NOROVIRUS:
TRAVELERS’ DIARRHEA AND MUCH MORE

Course # DL-996

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Approved for 2.0 CE
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OBJECTIVES
After completing this course, the participant will be able to:
1. Describe principal characteristics and classification of noroviruses.
2. Discuss noroviruses and their role in human disease.
3. Compare outbreaks of gastroenteritis with sporadic cases caused by noroviruses.
4. Outline epidemiology of norovirus infection.
5. Discuss clinical symptoms of norovirus gastroenteritis.
6. Explain the nature of susceptibility to norovirus infection.
7. Summarize methods used in laboratory diagnosis of norovirus gastroenteritis.
8. Discuss the role of the immune response in norovirus infections.

ABSTRACT
An epidemic of gastroenteritis took place in an elementary school in Norwalk, Ohio in 1968. Clinical samples that originated from this epidemic were eventually demonstrated to contain virus-like particles (the Norwalk agent). In the 1990s the genome of Norwalk agent was sequenced and the agent assigned to a new genus, *Norovirus*, in the *Caliciviridae* family.

Noroviruses are small, icosahedral, single-stranded RNA viruses. They are easily transmitted by the oral–fecal route through food, water, fomites, and person-to-person. These viruses are highly infectious and cause outbreaks of gastroenteritis on cruise ships, in nursing homes, schools, camps, and in many additional settings. The illness is generally mild and self-limited but may be severe in immunocompromised persons and in the elderly. The outbreaks are difficult to control due to high infectivity and ease of transmission of noroviruses.

Noroviruses are also important causative agents of sporadic gastroenteritis within communities, as well as playing an important causative role in diarrheal diseases that are common among travelers to developing countries (travelers’ diarrhea).

Norovirus infections are diagnosed by molecular methods, such as real-time reverse transcriptase polymerase chain reaction (RT-PCR). ELISA assays for specific antibody may also be useful in establishing diagnosis.

At the present time no specific treatment for norovirus gastroenteritis is available.

1. INTRODUCTION
Enteric infections are very common and may range in severity from mild to life-threatening. The etiology of these infections includes parasitic agents, bacteria, and viruses. Prior to discovery of diarrhea-causing viruses, an etiologic agent could be identified only among a limited number of persons with gastroenteritis. Advances in diagnostic methods resulted in identification of several viral groups as causative agents of gastroenteritis. These include the
Norwalk virus (norovirus), rotavirus, astrovirus, and enteric adenovirus. The ability to identify these viruses and to manage outbreaks of disease has been hindered by unavailability of routine diagnostic laboratory tests. In order to overcome the obstacles to laboratory diagnosis, clinical and epidemiologic criteria have been developed for identification of viral etiologic agents.

Among the diarrhea–causing viruses, the noroviruses appear to be responsible for the majority of outbreaks of non–bacterial gastroenteritis. It is estimated that in the United States there are more than 23 million norovirus infections every year. This represents approximately 60% of all gastroenteritis cases. According to the Centers for Disease Control and Prevention (CDC) noroviruses are involved in more than 50% of all food-borne disease outbreaks. Noroviruses also cause sporadic cases of gastroenteritis and are the most common viral agents involved in community-based enteric infections in developed countries. Less is known about the role of noroviruses as enteric pathogens in developing countries (1).

II. HISTORICAL BACKGROUND

The first description of outbreaks of nonbacterial gastroenteritis was by Zahorsky in 1929. The outbreaks occurred between September and March in the U.S. and involved students and institutional personnel. The principal symptoms were a sudden onset of nausea and vomiting accompanied, on occasion, by diarrhea and a mild fever. Zahorsky proposed the name “winter vomiting disease” for this illness. A number of similar reports followed and a 10-year family study in Ohio described epidemic gastroenteritis as the second most frequent disease experience that affected broad segments of the population. The disease was transmitted to volunteers but attempts to identify the causative agent were not successful.

In 1968 two epidemics of gastroenteritis in Ohio were investigated. On October 30 and 31, 1968, 50% of students and teachers at an elementary school in Norwalk, Ohio developed symptoms of gastroenteritis. The majority of cases occurred within a 24-hour period indicating a common source of infection. The symptoms included nausea, vomiting, and abdominal cramps. Weakness, diarrhea, and fever were also observed. Symptoms persisted for 12 to 24 hours for the majority of patients. Hospitalization was not required for any of the patients. Approximately 48 hours after the initial cases occurred, secondary cases among the patients’ family contacts were reported. The incubation time for this disease averaged 48 hours. An attempt to define the source of the epidemic was only partially successful. School lunches were eliminated as a source as they were prepared in a central kitchen and distributed to all city schools. There were no similar illnesses noted at the other schools.

Water could not be eliminated as the source of the epidemic since the chloride levels in drinking water were not bactericidal. The coliform counts of drinking water were negative. Stool samples and stool swabs were tested for parasites and bacterial enteric pathogens with negative results. Attempts to isolate viruses from the clinical samples were also negative and filtrates of stool samples were preserved by freezing for further testing at a later time.

A second epidemic of gastroenteritis occurred in Columbus, Ohio during the first week of December, 1968. An average of 25% of students in an elementary school was affected, although one second-grade class had an absentee rate of 88%. The illness spread to family members with a secondary attack rate of 30%. The source of the epidemic was not established. The disease could be passed to volunteers with filtrates of rectal swab specimens.

