Rotavirus

Introduction

- We have 5 virology lectures in total. This is the first one, and we're going to discuss the Rotavirus and its role as a causative agent of Gastroenteritis (GIT infection).

The criteria for detection of viruses which cause diarrheal diseases:

1- **Previously, viruses have being diagnosed using the "diagnosis by exclusion":**we had to exclude the bacteria, and then the protozoa so as to become able to think of viruses.

- But in order to make sure, they had to take – for example - a stool specimen, spin it down to make it cell-free, and by this, the viruses would become supernatant. Then, all what they had to do was to give volunteers (non-immune humans or anime hosts) this supernatant virus orally and see if it's associated with Gastroenteritis illness. This method is perfectly helpful in detection of Gastroenteritis cases, but is most difficult to achieve as it's impractical.

*We have Rotaviruses that infect humans as well as animals. We can use the previous method on experimental animals and see the outcomes(but it may not mimic it). Paradoxically, if an animal Rotavirus infects a human, what is the result?

- An asymptomatic patient. This was used in generating a vaccine for the virus. With the use of the vaccine, the virus can still replicate in the small intestines, but it doesn't cause a symptomatic illness.

Ex: Animal rotavirus is a vehicle to the vaccine.

2- **Virus is significantly detected in ill patients more than asymptomatic controls, and virus shedding correlates with symptoms:** we can take controls (asymptomatic patients) and symptomatic ones, and try to isolate the virus from the feces. Asymptomatic patients may shed the virus but in lower amounts, and this is why we should link the presence of the virus in higher amounts along with the symptoms (detection is easier symptomatic patients).

3- **Significant humoral or secretory antibody response or both in patients shedding the disease:** shedding the virus in the stool is also associated with elevated titers of antibodies against Rotavirus in the serum (IgG) or in the secretions (secretory IgA).

4-Reproduce the disease by experimental inoculation of nonimmune human or animal hosts (we said it's the most difficult to achieve).

5- Exclude other causes of diarrhea such as bacteria, bacterial toxins and protozoa.

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***The following table shows different viruses causingGastroenteritis:**

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

- Notice that all of these viruses are naked viruses except for Torovirus which is enveloped (belongs to Coronaviridae family)

- Notice that all of them are transmitted through the fecal-oral route.

- Notice that all of them are incapable of replication in a cell culture. An exception is Rotavirus which can partially replicate in a cell culture, but the cycle of replication won't be completed.

- Notice that all of them are RNA viruses except for Adenovirus which is DNA virus.

- Adenovirus is dsDNA virus, Rotavirus is dsRNA virus. The others are ssRNA viruses.

- The shape of Rotavirus is unique because its capsid has double shells, so it appears as a wheel under the electron microscope (hence the name"Rota" (means "wheel" in Latin)).

- The commonest serotypes for Adenovirus in Gastroenteritis cases are 40&41.

-the doctor read the number of serotypes from the table.

-the sites of infection : Rotavirus→ duodenum & Proximal jejunum
calicivirus→jejunum the rest→small_intestines

-immunity:

Rotavirus : the serum (IgG) or in the secretions (secretory IgA).

Rotavirus →mostly seen in winter.

- Rotavirus infects infants and children up to 2 years. By the age of 4, if you do screening, you'll find that 90% of the children have seroconversion (antibodies against Rotavirus).

-Calicivirus infect Older children and adults.

- IP for these viruses is short, ranging from 6-12 hours up to 3-4 days.

- Diagnosis can be achieved by electron microscope, PCR or serological tests.

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Rotavirus

- Family: Reoviridae.

