GENERAL AND SYSTEMIC VIROLOGY
(MICRO – 303)

PRACTICAL NOTES

FOR

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VIRO LECTURE 1

VIROLOGY – Study of viruses.

VIRUSES

Viruses can be defined as the minute infectious particles containing only one or several molecules of RNA or DNA usually covered by a coat made up of one or several proteins and sometimes by an envelope containing lipid, protein and carbohydrate. OR

Viruses are intracellular parasites which are composed of genetic material of either DNA, or RNA covered by a protein coat.

CHARACTERISTICS OF VIRUSES

- They are able to grow and multiply only in living tissues or cells using the host's enzyme apparatus.
- They are very active inside the host cell but outside the cell, they are inert.
- Viruses are metabolically not active and they can be killed by UV light exposure.
- Heat or other chemicals may act on nucleic acid of the virus and make it inactive or killed.

    - Proteins corresponding to the genetic material will be different for each virus.
    - A virus infected cell may release millions of viruses and then ultimately lysed.

    Naked Virus – The virus lack lipid bilayer or envelope. It is somewhat resistant as compared to other viruses.

DNA – found in viruses is usually double stranded but single stranded DNA may found in certain viruses.

RNA – found in viruses is usually single stranded but certain viruses may contain double stranded RNA.

Nucleic Acid _____ RNA _____ viroids (causes plant diseases)

Protein _____Infectious ___ mad cow disease. Infectious proteins also named Prions, may lack genetic material.

SIZE

Viruses have a very simple structure and occur in many shapes and size.

The unit used for measurement of viruses is nanometer (nm). It is one billionth part of a meter. (10^{-9})

The smallest virus ---- 18-20 nm

The Largest virus ----- 300nm

For Example

Picornavirus ……. The smallest RNA containing virus. RNA is 60bp and there are four proteins in its structure. The unit for protein is Daltons (Da) or Kilodaltons (KDa).

Pox virus ……. The largest DNA containing virus.

Filovirus ……. They are very long filamentous having thickness - 20 nm and length – up to 400nm.

EVOLUTION OF VIRUSES

It is believed that the viruses may be evolved from the bacterial plasmid.

VIRO – LECTURE 2

PURIFICATION OF VIRUSES

To study the virus, it is the first and foremost thing to take the virus come out of the host cell.

a) Disruption of Cell

Following are some processes involved in the Interruption of Cells containing virus.

1. Grinding / Pestle-Morter

   This process can be performed by a Blender/Homogenzer.

2. Ultrasonification – Sound waves.
3. Alternate Freez-thaw
4. In case of bacterial cell, treat the cell wall and then Lysozyme with EDTA.

1 gram of tissue + 9 ml normal saline solution (1:10)

b) Primary Centrifugation

i) High speed Centrifugation: ---- Less than 100,000 rpm.

ii) Ultra speed Centrifugation: ---- more than 100,000 rpm

- The methods used for purification of viruses are;
  1. Differential centrifugation
  2. Precipitation with ammonium sulphate or polyethylene glycol
  3. Density gradient centrifugation
  4. Equilibrium centrifugation

1. Differential centrifugation

- In this method, the virus containing solution is usually centrifuge at low speed centrifugation to eliminate the cell debris.
- The supernatant is then centrifuge at high speed centrifugation for 1 to 3 hours. During the period the virus particle usually settled and form pellet at the bottom of the centrifuge tube.

2. Precipitation with Ammonium Sulphate or Polyethylene Glycol

- Precipitation of viruses with ammonium sulphate or polyethylene glycol is done by gradually adding increasing amounts of water saturated ammonium sulphate to virus containing material when a precipitate appears, the precipitate is separated by low speed centrifugation and more of the salt is added slowly to the supernatant until the precipitate forms, which is centrifuged off.
- To precipitate the viruses that are sensitive to salt or ammonium sulphate, the polyethylene glycol of good quality should be used.

3. Density gradient centrifugation

- Using different concentrations of sucrose does purification of viruses by the density gradient centrifugation.
- Sucrose (25 to 5 percent) gradients can be prepared easily and they afford good separations of viruses and other particles of various sizes and densities.

- Viruses usually form bands, which can be revealed by illuminating from the top with a strong light.

4. Equilibrium centrifugation

- In this method, the concentration of cesium chloride (CsCl) does purifications of viruses by the equilibrium centrifugation. Viruses usually form bands or phases, which can be revealed by illuminating with a strong light.

SIZE DETERMINATION

In order to determine the size of the viruses, usually filtration technique is used.

Filtration

In this method, we use the filter paper possesses very minute pores of size 20nm to 300nm. Thus, all the viruses which have more than 20nm and less than 300nm can be filtered. By knowing certain expected size of viruses, we use filter paper of known size to trap these viruses.

Centrifugation during purification of viruses also helps in determination of size of different viruses.

Electron Microscopy: The exact size of a virus can only be measured by using electron microscope.
In this method, viruses + known particle size are put on the copper mesh/grid of electron microscope. Then by comparing the size of known particle with the virus, we can determine the exact size of the virus.
By using electron microscope, we can determine the size as well as shape of the viruses.

SHAPE

Viruses exhibit a variety of shapes;
They may be spherical, cubical or icosahedral, helical or of complex form.

NUCLEIC ACID VISUALIZATION OF A VIRUS

Break the virus → separate the nucleic acid and proteins →Agrogel Electrophoresis → Stain with ethedium bromide → the bands will be visible under UV light → Use marker & know molecular weight.

PROTEIN VISUALIZATION OF A VIRUS

Break the virus → separate the protein from nucleic acid → Dip the protein in SDS-PAGE (Sodium dodecylsulphate poly acrylamide gel elctrophoresis) → Different bands of proteins named M1, M2, M3 and HA will appear. → Stain it and use marker

PROTEINS

- Each protein in the virus has its own genome or a part of genome.
- Protein Types
- Nucleoproteins ii) Capsomeres iii) HA iv) Neuraminidase v) Fusion protein
- The envelope of the virus is made up of phospholipids. In between the envelope and capsomers, there is another protein named M protein (Matrix protein) that supports the envelope.
- The glycol-lipids and glyco-proteins (like HA) may be present on the surface of envelope of virus.

ENZYMES

- Usually there is no enzyme found inside the virus, but certain viruses possess certain enzymes.
- For Example; an enzyme "Reverse transcriptase" is present inside the Retroviruses.
- RNA dependant & RNA polymerases; useful for replication, can be found inside the large viruses.
- The enzymes, like proteins, can be visualized with SDS-PAGE.

VIRO - LECTURE 3

CULTIVATION OF ANIMAL VIRUSES

Viruses can not replicate in synthetic media and require living cells for their growth.

The living systems that are commonly used for cultivation of animal viruses are as follows;

i) Susceptible Host Inoculation
ii) Embryonated Eggs
iii) Cell Culture

Whatever system is adopted for cultivation of viruses, it should be free from bacteriological contamination. This can be achieved by passing the suspension through membrane filters (0.2um) or by treatment with antibiotics e.g. penicillin, streptomycin, etc.

The process of viral replication often by no means always destroys the infected living cells but it may result in formation of disease lesions or other abnormalities in the tissues.

SUSCEPTIBLE HOST INOCULATION

A wide range of viruses are host specific, therefore, the use of a susceptible animal specie for the cultivation of a specific type of virus is obligatory.

For virus cultivation, laboratory animals are commonly used. They are susceptible to a wide range of animal viruses and are useful not only for isolation, and studying the pathogenecity, but are invaluable for production of anti-viral sera.

Why this system is not most preferable for virus cultivation?

Except for raising antiviral sera, the use of laboratory animals have now been restricted because of high cost of breeding, difficulty in maintaing suitable colonies of animals and ethical reason.

Animals used for virus cultivation

The commonly used laboratory animals in virology are mice, guinea pig, rabbits and hamsters. The laboratory animals must be healthy before they are used and they should be properly cared for during the course of the experiment.

Animal/Birds of Choice for this System

Especially, for this system of virus cultivation, SPE birds are trialed. Such SPE birds and animals are named as *Gnotobiotic* (germ-free). (*SPE stands for Specific Pathogen Free*)

Routes of Inoculation

Various routes such as intracerebral, intradermal, intraperitoneal, intravenous and intramuscu-larly, have been used to inoculate animals with virus suspected material. The choice of a rout depends on the type of the virus.

Consequences of Virus Growth

Growth of a virus in the laboratory animal is characterized by;

i) Death of the animal

ii) Symptoms and development of lesions

Examination of Inoculated Animal

Experimentally inoculated/infected animals are examined daily for;

i) Clinical signs of disease, respiratory distress, CNS involvement, and visible lesions on skin and membranes.

ii) Abnormal behaviour of the animal

iii) Blood samples are taken daily for antibodies titer determination.

iv) Death of the animal

Examination of Biopsy Sample

Biopsy material or tissue specimens should be examined for;

a) Microscopically for lesions
b) Histopathologically for pathological changes
c) Serologically for presence of specific viral antigens by, e.g. gel diffusion, CFT etc.
d) By electron microscope, for identification of viral particles

EMBRYONATED EGG

Since the early 1950 the Embryonated hen's eggs have been used widely for cultivation of animal viruses. Embryonated egg however does not support the growth of all animal viruses. Most of the avian viruses grow in the embryonated eggs.

