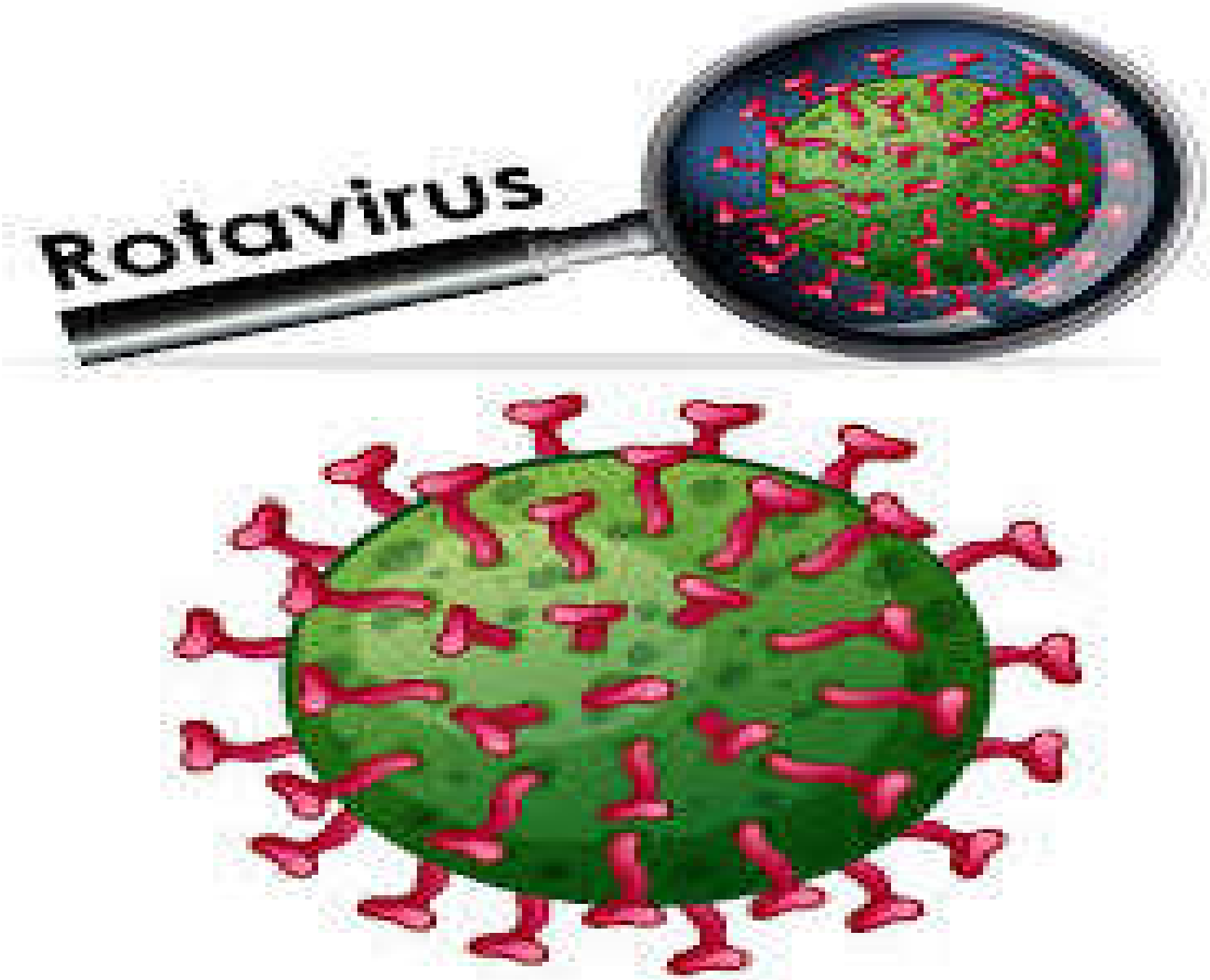
Rota & Rabies viruses

By Dr. Ekram Abd El Rahman

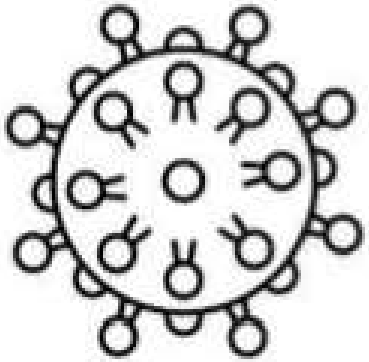

Rotavirus

Key issues

1. What is rotavirus ?
2. What are the signs and symptoms of rotavirus?
3. How is rotavirus spread?
4. Who is most at risk?
5. What are rotavirus prevention strategies?

What Is Rota virus disease?

- Rotavirus is the most common cause of diarrheal disease among infant and young children
- It is double stranded RNA virus
- The name rotavirus comes from the wheel-lik appearance of the virus under the microscope
- Nearly every child in the world is infected witl at least once by the age of 5
- Accounts for 1/3 of hospitalization for severe diarrhea in infants and children
- Causes about 37 % of death of children from diarrhea
- Out of 8 types species, Rotavirus A is most common (> 90 %) in human
- Infections primarily in cool and dry season



Virology- RV is double stranded RNA

Eight species (A,B,C,D,E,F & G)

Human - A,B & C

Pig - E & H

Birds- D,F & G

Two serotypes:

1. G Serotypes (G1,G2)
2. P Serotypes (P8,P6 & P4)

Viral proteins (VP)- called virion - 6 VP

VP1,VP2,VP3,VP4,VP6 and VP7

VP4 and VP7 involved in immunity to infection

Common Strain in India

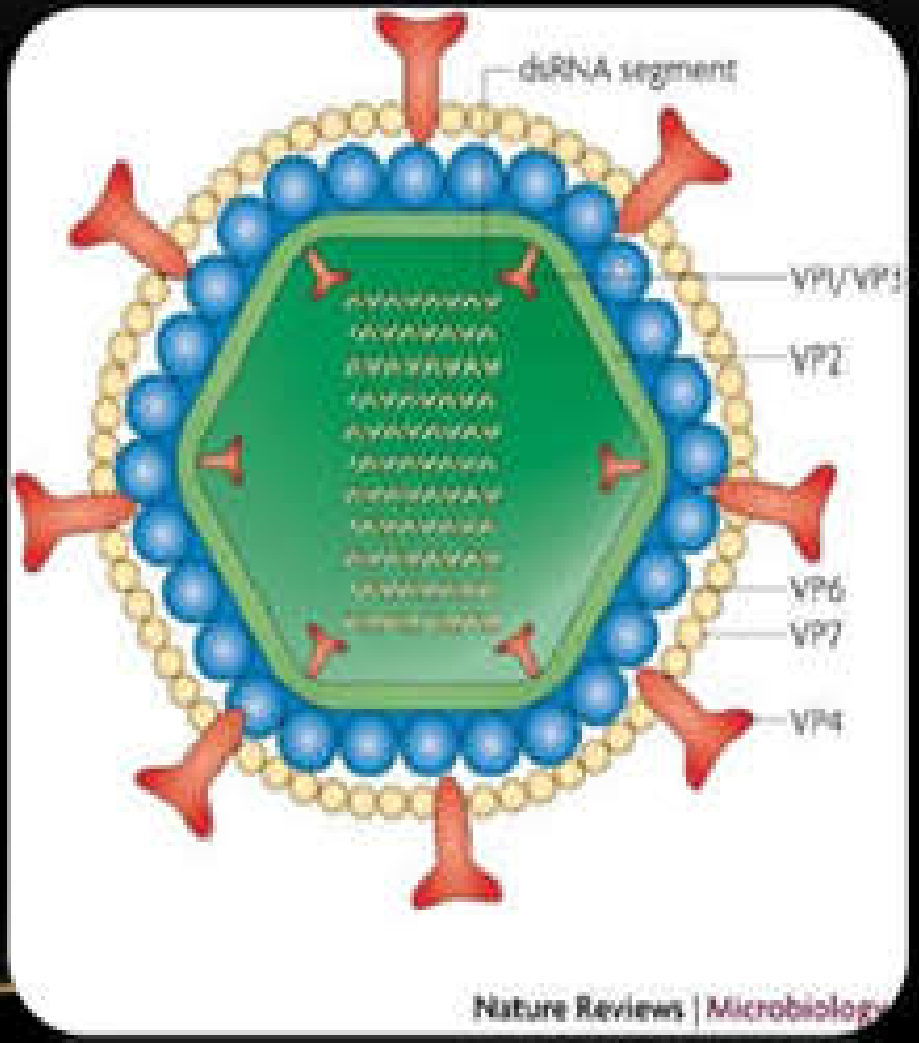
1.G1P(8)- 24.7 %

2.G2P(4)- 23.4 %

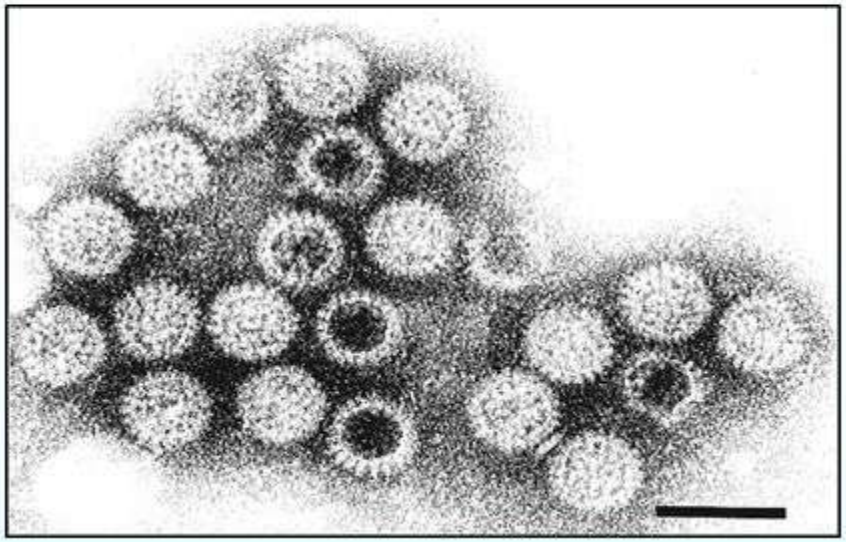
3.G9P(8)- 8.5 %

CHARACTERS OF ROTAVIRUS

- A rotavirus has a characteristic wheel-like appearance when viewed by electron microscopy (the name rotavirus is derived from the Latin rota, meaning "wheel"). Rotaviruses are non enveloped, double-shelled viruses. The genome is composed of 11 segments of double-stranded RNA, which code for six structural and five non-structural proteins. The virus is stable in the environment



Morphology



- Family Reoviridae
- 70-85 nm diameter
- Nearly spherical icosahedral particle
- Non-enveloped, double-shelled viruses
- Wheel-like distinct appearance under EM

(Picture Source: www.nlv.ch/Rotavirus/Rotafactsheet.htm)

Rotavirus

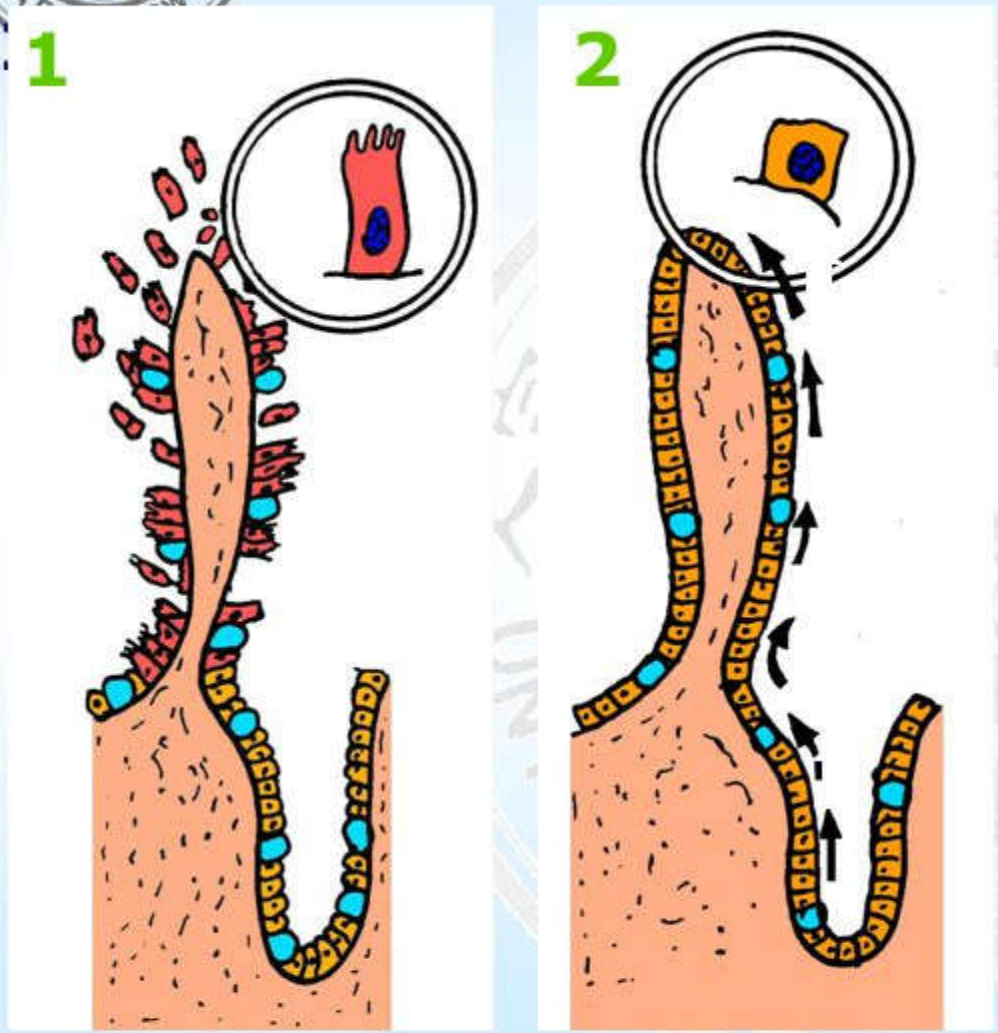
- Reovirus (RNA)
- VP7 and VP4 proteins define virus serotype and induce neutralizing antibody
- 5 predominant strains in U.S. (G1-G4, G9) and accounted for 90% of isolates
- G1 strain accounts for 75% of infections
- Very stable and may remain viable for weeks or months if not disinfected

Clinical Presentation

Pathogenesis

- The virus causes diarrhoea by three principle mechanisms:
  - infection of villus epithelial cells causes cell destruction, decreased absorption of salt and water, and decreased disaccharidase activity, increasing the osmotic load in the gut lumen
  - stimulation of the enteric nervous system, leading to increased fluid secretion
  - direct enterotoxin effects of nonstructural protein 4 (NSP4), the first viral enterotoxin to be described
- The osmotic load in the gut and increased fluid secretion lead to diarrhoea and, if unchecked and without fluid replacement, can ultimately lead to dehydration and acidosis

Pathogenesis of Rotavirus Diarrhea



1- Infected *absorptive enterocytes* are killed causing patchy epithelial cell destruction and villous shortening

2- Destroyed absorptive cells are rapidly replaced by cells that migrate from the crypts.

