hour. *TrueNAT* has the advantage of minimal requirements and can be run in a primary health care system.

What is the role of serology and viral culture in diagnosis of COVID- 19?

A.5 Viral culture doesn't have much role in diagnosis, however has a role in studying the pathogenicity of the disease and in vaccine development. Serological tests identify the presence of humoral response to SARS-CoV-2. Antibodies of IgA, IgM, and IgG isotypes specific to different virus proteins are detected by ELISA or chemiluminescence immunoassays (CLIA). IgM antibodies appear usually from 5th day of symptoms, whereas IgG usually appears from tenth day of symptoms onset. These tests are not appropriate for the early diagnosis of COVID-19. They are, however, relevant when RT-PCR is not available or is negative in the face of a suggestive clinical picture, or to assist in the diagnosis of COVID-19-related multisystemic inflammatory syndrome. Antibodies correlation with the virus's neutralization capability is yet to be fully understood.

Is there any definitive therapy available for COVID- 19?

A.6 There is no definitive therapy available for COVID-19. Remedesivir (which inhibits viral RdRp), Lopinavir/Ritonavir (protease inhibitors) have been used but their efficacy and safety needs to be further established. Recently monoclonal antibodies as Casirivimab have been approved by DCGI for emergency usage.

- Initially, hydroxychloroquine was suggested to be helpful for treating hospitalized patients with mild cases of COVID-19, though the current consensus from national health institutions state that it does not work for preventing or treating COVID-19.
- Convalescent plasma therapy (involved collecting plasma from people who have recovered from COVID-19 and transfusing into patients with severe coronavirus disease) was thought to help fight the coronavirus infection but did not give good results.

Mention about the currently available vaccines against coronavirus available in India.

A.7 (a)

	Covaxin	***Covishield***
Developed by	Bharat Biotech,ICMR	Serum Institute of India
Type	Inactivated	Non replicating viral vector
Efficacy	81% approx	91% approx
Duration of protection	6 months to 1year	6 months to 1 year
Monitoring efficacy of vaccine	Anti spike neutralizing antibody profile useful	Antispike neutralizing antibody profile useful
Effectivity against mutants	Present	Present
Age approval	18 years and above	18 years and above
Side effects	+	+
Approval	Emergency usage	Emergency usage(greater acceptance internationally)
Doses	2	2(gap of 2-3 months)
Adminstration	i/m	i/m
Cost	Borne by GOI	Borne by GOI

Mention about a concern of a mRNA Covid-19 vaccine.

A.7 (b) A mRNA vaccine manufactured by Pfizer based on gene editing mRNA technology is available. There is concern about it having a bearing on the human genome.

Integrated Clinical Based Study on SARS-CoV-2, Case 2

11

During March 2020, Sally (name changed) returns from USA to visit her parents in Civil Line area of Delhi. After 10 days, her mother who is a diabetic, becomes sick and is diagnosed to be having COVID -19. Testing also reveals Sally to be infected with COVID- 19 but she remains healthy. Her mother gets admitted in ICU of a leading private hospital. Despite best treatment, she succumbs to the infection.

'चमगादड़ का सूप चीन के लोग पीते रहे और परेशान उन लोगों को कर दिया जो ज़िंदगी भर पूछते रहे केक में अंडा तो नहीं है।'

Message on social media 2020

The case has been contributed by Dr Garima Gautam, Jr. Consultant in LHMC, New Delhi.

Linkages: See linkages of Chapter 10 (p. 801-803)

Discuss the above scenario.

A.1 (a) Sally is an asymptomatic carrier of COVID-19 infection. The infection likely spread to her mother from herself. Her mother had co morbidity condition of old age and diabetes, so gets the COVID-19 disease and succumbs to the infection.

How does COVID- 19 get transmitted?

A.1 (b) SARS-CoV-2 is transmitted primarily by droplet (respiratory) transmission. Contact transmission directly or indirectly (through inanimate objects) is also known. However, aerosol transmission is not documented.

Briefly describe the classification and structure of SARS-CoV- 2.

A.2 (a) (i) SARS-CoV-2 belong to the family *Coronaviridae*, which has been divided into two subfamilies, namel *Coronavirinae* and Toronavirinae. The former comprises of four genera- α, β, γ and δ. Most of the key coronaviruses belong to Betacoronavirus.