Point of Interest

The eggs should be free from any kind of germ. Thus, for this system of cultivation, usually *SPE-Eggs* laid by the SPE-birds are trialed.

Major Advantages

The major advantages of embryonated eggs over other systems are;

i) Easily available, economical and convenient to handle.
ii) Relatively free from bacterial and many latent viruses infections.
iii) Generally free from natural factors of defence.

This system provides the most suitable means for primary isolation and identification and production of viral vaccines.

Routes of Inoculation

The routes commonly used are i) yolk sac (intra-yolk) ii) chorio allantoic membrane (CAM)
iii) allantoic cavity (intra-allantoic).

ROUTES	AGE OF EMBRYONATED EGGS	EXAMPLES OF ANIMAL VIRUSES
Yolk Sac	6-8 days	RP-virus, BVD-virus, BT-virus
CAM	9-11 days	Canine distemper virus, IB-virus
Allantoic cavity	10-12 days	ND-virus, Avian Influenza virus

Consequences of virus growth

The presence of virus may be regarded by;

1. Death of the embryo (Toga virus)
2. Deformities such as dwarf growth (IB-virus)
3. Hemorrhages (ND-virus)
4. Oedema and pock lesions on CAM (Cow pox, Herpes B-virus)
5. Intracytoplasmic inclusion bodies (Herpes virus)

CELL CULTURE

Cell culture (earlier called *tissue culture*) is the most widely used method for cultivation of viruses.

Cell culture allows the primary isolation of viruses, performance of infectivity assays and biochemical studies and the production of viral vaccines.

Advantages

The main advantages of cell culture method over the other two systems are;

1. Growth of most viruses can be detected easily in cell culture.
2. Viruses can be grown in bulk.
3. Cells can be stored for longer period of time.

Disadvantages

1. Requirement of good facility.
2. More costly as compared to the embryonated eggs.
3. There are chances for the presence of latent viruses in the cultured cells.

PROCEDURE

Step1: To obtain a primary cell culture, tissue or organs preferably from embryonic or infant (e.g. chicken embryo, embryonic liver) are cut up in small fragments.

Step2: These fragments are mixed with TRYPSIN, which will enzymatically digest to individual cells. *As trypsin get dissolve the connective tissue, thus cells becomes separated.*

Step3: The cell suspension and appropriate growth medium are then added to a flat bottomed tissue culture flask and placed in a CO_2 incubator at 37^0C.

Step4: After a period of time, the cells attach to the bottom of the flask and start dividing until a monolayer is formed. This kind of cell culturing is known primary cell culture.

Step5: The inoculum suspected to contain a particular virus type is inoculated and allowed to absorb on the cell monolayer.

Step6: After absorption, the excess inoculum remained on the cell surface is removed.

Step7: Add an adequate amount of maintenance medium and incubate the flask at 37^0C.

Consequence of virus growth

The growth of the virus in the cell culture is observed daily by examining the inoculated flask for the CPE (Cytopathogenic Effect). The CPE depends upon the type of viruses. The effect may be any one of the followings;

1. Death of the cell
2. Ballooning appearance of the cell

3. Shrinkage of the cell
4. Synctial formation(e.g. respiratory virus having this prop. named bovine synctial virus)

Demonstration of Inclusion Bodies

INCLUSION BODIES*: These are the remnants of viruses that aggregate the protein and N.A.*

The inclusion bodies are characteristics morphological changes in the cells infected by certain viruses, which can be recognized by light microscopy.

Depending on the viruses, inclusion bodies may be intranuclear or intracytoplasmic, single or multiple, large or small, acidophilic (pink stained by eosin) or basophilic (blue stained by haematoxylin)

Cultivation of Viruses

The sewage water is full contaminated of bacteriophages. For example, *E.coli* can be grown on some media in the petridish --- the growth will be known as *Lawn of E.coli growth*.

VIRO - LECTURE 4

QUANTIFICATION OF VIRUSES

The quantification of viruses in a viral suspected sample/suspension is of marked importance.

For experimental purposes, it is foremost thing to know the intensity of the viral sample. We can only move forward for vaccine preparation, virus cultivation etc. if we first, quantify the viruses in a virus inoculum.

Methods of Quantification

The methods of quantification are divided into two categories;

i. Physical Method
ii. Biological Method

I. PHYSICAL METHOD

In this method, electron microscope is used for quantification.

Electron Microscopy

Through EM, besides the size and shape of viruses, we can quantify/count the viral particles.

Step1: Mix the known no. of *latex beads* with the diluted purified virus suspension.

Step2: Put this suspension on the copper grid/mesh of the microscope.

Step3: Examine it under microscope and count the particles in some specific area.

Step4: Find a ratio between the latex beads and virus particles being seen under microscope.

Conclusion

The counted virus particles can be expressed as ……. No. of virus particles/µl

II. BIOLOGICAL METHOD

This method includes a number of important techniques used for quantification of viruses.

Plaque Assay

Step1: Purified virus suspension is inoculated on the monolayer cell culture in vitro.

For bacteriophages, bacterial colonies are used for culturing process.

Step2: Make two fold diluted suspension of bacteriophage in separate test tube such as;

1:2	1:4	1:8	1:16
10^{-1}	10^{-2}	10^{-3}	10^{-4}

Step3: Allocate a petridish to each test tube and take measured volume (µl) of suspension.

Step4: Put a swab of *E.coli* culture on it. This type of bacterial culturing in the petridish is known as Lawn of E.coli.

Step5: Incubate them and examine each petridish for plaque formation.

Step6: Count the plaques formed in each petri dish.

CONCLUSION

Virus particles = Plaque number x reciprocal of dilution x reciprocal of volume in ml.

For example: If there is a mean of 50 plaques in 0.01ml of a 10^{-4} dilution of virus inoculum, then the PFU/ml will be 50 x 10^4 x 100 = 5 x 10^7 (*PFU stands for Plaque Forming Units*)

Only Cytopathogenic viruses can form plaques and the test cell must form a confluent monolayer and should be in good condition for the assay.

Pock Assay

In the previous technique we have to count the plaque numbers, but in this technique, pock lesions should be counted form on a surface exposed to the virus inoculum.

The counted pocks can be expressed as…Pock FU/ml. (Pock FU *stands for Pock Forming Units*.

Haemagglutination Assay

Many families of the viruses have the property to bind to erythrocytes (RBCs) of different species through complementary receptor sites on the erythrocytes surface. The reaction involves only virus and erythrocytes and no antibody is involved.

Haemagglutination is the one of the simple tests for the detection and quantification of some viruses or viral particles.

Step1: Add a suspension(2%) of washed erythrocyte of appropriate species to the virus infected cell culture supernatant in a normal saline solution.

Step2: Make two fold diluted suspension as follows;

1:2	1:4	1:8	1:16
10^{-1}	10^{-2}	10^{-3}	10^{-4}

Step3: Presence of haemagglutinating virus causes agglutination (clump formation) of the erythrocyte after about half hour incubation.

Step4: No agglutination of erythrocyte indicates the formation of button.

Conclusion

The clump formation/haemagglutination ability of a virus is known as HA titre.

The "HA titre" can be expressed as …… reciprocal of dilution / 10µl (e.g. 10^4/10µl)

For example, if a virus form clumps up to diluted suspension 10^{-3} and there is no agglutination (RBCs float freely) in the diluted suspension 10^{-4} and a button formation is here, then we can concluded that the HA titre of such virus will be …… 10^{4} /10µl.

Greater the ability of clump formation ---- Stronger will be the virus.

Lower the ability of clump formation ------ Weaker will be the virus.

Enzyme Linked Immunosorbent Assay (ELISA)

The principal of ELISA is the detection of solid phase bound antigen and antibody complex by a color reaction using specific enzymes and substrate.

An antigen or antibody, which is usually protein in nature can be adsorb passively on solid phase. If the bound antigen is specific to the antibody, an antigen, antibody and enzyme complex will form and this can be detected by adding chromogen and substrate specific to the enzyme. In this technique, we will make the different dilutions such as;

1:2	1:4	1:8	1:16	1:32
10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}

The antigens (on the surface of virus) form complex with the known antibodies, on the basis of this property of virus, we can experimentally quantify the viruses.

Polymerase Chain Reaction (PCR)

PCR is a highly sensitive technique by which minute quantities of specific DNA and RNA sequences can be enzymatically amplified to a sufficient quantity of material for detection.

Real-time PCR: This mode of PCR is helpful in the quantification of viruses.

ASSAYS FOR VIRAL INFECTIVITY

Two types of assays viz. i) *quantitative assays* and ii) *quantal assays* are used to determine the viral infectivity primarily in cell cultures and occasionally in other systems.

QUANTITATIVE ASSAYS

These assays quantify the number of virus particles in an inoculum. The commonly used assay in cell culture is *Plaque Assay*. In addition to plaque assay, *Pock Assay* is also used.

QUANTAL ASSAYS

These assays don't count the number of infectious virus particles present in an inoculum.