Villi become covered with immature *non-absorptive secretory* cells having:

-no brush border

- no brush border enzymes

How does rotavirus spread?

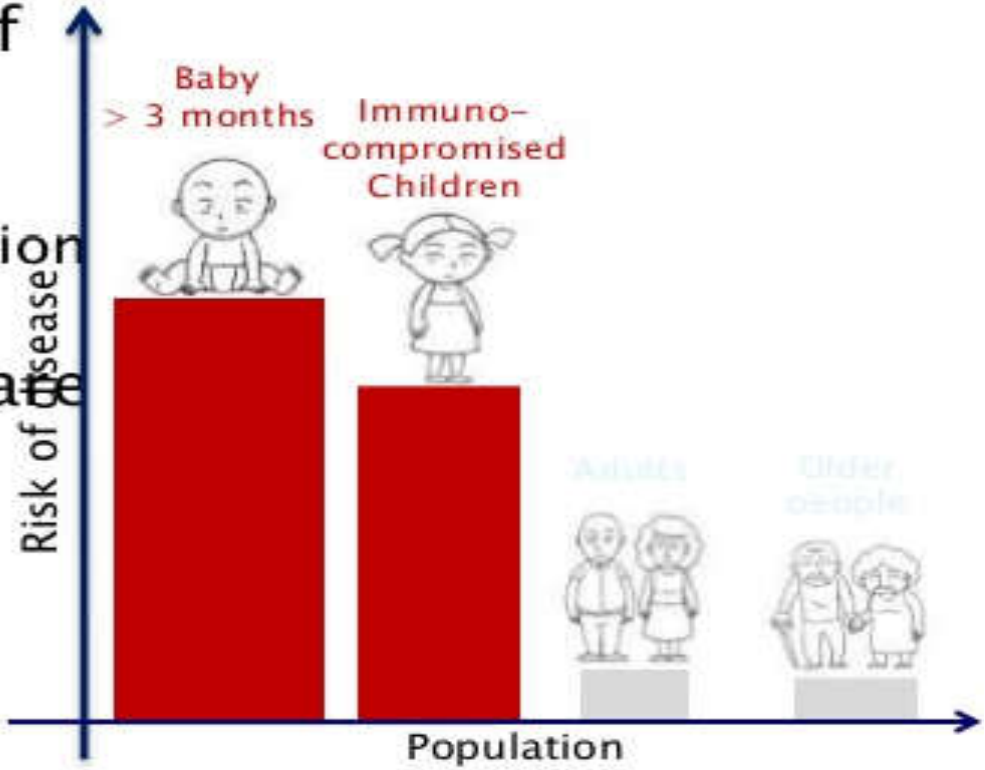
- Rotavirus infection is highly contagious
- Rotavirus spread by fecal-oral route
  - The primary mode of transmission of rotavirus is the passage of the virus in stool to the mouth of another child

Transmission

- By Faeco – oral route via contact with contaminated hands, surface and objects
- Highly contagious
- More than 10 trillion infectious particles per gram of infected person faeces.
- Fewer than 100 of these required to transmit infection from one to another
- Stable in environment and survive between 9 – 19 days
- Sanitary measure adequate for eliminating bacteria and parasites seems to be ineffective in control of rotavirus. Reason why rotavirus infection is similar in both high and low health standard country.

Who is a most risk

- Infants after the age of 3 months to 2 yrs
  - Low to no immunity
  - Vulnerable to dehydration
- Older children if they are immunocompromised

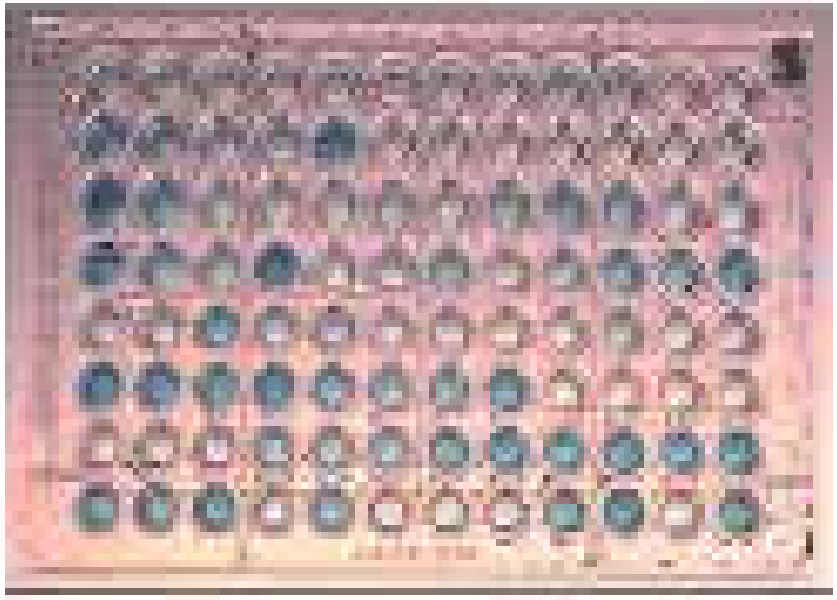


What are the signs and symptoms of rotavirus infection?

- Three main symptoms of rotavirus infection:
  - Fever – low grade
  - Nausea & Vomiting
  - Watery diarrhea
- Abdominal pain may also occur
- Diarrhea usually Extends from 4 to 8 days
- Dehydration is more common in RV than other causes and Most common cause of death related to Rotavirus.
- First infection usually produces symptoms but subsequent are are typically mild or asymptomatic.
- 40 % Protection after a single infection and 75-88 % after subsequent infections
- Age group- between 6 month to 2 years most severely affected

LABORATORY DIAGNOSIS

- Electron microscopy (EM)
- Immune electron microscopy (IEM)
- Detection of viral antigen by ELISA more sensitive than IEM
- PCR of viral genome most sensitive (Rotavirus)
- Serology- IgG and IgM antibodies against viral antigens



Treatment

- Treatment of Gastroenteritis is supportive
- Correction of loss of water and electrolytes remain the goal treatment
- Failure for prompt correction of dehydration leads to

  Acidosis

  Shock

  Death
- Lesser deaths if effective fluid replacement therapy is timely initiated

Is there a vaccine against rotavirus?