An attempt to define the presence of a viral agent in specimens from epidemics of non-bacterial gastroenteritis was made in 1972 by Albert Kapikian and his co-workers. Specimens in these studies were stool filtrates from volunteers who developed gastroenteritis after oral
administration of an inoculum originally derived from the Norwalk outbreak. The stool filtrate was mixed with inactivated convalescent serum from experimentally infected volunteers as a source of specific antibody and examined by immune electron microscopy. Viral particles were observed. These particles appeared to have cubic symmetry and measured approximately 27 by 32 nanometers (nm). Their morphology resembled picornaviruses and parvoviruses. This was the first observation of the Norwalk agent, which was later identified as a member of the noroviruses.

III. TAXONOMY OF NOROVIRUSES

In the early 1990s the genome of the Norwalk agent was cloned and sequenced. Based on the structure of its genome the Norwalk agent was placed with the *Caliciviridae* family of viruses. The caliciviruses are a very large and heterogeneous group of viral agents. They are widely spread in the environment and are found world-wide. Caliciviruses are major veterinary pathogens: they infect cattle, dogs, cats, swine, primates, rabbits, donkeys, foxes, horses, reptiles, seals, sea lions, whales, dolphins, fish, as well as humans. Marine caliciviruses are present in oceans in large numbers. Infection of sea mammals creates a large viral reservoir. For example, an infected whale is estimated to have a million caliciviruses per gram of feces. Marine caliciviruses remain viable more than 14 days in seawater and may move from ocean reservoirs to terrestrial hosts. The best-documented example of marine caliciviruses causing disease in terrestrial species is the disease known as vesicular exanthema of swine. The calicivirus that causes this disease originated with San Miguel sea lions. The virus causes abortion and produces lesions on flippers in sea lions. In swine, the vesicular exanthema virus causes vesicular lesions similar to foot-and-mouth disease.

A. Current Classification

At the present time five genera are recognized within the family *Caliciviridae*. These five genera are:

- **Genus Vesivirus**
- **Genus Lagovirus**
- **Genus Sapovirus**
- **Genus Norovirus**
- **Genus Nebovirus**

A recent isolation of a novel calicivirus from captive juvenile rhesus macaques prompted a suggestion for creation of a fifth genus in family *Caliciviridae*. The name proposed for the new genus is Recovirus, for rhesus enteric calicivirus. The virus was isolated from macaques at the Tulane National Primate Research Center and was named Tulane virus. The virus can be cultured in a monkey kidney cell line and produces a typical cytopathic effect in cultured cells (2).

The Vesivirus group includes viruses that infect cattle, dogs, primates, seals, swine, and other species. The prototype virus is the vesicular exanthema virus, which produces lesions similar to those of foot-and-mouth disease, as well as other clinical syndromes such as encephalitis, abortion, myocarditis, hemorrhagic disease, and diarrhea.

The lagoviruses infect rabbits and European brown hares causing hemorrhagic disease.

The sapoviruses infect humans as well as other animal species. These viruses cause gastroenteritis in infants and young children, although outbreaks in adults have been reported recently. Most of the adult patients are in the over 60 age bracket.

The noroviruses represent a diverse group of viruses currently classified into six groups.
These groups are GI, GII, GIII, GIV, GV and GVI. Human norovirus strains belong to groups GI, GII, and GIV. Norovirus that infect humans belong to three genogroups (G1, GII, and GIV). Variants of GII.4 genotypes are the most common causes of norovirus illness. Bovine strains are classified in GIII and murine strains in GV groups. In addition to human strains, genogroup I also contains porcine strains. The classification is in constant development due to the discovery of new strains.

The neboviruses are known to infect cattle.

Many animal species can be infected by noroviruses, including swine, lions, mice, monkeys, and dogs. Swine are susceptible to infection with norovirus GII strains. Within a genogroup, strains are further subdivided into genotypes. There are at least eight genogroup I genotypes, nineteen genogroup II genotypes, and two genogroup III genotypes. The original Norwalk agent belongs to genogroup I, genotype 1. Variants of the GII.4 genotype have been the most common cause of Norovirus outbreaks since 2002.

B. Principal Characteristics of Noroviruses

The Norwalk agent was originally described morphologically as small, round, structured virus particles. The sequencing of the Norwalk agent genome revealed a single strand of ribonucleic acid (RNA) that carried all genomic information as well as performed the function of messenger RNA. This categorized the agent as a “positive single-strand RNA virus.” The genome is approximately 7.7kb in size, enclosed in a protein coat with distinct cup-shaped depressions. There is no envelope surrounding the protein coat. The size of the viral particle measures approximately 38 to 40 nanometers in diameter. These characteristics placed the Norwalk agent, as a new genus, in the family Caliciviridae. The name of the family is derived from “calyx,” which means cup in Greek.

The viral protein coat (capsid) consists of identical protein subunits arranged in a highly symmetrical form. These subunits self-assemble and form an icosahedron—a geometric figure that consists of 20 identical triangular faces. When genes that encode the capsid proteins are expressed in an insect virus, the baculovirus, virus-like particles are produced that are similar to wild-type virus. These virus-like particles have become important reagents in studies of viral structure, replication, in the development of diagnostic techniques, and in vaccine research.

