GIS 5

MICROBIOLOGY

Done by: Dena Kofahi

Scientific Correction: Nour Awamleh

Gramatical Correction:

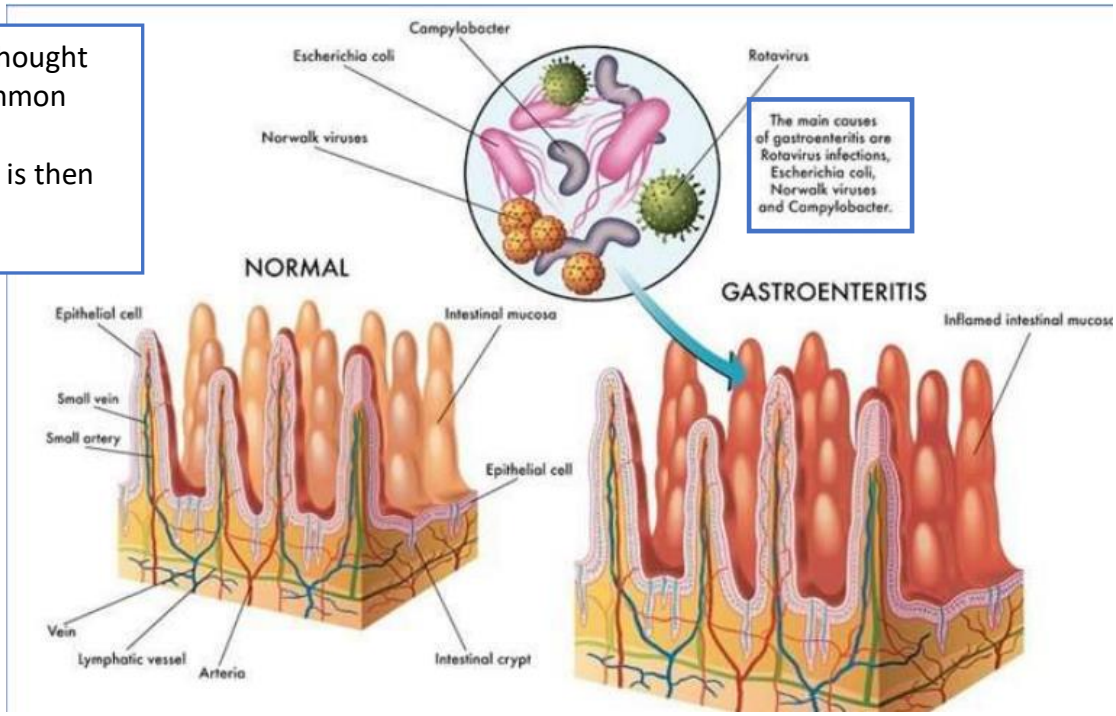
Doctor: Dr. Anas

The topic of this sheet is viral gastroenteritis. Please make sure to study the diagrams and pictures, especially where boxes and lines were added, as the professor spent a lot of time explaining them.

Overview

- Gastroenteritis is inflammation of the gastrointestinal tract— especially the stomach and small intestine.
- Has infectious and non-infectious causes:
  - Infectious (more common): **Viruses**, bacteria, fungi and parasites.
    - Viruses are the most common cause of gastroenteritis. Although there is regional and seasonal variation in the etiological agents, viruses are probably the most important agent in causing gastroenteritis.
  - Non-infectious: Drugs (such as NSAIDs), certain foods (people with Celiac disease cannot tolerate gluten), etc.
- Gastroenteritis is characterized by two major symptoms: **vomiting** and **diarrhea** (we can also have other symptoms that will be discussed later).

Campylobacter is thought to be the most common bacterial cause of gastroenteritis and is then followed by E. Coli.

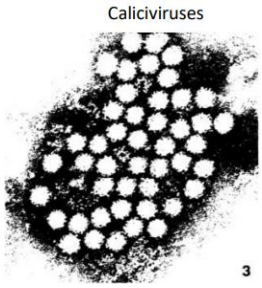


History

It was previously thought that bacteria are the main cause of gastroenteritis. Any other case was referred to as non-bacterial gastroenteritis, as they could not find a causative agent. However, in the 1970s, a group of articles were published that showed that nanometer sized particles were associated with acute infectious nonbacterial gastroenteritis. Although viruses were described in the early 1900s, it was not until the 1970s that they were found to be an important cause of gastroenteritis. This may be due to their smaller size or more difficult culture techniques.

