

Viral Infections of GIT

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Required

VIRAL AGENTS CAUSING GASTROENTERITIS

- Pathogens discussed in our lectures
 1. Rotavirus
 2. Enteric adenoviruses
 3. Caliciviruses
 4. Astroviruses
 5. Toroviruses

Viruses as a causative organism of diarrheal disease

Detection of a specific virus in the stool of symptomatic patients is not sufficient to establish the role of the virus in causing disease. These criteria need to be fulfilled:

1. Virus is detected in ill patients significantly more than asymptomatic controls and virus shedding correlate with symptoms
2. Significant humoral or secretory antibody response or both in patients shedding the disease.
3. Reproduce the disease by experimental inoculation of nonimmune human or animal hosts
4. Exclude other causes of diarrhea such as bacteria, bacterial toxins and protozoa.

Feature	Rotavirus	Calicivirus	Astrovirus	Adenovirus	Torovirus
Nucleic acid	DS RNA	SS RNA	SS RNA	DS DNA	SS RNA
Shape	Naked, Double shelled capsid	Naked, round	Naked, star shaped	Naked, icosahedral	Enveloped, donut shaped
Replication in CC	Usually incomplete	None	None	None or incomplete	None
Serotypes	5	>4	8	unknown	unknown
Site of infection	Duodenum, jejunum	Jejunum	Small intestine	Small intestine	Small intestine
Immunity	Local IgA	unknown	unknown	unknown	unknown
Seasonality	winter	Not known	Not known	Not known	Not known
Ages primarily affected	Infants, < 2 yrs	Older children, adults	Infants, children	Infants, children	Infants, children
Transmission	Fecal-oral	Fecal-oral	Fecal-oral	Fecal-oral	Fecal-oral
IP (days)	1-3	0.5-2	1-2	8-10	----- -
Dx	EIA, EM	IEM, PCR	EM, PCR	EIA, EM	EM, ELISA

ROTAVIRUS

- Family *Reoviridae*
- Genus *Rotavirus*
- Other genera *Orthreovirus, Coltivirus, orbivirus*
(*sheep*)

Note: Only immunity, seasonality, ages and transmission are required from last slide

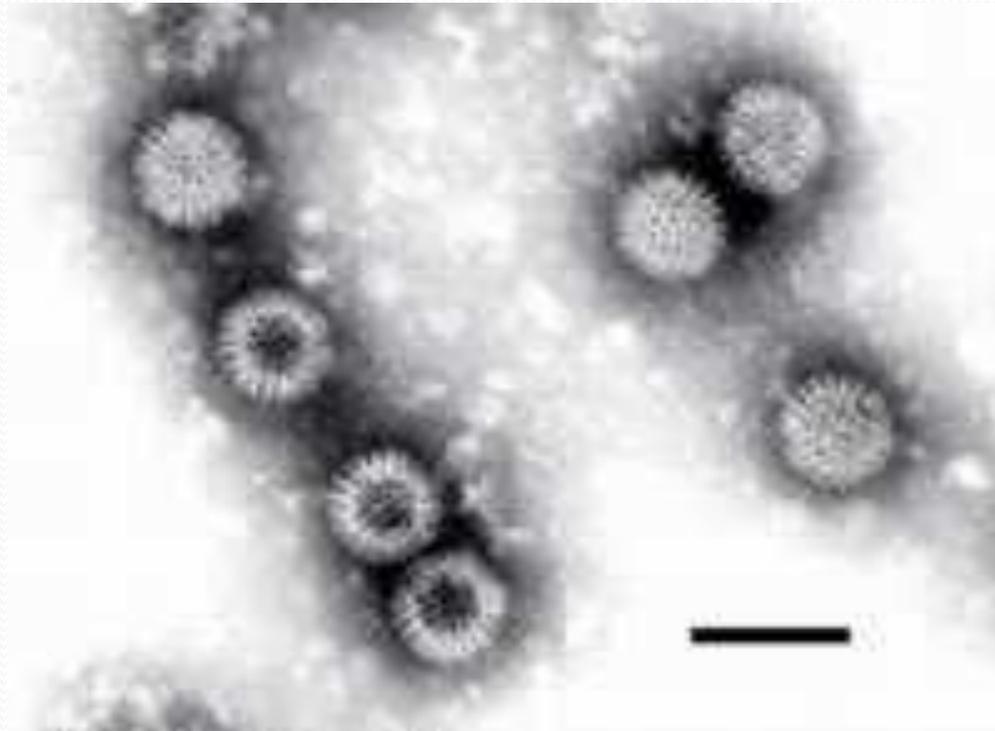
ROTAVIRUS- discovery

- First isolated in 1973 in Australia by Ruth Bishop at the Royal Children's Hospital in Melbourne.
- EM identification from duodenal biopsies from children with diarrhea.
- "Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis," *Lancet*, 1:1281-3, 1973.
- Described in stool samples from children by Albert Z. Kapikian, in the US
- Human and animal strains are recognized



