Introduction, Scope and Historical Development of Veterinary Microbiology

Introduction

- Microbiology is the study of living organisms of microscopic size. French chemist Louis Pasteur (1822-95) introduced the term 'Microbiology'.
- Sedilot first used the term *Microbe* in 1878. Microorganisms include bacteria, viruses, fungi and protozoa.
- Microbes were probably the first living things to appear on the earth and the study of fossil remains indicates that microbial infections and epidemic diseases existed thousands of years ago.
- Egyptian writing between 2000 B.C. and 1500 B.C. dates the earliest records and refer to descriptions of diseases such as anthrax, tuberculosis, sheep pox and rabies and the attempts to control the deadly and serious infectious diseases at that time.

Scope of Veterinary Microbiology

- Veterinary microbiology is concerned with the etiology (causation), pathogenesis (Mechanism of attack on tissues), laboratory diagnosis and treatment of infection in individual and with the epizootology (study of mass disease among the animals) and prevention and control of infection in the community. Hence, it has close links with other veterinary disciplines such as pathology, medicine, surgery, pharmacology and preventive medicine.
- Microbiology has a close link with curative medicine. Due to the common symptoms irrespective of pathogens, the veterinarian needs the casting vote of microbiologist.
- Role of microbiology is unparallel in the suggestion of exact/suitable drug by performing drug sensitivity test.
- Knowledge of veterinary microbiology is essential for the development of rapid, sensitive diagnostics and suitable, effective vaccines for diseases of livestock.

Theories of Disease Causation

- Ancient man had thought the infectious diseases to be cause of divine wrath and supernatural powers. From merely times, there have been many suggestions/ theories put forth for the cause of disease.

1. Theurgical theory of disease

- Disease was due to wrath of divine spirits for punishment of individual sins.

2. Miasmatic theory

- Disease was due to emanations from the earth, influence of stars, the moon and the wind, the water and seasons (4 elements).

3. **Bible**

- Jews required leprous people to be segregated indicates that they were aware of the fact ' that disease spread by contact', but the cause always was considered to be supernatural. Infected were required to shield their faces with their hand.

4. **Hippocrates**

- Related the disease to different water, direction of wind, and slope of land. Didn't think that disease caused by transmissible agents.

5. **Pore theory**

- Asclepiades (124 B.C.), Themison and Thessalus.
- According to this, symmetry or proportion of pores resulted in health and disease.
- Pores should be changed in afflicted parts to assure recovery from diseases.

6. **Galen (120-200 A.D.)**

- Excess or diffraction of four elements resulted in disease.

7. **Boccaccio (1313-75)**

- Infectious nature of disease. Transmission of plague-Two hogs dragged off a poor man died of plague . Rooted and tossed of their jaws and fell down taking as poison

8. Fracastorius (1478-1553)

- Advanced concept of *contagium vivum* as the possible cause of disease (infectious). Earliest doctrine of infection (Closest to truth). Was based on observations on epidemics of plague, syphilis, typhus of man and FMD in Italy.
- Contagion (The direct cause, such as a bacterium or virus, of a communicable disease) was attributed to:
    - Contagion by contact
    - Contagion by fomite
    - Contagion by distance
    - Contagion by seminaria (seeds of disease)

8. **Athanasius Kircher (1602-80)**

- Reported minute 'WORMS' in the blood of plague patient, but the equipment available to him it is more likely to that what he observed were only blood cells. Credit of using microscope of magnification 32 power.

10. Von Plenciz (1762)

- Each disease caused by separate agent.

11. Spontaneous Generation Theory

- Aristotle (384-322 B.C.) proposed that some of the simple invertebrates could arise by spontaneous generation.
- In 1748, the English Priest, John Needham conducted the experiment by boiling mutton broth and then tightly stoppered the flasks. On incubation, could found growth of microorganism in the flasks. Proposed that organic matter contained a vital force that confers the properties of life on non-living matter.