The professor placed the names of multiple articles in slides 2+3. The article in slide 3 was one of the first articles to describe viruses as the cause of gastroenteritis, and they did

so by using electron microscopy. This was done by observing an outbreak in Norwalk, Ohio and therefore the viruses were named Norwalk viruses and they caused Norwalk gastroenteritis. They later on discovered that these viruses are part of the Calicivirus family and that they cause epidemics in other parts of the world. Therefore, they are now more commonly known as Noroviruses.
Other caliciviruses can also cause gastroenteritis, including sapoviruses.



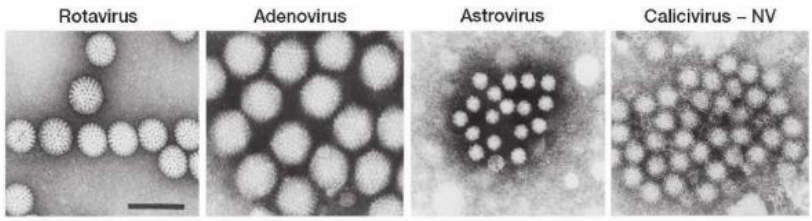
Etiology

VIRAL CAUSES OF GASTROENTERITIS AMONG HUMANS

VIRUS	FAMILY	GENOME	PRIMARY AGE GROUP AT RISK	CLINICAL SEVERITY	DETECTION ASSAYS
Group A rotavirus	Reoviridae	Double-strand segmented RNA	Children <5 years	+++	EM, EIA (commercial), PAGE, RT-PCR
Norovirus	Caliciviridae	Positive-sense single-strand RNA	All ages	++	EM, RT-PCR
Sapovirus	Caliciviridae	Positive-sense single-strand RNA	Children <5 years	+	EM, RT-PCR
Astrovirus	Astroviridae	Positive-sense single-strand RNA	Children <5 years	+	EM, EIA, RT-PCR
Adenovirus (mainly types 40 and 41)	Adenoviridae	Double-strand DNA	Children <5 years	+/++	EM, EIA (commercial), PCR

Abbreviations: EIA, enzyme immunoassay; EM, electron microscopy; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; RT-PCR, reverse-transcription PCR.

Notes on the table:

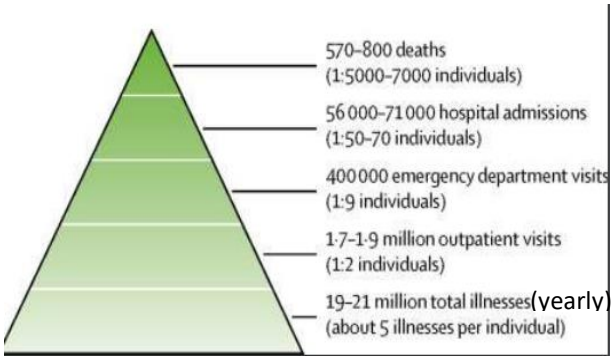


- Two of the most important viral causes are **rotaviruses** and **noroviruses**, and they will be the focus of this sheet.
- Most of the viruses are **RNA viruses** (except for adenovirus which is a double stranded DNA virus).

Epidemiology

- Acute infectious gastroenteritis is a common illness that affects persons of **all ages worldwide**.
- It is a leading cause of mortality among **children in developing countries** (where rehydration therapy is not as accessible as it is in the developed world), accounting for an estimated 0.7 million deaths each year, and is responsible for up to 10–12% of all hospitalizations among children in industrialized countries.
- **Elderly** persons, especially those with debilitating health conditions, also are at risk of severe complications and death from acute gastroenteritis.
- Among **healthy young adults**, acute gastroenteritis is **rarely fatal**. (can be managed with rehydration).

- The figure indicates that out of around 20 million cases yearly:
  - The ratio of individuals that go to the outpatient clinic versus those who don't is (1:2).
  - 1 of 9 cases will go to the emergency department.
  - 1 of 50 cases will be admitted to the hospital.
  - Around 1 of 5000 cases will die.