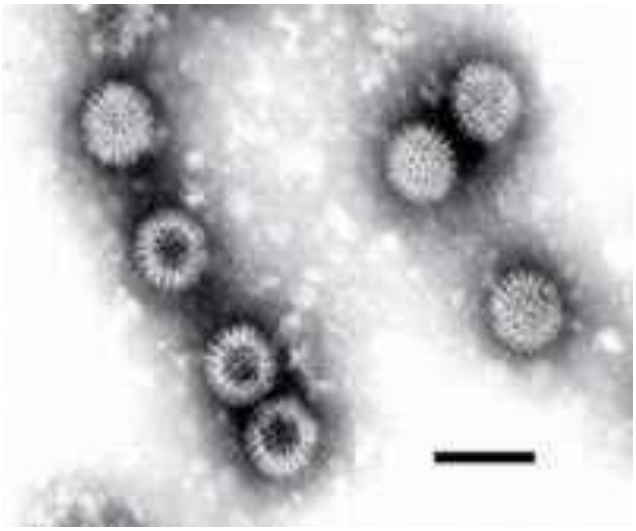
Genus: Rotavirus. (There are other genera that belong to this family)

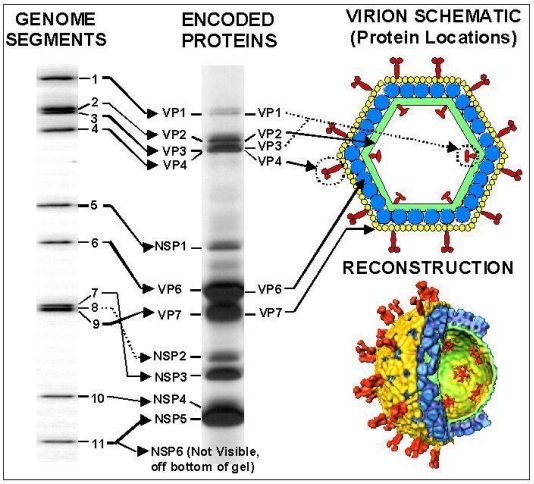
- It was first isolated in Australia in 1973 by taking samples from the duodenum of children with diarrhea. Then, it has been described in stool samples from children. After that, human and animal strains have been recognized.

Structure

- The left picture shows how the virus appears under the EM. Notice the wheel-shaped, double-shelled, icosahedral capsid (naked virus).It has outer and inner capsid.

Now for the right one, the outer shell(capsid) is VP7 structural protein, the inner shell is VP2. In between, we have the structural protein VP6 which is an important antigenic determinant, because the virus' groups (A-G) and subgroups (I-II) are classified based on VP6 structural differences.





-dsRNA segmented virus; 11 segments (Influenza virus is also segmented; 8 segments).

- The capsid is cleaved by trypsin to form Intermediate/Infectious sub-viral particle (ISVP). This indicates that the virus isn't infectious by itself;it rather needs activation by proteases (trypsin) in the small intestines. A cell culture lacks proteases, and this is why the virus cannot fully replicate there.

- There is also the surface protein VP4. VP4 and VP7 are the most antigenic, and the antibodies are formed against them. (Remember that VP6 is important in determining the groups and the subgroups **NOT** in evoking the immune response).

- Inner structural proteins contain VP1, VP3, VP6 in addition to the so-called VP2.

- All the VP proteins are structural. There are non-structural proteins which are the NSP proteins. The most important non-structural protein is NSP4. The importance of it comes from the fact that it was the first viral enterotoxin to be discovered. Remarkably, this is why the diarrhea is much severe in Gastroenteritis caused by Rotavirus than other viruses.

- virus' groups (A-G) and subgroups (I-II) are classified based on VP6 structural protein. Group A is the most common, and group C is found worldwild.

Replication cycle

1- Once the virus is ingested, it reaches the small intestines (duodenum and jejunum), exactly the mature enterocytes that line the tips of the intestinal villi. The replication cycle starts by the attachment of VP4 (with the help of VP7) to the receptors on the target cells.

2- Trypsin cleaves the outer capsid (VP7). Here, the virus is converted into the infectious sub-viral particle (ISVP).

3- The ISVP is internalized into the cell via receptor-mediated endocytosis. It's now in the cytoplasm.

4- Usually and after entering the cell, the uncoating of the virus occurs. In the case of Rotavirus, we have what we call "RNA interference" which are small RNA sequences that bind to the viral dsRNA and cause destruction of it, thus, preventing the viral replication. So, the transcription and replication (via viral RNA-dependent RNA polymerase) occur within the sub-viral particle (without uncoating-RNA doesn't exit the coat to the cytoplasm) where the plus-strand of the dsRNA serves as a template for the (negative-) minus-strand synthesis. And for the proteins:transcription > early mRNA > early protein synthesis>late mRNA > late protein synthesis.

