Enteric Viruses

(Polioviruses, Coxsackieviruses, Rotaviruses, Noroviruses and Hepatitis A virus)

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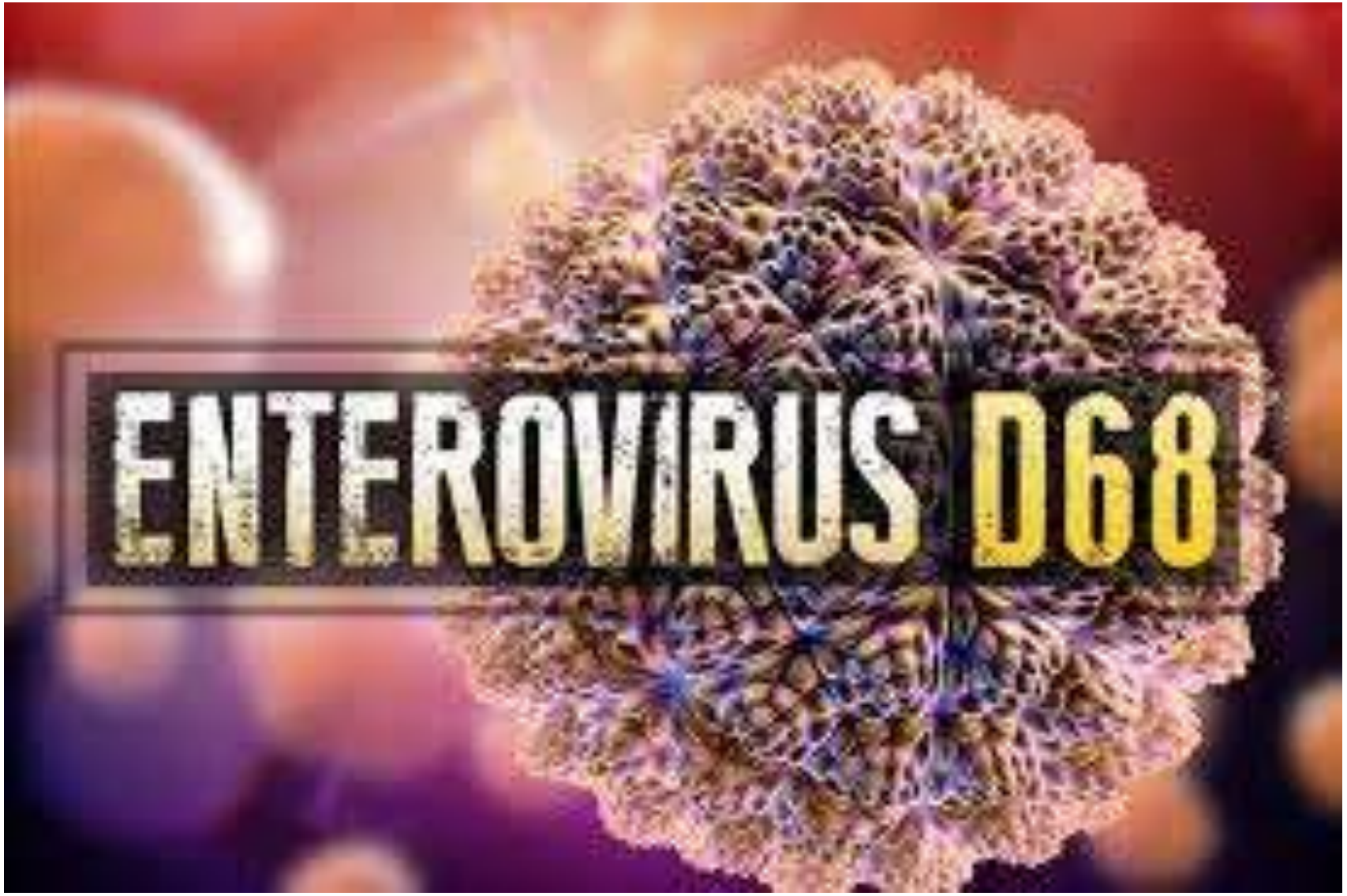
Enteric viruses

- **Picornaviruses (Enteroviridae)**
  - **Single stranded positive sense RNA viruses**
    - **(Picornaviridae) Picornaviruses** (polioviruses, coxsackievirus, rhinoviruses, Hepatitis A virus)
    - Naked polyhedral viruses (18-38nm)
    - Smallest RNA viruses
    - Enteric viruses (polioviruses), common cold viruses (rhinoviruses)
- **Reoviruses (Reoviridae)**
  - **Double stranded RNA viruses**
    - **Rotaviruses**
- **Caliciviruses (Caliciviridae)**
  - Norwalk viruses cause gastroenteritic, Hepatitis E virus cause hepatitis, *norovirus* also causes gastroenteritis.

Entroviridae (Picornaviridae)

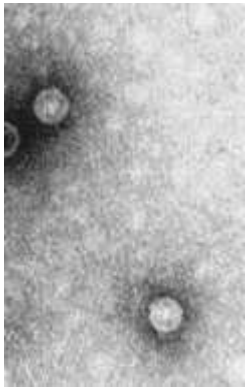
- Picornaviruses represent a very diverge viral group
  - Smallest virion size
  - Great genetic complexity
- Picornaviruses include
  - Enteroviruses: inhabitants of the GIT tract
  - Rhinoviruses: inhabitants of the nose and throat
- Human diseases ranging from paralysis (**poliomyelitis**), aseptic meningitis, pleurodynia, myocarditis, vesicular and exanthematous skin lesions, mucocutaneous lesions, respiratory illness, undifferentiated febrile illness, conjunctivitis, and severe generalised disease in infants and **common cold** in adults
- Subclinical infections are more common and clinical diagnosis is a challenge be since similar syndromes may be produced by different viruses and a single virus may produce different syndromes.

Fig. 2. Acute haemorrh

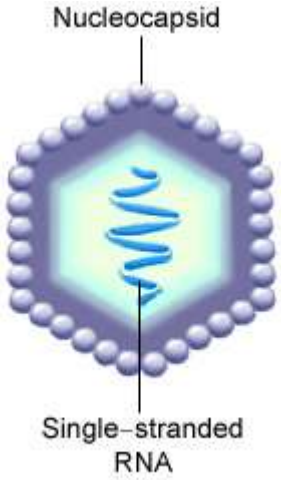

ENTEROVIRUS D68

Properties of Picronaviruses

- Virion
  - Icosahedral, 28-30nm, 60 subunits



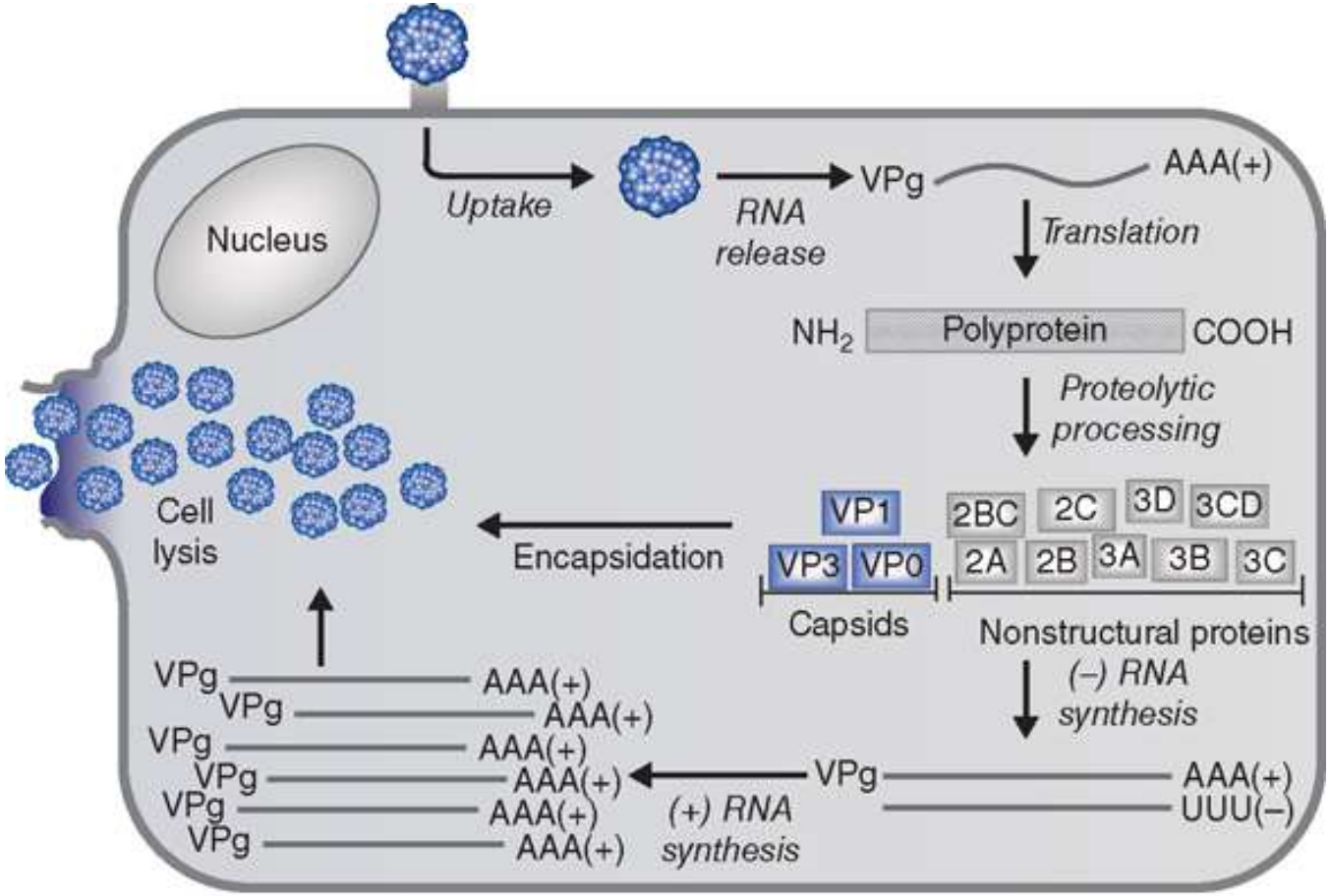
- Composition
  - RNA; 30%, protein; 70%
- Genome
  - Single stranded RNA-linear, positive sense, 7.2-8.4Kb in size, infecious
- Proteins
  - Four protiens cleaved from a single polyprotein; surface capsid protiens VP1 and VP3 are major antibody binding sites and VP4 is an internal protien
- Envelop
  - None
- Replication
  - Cytoplasm



Classification of Entroviridae

- Enteroviruses
  - Polioviruses 1, 2, 3
  - Coxsackievirus group A, types 1-24 (no type 15,18,23)
  - Coxsackievirus group B; types 1-6
  - Echoviruses types 1-33 (no type 8,10,22,23,28 and 34
  - Enteroviruses types 68-116 (no type 72)

Replication of Entroviruses



Source: Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA: *Jawetz, Melnick, & Adelberg's Medical Microbiology*, 26th Edition: www.accessmedicine.com

Coxsackieviruses

- Classified into two groups: Cox A and Cox B
  - Now classified under Human Entorovirus E (Group A, B and C)

- Cause a variety of human diseases
  - Aseptic (viral) meningitis
  - Respiratory and febrile illnesses
  - Hand-foot-and mouth disease, acute hemorrhagic conjunctivitis (Cox A
  - Myocarditis, Pericarditis, severe generalised disease in infants (Cox B)
  - Meningococcal encephalitis and paralysis

Clinical features

- Incubation period of 2 to 9 days

- Diverse clinical features ranging from mild febrile illnesses to CNS, skin, cardiac and respiratory diseases
  - Aseptic meningitis
  - Severe pharyngitis
  - Hand-foot and mouth disease
  - Myalgia
  - Myocarditis
  - Generalised disease of infants

Diagnosis

- Recovery of virus and culture
  - Tissue culture and mice
- Nucleic acid detection
  - Reverse transcriptase PCR (RT-PCR)
- Serology
  - Antibody detection by immunoflourescence
    - Difficult to evaluate
  - Antigen detection

Treatment and control

- No vaccine
- No antiviral drugs available

Echoviruses

- Enteric cytopathogenic human orphan viruses (ECHO Viruses)
  - Found in humans only
  - Can be cultured by inoculation of only certain tissue cultures
- 30 serotypes known
- Associated with aseptic meningitis, encephalitis, febrile illness with or without rash, common colds and ocular disease

Diagnostic criteria

- Recovery of virus from patients
- Detection of antibodies against virus during disease
- Virus isolation from body fluids (e.g. CSF for meningitis) or tissues manifesting lesions
- Methods
  - Molecular methods (nucleic acid detection (PCR)
  - Virus isolation

Control

- No effective antivirals or vaccination (except for polio)
- Avoidance of contact with infected patients
- Enteroviruses in the environments
  - Shed in faeces longer than from tissues/fluids
  - Transmission in faecal-oral (contamination of water, environment, good, utensils etc)
  - Present in sewerage (survive treatment and chlorination)

Introduction

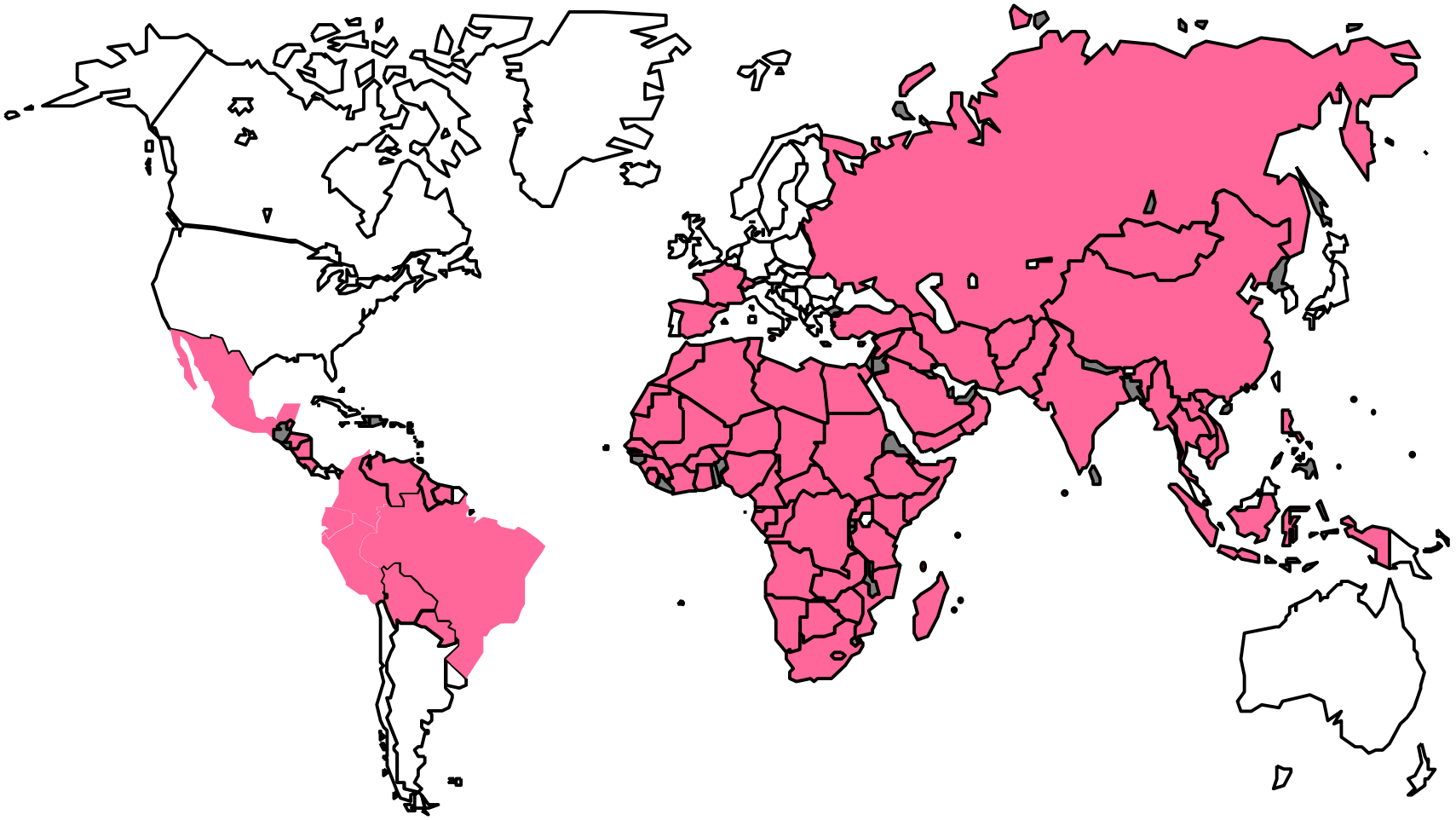
- Poliomyelitis (Greek)
  - Polio-grey
  - Myelon-marrow
- Major cause of paralytic disease in temperate regions in 19th century
- Less important as sanitary conditions improve
- Risk of paralysis increases with age