(ii) Coronavirus particles are spherical with a diameter of approximately 80 to 160 mm (Figures: 17.11.1 and 17.11.2). The envelope surface is covered with spike (S) protein, and the membrane (M) proteins and envelope (E) proteins. The spiral nucleocapsid (N protein) is located within the envelope. The coronavirus genome is comprised of a ss positive sense RNA about 30 kb pair in length.

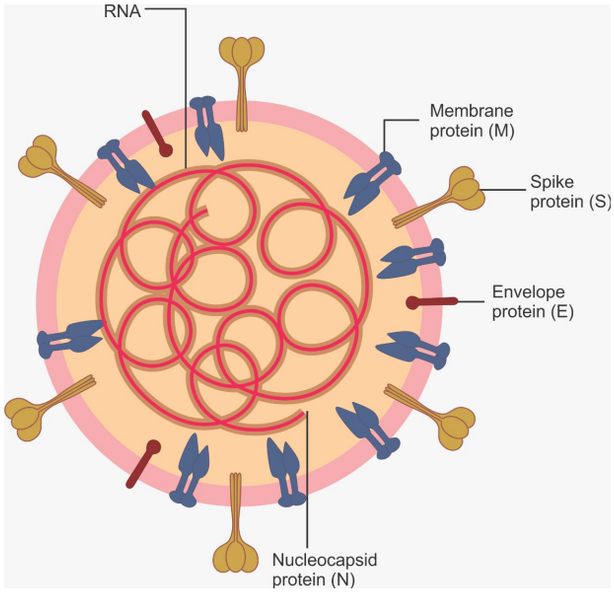

Fig. 17.11.1: Diagrammatic representation of SARS-CoV-2

What are the clinical features of COVID-19 infection?

A.2 (b) (i) Fever, muscle/ body aches with respiratory symptoms such as cold, sore throat, cough, chest pain and shortness of breath.

(ii) Gastrointestinal symptoms are seen in 2-40% of patients, with diarrhoea being most common manifestation.

(iii) Taste or olfactory disorders such as new anosmia seen in up to 55% of the cases.

(iv) Cardiovascular events that have been associated with covid-19 include myocardial injury, myocarditis, cardiac arrhythmias, heart failure (especially in patients with severe infections).

(v) Some neurological syndromes have also been associated with COVID-19 disease such as ischaemic or haemorrhagic stroke, dizziness, headache, musculoskeletal disturbance and altered mental state.

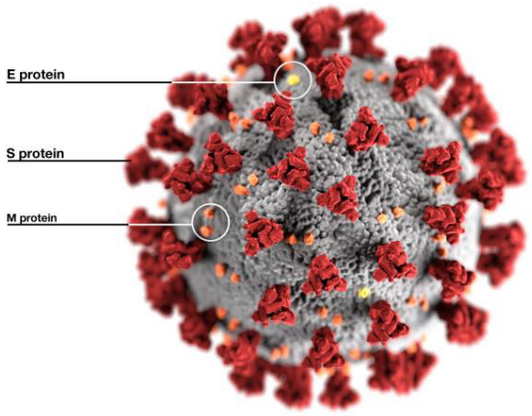

Fig.17.11.2: Illustration of SARS-Cov-2,depicts the spikes on the outer surface of virus, imparting the look of corona surrounding the virus. Protein partcles E,S and M are present on the outer surface of the particle.

Courtesy: Alisa Eckert ,MSMI and Dan Higgins, MAMS/CDC

Outline the key events of the COVID- 19 pandemic.

A.3 (i) Emergence of coronavirus disease in December 2019 in China. (majority of cases had direct/ indirect contact with Wuhan seafood market).

(ii) Outbreak recognised, viral genome sequenced and shared internationally.

(iii) SARS-CoV-2 nomenclature announced by ICTV-II – Feb-2020.

(iv) In January 2020, India reported its first case of COVID-19. Also declared as Public Health Emergency of International concern by WHO.

(v) WHO declares COVID-19 as a pandemic in March 2020.

(vi) Epidemic disease act (1897) enforced in India.

(vii) National Disaster Management Act (2005) invoked (in India). State governments took various measures to contain the spread of the virus. On 13 March, 52 labs were recognized to conduct the viral testing.

(viii) Nationwide lockdown on 24th March, 2020 (one of the biggest in the world).