1. Viral genome and structure of virus particle

Viral RNA is estimated to have 7654 nucleotides. A viral protein, VPg, is covalently linked to one end of the RNA molecule. The genomic RNA of human noroviruses encodes three proteins, although a murine norovirus was recently reported to encode an additional protein of unknown function. The first protein to be translated is a large polyprotein that is subsequently cleaved by the viral protease into six proteins. These proteins include VPg, protease, and proteins needed for viral replication. The newly translated proteins function in the replication process by copying the genomic RNA into a negative-sense copy, which is then used as a template for sub-genomic RNA molecules. The sub-genomic or progeny RNA is translated to produce the major capsid protein (VP1) and a few molecules of a second capsid protein (VP2). These proteins can self-assemble to form a capsid. They can also assemble into virus-like particles in experimental systems. The capsid protein of norovirus consists of 180 protein subunits arranged as dimers and forming an icosahedral structure. Some of the proteins form the shell of the capsid while others protrude from the shell. These protruding domains are known as P1 and P2. The P2 domain is positioned at the most exposed surface of the capsid and forms binding clefts for cell receptors. This region also binds neutralizing antibody and is highly variable in its amino acid content. The high
variability of viral surface receptors plays a major role in facilitating periodic epidemics of norovirus gastroenteritis.

2. Viral Replication

Replication of norovirus involves a number of steps. Initially, the virus must attach to host cell receptors and enter the cell. Virus entry may involve endocytosis of the viral particle. Once inside the cell, the norovirus must be uncoated and the viral genome released into the cytoplasm. At this stage the viral protein VPg is removed from the RNA.

At the next stage of viral replication, the viral genome is translated into a polyprotein in order to yield the non-structural proteins required for viral replication. Genomic RNA is used as a template for transcription of complementary RNA strands, which, in turn, are transcribed to progeny or sub-genomic RNA. Newly synthesized RNA encodes structural proteins which will constitute the capsid.

The proteins self-assemble around the genomic RNA, forming an icosahedral capsid. Assembled virus particles are released from the cell, attach to adjoining susceptible host cells, enter the cells, and initiate a new cycle of infection.

IV. NOROVIRUSES AND HUMAN DISEASE

Noroviruses are the most common cause of epidemic nonbacterial gastroenteritis outbreaks worldwide. These viruses are now recognized as playing a major role in sporadic gastrointestinal illnesses as well. Infection with noroviruses is responsible for thousands of emergency room visits and for hospitalization of young children because of severe diarrhea. What are the characteristics that make these viruses successful pathogens?

1. Noroviruses are highly infectious. Estimates of the smallest infectious viral dose vary, but it had been suggested that as few as 10 to 100 viral particles are sufficient to initiate infection.
2. Noroviruses are easily transmitted person-to-person as well as through food, water, and a variety of environmental sources.
3. Noroviruses are highly resistant to adverse environmental conditions. They survive at temperatures that range from freezing to 60˚C and are not affected by a number of common disinfectants. Infected persons shed viral particles for as long as a month and frequently longer after recovery from illness. After being shed from the infected host noroviruses may survive in the environment for long periods of time.
4. A large reservoir of susceptible individuals is available.
5. Immunity to noroviruses is transient and strain-specific.
6. Noroviruses are highly variable: there is a great diversity of antigenic types. New antigenic types are continually evolving. Amino acid substitutions are very common in the norovirus P2 protein, which protrudes from the capsid and functions as a host cell receptor. Changes in P2 protein affect its antigenic specificity and result in the emergence of new norovirus subtypes. New epidemic strains of norovirus appear to arise every two years. Several factors may contribute to evolution of variants: errors in RNA replication, genetic recombination, and population immunity. Genetic recombination is common among RNA viruses. When host cells are infected by more than one viral strain, genetic exchange between these strains is likely to take place. Immunity to noroviruses is strain-specific and antigenic variants have a distinct advantage for successful infection of host cells. The emergence and spread of new norovirus subtypes is often associated with greater magnitude of norovirus
epidemics. At the present time, the noroviruses in genogroup II, genotype 4 are the leading cause of acute norovirus gastroenteritis (3).

Genetic variation reported for noroviruses is similar to that known to occur in the influenza group of viruses.

**Noroviruses and gastroenteritis outbreaks**

Since the first demonstration of the Norwalk agent in specimens from a gastroenteritis outbreak at an elementary school, the noroviruses have continued to remain a major contributing cause of such epidemics. The numbers of reported outbreaks of gastroenteritis that implicate norovirus as causative agent have increased dramatically since 2002. The outbreaks occur in a variety of settings: restaurants, schools, hospitals, nursing homes, cruise ships, resorts, parks, community pools, shelters, houseboats, and recreation centers. These outbreaks occur worldwide, with reports of epidemics in Australia, Canada, Austria, Scotland, Sweden, Finland, New Zealand, Holland, United States, and many other countries. Multiple strains of noroviruses have been isolated in some of the outbreaks, posing the risk of genetic recombination. Food such as raw vegetables and shellfish has been the reported sources of infection in several outbreaks. In other instances, the source of the infecting agent was water or infected food handlers. Sometimes well water becomes contaminated. In other outbreaks, there may be maintenance failure of a public pool.

Secondary cases of gastroenteritis are common because of high infectivity of norovirus and its ease of transmission (4).