Introduction

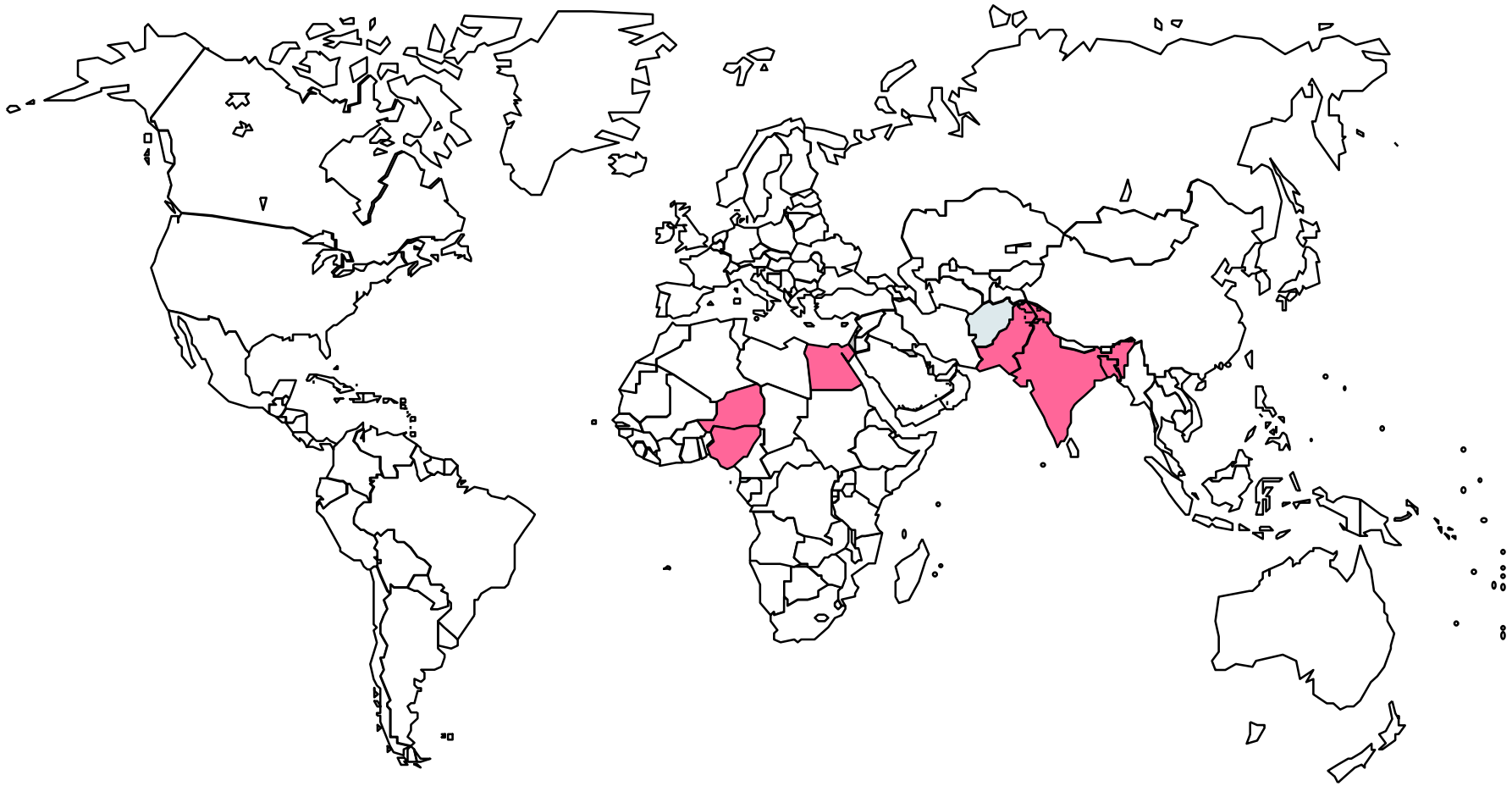
- Polio peak in the US was in 1952 (21,000 cases)

- Effective vaccines
  - Reduction of cases
  - Global eradication campaign

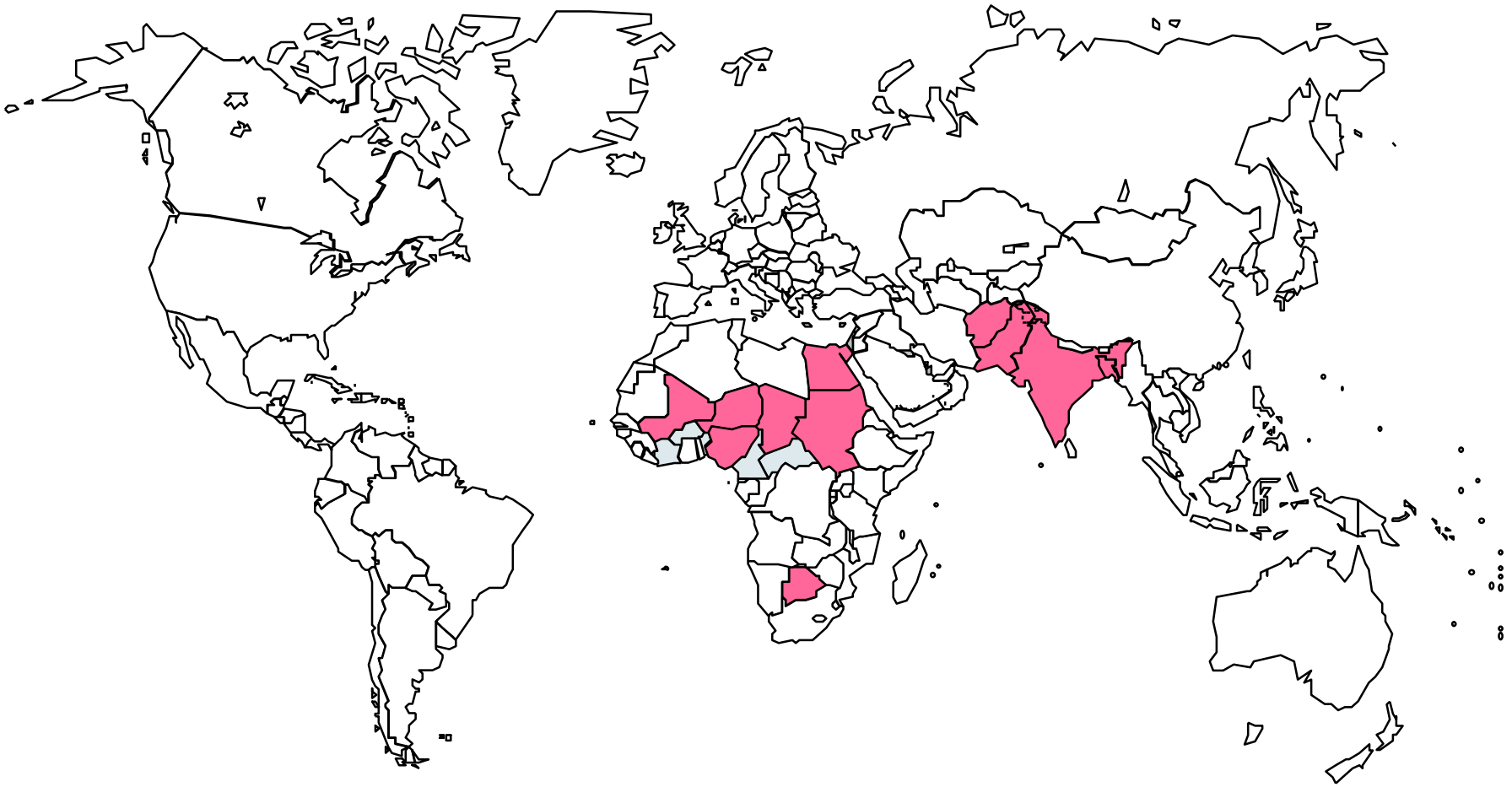
Wild Poliovirus 1988



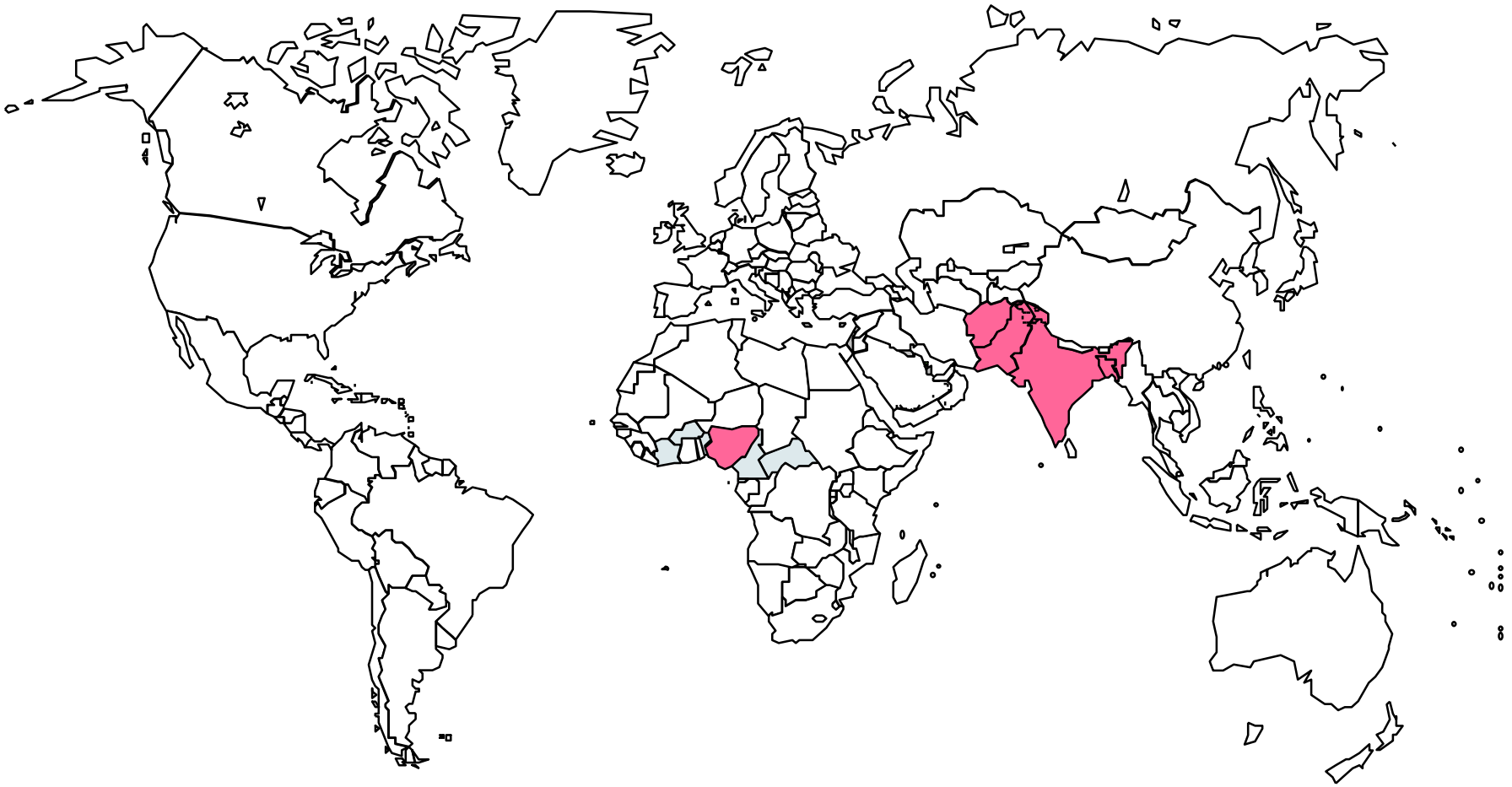
Wild Poliovirus 2003



Wild Poliovirus 2004



World Poliovirus 2006

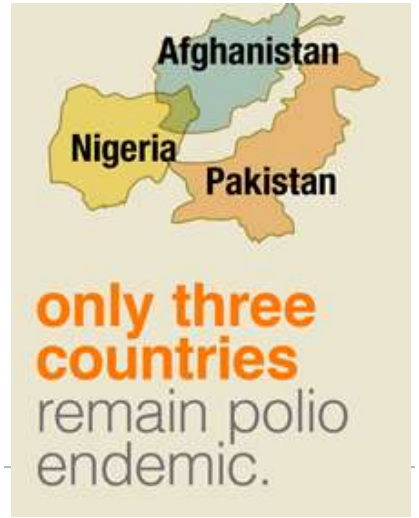


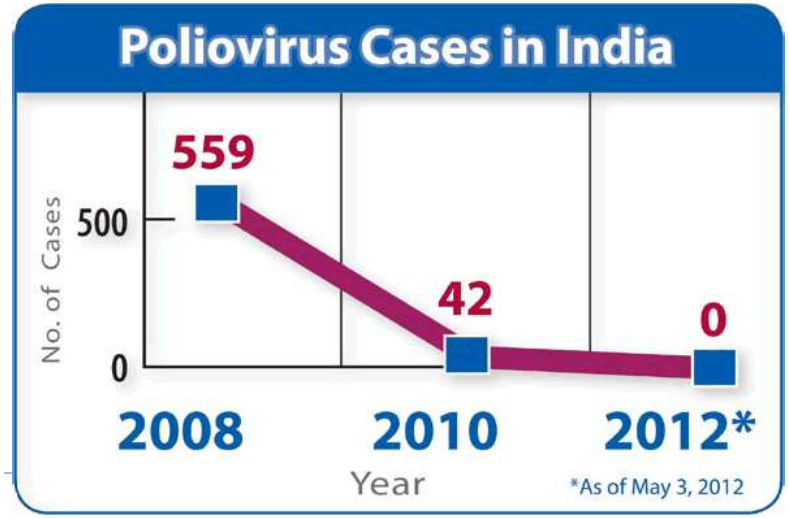
2006

- Only four countries in the world were reported to have endemic polio.
  - Nigeria
  - India
  - Pakistan
  - Afghanistan
- Cases in other countries are attributed to importation.
- Nigeria accounted for two-thirds of cases in 2006


1988
Countries that have never eliminated polio
Countries that have eliminated polio


2012*
Countries that have never eliminated polio
Countries that have eliminated polio
*As of May 3, 2012


Afghanistan
Nigeria
Pakistan
only three countries
remain polio endemic.


Poliovirus Cases in India
559
42
0
500
0
No. of Cases
2008
2010
2012*
Year
*As of May 3, 2012

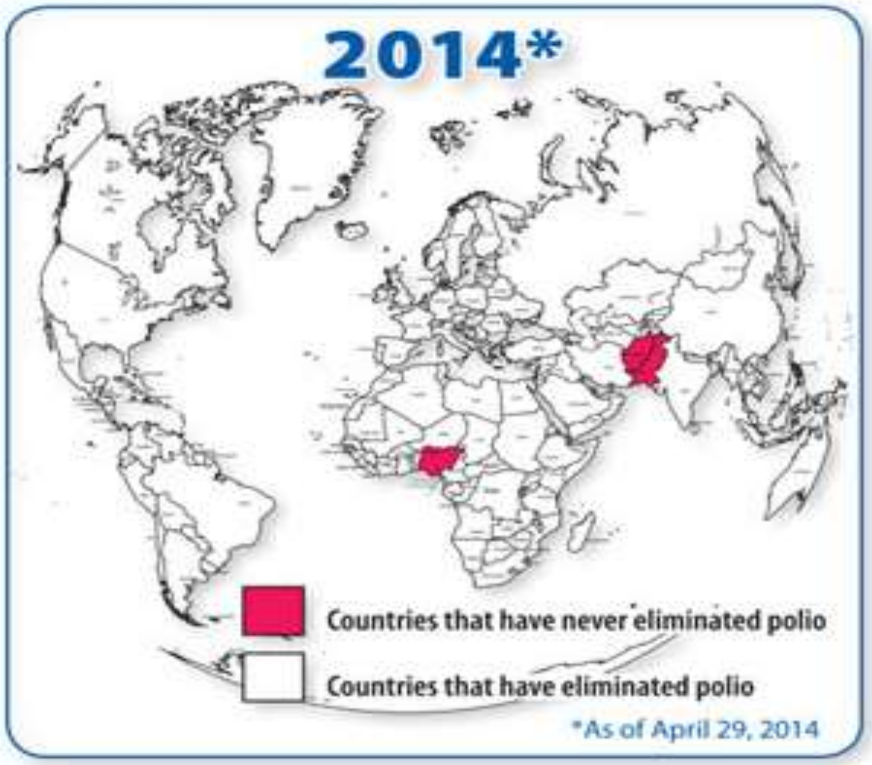
Polio as of 2014

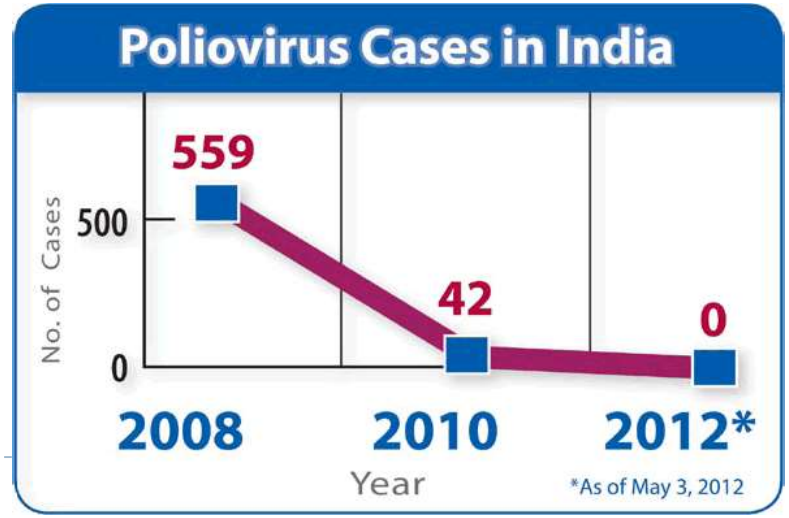
- The number of worldwide polio cases has fallen from an estimated 350,000 in 1988 to 407 in 2013—a decline of more than 99% in reported cases.