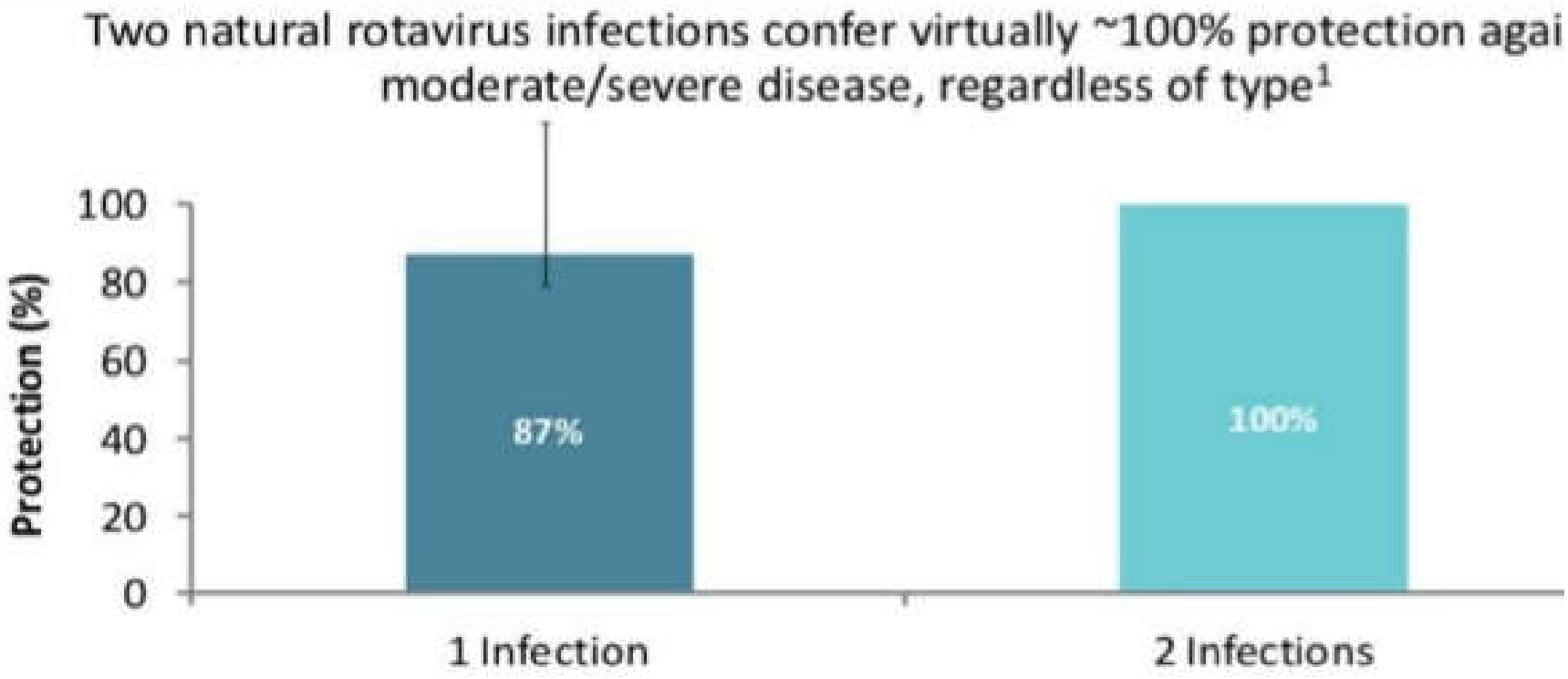
Currently There are 3 rota virus vaccines:

- Rotarix
- RotaTeq
- Rotavac

Rotarix

- Monovalent RV1, live attenuated rotavirus vaccine containing human rotavirus G1P(8) strain
- Prevention from GE caused by G1 and Non G1 Types(G3,G4 & G9)
- 2 Doses required.
- Viral shedding > 50 % after 1 st dose like natural infection
- Replicates well in gut
- Provide cross protection against most other serotypes.

Natural infection: a model for vaccine development with 2 doses

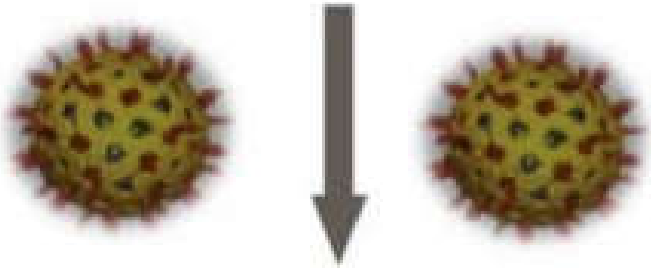


A realistic goal for a rotavirus vaccine is to establish the degree of protection against disease that follows natural infection[2]

The Human G1P8 strain in Rotarix mimics natural Rotavirus infection

2

Natural Human Rotavirus infections



~100% protection against subsequent moderate – severe RVGE **(regardless of the RV type)**

2

doses

Human RV vaccine contain

Human G1P8 strain

Mimics natu infection

Broad protection

The NEW ENGLAND JOURNAL of MEDICI
354;1 Jan5, 2006 75

Roger I. Glass & Umesh d. Parashar

Rotarix

Rotavirus Vaccine (RIX 4414) Live, Attenuated

Human RV

- **Human G1P[8]** strain RV vaccine
- 2 dose oral vaccine
- Vaccine strain replicates well in the gut
- Viral shedding > 50 % after the 1st dose *(like natural RV infection)*
- Provides cross protection against most

Human-Bovine Rotavirus Vac

- RV5 is a pentavalent vaccine on a bovine strain (WC3).
  - 3 dose oral vaccine
- Bovine Virus (WC3):
  - grows less well in the g
  - Viral shedding is infrequ
  - not broadly cross protec

Rotavirus: Current Vaccines

- The monovalent human rotavirus vaccine (Rotarix™)
- Multiple passages in tissue culture resulting in attenuated vaccine strain, RIX4414

- The pentavalent bovine–human reassortant rotavirus vaccine (RotaTeq™).
- Contains 5 reassortant rotaviruses developed from human and bovine (WC3) parent rotavirus strains.