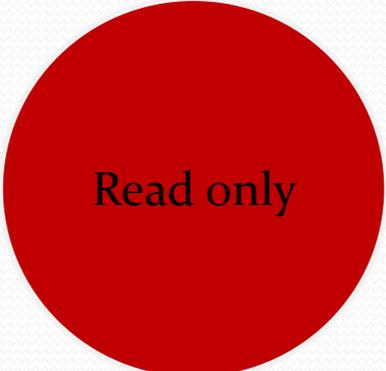
Important

ROTAVIRUS EM STRUCTURE



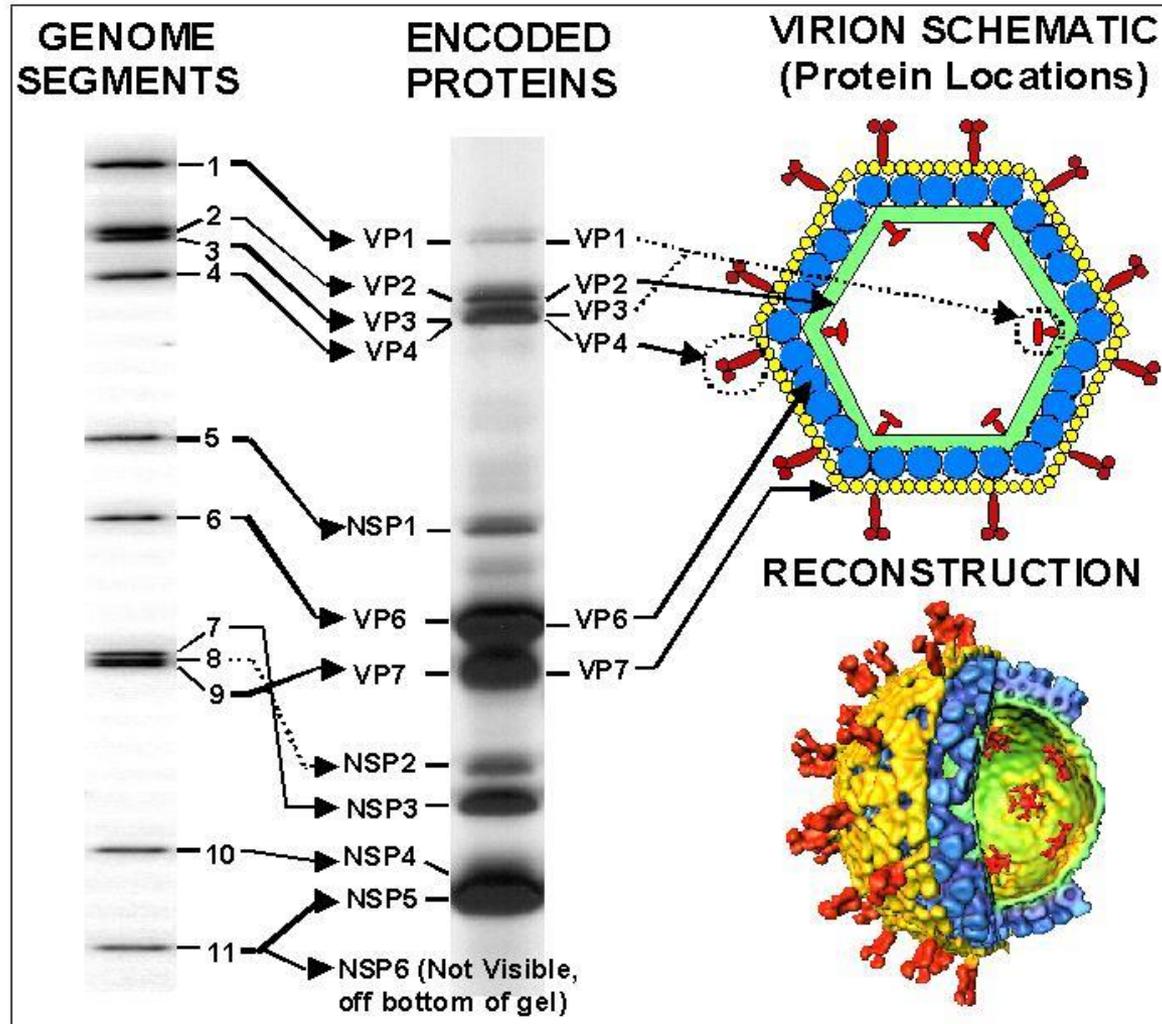
STRUCTURAL FEATURES OF ROTAVIRUS

- 65-75nm in size
- Non-enveloped virus (naked)
- EM appearance of a wheel with radiating spikes
- Icosahedral symmetry
- Double capsid (outer and inner capsid)
- Double stranded (*ds*) RNA in 11 segments
- Core with genome
- Capsid is cleaved by trypsin to form ***ISVP***
[intermediate/infective sub-viral particle]



Read only

Rotavirus structure



VIRAL STRUCTURAL PROTEINS (VP)

- Outer structural proteins - VP7 and VP4
 - VP7** - Glycoprotein
 - VP4** - protease-cleaved, P protein, viral hemagglutinin; forms spikes from the surface
- Inner core structural proteins VP 1, 2, 3, 6
- VP6 is an important antigenic determinant

Required

CLASSIFICATION- Groups

- 7 Groups (A through G) and 2 subgroups (I and II) based on VP6 differences
- Group A is the most common
- Group B (outbreaks in China)
- Group C (worldwide)

CLASSIFICATION - Serotypes

- Serotypes based on viral capsid proteins inducing neutralizing Ab
- 14 **G** serotypes based on G protein (VP 7) differences
 - 5 predominant strains in U.S. (G1-G4, G9) account for 90% of isolates
 - Strain G1 accounts for 73% of infections
- 20 **P** serotypes based on P protein (VP4) with P4/P8 predominance
- Common **PG** combinations are: P8G1, P8G2, P4G2, P8G4

ROTAVIRUS - Properties

- Virus is stable in the environment (months)
- Relatively resistant to handwashing agents
- Susceptible to disinfection with 95% ethanol, Lysol, formalin

PATHOGENESIS

- Targeted host cells - mature enterocytes lining the tips of intestinal villi
- Intermediate/infective sub-viral particle (ISVP) produced through proteolysis
- Enter host cell by endocytosis
- Virus replicates in the host cell cytoplasm

REPLICATION

- mRNA transcription with viral RNA polymerase
- Capsid proteins formed, assembled into immature capsid
- RNA replicated to form double stranded RNA genome

HISTOPATHOLOGY

- Mature enterocytes lining the tips of intestinal villi are affected
- Villous atrophy and blunting
- Death of the mature enterocytes
- Infiltration of lamina propria with mononuclear cells
- Repopulation of the villous tips with immature secretory cells [*crypt hyperplasia*]

