

## Lecture outline: **Gastrointestinal viruses** - 16.2.2016

*Prof. N.P. Sunil-Chandra, Senior Professor of Microbiology, Faculty of Medicine, University of Kelaniya*

### **Gastrointestinal viruses**

#### **INTRODUCTION**

**Paediatric diarrhoea** remains one of the major causes of death in young children. This is especially so in Asia, Africa and Latin America where it causes millions of deaths in the age group 0-4 years.

The main factors for high incidence and mortality are **unsafe water or inadequate sanitation**, requiring social, economic and political solutions.

#### **Non bacterial gastroenteritis:**

Gastroenteritis due to viruses, parasites and non-infectious causes. Although viruses appear to be the common cause of gastroenteritis it is indistinguishable from other types of gastroenteritis.

#### **Global importance of viral gastroenteritis:**

Viral gastroenteritis seen in all parts of the world especially in infants & young children but may occur in adults. Viral gastroenteritis is a major cause of death in developing countries. Impact is overwhelming in Asia, Africa & Latin America; 5-10 million die every year. Major effect is on nutritional status & growth

#### Historical background

1945: Non bacterial gastroenteritis was 1<sup>st</sup> demonstrated.

1972: Viral particles in faeces identified by EM was Norwalk virus.

1973: Bishop et al in Australia, Flewett *et al* in England & Middleton et al in Toronto, Canada identified rotaviruses associated with acute infantile gastroenteritis by EM.

#### **Acute infantile viral gastroenteritis**

A syndrome of vomiting & diarrhoea of acute onset often accompanied by fever. Infective in origin & not secondary to a primary disease outside the alimentary tract. Many aspects of viral gastroenteritis are similar, usually self-limited & non-inflammatory & **bloody diarrhoea does not occur**.

Peak incidence of diarrhoea occurs 6 -24 months age. Seasonal variation occurs in temperate countries with peak incidence in winter but not in tropics.

Geographical distribution: worldwide, highest incidence in conditions of poor sanitation, overcrowding & poverty.

Age: mainly affect infants & children aged 6 months & 2 years. Older children, rarely adults can be affected.

### **Clinical features**

Symptoms: Acute onset of diarrhoea often with vomiting (may be projectile)

Sometimes with fever & abdominal cramp

Dehydration with hypo-natremia is a common complication

(Winter vomiting disease is a form of viral gastroenteritis in which **vomiting** is much more prominent feature than diarrhoea. Associated with caliciviruses/Norwalk virus)

### **Epidemiology**

Endemic infection is wide spread

Epidemics or outbreaks often associated with some viruses. i.e. Norwalk virus, SRVs than others

### **Diagnosis**

Expensive battery of diagnostic tests are not justified.

Diagnostic support is important in epidemics & severe infections but best by reference laboratories.

### **The gastroenteritis viruses**

1. Rotaviruses

2. Adenoviruses

3. Astroviruses

4. Caliciviruses

5. *Small round viruses*

## Rotaviruses

The most important cause of viral diarrhoea

**Rotaviruses** have been estimated to cause 30-50% of all cases of severe diarrhoeal disease in man.

### Classification of Rotaviruses

**Family : Reoviridae      Genus: Rotavirus**

**Group A** subtypes 1, 2, 3, 4 (main human pathogens)

(Further 7 subtypes) also infect animals (monkey, calf, mouse)

**Group C** *Infects Pigs (Occasionally Man)*

### **VIRUS MORPHOLOGY:**

Particles are 70 nm round, **double shelled**, enclosing a genome of **11 segments of double stranded RNA**.

The virus is hardy and may even survive in sewage, despite stringent treatment.

Human rota virus has proved difficult to culture *in vitro*, but the serologically related monkey and calf rotaviruses grow easily in cell culture.

Refer EM of Rotavirus particles

### **Rotavirus particles**

Rotavirus particles are approximately 75nm in diameter.

They have icosahedral symmetry and particles possess two concentric protein shells, or capsids.

The term "rota", meaning wheel, is derived from the appearance of the complete double-capsid particle when viewed by negative staining in a position where the 5-fold axis of symmetry is acentric.

Apparent spoke-like components are then visible on one side of the virus particle

### **EPIDEMIOLOGY**

**Infection** is found world-wide. All ages can be infected and reinfection can occur (usually asymptomatic).

**Age:** 6 months to 2 years is common. Infections at < 6 months age and > 5 years of age tend to be **asymptomatic** and give degrees of protection against diarrhoeal infection.

Maternity hospitals commonly have resident strains which readily cause asymptomatic infections of newborns.

**Seasons:**

In temperate '1st world' populations rotavirus is the main cause of **winter gastroenteritis** .

In tropical and developing countries, rotavirus diarrhoea occurs all the year round , but with a **peak in summer**. However, it is only one of a variety of pathogens causing diarrhoea

**CLINICAL SIGNIFICANCE**

Common nosocomial infections including epidemics in children.

Essentially an ingestion disease (faecal-oral route)

**Incubation** is short. 1 to 3 days.

**Illness:** Sudden onset watery diarrhoea, with or without vomiting. May last up to 6 days (or longer if immunocompromised).