Pasteur and Tyndall Settled the Spontaneous Generation Controversy

- **Theodore Schwann (18810-1882)** conducted experiment and allowed air to enter the flask containing sterilized nutrient broth after the air had passed through red hot tube, no growth was observed in the sterile medium. Similarly**, George Friedrich Schroder and Theodor van Dusch** allowed to enter the air in a sterile nutrient broth passing through sterilized cotton wool, even though hot air was not used, no growth of microorganisms was observed in the sterile medium.
- 1860's **Louis Pasteur - "Father of Microbiology**" first filtered air through cotton and found that cotton when incubated in broth yielded bacterial growth. Next he used swan necked flasks to disprove spontaneous generation. He placed nutrient solutions in flask, heated their necks in a flame and drew them out in a variety of curves, while keeping the ends of the neck open to air. Pasteur boiled the solutions for few minutes and cooled. No growth occurred even though the contents were exposed to the air. Pasteur pointed out that no growth of microbes occur because the dust and germs had been trapped on the walls of the curved neck .
- **John Tyndall (1877)** demonstrated that dust carries germs, if dust was absent, nutrient broth remained sterile even exposed to air. Also provided the evidence for existence of most heat resistant forms of bacteria.
- The credit of discovery of heat resistant bacterial endospore goes to Ferdinand Cohn (1828-1898).

12. Germ Theory of Disease

- Disease arises from germs attacking the body from outside is called the germ theory of disease. Louis Pasteur proved the germ theory of disease. During 1857 - 1876, Pasteur demonstrated such fermentations were microbial processes.

Development of Microscopes

Lenses were first made during 13th century with limitation of degrees of magnification they provided. The art of grinding lenses was developed in Italy and spectacles were made to improve the eyesight. IN 16th century compound lenses were introduced with increased magnification and formed the basis for development of microscopes. In **1590,** Two Dutch spectacle makers, Zacharias Jansen and his father Hans started experimenting by mounting two lenses in a tube, the first compound microscope has evolved. **In 1609,** Galileo Galilei developed a compound microscope with a convex and a concave lens. In 1665, Robert Hooke's book called *Micrographia* officially documented a wide range of observations through the microscope including **sections of cork,** moulds, but there was no evidence that he observed bacteria.

Antony Van Leeuwenhoek (1632-1723)

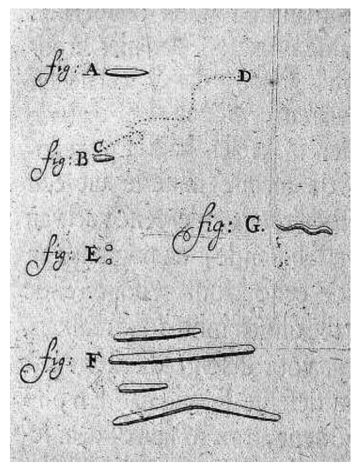
Antony Van Leeuwenhoek was a draper from Delft, Holland. His hobby was to grind the lenses and observe the diverse material through them. Antony Van Leeuwenhoek must be ascribed the credit of placing the science of microbiology on the firm basis of direct observation of microbes through his microscope. In 1675, Anton van Leeuwenhoek used a simple microscope with only one lens to look at blood, insects and many other objects. He was first to describe bacteria and cells, seen through his very small microscopes with, his time, extremely good lenses. In 1683, his detailed reports and drawings were submitted to the Royal Society of London, from these it was possible to recognize the first descriptions of protozoa, including coccidia, bacteria as cocci, bacilli and spirochaetes for the first time.

The importance of these observations was not realized then and to Leeuwenhoek the ‘World of Little Animalcules’ represented only curiosity of nature. The importance of Antony Van Leeuwenhoek’s observations in medicine and in other areas of biology was recognized two centuries later.

Antony Van Leeuwenhoek is known as **‘Father of Bacteriology’** because it was he who first accurately described the different shapes of bacteria (coccal, bacillary and spiral) and pictured their arrangement in infected material. Leeuwenhoek´s drawing of short rods of bacilli and bacteria, the spheres of micrococci, and the corkscrew spirillum were published in First edition, **Delft** in **Holland**, 12 September 1683, to Francois Aston, Pag.11- as description of microbes in tooth plaque).