To perform the assay; *Step1:* Serial dilution of a virus inoculum is made and is inoculated into tubes containing cell monolayer. *Step2:* After incubation, the incubated tubes are examined for virus infection i.e. by looking to the changes (e.g. CPE) in the inoculated cells, the titre of the inoculum is determined.

This method of Reed and Muench is widely used to calculate the 50% end point.

Conclusion

The *titre* can be defined as the reciprocal of the dilution of the inoculum that infects 50% of the inocultaed cells and the titre is expressed as the 50% tissue culture infective dose/$TCID_{50}$.

The *titre* expressed as the 50% lethal dose/LD_{50} tells us the infectivity of the inoculum that kills the 50% inoculated cells.

VIRO LECTURE 5

INACTIVATION OF VIRUSES

Physical Methods

Nature has provided some mechanism to inactivate the viruses.

1. Temperature

Most viruses are sensitive to heat and can be inactivated by heating at 50-60 C for 1-2 hours. Enveloped viruses are usually more heat labile than the non-enveloped viruses. For example, the non-enveloped *infectious bursal disease* virus can survive for 5 hours at 56 C, where as enveloped infectious bronchitis viruses of birds can be destroyed at 56 C within 15-30 minutes.

2. Radiations

Direct sun light is destructive to many viruses. Ultra violet light is responsible for the formation of Thiamine complex. Ionizing radiations (X-rays, gamma rays) inactivate viruses by causing damage to the nucleic acid.

3. pH

Ideal pH for viruses is 5.5 – 6.5. Certain viruses that are present normally in the intestinal tract (Rotaviruse) can survive even at pH 3. Strong acids and alkalis can denature viral proteins and are very effective in destroying many viruses.

Chemical Methods

In the chemical method of inactivation of viruses, certain chemical agents are used as follows;

a) Oxidizing agents

Oxidizing agents such as hypochlorite, iodophores, acetic acid and par acetic acid vapor etc. can inactivate most viruses. Iodophore disinfectants are widely used in the dairy industry and par acetic acid vapor is commonly used to disinfect plastic isolators.

b) Alkylating agents

Alkylating agents such as formaldehyde, gluteraldehyde cross link the viral proteins and also inactivate the viral nucleic acid.

c) Protein denaturants

Protein denaturants like alcohol, phenol etc. are poor disinfectants of many viruses because they leave the nucleic acid infectious. These imparts effect on the viral proteins thus, virus can not replicate.

d) Nucleic acid denaturants

Nucleic acid denaturants like beta-propiolactone, acetyl ethyleneimine and ethyleneimine denatured viral nucleic acid by cross linking and they do not denatured proteins.

e) Detergents

Detergents like sodium deoxycholate can lyses and inactivate most enveloped viruses.

f) Lipid Solvents

Lipid solvents such as ether and chloroform have effect on enveloped viruses.

PRESERVATION

Repeated freezing and thawing is harmful for some viruses and such viruses or the virus containing material can be preserved at 4 C for few days. For example, if we have a virus suspension and we want to inactivate the viruses then place it in the refrigerator at 4 C.

Preservation for 1 week:

If we have to study it within One week then don't freeze because due to freezing and thawing, there are chances of loss of 1 log titre and activity may lost.

Preservation for more than 1 week:

If we have to keep this virus suspension for some longer period of time (2 weeks or 1 month) then freeze it at -20 C for few weeks and by addition of glycerol, integrity of virus is maintained and thus we can preserve it for several months. If we have to keep these viruses for years then add glycerol in it and preserve at -70 C or at -190 C (liquid N_2).

Another method of preservation of viruses is the freeze drying or lyophilization. Purified virus suspended in cell culture medium can be lyophilized by adding 10% fetal bovine serum or casein. After lyophilization, the lyophilized virus-containing ampoule can be stored at 4 C. Logic: In the lyophilization, we get the protein and nucleic acid of viruses only and other parts becomes dry or in powder form at vacuum. The protein and N.A. are sealed under vacuum thus; the viruses can alive for many years.

This method is most successful for long-term preservation particularly for live viral vaccines.

CLASSIFICATION OF VIRUSES

For the nomenclature and taxonomic purposes, there is an International Committee for Taxonomy of Viruses (ICTV). There are certain criteria to classify the viruses such as on the basis of ….

NATURE OF GENOME: THERE ARE SEVEN GROUPS IN WHICH VIRUSES ARE DISTRIBUTED.

DNA-containing viruses

Group I	ds DNA viruses	i) Circular	a) Naked (Papovavirus) b) Enveloped (Baculovirus)
		ii) Linear	a) Naked (Adenovirus) b) Enveloped (Herpesvirus)
Group II	ss DNA viruses	i) Circular	(Circovirus)
		ii) Linear	(Parvovirus)

RNA – containing viruses

Group III	ds RNA viruses	i) 8-12 Segmented	Reoviridae (Rotavirus)
		ii) 2 segmented	Birnaviridae
Group IV	+ve sense ss RNA viruses	(Picornavirus) and (Flavivirus)	
Group V	-ve sense ss RNA viruses	(Thomyxoviridae, Paramyxoviridae and Rhabdoviridae)	
Group VI	Reverse transcripting RNA viruses - e.g. Retroviruses (HIV)		
Group VII	Reverse transcripting DNA viruses – e.g. Hepadnavirus (Hepatitis B virus)		

VIRUS TRANSMISSION

Viruses are present on every surface; may transmit from one animal to another and one human to other human.

Types of Virus Transmission

1. Horizontal Transmission

Transmission of viruses from one place to another, on farm to other or one individual to other individual

2. Vertical Transmission

Transmission from parents to offspring e.g. via milk secretions and even some viruses can cross the placental barrier and infect the fetus. It also includes the transmission from infected egg to hatched chick.

3. Vector Transmission

Transmission of viruses by insects/vectors. They may act as mechanical or biological vectors.

Inanimate vectors e.g. Needle, Razor. Viruses can be transmitted through skin, respiratory tract, GIT, genital tract, conjunctiva (Newcastle disease).

VIRO LECTURE 6

MULTIPLICATION/REPLICATION OF VIRUSES

There are certain stages involved in the viruses' multiplication/replication.

1. Adsorption

Virus has specific proteins on its surface called "virus attachment proteins" and the cells (may be plant or animal) have specific receptors (ligands) for the attachment of viruses.

The specific proteins of viruses are usually F-proteins or Glycoprotein in nature.

Inhibiting Agents

The attachment of virus can be inhibited by certain antiviral agents. If there is no attachment then there is no entry of the virus into the host's cell. 1) Specific antibodies play a pivotal role by occupying antigenic mass on the virus proteins. 2) Heparin and 3) Dextron Sulphate also inhibit the attachment of virus.

2. Penetration

After the attachment of virus on the host's cell, penetration is the next goal of the virus.

There is a marked variation in the penetration pattern of different viruses. Some viruses only inject their genome inside the host's cell. On the other hand, some viruses penetrate as a whole and reside in the cell.

Due to attachment of virus on the cell's surface, receptor-mediated endocytosis gets activated → Endocytic vesicle formed → virus penetrate though invagination → capsid proteins are dissolved → Genome get free inside the host's cell. → Penetration has been completed.

Inhibiting Agents

There are certain chemicals that can inhibit the penetration of virus. E.g. Tromantidine.

3. Uncoating

If penetration is done successfully then uncoating of the virus takes place. Enveloped viruses, which enter by endocytosis, the nucleocapsid is released directly into the host cell cytoplasm. But some of the enveloped viruses are uncoated by the synthesis of virus coded uncoating proteins. In some naked viruses, the attachment step itself triggers the process of uncoating.

Inhibiting Agents:

The antiviral drugs such as Amantadine and Arildone inhibit (stop) the uncoating of viruses.

4. Biosynthesis

The viral genome consists of two parts; named as early genome and Late genome.

i) Early genome → Early mRNA → Early protein

The early proteins are non-structural proteins/enzymes (polymerase). These proteins and enzymes are further used for the replication of viral genome.

ii) Late genome → Late mRNA → Late proteins

The late proteins are structural proteins and make the infrastructure of the virus capsule.

Inhibiting Agents

a) Interferon: inhibit the protein synthesis as well as interfere the function of polymerase.
b) Ribavirin (analogue of guanosine): inhibit mRNA synthesis.
c) Analogues of certain nucleus such as Azidothymidine (AZT) and 5-flurouracil: Inhibit the polymerization.

5. Assembly

In this step, the genome of virus gets inside the protein coat. If these genome and proteins are not assembled so, then we called them "inclusion bodies". These are aggregates of those contents which are not used in assembly.

Inhibiting Agents

Certain factors like Indinavir and Protease inhibitors i.e. Tamiflue inhibit the assembly of the viruses.

6. Release

It is the final step in the completion of viruses replication. Two mechanisms viz. lysis and budding are primarily involved for the release of mature virions from the infected cell. Most of the non-enveloped viruses are released by the lysis of the cell. Most of the enveloped viruses release by exocytosis (i.e.budding).

One Step Growth Curve

Definition: The usual experimental procedure for studying viral replication is called a "one step growth curve".