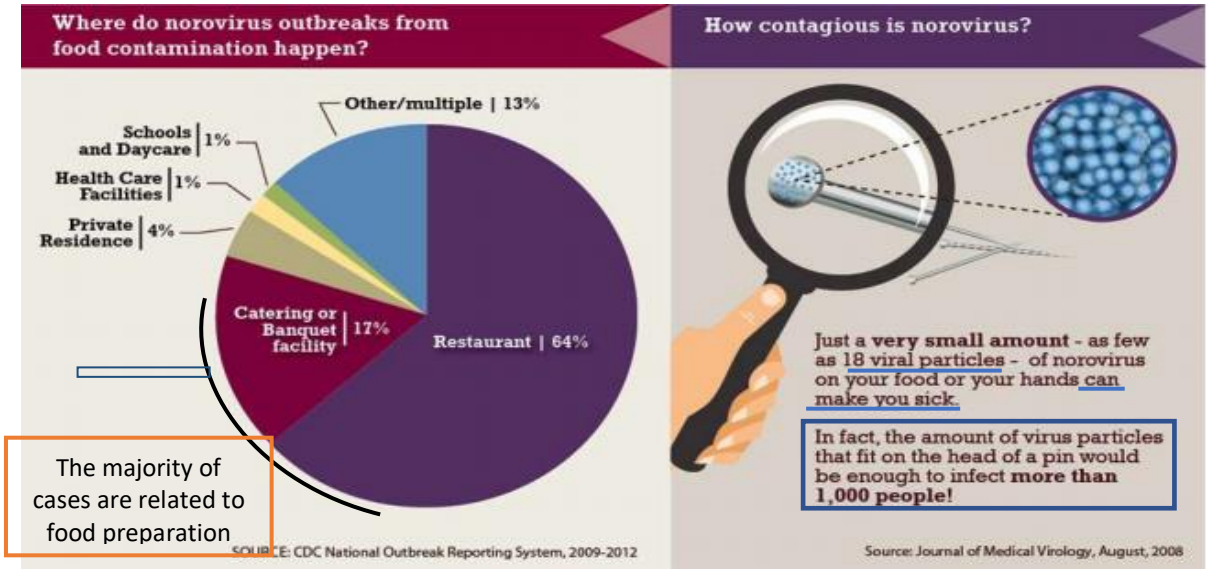


- Infections with the Norwalk and related human caliciviruses are common worldwide.
- Due to the large number of infections, **most adults worldwide have antibodies to these viruses**.
- Noroviruses are the leading cause of outbreaks from contaminated food (by either bacteria or viruses).
- Most of these cases will come from infected food workers.

Noroviruses

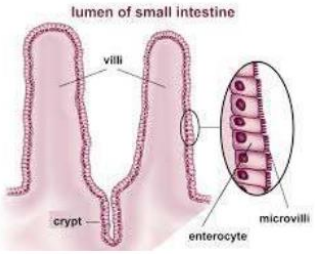
Transmission

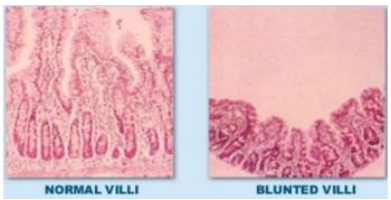
- The virus is transmitted predominantly by the **fecal-oral** route but is also present in **vomitus** (anyone who comes in contact with the vomit can also get gastroenteritis).
- This is a very important point, as very few viruses can be so infectious. Since it is so contagious, it is able to cause major outbreaks. These outbreaks most frequently occur in restaurants.
- Because an inoculum with **very few viruses can be infectious**, transmission can occur by **aerosolization** (virus particles in the air, person inhales and swallows them), by contact with **contaminated fomites** (on clothing or a chair, maybe there was vomit on it) and by **person-to-person contact** (shaking hands with someone who did not properly wash them).



Pathogenesis

- In the first step of viral infections, usually the virus will attach to a receptor on the cell to be internalized and cause its cytopathic effects. With noroviruses, a group of carbohydrates present on the gastroduodenal epithelium (upper GI tract) may serve as ligands for attachment.
  - These carbohydrates are similar to those that make up the blood groups. It seems that some individuals are more susceptible to norovirus infection than others, due to the presence of those specific carbohydrates on their epithelium.
- After attachment to the epithelium and internalization, the norovirus can then cause its effects (including cytopathic effects):
  - Lesions are noted in the upper jejunum, with broadening and blunting of the villi, and shortening of the microvilli → So, there will be less absorption.
  - Vacuolization of the lining epithelium
  - Crypt hyperplasia → So, there will be more secretions, which will manifest as diarrhea.
  - Infiltration of the lamina propria by polymorphonuclear neutrophils and lymphocytes that come to the site following infection by the virus.





- So, the GIT is in a general inflammatory state. A similar picture of blunted villi can occur when a person with Celiac disease ingests gluten, which will cause an inflammatory state quite similar to the one caused by norovirus with blunting, shortening of villi, and crypt hyperplasia.
- No histopathologic changes are seen in the stomach or colon usually.

Clinical Manifestations

The histopathological changes will show in the clinical manifestations.