Antony Van Leeuwenhoek

Antony Van’s Microscope



Leeuwenhoek´s drawing of bacteria

More details log on www.vanleeuwenhoek.com

In 1878, Ernst Abbe formulated a mathematical theory correlating resolution to the wavelength of light and made calculations of maximum resolution in microscopes possible. The theoretical minimum size able to be viewed by an optical microscope is 200nm (as defined by Abbe), since optical microscopes are only able focus on objects that are at least the size of a wavelength of light (usually, a wavelength of around 550 nm is assumed).

1903 – Richard Zsigmondy developed the ultramicroscope and is able to study objects below the wavelength of light. Awarded the Nobel Prize in Chemistry 1925.

In 1932, Frits Zernike invented the phase-contrast microscope that allows the study of unstained live preparations of bacteria, protozoa and moulds. Awarded noble prize in Physics in 1953.

In **1938,** Ernst Ruska develops the electron microscope. The ability to use electrons in microscopy greatly improved the resolution and greatly expanded the borders of exploration. Awarded the Nobel Prize in Physics 1986.

In 1981, Gerd Binnig and Heinrich Rohrer invented the scanning tunneling microscope with three-dimensional images of objects down to the atomic level.

Edward Jenner

During 18^{th} century, smallpox was a greatly feared and deadly disease. The majority of its victims were infants and children. The disease caused a high fever, sores and sometimes death. It had been the greatest killer of man for centuries prior, until declared recently the global eradication of small pox disease.

Born in 1749, Edward Jenner was the youngest son of the vicar of Berkeley. He began studying medicine at the age of 14. By 23, he was a practicing doctor.

One day, Jenner overheard a milkmaid mesmerizing about cowpox, a relatively harmless disease contracted from cows. She said that people who had contracted cowpox never got smallpox. This sparked an idea in Jenner's mind. He theorized that if a person was purposely infected with the cowpox disease, that person would be immune to smallpox.

Jenner tested his theory on an 8 year-old boy named James Phipps. He got pox lesion from a milkmaid infected with cowpox and injected it into the boy (the type of experimentation would never occur in modern medicine). James contracted cowpox, but was soon well. After a few months, Jenner challenged him with smallpox. The boy never developed the disease and the modern vaccine against small pox was born.

The last case of naturally occurring smallpox was found in Somalia in 1977. In 1980, the World Health Organization declared the global eradication of smallpox.

Sir Alexander Fleming

Born at Lochfield near Darvel in Ayrshire, Scotland on August 6^{th}, 1881. He attended Louden Moor School, Darvel School, and Kilmarnock Academy before moving to London where he attended the Polytechnic.

Early in his medical life, Fleming became interested in the natural bacterial action of the blood and in antiseptics. He was able to continue his studies throughout his military career and on demobilization he settled to work on antibacterial substances which would not be toxic to animal tissues.

In 1921, interesting story led to the discovery of Lysozyme. While Sir Alexander Fleming was working in the laboratory, his wife came complaining about the domestic problems and started weeping. The culture plates were kept open on the table. While weeping the tears from the eyes were dropped down on the culture plate. After incubation, on very next day, at that drop down place, Sir Alexander Fleming didn't find any growth of organism. And, he discovered in 'tissues and secretions' an important bacteriolytic substance by serendipity, which he named Lysozyme.

About this time, he devised sensitivity titration methods and assays in human blood and other body fluids, which he subsequently used for the titration of penicillin.

In 1928, while working on influenza virus, he observed that mould had developed accidently on a *Staphylococcus* culture plate and that the mould had created a bacteria-free circle around itself. He was inspired to further experiment and he found that a mould culture prevented growth of staphylococci, even when diluted 800 times. He named the active substance penicillin**-Wonder Drug-the first antibiotic,** which saved the life of millions of soldier.

