CHAPTER 1: HISTORY AND INTRODUCTION TO MICROBIOLOGY

Introduction and Historical Development of Microbiology

It is the science which deals with the study of minutes living organism's causative of disease. These cannot be seen by the naked eye but require magnification with the use of lens. Microbes, also called microorganisms, are minute living things that are usually too small to be seen with the unaided eye. The group includes bacteria, fungi (yeasts and molds), protozoa, and microscopic algae. It also includes viruses. The majority of microorganisms make crucial contributions by helping to maintain the balance of living organisms and chemicals in our environment. Soil microbes help break down wastes and incorporate nitrogen gas from the air into organic compounds, thereby recycling chemical elements between the soil, water, life, and air. Humans and many other animals depend on the microbes in their intestines for digestion and the synthesis of some vitamins that their bodies require, including some B vitamins for metabolism. Microorganisms also have many commercial applications. They are used in the synthesis of such chemical products as vitamins, organic acids, enzymes, alcohols, and many drugs. Only a minority of microorganisms are pathogenic (disease-producing). Pathogenic organisms are parasites or saprophytes that cause disease. The processes by which they establish themselves in a host individual is infection, but infection need not be followed by clinical illness. The term virulence is sometimes used to mean pathogenic but sometimes to express degree of pathogenicity.

Types of Microorganisms

Prokaryotic and Eukaryotic: Prokaryotes (pro– primitive, Karyotes- nucleus) which means cells with primitive nucleus. A prokaryotic cells is a cell that does not have a true nucleus. The nuclear structure is called a nuclenid. The nuclenid contains most of the cell's genetic material and is usually a single circular molecule of DNA. A prokaryotic organism, such as a bacterium, is a cell that lacks a membrane-bound nucleus or membrane-bound organelles. The exterior of the cell usually has glycocalyx, flagellum, fimbriae, and pili. Eukaryotes (Eu- advanced), which means cells with true or advanced nucleus. Eukaryotes have nucleus with nuclear membrane.

Bacteria are relatively simple, single-celled (unicellular) organisms. Because their genetic material is not enclosed in a special nuclear membrane, bacterial cells are called prokaryotes. Bacteria are enclosed in cell walls that are largely composed of a carbohydrate and protein complex called ***peptidoglycan.*** (By contrast, cellulose is the main substance of plant and algal cell walls.) Bacteria generally reproduce by dividing into two equal cells; this process is called ***binary fission***. For nutrition, most bacteria use organic chemicals, which in nature can be derived from either dead or living organisms.

Fungus is eukaryotes, organisms whose cells have a distinct nucleus containing the cell's genetic material (DNA), surrounded by a special envelope called the **nuclear membrane**. Organisms in the Kingdom Fungi may be unicellular or multicellular. Large multicellular fungi, such as mushrooms, may look somewhat like plants, but they cannot carry out photosynthesis, as most plants can. True fungi have cell walls composed primarily of a substance called ***chitin***. The unicellular forms of fungi, ***yeasts***, are oval microorganisms that are larger than bacteria. The most typical fungi are ***molds.***

Protozoa are unicellular eukaryotic Microbes. Protozoa have a variety of shapes and live either as free entities or as ***parasites*** (organisms that derive nutrients from living hosts) that absorb or ingest organic compounds from their environment. Protozoa can reproduce sexually or asexually.

Algae are photosynthetic eukaryotes with a wide variety of shapes and both sexual and asexual reproductive forms. The algae of interest to microbiologists are usually unicellular. The cell walls of many algae, are composed of a carbohydrate called ***cellulose.*** Algae are abundant in fresh and salt water, in soil, and in association with plants. As photosynthesizers, algae need light, water, and carbon dioxide for food production and growth, but they do not generally require organic compounds from the environment. As a result of photosynthesis, algae produce oxygen and carbohydrates that are then utilized by other organisms, including animals. Thus, they play an important role in the balance of nature.

Viruses are so small that most can be seen only with an **electron microscope**, and they are **acellular** (not cellular). Structurally very simple, a virus particle contains a core made of only one type of nucleic acid, either DNA or RNA. This core is surrounded by a protein coat. Sometimes the coat is encased by an additional layer, a lipid membrane called an envelope. All living cells have RNA *and* DNA, can carry out chemical reactions, and can reproduce as self-sufficient units. Viruses can reproduce only by using the cellular machinery of other organisms. Thus, viruses are considered to be living when they multiply within **host cells** they infect. On the other hand, viruses are not considered to be living outside of living hosts.

Development of microbiology

Generally, development of microbiology can be divided into three stages: these are:

The first stage: Based on the use of microscope, Mr. Hooke (1632-1723 AD) observed the shape, arrangement and size of microbes, which is the first time for man to observe microbes. However, the knowledge about microbes is limited to morphology for centuries due to the understanding of nature.

The second stage: also called as the stage of physiology and immunology, it lasted from 1870 to 1920 AD. During this period, scientists understood that there is metabolism inside the bacteria, wine is products produced by microbes, the infection after surgical operation is caused by bacteria, there is a

relationship between antigen and antibody, and some disease can be prevented by inoculating vaccine.

The third stage: Since 1920 AD, there is a better understanding of that the genetic material is DNA or RNA, rather than protein; the substance (antibiotics) produced by bacteria can be used to prevent and treat disease; a much smaller microorganism, virus can cause disease in both man and animal. The use of electron microscope and development of technologies for cell culture, purification of protein and nucleic acid, and antigen/antibody labeling contributed much to this great approach.

Subdivisions in microbiology

Nowadays, there are many characteristics for microbiology. They are: the understanding is largely based on the knowledge of molecular biology; most types of testing can be conducted in short time due to the use standardized, commercialized products and automatic, computerized facilities; microbiology has developed into a science which cross different subjects in different fields; the knowledge of microbiology has been widely used in almost each life circle around the world; and so on.

Microbiology can be defined as a science studying shape, structure, metabolism, classification, identification of microorganism, reaction between antigen and antibody, and the use of knowledge. Different applied microbiologies have been developed according to different purposes, such as agriculture microbiology, industry microbiology, oil microbiology, dairy product microbiology, food microbiology, aquatic product microbiology, medical microbiology, veterinary microbiology, and so on.

Veterinary Microbiology is a science studying the shape, structure, metabolism, classification, identification of microorganism which cause disease in animal, reaction between antigen and antibody, and the use of the knowledge. Veterinary Microbiology is to study the relationship between microorganism and disease in animal, and make diagnosis of disease, prevent and treat disease in animal by using the knowledge of microbiology and immunology.