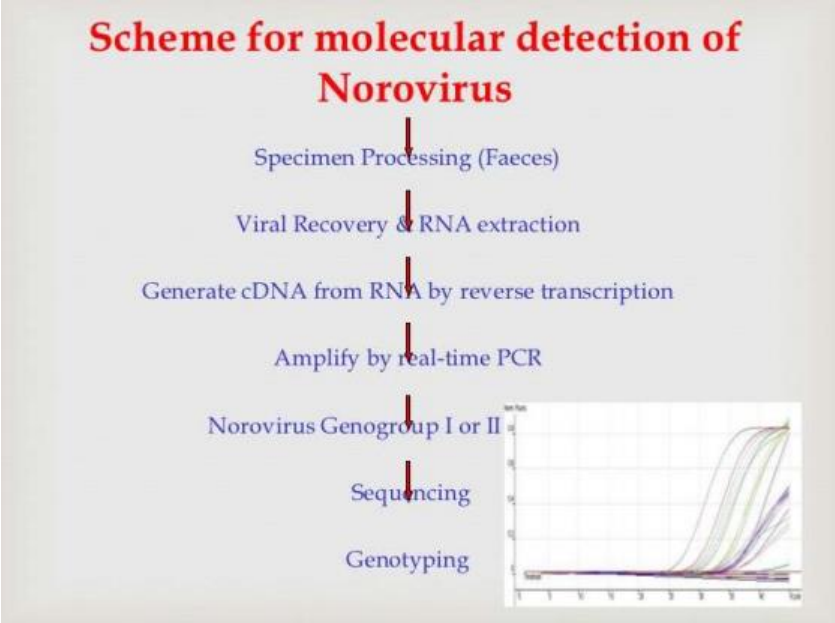
- Gastroenteritis caused by Norwalk and related human caliciviruses has a **sudden onset** following an average **incubation period of 24 hrs.**
- The illness generally lasts 12–60 hrs <u>(short duration)</u> and is characterized by one or more of the following symptoms: **nausea, vomiting, abdominal cramps,** and **diarrhea.** (main two: diarrhea and vomiting).
  - Within 24 hours of ingesting the contaminated food, the person will start vomiting (one of the more prominent features). They may also have abdominal cramps and diarrhea. This should make you think of gastroenteritis. Afterwards, you need to determine if the cause is bacterial or viral.
- Vomiting is more prevalent among children, whereas a greater proportion of adults develop diarrhea.
  - In general, in viral gastroenteritis, vomiting is more prominent.
- Constitutional (generalized) symptoms are common, including headache, fever, chills, and myalgias.
- The stools are characteristically loose and watery, without blood, mucus, or leukocytes.

Diagnosis

- It is probably going to be a diagnosis of exclusion. This means you'll culture for the bacteria you think it is. If you don't find anything, then it's non-bacterial gastroenteritis.
- If you would like to go further and determine what subtype of norovirus (or in general what virus) it is, you can do several tests.

One such test is PCR. Cloning and sequencing of the genomes of Norwalk and several other human caliciviruses have allowed the development of assays based on polymerase chain reaction (PCR) or detection of virus in stool and vomitus. With PCR, the aim is to detect the RNA (nucleic acid). The process can be seen below:

1. Stool is taken from the patient.
2. Recovery of the virus and extraction of RNA.
3. Complementary DNA is generated from the extracted RNA
4. If the virus is present, after doing PCR, the curve will start to appear in the graph like the one in the image. This indicates that viral RNA was present.
5. Afterwards, you can take the cDNA and sequence it to know what subtype of norovirus it is.



*Sequencing is usually done for the purpose of research or to document the specific cause of an outbreak of disease.

Another test can be used to look for antigens on the virus particles by using enzyme **immune assays (EIAs)**. Antibodies are used against specific antigens to determine if viral particles are present in the stool or not.

No currently available single assay can detect all human caliciviruses because of their great genetic and antigenic diversity. Genetic diversity makes it so that the primers we use will not necessarily be amplified (the primer may be correct for one virus but not another), and with antigenic diversity in EIA you may not find the antigen you are looking for.

For research purposes, electron microscopy can also be used. It is typically not used for diagnostic purposes.

Treatment

- The disease is **self-limited** and oral **rehydration** therapy is generally adequate.
- If severe dehydration develops, **IV fluid** therapy is indicated. An example is a child who keeps vomiting, oral rehydration may not be enough, especially if the child can't hold down any water.

- **No specific antiviral therapy** is available.
- The infected individual should stay home so as to not infect anyone else.
- One should also try to prevent spread through good hygiene.
- If there is pain, such as myalgia, or other constitutional symptoms (like fever) you can give paracetamol.