- Four regions of the world are certified polio free—the Americas, Europe, South East Asia and the Western Pacific. Only three polio-endemic countries (countries that have never interrupted the transmission of wild poliovirus) remain—**Afghanistan, Nigeria, and Pakistan**.

- January 13, 2014 marked three years since a child was paralyzed by wild poliovirus in **India**.

- The country was once considered the most complex challenge to achieving global polio eradication.
  - On March 27, 2014, the country of India, along with the other 10 countries in the WHO South East Asia Region, was certified polio-free.
  - 80% of the world's people now live in polio-free areas.





http://www.cdc.gov/polio/progress/

The polio endgame

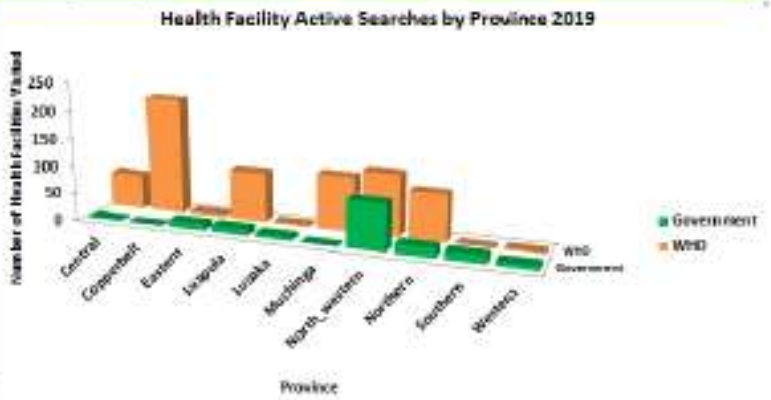
Since 1988, when the WHO resolved to eradicate polio, its footprint has shrunk dramatically. It is only considered endemic in Afghanistan, Pakistan and Nigeria (which hasn't seen a case since 2016). Last year there were only 22 new cases reported.

	1988	2017
Endemic countries	125	3

SOURCE: World Health Organization
TORONTO STAR GRAPHIC

Summary of new viruses this week
(AFP cases and environmental samples):

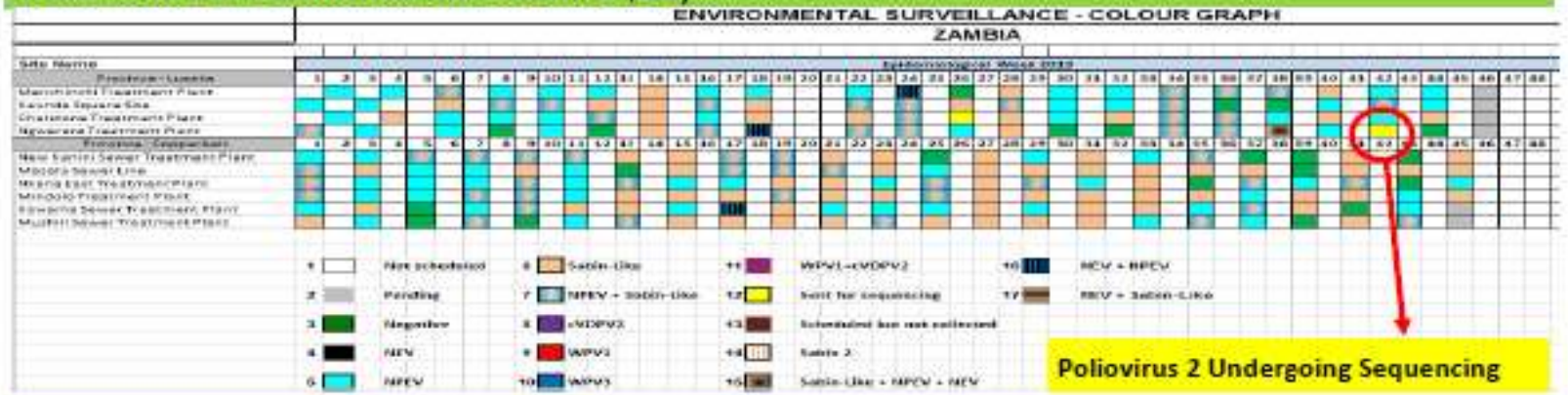
Afghanistan: one WPV1 positive environmental sample
Pakistan: two WPV1 cases and 14 WPV1 positive environmental samples
Central African Republic: one cVDPV2 positive environmental sample
Benin: one cVDPV2 caseUnknown date
Ghana: six cVDPV2 cases and two cVDPV2 positive environmental samples
Togo: two cVDPV2 cases



- 897 active searches conducted this year are less than 10% of expected searches
- Provinces are to conduct weekly active surveillance in high priority sites (al hospitals

Number of Health Facilities Visited by Priority Level	
Priority Level	**Number of Visits**
Highest	253
High	233
Medium	319
Low	79
Grand Total	**897**

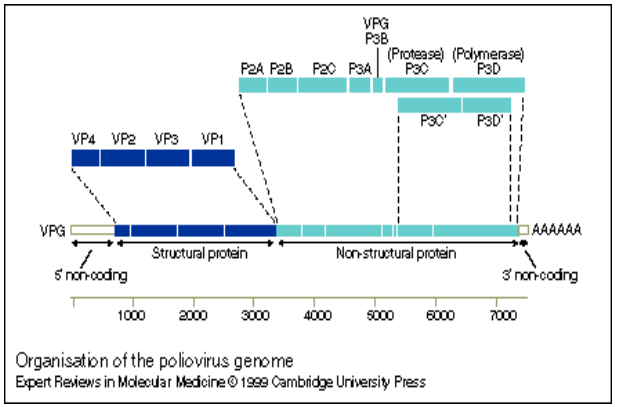
Poliovirus Environmental Surveillance (ES)



- Scheduled collections from the Copperbelt Province ES sites were conducted
- One type 2 poliovirus isolate from the Ngwerere Treatment Plant is undergoing sequencing
- Non Polio Enteroviruses detection for ES is ≥50% isolation & 7.7% among AFP cases

Virology

- Enteric virus, family Picornaviridae
- Naked virions with icosahedral symmetry
- non-enveloped capsid comprises 60 copies of capsid proteins (VP1,VP2,VP3,VP4)
- + sense, single-stranded RNA genome
- 3-D structure reveals 'canyons'



Organisation of the poliovirus genome

Virology

- Replication in host cytoplasm, ceases host cell protein synthesis and causes cell lysis

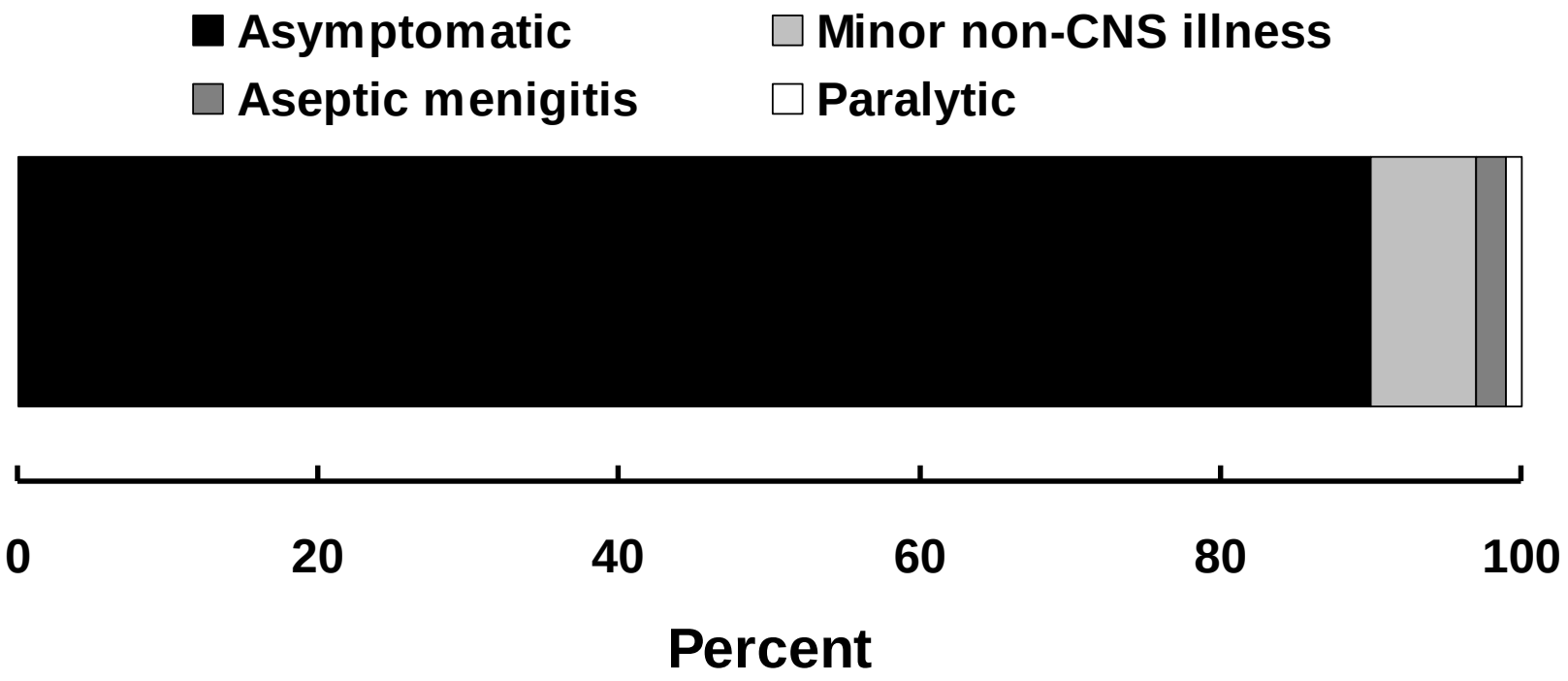
- Three serotypes with little heterotypic immunity (P1,P2 and P3)

- Rapidly inactivated by heat, formaldehyde, chlorine, UV

Virology

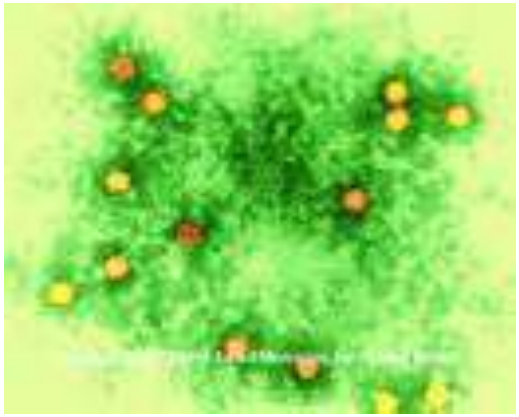
- Fecal-oral transmission (Human reservoir)
- Incubation period: 3-35 days on average
- Initial replication in pharynx and GI tract
  - Shed in feces
- Invasion of local lymphoid tissue (Alimentary mucosa, tonsils and peyer's patches)
  - Entry into blood stream
  - Entry into CNS
- Paralysis: replication of virus and spread along nerve fibers to motor neurons

Outcomes of Poliovirus infection



Diagnosis

- Isolation of Poliovirus from stool or pharynx
- Oligonucleotide typing
  - Wild type
  - Vaccine derived



Polio vaccine

Sabin polio vaccine:

A live, attenuated poliovirus vaccine

Salk polio vaccine:

An inactivated poliovirus vaccine

Polio Eradication Campaign

- Worldwide polio eradication target date: end of 2005
  - 1995: 3000 million children immunized worldwide
  - Strategy
    - Achieving & maintaining high routine vaccine coverage
    - Giving supplemental vaccine doses during National Immunization Days to interrupt wild poliovirus transmission
    - Developing sensitive surveillance systems
    - Conducting mopping-up immunization campaigns

Polio Eradication Campaign

- Current status (Jordan Report, 2002)
  - Number of reported polio cases decreased by > 99% since 1988
    - 350,000 (1988) to < 1,000 (1988)
    - Only 10 countries with indigenous transmission of wild poliovirus in 2001
    - Western Hemisphere – last case of paralytic poliomyelitis in Peru in 1991
    - Challenges
      - Ongoing transmission in heavily populated countries with poor health infrastructures (eg. India, Pakistan, Nigeria, Chechnya)
      - Continued importation of wild poliovirus into polio-free areas
      - Detection of circulating vaccine-derived poliomyelitis cVDPV
  - 2000: Global vaccination coverage with 3 doses OPV 82% in children under 12 mo of age

Polio Eradication Campaign

- Vaccine-derived poliovirus infections: *reversion of attenuated vaccine*
  - 2000-2001 cluster of cases in Haiti, Dominican Republic, Philippines
  - Circulating vaccine-derived poliovirus (cVDPV) type 1
    - Revertant Sabin virus – 2% difference from parent, vaccine virus → neurovirulence
    - Circulation probably due to low vaccination coverage in regions
    - National OPV vaccination campaigns underway to control cVDPV outbreaks

Polio Eradication Campaign

- Vaccine-associated paralytic poliomyelitis
  - Associated with transmission of vaccine strain to immunocompromised contacts
  - Risks/benefits of OPV vs. IPV
    - OPV (Sabin) – live vaccine, administered orally, single dose
      - Superior ability to induce intestinal/mucosal immunity (IgA) as well as serum IgG & to prevent spread among close contacts
      - Vaccine of choice in areas where wild poliovirus still present
      - Risk of vaccine-virus induced polio disease
    - IPV (Salk) – inactivated poliovirus vaccine, administered parenterally, multiple doses (3)
      - Effective in inducing circulating antibody titers (IgG) but not mucosal immunity
      - No risk of transmission to contacts
      - Routine vaccination in non-endemic areas (eg. US)

Vaccines

- Inactivated Polio Vaccine (IPV) in 1955, Salk Vaccine
  - Contains all three serotypes
  - Inactivated with formaldehyde
  - Administered IV or IM
  - Introduced in Zambia in 2018

- Advantages:
  - Protection from paralysis with neutralizing Ab (IgG)
  - No risk of vaccine-associated paralytic poliomyelitis (VAPP)

- Disadvantages:
  - Less GI mucosal immunity
  - Recipients of IPV are readily infected with wild-type Polio virus
  - Duration of immunity not known

Vaccines

- Oral Polio Vaccine (OPV) 1963, Sabin Vaccine
  - Contains all three live-attenuated serotypes
- Advantages:
  - Life-long immunity
  - Excellent GI mucosal immunity (IgG and IgA)
  - Prevent wild-type Poliovirus transmission (Herd immunity)
- Disadvantages:
  - Vaccine virus replicate in intestine mucosa and local draining lymph nodes
  - shed in feces up to six weeks post immunization
  - May be transmitted via fecal-oral to close contacts causing VAPP
  - Risk to the immuno-compromised
  - Does not grow/Disseminate well to the CNS

When should we stop OPV?

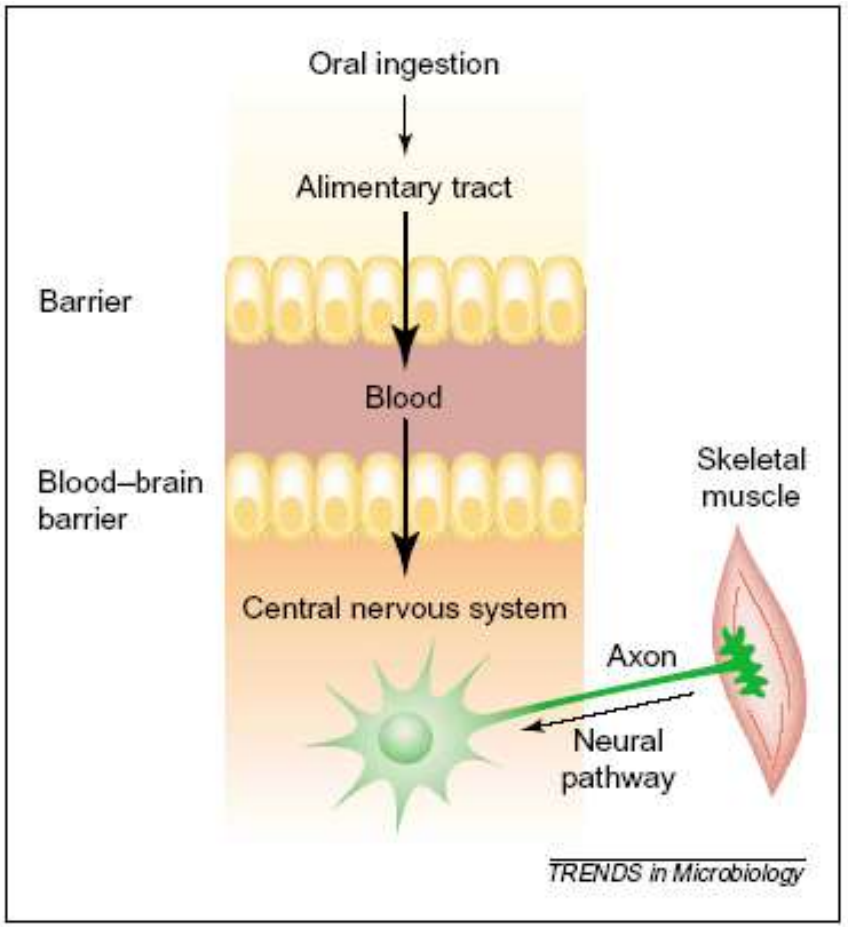
Should we continue indefinitely with IPV or stop all immunization after polio is considered "eradicated" (*a la* smallpox)?