Rota Teq vaccine

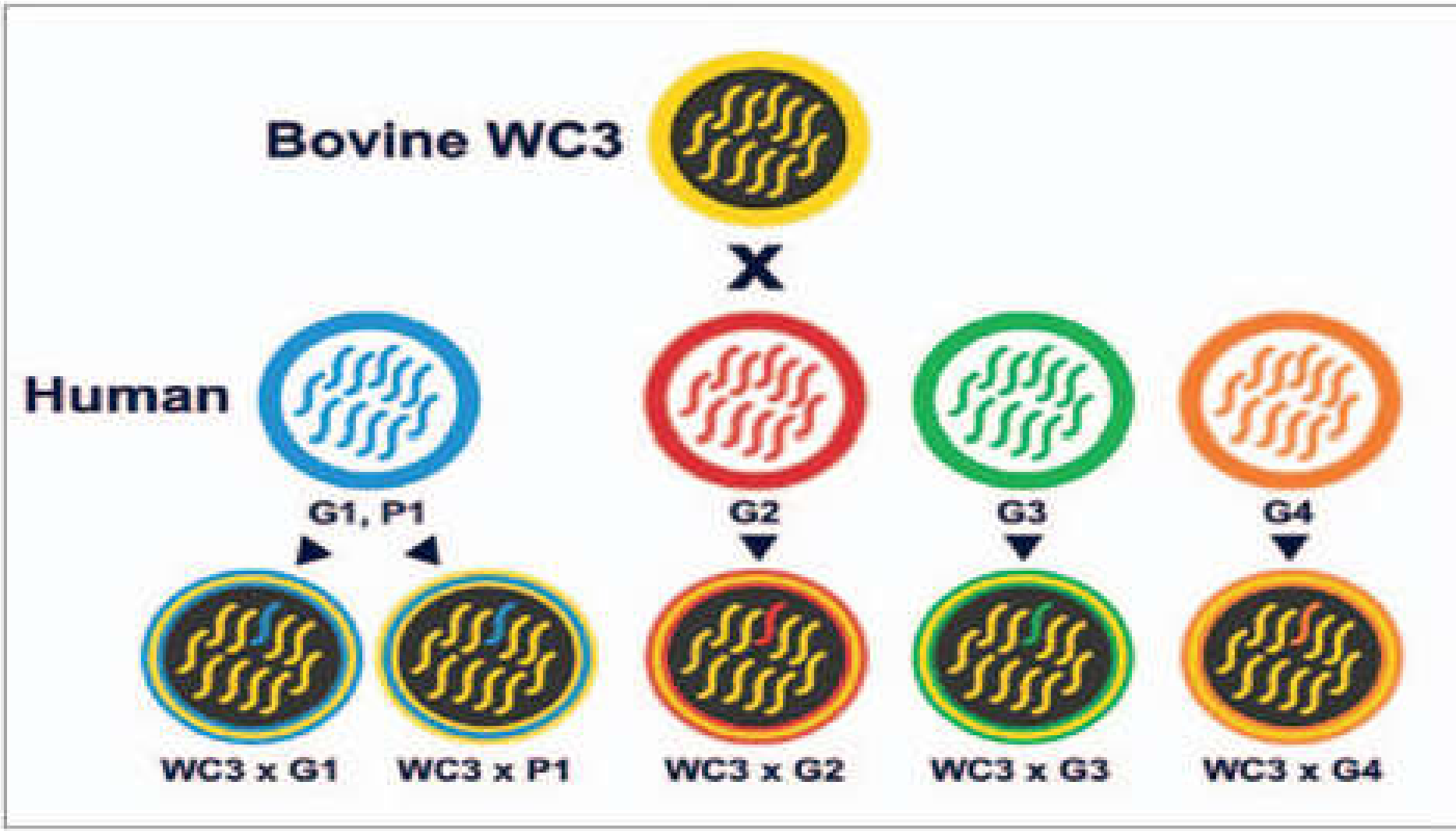
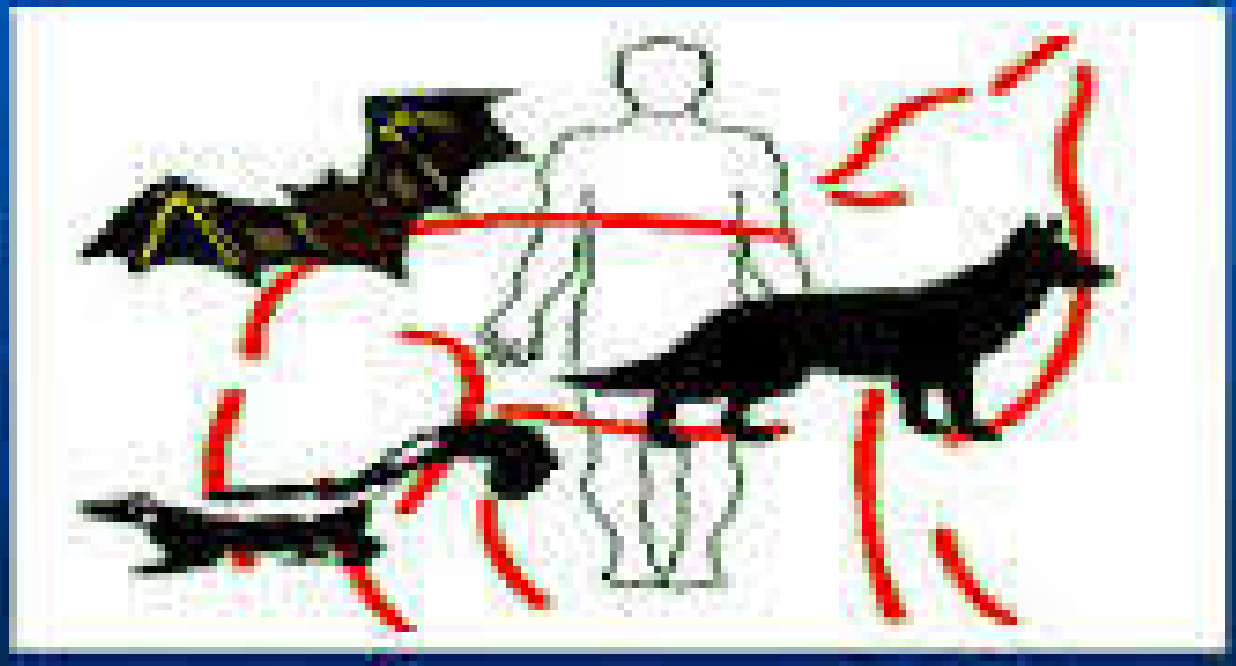


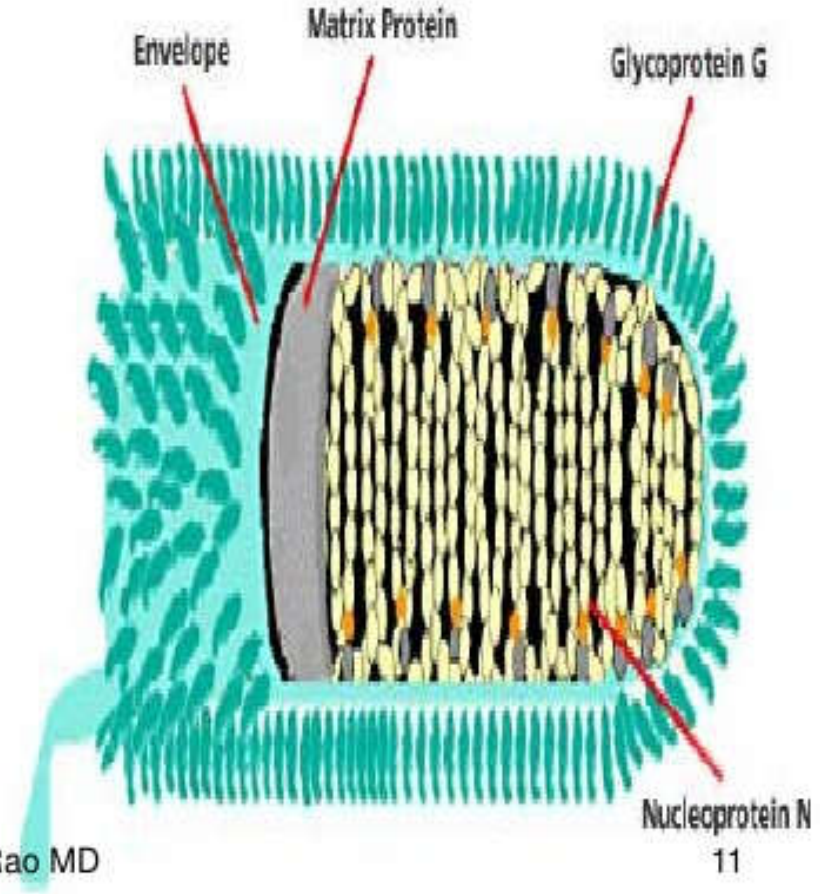
Figure 2. The bovine-human reassortant rotavirus vaccine (RotaTeq) contains five different reassortant strains made by combining bovine strain WC3 with human strains representing serotypes P1, G1, G2 G3 and G4. Vaccine strains have both the attenuated virulence characteristics of WC3 and the human surface proteins that evoke virus-specific neutralizing antibodies.