The Germ Theory of disease

Until the late 1700s, not much was really known about diseases except their impact. It seemed that anyone who came in contact with an infected person contracted the disease. A disease that is spread by being exposed to infection is called a contagious disease. The unknown agent that causes the disease is called a contagion. Today we known that a contagion is a microorganisms, but in the 1700s many found it hard to believe something so small could cause such devastation.

Koch made some observations on the disease caused *Bacillus anthracci* called anthrax. Based on his findings, Koch developed the Germ Theory. Koch’s Postulates states the Germ Theory that:

Four steps used by Koch to study microorganisms are referred to as Koch's Postulates. These are:

1. The microorganism must be present in the diseased animals and not presence in the healthy animal.
2. Cultivate the microorganism away from the animal in a pure culture.
3. Symptoms of the disease should appear in the healthy animal after the healthy animal is inoculated with the culture of the microorganisms.
4. Isolate the microorganism from the newly infected animal and culture it in the laboratory. The new culture should be the same as the microorganism that was cultivated from the original diseased animal.

Microscope and microscopy

Microorganisms are much too small to be seen with the unaided eye; they must be observed with a microscope. The word *microscope* is derived from the Latin word *micro,* which means small, and the Greek word *skopos,* to look at. Because microorganisms and their component parts are so very small, they are measured in units that are unfamiliar to many of us in everyday life. When measuring microorganisms, we use the metric system. The standard unit of length in the metric system is the meter (m). A major advantage of the metric system is that the units are related to each other by factors of 10. Thus, 1 meter equals 10 decimeters (dm) or 100 centimeters (cm) or 1000 millimeters (mm).

Microorganisms and their structural components are measured in even smaller units, such as micrometers and nanometers. A micrometer (μm) is equal to 0.000001 m (10^{-6} m). The prefix *micro* indicates that the unit following it should be divided by 1 million, or 10^{6}. A nanometer (nm) is equal to 0.000000001 m (10^{-9} m).

Microorganisms are measured using the metric system. In order to give you some idea of the size of a microorganism, let's compare a microorganism to things that are familiar to you.

A human red blood cell	100 micrometers (μm)
A typical bacterium cell	10 micrometers (μm)
A virus	10 nanometers (nm)

Microscope and its Parts

1. **Microscope**:- (Magnifying instrument) or a device that uses a lens or system of lenses to produce a greatly magnified image of an object. An optical microscope uses transmitted or reflected light to obtain the image. An electron microscope uses a bean of electrons and a system of electron focusing lenses to obtain image.
2. **Microscope slide**:- is a specimen holder a small glass plate on which a specimen is mounted for viewing under a microscope

3. **Cover slip (cover for microscopes specimen)**:- a piece of thin glass used to cover a specimen on a microscope slide.
4. **Eye piece**:- where you look through to see the image of your specimen.
5. **Body tube** :- the long tube that holds the eye pieces and connects it to the objectives
6. **Nose piece**: - the rotating part of the microscope at the bottom of the body tube. It holds the objectives.
7. **Objective lenses** :- (low, medium, high, oil immersion) the microscope may have 2, 3 or more objectives attached to the nosepiece they vary in length (the shortest is the lowest power or magnification: the longest is the highest power or magnification).

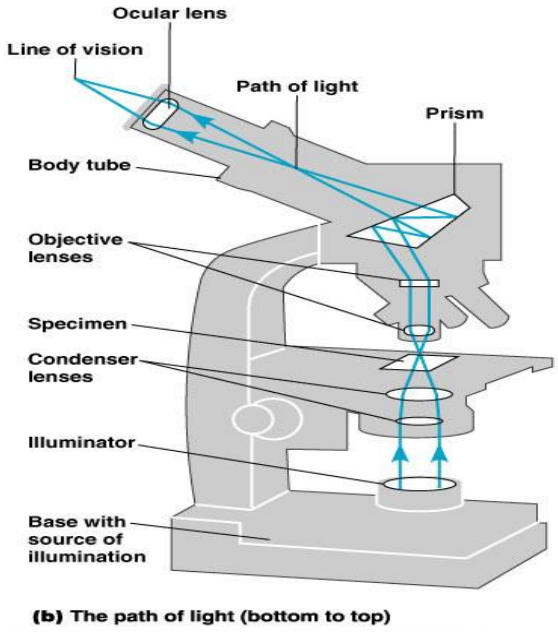


Figure 1. Compound microscope

8. **Arm**: - part of the microscope that you carry the microscope with.
9. **Coarse adjustment knob:** large round knob on the slide of the microscope used for focusing the specimen: - It may move either the stage or the upper part of the microscope.
10. **Fine adjustment knob**:- Small, round knob on the side of the microscope used to fine tune the focus of your specimen after using the coarse adjustment knob.
11. **Stage:**- large flat area under the objectives. It has a hole in it that allows light through the specimen/ slide is placed on the stage for viewing.
12. **Stage clips**:- shiny, clips on top of the stage which hold the slide in place.
13. **Light or mirror**: - source of light usually found near the base of the microscope. The light source makes the specimen easier to see.
14. **Diaphrghm**:- controls the amount of light going through the aperture.

CHAPTER 2: CLASSIFICATION, MORPHOLOGY AND STRUCTURE OF BACTERIA

2.1 Classification of bacteria

The basis of bacterial identification is rooted in taxonomy. Taxonomy is concerned with cataloging bacterial species and nowadays generally uses molecular biology (genetic) approaches. It is now

recognized that many of the classical schemes for differentiation of bacteria provide little insight into their genetic relationships and in some instances are scientifically incorrect. New information has resulted in renaming of certain bacterial species and in some instances has required totally re-organizing relationships within and between many bacterial families. Genetic methods provide much more precise identification of bacteria but are more difficult to perform than physiology-based methods.

The taxonomy system for all living organisms includes the followings: Kingdom, Phylum, Class, Order, Family, Genus, Species, Type, (e.g. biotypes, serotypes), and Strain. The most commonly used term is the species name (e.g. *Streptococcus pyogenes* or Streptococcus pyogenes abbreviation *S. pyogenes*). There is always two parts to the species name, one defining the genus in this case "*Streptococcus*" and the other the species (in this case "pyogenes"). The genus name is always capitalized but the species name is not. Both species and genus are underlined or in italics.

2.2 Morphology and structure of bacteria

Size: Bacterial cell have a wide range of sizes. The units of measurement for recording the size of microorganisms are: 1 micrometer (μm) = 10^{-6}m, 1 nanometer (nm) = 10^{-9}m. coccus commonly measures 0.5-2 μm in diameter, bacterium 3-8 μm in length and 1-1.25 μm in width for larger ones, 2-3 μm and 0.5-1 μm for middle, 0.7-1.5 μm and 0.2-0.4 μm for smaller, and leptospira 2-20 μm long and 0.2-0.4 μm wide.