Prevention

The important part of gastroenteritis is to prevent it from occurring from the start in order to prevent epidemics. A focus is on food preparation, as most cases are from restaurants or other services related to food such as catering. Epidemic prevention relies on situation-specifc measures, such as:

1. **Control of contamination of food and water and exclusion of ill food handlers**.
    a. Clean fruits and vegetables
    b. Cook food, especially shellfish, thoroughly.
    c. Food service workers should stay home when sick, wash their hands, and avoid touching food with their bare hands.
    d. Clean and sanitize surfaces and utensils
2. Reduction of person-to-person spread through good personal hygiene and disinfection of contaminated fomites.

Clinical Case (very interesting!)

> An outbreak of gastroenteritis followed a meal in a large hotel during which one of the diners vomited. The clinical features of the illness suggested Norwalk-like virus (NLV, small round structured virus) infection, and this was confirmed by electron microscopy and reverse transcriptase polymerase chain reaction (RT-PCR) of stool samples. Further characterization of the virus by nucleotide sequence analysis of the PCR amplicons revealed identical strains in all the affected individuals. The foods served at the meal could not be demonstrated to be the cause of the outbreak. Analysis of attack rates by dining table showed an inverse relationship with the distance from the person who vomited. No one eating in a separate restaurant reported illness. Transmission from person-to-person or direct contamination of food seems unlikely in this outbreak. However, the findings are consistent with airborne spread of NLV with infection by inhalation with subsequent ingestion of virus particles.

Considering that an identical strain was identified in all individuals, it would be assumed the food was contaminated with the virus. However, this wasn't the case, so they analyzed the case by drawing out the area in relation to the vomiter (next page)

Table 1 and 3 had the highest attack rates because they are closest to table 2.

It turns out after the person vomited, the virus was aerosolized (spread in the air) around the restaurant and others got infected. The closer they were to the person in table 2, the higher the rates of gastroenteritis due to the higher chance of being infected by the virus. This proves how infectious norovirus is, and why it is often associated with outbreaks.

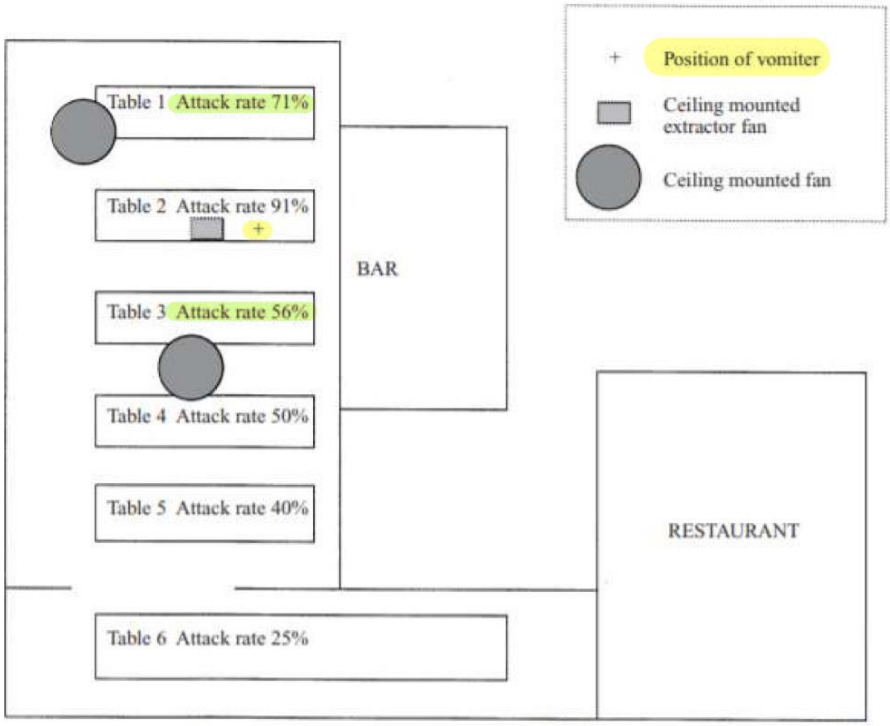


Fig. 3. Plan of the layout of tables in the restaurant. The locations of the index case and those who subsequently became ill are indicated.

Rotavirus

Rotaviruses also cause gastroenteritis. Really, there are more similarities than differences in the gastroenteritis caused by rotaviruses and noroviruses. Rotaviruses affect people of all ages, but they are an important cause of gastroenteritis in children.