Louis Pasteur (1822-95) France

Pasteur, Louis (1822-1895), French chemist and biologist, founded the science of microbiology, proved the germ theory of disease, invented the process of pasteurization and developed vaccine for several diseases, including fatal disease like rabies. His studies on fermentation led him to take interest in microorganisms.

Louis Pasteur was born in Dôle (27, December, 1822), the son of a tanner and grew up in the small town of Arbois. In 1847, he earned a doctorate at the École Normale in Paris, with a focus on both physics and chemistry. His studies on fermentation led him to take interest in microorganisms.

Major contributions include:

- Basic principles and techniques of microbiology
- Techniques of sterilization (Hot air oven, Steam sterilizer)
- Growth needs of different bacteria
- Coined the term 'Vaccine' for prophylactic preparations
- Fermentation- Pasteur demonstrated fermentations were microbial processes
- Silkworm Studies
- Disproof of Spontaneous Generation

Germ Theory of Disease: The concept of 'Disease arises from germs attacking the body from outside' is called the germ theory of disease. Louis Pasteur proved the germ theory of disease. During 1857 - 1876, Pasteur demonstrated such fermentations were microbial processes.

Pasteurization: Pasteur extended these studies to such other problems as the souring of milk, and he proposed a similar solution: heating the milk to a high temperature and pressure before bottling. This process is now called pasteurization.

Anthrax Vaccine: Accidental observation led to discovery: Chicken cholera bacillus cultures left on the bench for several weeks lost their pathogenicity but retained their ability to protect the birds against subsequent specific infections led to the discovery of process of attenuation and development of live vaccines. He proved that anthrax is caused by a particular bacillus. He attenuated the culture of Anthrax bacilli at 42-43^{O}C and proved specific protection. Demonstrated the success on farm at *Poxiley Le Fort* (1881) vaccinated animals (sheep, oat and cow) survived and controls died. Pasteur and Chamberland developed attenuated Anthrax Vaccine.

Rabies vaccine development

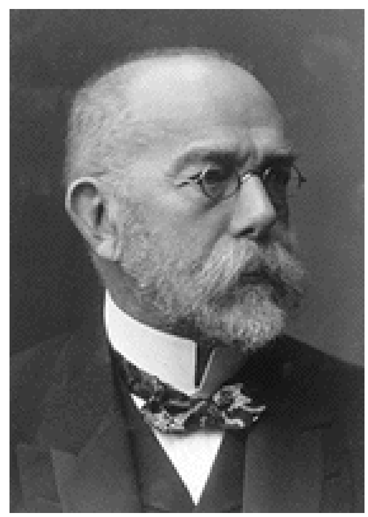
Pasteur is best known for his investigations concerning the prevention of rabies, otherwise known in humans as hydrophobia **Pasteur concluded that the disease rests in the nerve centers of the body; when an extract from the spinal column of a rabid dog was injected into the bodies of healthy animals, symptoms of rabies were produced**. By studying the tissues of infected animals, particularly rabbits, Pasteur was able to develop an attenuated form of the virus that could be used for inoculation.

In 1885, a young boy and his mother arrived at Pasteur's laboratory; the boy had been bitten by a rabid dog and Pasteur was urged to treat him with his new method. At the end of the treatment, which lasted ten days, the boy was being inoculated with the most potent rabies virus known; he recovered and remained healthy. Since that time, thousands of people have been saved from rabies by this treatment.

Pasteur's research on rabies resulted, in 1888, in the founding of a special institute in Paris for the treatment of the disease. This became known as the **Institut Pasteur**, and Pasteur himself directed it until he died. He died in Saint-Cloud on September 28, 1895. He was given a state funeral at the Cathedral.

Robert Koch (1843-1910) Germany

- Robert Koch was born on December 11, 1843, at Clausthal in the Upper Harz Mountains. The son of a mining engineer. Awarded the Nobel Prize in Physiology or Medicine 1905.