Eclipse Period: After uncoating, it is the period during which no infectious virus particles can be demonstrated even intracellularly. Latent Period: In this period, no virus can be found inside the cell and it lasts till the release.

Latent Period in FMD virus = 5 hours

Latent Period in Influenza virus = 12 hours

DRAW ONE STEP GROWTH CURVE HERE

VIRO – LECTURE 7

ANTIVIRAL DRUGS

Definition: Antiviral drugs are the substances that can either prevent the penetration of a virus in the host's cell or specifically inhibit some essential steps in virus replication and maturation etc.

Antiviral Agents	Mode of Action
Antibodies	Inhibit adsorption of viruses
Heparin	Inhibit adsorption of viruses
Dextron Sulphate	Inhibit adsorption of viruses
Tromantidine	Inhibit penetration of viruses
Amantidine	Inhibit uncoating of viruses
Arilidine	Inhibit uncoating of viruses
Interferon	Inhibit mRNA synthesis (Transcription) and Protein synthesis (Translation).
Anti-sense analogues	Inhibit mRNA synthesis (Transcription)
Trfluorothymidine	Inhibit DNA replication
Protease inhibitors (i.e. Tamiflu)	Inhibit assembling of viruses

POSSIBLE OUTCOME OF VIRUSES INFECTION

There are a number of possibilities as a result of viruses' infection. From which more frequent and commonly observed consequences are listed below;

1. Productive Infection:

   Virus → Normal Host Cell → Virus-infected cell → Virus replicate by using host's cell machinery;

   → Cell death

   → CPE (Cytophathogenic effects) like

   - Syncytial formation: Cell membrane is lost & many cells look like a gathering mass with numerous nucleus
   - Ballooning of the cells - Presence of Inclusion bodies.

2. Persistently Chronic Infection

   Virus may persist in the cell for many years and causes persistently chronic infection. The cell lyses take a longer period of time in this case.

   e.g. Hepatitis C virus: It may constantly persist inside the cell for about 10 years without causing cell lyses.

3. Latent Infection:

   In this type of infection, virus integrates into the host's cell. The genome of virus occupies the host's cell genome and the necessary configuration occurs in the host's cell genome. Host's cell genome recognizes the virus's genome as its own and thus, makes it the part of its won genome. → Transmission occurs from one progeny to other.

   But 10-15 years later, the virus genome again gets activated and will start causing lyses of host's cells.

   e.g Herpes virus: It often lives inside the neuronal cells calmly and after a longer period of time, gets activate.

4. Abortive Infection

   Such type of infection is carried out by the defective viruses. The genome of such viruses has not been full potential to replicate because their genome is incomplete.

   - During biosynthesis, early proteins may be formed but the structural proteins are not formed, due to which these viruses known as defective viruses. They lacking something in their genome; thus replication is incomplete. On the other aspect, there are certain viruses which have a capability to complete the genome of such defective viruses; are known as helping viruses.

5. Apoptosis-inducing Protein Infection:

   Many viruses have the ability to modulate the apoptotic pathways through the manipulation of a variety of apoptotic-inducing proteins. These proteins are responsible for enhancing apoptosis.

   Apoptosis: It may be defined as "the programmed cell death"

   Mechanism: Due to early apoptosis of the host's cell, viruses avail a chance of limited replication. As soon as cell lysed, viruses lost the host's cell machinery.

   Examples; Apoptosis-inducing proteins are; E1A and E4 produced by adenovirus, 5EL produced by chicken anemia virus.

VIRUS - HOST CELL INTERACTION

In the previous section, various responds of virus towards host's cell has been explained with all the possible outcomes of viruses infections. But here only those factors will be described that are exhibited by the host in the viruses infection.

1. Non- Specific Immunity i.e. Innate Immunity

It is naturally present immunity in an individual which have a great impact on viruses infection. This factor varies from species to species, individual to individual.

a) Species Variation:

FMD virus only infects the Cloven Footed animals while it doesn't affect the Equines and Poultry birds.

ND virus causes New castle disease in poultry but doesn't transmit infection to all other animals.

b) Individual Variation:

This variation can be observed in the individuals of the same species. Such type of variation against the viruses infection exists due to developed innate immunity.

Complement System

It is present in every body which gets activated against the infections particularly for viruses infection. Serum proteins (may be formed in liver) present in the blood start making the antibodies against the recognized antigens of the viral particles. Antibody + virus antigen reaction → Lysis of virus particles

Dose of Inoculum

It is also a very important factor. With a smaller dose of inoculum, there may be destruction of half of the cells but with a large dose, destruction of all the cells in a particular area may be observed.

Cell → Neutrophils, Monocytes and Macrophages – These defensive cells may cover the virus infection but if the infection is more virulent then infection may persist.

INTERFERON

Definition: 1) Interferons are antiviral substances produced naturally by some body cells of vertebrate in response to viral infection and a variety of other agents. OR

2) Interferons are protein or glycoprotein in nature produced in body cells in response to viruses infection.

Commercially, interferon is available in the market in injections, may be formed synthetically by using E.coli.

MECHANISM OF ACTION

1. Genome Degradation

   Interferon is injected into virus-infected cell → INF also affects the neighboring cells → These cells have restriction enzymes → INF bring a change in the enzymatic system and get activate. → INF penetrate into the virus and degrade its genome i.e. may be RNA.

2. Protein Synthesis Inhibition

   INF inhibits the protein synthesis mechanism by disrupting the genome of the virus.

CHARACTERISTICS OF INTERFERONS

i) INFs are relatively thermo-stable.
ii) INFs are active through a wide range of pH.
iii) INFs are Weakly antigenic
iv) INFs can be inactivated by proteolytic enzymes like trypsin
v) INFs are not destroyed by receptor destroying enzymes like RNase, DNase or periodate.

TYPES OF INTERFERONS

Four types of interferons have been recognized and specific host cells produce each.

1. Alpha (α) interferon: They are produced primarily by B-lymphocytes and macrophages.
2. Beta (β) interferon: This type of interferon is primarily produced by fibroblasts and epithelial cells.
3. Gamma (γ) interferon: This type of interferon is produced by T lymphocytes.

4. Omega (ω) Interferon: It is produced by the leukocytes.

Interferons play very important role to check (stop) the replication of viruses.

2. Specific Immunity i.e. Acquired Immunity

Specific immunity response is also very appreciable and of great value in the viruses infection. In this type of response, different defensive cells are involved like B-Lymphocytes and T-Lymphocytes.

Mode of action of B-cell

B-cells → Effectors of B-cells → Abs production → Abs are very specific to virus antigens. The vaccine response mostly depends upon the Abs production.

Mode of action of T-cell

T-cells → Found in the spleen and blood circulation → If body is suffering from any viruses infection → T-cells become activated and known as "Activated T-cells" or "Sensitized T-cells". → In this case, the population of Cytotoxic T-cells will be activated → Such type of response is known as cell mediated response (cells are involved)

Premature Lysis of Host's Cell

Virus infect the specific cells → These cells will now known as target cells for T-cells → T-cells will produce the "Porin" protein → As a result, the target cells will lysed thus, biosynthesis or replication of virus being present in these cells will be stopped. → Such type of target cell lysis is known as " *Pre-mature lysis of the cells*".

LECTURE 8

ACQUIRED IMMUNITY

Define: Immunity obtained either from the development of antibodies in response to exposure to an antigen, as from vaccination or an attack of an infectious disease, or the injection of antiserum.

The acquired immunity is of two types;

1) Active Acquired Immunity
2) Passive Acquired Immunity

1. Active Acquired Immunity

It is further divided into two subtypes.

i) Natural Acquired: It is usually developed after low or virulent type of infection. The antibodies and sensitize T-lymphocytes impart a major role to develop this type of immunity.

ii) Artificial Acquired: This type of immunity can be developed by vaccination. Inactivated viruses or any part of these viruses having an immunogenic property is introduced for this purpose.

Vaccines: These are of two types; a) Live vaccines b) Killed vaccines

a) Live Vaccines: (e.g. Polio Vaccine)

Feature: i) Easy to inoculate; can be given in the drinking water, feed or via spraying.

ii) Virus multiplication is very quick and efficient iii) Smaller doses are sufficient to get the optimum response. iv) There is no need of boosting dose. v) Cold chain is required to revert the response. vi) These are regarded as attenuated because of chance of infection.

b) Killed Vaccines

Features: i) Viruses are kept at low temperature in order to keep them inactive ii) Larger doses are needed to get the optimum response. iii) There is no need of cold chain to revert the response. iv) Killed vaccines can be inoculated via I/V and I/M routes.

2. Passive Acquired Immunity

Like active acquired, it is also further subdivided into two subtypes.

i) Natural Passive: Colostrum feeding, immune cells in pregnancy from mother to the fetus and Egg yolk for poultry chick result into development of this type of immunity.

ii) Artificial Passive: Hyperimmune serum (full of readymade Abs) can be used for many life threatening viral diseases like FMD in animals, Snake bite and Rabies in human.

VIRAL GENETICS

In this section we will discuss the following aspects of viral genetics.