(ix) Ordinance (law) passed in parliament on 23th April, 2020 for 7 years jail/ five lakh rupees fine for attack on health care workers.

(x) Rollout of national Coronavirus vaccine on 16th January, 2021.

What is the global and Indian situation (as of early June 2021)?

A.4 Globally more than 172 million (approximately) cases of coronavirus have been reported with more than 3.7 million deaths. In India, more than 28.4 million cases of infection with more than 3.38 lakh deaths have occurred.

What have been the key social consequences (including economical) consequent to the occurrence of this pandemic?

A.5 (i) Thermal scanning and hand sanitization practices started at metro, malls, restaurants etc. Public transport started getting sanitised daily.

(ii) Prisoners at some places freed conditionally as in Iran and India.

(iii) Many cruises stranded after passengers test positive, such passengers were offloaded and deferred from flying (who tested positive).

(iv) FIRs were registered against persons for concealing information or refusing to get quarantined.

(v) e-learning started due to countrywide lock-down of schools and colleges. Revised guidelines for conducting examinations released which included maintaining a social distance of 1 metre and not more than 24 students in one class.

(vi) No biometric attendance (to decrease finger contact).

(vii) Film shooting stopped in Mumbai. Cinema halls were shut down by most state governments.

(viii) Concept of work from home started.

(ix) Electronic transaction of money encouraged (many places refused to handle currency notes as Metro stations).

(x) Ventilator splitting was permitted in US and other countries due to shortage of this equipment.

(xi) For a period, Yuan became on par with US dollar.

(xii) All mail/courier/newspaper & magazines distribution got stopped for some time.

(xiii) Many hospitals got totally closed after a staff member unknowingly got exposed to COVID-19 case/tested positive.

(xiv) Mass exodus of workers on foot, as no transport was available (many walked thousands of kilometres to go back to their native villages).

(xv) Universal screening for SARS-CoV-2 started before conducting surgical procedures including deliveries.

(xvi) Online (video) medical consultation services started.

(xvii) Ban on tobacco products suggested to curb spitting.

(xviii) When parks were opened, 65yr and above were not permitted.

(xix) Virtual gym classes, e-court services at places (i.e., no physical appearance required). At places compulsory COVID testing required before producing accused to the judge.

(xx) Hybrid session for monsoon 2020 session of parliament held (both online & offline).

(xxi) Train coaches converted into isolation wards for COVID-19 patients.

(xxii) Dentistry speciality almost got closed.

(xxiii) In IPL 2020 (in Abu Dhabi) a concept of Bio-secure bubble environment introduced including wearing of headbands to avoid flicking sweat onto the ball, digital cheerleaders and canned screening of joy and agony.

(xxiv) A UN report estimated a trade impact of US$348 million on India due to the outbreak, making India one of the 15 worst affected economies across the world. Indian stock markets suffered their worst crash since June 2017.

What were some events that caused scare during COVID-19 pandemic?

A.6 (i) There were rumors that in certain part of the world, there were forcible rounding of sick cases (having fever) and putting them in detention.

(ii) It was also rumored that in one place a court approval was taken to kill thousands of Covid-19 patients to avoid further spread of infection (detection of unusual sulfur emissions in the area pointed towards mass cremation).

Was the spread of infection to Sally's mother preventable? If so, how?

A.7 Yes. Sally should have got herself tested for COVID-19 on returning to India. She should have isolated herself on turning out to be positive for the infection.

What are the four main stages in a disease outbreak?

A.8 (a) (i) Cases are of imported origin (not local)

(ii) Local transmission of cases get reported

(iii) Community transmission occurs

(iv) Epidemic gets declared (spreads to entire population)

What is R 0?

A.8 (b) 'R- naught' is the number of people one coronavirus patient will infect on an average. For Covid-19, the value varies from 1-4 to 1-8, whereas for SARS, it is in the range of 2-4 and for measles, it is estimated to be as high as 18.

How do you define a COVID-19 contact?

A.8 (c) A contact is a person who has experienced any one of the following during the first two days before and till fourteen days after the onset of symptoms of a probable or confirmed case

(i) face to face contact within 6 feet of an infected person for a cumulative total of 15 minutes or more over a 24-hour period.

(ii) direct physical contact with probable or confirmed case

(iii) Direct care for a patient with probable or confirmed COVID-19 disease without using proper protective equipment, or other situations as directed by local risk assessment.