The doctor said :""It's a double stranded RNA, But in replication the negative strand is used, so it behaves like a negative stranded RNA Virus.""

5- After the synthesis of structural and non-structural proteins, the virus starts the assembly process where the coat of the virus attaches to non-structural protein 28.

6- It then goes to the endoplasmic reticulum and acquires a membrane. After that and during the journey to the Golgi apparatus and the cytoplasm, it loses its membrane (it's a naked virus).

7- The new virion exits the cell after lysis of the cell **NOT** via exocytosis.

Slide15:
mRNA transcription with viral RNA polymerase.
Capsid proteins formed, assembled into immature capsid .
RNA replicated to form double stranded RNA genome .

Classification

- Serotypes are based on viral proteins including neutralizing antibodies:we have G serotypes which are based on VP7 and the P serotypes which are based on VP4. Also, we have PG combinations between these serotypes (like Influenza virus),Ex: P8G1, P4G2 ...etc.

Properties

- Since it's a naked virus, it's much stable than enveloped viruses in the outer environment. It can tolerate high acidity as well as the effects of many chemicals. But it's susceptible to disinfectants like 95% ethanol, Lysol, and 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 .

***Histopathology**

- **The effects of infection <u>of duodenum and proximal jejunum</u> by Rotavirus:** the infection leads to atrophy and blunting (shortening) in the villi as well as replacement of the secretory cells with immature ones. Those old secretory cells were responsible for production of disaccharides which are important in absorption. So, there is a loss in absorption of fats, sugars, water, and electrolytes.

- Infiltration of the villi by inflammatory cells follows.

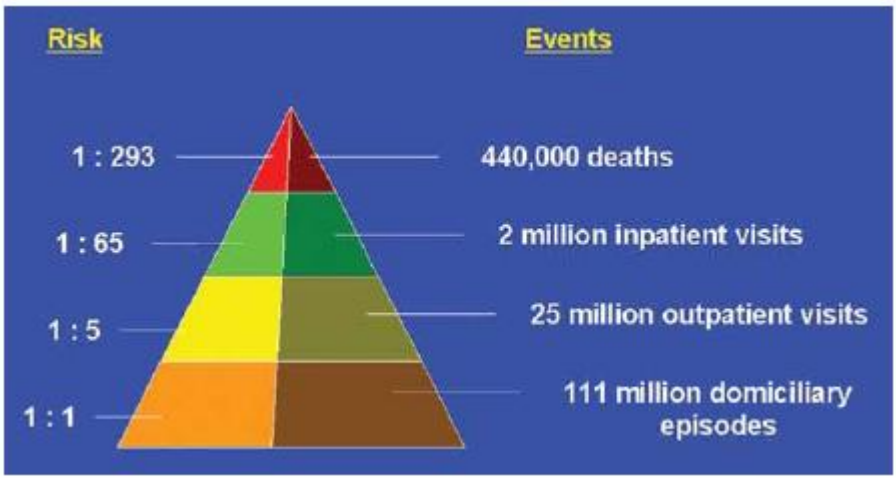
Epidemiology

- Rotavirus is a major cause of diarrhea-associated hospitalizations and deaths. There are 440,000 deaths, 2 million inpatient visits, 25 million outpatient visits, and over <u>100 million infections every year</u>.

*The death numbers are great. We're talking about <u>half a million deaths/year</u>. **Why?**

- Because of severe <u>dehydration</u>. If there is a good access to good health care services, those death numbers should dramatically drop. Actually, those large numbers mostly exist in developing countries unlike developed ones where they are low.

The doctor read the numbers :



- **Age**: it mostly infects infants and children(4 months up to 2-3 years).

*Why does it **NOT** infect younger infants?