HISTOPATHOLOGY



EPIDEMIOLOGY

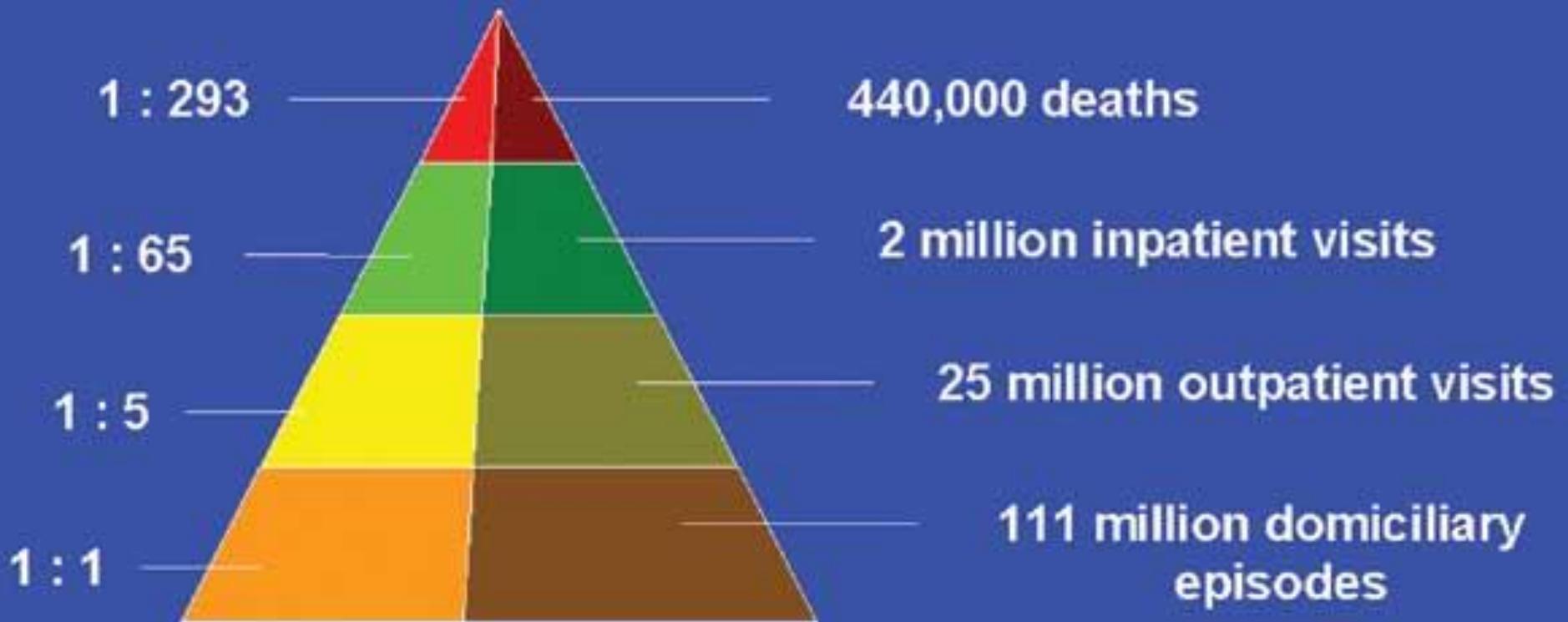
- A major cause of diarrhea-associated hospitalizations and deaths
- Sero-prevalence studies show that antibody is present in most (90%) by age 4 years

ROTAVIRAL DISEASE BURDEN

Worldwide

Risk

Events



EPIDEMIOLOGY

- ▶ Age- children 4 months - 2 years are most affected
Protection of younger infants through transplacental antibody transfer
- ▶ Asymptomatic infections are common, especially in adults
- ▶ Nosocomial infections
- ▶ Outbreaks
- ▶ Severe Disease young, immunocompromised
- ▶ Seasonality Winter months
- ▶ Incubation period - thought to be <4 days

Required

TRANSMISSION

- Mainly person to person via fecal-oral route
- Food and water-borne spread is possible
- Fomites
- Spread via respiratory route is speculated

Important

EPIDEMIOLOGY - spread

- Contagious from before onset of diarrhea to a few days after end of diarrhea
- Large amounts of viral particles are shed in diarrheal stools
- Infective dose 10-100 pfu

Rotavirus Immunity

- Type specific humoral antibody (VP7 and VP4) are partially protective (they last for years)
- Type specific secretory (IgA) antibodies are produced in the GIT
- First infection usually does not lead to permanent immunity
- Reinfection can occur at any age
- Subsequent infections generally less severe
- Breast feeding protect against rotavirus disease:
 - Colostrum and breast milk IgA antibodies
 - Breast milk mucin glycoproteins: bind rotavirus and inhibit their replication

CLINICAL FEATURES

- ▶ Incubation period - thought to be <4 days
- ▶ Fever- can be high grade (>39°C in 30%)
- ▶ Vomiting (1-3 days), nausea precede diarrhea
- ▶ Diarrhea
 - usually watery (no blood or leukocytes)
 - lasts 3-9 days
 - longer in malnourished and immune deficient individuals.
 - Necrotizing enterocolitis (NEC) and hemorrhagic GE seen in neonates
- ▶ *Dehydration* is the main contributor to mortality
- ▶ *Secondary malabsorption* of lactose and fat, and chronic diarrhea are possible



Important



Important

MECHANISM OF DIARRHEA

- Rotavirus localizes to duodenum and proximal jejunum leading to:
 - destruction of villous epithelial cells
 - blunting of the villi
 - Infiltration of the villi with inflammatory cells
- Watery diarrhea due to net secretion of intestinal fluid and loss of absorptive surface (recover in 3-8 weeks)
- Activation of the enteric nervous system
- Role of nonstructural glycoprotein (NSP4) peptide regions as an enterotoxin

DIAGNOSIS

- Antigen detection in stool
ELISA
- EM- non-Group A viruses also
- Culture- Group A rotaviruses can be cultured in monkey kidney cells
- Serology for epidemiologic studies

TREATMENT and PREVENTION

- ▶ **Treatment**

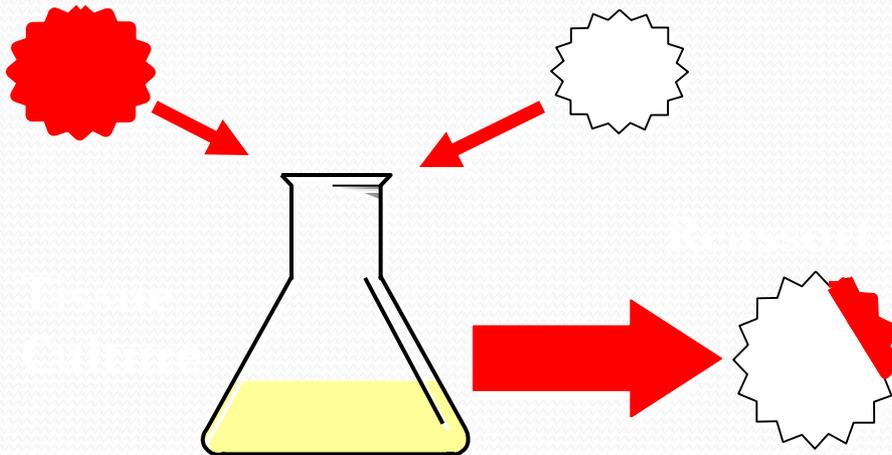
Supportive - oral, IV rehydration

- ▶ **Prevention**

Hand hygiene and disinfection of surfaces

- ▶ **Vaccine**

Rotavirus Vaccine (Rota)