Bioterrorism issues?

Luis Tenorio – the last polio case in the Western Hemisphere

Entry of Polioviruses into CNS

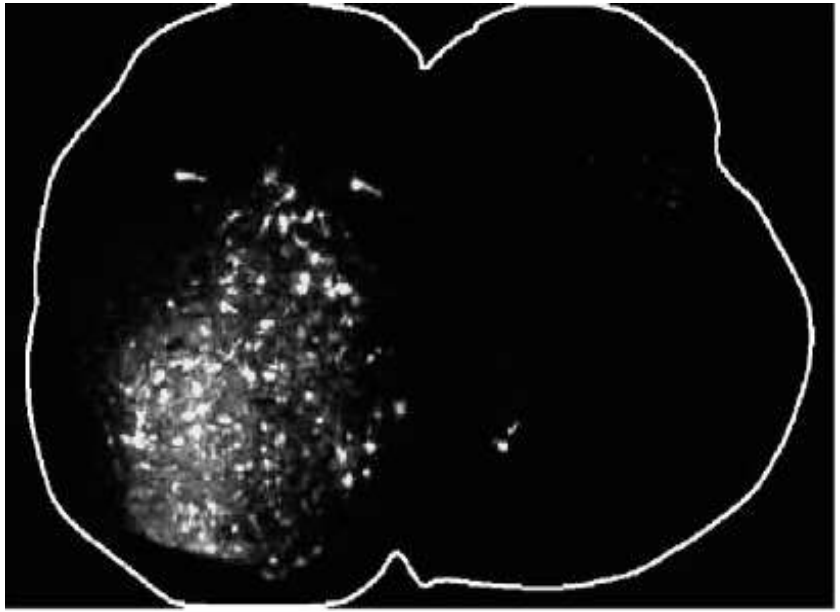
- Direct passage from blood into CNS
  - Initial viremia
  - Crossing the blood brain barrier
  - Independent of receptor
- Retrograde axonal transport from muscle to CNS
  - Viremia
  - Lymphoid tissue
- 'Trojan horse' mechanism?
  - Polioviruses shown to replicate in primary monocytes



Ohka *et al* Trends Microbiol, 2001. 9(10)

Retrograde Transport

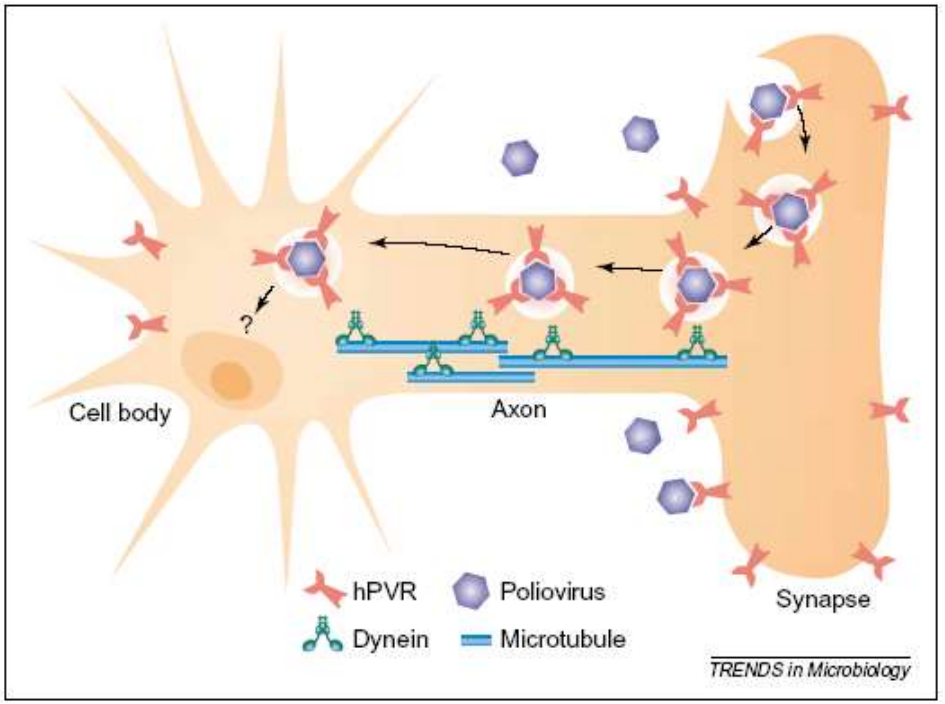
- IM injection of Poliovirus into CD155tg mice
  - left gastrocnemius muscle
  - Immunohistochemistry
- Virus found exclusively within motor neurons of the left anterior horn of the spinal cord



Mueller *et al.* Virus Res, 2005. 111(2):

Retrograde Transport

- hPVR-mediated endocytosis occurs at synapses
- Enclosure of intact poliovirions and interaction with cytoplasmic dynein
- Endosomes retrogradely transported along microtubules through the axon to the neural cell body
- Uncoating and replication
- Neurovirulence observed only in tg mice



Ohka *et al* Trends Microbiol, 2001. 9(10)

Entry via Blood-Brain Barrier

- Inoculation of Poliovirus into tail vein
- Systemic distribution
  - Spleen, liver, kidneys, small intestines, heart, lungs, muscle, cerebellum and cerebrum
- Distribution profile similar between tg mice vs non tg mice
  - hPVR does not play role in distribution
  - Distribution is not strain specific

Table 1. Accumulation rate of poliovirus into the mouse cerebrum[a,b]

Virus strains or control material	Accumulation rate		
	Tg mouse	Non-tg mouse	Rat
Virulent Mahoney	164	123	–
Attenuated Sabin 1	223	246	–
Albumin	–	1[c]	–
OX-26	–	–	650

[a]Abbreviation: Tg, transgenic.
[b]Modified from Ref. 23.
[c]0.001 μl min^{-1} g^{-1} tissue is regarded as 1.

Ohka *et al* Trends Microbiol, 2001. 9(10)

Neurovirulence

- Neurovirulence determinants
  - Nt 472, 480, 481
  - IRES (Translation initiation)
  - Mutants unable to propagate in neurons
- Viral uncoating
  - Escape endocytic vesicle
- Viral replication
  - Death of motor neuron
  - Flaccid paralysis

Questions for future research

- How does poliovirus cross the BBB and arrive at neurons?
- What are the mechanisms responsible for the protective reaction of neural cells against poliovirus infection?
- Why do poliovirus receptors exist only in primates?
- What are the differences in the functions of hPVR isotypes.
- Why does poliovirus replicate well in motor neurons?

Summary and Conclusion

- Polioviruses as one of the most well characterized viruses at molecular level
  - Successful vaccines
- Development of transgenic mouse model
  - Lack of adequate and affordable animal model
  - Save of old world monkeys
- Elucidation of neurovirulence is at starting point
- Global Polio Eradication?
  - Stop vs continue vaccination?
  - IPV vs OPV?
  - Stop research, destroy wild Polioviruses?
  - Bioterrorism?

Hepatitis A Virus (HAV)

Presentation Outline:

I. Characterization of virus

   a. Structure

   b. Pathogenesis & Clinical Significance

II. Diagnostics

III. Treatment

IV. Epidemiology

V. Prevention/Vaccine

HAV: Structure

- The hepatitis A virus is a positive-sense, single-stranded RNA virus of approx. 7.5 kb in length.
- It is the sole member of the genus Heparnavirus and part of the Picornavirus family.
- Like other members of the Picornaviridae, HAV is small with a naked, that is non-enveloped, capsid.
- There is only one serotype of HAV and it is only known to infect primates.

PICRORNAVIRADAE

Enterovirus

- Poliovirus
- Coxsackie A virus
- Coxsackie B virus
- Echovirus (ECHO virus)
- Enterovirus

Rhinovirus types

Cardiovirus

Aphthovirus

Heparnavirus

- Hepatitis A virus

HAV: Environmental Resistance

The capsid of Hepatitis A is icosahedral and extremely stable.

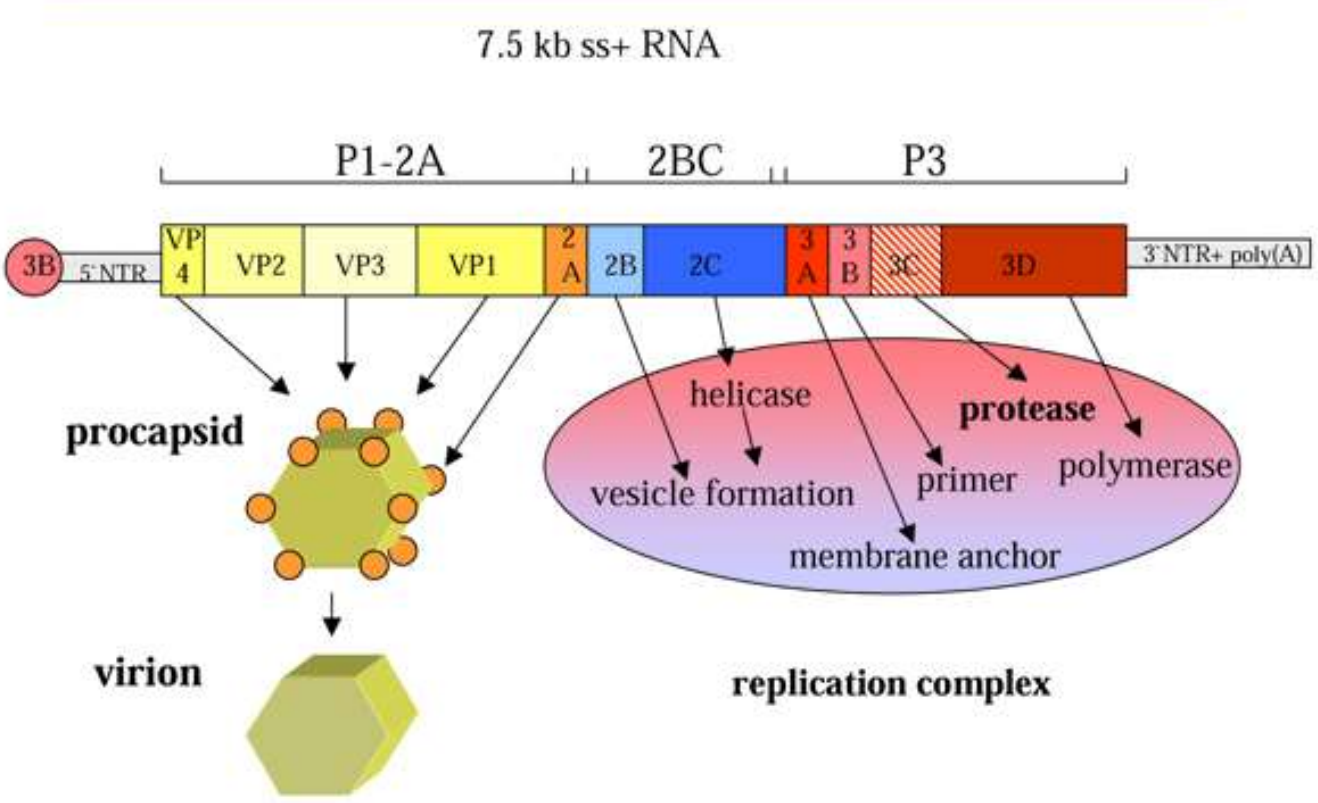
HAV is capable of survival in seawater, fresh water, and soil and shows long-term survival on hands and fomites, making them efficient vehicles of infectious transmission.

- Stable to:
  - Acid at pH 1
  - Solvents (ether, chloroform)
  - Detergents
  - Salt water, groundwater (months)
  - Drying (stable)
  - Temperature
    - 4°C: weeks
    - 56°C for 30 minutes: Stable
    - 61°C for 20 minutes: Partial inactivation
- Inactivated by:
  - Chlorine treatment of drinking water
  - Formalin (0.35%, 37°C, 72 hours)
  - Peracetic acid (2%, 4 hours)
  - β-Propiolactone (0.25%, 1 hour)
  - Ultraviolet radiation (2 μW/cm2/min)

Source: *Murray* et al. *Medical Microbiology, 5th ed.*

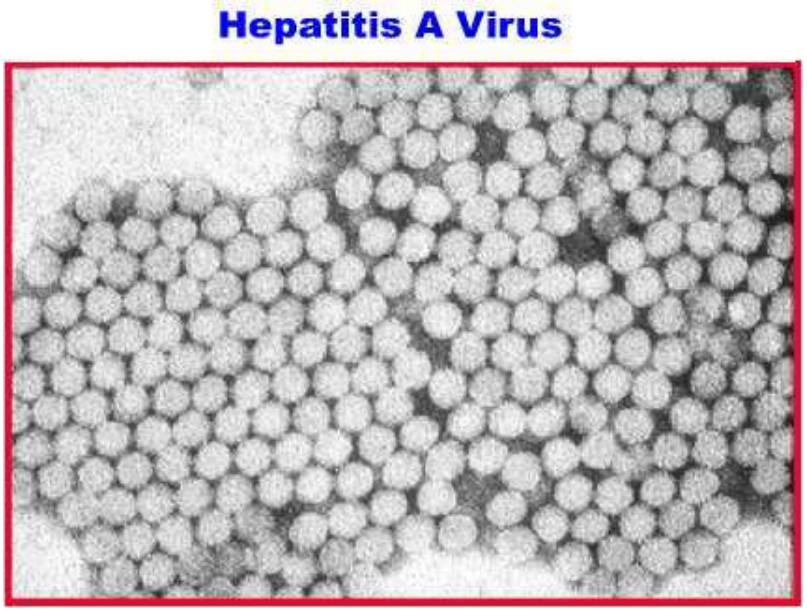
HAV: Genomics

1. 5’ noncoding region (NCR) is uncapped and covalently linked at the 5’ end to the protein VPg which may be involved in initiation of RNA synthesis.
2. Single open-reading frame encodes all viral proteins, both structural and non-structural.
3. Short 3’ NCR ends in a poly(A) tail.



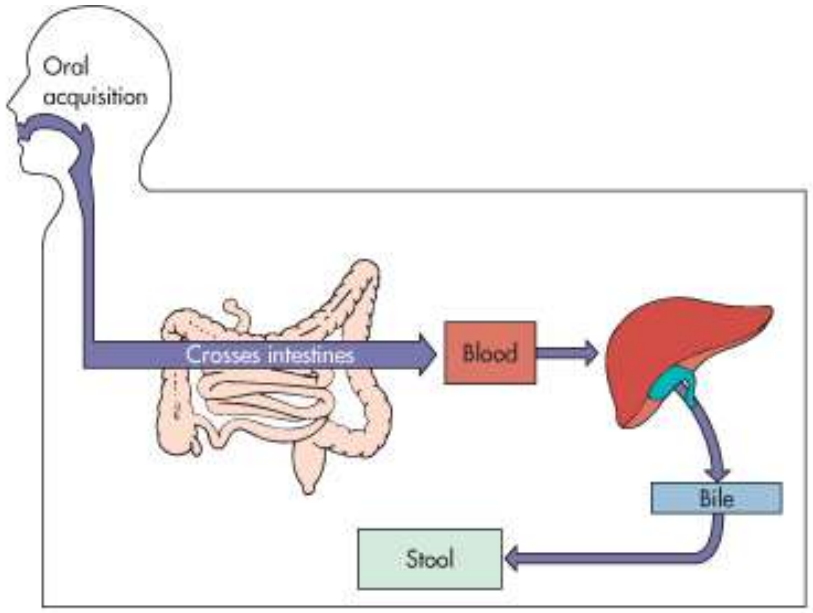
HAV: Transmission

- Close personal contact
  - Household member
  - Sex contact
  - Childcare centers
- Contaminated food or water
  - Fecal – oral contact
  - Contaminated shellfish
  - Infected food handlers
- Blood exposure
  - rare



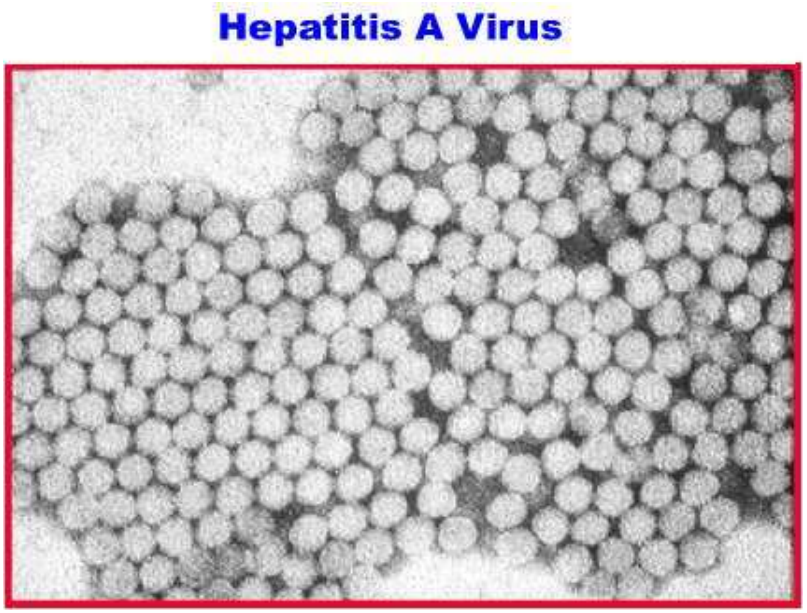
HAV: Transmission (cont.)