- Because of <u>protection by transplacental antibodies transfe</u>r (IgG) as well as <u>breastfeeding protection which is represented by antibodies</u> (IgA) and mucin glycoproteins. These structures <u>attach</u> to the virus and prevent it from infecting the cells. Another theory suggests that the p<u>roteases needed</u>

for cleavage and production of infectious sub-viral particle are **NOT** present yet in the immature infantile intestinal cells.

- Infected adults are commonly asymptomatic. Once the infection occurs, the production of the antibodies makes the following infections milder, but one infection is **NOT** enough for full protection. This concept is used in vaccination, and this is important in getting protection rates of 95% (we usually give 3 doses).

- It can cause nosocomial infections and effect nurseries. It can also be seen in outbreaks.

- It's much severe in the infants and immunocompromised patients.

- Mostly in the winter months.

- IP ranges from 1-3 days.(Below 4 days).

- Group A is the most common. Group B is seen in outbreaks (E.g: China). Group C is distributed worldwide.

Transmission

- Mainly fecal-oral route. It can also be transmitted by contaminated food or water.

- Fomites: direct contact with items of infected patients.

- Respiratory route: is questionable (speculated).

- The disease remains contagious from before the onset of diarrhea until the end of it. We're talking about 10-12 days because the diarrhea lasts for 3-8 days (add them to the IP).

- Large amounts of viral particles are shed in diarrheal stool, and we're talking about low infective doses (10-100 plaque-forming units). It's **HIGHLY** contagious!

Immunity

- The most immunogenic structural proteins, which are VP7 and VP4, generate IgG and IgA antibodies in the intestines.

Clinical features

*How does an infant with a Rotavirus GIT infection present?

- The first presentation is vomiting with a possible mild fever. Few hours-2 days later, diarrhea will develop. Vomiting lasts for 1-3 days, while diarrhea lasts for 3-8 days. The diarrhea is watery brown, but if it's severe, it'll become a clear watery diarrhea ("Hakori" shift from brown to clear watery: a Japanese term).

- Watery diarrhea may lead to loss of absorptive surface in the intestines, which needs from 3-8 weeks in order to get back to its pre-infected condition.

- The diarrhea lasts longer in malnourished and immune-deficient individuals.

- High mortalities are mainly due to dehydration. If you compensate for the fluid loss (rehydration), the patient will **NOT**have a high mortality risk.

- There might be chronic diarrhea and secondary malabsorption of lactose and fats.

- The so-called NSP4 plays a role as an enterotoxin. Remember that this is the reason behind the remarkably increased severity of the diarrhea caused by Rotavirus.

Diagnosis

1- Antigen detection in stool: achieved by ELISA.

2- Electron microscope.

3- Inoculation of the sample in a cell culture.

4- Serology for epidemiologic studies.

Treatment and prevention

- There is NO specific antiviral therapy for Rotavirus. This also applies to all viruses that cause Gastroenteritis.

- So, the treatment is supportive; orally in mild cases, IV rehydration in severe ones.

- Prevention - as in any viral infection - can be accomplished by disinfectants and hygienic means. You - as a pediatrician - must wash your hands continuously.

Vaccine

- The vehicle of the vaccine is an animal Rotavirus. The virus - as we said – can still replicate, but it does **NOT** produce symptoms.

- The vaccine is live, oral (2 drops in the mouth).

- **Mechanism of action of the vaccine:** we said that the virus used here was animal virus. Rotavirus' genome is segmented, so the accomplishment of the vaccine is achieved by **Reassortment** of the segments. We take the VP4 and VP7 from a human virus, and add them to be expressed on an animal one. Once the vaccine is given, the body produces antibodies against human VP4 and VP7 which are present on the ingested viral surface.

-It contains 5 reassortants (WC3 bovine strain with viral surface proteins of human serotypes G1-4 and P1A).

- It's given 3 times at the ages of 2, 4, and 6 months. The minimum age of first dose is 6 weeks, but should **NOT** go beyond 12 weeks of age because it will**NOT** initiate the pre-mentioned series after 12 weeks. **Why?** Search for it.

Please refer to the slides, this sheet doesn't include everything.

Sorry For Any Mistake