1. Mutation
2. Mutagens
3. Mutations at virus level

Genome: In modern molecular biology the genome refers to all of its hereditary information encoded in DNA or RNA which may be single stranded (ssDNA, ssRNA) or double stranded (dsDNA, dsRNA).

1. MUTATION

Definition: It is defined as a change of the nucleotide sequence of a gene. Gene is a function unit of genome. The mutation can occur spontaneously or can be induced by chemical or physical agents (called mutagens).

Consequences of Mutation: The changes in the gene can alter the properties of virus and also causes phenotypic changes of the virus.

Rate of Mutation:

The frequency of mutation either spontaneous or induced is higher with RNA than with DNA viruses.

Mutation Rate
RNA viruses: 10^{-3} to 10^{-6}
DNA viruses: 10-8 to 10^{-11}

In case of RNA viruses, the rate of spontaneous may vary from 10-3 to 10-6 per incorporated nucleotides. Whereas in case of DNA viruses, the rate of mutation varies from 10-8 to 10-11 per incorporated nucleotide.

Why Mutation rate of RNA viruses are higher than that of DNA viruses?

It is primarily due to the difference in the replication enzymes. For example;

- RNA viruses having ssRNA or dsRNA, use "*RNA dependent RNA polymerase*" enzyme whereas DNA viruses having ssDNA or dsDNA use "*DNA dependent DNA polymerase*".
- DNA dep. DNA polymerase has an ability of proofreading while the enzyme for RNA viruses lacks this ability, due to which they exhibit high mutation rate.

Spontaneous Mutation: it is the change occurs by nature in the nucleotide sequence of the gene.

Induced Mutation: the change in the nucleotide sequence carried out by mutagens. The main purpose of such mutation is to manipulate various kinds of vaccines.

2. MUTAGENS:

The agents may be physical or chemical which can induce the mutation.

MUTAGENS OF PHYSICAL TYPE

1. UV-light: It result into "dimer formation" of nucleotide bases i.e. pyrimidine etc.
2. Temperature (Heat): It causes deamination of nucleotides.
3. Ionizing Radiations i.e. X-rays: In RNA viruses, make trimer formation by breaking up chemical bonds.

MUTAGENS OF CHEMICAL TYPE

Some of the important mutagens are enlisted as;

1. Base Analogues such as;
    a) 5-bromouracil (replaces "Thymidine" base)
    b) 5-bromo-deoxyuridine (for DNA virus)
2. Alkylating agent: e.g. diethyl sulphate
3. Deaminating agent: e.g. nitrous acid

TYPES OF MUTATION

Point Mutation

It is the type of mutation which occurs through single nucleotide replacement or substitution of one base by another base. It results into change of nucleotide sequence without any change in molecular size.

a) Transitional Mutation:
   The mutation in which a base is replaced with another but of the same group. For example: purines are replaced with purines or pyrimidine with pyrimidine.
b) Transvertional Mutation:
   In this mutation, a purine base is replaced with a pyrimidine and vice versa.

Frameshift Mutation

In this mutation, there is multiple deletion and insertion of nucleotide sequence.

Inversion Mutation: When a group of bases is inserted or deleted from the nucleotide sequence, then such type of mutation is called inversion mutation.

Consequences/Effects of above described Types of Mutations.

i) Silent Mutation: It is also known as Neutral mutation. In this, a few change may occur in the base sequence but there is no change in amino acid expression sequence.
ii) Mis-sense Mutation: Point mutation leading to a change in the structure and character of a protein through replacement of an aminoacid. The properties of A.A may be conservative (i.e. aminoacid sequence properties of a new proteins are similar to that of the previous one protein).
iii) Nonsense Mutation: The point mutation when it causes non-formation of a protein through production of a termination codon.
iv) Null Mutation: The mutation which destroys the gene function completely; due to extensive insertion or deletion and result into major/gross rearrangement in the genome.

MUTATIONS AT VIRUS LEVEL

1. Lethal Mutation: Mutation, which leads to non-production of viable progeny of a test organism. Viruses are unable to replicate and even to survive and cann't isolate from cell culture.
2. Deletion Mutation: Mutation, which result into loss of a portion of gene or loss of its function.
3. Plaque Mutants: These are some wild type of virus mutate its infectivity by changing the plaque shape.

4. Host range Mutants: Difference in the host tissue tropism from the wild type virus; for example if a virus itself not affect the poultry then certain mutants may involve in this mechanism and causes infection in the poultry.
5. Attenuated Mutants: Viruses have mutation to become attenuate, so they become less virulent and pathogenic. We can do attenuation by growing these viruses in the cell culture of non-natural host which make them less virulent.
   e.g. Rabies virus, no doubt, is very infective but if it is inoculated again and again in embryonated egg (non-specific host), its infectivity in the natural host may lost to an appreciable extent.
6. Temperature Sensitive (Ts) Mutants: Mutants bring some changes in order to induce the capability to grow at very low as well as at very high temperature in the virus.

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VIRO LECTURE 9

LABORATORY DIAGNOSIS OF VIRAL DISEASES

For sampling of virus, condition and stage of the disease is very important; for example;

In the early stage of disease, look for the virus in the different suspected tissues. But in the later stage (chronic or convalescent form), virus isolation is very difficult and its chances are very much low due to dominant population of Abs produce in response to this viral infection, present in the serum. If virus no. is quite high in the sample, then go for direct demonstration.

1. Direct demonstration of virus:
   For this purpose, electron microscope (EM) is used by which we can observe the images of viruses to determine the shape and even size of the viruses.
2. Isolation of viruses
   Use any virus cultivation system either of three i.e. animal host, embryonated egg or cell/tissue culture system for isolation of viruses.
3. Detection of Virus specific antigen
   We can detect virus specific antigens by conducting a variety of laboratory test. The virus proteins like Glycoprotein (G-protein) and Fusion protein (F-protein), are usually immunogenic and antigenic in nature and we can observe it via HA test, Immuno-diffusion test etc. Most commonly used tests are described below.

Immuno-diffusion (ID)

In this test, the specificity of the antigen to the known antibodies is observed and vice versa. For example, if the known antibodies are specific to the antigen, then a line of precipitation is formed which indicates the reaction.

PROCEDURE

If we have known antibodies then, we can find the antigen specific to it and vice versa.

Step1: Take a glass petri-plate and pour agar agar medium in it.

Step2: Make wells in the medium.

Step3: Add antibodies in the central well.

Step4: Add antigens in the other peripheral wells (as seen in fig.)

Step5: Incubate for some time.

OBSERVATIONS & RESULT

If known antibodies are specific to the antigen then a line of precipitation will formed in between both the wells.

For this test, specific antibodies for certain antigen should be available.

Complement Fixation Test (CFT)

This test is based on the antigen and antibody reaction to which complement binds and form antigen, antibody and complement complex.

Indicator System: An indicator system comprising of sheep RBCs and anti-sheep RBCs is used to detect the presence of such complex. Amboceptor: The anti-sheep RBCs are also called Amboceptor. It is a serum which contains sheep RBCs antibodies. How to get Complement? Rabbit's serum is a good source of complement.

PROCEDURE

This conventional CFT is performed in two stages;

FIRST STAGE

Step1: Mix the known viral antibodies with unknown antigen and measured complement.

If the unknown antigen is specific to the antibody; Then antigen-antibody complexes are formed and complement will be fixed, inactivated and depleted.

If antigen is not specific to the antibody; antigen-antibody complex is not formed, the complement will not be fixed and will remain free in the mixture.

SECOND STAGE

A suspension of sheep RBCs and anti-sheep RBCs is added to the first stage mixture. Complement being a lytic agent, active free complement (in absence of antigen-antibody complex) will cause lysis of this suspension.

Conversely, absence of hemolysis indicates a positive test that means complement has fixed to the antigen-antibody complex.

CONCLUSIONS:

No lysis of sheep RBCs + anti-sheep RBCs indicates that antigen is –ve for antibodies and it results into bead formation. If lysis of sheep RBCs + anti-sheep RBCs occurs, fluid will be transparent, no RBCs will be there and no bead formation.

Fluorescent Antibody Test (FAT)

The principal of FAT is based on the antigen and antibody reaction to which a fluorescent dye labeled with antibody is applied and examined under fluorescent microscope.

If the antibody is specific for the antigen, antigen-antibody complex form and labeled dye giving a visible color under the microscope.

Dyes Used in FAT

The two fluorescent dyes (known as fluorochromes), commonly used are rhodamine, which fluoresces raddish orange, and fluorescein isothiocyanate, which fluoresces yellowish green.

This technique is used commonly to detect viral antigens in clinical specimens directly.

PROCEDURE

The steps involved in the test are;

Step1: Apply virus specific antibody directly on the acetone fixed tissue smear.

Step2: Incubate for 30 min.

Step3: Go for washing and then apply optimally diluted antispecies antibody over it.

Step4: Finally wash the smear, keep it dry and then examine under fluorescent microscope using a powerful ultraviolet/blue light source.

CONCLUSION

If the smear remains intact after washing then it will appear fluorescence. The presence of fluorescence indicates positive result.