Major contributions include:

- Perfected bacteriological techniques.
- Introduced staining techniques
- Methods for pure culture of bacteria.
- Discovered bacillus of Tuberculosis (1882)
- Discovered bacteria *Chlorea vibrio* (1883).
- Use of solid media for growing bacteria

Henle-Koch's postulates:

- Different investigators were reporting causative agents of various infectious diseases as, there should be some criteria for the claims. First indicated by Jacob Henle were enunciated by Koch.

According to Koch's postulates microorganisms can be accepted as the causative agent of infectious disease only if the following conditions are satisfied:

1. Bacteria should be constantly associated with the lesions of disease.
2. Should be possible to isolate the bacteria in pure culture.

3. Inoculation of such pure culture into suitable lab animals should reproduce the lesions of the disease.
4. Should be possible to re-isolate the bacteria in pure culture from the lesions produced in the experimental animal.
5. Additional criteria: Specific antibodies to the (subsequently introduced) infection in serum.

Koch's phenomenon:

- When tubercle bacillus (or its protein) was injected into a guinea pig already infected with mycobacteria - an exaggerated response i.e., hypersensitivity reaction known as Koch's phenomenon was recorded.

Time Line

Year	Scientist	Contribution
384-322 B.C.	Aristotle	Some of the simple invertebrates could arise by spontaneous generation
98-55 B.C	Lucretius	Suggested disease caused by invisible living creatures
100 BC	Varo and Columella	Postulated that minute invisible beings are responsible for disease.
1546	Facastorius of Verona	*Contagium vivum* as a cause of infectious disease
1665	Robert Hooke	First drawing of microorganism published in *Micrographia*
1659	Kircher	Found minute worms in blood of plague victim.
1748	John Needhem	Spontaneous Generation Theory
1713-1781	Francesco Redi	Disapproved the proposal of Spontaneous Generation by proving generation of maggots by decaying meat resulted from the presence of fly eggs
1765-1776	Lazzaro Spallanzani	External air carries the -modified proposal of spontaneous generation.
1798	Edward Jenner	Smallpox vaccine (Used lesions from Cowpox)
1838-39	Schwann & Schleiden	Cell Theory
1828-98	Ferdinand Cohn	Existence of Heat resistant bacterial spores
1834-1844	Agostino Bassi	Discovers Silkworm Disease caused by fungus
1847-1850	Semmelweis	Introduces antiseptics to prevent diseases
1822-1895	Louis Pasteur	Fermentation (1857) Disapproves spontaneous generation theory (1861) Attenuated chicken cholera vaccine (1879) Anthrax vaccine (1881) Rabies vaccine (1885) Fowl Cholera vaccine
1867	Joseph Lister	Antiseptic Surgery- developed aseptic surgical techniques, used carbolic acid (phenol) to treat dressings
1843-1910	Robert Koch	Staining techniques, Isolation of bacteria in pure culture , Cultures bacteria on gelatin (1881) Discovers *Mycobacterium tuberculosis* (1882)

		Discovers *Cholera vibrio* (1883) Demonstrates *Bacillus anthracis* as cause of Anthrax Koch's Postulates (1884) Hypersensitivity (Koch's Phenomenon) (1890)
1846-1934	Fannie Eilshemius & Walther Hesse	Use of agar in culture medium
1884	Charles Chamberland	Porcelain Bacterial Filter. Autoclave developed (Greek auto meaning self, and Latin clavis meaning key, thus a self-locking device)
	Christian Gram	Gram Stain Developed
	Escherich	Discovers Escherichia coli
1887	Richard Julius Petri	Petri dish (Plate)
1888	Pierre Roux & Alexandre Yersin	Bacterial toxins
1888	George Nuttall	Bactericidal action of blood
1887-1890	Winogradsky	Studies sulphur and nitrifying bacteria
1889	Beijerinck	Isolates root nodule bacteria
1892	Iwanowski	Showed an agent passing through a filter caused a disease in plants. Proved existence of ultra microscopic microbes (Viruses) by inoculating bacteria free filtered leave juice from diseased plants into healthy
1894	Shibasaburo Kitasato & Yersin	*Yersinia pestis*
1896	Van Ermengem	*Clostridium botulinum*
1897	Buchner	Discovered enzymes
1899	Beijerinck	Virus causes Tobacco mosaic disease Coined the term 'Virus'
1900	Reed	Yellow Fever transmitted by mosquitoes
1908	Paul Ehrlich	Treatment for syphilis (Salvarsan = arsenic derivative) Coined term 'Magic bullet' for antimicrobial
1915-17	D'Herelle & Twort	Bacterial viruses-Bacteriophage
1921	Alexander Fleming	Lysozyme
1923	Bergey	First Edition of Bergey's Manual
1928	Griffith	Bacterial Transformation
1929	Alexander Fleming	Penicillin-Antibiotic
1930	Goodpasture	Isolation of virus in chicken eggs.
1933	Ruska	Electron Microscope
1935	Gerhard Domagk	Sulfa Drugs
1939	Rene DUBOS	Gramicidin- used against skin infections
1941	Beadle & Tatum	One gene one Enzyme theory
1944	Selman Waksman & Albert Schatz	Streptomycin – First TB drug Waksman-Coined the term 'Antibiotics'
1949	Enders, Weller & Robins	Grow Poliovirus in human tissue culture
1953	Watson & Crick	DNA Double Helix