What is the difference between isolation and quarantine?

A.8 (d) Isolation separates the sick persons with a contagious disease from people who are not sick. Quarantine separates and restricts the movement of people who are exposed to a contagious disease to see if they become sick. It aims to prevent the disease ,if they develop subsequently.

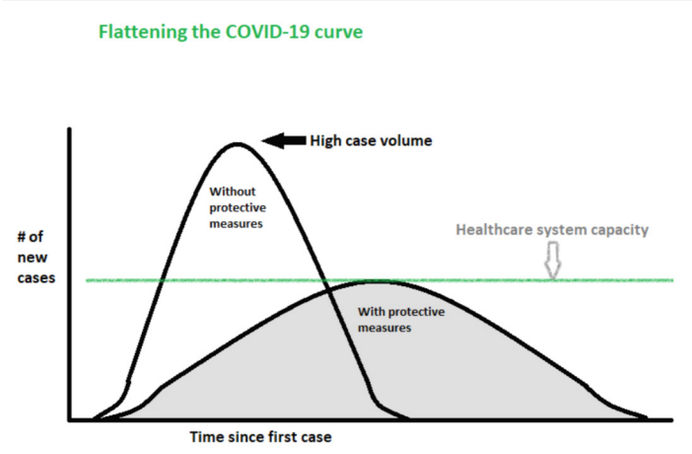

Fig. 17.11.3: Concept of 'flattening the curve'

What is the basis strategy to control COVID-19 pandemic?

A.9 (a) The strategy is to have early identification of community transmission of infection and enforce effective containment to slow spread of infection i.e., test, trace, isolate/quarantine and treat.

What is the concept of 'flattening the curve' in the control of COVID-19?

A.9 (b) It implies that in a community the curve of spread of infection should become flat instead of being a steep one (Fig: 17.11.3). That means if the same number of people get sick, the infections should occur over a longer span of time, so hospitals would not get packed with patients and could treat everyone.

At the global level, at some places there was a difference in the strategy to deal with the COVID 19 pandemic. What is the unique concept in the UK approach?

A.10 (a) It states that at least 70% of the population must contract COVID-19 in order to develop herd immunity and prevent transmission in future. So, it implies that strict lockdowns are only acting as temporary measures, as soon as they are relaxed, infections are bound to occur with a strong comeback. So, one needs to protect mainly the vulnerable population.

What is the objective of lockdown?

A.10 (b) Lockdown implies limiting of movements of the entire population as a control measure against a pandemic.

What is the concept of cluster containment?

A.10 (c) It aims at containing the disease within a defined geographical area by early detection of cases, breaking the chain of transmission and so preventing the disease spread to new areas. It involves locating the index case/cluster and listing and mapping of contacts.

What is the basis of 20 seconds hand wash with soap foam and hand rub with alcohol?

A.11 Soap foam is believed to dissolve the lipid covering around the SARS-CoV-2. Seventy percent alcohol destroys the viral protein.

What was the silver lining of the Covid-19 pandemic?

A.12 (i) Less accident deaths, as decreased traffic on roads.
(ii) Decrease in respiratory illness, as less pollution.
(iii) Boost to infection control, as increased hand hygiene, decreased outside eating etc.
(iv) Increased home temples, as community temples were closed.

Compare and contrast the characteristics of SARS, MERS & COVID-19.

A.13

	SARS	MERS	COVID-19
• **Etiological agent**	SARS-CoV**	MERS- CoV	SARS-CoV-2
• **Year of detection**	2002	2012	2019
• **Place of origin**	China (Guangdong)	Saudi Arabia	China (Hubei)

• **Possible source**	Bats	Dromedary camels	?
• **Transmission**	Droplets	Zoonotic (direct or indirect)	Droplets and contact
• **I.P**	2-7 days	2-14 days	2-14 days
• **Primary receptor**	ACE 2	Dipeptidyl peptidase 4	ACE 4
• **Pathogenicity**	Involves Respiratory tract (upper & lower) and GIT	Involves Respiratory tract (upper & lower)	Involves Respiratory tract (upper & lower) and GIT, cardiac system
• **Mortality rate**	10%	37%	1.5%
• **Treatment**	Symptomatic	Symptomatic	Various drugs under trial
• **Vaccine**	NA	NA	Available

**Dr Carlos Urbani diagnosed the first case of SARS. His commitment to contain the disease and case management led to his exposure to the virus, resulting him contracting the disease and succumbing to the dreaded disease.

nb: ACE 2 is Angiotensin converting enzyme 2.