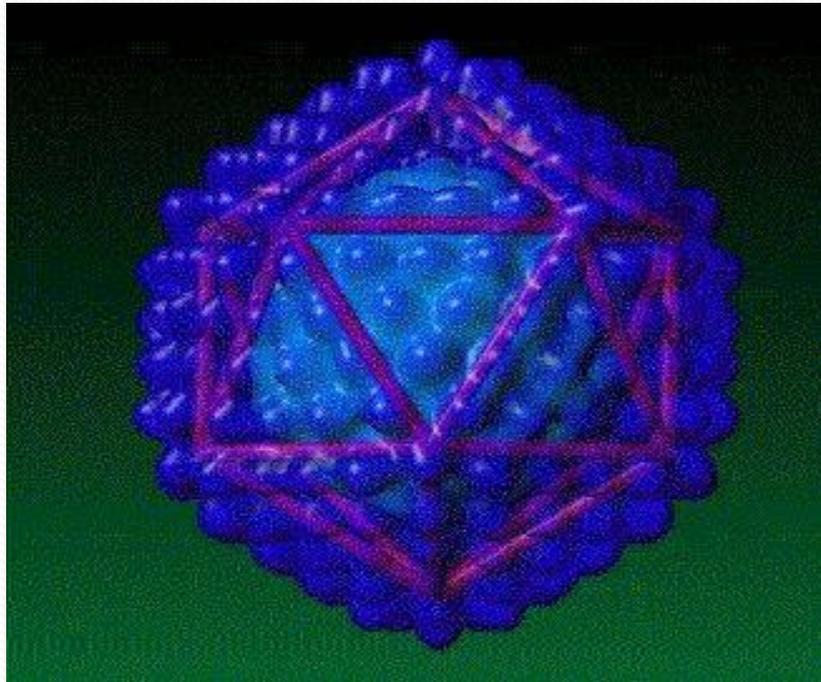


- Created by genetic reassortment
 - Causes nonhuman rotavirus strains to express human rotavirus antigens on their surface
 - Nonhuman rotaviruses have low pathogenicity for humans
 - Replicate but do not cause disease

RotaTeq[®] (Merck)

- Live oral vaccine licensed 2006 in US
- Contains 5 reassortants (WC3 bovine strain with viral surface proteins of human serotypes G1-4 and P1A)
- Contains no preservatives or thimerosal
- 3-dose schedule – age 2,4,6 month
- Minimum age of first doses is 6 weeks
- First dose should be administered between 6 and 12 weeks of age (until age 13 weeks)
- Do not initiate series after 12 weeks of age

GASTROENTERITIS DUE TO ENTERIC ADENOVIRUS

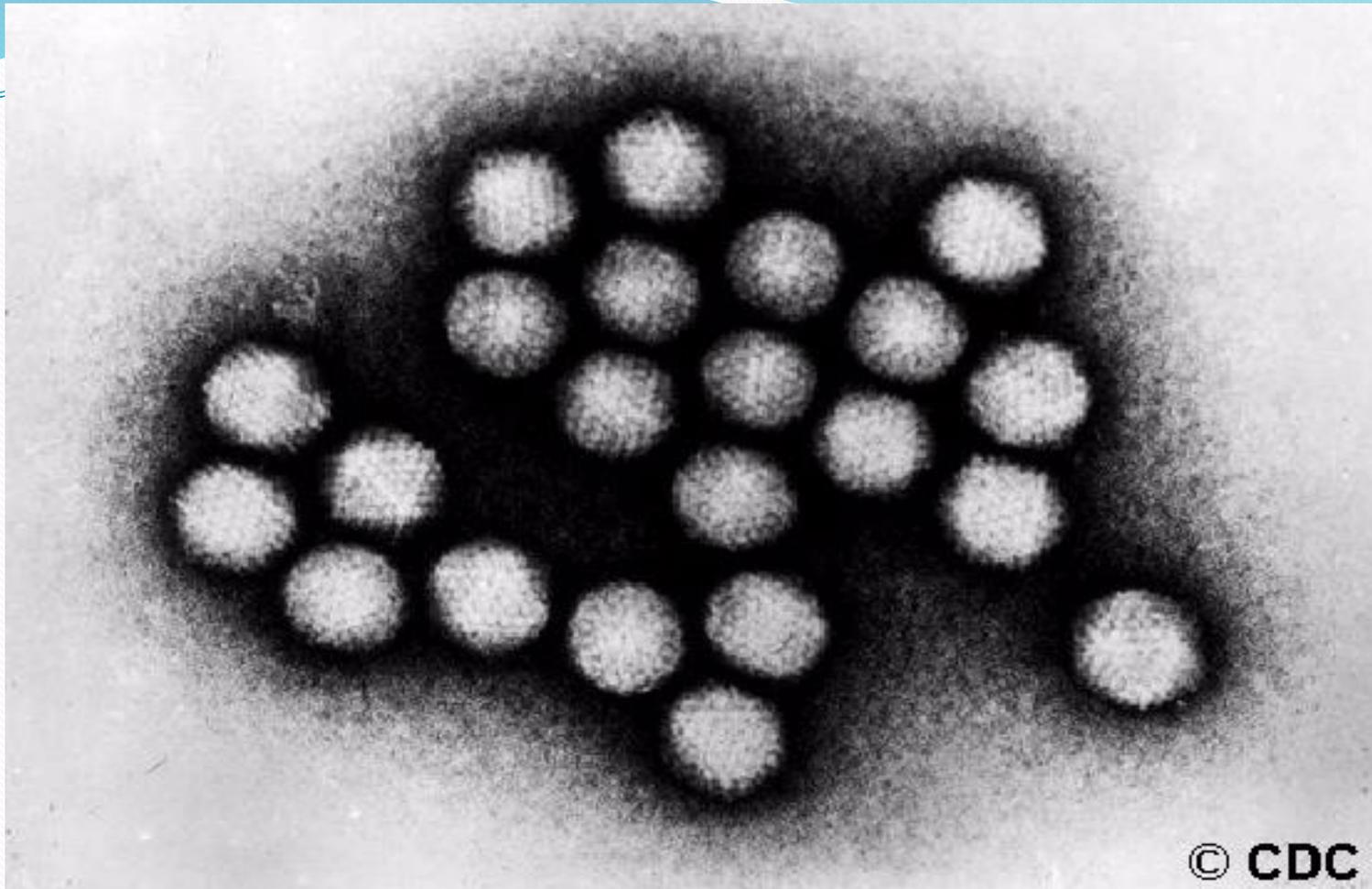


Adenovirus Gastroenteritis

- Isolated in 1953 by Rowe from adenoidal tissue
- Virion:
 - Icosahedral, non-enveloped, 70-90 nm in diameter, 252 capsomeres; fibers project from each other
 - Composition: DNA (13%), protein (87%)
 - Genome: Double-stranded DNA, linear, 26-45 kbp, protein-bound to termini, infectious
 - Proteins: Important antigens (hexon, penton base, fiber) are associated with the major outer capsid proteins
 - Replication: Nucleus
 - Virus classification: Group I: ds DNA; Family: Adenoviridae; Genus: Mastadenovirus; Species: Human adenovirus (H Ad)
- At least 51 serotypes are known
- classified into 6 subgenera: A to F