**Noroviruses and travelers’ diarrhea**

Gastroenteritis is the most common illness experienced by persons from industrialized nations who travel to developing areas of the world. In these regions, hygienic standards and sanitation may be inadequate and fecal contamination of the water supply and of locally grown crops is not unusual. These conditions facilitate transmission of enteric pathogens. Enteric bacterial pathogens such as certain strains of *E. coli* are involved most frequently. However, as many as 40% of cases of travelers’ diarrhea are never diagnosed with respect to the etiologic agent. The role of noroviruses in the etiology of travelers’ diarrhea is not well documented. A recent study by Koo and his co-workers investigated the prevalence of norovirus infection in groups of travelers from United States and Europe who were visiting Mexico, Guatemala, and India (5).

All of the subjects included in this study were suffering from diarrhea. Nausea and abdominal cramping were also present in the majority of the ill individuals. The study groups consisted of persons between the ages of 16 and 40, predominantly male. A number of enteric pathogens, including norovirus, were demonstrated in stool samples taken from subjects ill with gastroenteritis. The prevalence of norovirus infection in travelers with diarrhea was 10.2%. Frequently other enteric pathogens were also present as co-infecting agents. The enterotoxigenic and enteroaggregative strains of *E. coli* were found to be the most common co-pathogens.

Additional enteric pathogens isolated from the travelers with diarrhea were *Salmonella*, *Giardia*, *Shigella*, *Aeromonas*, *Plesiomonas*, and *Cryptosporidium*.

Visitors to Guatemala had a 17% prevalence of norovirus infection, while the group visiting India showed 11.9% prevalence of norovirus gastroenteritis. Two norovirus genogroups were involved in the gastroenteritis cases in both countries: strains from genogroup I and from genogroup II. The prevalence of norovirus gastroenteritis in visitors to Mexico varied from 3% to 12%, depending on the specific year of the study. Year 2006 had the highest prevalence of norovirus infections (12%), with majority of strains belonging to the GII genogroup.
It appears likely that travelers acquire norovirus gastroenteritis from local residents, either directly or indirectly. When very young children in a rural community in Guatemala were screened for norovirus infection 72% were shown to have prior exposure to the virus. Studies in India show that prevalence of norovirus gastroenteritis in hospitalized children ranged from 10.9% to 18.9%. Additional studies in other high-risk developing regions of the world may demonstrate that noroviruses play an important role as causative agents of gastroenteritis in travelers to these regions.

**Noroviruses and sporadic cases of gastroenteritis**

It is difficult to determine the precise number of sporadic cases of norovirus gastroenteritis that may occur within a community because many of these illnesses are mild and remain unreported. Children as well as adults not infrequently suffer from diarrheal illness. If the illness becomes severe, hospitalization may be required, and the etiologic agent responsible for the illness may be identified. The same is true for patients who seek medical help at community medical facilities. Transmission of norovirus in sporadic cases may occur through contaminated food, drinking water, swimming areas contaminated with enteric pathogens, person-to-person contact, fomites, aerosols from ill individuals, and any other route that results in ingestion of infectious material.

A number of studies of the etiology of sporadic gastrointestinal disease have been published and a considerable amount of information is available on the role of norovirus as causative agent of diarrhea in patients who are hospitalized or seek community-based medical help. According to these studies the prevalence of norovirus infection ranges from 4% to 48%. The highest prevalence was in hospitalized children and had been reported from Italy. Studies from Iraq and Brazil also show a high prevalence of norovirus infection in hospitalized children. These prevalence rates are 30% and 40%, respectively. It is interesting that a low 4% prevalence rate of norovirus infection was reported in hospitalized children in Bangladesh.

In spite of considerable variation in the prevalence of norovirus gastroenteritis, it is clear that the noroviruses are an important cause of gastrointestinal illness worldwide.

**V. CLINICAL SYMPTOMS OF NOROVIRUS GASTROENTERITIS**

Clinical symptoms in infected persons may vary. Studies with volunteers have shown that approximately one-third of individuals infected with norovirus do not show any symptoms. Other infected volunteers develop only diarrhea or only vomiting. Early in the history of norovirus gastroenteritis this illness was known as the “winter vomiting disease.”

The incubation period is generally 10 to 51 hours. Initial symptoms may be vomiting, followed by abdominal cramps and diarrhea, which is watery and may occasionally show the presence of blood and mucus. Additional symptoms may be present, such as headache, myalgia, fever, chills, fecal urgency, and flatulence. The duration of illness is generally 2 to 3 days but can be 4 to 6 days. Longer duration of illness is more common in children younger than 11 years of age.

Virus may be shed prior to appearance of symptoms and up to 8 weeks or longer in previously healthy persons after resolution of the illness. Viral shedding may continue for more than a year in immunocompromised persons and in patients who had undergone organ transplantation.

**Severity of disease:**

The gastroenteritis is usually relatively mild and self-limited. Dehydration can occur and fatalities have been reported in association with gastroenteritis cases among the elderly in
nursing homes. In the United Kingdom, it has been reported that close to 80 deaths per year take place among persons older than 64 years of age. The severity of norovirus gastroenteritis is greater in immunocompromised patients.

Role of norovirus in other clinical syndromes:
Recent reports link norovirus infections to necrotizing enterocolitis in newborns and to seizures in infants. Norovirus may have an adverse effect on the course of inflammatory bowel disease in pediatric patients.

VI. PATHOGENESIS OF INFECTION
Much of the information on pathogenesis of norovirus infections comes from studies of more than 1,000 volunteers who were experimentally infected with norovirus. The subjects who developed symptoms of illness underwent intestinal biopsies in order to determine the site and progress of infection.