Vaccines

15

– **'Childhood vaccines are one of the greatest triumphs of modern medicine.Indeed parents whose children are vaccinatedno longer have to worry about their child's death or disability from whooping cough, polio, diphtheria, hepatitis and a host of other infections'.**

— Ezekiel Emanuel

Let's study the BACTERIAL vaccines organized in a tabular fashion..

Bacterial Vaccine Types	Composition		Indications	Route & Dosage Schedule	Mechanism	Effectiveness	Adverse Effects	Contraindication/Special Precaution*
Pneumo-coccal	• Monovalent composed of single Serotype -23 valent 23 serotype (most prevalent)-PPSV-23 -Heptavalent protein (7 serotype) conjugated to protein	• Capsular polysac-charide	• Those at increased risk adults > 65 years old adults & children > 2 yrs. with chronic disease of Heart, Lung, Diabetes, alcoholism, asplenia & sickle cell disease; • Immunocompromised individuals for example with Hodgkin's disease, lymphoma, multiple myeloma, AIDS	• Parenteral s/c or i/m 25 µg/ml of each type,single dose	• Antibodies elicited against the different serotypes, opsonizes the bacteria, which are then more efficiently phagocytosed. Also effective in children below 2 years	• Immunity appears after few weeks of vaccination & lasts for 5 years, Boosters not considered necessary. Some studies, efficacy of >90% in reducing invasive pneumococc-cal disease. Reduction also in Otitis media & penicillin resistant pneumococcal strains	• Local reaction: swelling redness, pain • General: Fever, rarely neural disorders as Guil-lain Barre syndrome, anaphylaxis (rarely)	• Acute febrile illness • Severe reaction; as neurological or anaphylactic to any component of vaccine • Pregnancy, Children less than 2 yrs. age (unsatisfactory response as is a polysaccharide component) • Malignancies; as lymphomas
Meningo-coccal	• Bivalent A,C • Tetravalent A-C-Y-W135 • Conjugate protein poly-saccharide tetravalent vaccine	• Capsular Polysac-charide (50 µg of each antigen)	• Population at risk during outbreak (children above 2 years, below this age the vaccine is poorly immunogenic except for group A>- 3months) • All pilgrims to Mecca for Haj -Miltary recruits -Individuals with diseases as sickle cell anaemia	• parenteral i/m	• Antibodies elicited, opso-nizes bacteria, which are readily phagocytosed	• Immunity appears within few days of vaccination & lasts for 3 years. Boosters are mandatory after 3 years Cost is a limiting factor in use Effective also in children below 2 years (conjugate vaccine)	• Local: Swelling, redness, pain • Systemic: rarely anaphylaxis	• Allergic severe reaction to dry natural rubber • Acute febrile illness • Children <2 years of age, unless conjugate vaccine • Age>55 years • H/o GBS (Precaution)
Diphtheria	• Toxoid (25Lf) • Other forms In combination with per-tusis & tetanus (DPT) • Future: CRM197 (Cross reacting material) recom-binant, vaccine, (synthetic antigen)	• Diphtheria Toxoid	• Routine in Immunization programme	• Parenteral, i/m Children 3 doses starting at 6 weeks of life Booster at: 18 months and 5 years	• Does not eliminate carriage of organism (*C. diphtheriae*) in pharynx or skin • Antibacterial antibody is of no significance • Immunity depends solely on the presence in blood & intestinal fluid of an IgG anti-toxin which forms an antigen - antibody complex, which prevents the toxin from binding to target cell. This complex is readily phagocytosed	• Nearly 100% Schick conversion rate	• Children, Allergic-hyper-sensitivity • Adults: Same as in paediatric except that incidence & severity is far greater in adult population	• No special contraindication except that should be restricted after 6 yrs. older children as are more likely to be sensitized to diphtheria antigens, thus offering a higher rate of adverse effects

Contd.