Shape: Bacteria may be spherical, rod-shaped, or spiral in shape.

Arrangement: the arrangement may be in pairs, clusters and chains

Structure: Bacteria can be divided into two groups on the basis of staining with the Gram stain; Gram positive bacteria remain stained by crystal violet on washing, Gram negative do not. All bacteria have a cell membrane where oxidative phosphorylation occurs (since there are no mitochondria). Outside the cell membrane is the cell wall which is rigid and protects the cell from osmotic lysis. In Gram positive bacteria the cell wall peptidoglycan layer is a much thicker layer than in Gram negative bacteria. Gram negative bacteria have an additional outer membrane. The outer membrane is the major permeability barrier in Gram negative bacteria. The space between the inner and outer membranes is known as the periplasmic space. Gram negative bacteria store degradative enzymes in the periplasmic space. Gram positive bacteria lack a periplasmic space and thus can not store degradative enzymes. In both cases digestive enzymes perform extracellular digestion. Digestion is needed since large molecules can not readily pass across the outer membrane (if present) or cell membrane.

Flagella: Some bacterial species are mobile and possess locomotory organelles- flagella. Those that do are able to taste their environment and respond to specific chemical foodstuffs or toxic materials

and move towards or away from them (chemotaxis). Flagella are embedded in the cell membrane, extend through the cell envelope and project as a long strand. Flagella consist of a number of proteins including flagellin. They move the cell by rotating with a propeller like action.

Pili (synonym; fimbriae): The types of pili varies both among and between species. Pili are hair-like projections of the cell. Allow adhesion to host epithelial surfaces in infection.

Cell wall: Prevents osmotic lysis and is made of peptidoglycan (in bacteria)

2.3 Bacterial growth, reproduction and metabolism

Bacterial requirements for growth includes **oxygen (or its absence), nutrients, optimal temperature and pH.**

A. Oxygen Requirements
   - Obligate aerobes must grow in the presence of oxygen; they cannot carry out fermentation.
   - Obligate anaerobes do not carry out oxidative phosphorylation; furthermore, they are killed by oxygen.
   - Aerotolerant anaerobes are bacteria that respire anaerobically, but can survive in the presence of oxygen.
   - Facultative anaerobes can perform both fermentation and aerobic respiration. In the presence of oxygen, anaerobic respiration is generally shut down and these organisms respire aerobically.
   - Microaerophilic bacteria grow well in low concentrations of oxygen, but are killed by higher concentrations.

B. Nutrient Requirements include sources of organic carbon, Nitrogen, Phosphorus and Sulfur

C. Temperature

   Bacteria may grow at a variety of temperatures from close to freezing to near to the boiling point of water. Those that grow best at the middle of this range are referred to as mesophiles; which includes all animal pathogens and opportunists. Those having lower and higher temperature optima are respectively known as psychrophiles and thermophiles.

D. pH: Many bacteria grow best at neutral pH; however certain bacteria can survive and even grow in quite acidic or alkaline conditions.

Bacteria growth curve

1. Lag - microbes are growing but not dividing. During this phase, bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.

2. Log (exponential) - cells are dividing at a constant speed. The relation between the number of living cells and time is exponential. There are more than enough nutrients to allow the cells to grow.

3. Stationary - the number of cells is stable. Here the growth rate slows down due to the lack of nutrients and the accumulation of metabolites.

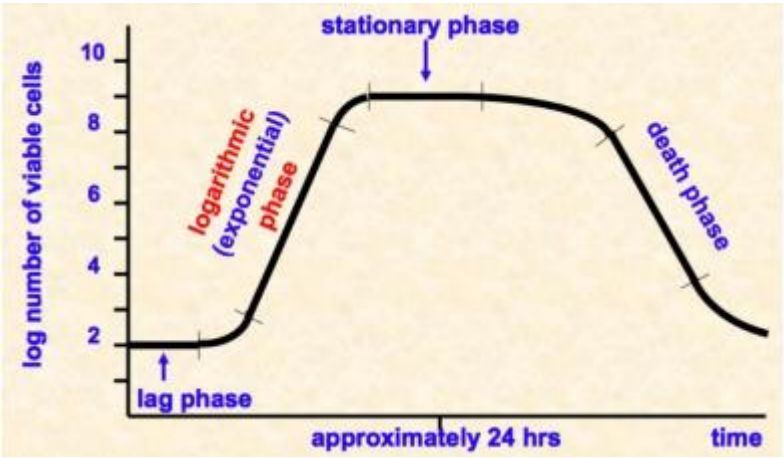


Fig.1 growth curve of bacteria

4. Death - number of cells dying > number of cells being produced. Here the bacteria have run out of nutrients and die.

2.3 Bacterial pathogenicity

Terminologies

Pathogenicity: Pathogenicity is the capacity of a microorganism to produce various enzymes and toxins, by which an infection is established. Pathogenicity depends on the immune status of the host, the nature of the bacterial species or strain and the number of organisms in the initial exposure.

Virulence: Virulence is to measure the degree of pathogenicity of different strains in the same species of pathogen. It depends on the invasiveness (by various enzymes) and toxication (by both exotoxin and endotoxin) of the strain.

Invasiveness: Invasiveness is the ability for a microorganism to pierce into the defence barrier of animal body, grow at local site, and spread to other parts from the initial site.

Dosage: Dosage is a quantity of individual number and virulence for a pathogen, in terms of LD_{50} and ID_{50}. ID_{50} means the Infectious dose for 50% of the test population and LD_{50} means Lethal dose (of a toxin) for 50% of the test population.

Infection: Infection is a process for a microorganism to invade the defense barrier of host, and propagate in the invaded site, which result in the physiological response of host.

Pathogenesis

1. **Transmission:** Specific bacterial species (or strains within a species) initiate infection after being transmitted by different routes to specific sites in the animal body. For example, bacteria are transmitted in airborne droplets to the respiratory tract, by ingestion of food or water, or by

sexual contact.

2. **Adhesion:** Bacterial infections are usually initiated by adherence of the microbe to a specific epithelial surface of the host. Otherwise the organism is removed by peristalsis and defecation (from the gut), ciliary action, coughing and sneezing (from the respiratory tract), or urination (from the urogenital tract). Adhesion involves interactions between external constituents on the bacterial cell (adhesins) and on the host cell (receptors).
3. **Penetration and spread:** Some bacterial pathogens reside on epithelial surfaces. Other species are able to penetrate these cells, but remain locally. Others pass into the bloodstream, or from there onto other systemic sites.
4. **Survival in the host:** Many bacterial pathogens are able to resist the cytotoxic action of plasma and other body fluids involving antibody and complement or lysozyme. Killing of extracellular pathogens largely occurs within phagocytes after opsonization (by antibody and/or complement) and phagocytosis. Circumvention of phagocytosis by extracellular pathogens is thus a major survival mechanism.
5. **Tissue injury:** Bacteria cause tissue injury primarily by several distinct mechanisms involving, for example, many bacteria produce exotoxins and endotoxins.