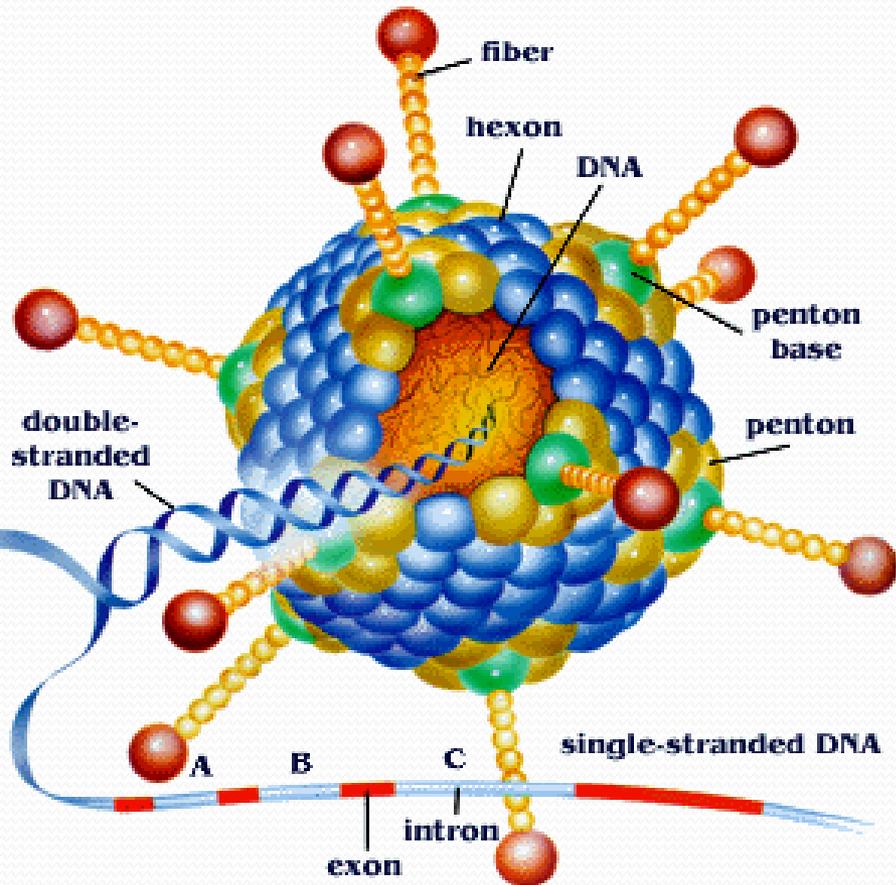


Only this is required



- Known oncogenic potential of some serotypes
- Commonly used as vectors in gene therapy and vaccine delivery

Adenoviral structure



- Outstanding characteristics
 - Excellent **models for molecular & biochemical studies** of eukaryotic cell processes; a few models serve as models for cancer induction in animals
 - Largest viruses (maximum size able to be transported through the endosome)
 - virion has unique "spike" or fiber associated with each penton base of the capsid that aids in attachment to the host cell via the coxsackie-adenovirus receptor on the surface of the host cell
 - Adenovirus has tropism for **cells of epithelial origin**
 - Replicative cycle is sharply divided into EARLY & LATE events

Adenovirus pathogenesis

- Virus replicate in epithelial cells producing cell necrosis and inflammation
- After acute infection; the virus may remain in tissues (tonsils, adenoids, peyer's patches) and become reactivated and shed asymptotomatically 6-18 months.
- Reactivation enhanced by stressful events
- Integration of adenoviral DNA into host cell genome may occur leading to latency in tonsils and peripheral lymphocytes
- Some times smudgy intranuclear inclusions may be seen

Required

Adenovirus infection leads to the following:

- Pentons have toxic effects on host cells
- Encode a protein in the E₃ genomic region that binds class I MHC antigens in the ER which restricts their expression on the surface of infected cells and interfere with recognition and targeting by cytotoxic T cells. (Helps in latency)
- E₁A protein has been associated with increased susceptibility of infected cells to destruction by TNF and other cytokines.
- Adenovirus death protein; important for efficient lysis of infected cells and release of progeny virions.

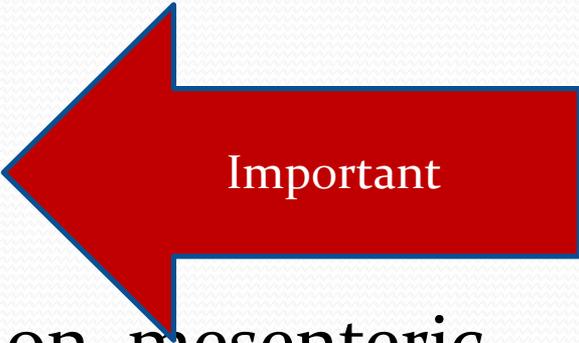
Diarrhea due to Enteric Adenovirus

- Cause 5-15% of all viral gastroenteritis
- Belong to serogroup F (Types 40, 41) 38??
- Age <4 years
- Year round
- Spread via fecal-oral route
- Isolation requires special media-Graham 29
- ELISA for rapid detection is available

CLINICAL FEATURES -

Adenovirus gastroenteritis

- Incubation period 3 -10 days
- Diarrhea lasts for 10 -14 days
- Can also cause intussusception, mesenteric adenitis, appendicitis
- Treatment with cidofovir promising for severe disease in immunocompromised