Norovirus is an intestinal pathogen. Once the virus is ingested, it localizes in portions of the small intestine. The stomach and the large intestine do not appear to be affected, although gastric emptying is delayed. The reduced gastric motility may be responsible for the nausea and vomiting associated with gastroenteritis.

The first step in the infectious process is the attachment of norovirus P2 protein to specific host cell receptors found on the mucosa of the small intestine. Biopsy samples from the small intestine (the jejunum) show cellular damage and infiltration of the mucosa by polymorphonuclear and mononuclear cells. There is no indication of viral spread from the small intestine to other organs.

Additional information on norovirus pathogenesis comes from studies in germ-free calves and pigs. The animals were infected with the human norovirus GII/4 strain. In studies with germ-free pigs 74% percent of the inoculated animals developed mild diarrhea. Infection of patches of mucosal cells in the duodenal and jejunal portions of the small intestine was demonstrated by immunofluorescent microscopy. Transmission electron microscopy of the small intestine showed disrupted cells containing virus particles that were 25 to 40 nanometers in diameter. Immunological tests and molecular tests of rectal swab fluids confirmed infection of pigs with the human norovirus strain GII/4. Similarly, infection of germ-free calves with the human norovirus GII/4 resulted in intestinal lesions and in the demonstration of viral capsid antigen in the jejunum.

VII. EPIDEMIOLOGY
Sporadic cases of norovirus gastroenteritis occur throughout the year independent of seasons. Norovirus outbreaks have a tendency to cluster in late autumn and in the winter months (thus the term “winter vomiting disease”).

Reservoir of infection
Humans are the only proven source of norovirus infections. Recent findings have raised the possibility of zoonotic transmission as well. The genogroups GII and GIV contain both human and animal strains. Mixed infection with more than one strain accompanied by genetic recombination between viral strains had been demonstrated. Human norovirus strains do not have strict species specificity, as demonstrated by experimental infection of pigs and calves with human norovirus strains. A study in Montreal, Canada reported the presence of viruses resembling human GII/4 strains in pig stool samples and in retail meat in Canada. Historically,
viruses from the calicivirus group have been known to cross the species barrier. For example, the San Miguel sea lion virus infects swine and the feline calicivirus can cause disease not only in cats but also in dogs and in sea lions.

**Transmission of infection**

Transmission of norovirus infection can occur through a variety of routes. These include person-to-person spread, transmission through ingestion of contaminated food or water, spread of infection by the fecal-oral route or through fomites due to poor hygiene, and spread through aerosols. In some cases, persons become ill without having direct contact with the index case. For example, aerosol transmission had been shown for persons who walked through an emergency department where a person was vomiting. Similarly, infection through aerosols may occur in airplanes and in restaurants, even when the person vomiting is seated at a separate table. Food is an important vehicle for transmission of infection. Infected food handlers may be involved or the food itself may be contaminated, such as shellfish from waters contaminated with sewage or raw vegetables grown in fields fertilized with untreated sewage. In all instances, norovirus must enter the digestive tract in order to initiate infection. Respiratory transmission for norovirus infection had not been demonstrated.

**Host factors in susceptibility to infection**

**Demographic factors**

1. **Age:** norovirus infections occur in persons of all ages. The illness appears to be more severe in persons over the age of 65. The course of the disease may be prolonged in older patients, with a greater possibility of serious complications.
2. **Gender:** there is no apparent difference in the prevalence of infection in persons of either sex.
3. **Race:** a single, large-scale study among military personnel found that blacks have a lower risk of norovirus infection than the non-black members of the study group.
4. **Immunity:** the occurrence of norovirus infections at all ages indicates that either the immunity to re-infection does not last or that there is a large diversity of norovirus strains. Circulating antibody to norovirus can be demonstrated in infected persons. It may provide some protection against re-infection with the same strain of virus, but such protection is transitory and does not extend beyond a few weeks. Mucosal antibody, when present, offers greater protection against re-infection with the same strain of norovirus.
5. **Genetic resistance to infection with norovirus:** studies using volunteers demonstrate that some individuals are repeatedly susceptible to infection with norovirus while others are consistently resistant. Further studies have shown that an individual’s ABH (ABO) blood group antigens are involved in susceptibility or resistance to norovirus infection. The human ABH blood group antigens are complex carbohydrates present on red blood cells and on the mucosal layers of digestive, respiratory, and genitourinary tracts. These antigens are organized into several families, are highly polymorphic and are controlled by multiple gene families. In the majority of individuals these antigens are present not only on red blood cells and epithelial surfaces of various organs but also in the biologic fluids associated with the mucosal cell layers. Such individuals are called “secretors” and constitute approximately 80% of population. In about 20% of the population some of the blood group antigens are not expressed on the mucosal surfaces of their organs and are not present in soluble form in body fluids. Such individuals are called “non-secretors.”
ABH blood group antigens are important in epidemiology of norovirus infections because these antigens serve as host cell receptors for noroviruses. Detailed studies of viral binding to host cells have used sub-viral particles generated in *E. coli* expression systems. The protruding (P) portion of the viral capsid participates in cell binding. With the use of X-ray crystallography, it had been possible to demonstrate formation of hydrogen bond networks between sites on the ABH blood group antigens and the viral P protein. The binding sites are strain specific.

If an individual is a non-secretor, he may lack cell receptors for noroviruses on the surface of the intestinal mucosa. Such persons may be resistant to norovirus infection.