- Upon ingestion, viral particles reach target, the parenchymal cells of the liver.
- HAV replicates in hepatocytes and gastrointestinal epithelial cells and is released through exocytosis (rather than cell lysis) into bile and from there into stool.

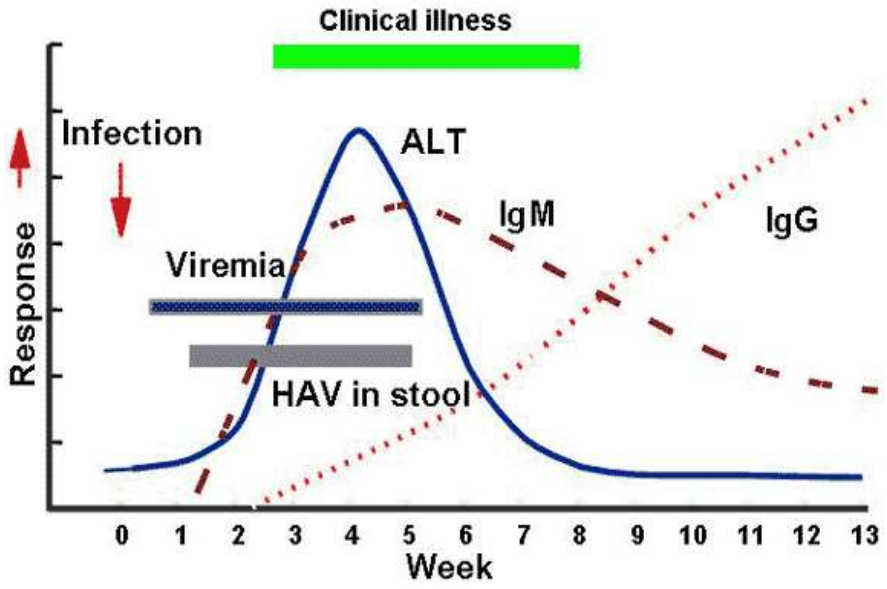


HAV: Clinical Features

- Incubation period is usually about 30 days after exposure, range is 15 – 50 days
- Jaundice (turning yellow) is most commonly seen in the older patients
  - Under 6 years old (10%)
  - 6 to 14 years old (40 – 50%)
  - Greater than 14 years old (70 – 80%)
- Fatigue
- Dark urine
- Fever
- Nausea and vomiting
- Abdominal pain
  - Complications of this type of viral infection include rare liver failure and relapsing hepatitis
  - Chronic sequelae are not seen
- 33% of the US population has evidence of past infection and thus immunity



HAV: EVENTS IN INFECTION



ALT = Alanine transaminase

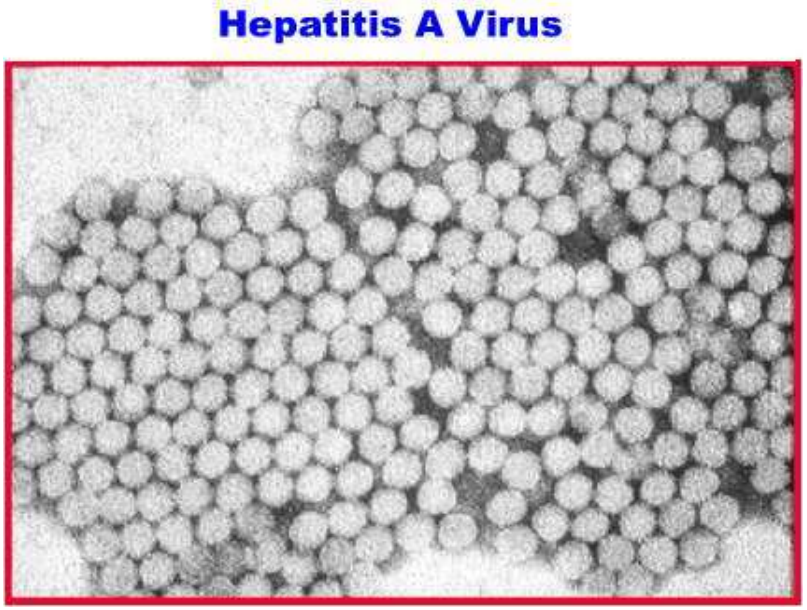
- As the immune system responds to the infection, the amount of virus in the blood (viremia) and in the stool (HAV in stool) disappears.
- The liver enzyme, ALT goes up at the beginning of the infection, but descreases to normal at about 8 weeks.
- IgM shows acute infection and IgG is positive long – term.

HAV: Diagnosis

- Time course of clinical symptoms
- Identification of known source
- Hepatitis Panel
  - IgM anti-HAV detectable in blood as early as 2 weeks after the initial infection. Antibodies disappear 3 to 12 months after the infection.
  - IgG anti-HAV appear approximately 8 to 12 weeks after initial infection. Antibodies remain in the blood for lifelong protection (immunity) against HAV and indicate previous infection.

HAV: Treatment

- HAV Treatment
  - No specific medical treatment
  - Avoid alcohol and all medications that are metabolized in the liver
  - Manage symptoms
  - If the spleen is enlarged avoid activities that could lead to abdominal pressure or injury

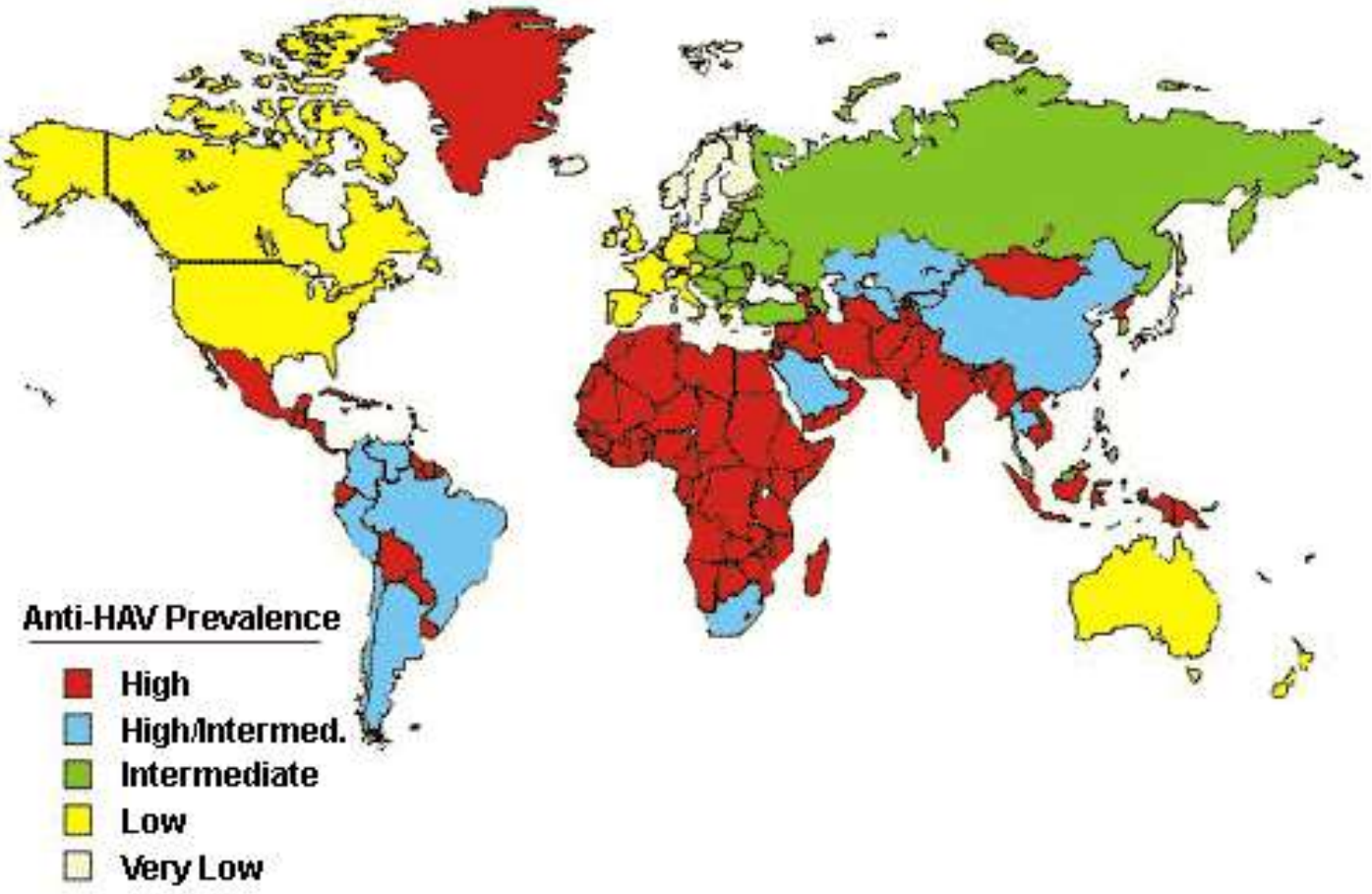


HAV: Epidemiology Summary

Risk Factors include:

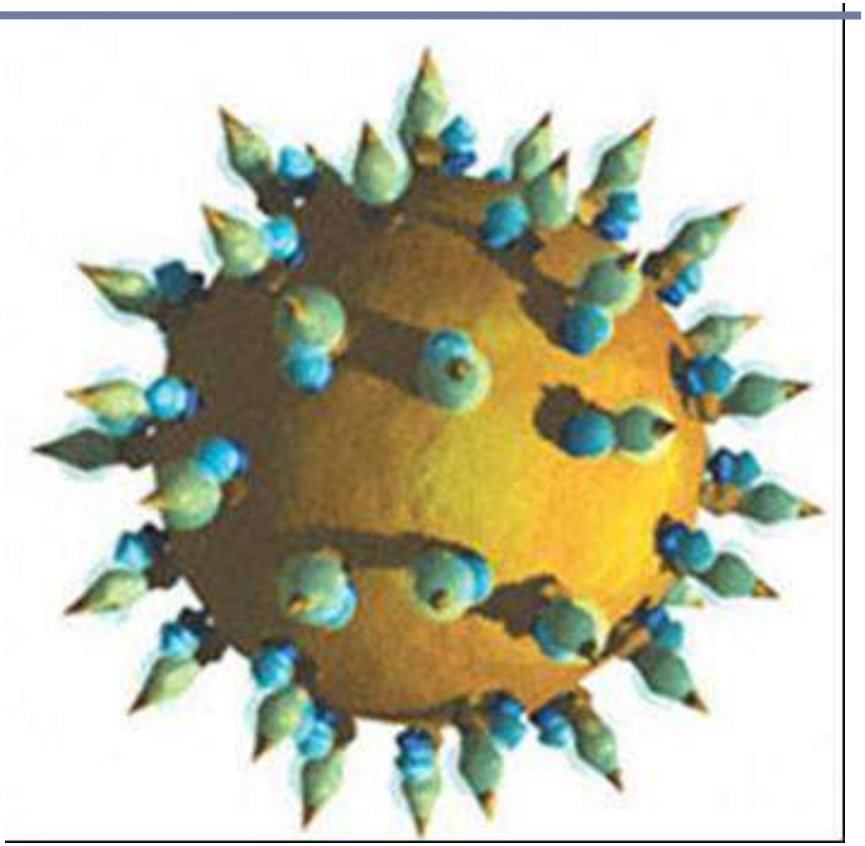
- Travel to endemic areas (30%)
- MSM (18%)
- Household contact (10%)
- IDU (6%)
- Homeless (5%)

HAV: Worldwide Prevalence



HAV: Prevention

- Wash hands
- Use gloves when appropriate
- Risk reduction if involved in oral/anal sexual practices
- Risk reduction if involved in intravenous drug use
- Vaccination



Hepatitis A virus. Courtesy of PRN Notebook Online (www.prn.org). Model created by Dr. Louis Henderson PhD, Frederick Cancer Research Center.

HAV: Prevention (continued)

- Immune Globulin (IG)
  - Sterile preparation of concentrated antibodies (immunoglobulins) made from pooled human plasma
    - Only plasma tested negative for hepatitis B, HIV, and hepatitis C are used
  - Provides protection against hepatitis A through passive transfer of antibody
  - When administered within 2 weeks after an exposure to hepatitis A virus, IG is 80 – 90% effective in preventing hepatitis A
  - IgA deficiency has been known to cause anaphylaxis after repeated intramuscular administration of IG

HAV: Vaccination

- HAV Vaccines first licensed in 1995 in the US (1992-1994 in other places)
  - Vaccines created from inactivated virus
  - Vaccines are highly immunogenic where 100% of those vaccinated with 2 doses will seroconvert to a protected level
  - 2005 recommendations: routine vaccination of all children in US beginning at 1 year of age
- Pre-exposure Vaccination
  - Persons at increased risk for infection:
    - Travelers to intermediate and high HAV-endemic countries
    - Homosexual and bisexual men (men who have sex with men)
    - Persons with HIV/AIDS
    - Drug users
    - Persons with chronic liver disease including Hepatitis C
    - Persons with a diagnosis of clotting factor disorder
    - Persons with occupational risks
  - Communities with high rates of hepatitis A [e.g., Alaska Natives, American Indians]
  - Routine childhood vaccination

HAV: Vaccination Problems

- Case Report of Vaccine-induced pancreatitis

Patient History:

-63-year old male

-received HepA & HepB (combination vax)

-followed accelerated regime (0, 7, 21 days +booster)

-came to hospital with epigastric pain and severe vomiting

-no history of drug abuse, blood transfusion, pancreatitis, recent illnesses, or contact with ill people

-no alcohol consumption, no known chronic or autoimmune diseases

-negative blood and urine cultures

-negative for common causes of pancreatitis (gallstones, neoplasms, or congenital anomalies)

-3 months after vax, immunity to Hep A but not Hep B

HAV: Vaccination Problems

- Patient HLA genotype and incongruent immune response a possilbe link
- Other ideas:

-activation of pancreatic zymogens by serum factors can initiate acinar cell injury

-molecular mimicry

-polyclonal activation (adjuvant reaction) of lymphocytes

-"bystander activation" of self-reactive lymphocytes or somatic mutation of immunoglobulin variable genes

-vaccine-induced vasculitis or the release of anaphylactic mediators such as histamine and leukotrienes induced by antigens

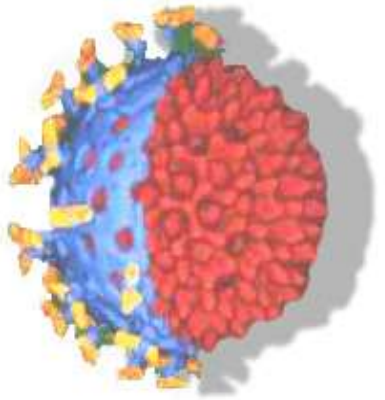
**HLA genotype of patient most likely responsible:

HLA-DRB1*0401, HLA-DQB1*0301, HLA-Cw5, HLA-B44, and DR4

HLA genotyping could provide useful info

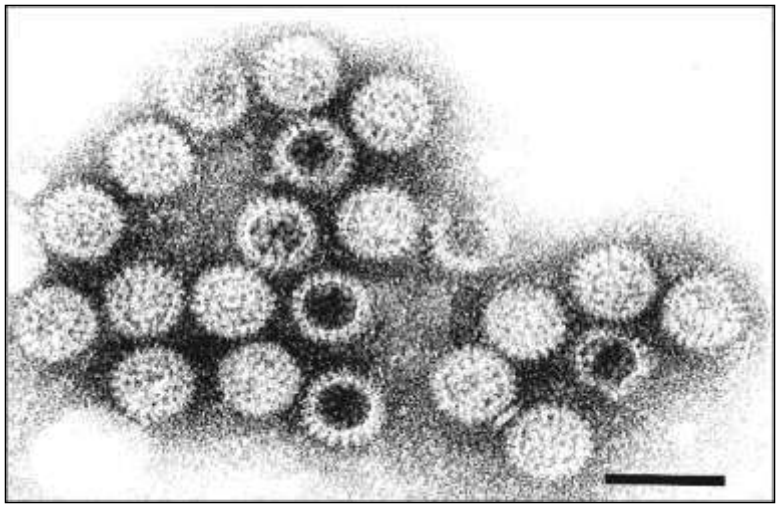
Rotaviruses

- **Double stranded RNA viruses**
  - **Reoviruses (Reoviridae)**
    - Naked polyhedral viruses (60-80nm)
    - Involved in upper respiratory tract infections
    - Rotaviruses cause diarrhea in humans