Conjugated Antibody: Antibody against viruses can also be conjugated with biotin and after application such conjugated antibody on tissue smear, the antigen-antibody reaction can be detected by avidin labeled with fluorescent dye.

Radioimmunoassay (RIA)

The principle of RIA is similar to ELISA except that the antibodies are labeled with radio-isotopes. The assay can be used for both detection of unknown viral antigen and antibodies.

Sandwich RIA: It is type of RIA used for detection of unknown viral antigen.

PROCEDURE

The steps involved in the test are as follows:

Step1: Firstly, absorb unlabelled known viral antibodies to a solid phase support as capture or coating antibody.

Step2: After washing the clinical specimens, apply on this solid phase.

Step3: Incubate for 1 hour at 37 C.

Step4: Remove unreacted material by washing after incubation.

Step5: Add the antispecies gamma globulin labeled with radioisotope.

Step6: Incubate it again and then wash it carefully.

CONCLUSION

The radio-activity of the antibody bound to the solid phase (in positive case) is measured in a gamma counter and the results evaluated by comparison with proper controls.

If antigen is not specific to antibody, then there would be no radioactivity of the antibody on the solid phase. This activity is only visible due to the specificity for each other.

Enzyme Linked Immunosorbent Assay (ELISA)

The principle of ELISA is the detection of solid phase bound antigen and antibody complex by a color reaction using specific enzymes and substrate.

OBSERVATION

If the bound antigen is specific to the antibody, an antigen, antibody and enzyme complex will form and this can be detected by adding chromogen and substrate specific to the enzyme.

CONCLUSION

A positive reaction is indicated by the development of color, which can be detected visually or in spectrophotometer (ELISA reader).

Advantage of ELISA over RIA

ELISA is replacing other immunoassay like radioimmunoassay (RIA) beacuase it offers the same sensitivity and not requires expensive hazardous radioisotopes. The results in ELISA can be read visually, but in RIA results are read always with equipment.

Virus Neutralization Test (VN)

It is one of the sensitive and specific tests for detection of unknown virus using known antibodies or unknown viral antibodies using known virus. Application: This test is usually used to determine the titre of virus neutralizing antibodies in serum samples of a virus infected animals.

PROCEDURE

Step1: Make two fold serial dilution of the test serum samples.

Step2: Mix with the standard volumes of an infective reference virus.

Step3: Incubate the serum virus mixture at 37C for 1 hour to 1½ hour.

Step4: Inoculate this serum virus mixture into a susceptible host system.

The host system may be a cell culture, animal or embryonated eggs.

Note: The host system must be one in which the virus produce specific lesions or cause death in the absence of antibody.

CONCLUSION

Presence of neutralizing antibody in the test serum samples will indicate the virus non-infectious by absence of specific lesions in the host system after inoculation of serum-virus mixture is considered as positive test.

4. Detection of Virus specific antibodies.

The test for detection of virus specific antibodies is usually conducted in the last stage of the disease and we must know about the virus antigen.

How we can detect? We take antisera in the later stages of the disease from the animal and measure the antibodies titre present in the serum.

Westernblotting is a technique which is used to detect specific antibodies in the serum even some specific proteins of the virus.

5. Detection of Nucleic Acid

Nucleic acid can be detected by certain means; for this purpose, specific nucleic acid sequence is taken and then label with biotin radioisotope and DNA probe is made.

In addition to DNA probe, cDNA probe and PCR are also used to detect the nucleic acid.

DNA probe is also known as "Primer". Its specificity for a certain viral disease is of much importance. It is used in a specimen to detect a specific sequence of nucleotide.

Polymerase Chain Reaction (PCR)

PCR is a highly sensitive technique by which minute quantity of nucleotide sequences can be detected as well as enzymatically amplified to a sufficient quantity of material for detection.

In PCR, different Primers (specific nucleotide sequence) are used. What is primer? Primer is structurally composed of 18-20 bp; it can only be hybridized if it is specific for a certain nucleotide sequence. We can get millions copies of DNA or RNA sequence within half an hour. It is the beauty of PCR.

VIRO LECTURE 10

ORTHOMYXOVIRIDAE

The family name "orthomyxoviridae" derives from the words, *ortho*; means true and *myxo*; means mucus.

Members of this family have special affinity for *mucin* (of mucus membranes). They are responsible for a wide variety of upper respiratory tract infections.

Common Lesion

The common name of the lesion caused by orthomyxoviruses is "flue".

Morphological Characteristics

Orthomyxoviruses are highly pleomorphic, mostly spherical/ovoid ----- 80-120 nm in diameter.

The genome of the viruses is negative sense, single stranded RNA (-ve sense ssRNA) with 8 segments.

Genome: The genome of the virus is enclosed by nucleoprotein + capsid protein which are antigenic and group specific. Envelope: The virions are surrounded by an envelope which is made up of lipid bilayer. Haemagglutinin and Neuraminidase: From the envelope, two types of glycoprotiens i.e. rod shaped haemagglutinins (H) and mushroom shaped neuraminidase (N) are projected out in the form of spikes or peplomers. These proteins are antigenic in nature and sub-type specific. Matrix Protein: Beneath the envelope there is a matrix protein which is antigenic and genus specific.

Antigenic Properties

The major antigenic components of the orthomyxoviruses are the two antigenically variable envelope proteins viz. H and N and two major internal proteins viz. NP and matrix protein.

Haemagglutinin: In each virus particle, there is about 1000 haemagglutinin spikes. Haemagglutinin is responsible for their ability 1) to agglutinate RBCs and 2) to attach and penetrate host cells (simply called adsorption). Sialic acid is a receptor for attachment of HA virus.

Neuraminidase: It plays a reversal role to that of Haemagglutinin. Instead of agglutinating, it appears to be involved in the release of newly formed virus from the host cells.

The structural arrangement of these glycoproteins on the virus envelope is also of great account.

The spike or peplomer of neuraminidase is larger than that of haemagglutinin. After 1 spike of neuraminidase, 4 haemagglutinin spikes are arranged. Thus the ratio between H: N is 4: 1.

Genotypic Expression

The genome of these viruses is usually 8 segmented (Group C viruses have 7 segmented genome)

Each of these 8 segments codes for one type of protein as follows;

Segment 1, 2, 3 → Polymerase	Segment 4 → Haemagglutinin	Segment 5 → Neuraminidase
Segment 6 → Nucleoprotein	Segment 7 → Matrix Protein	Segment 8 → Enzymes

Remember: four segments codes structural proteins while the rest four codes non structural proteins.

Both H (haemagglutinin) and N (neuraminidase) carry the subtype antigens as follows;

Subtype antigen for haemagglutinin are H1, H2, H3, H4, ……. H16

Subtype antigen for neuraminidase are N1, N2, N3, …….. N9.

Thus, we can conclude that orthomyxoviruses may posses 144 serotypes. (16x9 = 44)

Serotype of orthomyxoviruses can be denoted as; $H_{n\ (1\text{-}16)}\ N_{n\ (1\text{-}9)}$

Reassortment: Reshuffling of segments of a genome is known as reassortment.

Antigenic Drift: It is the minor change in the H and N proteins usually due to point mutations.

Antigenic Shift: The major antigenic change involves the acquisition of a gene for a completely new H or N antigen or both H and N antigens by genomic segment reassortment.

The antigenic changes are usually due to reshuffling and reassortment of genomic segments.

Virulence Variation

The virulence is depending upon the phenotypic as well as genomic expression of the virus but it may depend on the host if he/she is immunocompromised or immunosuppressed.

The virulence may be more or less after antigenic drift/shift. It has been observed that the virus reach the host (like human) after passing from the swine proves more virulent.

For example; the progeny of two viruses with serotype H_7N_1 and H_6N_2 posses four different serotypes that may be more virulent from the parent serotype. (as follows).

Inclusion bodies Formation in the host's cell

In orthomyxoviruses, as some multiplicating/replicating processes occur in the cytoplasm and rest in the nucleus; therefore, intra-cytoplasmic and intra-nuclear inclusion bodies may be seen after replication in the host's cell. Release of viruses: The release of newly formed viruses from the host's cell is done by budding. The cell membrane (made up of lipid bilayer; same composition to that of envelope of the virus) buds out and form an envelope around the virion. *(Remember*: Release of non-enveloped or naked viruses occur by lysis of the host's cell). After release, glycoproteins are synthesized thereafter.

Various Groups

On the basis of nucleoprotein or riboncleoprotein, orthomyxoviruses are classified into three groups, viz:

1. Infleunzaviruses A: } can infect birds, animals and human as well.
2. Influenzaviruses B: }
3. Influenzaviruses C: can infect only human.

Viruses of Group A and B has 8 segmented genome while that of Group C has only 7 segmented genome. Segment # 5 which codes for neuraminidase is missing but the activity associated with this protein is present.

Symptoms and Pathogenecity

Tentative diagnosis can be made based on clinical symptoms. Infection with virulent strain may results viremia and production of lesions in liver, spleen, heart and kidney and hemorrhages on the comb and wattles of poultry. It may be characterized by high mortality rate depending upon the serotype.