**Norovirus gastroenteritis outbreaks**

Several norovirus outbreaks are described below. Each outbreak illustrates different aspects of norovirus epidemiology.

Shellfish is commonly involved in outbreaks of norovirus gastroenteritis. Mollusks such as oysters filter large quantities of water as part of their feeding activities, allowing them to concentrate enteric pathogens in their tissues. Sewage treatment does not eliminate all viral enteric pathogens. Consequently, contamination of coastal waters may occur with sewage that contains infectious enteric pathogens. Contamination of drinking water, vegetables, and shellfish is also possible.

Noroviruses has been shown to persist in oysters. The virus binds in a specific manner to oyster tissues such as gills and digestive glands. This binding is similar to attachment of norovirus to ABH blood group antigen receptors in the human digestive tract. Norovirus binding site for oyster cells is the same as in human infection. The binding of norovirus to carbohydrate receptors in oyster tissue can be inhibited by saliva taken from individuals who are blood group A secretors. The specific attachment of norovirus to oyster tissue makes it difficult to eliminate this virus from contaminated oysters.

**An outbreak of norovirus gastroenteritis traced to oyster contamination**

In February 2008, a lunch banquet was held for 80 persons in Brittany, France. The guests were in two separate rooms, with oysters served in only one room. The menu included fish, lobster, shrimp, oysters, cheese, salad, and fruit tarts for dessert. All persons who became ill with gastroenteritis ate oysters, with no sign of disease in people who did not consume oysters. Twenty-three persons out of thirty-four who ate oysters became ill. Symptoms developed in eight to fifty hours after the meal, with an average incubation period of 33.4 hours. The symptoms included vomiting and diarrhea. The duration of symptoms ranged from half a day to six days. Only two persons consulted a physician. Analysis of stool samples from patients yielded norovirus Group II, sapovirus, and Aichi virus (a picornavirus). Samples of oysters that were served at the restaurant contained norovirus RNA. Sequence analysis of norovirus RNA from oysters corresponded to the GII/4 strain detected in two stool samples.

The 33 persons who ate oysters were tested to determine their blood type and secretor status. It was found that the frequency of illness was lower among non-secretors than among secretors. When illness was observed in non-secretors it was associated with ingestion of larger portions of contaminated oysters, indicating that a higher viral infecting dose is needed for infection of non-secretors. There was less vomiting and nausea in non-secretors who became ill than in the secretor group of patients. Persons who were blood group A and were secretors had the same rate of infection as non-secretors and were less likely to show symptoms of diarrhea. Apparently, epithelial expression of blood group A antigen interferes with the recognition of the host cell receptor by norovirus.
In order to determine the source of oyster contamination, the oysters were sampled in the waters where shellfish were grown. Oysters involved in this outbreak were grown in an area designated class A (a production area with low coliform counts). Viral contamination was not found in oysters from the growth area. A police investigation followed and resulted in the arrest of a fisherman who illegally collected oysters from an area in a major harbor where oyster collection was forbidden. The fisherman eventually admitted selling the oysters to the producer involved in the outbreak.

This outbreak illustrates the public health risks connected with illegal harvest of oysters, the important function of viral receptors for host tissue cells, and the role of blood group antigens in norovirus epidemiology.

**Norovirus gastroenteritis outbreaks on cruise ships**

Epidemics of norovirus gastroenteritis are well known and have led to the term “the cruise ship virus” as another name for noroviruses. Transmission on cruise ships frequently occurs by many routes, including infected food, contamination of drinking water, person-to-person contact, and from contaminated environments. The infecting virus strain may be introduced on board ship by the crew or by passengers who are either asymptomatic or show symptoms of infection. Once introduced, noroviruses are difficult to eradicate from the ship’s environment. Outbreak strains may persist, leading to sequential epidemics on the same ship. Cruise ships have implemented control measures that include intensified ship cleaning, emphasis on proper hygiene for passengers and crew, food and water sanitation measures, disinfection of ships during cruises, and isolation of ill passengers and crew for 72 hours.

The investigation described below is an outbreak of norovirus gastroenteritis that affected six consecutive cruises and resulted in the spread of the infecting norovirus strain to the community.

The outbreak took place on a ship that was on a 7-day vacation cruise from Florida to the Caribbean. There were 2,456 passengers and 999 crewmembers on board ship. The outbreak began abruptly on the second day of the first trip and continued on cruise 2 with new passengers. Despite the fact that the ship was sanitized for one week after cruise 2, illness on board ship was reported during subsequent cruises.

An investigation of the original outbreak indicated that infection originated from food served in ship’s restaurants. The incubation period appeared to be in the range of 24 to 48 hours. The specific foods or food handlers involved in the initial infections were not identified. Some of the illnesses developed as late as the 5th day of the cruise and were most probably due to contact transmission of norovirus from other passengers who were already ill. Despite control measures, 4% of passengers on cruise 1 became ill. One of the passengers on cruise 1 was still ill at the conclusion of the cruise. When he returned to the long-term care facility where he was a resident, an outbreak of norovirus gastroenteritis took place in that facility. The outbreak continued on the second vacation trip of this ship, after which the ship was sanitized for one week. In spite of thorough cleaning, norovirus gastroenteritis occurred on subsequent trips 3, 4, 5, and 6.