Rabies virus



Rabies virus

- Bullet shaped virus
- Size is 180 x 75 nm
- Has Lipoprotein envelop
- Knob like spikes /Glycoprotein S
- Genome un segmented
- Linear negative sense RNA



Rabies

Epidemiology

- Acute rapid progressive & highly fatal viral disease of CNS caused by Lyssavirus type 1.
- Zoonotic disease of warm blooded animals (dogs, cats , bats, racoons, skunks, foxes)
- Transmitted to man by bite of rabid animal.
- Non-bite exposures : aerosols; generated in labs , caves with bats , corneal transplantation.
- Human to human transmission extremely rare.
- Worldwide endemic canine rabies : 55,000 deaths annually (India alone 20,000)
- Louis Pasteur and Emile Roux first developed rabies vaccine in 1885.

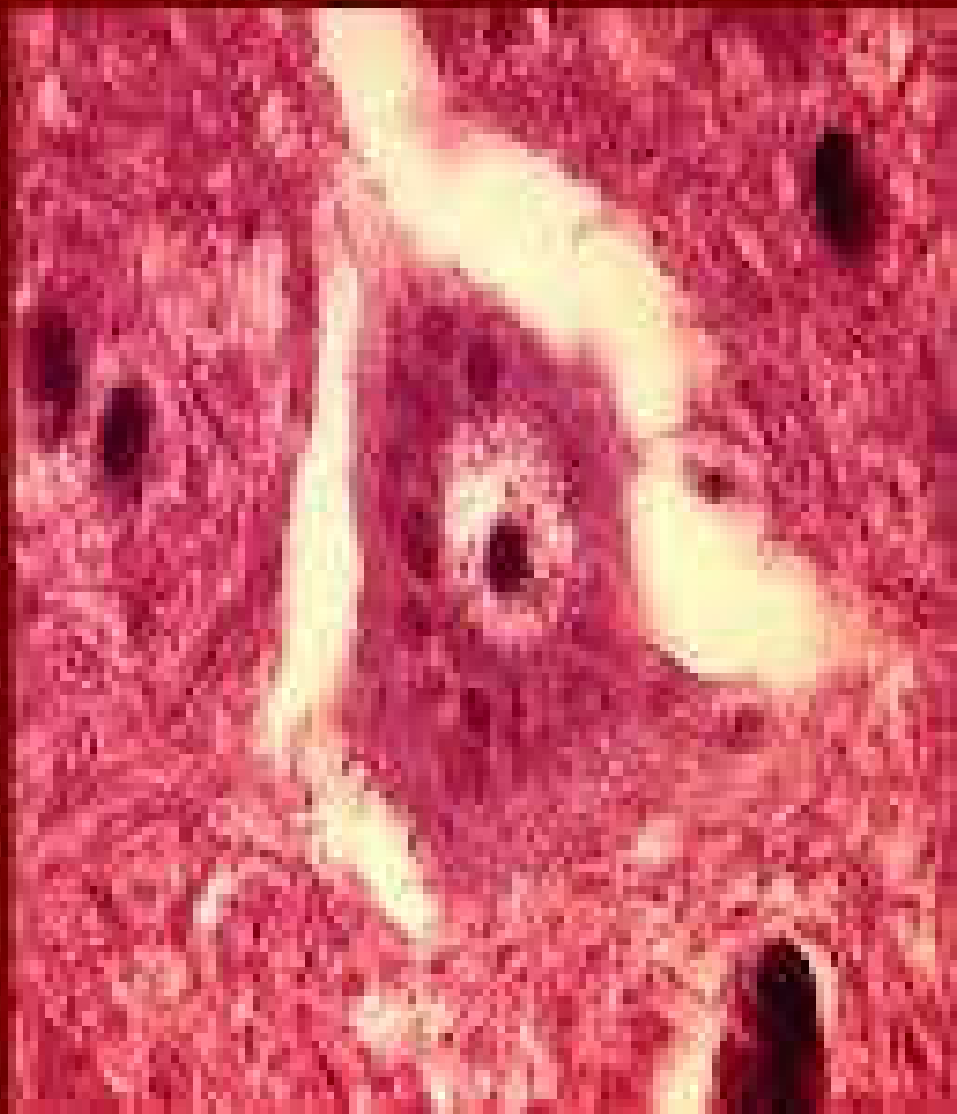
There is only one serotype of the virus but there are two biologic forms.