Epidemiology

- Worldwide, **nearly all children** are infected with rotavirus by 3–5 years of age.
    - So, even with the differences in sanitation between developed and developing countries, nearly every child is infected by rotavirus. (incidence is the same in developing and developed countries)
- **Neonatal infections** (within the first 3 months of life) are common but are often **asymptomatic** or mild, presumably because of protection by maternal antibodies given transplacentally during pregnancy or through breast milk.
- First infections after 3 months of age are likely to be **symptomatic** (diarrhea, vomiting), and the **incidence** of disease **peaks** among children **4–23 months** of age (before immunity develops properly).

- Because of suboptimal access to hydration therapy**, rotavirus is a leading cause of diarrheal death among children in the developing world.**
- All around the world, the incidence of rotavirus is the same. However, in some areas (red countries), such as Sub-Saharan African and some areas in Asia, there is a much higher mortality rate.

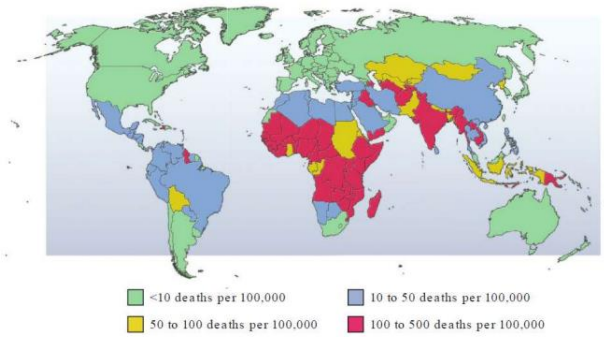
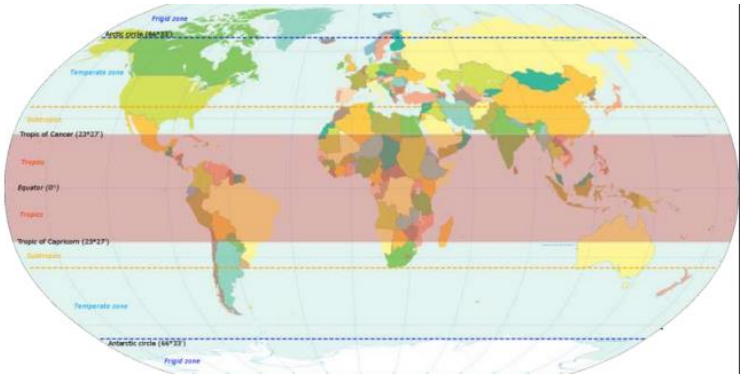


FIGURE 98-2
Rotavirus mortality rates by country, per 100,000 children <5 years of age. (Reproduced with permission from UDP Dis 200:S9, 2009.)

- In **tropical** settings (red shaded area), rotavirus disease occurs **year-round**, with less pronounced seasonal peaks than in **temperate** settings (area between the orange and blue dotted lines), where rotavirus disease occurs predominantly during the cooler (fall) and **winter months**.



- The implementation of **routine vaccination** of U.S. infants against rotavirus in 2006, was accompanied by substantial **declines in rotavirus detections** in symptomatic cases by a national network of sentinel laboratories.

In the graph, each colored line indicates a different year. Higher incidence is present in the winter months until early spring.

The black line indicates the number of cases before the vaccine was introduced.
After the introduction of the vaccine in 2006, there was a significant decline in rotavirus cases (colored lines).