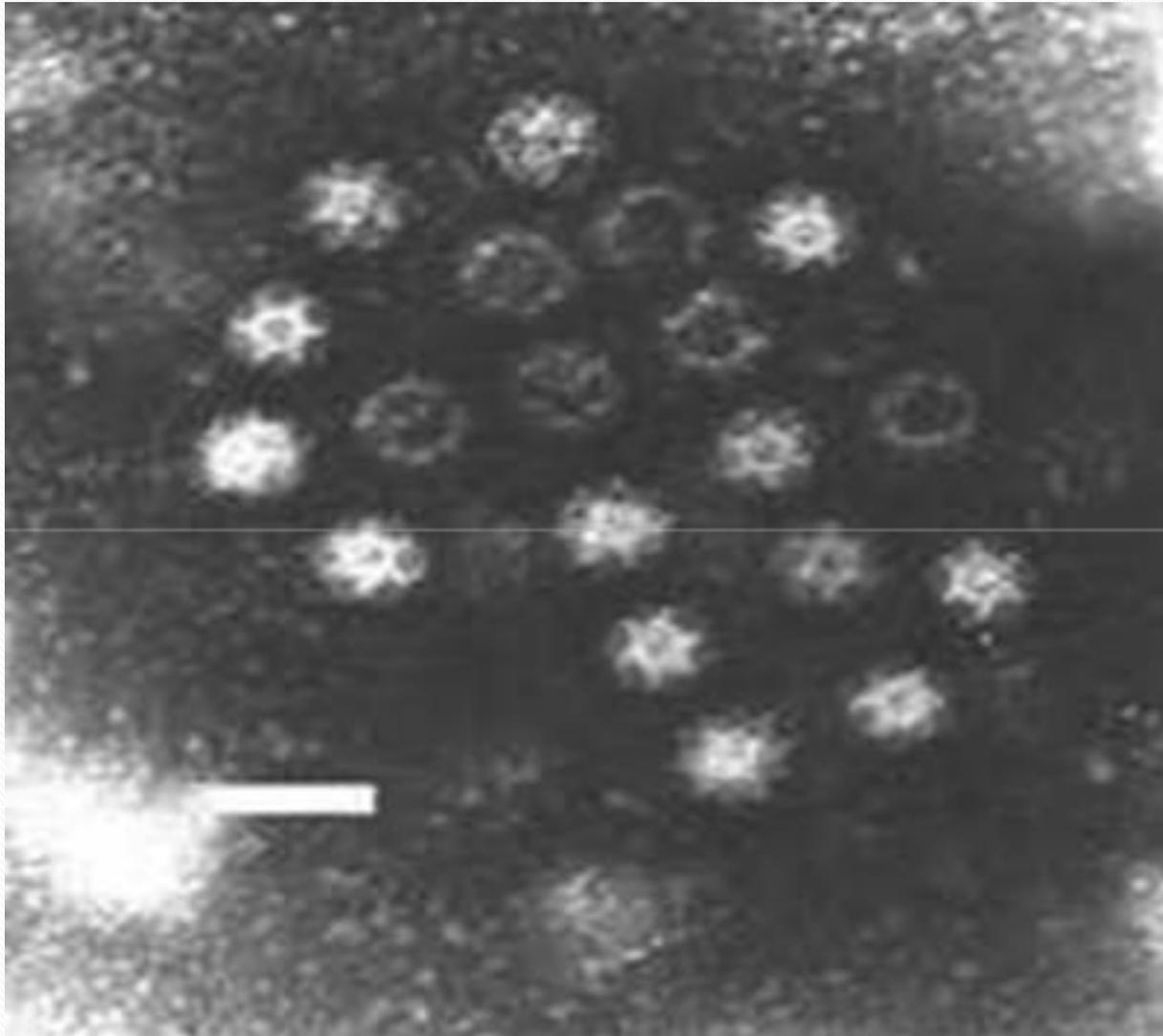


Important

HUMAN CALICIVIRUSES (HuCV)

- Family *Caliciviridae*
- Non-enveloped RNA viruses with ss [+] sense RNA
- 27-35 nm in size
- Icosahedral capsid
- Contains single capsid protein
- Resistant to acid, ether and heat
- Have not been effectively propagated in cell culture

HUMAN CALICIVIRUSES



Calicivirus

- Norwalk virus and “Norwalk-like”
- “Sapporo-like” viruses
- Vesivirus
- Lagovirus



Only this is required

NLV (Norovirus) round shaped

Norwalk virus

Hawaii virus

Snow Mountain virus

Montgomery county virus

Taunton (England)

SLV (Sapovirus) star shaped

Sapporo virus

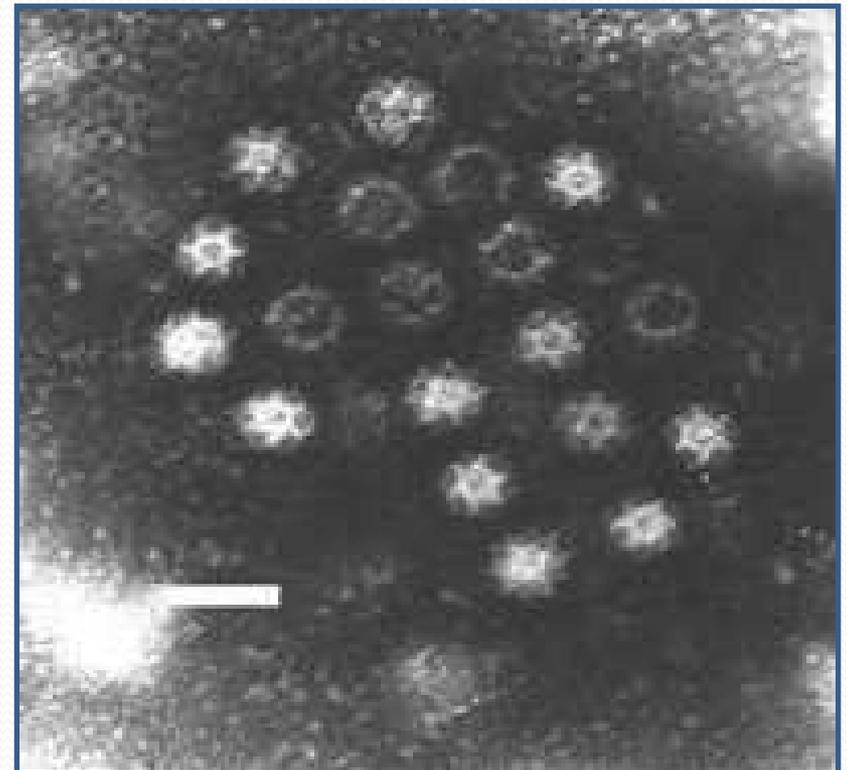
Manchester virus

Houston/86

London/92

MORPHOLOGY- typical

- 32 cup-like depressions
- EM appearance of “Star”
- 31-35 nm size
- E.g.- Sapporo-like viruses