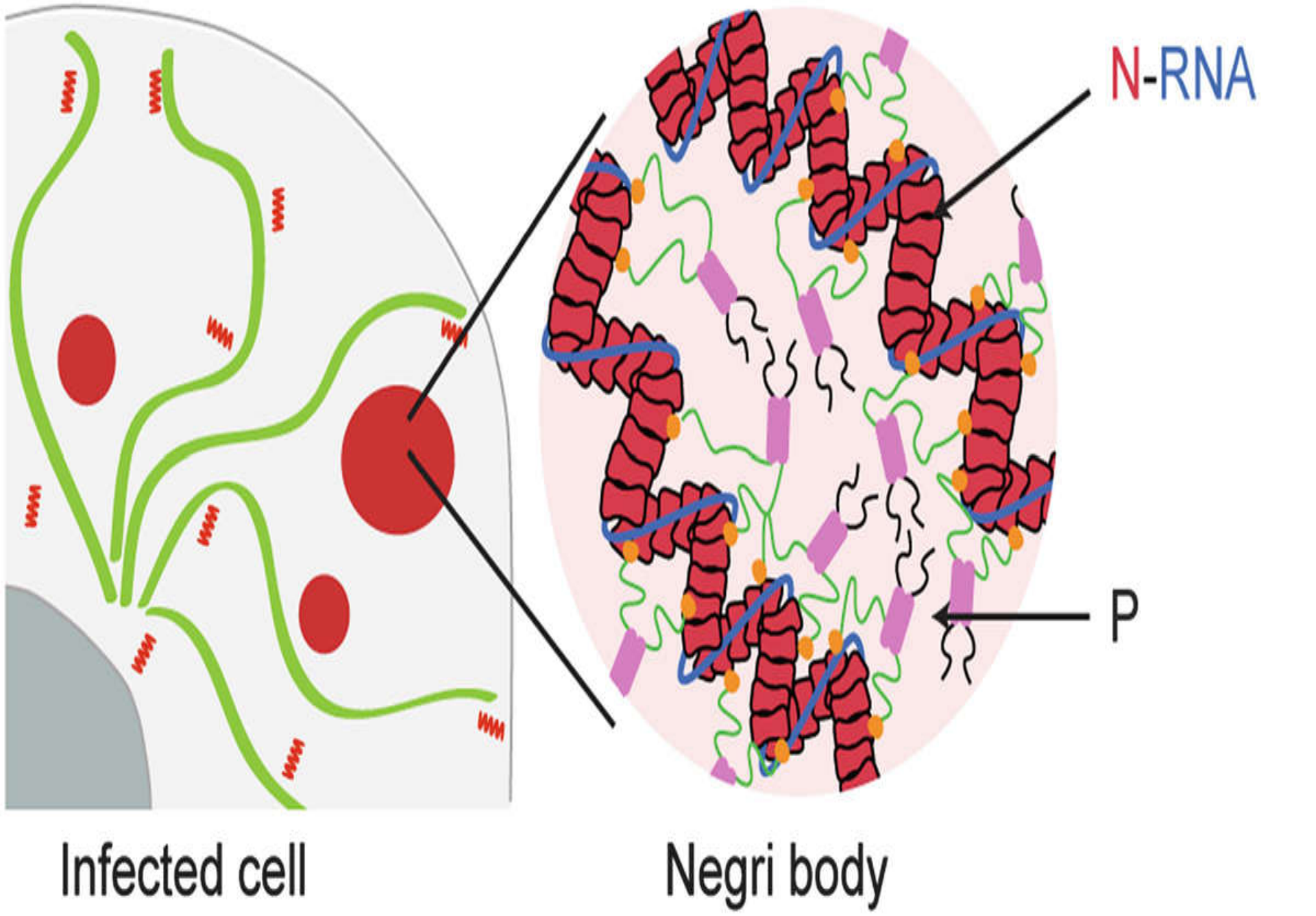
TYPES OF RABIES VIRUS

	STREET VIRUS	FIXED VIRUS
	The virus recovered from naturally occurring cases of rabies is called street virus.	The virus which has a short , fixed and reproducible incubation period is called fixed virus.
SOURCES	It is naturally occurring virus . It is found in saliva of infected animal	It is prepared by repeated culture in brain of rabbit such that its IP is reduced and fixed.
FEATURES	1. It produce negri bodies 2. Incubation period is 20 to 60 days. 3. It is pathogenic for all mammals 4. Cannot be used for preparation of vaccine	1. It does not form negri bodies 2. Incubation period is constant between 4-6 days. 3. It can be pathogenic for humans under certain conditions. 4. It is used to prepare anti-rabies vaccine.

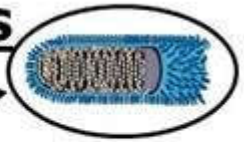
Negri bodies in Brain Tissue

- **Negri bodies**: round or oval inclusion bodies seen in the cytoplasm and sometimes in the processes of neurons of rabid animals after death.
- Negri bodies are Eosinophilic, sharply outlined, pathognomonic inclusion bodies (2-10 µm in diameter) found in the cytoplasm of certain nerve ..




N-RNA
P
Infected cell
Negri body

Pathogenesis of Rabies virus



5) Replications take place in the motor neuron of spinal cord & ganglia travels to brain

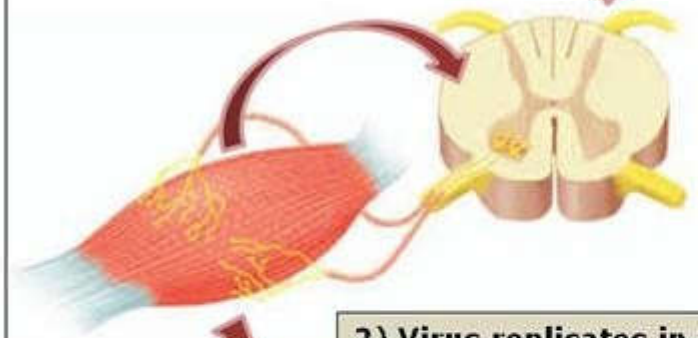
6) Infection of brain neurons leading to fatal inflammation

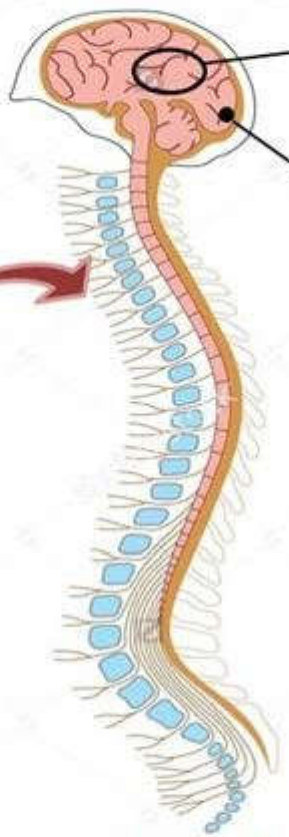
4) Virus travels within axons in peripheral nerves through axonal transport

Encephalitis is a condition where there is inflammation of the brain; this is caused by rabies viral infection

3) Virus binds to nicotinic acetylcholine receptors at neuromuscular junctions

7) Virus enters salivary glands & other organs of the victim





2) Virus replicates in the muscle

1) Viral inoculation from a rabid dog bite or a rabid animal

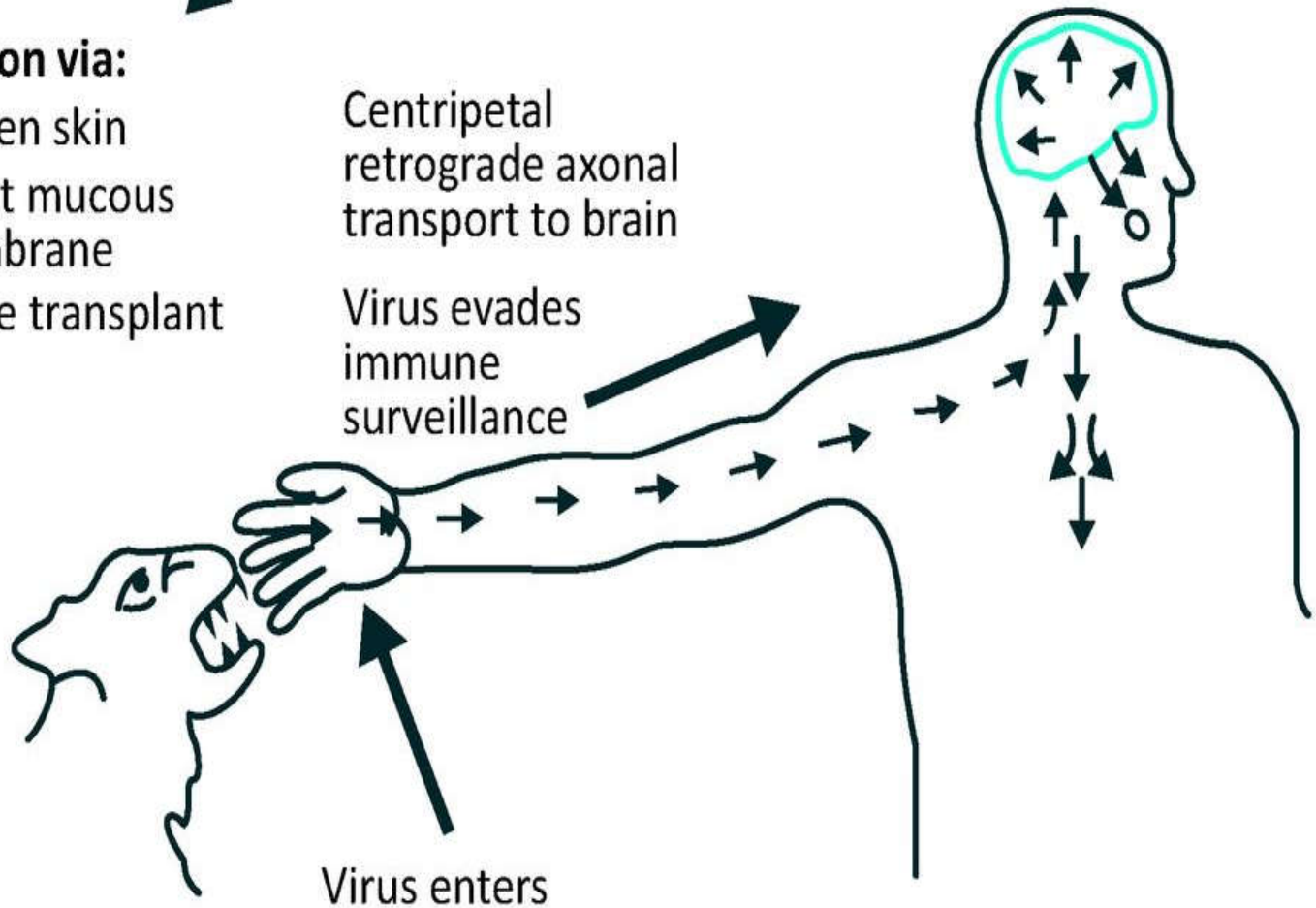
Incubation period
days to years usually
20–90 days

CNS infection:
> transynaptic spread
> viral replication
> inclusion (Negri) body formation
> neurons intact but dysfunctional

Infection via:
> broken skin
> intact mucous membrane
> tissue transplant

Centripetal retrograde axonal transport to brain

Virus evades immune surveillance



Virus enters nerve endings

Clinical signs of encephalomyelitis begin - immune response may be initiated

Centrifugal neuronal transport to: salivary glands (viral excretion)- virus carried to skin, heart, muscle, tongue etc, but no viraemia

- The incubation period (IP.) in humans varies depending on the distance between the site of the bite and the brain. It also depends on the severity of laceration and amount of virus introduced. The IP. is shorter in persons bitten on the face or head. The long IP. gives time for effective vaccination and prevention of the virus from reaching the CNS.