Cultivation and Growth Characteristics

Orthomyxoviruses can be cultivated in embryonated chicken eggs by allantoic and amniotic cavity routs. The viruses also grow in cell cultures of chicken and mammalian tissues and produce cytopathic effect.

Living animals prone to such viruses may be used for cultivation purposes.

Host Susceptibility

Influenza viruses A and B are known to infect poultry, animals and human as well. But influenza viruses C infect only humans. Wide range of wild water birds, water fowls, seagulls and ducks are susceptible and they may act as reservoirs of these viruses.

Isolation of viruses

Swabs of nasal discharge, tissues of upper respiratory tract. The virus may be isolated from the carcasses. If carcasses are chilled then the virus may stay there for about 10 months.

Laboratory Diagnosis

The most commonly used serological tests are as follows:

1. Haemagglutination Inhibition (HI) test is very specific test. Known antibodies are used to identify the virus serotype which is specific to those antibodies.
2. Virus Neutralizing (VN) test is also used in which known antibodies will neutralize the serotype specific to those antibodies.
3. PCR is an advanced technique in which by using specific primer, virus strain can be identified and multiple copies of its genome can be obtained.
4. CFT is also a very commonly used serological test to identify the virus/antigen.

Vaccination

For Influenzaviruses infection, live vaccines are not feasible at all because of active replicating process there are many chances of reassortment (reshuffling of genomic segments). Only killed vaccines can be used. But to go for vaccination, it is a hard job to identify the virus serotype among the total of 144. You may prepare vaccine against a fewer serotypes but you may not able to prepare for all. Thus vaccination tool is not so effective against orthomyxoviruses infection but has been trialed.

PAIRED SERUM TEST to check efficacy

A number of tests can be performed but the most reliable is paired serum test.

Take paired serum and after first day of vaccination check the titre (called base titre), suppose it was 2.

After 10-15 days, again check the titre, suppose it has been now 8.

It means that the titre has been raised 4 folds, (response has been boosted to four times). And we can say that antigenic mass has been replicated.

Prevention and Control

The best way to prevent the infections is to protect susceptible animals from infected animals. Notifiable should be burnt or slaughtered.

Antiviral Drug

To inhibit the uncoating of virus, Amantidine can be used as antiviral drug.

VIRO LECTURE 11

PARAMYXOVIRIDAE

Like orthomyxoviruses, paramyxoviruses have also affinity for mucin (mucous membrane).

Morphological Features

Members of the family are spherical in shape. They contain negative sense, single stranded RNA (-ve sense ssRNA) which is not segmented but also exists as linear molecule.

Nucleocapsid symmetry of the viruses is helical and it is characterized as "Herring Bone Structure".

Envelop: The viruses have lipid containing bilayer envelope with glycoprotein spikes or peplomers. On the structural basis, the peplomers are differentiated into two types; 1) HA+ Neuraminidase spikes 2) F-protein spikes

HA+ Neuraminidase spike: presents ability of both antigenic proteins via same spike.

Fusion protein spike: has an ability of fusion/adhesion and causes hemolysis.

Gene Expression

The genome of the virus is linear in shape

3" N P/C M FG L 5"

and the genes encodes for about 10 to 12 proteins but the important proteins are as follows;

N: Nucleocapsid (phospolip.)

P/C: Polymerase (phosphoprotein) and capsid protein (structural protein)

M: Matrix protein

FG: Fusion + Glycoprotein (esp. Haemagglutinin)

L: Large protein (It is involved in RNA transcription)

Replication/Multiplication of Virus in Host Cell

When the virus cell come in contact with host cell, the surface antigenic protein of virus i.e. F-protein get its receptor on the cell. After adsorption the virus get entry into the cell cytoplasm through receptor mediated endocytosis. This virion is now termed as endocytic vesicle → pH of the cytosol is lowered down → envelop get dissolve → nucleocapsid + protein are released → replication and other process are initiated → As a result, early proteins are formed which are enzymes and then, by the action of polymerase other structural proteins are formed. → Multiple copies of genome are also formed by replication → then, assembly occur → Consequently, after assembling, newly formed viruses are relaesed by budding. During budding, the cell membrane of the host cell is served as the envelope of the virion and after envelop formation, surface antigen proteins such as F-proteins are expressed.

For Expression of F-protein

Most of the non-enveloped viruses are released by the lysis of the cell but most of the enveloped viruses released by exocytosis (i.e. budding).

The virus should be enveloped and have surface antigenic protein. The envelop is not only virus but also host cell specific.

Classification

The family "paramyxoviridae" is very large family and is divided into two sub families based on H- protein.

1. Paramyxovirinae
2. Pnemovirinae

1. PARAMYXOVIRINAE

This subfamily contains many important genera of veterinary as well as medical importance.

i) Paramyxoviruses: (H^+N^+)

This genus contains viruses of every species but the virus of our interest is:

- Bovine Parainfluenza -3 (PI-3) virus (also known as shipping virus). It play very imp. role in HS.

ii) Rubulavirues: (H+N+)

This genus contains almost all the human viruses;

- Mumps virus
- Human Parainfluenza 2, 4a, 4b Viruses

iii) Avuloviruses: (H+N+)

- Newcastle disease virus (ND virus)

iv) Morbiliviruses:

Human virus like
- Measles virus (H^+N^-)

Animal virus like
- Rinderpest virus (H^-N^-)
- PPR virus (H^-N^-)

Fish virus like
- Phocin distemper virus (H^-N^-)

vi) Henipaviruses: (H^-N^-)

- Hendravirus
- Nephavirus

2. PNEUMOVIRINAE

This subfamily contains following two important genera;

i) Pneumoviruses (H^-N^-)

- Respiratory Synctial virus

ii) Metapneumoviruses: (H^-N^-)

This genus contains viruses of human, bovine as well as canine.

- Turkey's virus --- causes Rhinotrachitis

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VIRO LECTURE 12

NEWCASTLE DISEASE

The disease was first reported in Java, Indonesia in 1926.

Causing Agent

The disease is caused by the Newcastle disease virus (NDV) that belongs to the genus ---- *Avulovirus* of the sub-family ---- *Paramyxovirinae* and family ----- *Paramyxoviridae*.

Host Susceptibility

All the birds are more susceptible for this disease. Man is also susceptible --- poultry workers/poultry lab workers may experience "self limiting conjunctivitis".

Signs, symptoms and lesions of this disease vary according to the age and immune status of the bird and strain of the virus.

Age and Immune Status

Passive immunity plays very vital role in the immune status of the grower bird as well as newly hatched chick. Newly hatched chick is served with egg yolk as its nutrition for few days. If the titre of antibodies is very high in the egg yolk contents --- then a greater part of abs will be transferred from egg yolk to the bird. Consequently, the bird develop a very good immune status against the ND virus and other viral infections.

Strains of ND virus

The NDV strain varies in their virulence. The virulence of the virus strains is measured based on neuropathic index (NI) or mean death time. The NI is usually determined by intracerebral inoculation of day old chick.

Based on the virulency, the NDV strains can be divided into three groups:

i) Lentogenic ii) Mesogenic iii) Velogenic

1. Lentogenic strain

   This strain cause mil or inapparent infection but the mortality rate is 0%.

   Most important viruses of this strain are:

   Lasota Hitchner B_1 F ulster 2C Queensland V_4 (thermostable)

   - ► This strain is usually used as live vaccine because they cause no disease.
   - ► It is also known as "live vaccinal strain". Live vaccine is very economical in poultry. It can be administered via feed, drinking water or inhalation (aerosols).

2. Mesogenic strain

   It is "attenuated velogenic strain" of which mortality rate is as low as 25% but infection may be reported many times.

   The important viruses of the strain of this pathotype are:

   H Mukteswar Roakin Beaudette C

   This strain is also used for live vaccine. For attenuation, velogenic property is masked (inactivated) by using Formuline, β-propiolactone. After inactivation, the killed viruses can be used in vaccine. They can be injected I/M or S/C and you have to vaccinate the birds one by one ---- quite laborious method.

3. Velogenic strain

   This strain is responsible for 100% infection and results into 90-100% mortality.

   This strain is further divided into two forms;

   Viscerotropic (Doyle's form): virus strains of this viscerotropic form multiply in the intestinal tract and causes wide variety of GIT infections such as enteritis, diarrhea, dehydration etc.

   Neurotropic (Beach's form): Such virus strains multiply in the neuronal cells and cause various nervous problems such as encephalitis, pneumo-encephalitis, respiratory paralysis, torticollis, and star gazing etc. A number of velogenic strain viruses have been identified such as;

   GB Texas Herts 33/35 NY Parrotte Largo Cal O 83 Intaliana Milana

What is Attenuation?

If we grow NDV in any one of the cultivating systems (e.g. rabbit inoculation or cell lines) but other than its actual host (i.e. poultry) → the virus may loose its pathogenecity to some extent → This process is known as attenuation and such subjected virus will be called attenuated virus. To obtain mesogenic strain, velogenic virus strains are attanuated which then, used for vaccine preparation.

Isolation and Growth Characteristics

NDV multiplies in its natural host in the circulation, spleen, lung respiratory tract. Thus, it can be isolated from the spleen samples, swabs of respiratory secretions and fecal material. In case of all birds, spleen (may be swollen) is the main source of virus isolation.