Morphology of HuCV- atypical

- Smaller size- approx. 27 nm
- Rough, feathery surface but no internal pattern
- Small Round Structured viruses, e.g.-
Norwalk-like viruses

Norwalk virus

- “winter vomiting disease” 1968, Norwalk OH
(sometimes known as winter vomiting bug in the UK)
- Causes 40% of nonbacterial epidemics
 - 45% foodborne, 52% shell fish associated outbreaks
- Explosive epidemics
 - camps, cruise ships, nursing homes
- Food borne illness
 - raw shellfish

EPIDEMIOLOGY - Noroviruses

- Worldwide distribution
- Affects all ages
- >23 million cases/year in the U.S.
- Major cause of food-borne outbreaks of GE (>50%)
- Prevalence of antibodies reach ~50% by the fifth decade.
- Asymptomatic infections- seroconversion but asymptomatic shedding of virus
- Low infective dose (~10 pfu)
- Viral shedding 3-4 days
- Mucosal changes revert in ~2 weeks.
- Protective immunity short-lived
- NLV cross protection?

Norwalk virus: Clinical Features

- 24 hour (range 10-50hr) incubation period
- Vomiting prominent
- Headache, myalgia, fever
- Diarrhea 1-3 days, less severe than rotavirus
- Treatment symptomatic
 - rehydration, antidiarrheals
- Complications rare
 - immunocompromised

Required

SPREAD

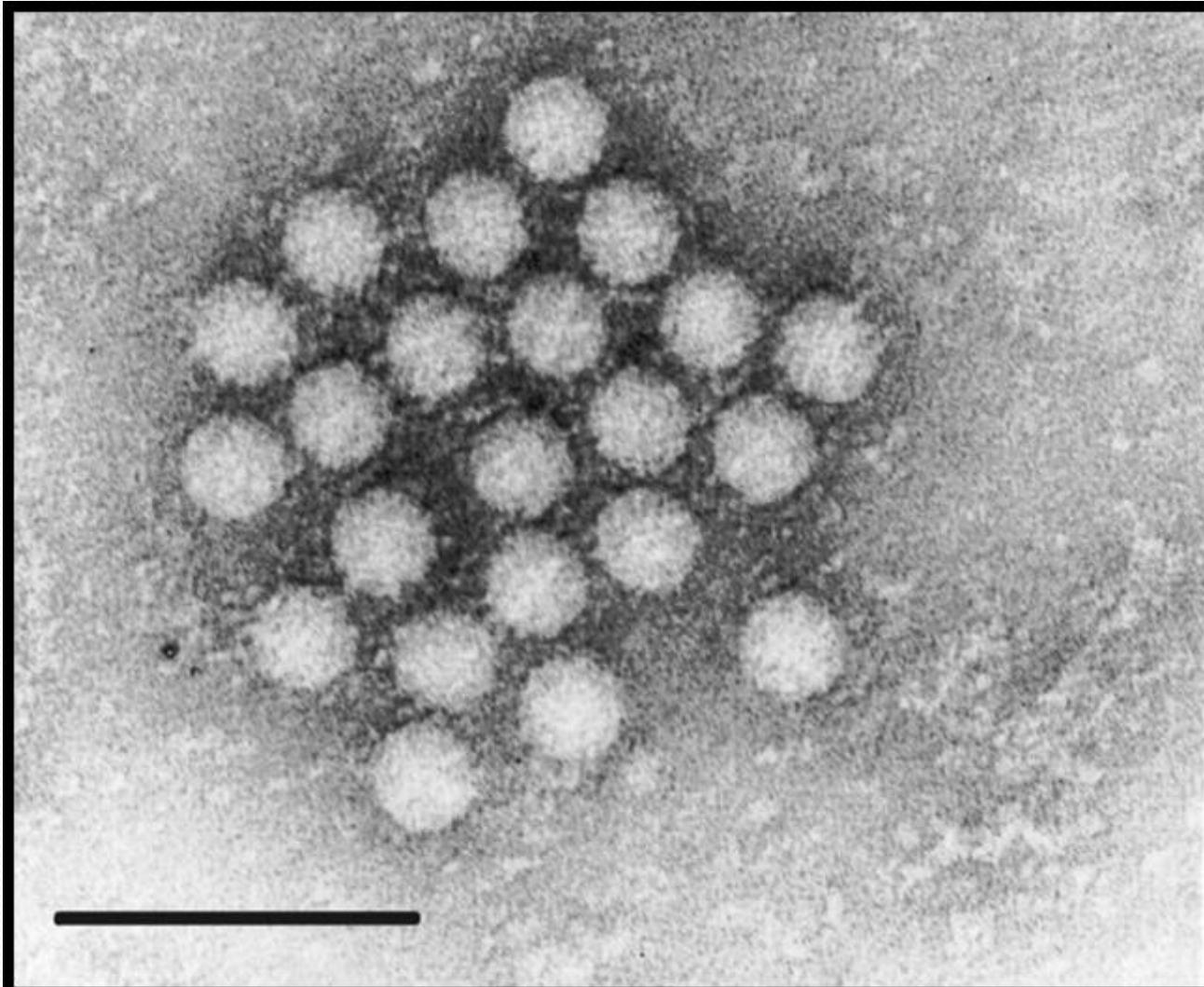
- Person-to-person fecal-oral spread (stool/vomitus)
- Fecal contamination of food or water (uncooked shellfish)
- Fomites (stool/vomitus)
- Ingestion of aerosolized particles
- Survive on surfaces for several days
- Survive in water chlorinated at routine levels (up to 10 ppm)
- Survive freezing, heating up to 60°C (30min)
- Evidently survive in steamed shellfish

Required

DIAGNOSIS

- Specimen- stool, vomitus, food, environmental swabs (during outbreak investigations)
- RT-PCR in state public health labs.
- Serology for epidemiologic purposes
- Immune EM is less used

HUMAN ASTROVIRUS



Required

ASTROVIRUS

- Described in relation to an outbreak of gastroenteritis in 1975
- Detected by EM
- Immunologically distinct from Hu CV
- Animal strains are known