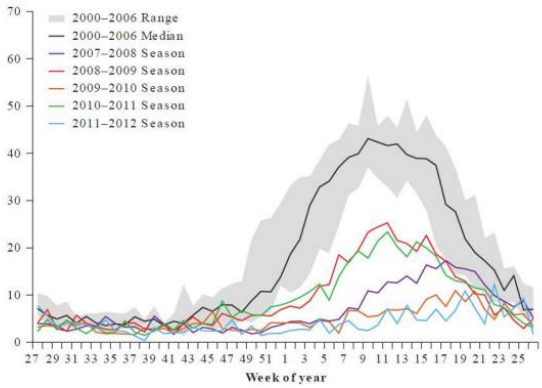


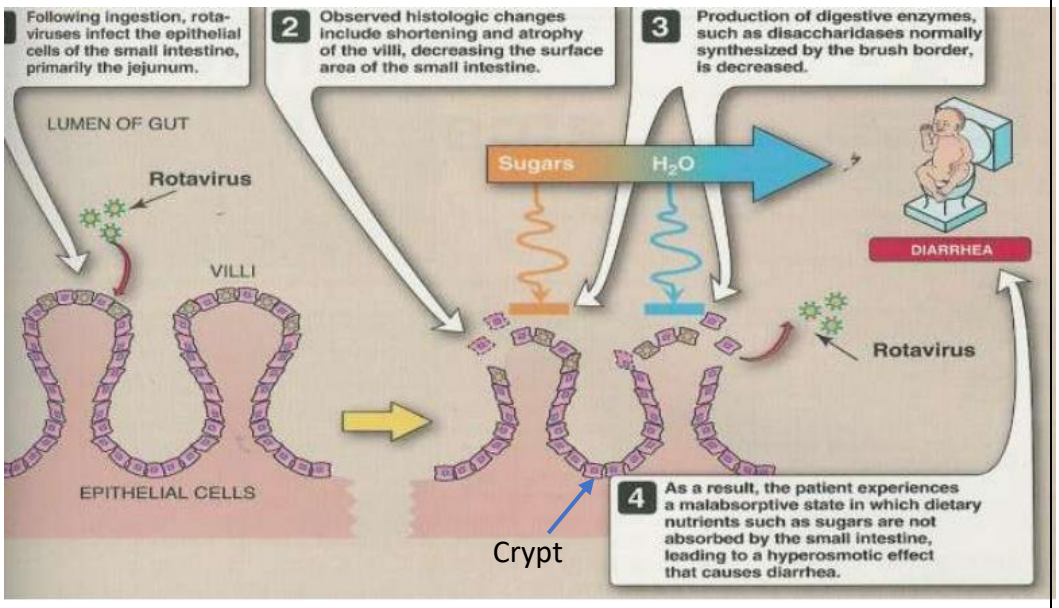
FIGURE 98-3
Percentage of rotavirus tests with positive results, by week of year, July–June, 2000–2012. The maximal or minimal percentage of rotavirus-positive tests for 2000–2006 may have occurred during any of the six baseline seasons. Data are from the National Respiratory and Enteric Virus Surveillance System. (Adapted from Centers for Disease Control and Prevention, 2012.)

Pathogenesis

The pathogenesis is also quite similar to that of noroviruses.

i. The rotavirus will go to the lumen of the gut and infect epithelial cells, especially in the jejunum (proximal small intestine).
ii. Destruction of villi due to cytopathic changes. Some of the epithelial cells shed off and die and the villi become blunted and shorter. The microvilli on the edge of the cell will also shorten. All these changes result in decreased absorption.
iii. Proliferation of crypt cells will occur, resulting in increased secretions.

Rotaviruses infect and ultimately destroy mature enterocytes in the villous epithelium of the proximal small intestine. The loss of **absorptive villous epithelium**, coupled with the **proliferation of secretory crypt cells**, results in **secretory diarrhea**.



Clinical Manifestations

- The clinical spectrum of rotavirus infection ranges from a subclinical infection to severe gastroenteritis leading to life-threatening dehydration.
- After an incubation period of 1–3 days (may be slightly longer than that of norovirus), the illness has an abrupt onset, with **vomiting frequently preceding the onset of diarrhea**.
- The stools are characteristically **loose and watery** and only infrequently contain red or white blood cells. Gastrointestinal symptoms generally **resolve in 3–7 days**. (So, the symptoms may also last longer than those of norovirus. Therefore, in areas with poor rehydration therapy, a baby after seven days may be dehydrated and this can lead to its death.)

Diagnosis

- Illness caused by rotavirus is difficult to distinguish clinically from that caused by other enteric viruses.
- Because large quantities of virus are shed in feces, the diagnosis can usually be confirmed by a wide variety of commercially available **EIAs** or by techniques for detecting viral RNA, like **PCR** or **probe hybridization**.
- The image shows an ELISA test kit. The processed stool samples are placed in the microwells. Then you add the antibodies against the antigens in the virus and if you detect a signal, the infection is probably caused by rotavirus.

Treatment

- Rotavirus gastroenteritis can lead to severe dehydration. Thus, appropriate treatment should be instituted early.
- **Standard oral rehydration therapy** is successful for most children who can take fluids by mouth, but **IV fluid replacement** may be required for patients who are severely dehydrated or are unable to tolerate oral therapy because of frequent vomiting.
- Antibiotics and antimotility agents (decrease the motility of the gut and inhibit diarrhea) **should be avoided**. This is especially the case after determining it is viral gastroenteritis.

Prevention

- Efforts to develop rotavirus vaccines were pursued because it was apparent—**given the similar rates in less developed and industrialized nations**—that improvements in hygiene and sanitation were unlikely to reduce disease incidence.
- In 2006, **promising safety and efficacy results** of **two new rotavirus vaccines** were reported. Both vaccines are now **recommended for routine immunization**, and their use has rapidly led to a > 70–80% decline in rotavirus hospitalizations and emergency department visits at hospitals.