CHAPTER 3: BASIC MICROBIOLOGICAL TECHNIQUES

Terminology

- Sterilization: Removal of all microbial life
- Disinfection: Removal of pathogens
- Antisepsis: Removal of pathogens from living tissue
- Degerming: Removal of microbes from a limited area
- Biocide/Germicide: Kills microbes
- Bacteriostasis: Inhibiting, not killing, microbes
- Sepsis refers to microbial contamination.
- Asepsis is the absence of significant contamination.

3.1 Sterilization

Sterilization means the foreign of an article from all living organisms, including viruses, bacteria and their spores, and fungi and their spores. Sterilization is required for instruments and materials used in procedures that involve penetration into normally sterile parts of the body, e.g. in surgical operations,

intravenous infusions, hypodermic injections and diagnostic aspirations. It is also required for media, reagents and equipment used in laboratory practice.

Methods:

- Heat - the only method of sterilization that is both reliable and widely applicable is by heating under carefully controlled conditions at temperatures above 100ºC to ensure that bacterial spores are killed. For example, Dry Heat Sterilization kills bacteria by Hot-air sterilization. Pasteurization reduces spoilage organisms and pathogens. Equivalent treatments are 63°C for 30 min or High-temperature short-time (72°C for 15 sec). However, Thermoduric organisms survive Pasteurization temperature. The other is moist heat which can denatures proteins called Autoclave, Steam under pressure.
- Ionizing irradiation - irradiation are employed industrially for the sterilization of single use disposable items such as needles and syringes, latex catheters and surgical gloves, and in the food industry to reduce spoilage and remove pathogens.
- Filtration - filters are used to remove bacteria and all larger microorganisms from liquids that are liable to be spoiled by heating, e.g. blood serum.
- Sterilant gases - ethylene oxide is used mainly by industry for the sterilization of plastics and other thermolabile materials that cannot withstand heating. Formaldehyde in combination with subatmospheric steam is more commonly used in hospitals for reprocessing thermolabile equipment.
- Sterilant liquids - use of liquids such as glutaraldehyde is generally the least effective and the most unreliable method, only to be applied when no other sterilization method is available.

The method to use for sterilization depends on the resistance of the materials to various temperatures, humidity, chemicals, etc.

3.2 Culture media, pure culture method, cell count techniques

Reading assignment

3.3 Staining techniques

Not all specimens can be clearly seen under a microscope. Sometimes the specimen blends with other objects in the background because they absorb and reflect approximately the same light waves. You can enhance the appearance of a specimen by using a stain. A stain is used to contrast the specimen from the background. A stain is a chemical that adheres to structures of the microorganisms and in effect dyes the microorganisms so that can be easily seen under a microscope. Stains used in microbiology are either basic or acidic. Common basic stains are methylene blue, crystal violet, safranin, and malachite green. These are ideal for staining chromosomes and the cell membranes of many bacteria. Acid stains are used to identify bacteria that have a waxy material in their cell walls. This form of staining differentiates bacteria.

Different types of stains

1. Simple stains:
    - Most are basic. Crystal violet, and methylene blue are examples of simple stains.
2. Differential stains:
    - Bind to some types of bacteria but not others, therefore providing a method of differentiating between different types of bacteria.
    - Gram stain (gram positive vs. gram negative)
    - Acid-fast stain for bacteria that do not take up stain well *(e.g.:* _ Acid-fast stain for bacteria that do not take up stain well *(e.g.: Mycobacteria)*
3. Special stains
    - Capsule stain with India ink (wet mount) – Negative stain
    - Endospore stain
    - Flagella stain

3.4 Rickettsia

All the *Rickettsial* organisms are small strictly obligate intracellular parasites, generally parasitizing in reticuloendothelial cell and red cell. Like the *Chlamydia* these bacteria were once thought to be viruses because of their small size and intracellular life cycle. However, they are true bacteria structurally similar to Gram-negative bacteria. These bacteria are small Gram-negative coccobacilli that are normally stained with Giemsa since they stain poorly by the Gram stain. The size of the organisms is within 0.3-0.5 um, only few members of *Rickettsia* can grow on artificial medium, most can be sterilized by filtration, all contain both DNA and RNA, and they reproduce by separating the cell into two equal parts. All of these organisms are maintained in animal and arthropod reservoirs and, with the exception of *Coxiella*, are transmitted by arthropod vectors (*e.g.*, ticks, mites, lice or fleas). Humans are accidentally infected with these organisms. The reservoirs, vectors and major diseases caused by these organisms are summarized as the followings:

Disease	Causative organism	Distribution known	Principal animals involved	Means of spread
Q-fever	*Coxiella burnetii*	Worldwide, common	Sheep, cattle, goat, cats, other mammals	Mainly air-borne, ticks and milk occasionally
Heartwater (Cowdriosis)	*Cowdria ruminantium*	Africa	Cattle, sheep, goat, antelope	Amblyomma
Boutonneuse fever	*Rickettsia conorii Rickettsia spp*	Europe, Asia, Africa	Dogs, rodents, other animals	Bite of infected ticks

3.5 Chlamydia

Chlamydial organisms are strictly obligate intracellular parasites, they cannot grow on artificial medium (grow well in embryonated eggs and cell cultures). They can be examined through light microscope, but the size of the individual organism differs from different development stages.

Chlamydial organisms are spherical with the size of 0.2-1.5 um, they are gram-negative. Chlamydial organisms are sensitive to heat, they can only survive for 5-10 minutes at 56-60℃, and they are also sensitive to general fat solvents and detergents. Unlike Rickettsia, Chlamydial organisms cause diseases in animals without involvement of arthropod vectors. *Chlamydia trachomatis* and C*hlamydia psittaci* can cause abortion in various species of domestic animals, conjunctivitis in birds, and intestinal infection and pneumonia in cattle.

CHAPTER 4. THE MYCOPLASMA

Introduction and characteristics of mycoplasma

Mycoplasma are the smallest free-living bacteria. They range from 0.2 - 0.8 micrometers and thus can pass through some filters used to remove bacteria. They have the smallest genome size and, as a result, lack many metabolic pathways and require complex media for their isolation. The mycoplasmas are facultative anaerobes, except for *M. pneumoniae*, which is a strict aerobe. A characteristic feature that distinguishes the mycoplasmas from other bacteria is the lack of a cell wall. Thus, they can assume multiple shapes including round, pear shaped and even filamentous. The mycoplasmas grow slowly by binary fission and *prod*uce "fried egg" colonies on agar plates; the colonies of *M. pneumoniae* have a granular appearance. Due to the slow growth of mycoplasmas, the colonies may take up to 3 weeks to develop and are usually very small. The colonies of *Urea plasma* are extremely small and thus *Urea plasma* are also called T-strains (tiny strains).The mycoplasma all require sterols for growth and for membrane synthesis. The three species can be differentiated by their ability to metabolize glucose (*M. pneumoniae*), arginine (*M. hominis*) or urea (*U. urealyticum*). The fourth species *M. genitalium* is extremely difficult to culture.