Tuber-cular	• Bacillus calmette Guerin (attenuated *M. bovis* strain) (developed by Albert Calmette and Camille Guerin in 1906) • New vaccine candidates include recombinant BCG Protein & DNA vaccines	• Strain subcultured 239 times in glycerol-bile-potato medium over a period of 13 years	• Routine in Immunization programme • Revaccination at school entry or those who are tuberculin negative • All family contacts of open tuberculin cases, if tuberculin negative • Community wide vaccination of all individuals with tuberculin reaction to 5 T.U. of P.P.D. of less than 5 mm after 72 hrs. In India BCG manufacturered in Guindy, Tamil Nadu	• i/d (intradermal at birth or immediately after (in India)	• Specific cell mediated responses (beneficial)	• Efficacy has varied in different trials from 0% protection (Ch-ingleput, South India) to 80% (1935, North America) • Benefits: Disease, when occurs is mild. Severe disease forms, as miliary tuberculosis avoided	• Papule at site of vaccination may ulcerate due to inadvertent s/c infection or excessive dosage • Suppurative lymphadenitis • BCG osteitis • Disseminated BCG disease • IRIS (Immune reconstitution inflammatory syndrome)	• Acute febrile illness • Generalized eczema (vaccination can be given during remission) • Septic skin condition at site of vaccination • Immunodeficency conditions
Leprosy	• B.C.G alone • B.C.G. + heart killed M.leprae • Mycobacterium W • ICRC (Indian Cancer Research Centre, Bombay) • Currently 'Leprovac', marketed by Cadila Ltd.		• Useful in prophylaxis of contacts • Useful in Lepromatous cases	• i/d • i/d 3-4 times at of interval of few months	• Cross reactivity with *M.leprae* • Mycobacterium W is a fast growing saprophyte (non-pathogen) mycobacterium of the soil which cross-reacts with M.leprae	• Good lepromin conversion rate, which varies with different vaccines		
Tetanus : Toxoid : Serum	<25 Lf toxoid in single + preservative + aluminum & calcium compounds as adjuvant • Available as single vaccine or Double (DT. PT) or triple (DPT) or Quadruple (DPT+polio)	• Tetanus Toxoid with reduced amounts of diphtheria (d) or/and • Acellularpertusis (ap)	• To children as part of immunization schedule • Pregnant women Individuals who have suffered injury suspected to be contaminated with tetanus spores • Non-immune individuals • Workers with greater than usual risk of injury; as military personnel • Replacement of TT with Td after 7 years of age • Tdap to be given as one time booster during 11-18 years followed by Td every 10years	• i/m or s/c 3 doses + booster • 2 doses • 3 doses at 0,1 & 6-12 months & then booster at 10 yrs.	• IgG antitoxic antibodies are formed after vaccination, which are present in the blood & extravascular fluids. These can neutralize the toxin, which is released locally, in lymph & blood (by the bacteria) before it becomes fixed to the ganglioside in the nervous system. Vaccination during pregnancy (5th & 8th month) results also in the enhancement the response of these infants to subsequent immunization. In tetanus there is no natural immunity following disease or sub-clinical infection (but natural attack of pertusis confers immunity)		• *Local:* Swelling, redness & pain up to 10 days after injection • *Systemic:* Pyrexia, headache, malaise, myalgia, urticaria, acute anaphylaxis, peripheral neuropathy, elevated IgE levels Frequent boosters may be associated with local arthrus type & urticarial reactions.	• GBS < 6 weeks, after previous dose • History of arthrus type hypersensitivity after previous dose of TT containing vaccine.