The disease is self limiting.

**Complications:**

Dehydration may result, this can be severe and life threatening in young children.

Chronic rotavirus infection occurs in immuno-suppressed children

(Adults – asymptomatic infection occurs)

**Treatment:**

No specific treatment of viral infection is available nor is it really required.

Treatment is aimed at prevention and/or **treatment for dehydration** by oral and/or intravenous fluids and electrolytes

**Diagnosis:**

Detection of virus in stools (peaks at day 3 or 4 of diarrhoea):-

- Latex agglutination (antigen)

- ELISA (antigen)

- Electron Microscopy (labour intensive, relatively insensitive) – Morphology

- Electrophoresis of RNA segments

- (Antibody can be detected but is not clinically useful)

Virus can be grown in MA104 cells. Trypsin pretreatment and roller cultures facilitates growth

### **Prevention:**

*Non specific factors:* improved hygiene, education, clean water

*Specific* - Breast feeding helps to provide passive immunity in the newborn (from maternal antibodies),

Vaccination is still experimental

### **Vaccine:**

In view of the major role of dehydration from diarrhoea as a cause of childhood death, the World Health Organisation has waged an intensive campaign for

(1) oral rehydration solutions to prevent or treat dehydration

(2) Rotavirus Vaccine is currently used in some countries,

The **prevention of severe dehydration** is the main aim, rather than totally preventing infection

## **Adenoviruses**

74nm in size, ds DNA genome, non-enveloped,

Classical icosahedron with rounded capsomeres.

Two strains of **adenovirus (40 and 41)** have also been associated with diarrhoeal disease

Refer EM of adenovirus particles

They do not grow in cell cultures and were discovered by Electron Microscopy. ( Recently there has been limited success in special cell culture systems - i.e. Graham 293 cells).

Use of EM can detect large number of particles ( $10^{11}$ particles/gram faeces)

Apart from this 40/41 group, **other adenoviruses** (type 1, 2, 5, 6 &7) may be found in the stools of **asymptomatic** children.

They are regularly isolated in tissue culture

## **DIAGNOSIS**

Viruses can be isolated from stools, as well as throat and respiratory secretions.

Adenoviruses in stools can be detected by ELISA, latex agglutination, and the 40/41 strains can be detected by specific molecular techniques.

## Astroviruses

Astroviruses – EM picture

28nm, ss RNA viruses, star shaped structure

5 serotypes are common, associated with mild childhood diarrhoea.

Virus can be found in large numbers in faeces more commonly in diarrhoeal patients than normal persons.

High percentage of seropositives in different age groups.

Virus observed in ¼ frequency to that of rotavirus.

Concurrent infections can occur with rotaviruses or caliciviruses. But do not make more ill.

Difficult to cultivate *in vitro* except few strains.

Less pathogenic to Norwalk virus

Epidemic disease in young children in paediatric wards, day care centres, nursery homes.

Diagnosis is by EM.

## GASTROENTERITIS IN OLDER CHILDREN AND ADULTS

Apart from the severe problem of diarrhoea in young children there have been outbreaks of **infectious gastro-enteritis in adults**.

Electron microscopy (of the stools) has revealed two main groups of virus particles which do not grow in cell culture.

(1) **Calici viruses** (ssRNA) including **Noroviruses**, Norwalk and related agents ('Hawaii'; Ditchling; 'W')

(2) **"small round viruses"** about which very little is known

## **Caliciviruses**

33nm (31-35nm) in size, ssRNA genome

Cup shape (chalice) indentation

Primarily in epidemic & sporadic gastroenteritis in young children.

Cause winter vomiting disease

Not a new virus observed by EM. Animal caliciviruses were recognized before.

1<sup>st</sup> human calicivirus described in 1976 from diarrhoeic faeces of babies.

Subsequently, found in community & nosocomial common-source outbreaks of vomiting & diarrhoea in children and adults

Refer : Caliciviruses EM

Rarely seen in stools of normal babies

Some serotypes are more likely associated with outbreaks

Majority of adults and older children possess antibodies to caliciviruses

Symptomatic illness often occurs in young children not in neonates.

## **Norwalk and related viruses**

27nm in size, + ss RNA genome. Unclear surface structure

Originally described in faecal material from a school outbreak in Norwalk, Ohio, USA

Norwalk virus is an important pathogen in epidemic gastroenteritis associated with outbreaks/epidemics of waterborne, food borne, and shellfish associated food poisoning.

'*Common source*' type of explosive outbreaks of gastroenteritis, with limited secondary spread to household contacts, have been described. .

These often occur in institutions, or follow common source ingestion episodes e.g. celebratory feasts.

It is a cause of winter vomiting disease.

It causes infection older children & adults. Uncommon in infants and toddlers

Vomiting with cramps are more common symptoms than the diarrhoea

Norwalk viruses differ from rotaviruses, astroviruses & other caliciviruses

1. found only in outbreaks
2. no equivalent animal strain recognized.
3. amount of virus present in diarrhoeic stools very much less.
4. With few exceptions it has been found only in USA.