CHAPTER 5. VIROLOGY

5.1 Introduction

Viruses are obligatory intracellular infectious agents of sizes ranging from 20 to 300 nanometere with an absolute dependence on living cells for their replication. Viruses contain: a nucleic acid genome (RNA or DNA), a protective protein coat (called the capsid). The nucleic acid genome plus the protective protein coat is called the nucleocapsid.

5.2 Virus structure

1. Size: viruses are very small retaining infectivity after passing through filter with pore size small enough to hold back the smallest bacteria. Bacteria are measured in terms of micrometer (μm, 10^{-6} of a meter) whereas viruses are measured in nanometer (nm, 10^{-9} of a meter). Viruses range in size from 20nm to 300nm. The picornaviruses (e.g. Foot-and-Mouth-Disease virus) are the smallest viruses (20nm) while the poxviruses are the largest viruses (300nm). Viruses can not be seen by light microscope because of their small size. They are seen only by the aid of electron microscope.
2. Organelles: Viruses do not possess cellular organization and do not have organelles.
3. Viruses are completely dependent on living cells for replication and existence. They do not grow in inanimate/non-living media.
4. Viruses have their genetic information in either DNA or RNA. A virus possesses only one species of nucleic acid either DNA or RNA but never both.
5. Viruses do not multiply by binary fission but by a complex process involving protein synthesis and nucleic acid production
6. Viruses are unaffected by antibiotics

5.3 Classification of virus (viral taxonomy)

Following the discovery of viruses, earliest studies on them were based on their filterability, and observations on the diseases which they caused. Early classification systems were premised on pathogenic effects of the viruses and their transmission patterns. However, with the invention of electron microscope and sophisticated molecular techniques that permitted ultra-structural studies, details of the structures and compositions of viruses began to emerge. Thereafter, it became possible to group viruses on the basis of shared features of the virions. Consequently, the following general parameters are have been used for classification of viruses:

1. Pathogenicity
2. Ecological characteristics
3. Physico-chemical characteristics

In this classification, viruses affecting same tissues producing similar syndrome and pathological manifestations are grouped together.

- Viruses affecting the respiratory tract: eg. Influenza
- Vesicular viruses: eg. Foot-and-Mouth-Disease, vesicular stomatitis
- Central nervous system viruses: eg. rabies
- Mucous membrane viruses: eg. Myxomaviruses
- Enteric viruses: eg. rotaviruses,

Limitations of this classification system: some viruses affect more than one system of the body and they will belong to several groups. Pantropic viruses affecting multiple systems such as canine

distemper, Newcastle disease, rinderpest, pestes des petits ruminants will belong to respiratory, enteric and CNS groups.

Ecological characteristics:

Ecological features of viruses such as the involvement of vectors or vertebrate reservoirs in their transmission cycles and maintenance in nature can be used for classification. Viruses are classified into arboviruses and non-arboviruses or roboviruses and non-roboviruses.

Arboviruses: these are viruses that are transmitted biologically between blood sucking arthropods (such as ticks, culicoides, mosquitoes) and vertebrate hosts. They cause disease in the vertebrate host but not in the arthropods. Examples include African swine fever virus (soft ticks), Yellow fever virus (mosquitoes), Equine encephalitis virus (mosquitoes), African horse sickness virus (culicoides).

Roboviruses: These are viruses with rodent reservoirs. Infected rodents are asymptomatic. They shed the virus in their urine and contaminate the human surroundings, food, drinks and formites. Example include Lassa fever virus with rat as reservoir.

Physico-chemical characteristics:

These are the most reliable, verifiable and satisfactory parameters for classifying viruses.

Viruses are classified based on the following criteria:

- Type of nucleic acid (RNA or DNA)
- Number of strands of the nucleic acid (single or double stranded)
- Physical construction of the nucleic acid (linear, circular, circular with break, segmented, non-segmented)
- Presence or absence of envelope
- Size of the virus
- Antigenic and chemical compositions
- Susceptibility to physical and chemical changes

Based on these criteria, viruses are grouped into families, subfamilies and genera

5.4 Replication of virus

Viruses rely completely on living host cells for their replication. The small genome size put them at disadvantage. Also, they lack organelles and other machineries required for protein synthesis. Although some viruses enter the host cell with few virus-encoded enzymes, others do not possess any protein of their own and therefore depend completely on those produced by the host cell. Virus replication is facilitated by the host cell which provides the required energy and synthetic machinery and sometimes essential enzymes for replication and also by the viral nucleic acid which carries the genetic information required for the synthesis of viral components.

CHAPTER 6. MYCOLOGY

6.1 Introduction

Mycology is the study of fungi. Fungi have several features that distinguish them from other organisms

1. They have a filametous branching of system of cells with apical growth, lateral branching and heterotrophic nutrition.
2. They are characterized by a life-cycle that begins with germination from spore or a resting structure, followed by a period of growth a the substrate is exploited to produce a biomass
3. Finally, there is a period of sporullation that can be disseminated from the parent mycelium.

Importance of Fungi

1. They are vital to the biosphere for many reasons not the least, for their decomposing activities on dead substrate that ensure the release of nutrients like carbon, minerals and nitrogen back into the atmosphere (i.e. act as decomposers of complex organic materials in the environment)
2. They are major cause of plant diseases
3. They also cause many diseases of animals and man.
4. Fungi, especially the yeasts are essential to many industrial processes involving fermentation e.g. bread, wine and beer making.
5. They are important in the manufacture many antibiotics.
6. Fungi are important research tool in the study of fundamentals of biological process.

6.2 classification of fungi

Fungi are eukaryotic organisms that do not contain chlorophyll, but have cell walls, filamentous structure, and produce spores. These organisms grow as saprophytes and decompose dead organic matter. There are between 100,000 to 200,000 species depending on how they are classified. About 300 species are presently known to be pathogenic for man and animals

Medically important Fungi are in four phyla.

1. Ascomycota- Sexual reproduction in a sack called an ascus with the production of ascospores
2. Basidiomycota- Sexual reproduction in asack called basidium with the production of basidiospores
3. Zygomycota- Sexual reproduction by gametes and asexual reproduction with formation of zygospores
4. Mitosporic Fungi (Fungi imperfect)- no recognizable form of sexual reproduction, includes most pathogenic Fungi.