Morphology

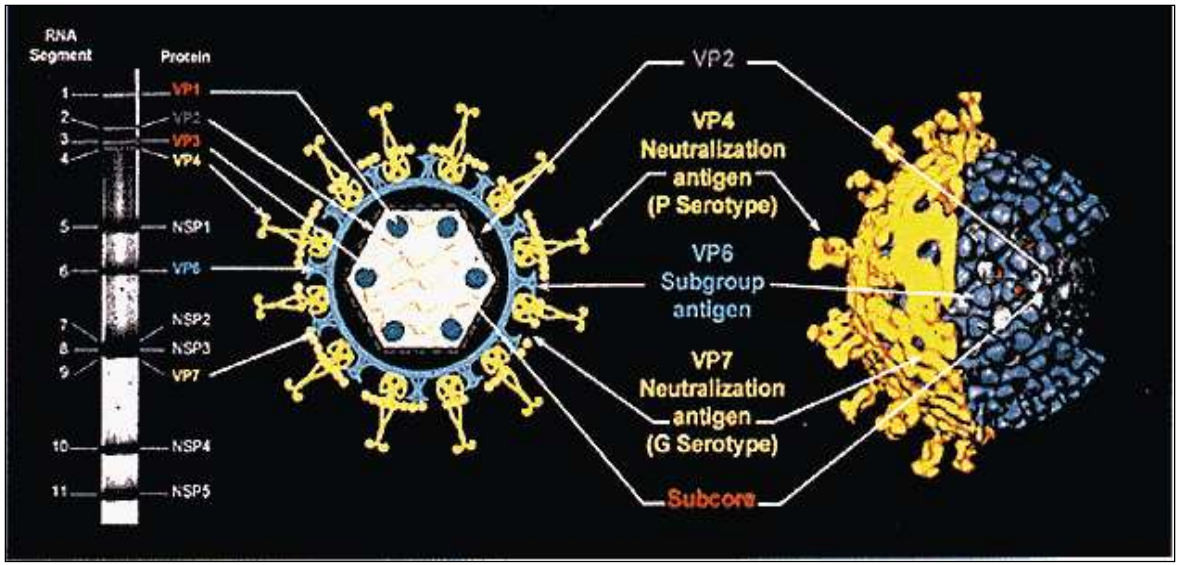
- Family Reoviridae
- 70-85 nm diameter
- Nearly spherical icosahedral particle
- Non-enveloped, double-shelled viruses
- Wheel-like distinct appearance under EM
- Heat stable and up to pH 3



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

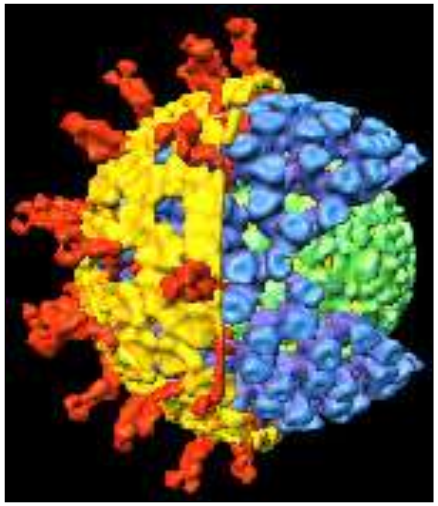
Genome

- 11 segments of double-stranded RNA
- structural viral proteins (VP):
  outer/inner capsids: VP4 and VP7
  core: VP2, VP6, VP1, VP3
- nonstructural proteins (NSP): NSP1-5



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

Classification



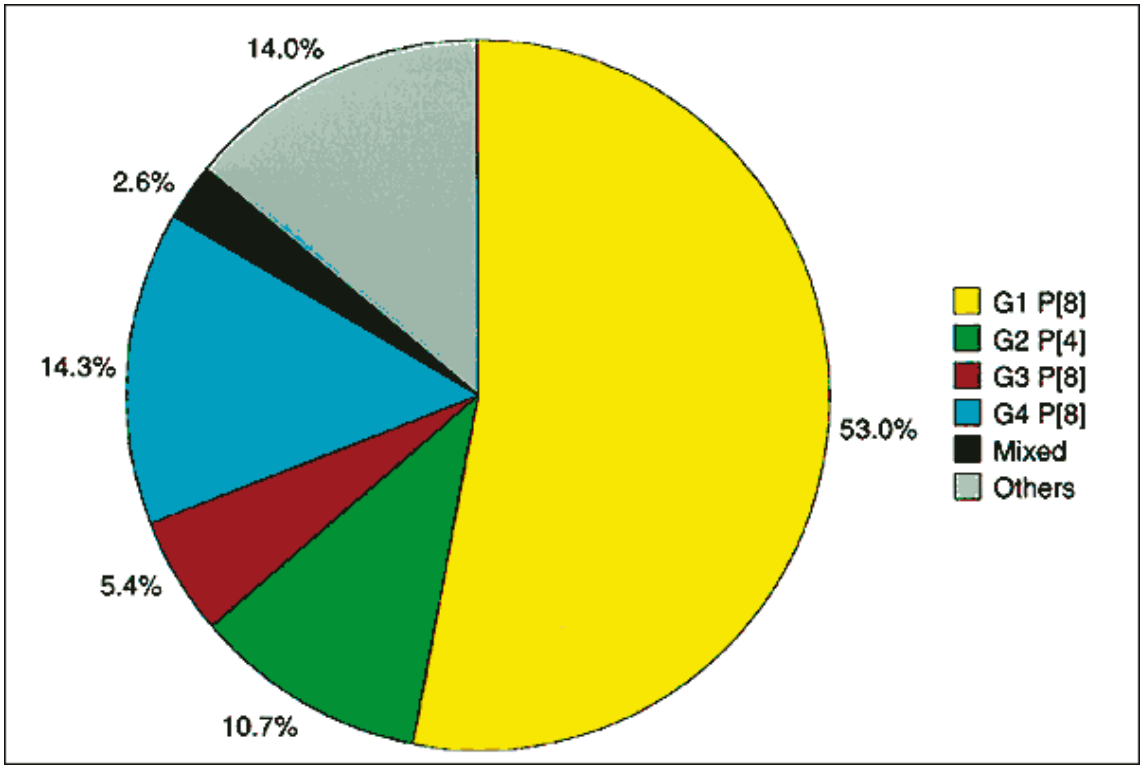
- defined by cross-neutralization with polyclonal antibodies of antigenic specificities (glycoproteins)

- VP4 antigen: P serotype; 8 human rotaviruses
  - Hemagglutinin
  - Cell attachment

- VP7 antigen: G serotype; 10 human rotaviruses
  - Glycoprotein

- commonly found strains: P[8]G1; P[8]G3; P[8]G4; and P[4]G2

Classification (cont.)



Distribution of rotavirus strains from a global collection of 2,748 strains. "Others" includes strains that were not typable.

(Picture Source: http://www.cdc.gov/ncidod/EID/vol4no4/parasharG.htm#fig%204)

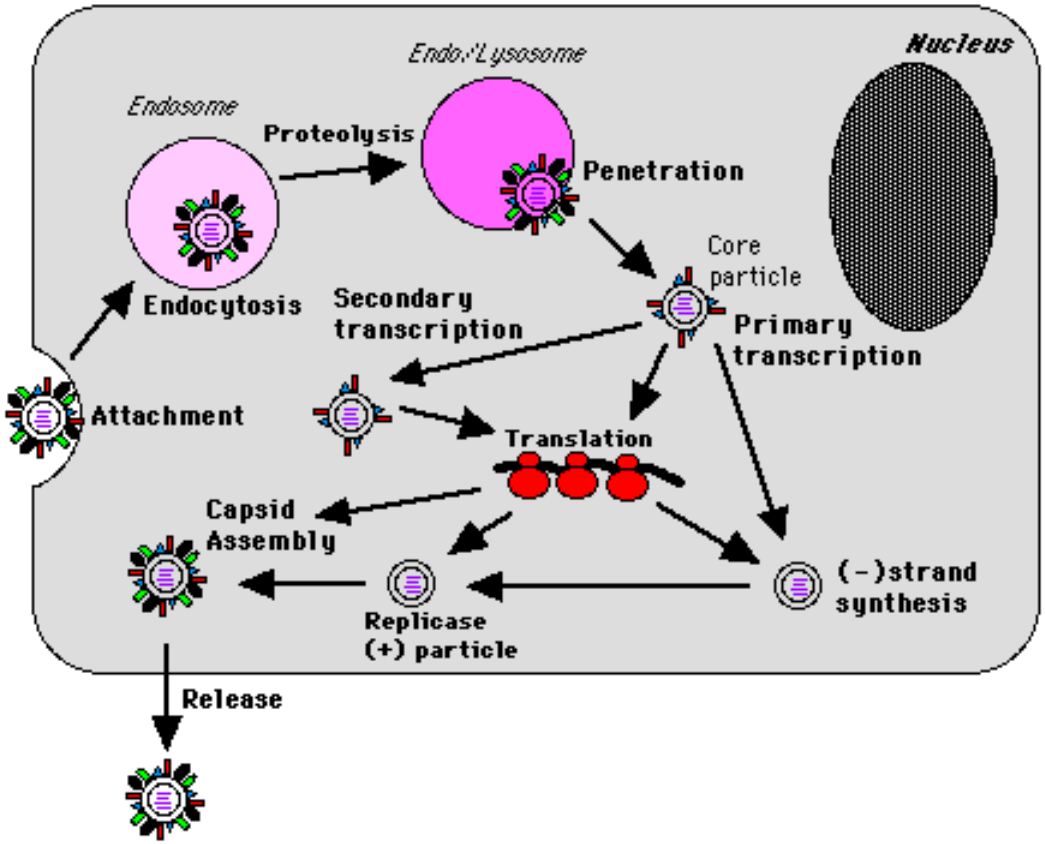
Replication

- Attached to cell receptors contained sialic acid
- Internalized and uncoated via endolysosomes
- Early transcription by viral RNA polymerase occurs inside sub-viral particle
- Resulted in synthesis of (+) mRNAs and are translated in the cytoplasm.
- Reassortment occurs during Early transcription.

Replication (cont.)

- Secondary transcription occurs in cytoplasm in later infection in a conservative fashion.
- Uncapped non-polyadenylated transcripts
- Particles assemble in the cytoplasm 6-7 h after infection
- Budding from the E.R. into internal spaces & are eventually released when the cell lyses.

Replication (cont.)

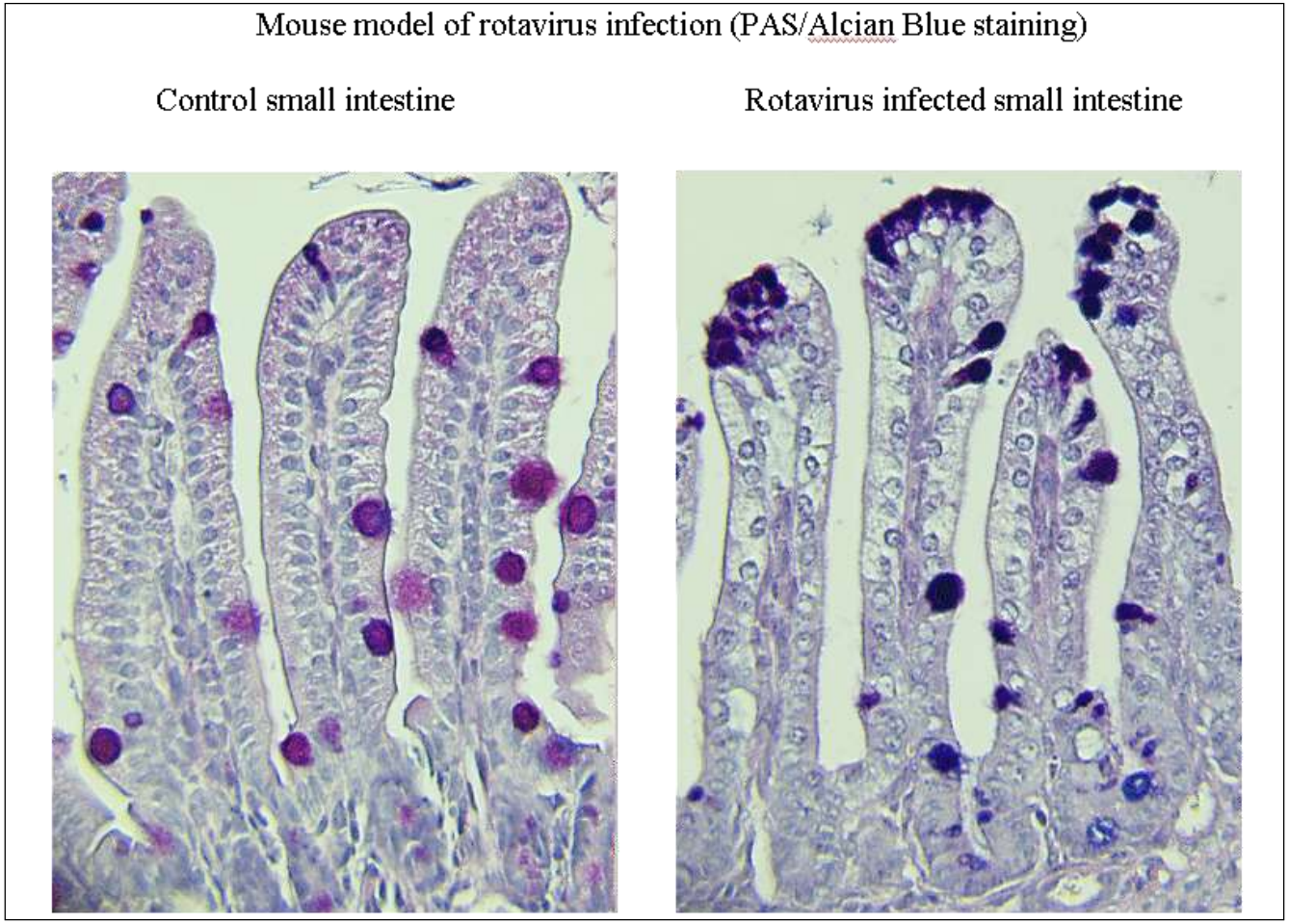


(Picture Source: http://www.tulane.edu/~dmsander/WWW/335/Diarrhoea.html)

Pathogenesis

- infect upper two-third of duodenal epithelial cell
- infectious particles are released to intestinal lumen and undergo further replication in distal areas
- Virus causes shortening or destruction of microvilli and impairs adsorption
- cause severe diarrhea, vomiting and abdominal pain among children
- 2 days incubation with 3-8 days watery diarrhea
- death of over 600,000 children annually worldwide

Pathogenesis (cont.)



(Picture Source:www.eur.nl/fgg/kgk/gastro/ rotavirus.gif)

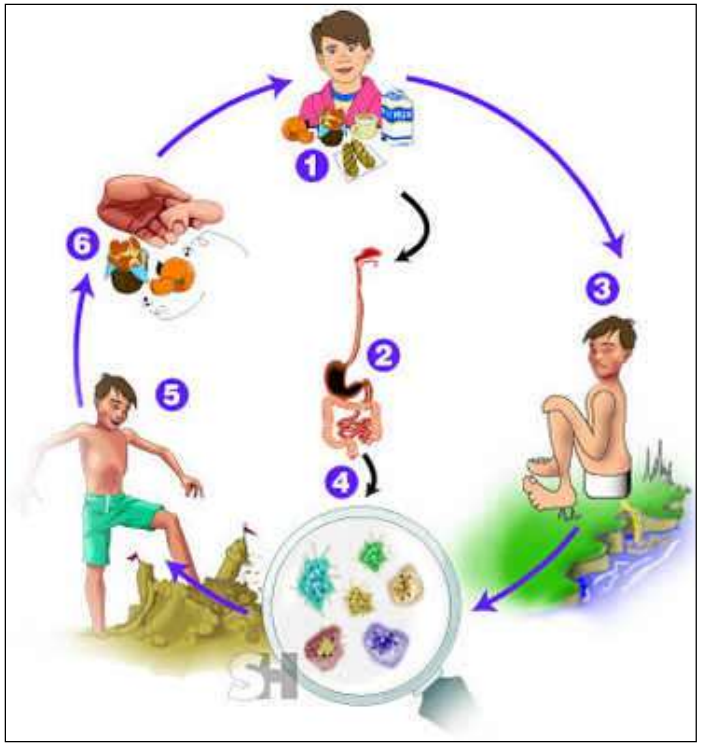
Epidemiology

- Primary transmission mode is fecal-oral

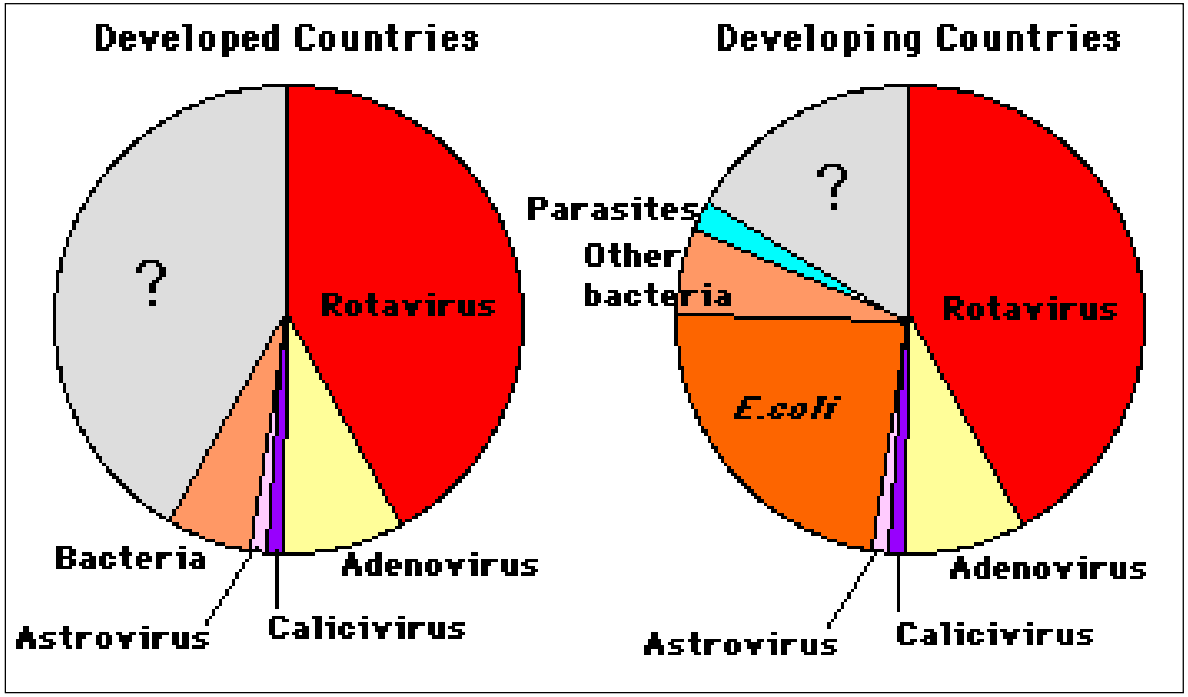
- ingestion of contaminated food or water and contact with contaminated surfaces