Stool samples were tested from passengers on all cruises. Norovirus was identified in 45% of samples and belonged to 6 strains. Genetic sequences of norovirus strains found in specimens from the first two outbreaks were identical. The implicated strain was norovirus group II, genotype 4. The same virus strain was demonstrated in specimens collected from outbreak 3, indicating a failure in ship sanitizing procedures. However, additional virus strains with different genetic sequences were also present in stool samples collected from outbreaks 3, 4, 5, and 6.
Apparently, new viral strains were introduced on board ship by passengers on cruises 3, 4, 5, and 6.

This outbreak is an illustration of an epidemic of norovirus gastroenteritis that affected several hundred people, was transmitted by multiple modes, and recurred on subsequent cruises. Spread of the infecting strain to the community was documented. Sanitizing measures did not prove effective. Multiple strains of norovirus were involved as new passengers boarded the ship, but the original epidemic strain belonged to norovirus genogroup II, genotype 4. A full epidemiologic study was done only on cruises where the number of ill persons exceeded 3%. A widespread outbreak in a temporary shelter among evacuees of Hurricane Katrina.

This report describes a very large outbreak of norovirus gastroenteritis in a shelter that housed thousands of people. On August 29, 2005, Hurricane Katrina landed on Louisiana coast. The hurricane caused catastrophic damage, leaving thousands of people homeless and displaced. New Orleans was badly damaged and many residents were placed in temporary shelters. As conditions in New Orleans deteriorated residents were moved to shelters in other cities. On August 31, 2005, buses began relocation of New Orleans shelter residents to Houston, Texas. The evacuees were housed in the Reliant Park Complex, a large, multifacility sporting area. Approximately 27,000 evacuees were relocated to Reliant Park Complex and were provided with clean food, water, clothes, sleeping accommodations, access to toiletries and showers, and other necessities. Two days after arrival at the shelter a large number of evacuees began coming to the Reliant Clinic with symptoms of gastroenteritis that included vomiting and diarrhea. Both adults and children were affected, with approximately 59% of patients among adults. The entire outbreak affected more than 1,000 persons over an 11-day period. Stool and vomitus specimens were collected and tested for bacterial enteric pathogens, ova and parasites, and enteric viruses, including norovirus and rotavirus. Norovirus was identified by reverse transcriptase–polymerase chain reaction (RT-PCR) in 45% of specimens that were submitted for testing. The tests identified at least three different norovirus strains. The predominant strain belonged to the GII.17 group with seven different variant sub-groups. A common source of infection could not be identified. Transmission was mostly person-to-person or from aerosols that contained virus particles. The presence of multiple norovirus strains suggested multiple introductions of infecting strains, multiple sources of infection, and a continuing epidemic with the cases appearing daily. Control measures that were introduced to limit the outbreak did not appear to have a major effect. These control measures included installation of additional portable bathrooms and sinks, distribution of alcohol-based sanitizers, isolation of ill persons with active symptoms of gastroenteritis, implementation of environmental controls, such as increased maintenance of all public areas used by patients, and a recommendation of a change to bleach-based cleaning products.

This outbreak illustrates the difficulties involved in the control of large epidemics where many persons live in close quarters and under crowded conditions. The presence of multiple norovirus strains indicates the wide distribution of these viruses in general population and the potential for occurrence of gastroenteritis outbreaks under conditions favorable to spread of infection.

Surveillance of norovirus outbreaks in the United States

Occurrence of outbreaks is reported by each state to the Center for Disease Control and Prevention (CDC). Surveillance for norovirus outbreaks is conducted through the recently implemented National Outbreak Reporting System (NORS). Norovirus outbreaks may also be
reported to CDC’s National Calicivirus Laboratory when specimens are submitted for testing or sequencing.

VIII. TREATMENT AND PREVENTION

Treatment of norovirus gastroenteritis is similar to that of other diarrheal illnesses, relying on hydration with fluids and electrolytes. Antimotility and antisecretory agents may be useful in adults to decrease diarrhea. At the present time, antiviral drugs for norovirus gastroenteritis are not yet available. Studies leading to development of antiviral medications are in progress.

Prevention of norovirus gastroenteritis can be difficult since the noroviruses are quite resistant to heat, cold, and chemicals. They are able to survive temperatures as high as 60˚C as well as freezing temperatures. There are reports of steamed oysters transmitting norovirus infection. Noroviruses survive in chlorinated drinking water and are only moderately affected by alcohol-based disinfectants. In spite of these attributes of noroviruses, one of the most important means of preventing norovirus infection is by frequent and thorough hand washing. High level of personal hygiene, prevention of sewage contamination of food and water, and disinfection of surfaces that may have been contaminated by norovirus are all of prime importance. Food handlers who are ill should not be allowed to remain on the job. A study of norovirus infection in food handlers in Japan reported high rates of norovirus infection in food handlers. These individuals were asymptomatic but continued to shed norovirus for prolonged periods of time.

Vaccine development

Outbreaks of norovirus gastroenteritis are difficult to control and a norovirus vaccine could limit spread of norovirus infection. Current vaccine development is focused on virus-like particles expressed in experimental systems. Studies in mice have shown that virus-like particles administered to mice by the oral, intranasal, or parenteral routes are highly immunogenic. In another study, human volunteers were immunized with virus-like particles expressed in plants and in baculovirus; the experimental vaccine was both safe and immunogenic. It is not known whether these vaccine preparations offer any protection against disease or the duration of any protection that may follow vaccination. Clinical studies of the effectiveness of these vaccines are continuing.