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Typhoid	• T.A • T.A.B • Ty21a (Typhoral)	• Has killed organisms of S. Typhi & S. Paratyphi A (phenol or acetone inactivated) • Is live attenuated strain (mutant of S.Typhi 21a, which lack galE gene, as well as Vi antigen	• High risk	• i/m one dose • oral 3 doses preferably taken one hour before food on alternate days with cold/ lukewarm water	• With live attenuated strain which lacks gal E & Vi antigen gene, cellular & secretory IgA response in the intestinal tract is initiated/ latter prevents infection (strain is mutant developed by genetic manipulation, which takes UDP-galactose - 4-epimerase, the enzyme responsible for incorporation of galactose into cell wall lipopolysaccharide.		• Nausea, vomitting & diarrhoea, (local), and systemic side-effects ''Typhoral' indicated after 6 years of age	• Like those of any live vaccine (for Ty21a) *Precaution:* • time gap of 2 weeks between oral polio vaccine & Ty21a • Minimum gap of 12 hours between administration of vaccine & mefloquine, as may inhibit replication • Vaccine not licensed for Children <18 months, as there may be
	• V_i (Typhim)	• Vi polysaccharide antigen based		• parenteral dose,>2 years of age	• Elicits IgG Vi antibody (poorly immunogenic in infants)	64-72% - 3 years (for *typhiml*)	• Typhim Vi' given only after 2 years of age sub-optimal response in infants) interferes for screening for serum Vi antibody	
	• Ty800, CVD908/909	• Live attenuated vaccine	• Trial phase		• Highly immunogenic	Higher efficacy		
	• Conjugated vaccines	• Vi antigen bound to carrier protein						
Plague	• Killed vaccine (modification of original Haffkine vaccine)		• High risk group: Field workers as ecologists, geologists in area known to have plague: • Lab personnel working with infected material. • In outbreak limited role (even vaccinated persons with adequate level titre must be given prophylactic antibiotic	• Parenteral s/c - two doses at interval of 4 weeks - boosters given every 6 months	• Mediated by circulating antibodies directed against Fraction 1 (i.e., capsular antigen in *Y.pestis,* which stimulates antibacterial immunity) As a consequence, vaccination reduces the risk of infection spread from rat to man by fleas (bubonic plague) but it's effect against airborne infection (primary pneumonic plague) is unknown	• Protection is 50% fir 3-6 months Immunity appears 5-7 days after vaccination May not give protection against air borne pneumonic plague	• Erythema, induration at injection site, fever, headache, malaise, lymphadenopathy	
	• Subunit/attenuated mutant strain vaccines	• Newer vaccine, subunit aling with fraction F1 or live attemuated				Higher efficacy		
Cholera	• Killed, whole cell (heat killed, phenol preserved) prepared from *V cholerae* type Ogawa & inaba		• Individual of all ages who live in high risk areas, during cholera outbreaks, Travellers to endemic areas	• s/c parenteral 2 doses at intervals of 4-6 weeks orally at >1 year of age	• Elicits high blood level of vibriocidal antibodies of IgG class. Small amount of the antibody may reach gut.	• Limited protection for few months • Variable • Transient protection in children • High cost of oral vaccine because of acid sensitivity of B subunits vaccine administered together with NaHCO3, citric acid, buffer solution to ensure adequate neutralization of stomach acidity for preservation of vaccine, while passing through stomach.	• *Local:* Transient redness, swelling, pain • *General:* Headache, pyrexia, reaction	
	• B subunit & whole cell cholera consists of purified B subunit from cholera toxin & formalin/heat inactivated classical & El tor *V. cholerae* of Inaba & Ogawa serotype	• Utilizes recombinant B Subunit with whole cell vaccine		• 3 dose	• Vaccine is prepared from classical biotype, but carries equal protection against Eltor biotype Significant rise in serum vibriocidal antibodies & antitoxin levels			