### **Clinical features:**

25-50% cases, manifest – chills, headache, myelgia, fever as well as nausea, vomiting & diarrhoea.

Sero positives increases with age but much lower percentage than for rotaviruses & astroviruses.

Similar viruses found in diarrheic stools are – Hawaii agent, Montgomery county agent

### **Diagnosis**

At first, the virus was seldom identified as there were no easy diagnostic tests - only expensive electron microscopy of stools.

Serology was limited, as the only antigen available was prepared from known infected stools - not in plentiful supply!

Recently, molecular techniques have shown that many of these agents from different parts of the world are essentially similar. Molecular techniques have also enabled the expression of viral antigens that can be used in serological surveys

## **Small round viruses (SRVs) & Small round structured viruses (SRSVs)**

Variety of virus like particles observed by EM

Size & shape resembles enteroviruses

No virus can be grown in tissue culture & their identity is not known

SRVs divide in to 2 groups depend on outer surface; smooth & rough

Within each group there is variation in size

But particles in any one stool in constant in size

Associated with common source outbreaks of diarrhoea



Different names are given according to the location, vehicle of transport; i.e. W- agent, cockle virus, ditchling agent

Cockle virus may have concentrated in molluscs. Often implicated in diarrhoea after eating sewage contaminated cockles & mussels.

### **What are noroviruses?**

Noroviruses belong to a family of viruses that cause the "stomach flu." Noroviruses are classified into Family Caliciviridae. Stomach flu is also called gastroenteritis, food infection, food poisoning, and acute nonbacterial gastroenteritis

## **Coronaviruses**

80-180nm, enveloped, pleomorphic, club shaped projections.

Until recently, coronaviruses known as the cause of common cold syndrome, although similar viruses cause diarrhoea in piglets & calves.

Coronavirus –like particles (CVLP) found in stools of adults rather than children

Prolonged excretion lasting up to several months

Rarely described in diarrhoeal disease.

Refer Coronaviruses – EM picture

**List of gastroenteritis viruses:**

| <b><i>Virus</i></b>   | <b><i>Nucleic acid (genome)</i></b> | <b><i>Particle</i></b>  | <b><i>Number of sero-types</i></b>      | <b><i>Age.disease</i></b>  |
|---|-------------------------------------|---|---|--|
| <b><i>Rotaviruses (Reoviridae)</i></b>  | <b><i>ds RNA, 11 segments</i></b>   | <b><i>Nonenveloped, icosahedral, 70nm, double shelled wheel-like</i></b>        | <b><i>9 serotypes</i></b>               | <b><i>6-24 months<br/>Acute diarrhoea in infants &amp; children</i></b>                                |
| <b><i>Adenoviruses (Adenoviridae)</i></b>   | <b><i>ds DNA</i></b>                | <b><i>Nonenveloped, Classical icosahedron with rounded capsomeres, 74nm</i></b> | <b><i>2 serotypes (40 &amp; 41)</i></b> | <b><i>Acute diarrhoea in young children</i></b>  |
| <b><i>Astroviruses</i></b>  | <b><i>+ssRNA</i></b>                | <b><i>28nm, 6 point star shaped structure</i></b>                               | <b><i>5 serotypes</i></b>               | <b><i>Mild childhood diarrhoea</i></b>   |
| <b><i>Caliciviruses (Caliciviridae)</i></b>   | <b><i>+ssRNA</i></b>                | <b><i>33nm, 6 point star surface structure with central hole</i></b>            | <b><i>4 serotypes</i></b>               | <b><i>Major cause of winter vomiting disease, Common source outbreaks of diarrhoea in children</i></b> |
| <b><i>Norwalk virus (Caliciviridae)</i></b>   | <b><i>+ssRNA</i></b>                | <b><i>27nm</i></b>  | <b><i>Not known</i></b>                 | <b><i>Cause outbreaks of diarrhoea</i></b>   |
| <b><i>Small round viruses(SRVs)</i></b>   | <b><i>+ssRNA</i></b>                | <b><i>22-25nm, small &amp; round, featureless</i></b>                           | <b><i>Not known</i></b>                 | <b><i>Cause outbreaks of diarrhoea</i></b>   |
| <b><i>Small round structured viruses (SRSVs) (Caliciviridae)</i></b><br><i>i.e. UK-2, Norwalk virus; UK-1, Taunton agent; UK-3, Hawaii agent; and UK-4, Snow Mountain agent</i> | <b><i>+ssRNA</i></b>                | <b><i>35nm, ill defined surface structure</i></b>                               | <b><i>Not known</i></b>                 | <b><i>Cause outbreaks of diarrhoea</i></b>   |
| <b><i>Coronavirus (Coronaviridae)</i></b>   | <b><i>+ssRNA</i></b>                | <b><i>80-180nm, enveloped, pleomorphic, club shaped projections of 20nm</i></b> | <b><i>Not known</i></b>                 | <b><i>No clear association with gastroenteritis</i></b>  |

**\*Norwalk virus is a SRSV (UK2 group) - classified into Caliciviridae**

*Prof. N.P. Sunil-Chandra, Senior Professor of Microbiology, Faculty of Medicine, University of Kelaniya*