6.3 Morphology of fungi

Pathogenic fungi can exist as yeasts or as hyphae. A mass of hyphae is called mycelia. Yeasts are unicellular organisms and mycelia are multicellular filamentous structures, constituted by tubular cells with cell wall. The yeasts reproduce by budding. The mycelia forms branch and the pattern of branching is an aid to the morphological identification. If the mycelia do not have SEPTA, they are called coenocytic (nonseptate). The terms "hypha" and "mycelium" are frequently used interchangeably. Some fungi occur in both the yeast and mycelial forms. These are called dimorphic fungi.

Dimorphic Fungi

The dimorphic fungi have two forms

1. YEAST- (Parasitic or Pathogenic form). This is the form usually seen in tissue, in exudates, or cultured in an incubator at 37^0C .
2. MYCELIUM- (Saprophytic form). The form observed in nature or when cultured at 26^0C. Conversion to the yeast form appears to be essential for pathogenicity.

CHAPTER 7: IMMUNOLOGY

7.1 Types and forms of immunity

Immunization: Immunization is the means of providing specific protection against pathogens. Specific immunity can be acquired either by passive or by active immunization and both modes of immunization can occur by natural or artificial processes.

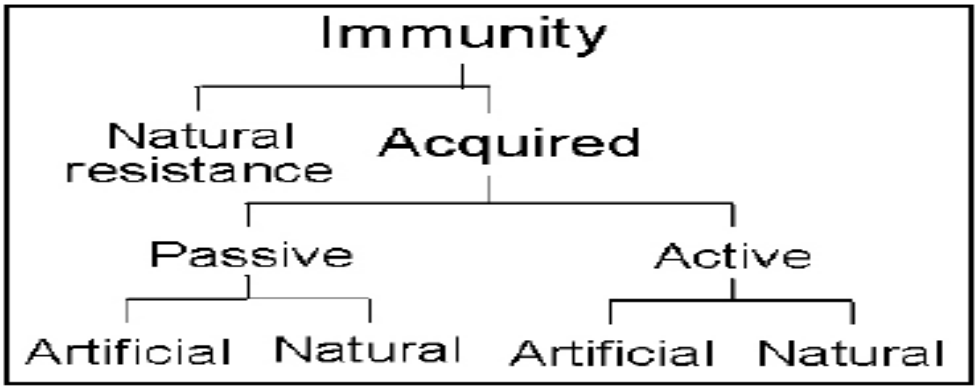
Passive Immunity: Immunity can be acquired, without the immune system being challenged with an antigen, by transfer of serum or gamma globulins from an immune donor to a non-immune individual. Alternatively, immune cells from an immunized individual may be used to transfer immunity. Passive immunity may be acquired naturally or artificially.

Naturally acquired passive immunity: Immunity is transferred from mother to fetus through placental transfer of IgG or colostral transfer of IgA. A newly-born mammal acquires specific immunity through colostral transfer of IgA and IgG transfer on milk during the first stage of life. A newly-hatched bird acquires specific immunity by antibody in yolk.

Artificially acquired passive immunity: Immunity is often artificially transferred by injection with gamma globulin from other individuals or from an immune animal. Passive transfer of immunity with immune globulin or gamma globulin is practiced in numerous acute situations of infections, poisonin, and as a prophylactic measure. In these situations, gamma globulin of same animal species origin is preferable although specific antibodies raised in other species (usually horse and goat) are effective and used in some cases. While this form of immunization has the advantage of providing immediate protection, heterologous gamma globulin is effective for only a short duration and often result in pathological complications

(so-called serum sickness) and anaphylaxis. Homologous immunoglobulin carries the risk of transmitting infectious diseases. In China, yolk and anti-sera with high-titer antibody are widely used to treat animals with Infectious Bursal Disease, Classical Swine Fever, or other infectious diseases, different kinds of monoclonal antibody preparation are also used for the same purpose.

Passive transfer of cell-mediated immunity (immunity that is transferred by cells and not antibody) can also be accomplished in certain diseases (cancer, immunodeficiency). However, it is difficult to find histocompatible (matched) donors and there is severe risk of graft versus host disease.



Active Immunity: This refers to immunity produced by the body following exposure to antigens.

Naturally acquired active immunity: Exposure to different pathogens leads to sub clinical or clinical infections, which result in a protective immune response against these pathogens. The recovered animal individuals in group acquire specific immunity against specific infectious disease for a period or even the whole life.

Artificially acquired active immunity: Immunization may be achieved by administering live or dead pathogens or their components. Vaccines used for active immunization consist of live (attenuated) organism, killed whole organism, microbial components or secreted, detoxified toxins (detoxified).

Live vaccines: Live organisms are used for immunization against a number of viral infections. Live vaccines for Classical Swine Fever, Newcastle Disease, Infectious Bursal Disease and Fowl Pox (varicella) are routinely used all over the world. Kinds of live bacterial vaccine are also used against several bacterial infectious diseases in many countries, especially in developing countries. Live vaccines normally produce self-limiting non-clinical infections and lead to subsequent immunity, both humoral and cell-mediated, the latter being essential for intracellular pathogens. However, they carry a serious risk of probably causing disease, since live vaccines are often attenuated (made less pathogenic) by passage in animal, embryonated egg, or thermal mutation, they can revert to their pathogenic form and cause serious illness. It is for this reason, polio live (Sabin) vaccine, which was used for many years, has been replaced by the inactivated (Salk) vaccine.

Killed vaccines: These consist of whole organisms inactivated by heat, chemicals or UV irradiation treatment. Many killed viral and bacterial vaccines are available. They are used to immunize domestic animals for ether routine vaccination (*e.g.* Classical Swine Fever, Newcastle Disease, Infectious Bursal Disease and Fowl Pox, Infectious Bronchitis, etc.) or emergency (*e.g. Avian Influenza, Foot-mouth-disease, etc)*.

Sub-unit vaccines: Some vaccines consist of subcomponents of the pathogenic organisms, usually proteins or polysaccharides. Since polysaccharides are relatively weak T-independent antigens, and produce only IgM responses without immunologic memory, they are made more immunogenic and T-dependent by conjugation with proteins, rabies vaccines consist of antigenic proteins cloned into a suitable vector (*e.g.*, yeast). These subunit vaccines are designed to reduce the problems of toxicity and risk of infection. When the pathogenic mechanism of an agent involves a toxin, a modified form of the toxin (toxoid) is used as vaccine. Toxoid, while remains immunogenic, loses its toxicity. In most situations, they are produced by biological engineering.