Signs And Symptoms

- Lack of appetite
- Headache
- Irritability
- Anxiety
- Paralysis of the jaw, mouth and throat muscles
- Excessive sweating
- Hydrophobia
- Increased production of saliva
- Double Vision

Diagnosis of rabies in human

DIAGNOSIS

1. History
2. Signs and symptoms
3. Clinical examination
4. Detection of antigen by taking skin biopsy using immunofluorescence
5. virus isolation from saliva & other secretions.
6. CSF analysis and CT scan
7. ELISA
8. RT-PCR
9. DFA testing
10. Negri bodies

• Diagnosis in animals:

- Immediately after the bite, the animal should be captured and observed for 10 days. If no symptoms appear during this period the diagnosis of rabies is excluded. If the animal dies or symptoms appear the animal is sacrificed, and diagnosis is done as follows:
- **1- Antigen detection.**
- **2- Detection of "Negri bodies" in stained brain smears.**
- **3-** Isolation of the virus from brain or saliva
- 4- RT-PCR applied to brain tissues.

TREATMENT

- No specific treatment
- Case management
  - Isolation in a quiet room protected as far as possible from external stimuli to prevent spasms and convulsions
  - Relieve anxiety and pain by use of sedatives
    - Morphia 30-54 mg
  - If spastic muscle contractions present use drugs with curare like action
  - Ensure hydration and diuresis
  - Intensive therapy in the form of respiratory and cardiac support

Management of rabies

*If the animal is available, diagnosis is done as mentioned above. If the biting animal is not available, the case is managed as impending rabies.

*The best treatment is prevention

1. Immediately wash the wound well with soap under running water for at least 15 minutes.

2. Disinfect the wound with alcohol or iodine solution. (If available)

3. Immediately consult a doctor or go to the nearest local health facility.

4- Administer tetanus vaccine and antibiotics

5- Post-exposure prophylaxis by vaccination and passive immunization with rabies immune globulins is required in the following situations:

- A bite by domestic or wild animals and the animal was not captured.
- If the diagnosis was established in the captured animal.

- In severe bites on the head and neck, even if the animal is available due to short incubation period, which may be as short as 2 weeks. Vaccination should start immediately to be stopped if the animal proved free of rabies.
- Bite or non-bite exposure to bats.
- Non-bite exposure of family members and health care staff to patients´ saliva through mucous membrane contact, scratches or abrasions.

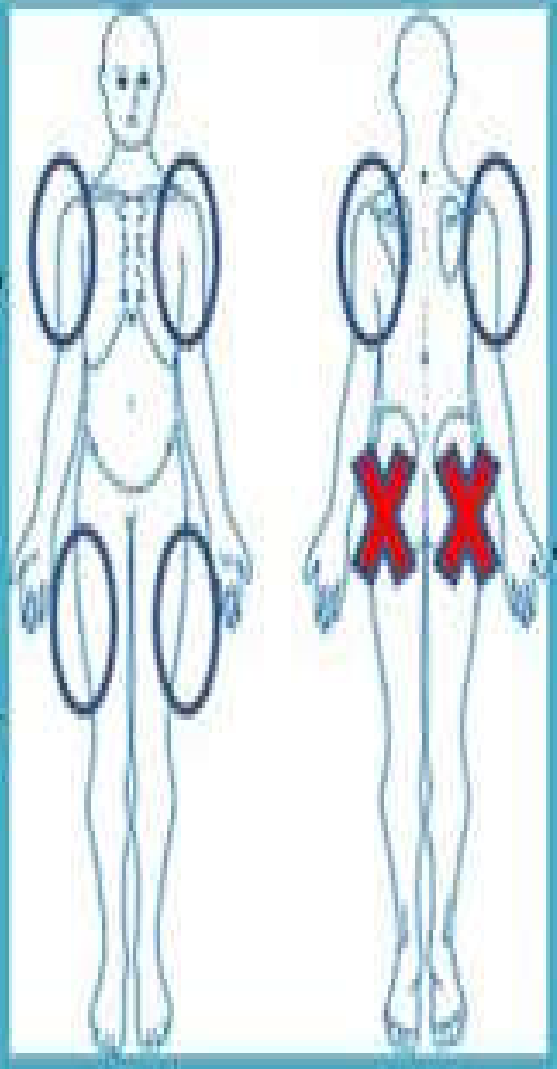

Rabies Vaccine,
HUMAN
U.S.P.

- **Types of vaccines:**
- All vaccines for human use contain inactivated rabies virus (by β-propionolactone).
- a) Human diploid cell vaccine (HDCV): rabies virus adapted to grow in human normal fibroblast cell line and inactivated by β-propiolactone.

- b) Rabies vaccine adsorbed (RVA): prepared in diploid cell line derived from rhesus monkey fetal lung, inactivated by β-propiolactone, and adsorbed on aluminum phosphate.
- c) Purified chick embryo cell vaccine (PCEC): prepared from fixed rabies virus strain (Flurry LEP) grown in chicken fibroblasts and inactivated with B-propiolactone, then purified by zonal centrifugation.

- The three vaccines are currently used. They induce immunity in 7 days which lasts for 2 years. Five doses 1 ml each are given I.M in the deltoid area in adults or in the anterolateral aspect of the thigh in children, on days 0, 3, 7, 14 and 28 post-exposure. It should not be given in the gluteal area.

Human Rabies Immune Globulin and Rabies Vaccine Administration Sites



Deltoid IM for adults and children

Anterolateral thigh IM OK for infants and small children

DO NOT GIVE RABIES VACCINE OR HRIG IN GLUTEAL

- **NB.** The old nerve tissue (prepared form infected sheep brain) & duck embryo vaccines are not recommended as they are less immunogenic require 25 injections and cause complications

Passive immunization

- administration of (HRIG) (human rabies immunoglobulin) 20 IU per kg of body weight. Half of the dose is given around the wound and the other half is given I.M. The antibodies delay multiplication of the virus and give more chance of diagnosis and for the vaccine to stimulate active immunity before the virus reaches the CNS.

- **Control measures:**

- 1-Pre-exposure vaccination is recommended for persons at risk including veterinarians
- HDCV is given in 3 doses on days 0, 7, and 21 or 28. Booster doses needed every 2-3 years to maintain antibody titer of 1: 5.
- 2- Destruction of stray animals and quarantine of imported dogs.
- 3- Vaccination of dogs and other pets.


THANK
YOU
VERY
MUCH