- Annual epidemics occurring from November to April

- high rates of illness among infants and children below 2 years old, but mild among adults



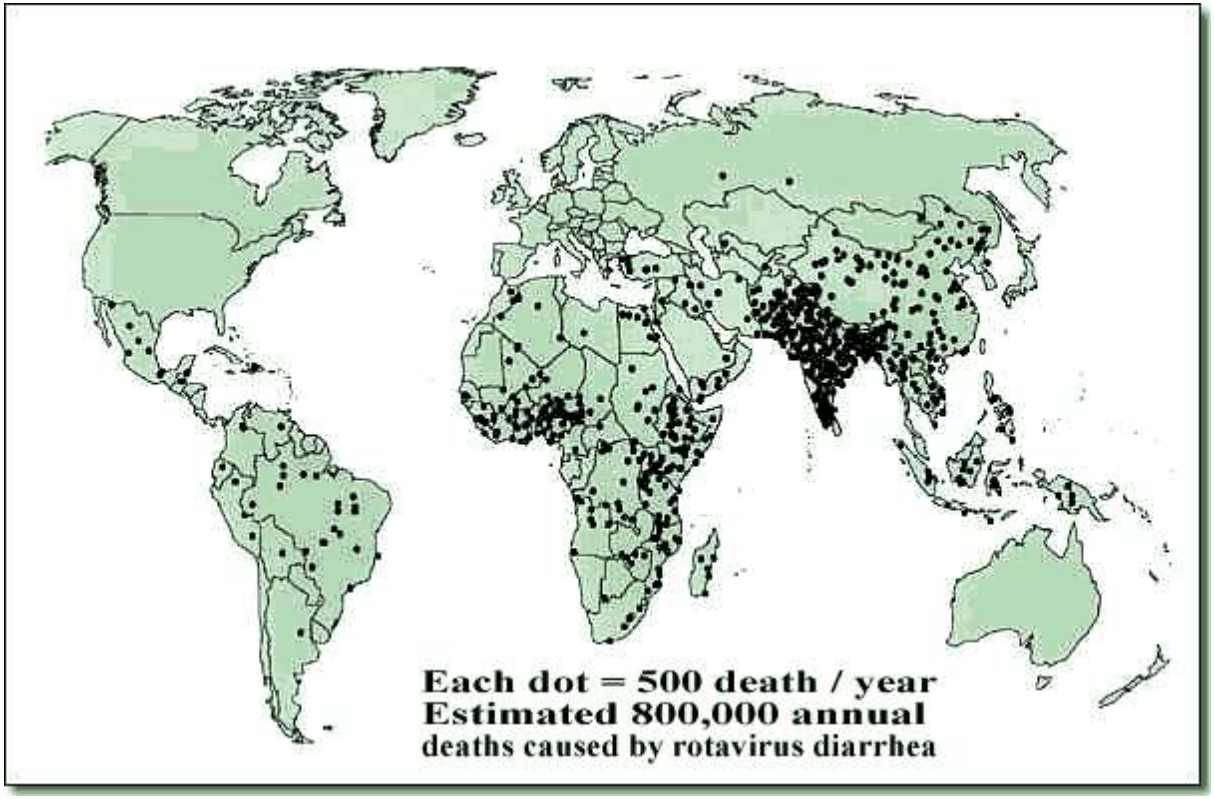
Epidemiology (cont.)



Rotavirus causes the highest level of diarrhea among developed and developing countries

(Picture Source: http://www.tulane.edu/~dmsander/WWW/335/Diarrhoea.html)

Epidemiology (cont.)



Estimated global distribution of the 800,000 annual deaths caused by rotavirus diarrhea.

(Picture Source: http://www.nlv.ch/Rotavirus/graphics/rotavirusdistribution.gif)

Diagnosis and Treatment

- Antigen Enzyme Immunoassay (EIA) of stool specimens
- RT-PCR; not commonly done
- Non-specific treatment: oral rehydration therapy to prevent dehydration
- Intravenous fluid is required in severe infant cases
- Immunization by vaccines

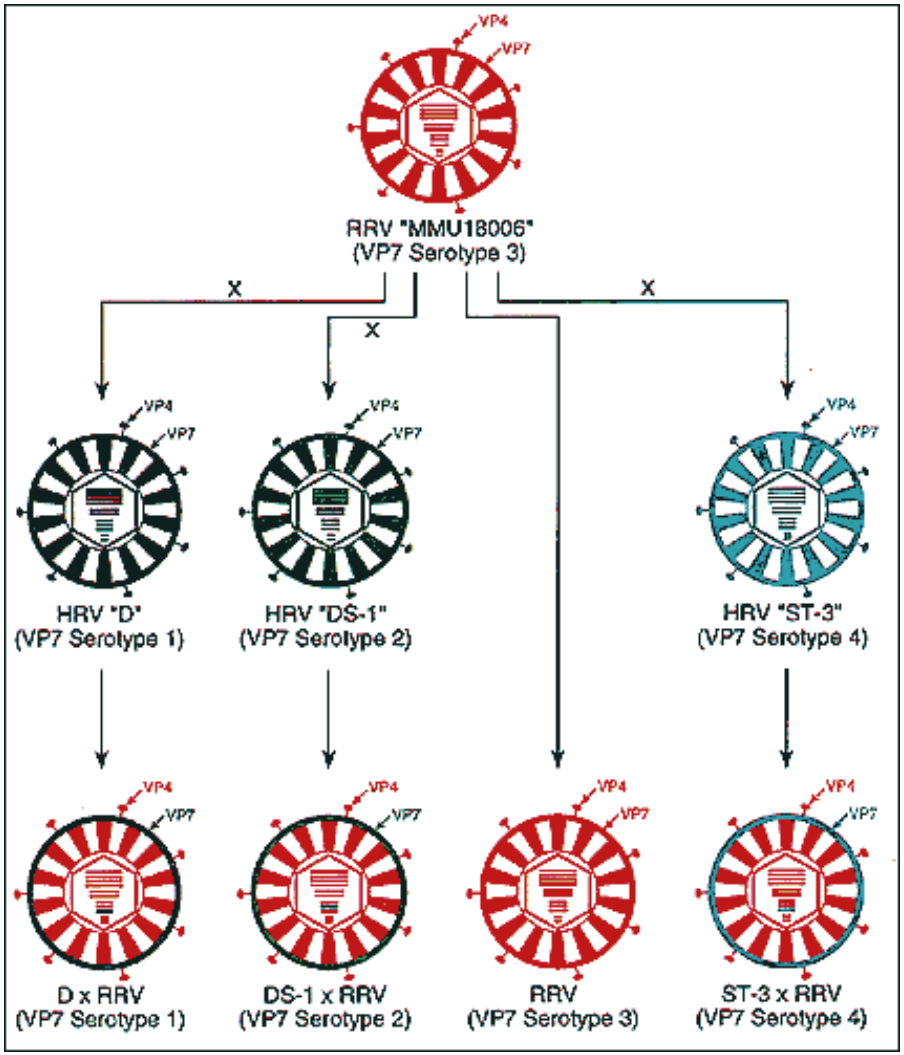
Rotavirus vaccine

- Monovalent Vaccines
  - Live attenuated vaccine derived from nonhuman host rotaviruses such as bovine and rhesus

- Reassortant Vaccines
  - animal-human reassortants expressing VP7 proteins used as immunogens such as rhesus-human reassortant or bovine-human reassortant vaccines

(Picture Source: www.ismp.org/Images/ rotavirus.gif)

Rotavirus vaccine (cont.)



- Production of reassortant tetravalent vaccine with VP7 serotype 1-4 specificity
- co-infection of RRV with HRV serotype 1,2, and 3
- safe and immunogenic

(Picture Source: http://www.cdc.gov/ncidod/EID/vol4no4/parasharG)

Prevention

CDC program

- Hand washing
- Proper sanitation
- Safe drinking water and food

"Boil it, cook it, peel it, or forget it"

Noroviruses (Overview)

- Belongs to Calicivirus Family
- Common cause of gastroenteritis in hospitals and geriatric institutions
- Outbreaks associated with travelers (hotels, cruise ships), schools, indoor and outdoor sport facilities, military camps, aircraft carriers
- Major cause of acute non-bacterial gastroenteritis
- 23 million cases in the US annually
- Difficult to control outbreaks in hospital and healthcare settings

Noroviruses (History)

- Caliciviruses first recognized in humans in 1972 by electron microscopy (fecal specimens from Norwalk, Ohio)
- AKA Norwalk viruses, Norwalk-like viruses (NLV), small round-structured viruses (SRSV), or Snow Mountain virus
- Calciviridae divided into four genera
  - -Vesivirus and Lagovirus (affecting animals)
  - -Norovirus and Sapovirus (affecting humans)
- Classification into genetic groups, genotypes, and variants based on genomic sequence analysis
- Nomenclature is drifting away from site of recognition to country of recognition, genus, genogroup, and year of recognition (i.e. US/Norovirus/I/1968)

Noroviruses (Structure and Genome)

- Non-enveloped, 27-30nm virions
- Single structural capsid protein (icosahedral symmetry with cup-shaped depressions)
- (+) sense, single stranded, poly A RNA of 7500 bp
- Genome is organized into 3 ORFs
- ORF1 (NS proteins)
- ORF2 (structural capsid protein)
- ORF3 (minor nucleocapsid protein)

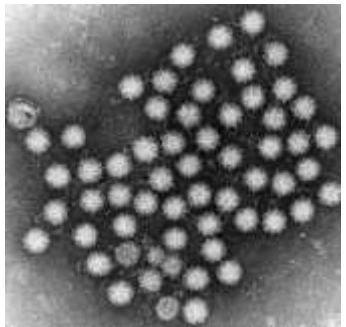
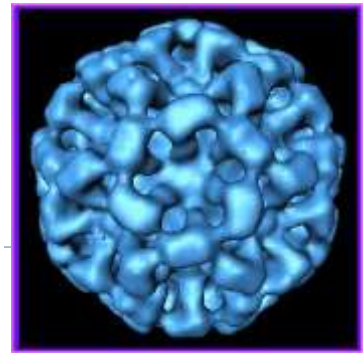


Fig. 1. Organization of the *Norovirus* genome. The arrow indicates the most frequently investigated part of the genome for revealing recombinant *Norovirus* strains.



Noroviruses (Genetic variability)

- Unknown genomic and antigenic diversity
- RNA recombination prominent
- Strain characterization based on polymerase region insufficient in determining host susceptibility
- Calciviruses in swine and cattle appear to be related to human strains (animal-to-human transmission?)

Noroviruses (Pathogenesis)

- Likely attach to H type 1 or 3 Ags on gastroduodenal epithelial cell surfaces
- ABO phenotype associated with risk of infection
- B histo-blood group showed less symptomatic disease and infection
- Conflicting observations?
- Perhaps strain variability plays a role
- Pigs show similar infection outcomes as with HBGAs
- SMV infection was not dependent on blood group secretor status

Noroviruses (Immunology & Clinical)

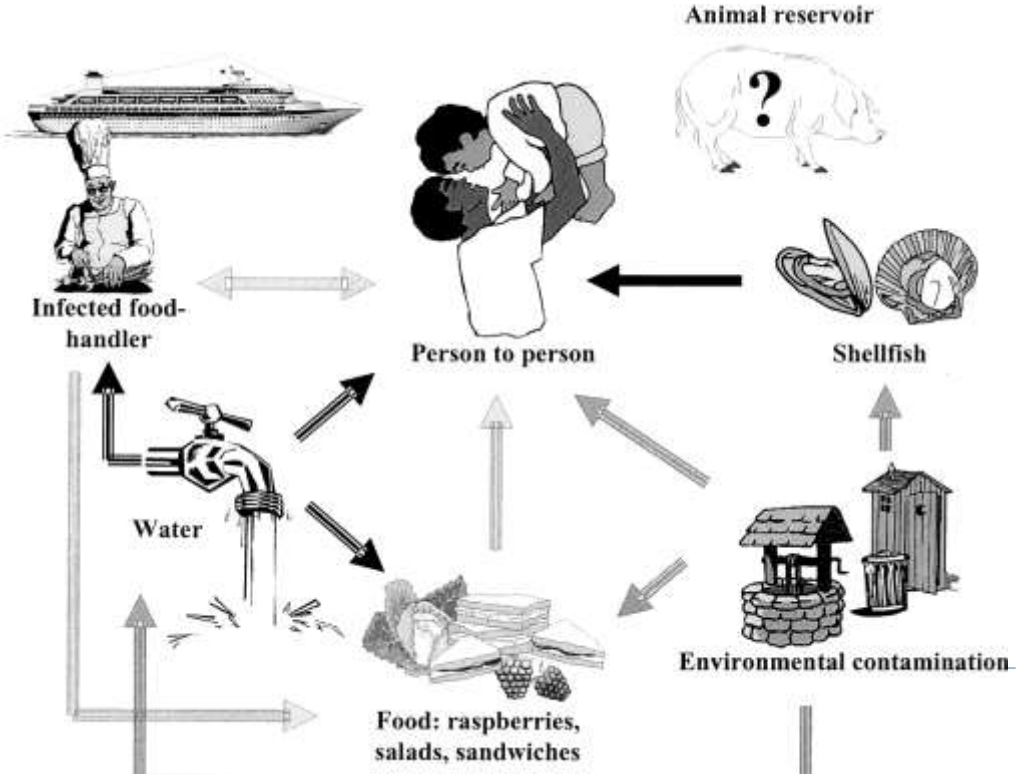
- Infection induces specific IgG and IgA Ab responses
- IgM response occurs even if previously exposed
- Long-term immunity not seen
- Gastroenteritis cannot be distinguised
- Symptoms occur 24-48 hrs post-infection and subside 12-72 with acute onset and rapid recovery
- Symptoms include: abdominal cramps, vomiting, malaise, headache, flu-like, chills, low-grade fever, dehydration
- Unlike rotaviruses, enteric adenoviruses or astroviruses, adults and school-aged children are the groups most affected

Noroviruses (Epidemiology)

- Physician awareness of human caliciviruses very limited
- Definitive diagnostic tests rarely conducted
- 20% of sporadic GE, 60% of outbreak GE, 90% vomiting and stool samples (negative for bacterial pathogens)
- Epidemic spread associated with contaminated food
- Appears during "winter vomiting" seasons
- Infectious dose is <100 virions
- Route of transmission can be contaminated food, fecal-oral, and vomiting
- Tap water contamination found during hospital outbreak

Noroviruses (Epidemiology)

- Common foods associated are shellfish (oysters), cold foods (salads, sandwiches, fruits)
- Virus can be shed and remain contagious for 7-10 days
- Max shedding occurs 24-72 hours post-exposure



Noroviruses (Epidemiology)

- Predominantly found in hospital outbreaks
- Patient care setting is ideal for transmission
- 3 factors contribute to impact of disease:
  - large human reservoir of infection
  - very low infective dose
  - ability to transmit by a variety of routes
- Diagnosis can be based on clinical and epi features
  - explosive onset with projectile vomiting (>50%)
  - illness in patients and staff
  - short incubation period and duration of illness

Noroviruses (Epidemiology)

- Route of transmission is person-to-person
- Airborne transmission due to vomiting and aerosols

Table 2. Year, duration, attack rates among patients and staff and the most probable source of infection of gastroenteritis in hospital outbreaks associated with noroviruses in different countries.