	• rBS_WCV (Dukoral or Colorvac) • CVD 103_HgR (Mutacol or Orochol)	Oral live attenu- ated (lack gene for cholera toxin) Live attenuated		Two oral dose immunization regimen 10-14 day apart • Single dose	• Protection atleast for 6 months			
H. influenzae	• *H.influenzae* type b polysaccharide vaccine (unconjugated) • Conjugated Vaccine, Combinations available	• Antigen based	• Routine,	• i/m,primary series of 3 doses at 6,10,14 weeks of age with booster at 16-18 months Parenteral 2 doses with one booster	• Type b is used, as, 90% of infections are caused by it, combination may be used	-Good protection -Reduction in nasopharyngeal carriage of Hib -Induction of herd immunity	• *Local* - slight reaction, elevation of temperature	• Severe allergic reaction after previous dose • Severe acute illness
Anthrax In Man In Animal	• Protective antigen (PA) adsorbed on aluminum hydroxide (alum precipitated toxoid) rPA (recombinant Protective antigen) is a current approach • Spores of a nonvirulent strain (Sterne vaccine)		• Individuals likely to be exposed to anthrax(18-65 years of age)	• i/m, 3 doses at intervals of 6 weeks & booster at 6 months,parenteral. • Single dose,single dose ,revaccinate every 6 months in highly vaccinated area	• Antibodies to PA have a protective role probably by blocking binding of lethal factor to cell surface Immune response against spore, which is an infective form	• Booster dose may be administered after 1 year, Effective for a year,approximately 92.5% efficacy		• Severe allergic reaction after previous dose • Severe acute illness
Pertusis	• Whole cell pertusis vaccine • Tdap • Acellular pertusis (using recombinant technology)	• Smooth, encap- sular, virulent, phase I strain of *B pertusis* (Killed) having 3 principal, agglutino- gens is used • Tetanus, diphtheria, acellular pertusis • Contains pre- dominantly FHA, agglutinogens and inactivated PT.	• Routinely in immunization programme	• Parenteral given with Diphtheria and Tetanus. Three doses at intervals of 4-6 weeks, 6 months of age Boosters at 1½ and 4 years	• Vaccine induced immunity is mediated partly by circulating IgG antibody which reaches respiratory sercetion, the latter opsonizes the bacteria, which are easily phagocytosed. C.M.I. confers long- term protection	• Good protection (Ap- proximately 90%)	• Local swelling and redness, Fever, Persistent screaming.Hypotonic hyporesponsive episode (HHE),Shock, Encephalopathy, convul- sion	-Febrile seizure -Epilepsy,progressive neural disorder -severe reaction to previous dose as, convulsion *Precautions* if -H/o of Arthrus type hypersensitivity -Pregnancy -GBS within 6weeks of previous dose of TT containing vaccine

Contd.

Essentials of Microbiology

As per the latest CBME Guidelines | Competency Based Undergraduate Curriculum for the Indian Medical Graduate

An Integrated Clinical Based Approach *Including* Parasitology

has been written and designed in accordance with the latest CBME Guidelines. In a first level medical microbiology programme, a strict clinical system-wise study of the microbes would not only hinder the understanding and concept building of microbiology in the medical students but may imprint erroneous perceptions, as most microbes know no anatomical borders. Competency Based Undergraduate Curriculum for the Indian Medical Graduate advocates a horizontal and vertical integration of microbiology with other subjects on the basis of clinical systems. On this ground, microbiology has been a victim in the new CBME Guidelines. How does one approach this dilemma? The book has introduced more than 100 integrated clinical cases (including 23 on parasitic diseases); referenced clinical system-wise in exclusive infectious diseases section and dealt in the relevant microbiology category. So the book imbibes the spirit and word of the competency-based curriculum and yet retains the traditional approach of microbe learning for greater understanding. It has bidirectional linkages, which permit navigation from clinical cases to core microbiology and otherwise. The book has been designed primarily for induction of the subject to the medical undergraduates and not as a postgraduate entrance book, yet would be helpful in the latter respect.

Highlights

- More than 55 bacterial and fungal, 25 viral and 23 parasitic based integrated clinical-based cases to make a sound foundation of the infectious diseases in the budding doctors. Worked out in a systematic **Q** and **A** based format for clear understanding.
- More than 33 general microbiology/infection control and pandemic management and 15 immunology-based clinical vignettes/clinical cases worked out in a systematic fashion to make a sound foundation of medical microbiology in the budding doctors.
- All chapters in clinical infectious diseases/syndromes of various systems are provided with clinical details including collection, transport and processing techniques. Also provided are exclusive clinical cases including references of clinical cases.
- All sections on bacterial diseases have separate section for outline/classification of organisms, metabolic and microscopic features, media requirements and colonial characters (including diagnostic), clinical profile, laboratory diagnosis (of important bacterial diseases) and treatment. This is organized in an integrated tabular format.
- Sections dealing with DNA and RNA viral infections have exclusive tabulated and referenced chapters on clinical profile and laboratory diagnosis of such infections.
- Protozoology and helminthology sections in parasitic diseases have exclusive chapters on morphological profile, transmission/life cycle and host's profile, clinical profile, laboratory diagnosis profile and treatment profile.
- All sections have assessment/examination questions including MCQs (with references/answers).

The book also Includes:

- An exclusive section on pandemic management
- Two AETCOM scenarios with reference to microbiology
- More than 50 quotations to inspire the reader
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- "First ...microbiology book by an Indian author on clinical case format."
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- "Interactive reading as a result of clinical vignettes/cases makes study relevant, interesting and easily recapitulatable"
- "Is alternative (relevant and exciting) route to medical microbiology learning"

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