1955	Jacob & Wollman	F-factor Plasmid
1959	Yalow	Radioimmunoassay
1961	Jacob & Monod	Proposed lac operon
1970	Arber & Smith	Restriction Endonucleases
1973	Chakrabarty	Genetic engineering - Transferred genes from one organism to another
	Gilbert and Sanger	DNA Sequencing method
1981	Gerd Binnig & Heinrich Rohrer	Scanning Tunneling Microscope
1983	Karry Banks Mullis	PCR Technique

Relation of microbes and diseases

Year	Scientist	Contribution
1850	Davaine and Pollender	Observed Anthrax bacillus in blood smear of animals.
1876-77	Robert Koch	Demonstrates Anthrax caused by *Bacillus anthracis*
1898	F Loeffler & P Frosch	Discovered Foot & Mouth Disease Virus
1910	Ricketts	Rocky Mountain Spotted Fever caused by microbe
1911	Rous	Discovers virus can cause cancer
1983-84	Gallo & Montagnier	HIV isolated & identified

Contributions of eminent scientists in the field of Immunology

Year	Scientist	Contribution
1881	Pasteur	"Attenuation" of virus for immunization against rabies
1884	Elie Metchnikoff	Phagocytosis
1888	George Nuttall	Bactericidal Action of Blood
1891	Robert Koch	Delayed type hypersensitivity
1894	Richard Pfeiffer	Bacteriolysis
1890	Emil von Behring	Antitoxin for diphtheria and tetanus. Immunity in cell free portions of blood- humoral theory
1895	Jules Bordet	Complement and antibody activity in bacteriolysis
1900	Paul Erlich	Antibody formation theory
1901	Karl Landsteiner	A, B and O blood groupings
1902	Paul Portier & Charles Richet	Anaphylaxis
1906	Wassermann	Complement fixation test for syphilis developed
1910	Emil von Dungern, & Ludwik Hirszfeld	Inheritance of ABO blood groups
1910	Peyton Rous	Viral immunology theory
1917	Karl Landsteiner	Haptens
1940	Karl Landsteiner & Alexander Weiner	Identification of the Rh antigens
1941	Albert Coons	Immunofluorescence technique

1942	Jules Freund & Katherine McDermott	Adjuvants
1942	Karl Landsteiner & Merill Chase	Cellular transfer of sensitivity in guinea pigs (anaphylaxis)
1944	Peter Medwar	Immunological hypothesis of allograft rejection
1955-1959	Niels Jerne, David Talmage & Macfarlane Burnet	Clonal selection theory
1957	Ernest Witebsky	Induction of autoimmunity in animals
1957	Alick Isaacs & Jean Lindemann	Discovery of interferon (cytokine)
1959-62	Rodney Porter	Discovery of antibody structure
1975	Kohler and Milstein	Monoclonal antibodies used in genetic analysis

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