Embryonated Egg :

For growth purposes, we, preferably use embryonated egg contain a live embryo of age 10-12 days. → Inoculate the virus via chorio-allantoic or chorionic cavity route. → Wait 3-4 days for virus growth after inoculation → As a result of virus growth, ultimately embryo will kill. → Then, take allantoic fluid and go for isolation.

Vero Cell Line

In addition to embryonated egg, cell lines may be used for cultivation/growth of the virus. Vero cell line (isolated from green monkey) are used → Consequently, CPE will be: i) fusion of the cells ii) Synctial formation iii) Presence of Intracytoplasmic Inclusion bodies in the cells.

Laboratory Diagnosis

For initial conformation, slide agglutination test may be performed. In this test, agglutination of RBCs is observed due to haemagglutinating property of NDV.

For confirmatory diagnosis, various laboratory tests are being used in which HI (haemagglutination inhibition) test is very specific and simple test for NDV. Other tests are; ELISA, VN test etc.

Passive Immunity

- In case of poultry birds, it usually depends on the titre of the antibodies (Abs) in the parent flock.
- Flock Ab profile should be checked before go for vaccination.

Suppose in a flock, titre of abs is 1:128 – then there will be increased nutrilization of lentogenic strain while

If abs titre is 1:16 in another flock --- Mesogenic strain will be more susceptible for this titre because neutralization will be easy due to low titre and it will replicate profoundly because of its attenuated property.

TIME REQUIRED FOR DECLINE IN ABS TITRE

1:128 → 1:64 (4.5 days) 1:64 → 1:32 (4.5 days) and so on ……..

So we conclude that: Half life for Passive immunity decay is ------ 4.5 days

Examples of pathogenicity indices obtained for strains of Newcastle disease virus.

Parameters	Lentogenic	Mesogenic	Velogenic
Mortality rate	Zero	25%	100%
Mean Death Time	> 90 hours	60-90 hours	< 60 hours
Intracerebral Pathogenicity Index (day old chick)	0.4	0.6 – 1.6	> 1.7
Intravenous Pathogenicity Index (6 week old chick)	Zero	0.0 – 1.4	> 1.5

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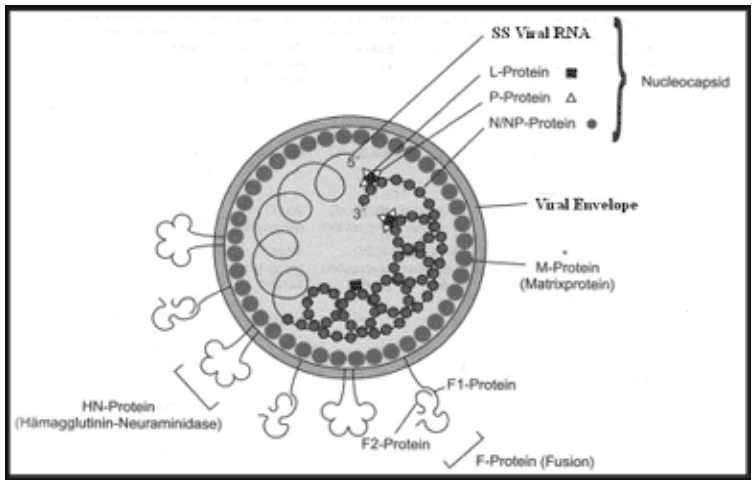

NEWCASTLE DISEASE VIRUS

IMPORTANT TERMINOLOGY

Antigen: It is a substance that prompts the generation of antibodies and can cause an immune response.

Antibodies: Antibodies are gamma globulin proteins that are found in blood or other bodily fluids, and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.

Vaccine: A vaccine basically contains an antigen (a virus or a bacterium or its component) which, when injected into an individual, produces an immune response or antibody and thereby makes the individual resistant or immune to the disease caused by the agent.

Live Vaccines: Live vaccines are those, which are usually prepared by using an attenuated or a laboratory modified or a naturally occurring avirulent strain.

Killed Vaccines: Killed or inactivated vaccines are those, which contain viruses that have been inactivated or killed by treatment with physical or chemical agents.

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IMPORTANT DEFINITIONS

Virus, Virology, Prion, Antigen, Antibodies, Acquired immunity, Passive immunity, Mutation, Antigenic shift, Antigenic drift, Vaccine, Live vaccine, killed vaccine, Antiviral drugs, interferon, inclusion bodies, Gnotobiotic, Blood plasma and serum

IMPORTANT NOTE

In reference to lecture# 5> Classification of viruses, please also consult printout notes (provided by sir) in which classification is done in tabulated form. Remember: These notes are also a part of lecture # 5.

REVIEW OF MID COURSE OF VIROLOGY (TABLE OF CONTENTS)

Lecture #	Contents	Ref. Notes
Lecture 1	Define Virology, Viruses and Characteristics of Viruses Naked viruses, size and shape of viruses, unit for measuring size. The largest and smallest virus in size. Evolution of viruses	WEEKEND WORK 1
Lecture 2	PURIFICATION OF VIRUSES a) disruption of host cell (different techniques) b) Primary centrifugation Other methods: i) Differential centrifugation ii) Precipitation with Amm. Sulph. and polyethylene glycol iii) Density gradient centrifugation iv) Equi. centrifug. Size Determination, by: Filtration , Centrifugation, and Electron Microscope Visualization and Characteristics of: Nucleic acid, Protein and Enzymes	WEEKEND WORK 1
Lecture 3	CULTIVATION OF ANIMAL VIRUSES a) Susceptible Host inoculation; Different animals used, routes of	VIRO WEEKEND 2

	inoculation, consequences of virus growth, Examination of inoculated animal b) Embryonated Egg: Major advantages, routes of inoculation and virus growth consequences c) Cell Culture: advantages and disadvantages, Procedure and growth consequences demonstration of inclusion bodies & usage of *E.coli growth* lawn for virus cultiva.	
Lecture 4	QUANTIFICATION OF VIRUSES Methods; i) Physical → a) Electron microscopy ii) Biological → a) Plaque assay b) Pock assay c) Haemagglutination assay d) ELISA e) PCR Assays for Infectivity of Viruses: a) Quantitative and b) Quantal assays	VIRO WEEKEND 2
Lecture 5	Inactivation of Viruses a) Physical methods (3 parameters) b) Chemical methods (6 types of agents) Preservation of Viruses Classification of Viruses; on the basis of the nature of their genome; a) DNA containing viruses b) RNA containing viruses Virus Transmission 1. Horizontal Transmission 2. Vertical Transmission 3. Vector Transmission	VIRO WEEKEND 4+5
Lecture 6	Replication of Viruses ; have various stages such as 1. Adsorption 2. Penetration 3. Uncoating 4. Biosynthesis 5. Assembly 6. Release One step growth curve (eclipse and latent periods)	VIRO WEEKEND 4+5
Lecture 7	Antiviral Drugs Possible outcome of Viruses Infection 1. Productive infections 2. Persistently chronic infection 3. Latent infection 4. Abortive infection 5. Apoptosis-inducing protein infections Virus – Host Cell Interaction : a) Innate immunity, Complement system and Effect of inoculum dose c) Specific or Acquired immunity, Mode of action of B and T cells -- What is meant by "premature lysis of host cell? Interferon (Define), Mechanism of action (2 mechanisms), Characteristics and Types of Interferon	VIRO WEEKEND 4+5
Lecture 8	Acquired Immunity Its Types: i) Active acquired ii) Passive acquired and their SUBtypes – Vaccines?	VIRO WEEKEND 6

	Viral Genetics i) Mutation, Rate, Types and consequences of mutation. Ii). Mutagens; its types Mutation at Virus Level	
Lecture 9	Laboratory Diagnosis of Viral Diseases a) Direct demonstration b) Isolation of virus c) Detection of virus antigen (dif. Tests) i) Immuno-diffusion test ii) CFT iii) FAT iv) RIA v) ELISA vi) VN test d) Detection of virus specific antibodies e) Detection of nucleic acid. Polymerase Chain Reaction (PCR)	VIRO WEEKEND 6
Lecture 10	ORTHOMYXOVIRIDAE Common lesion, Morphological characteristics, Antigenic properties, Genotypic expression, Virulence variation and inclusion bodies presence, Different groups of the viruses, Symptoms and Pathogenicity, Cultivation and Growth Characteristics, Host susceptibility, Isolation of viruses, Laboratory Diagnosis, Vaccination (usage of paired serum to check efficacy), Control and Prevention, Antiviral Drug	VIRO WEEKEND 7
Lecture 11	PARAMYXOVIRIDAE Morphological Features, Gene Expression, Replication/multiplication of virus, Classification; two subfamilies a) Paramyxovirinae (its genera) b) Pneumovirinae (its genera)	VIRO WEEKEND 8
Lecture 12	NEWCASTLE DISEASE Causative agent, Host susceptibility, Strains of ND virus (Lentogenic, Mesogenic and Velogenic), What is attenuation?, Isolation and growth characteristics, Laboratroy diagnosis, Passive immunity	VIRO WEEKEND 8