Other novel vaccines: A number of novel approaches to active immunization are in the investigative stage and are used only experimentally. These include anti-idiotype antibodies, DNA vaccines and immunodominant peptides (recognized by the MHC molecules) and may be available in the future. Anti-idiotype antibodies against polysaccharide antibody produce long lasting immune responses with immunologic memory. Viral peptide genes cloned into vectors, when injected transfect host cells and consequently produce a response similar to that produced against live-attenuated viruses (both cell-mediated and humoral). Immunodominant peptides are simple and easy to prepare and can provoke both humoral and cell mediated responses.

Adjuvants: Weaker antigens may be rendered more immunogenic by the addition of other chemicals. Such chemicals are known as adjuvants. There are many biological and chemical substances that have been used in experimental conditions. However, only Aluminum salts (alum), a kind of oil and bee mucus are commercially used for animal use only. Adjuvants used experimentally include mixtures of oil and detergents, with or without certain bacteria, most often BCG, or their components. Newer adjuvant formulations include synthetic polymers. Adjuvants normally function by either creating an antigen depot and/or by stimulating mononuclear phagocytes.
The protective immunity induced by a vaccine may be life-long (Rinderpest, Tuberculosis, etc.) or may last as little as a few months (cholera).

7.2 Innate (Non-specific) Immunity: Mechanisms of protection against infection and disease are diverse. Primarily they can be divided into two major categories: **non-specific** (innate) and **specific** (adaptive), which differ as follows:

Non-specific Immunity	Specific Immunity
Its response is **antigen-independent**.	Its response is **antigen-dependent**.
There is **immediate** response.	There is a **lag time** between exposure and maximal response.
It is **not antigen-specific**.	It is **antigen-specific**.
Exposure does **not** result in induction of **memory** cells.	Exposure results in induction of **memory** cells.

The elements of the innate immune system include anatomical barriers, secretory molecules and cellular components.

Anatomical barriers: Skin, intestinal movement, oscillation of broncho-pulmonary cilia, etc. prevent pathogens from entering and/or getting a foothold in the body.

Secretory molecules: These include organic acids in skin secretions, lysozyme in oro-naso-pharyngeal and lacrimal secretions, thiocyanate in saliva, low molecular weight fatty acids in the lower bowel; bile acids and low molecular weight fatty acids in lower GI tract; transferrin, lactoferrin, lysozyme, interferons, fibronectin, complement, acute phase proteins, etc. in serum; Interferons and tumor necrosis factor (TNF) at the site of inflammation.

Transferrin and **lactoferrin** deprive organisms of iron. **Interferons** inhibit viral replication and activate other cells which kill pathogens. **Lysozyme**, in serum and tears, breaks down the bacterial cell wall (peptidoglycan); **fibronectin** coats (**opsonizes**) bacteria and promotes their rapid phagocytosis. **Complement** components and their products cause destruction of microorganism directly or with the help of phagocytic cells. Acute phase proteins (such as CRP) interact with the complement system proteins to combat infections. TNF-" suppresses viral replication and activates phagocytes.

Cellular Components:

Phagocytic cells: Neutrophils (PMN), macrophages and monocytes are the most important cellular components of the non-specific immune system. **Neutrophils** (polymorphonuclear: PMN) are most important cellular components in bacterial destruction. They are relatively large and most abundant white blood cells with lobed nucleus and cytoplasmic granules (lysosomes). **Mononuclear phagocytes** are the other population of phagocytic cells and include monocytes in circulation, histiocytes in tissues, microglilal cells in the brain, Kupffer in liver, macrophages in serous cavities, and lymphoid organs, They also have granules similar to those in neutrophils, although not as abundant. All phagocytic cells have receptors for a variety of molecules.

Other cells: A number of other cells are also involved in non-specific resistance: they include natural killer cell (NK), antibody dependent cytotoxic cells (ADCC) also referred to as K-cells and lymphokine (proteins secreted by lymphocytes) activated killer (LAK) cells and eosinophils. **NK cells** are important in defense against viral infections and malignancies. They resemble lymphocytes in morphology but are larger and granular, hence also known as large granular lymphocytes (LGL). The granules contain cytolytic proteins such as perforin. NK

cells recognize the difference between normal and malignant or virus-infected cells in a nonspecific manner via sugar-lectin interaction and kill them following intimate contact. **K-cells** are morphologically undefined cells attach to target cells. Macrophages can also function as K cells without the aid of antibody. **Eosinophils** cause cytotoxicity to large multicellular parasites, analogous to K cells.
There is no memory or specificity in the components of non-specific immunity. However, cells of the non-specific immune system become functionally more efficient following exposure to a pathogen because of interaction with products of the specific immune system (e.g., antibodies and cytokines).

7.3 Specific immunity: A second line of defense is the **specific** or **adaptive** immune system which may take days to respond to a primary invasion (that is infection by an organism that has not hitherto been seen). In the specific immune system, B cells produce **antibodies** (soluble proteins that bind to foreign antigens) and T cells respond to the pathogen in which specific cells recognize foreign pathogens and destroy them. In the case of viruses or tumors, this response is also vital to the recognition and destruction of virally-infected or tumorigenic cells. The response to a second round of infection is often more rapid than to the primary infection because of the activation of **memory B and T cells**. Cells of the immune system interact with one another so that a coordinated response may be mounted by proteins such as **lymphokines** which are produced by cells of the lymphoid system, **cytokines** and **chemokines** that are produced by other cells in an immune response, and this stimulate cells of the immune system.

7.4 Specific immuno prophylaxis and immuno therapy

7.4.1 Specific immuno prophylaxis of infectious diseases: The immunologic defense of the animal body respond to antigen stimulation by production of antibodies or by activation of cell-mediated immunity, or both. Administration of a specific antigen (as in a vaccine) may result in development of a specific immune response to the inducing antigen only. Vaccines can provoke effective, and often specific, long-term immunity. When we establish practical vaccination programs in the field, many factors which influence the efficacy of vaccination should be considered. These factors include:

A) Choice of vaccine: For most infectious diseases, both inactivated (killed) vaccine and attenuated live vaccine are commercially available. The basic difference between them is that the attenuated live vaccine needs to propagate (replicate) to some limited degree in specific systems, organs, or tissues, but it does not produce disease because it has been attenuated; while the inactivated vaccine does not propagate inside the animal body. Therefore, the antigen amount of inactivated vaccine in dose is much larger than the live one, this is the reason why the former is more expensive than the latter. Generally, inactivated vaccines can induce antibody at higher level, and the induced antibody can last for longer time; but it is labor-costing, because it needs to be injected individually (so-called individual vaccination). The live vaccines can induce antibody in shorter time, and it’s easy to vaccinate to group of animals by means of water-drinking, spraying or in-feed (so-called mass vaccination). But the antibody induced by live vaccine is always at lower level (not high enough to protect animal

from infection in endemic area) and lasts for a shorter period (not enough for some species of animals for longer term production, also there is potential danger for attenuated viruses to reverse to virulence.