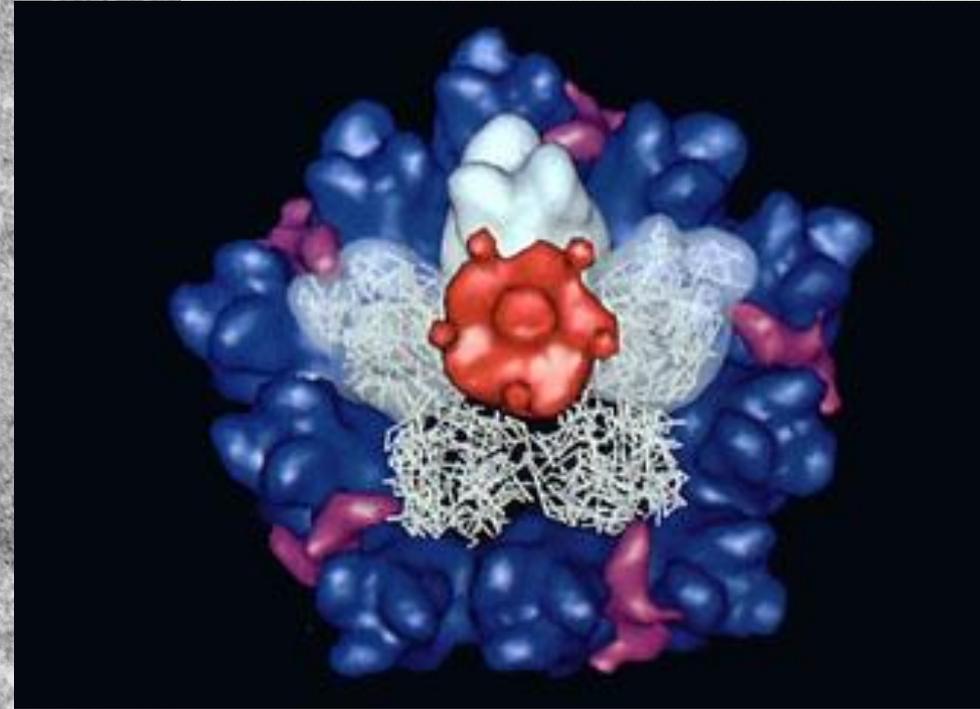
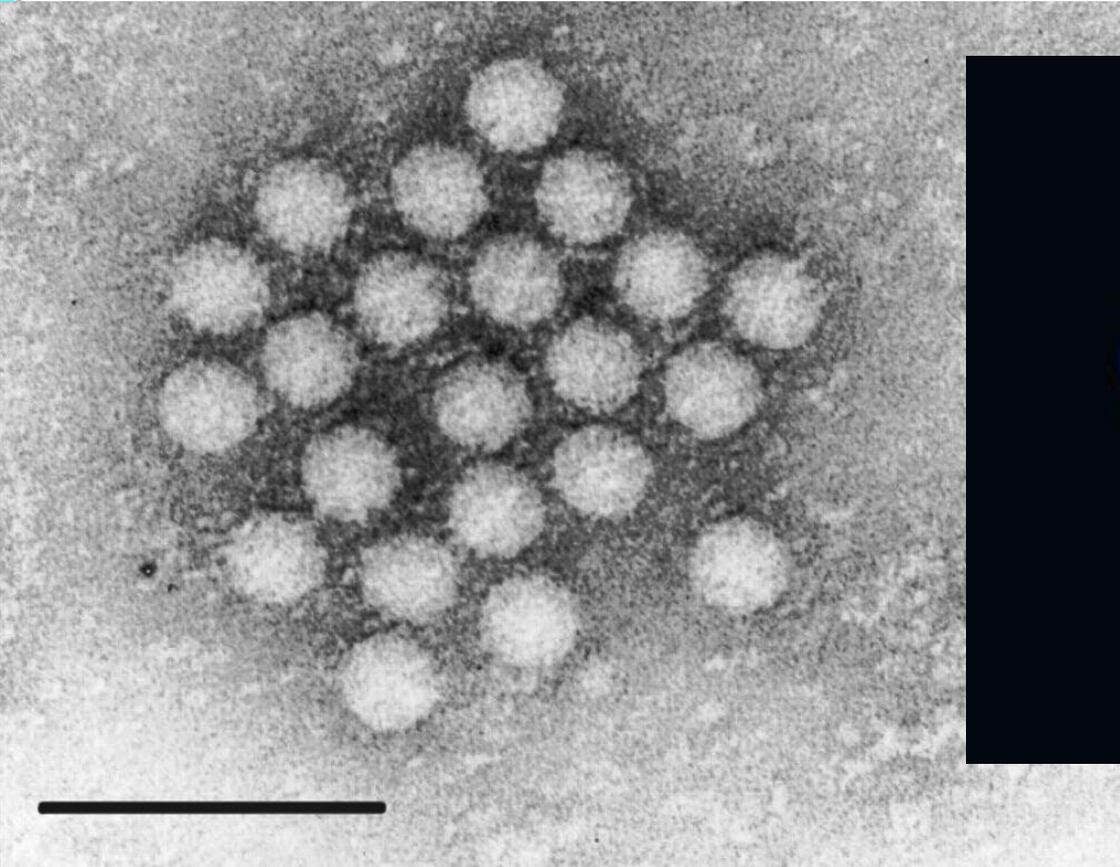
Classification of Astrovirus

- Family *Astroviridae*
- Genus *Astrovirus*
- Human serotypes: HuAstV 1-8

ASTROVIRUS- structure

- Small ss RNA virus
- Non-enveloped
- 27-32nm in size
- Round with an unbroken, smooth surface
- EM appearance of a 5 or 6 pointed star *within* smooth edge
- Contain 3 structural proteins
- Genome has been sequenced

ASTROVIRUS- STRUCTURE



- heat stable
- acid resistant
- Replication cycle not characterized

ASTROVIRUS - Clinical Features

- Infants and children are most often affected
- Elderly and immune compromised persons as well
- Short incubation period 1-4 days
- Nausea, vomiting, abdominal cramping and watery diarrhea
- Constitutional symptoms- fever, malaise, headache
- Symptoms last 3-4 days

ASTROVIRUS - Epidemiology

- Endemic worldwide
- Astroviruses are associated with 5%–9% of cases of gastroenteritis in young children
- Mainly in children <7 years of age
- Transmission- person-to-person [fecal-oral]
- Outbreaks due to fecal contamination of sea-food or water

Required

ASTROVIRUS - Diagnosis

- EM (virus shed in stool in great numbers)
- EIA
- RT-PCR

Toroviruses

- Family Coronaviridae
- Genus Torovirus
- They cause gastroenteritis in mammals, but rarely in humans
- Enveloped, ss (+) RNA virus
- Core “doughnut-shaped” (torus)
- Watery diarrhea in 2 – 12 months old
- Diagnosis: EM, ELISA, HI