Country	(Year)/duration (days)	Attack rate (%)		Source/transmission	Type of hospital	Reference
		Patients	Staff			
USA	(1981)/11	55	61	?/person-to-person	Psychiatric chronic-care hospital	Gustafson *et al.* [59]
USA	(1988)/50	55	25	?/person-to-person; airborne	Geriatric convalescent facility	Gellert *et al.* [6]
UK	(?)/12	15[a]	11[a]	Sick food-handler/ sandwiches	Four long-stay rehabilitation hospitals	Lo *et al.* [38]
UK	(1994)/17	62	46	?/person-to-person	Hospital for mentally infirm	Green *et al.* [70]
USA	(1996)/9	11	31	Sick nurse; person-to-person	Regional referral hospital	Cáceres *et al.* [5]
Sweden	(1996)/1 year[b]	13[a]	21[a]	–/person-to-person;	10 hospitals (medical and geriatric wards)	Billgren *et al.* [2]
Germany	(1999)/24	44	23	?/person-to-person	Mother-and-child health clinic;	Oppermann *et al.* [8]
	(2000)/14	57	41			
New Zealand	(2000)/14	57	41	?/person-to-persons	geriatric rehabilitation hospitals	Lynn, *et al.* [1]
Switzerland	(2002)/16	56	18	?/person-to-person	Nursing home; (elderly residents participating in a pilgrimage)	Fretz *et al.* [50]

[a]Median.
[b]1-year-long follow-up. ? No data or unknown.

Noroviruses (Diagnosis)

- 3D Cell Culture (EID, April 2007)

"Human noroviruses cause severe, self-limiting gastroenteritis that typically lasts 24–48 hours. Because of the lack of suitable tissue culture or animal models, the true nature of norovirus pathogenesis remains unknown. We show for the first time that **noroviruses can infect and replicate in a physiologically relevant 3-dimensional (3-D), organoid model of human small intestinal epithelium**. This level of cellular differentiation was achieved by growing the cells on porous collagen-I coated microcarrier beads under conditions of physiological fluid shear in rotating wall vessel (RWV) bioreactors. This level of cellular differentiation was achieved by growing the cells on porous collagen-I coated microcarrier beads under conditions of physiological fluid shear in rotating wall vessel bioreactors. Microscopy, PCR, and fluorescent in situ hybridization provided evidence of norovirus infection. Cytopathic effect and norovirus RNA were detected at each of the 5 cell passages for genogroup I and II viruses. Our results demonstrate that the highly differentiated 3-D cell culture model can support the natural growth of human noroviruses, whereas previous attempts that used differentiated monolayer cultures failed."

Noroviruses (Diagnosis)

- 3D Cell Culture (EID, April 2007)

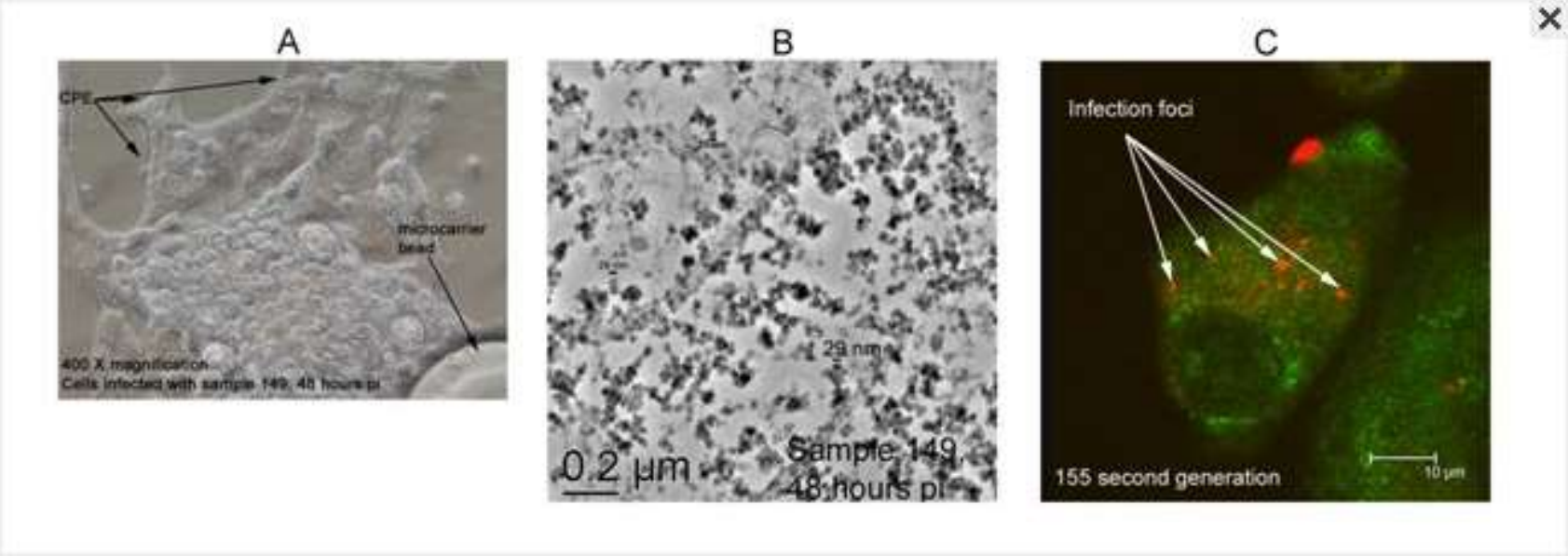


Figure 1. Human norovirus infection of 3-D small intestinal cells. A) Demonstration of host cellular cytopathic effect, B) transmission electron micrograph showing numerous particles that are the correct size for human noroviruses, C) fluorescent in situ hybridization demonstrating the presence of viral RNA. - Click on image to close

Tim Straub et. al

Noroviruses (Diagnosis)

- Factors influencing diagnostis

  - highest titer 2-3 days onset of symptoms

  - need to collect specimens during symptoms

  - need to examine specimens from multiple cases (6) to find potential outbreak pathogen

- EM and immunoelectron microscopy first used to visualize virus in 1972

- RT-PCR is more sensitive than EM but quasispecies indicate that single primer pairs may be insufficient

- Sequencing of amplicons are good for epi investigations

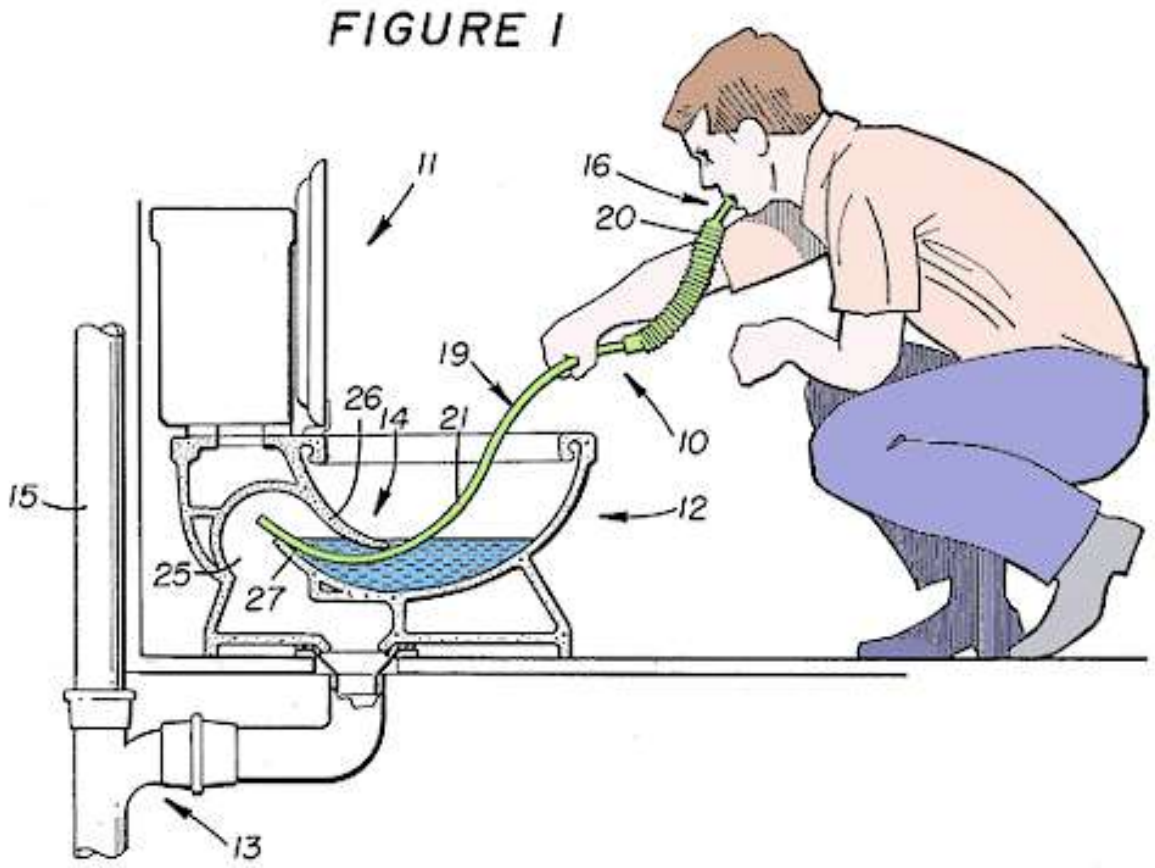
Noroviruses (Diagnosis)

- Recombinant capsid generated in baculovirus and E. coli
- EIA kits with monoclonal and polyclonal Abs are sensitive
- EIA platforms are relatively cheaper

Noroviruses (Prevention)

- Wash hands frequently
- Disinfect contaminated surfaces with bleach (promptly)
- Wash soiled clothing
- Avoid food and water from contaminated or suspect sources
- Cook shellfish completely
- Avoid contact with others if symptomatic especially in school, healthcare, and food-handling settings

Noroviruses???



Toilet Snorkel (Patent #4320756), formulated to provide a fresh air source during fires in high rise buildings. Patented in 1982. Norovirus transmission via fecal-oral route?

Norovirus Recombinant Vaccine

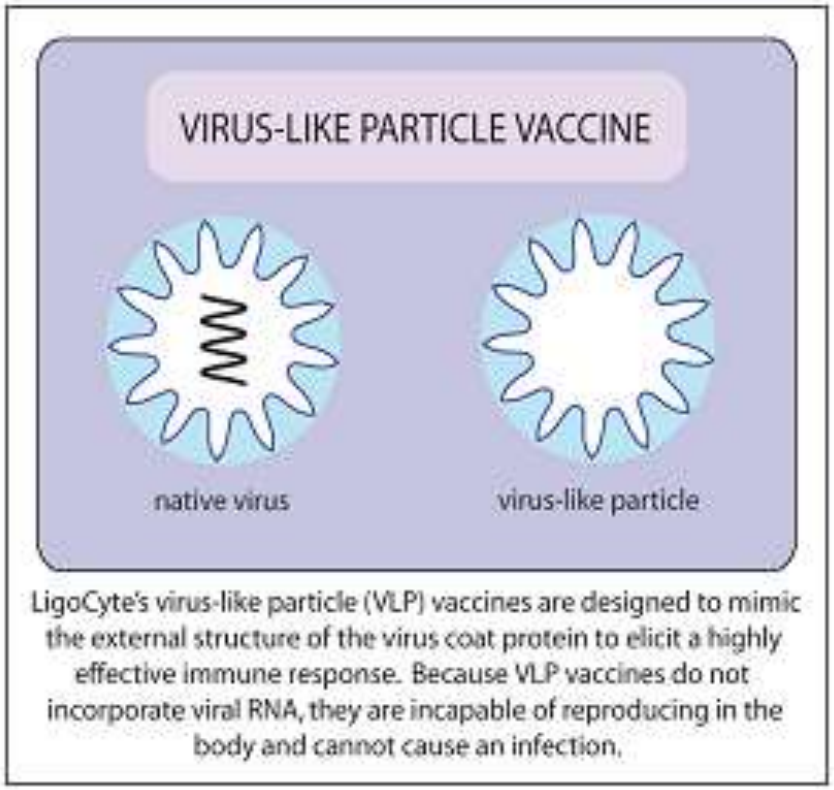
- Ligocyte Pharmaceuticals is developing a mucosal vaccine and on April 4th 2007 initiated Phase I Clinical Trials
- The Norovirus vaccine is a needle-free, dry powder formulation based upon virus like particles (VLPs), which are highly purified protein products.
- VLPs mimic the functional interactions of the live virus by preserving the authentic conformation of the viral capsid, while lacking the ability to reproduce or cause illness
- The adjuvant Monophosphoryl Lipid A is also included to enhance nasal delivery.
- An excipient (a carrier) is included to improve nasal delivery

Norovirus Recombinant Vaccine

- The vaccine induces an immune response at the gastrointestinal epithelium
- Designed to protect against multiple serovars
- Target groups are pediatric and geriatric populations, Navy personnel and cruise ship passengers

Norovirus Recombinant Vaccine

- The VPLs retain the external structure of the virus while the viral RNA is not included in the vaccine



LigoCyte's virus-like particle (VLP) vaccines are designed to mimic the external structure of the virus coat protein to elicit a highly effective immune response. Because VLP vaccines do not incorporate viral RNA, they are incapable of reproducing in the body and cannot cause an infection.

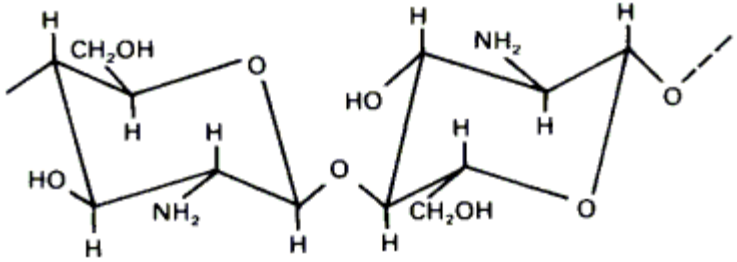
Norovirus Recombinant Vaccine

- TLR 4 is a toll-like receptor which detects lipopolysaccharides on gram-negative bacteria, leading to the activation of the innate immune system

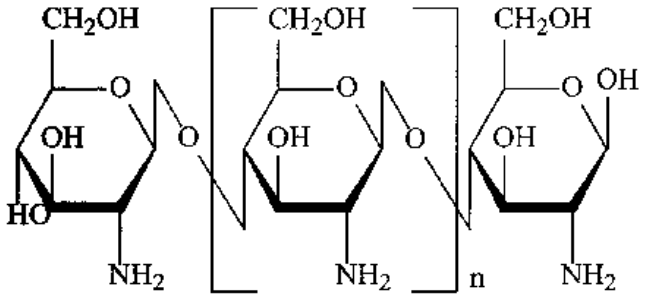
- The adjuvant MPL which is included in the vaccine is a TLR-4 agonist, and it rapidly initiates an innate immune response, providing protection from viral challenge while at the same time enhancing specific responses to the VLP

Norovirus Recombinant Vaccine

- The bioadhesive chitosan (partly deacetylated chitin) which is a positively charged polysaccharide increases uptake of the vaccine components by increasing the contact time of the vaccine with the nasal membrane



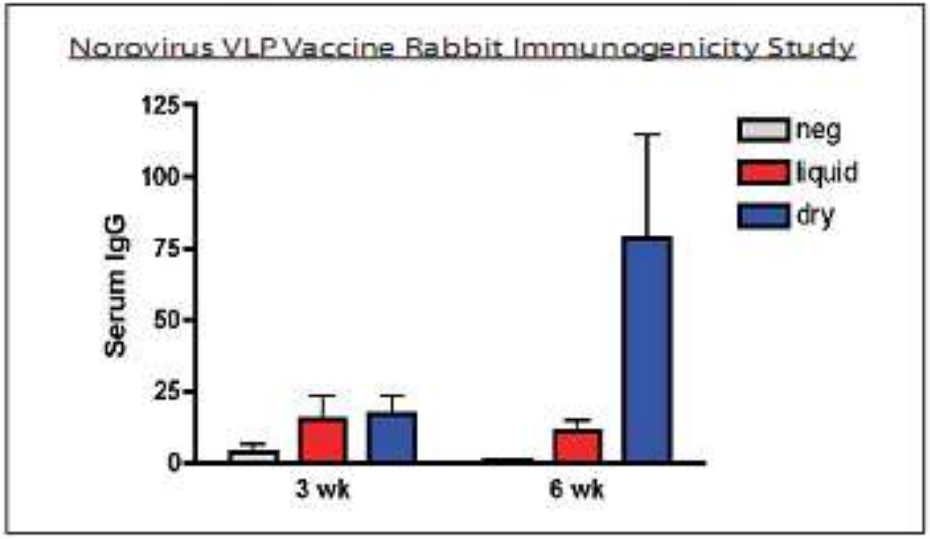
The structure of chitosan



Chitosan

Norovirus Recombinant Vaccine

- The dry vaccine proved to be more efficiet than a liquid vaccine with the same components



PRECLINICAL DATA

In an immunogenicity study, rabbits were challenged with either LigoCyte's norovirus dry powder vaccine candidate or a liquid version containing the same components. At three weeks, anti-VLP IgG serum levels were similar. At six weeks, the dry powder formulation yielded a considerable increase in the immunological response. Significantly, all dry-powder test subjects showed an increase in anti-VLP IgG from the three to six week time point.

Norovirus Recombinant Vaccine

- After Phase I safety and immunogenicity clinical trials are complete, LigoCyte will test the vaccine's efficacy against live virus challenge