B) Age of vaccination: The proper vaccination age differs from species of animals and kinds of vaccines. The newly-born animals do not develop the immune system into mature status, so they can not response to the stimulation by vaccines at utmost, even not response at all sometimes. Still, there are antibodies maternally-derived from either colostrums for mammals or yolks for oviparous animals; since the maternally-derived antibodies influence or restrict the propagation of inoculated vaccine viruses, finally, the efficacy of vaccination is very low, at least not at utmost. When we make practical vaccination programs in the field, we always face the situation, in which the immune system is not mature, the maternally-derived antibody is still relatively high, and the possible infection is present. In this situation, we consider the coming danger first, even the efficacy of vaccine is not perfect.

C) Times of vaccination: The first time of vaccination is called the primary vaccination, and second time of vaccination by using the same vaccine is called the boost vaccination. Generally, primary vaccination of most vaccines can only induce low-titer level of antibody, which lasts for only shorter period; therefore, a boost vaccination is required in order to induce high-titer and long-lasting antibody. Whether or not a boost vaccination is required largely depends on the better understanding of epidemiology and monitoring of antibody level.

D) Dose of vaccine: Generally, we just follow the instruction of vaccine supplied by vaccine producer, in which total doses contained in one bottle of vaccine has already be determined. However, if any loss in efficacy during vaccine production, transportation, storage and use occurs, dose should be increased by half to one time, e.g. 1.5 to 2 doses as determined by producer used for one single animal. For emergency use during infectious diseases outbreak, dose is increased by 2 to 4 times. According to immunological knowledge, the use of an amount of vaccine above threshold will result in immune paralysis, and the use of much amount of vaccine will result in side-effects, such as malabsorption of injected vaccine, inflammation in injection site, and inappetence for inactivated vaccines; fever, respiratory reactions, diarrhea and inappetence for live vaccines.

E) Route of vaccination: There are several routes of vaccination based on nature of vaccines and convenience of use. IM, IS, and IM/IS are conducted for all inactivated vaccines and some of live vaccines; eye-drop, nose-drop, and mouth-drop for some of live vaccines; and water-drinking, spraying, in-feed for some of live vaccines, and puncturing for a few of live vaccines (Fowl Pox Vaccine).

F) Local and systemic immunity: Local immunity plays important role for animals to protect from infection, this is because there are only a small amount of pathogen individuals (from several to thousands) at beginning of an infection establishment, if there is a specific immunity joined with innate immunity in local sites (e.g. eye, nose, mouth, and so on) at this stage, these pathogen individuals can be easily killed, so the pathogens can not propagate and spread to the deeper and wider parts of the animal body. Most species of live vaccines can induce local immunity in different sites of the body, for example, the live vaccine of Newcastle Disease can induce powerful and effective local immunity in the sites of nose, eye and mouth.

G) Proper way of vaccination: Vaccines are special goods, and all activities from production, transportation, storage and use should be conducted in proper way; otherwise, they will lose efficacy partly or even completely. Since almost all vaccines are kept in cold from production, transportation, storage and use, this storage system for vaccines is call **cold chain**. For inactivated vaccines, the users take them out of refrigerator or icebox, place them at room temperature for 1-2 hours, shake them vigorously to let the antigen disperse in the solution of bottle evenly, adjust the syringe to the exact volume, and inject into the site (make sure the injected vaccine is inside the site and it doesn't flow out).

H) Immunosuppression: There are several factors which inhibit partly (more or less) the function of immune system, as a result, the immune system response to the inoculated or injected antigens less effectively. These factors include chemical substances (rare in animal), some of antibiotics (chloramphenicol), toxins (aflatoxins), diseases (Equine Infectious Anemia, Infectious Bursal Disease, Chicken Infectious Anemia, Marek's Disease), and vaccines (attenuated live vaccine of Infectious Bursal Disease). In the field, try best to avoid these immunosuppression-caused factors if possible. For example, the attenuated live vaccine of Infectious Bursal Disease can result in poor response of chickens to the vaccination of Newcastle Disease if Newcastle Disease vaccine is inoculated within 3 days post-inoculation of attenuated live vaccine of Infectious Bursal Disease.

7.4.2 Immuno therapy of infectious diseases

Based on the understanding of immunology knowledge and technology, several methods of immuno therapy have been developed and used practically. These methods include:

A) Hyper immune serum: After a animal is naturally infected by a specific pathogen, or vaccinated by vaccine, or challenged by virulent pathogen, the serum produced from the animal containing antibody especially against this specific pathogen, the serum is known as antiserum; and if the titer level of the antibody is high enough (repeatedly by inoculations) for treatment purpose (passive immunity), the serum is known as hyper immune serum. The treated animal can receive a passive immunity by injection of hyper immune serum. The treatment is very effective if the titer level of antibody is high enough. The disadvantages of hyper immune serum include: a) the potential danger of serum containing pathogens in the same species of animals if the serum is produced in individuals within the same species; b) the possible anaphylaxis caused by the injected hyper immune serum, if the serum is produced in heterogeneous animals, which can result in severe reactions or even death. The hyper immune sera are used to treat sick animals suffering Classical Swine Fever, Canine Distemper, and so on.

B) Immunoglobulin: In order to decrease the potential danger and possible anaphylaxis caused by hyper immune serum as mentioned above, immunoglobulins are separated and roughly purified from other components in serum. Therefore, immunoglobulins are much safer than serum; but they are much more costing. The practical use of immunoglobulins as treatment method is very limited.

C) Hyper immune yolk: Hyper immune yolk solution is produced by layers in the same way as hyper immune serum mentioned above; and they are used wider than hyper immune serum, because they are cheaper and easily available in abundance. They are

used to treat sick birds with Newcastle Disease, Avian Influenza, Duckling Viral Hepatitis, and so on. But the most important disadvantage with hyper immune serum is that it causes anaphylaxis due to contamination by albumen in egg white; and this side-effect is very severe in some situations.

D) Monoclonal antibody: Monoclonal antibody is a highly purified antibody preparation produced by a clone of hybridoma cells, which is especially against a specific antigenic determinant of antigen of pathogens. However, monoclonal antibody is only used for experimental treatment of limited diseases because of the high cost.

E) Immune stimulants: There are some chemical substances, such as mucopeptide from bacteria, Vitamin E, and short corynebacterium, in the nature which can stimulate or increase immunity. Unlike adjuvant, immune stimulants are not necessary to be used at the same time with vaccines.