Rotavirus vaccination became a part of the Jordanian vaccination program in 2015.


THE JORDAN TIMES

WEATHER,

Home | Local | Region | World | Business | Sports | Features | Opinion | Letters | Biz Club

Home » Local » Rotavirus vaccination drive begins next Sunday

Rotavirus vaccination drive begins next Sunday

By JT - Feb 25,2015 - Last updated at Feb 25,2015

AMMAN — The Health Ministry on Wednesday said it will begin immunising children against the rotavirus on Sunday after its inclusion in the national vaccination programme.

Viral vs. Bacterial Gastroenteritis

Please note that these tables are very important, read through them carefully. Some extra notes not mentioned in the tables are placed below them.

CHARACTERISTICS OF GASTROENTERITIS CAUSED BY VIRAL AND BACTERIAL AGENTS

FEATURE	VIRAL GASTROENTERITIS	BACTERIAL GASTROENTERITIS
Setting	Incidence similar in developing and developed countries **(Especially in rotaviruses)**	More common in settings with poor hygiene and sanitation
Infectious dose	Low (10–100 viral particles) for most agents	High (>10^5 bacteria) for Escherichia coli, Salmonella, Vibrio; medium (10^2–10^5 bacteria) for Campylobacter jejuni; low (10–100 bacteria) for Shigella
Seasonality	In temperate climates, winter seasonality for most agents; year-round occurrence in tropical areas	More common in summer or rainy months, particularly in developing countries with a high disease burden
Incubation period	1–3 days for most agents; can be shorter for norovirus	1–7 days for common agents (e.g., Campylobacter, E coli, Shigella, Salmonella); a few hours for bacteria producing preformed toxins (e.g., Staphylococcus aureus, Bacillus cereus)
Reservoir	Primarily humans	Depending on species, human (e.g., Shigella, Salmonella), animal (e.g., Campylobacter, Salmonella, E coli), and water (e.g., Vibrio) reservoirs exist

- Of course, proper sanitation will help in preventing both viral and bacterial gastroenteritis. As seen with noroviruses, prevention was by proper sanitation methods. However, while the simplest methods can get rid of bacteria, not all can get rid of viruses. This is why there is similar incidence in developed and undeveloped countries of viral gastroenteritis, while there is a much higher incidence of bacterial gastroenteritis in developing countries.
- In bacterial gastroenteritis, the infectious dose depends on the bacteria causing it.
- For bacteria, the higher incidence in the summer may be due to more suitable temperatures for the growth of the bacteria on food.

CHARACTERISTICS OF GASTROENTERITIS CAUSED BY VIRAL AND BACTERIAL AGENTS		
FEATURE	VIRAL GASTROENTERITIS	BACTERIAL GASTROENTERITIS
Fever	Common with rotavirus and norovirus; uncommon with other agents	Common with agents causing inflammatory diarrhea (e.g., Salmonella, Shigella)
Vomiting	Prominent and can be the only presenting feature, especially in children	Common with bacteria producing preformed toxins; less prominent in diarrhea due to other agents
Diarrhea	Common; nonbloody in almost all cases	Prominent and occasionally bloody with agents causing inflammatory diarrhea
Duration	1–3 days for norovirus and sapovirus; 2–8 days for other viruses	1–2 days for bacteria producing preformed toxins; 2–8 days for most other bacteria
Diagnosis	This is often a diagnosis of exclusion in clinical practice. Commercial enzyme immunoassays are available for detection of rotavirus and adenovirus, but identification of other agents is limited to research and public health laboratories.	Fecal examination for leukocytes and blood is helpful in dif erential diagnosis. Culture of stool specimens, sometimes on special media, can identify several pathogens. Molecular techniques are useful epidemiologic tools but are not routinely used in most laboratories.
Treatment	Supportive therapy to maintain adequate hydration and nutrition should be given. Antibiotics and antimotility agents are contraindicated.	Supportive hydration therapy is adequate for most patients. Antibiotics are recommended for patients with dysentery caused by Shigella or diarrhea caused by Vibrio cholerae and for some patients with Clostridium dif cile colitis.

- In viral gastroenteritis, fever (and other constitutional symptoms) are common in the viral agents we took. In bacterial gastroenteritis, fever occurs in more severe cases of inflammatory diarrhea. Traveler's diarrhea, caused by E. Coli, will probably not have fever.
- Molecular techniques can be used to look for bacterial antigens.
- If you suspect dysentery (high fever, bloody stools, significant cramps/pain) you can give antibiotics, especially after knowing the pathogenic agent.