IX. IMMUNE RESPONSE IN NOROVIRUS INFECTION

Immunity to norovirus is strain-specific and appears to last only a few weeks after norovirus infection. The large number of norovirus strains and continued evolution of new strains are reasons why individuals are likely to be repeatedly infected throughout their lifetime.

Immune response to norovirus has been studied in experimental animal systems. Gnotobiotic (germ-free) calves were infected with a human norovirus strain. Antibody and cytokine profiles in serum, feces, and intestinal contents were determined by enzyme-linked immunosorbent assay 3 days and 28 days after infection with norovirus. The majority of calves (67%) developed serum antibody and all of the animals had antibody in the feces. Antibodies of the immunoglobulin A (IgA) class and of the immunoglobulin G (IgG) class were found. The highest number of antibody producing cells, IgG and IgA, were found in the intestines.

In another study, germ-free pigs were inoculated with a human norovirus strain. Serum antibody to norovirus could be demonstrated 21 days after the inoculation of animals.

X. DIAGNOSIS OF NOROVIRUS INFECTION

Clinical diagnosis
In cases where laboratory identification of the cause of infection is not possible, clinical characteristics may be used to arrive at a tentative diagnosis. In diagnosis of gastrointestinal disease, the following criteria are helpful: no bacterial agent is found, illness duration is an average of 12 to 60 hours, the incubation period is 24 to 48 hours, and more than 50% of ill individuals have vomiting as one of the symptoms of their illness. These disease characteristics are known as the Kaplan criteria. When all four criteria are present, it is highly probable that the illness is caused by noroviruses. However, about 30% of norovirus outbreaks do not meet these criteria and the possibility that an outbreak has viral etiology should not be discarded.

**Laboratory diagnosis**

Human noroviruses have not been grown in cell cultures and alternate techniques for identification of these viruses must be used. With the development of molecular diagnostic techniques, it became possible to identify norovirus RNA in fecal specimens, water, and food. The real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR) is the reference method for identification of norovirus. This method is available in all state public health laboratories. In order to detect the large variety of norovirus strains, a cocktail of primers is required for RT-PCR. This assay can detect norovirus RNA in swabs taken from environmental samples, stool specimens and fecal swabs, and in vomit samples. For best results stool specimens should be examined during the acute phase of illness (48 to 72 hours after initial symptoms) but positive results can be obtained as late as 5 days after onset of illness.

Sequencing of viral RNA from clinical and environmental samples is useful in epidemiologic studies. The RNA sequences can be entered into CaliciNet, a national network of public health laboratories that keeps track of the different sequences of noroviruses involved in infections. This system allows rapid identification of new strains as they arise.

Demonstration of antibody to norovirus may be helpful in diagnosis of norovirus infections, particularly in outbreaks of gastroenteritis. The enzyme-linked immunosorbent assay (ELISA) is used to test for the presence of specific antibody to norovirus. The tests should be done during the acute as well as during the convalescent stages of the illness in order to detect any change in antibody titer. A four-fold increase in titer indicates a current infection.

The ELISA assay can be modified to detect the presence of norovirus antigen in feces or other types of clinical samples. In general, ELISA (enzyme immunoassays) have low sensitivity (50-75%). They are mainly useful for preliminary identification of norovirus when testing multiple specimens.

**Commercial assays: multiplex gastrointestinal platforms**
Commercial assays for detection of multiple gastrointestinal pathogens, including norovirus, are now available. Sensitivity of these assays is similar to reverse transcriptase assays. These assays include the following:

- FilmAssay Gastrointestinal Panel
- TAG Gastrointestinal Pathogen Panel
- Verigene Enteric Pathogens Test

The presence of norovirus particles in clinical specimens can be demonstrated by electron microscopy, including immuno-electron microscopy.

**IX. ANIMAL MODELS**

Models of norovirus gastroenteritis have been developed in gnotobiotic pigs and calves. Both animal models have been used to study the pathogenesis of norovirus gastroenteritis and the development of immune response to norovirus infection.
The recent discovery of murine norovirus-1 has provided an efficient cell culture model for the study of norovirus replication. The murine norovirus-1 replicates in murine macrophages and dendritic cells in contrast to human noroviruses for which a routine cell culture system is not available.

X. SUMMARY

Norovirus gastroenteritis is highly prevalent worldwide. In the United States, there are more than 23 million norovirus infections per year. More than 50% of all food-borne disease outbreaks may be caused by noroviruses according to the CDC.

Outbreaks of non-bacterial gastroenteritis had been reported for many years but the causative agents of these outbreaks were not known. Viral particles could be demonstrated in fecal specimens from gastroenteritis patients by electron microscopy. In the 1990’s the genome of an infectious agent involved in a gastroenteritis outbreak in Norwalk, Ohio was sequenced and identified as a virus from the Caliciviridae family. The Norwalk agent was placed in a new genus named Norovirus.

The noroviruses are intestinal pathogens that infect many species of animals, including pigs, lions, mice, dogs and monkeys. These viruses are genetically diverse and are classified in five groups with a large number of sub-types. Human noroviruses have been placed in genogroups I, II, and IV. Noroviruses are approximately 38 to 40 nm in diameter and have an icosahedral shape. The norovirus genome consists of single-stranded RNA. The icosahedral capsid is constructed from identical protein subunits. Some of the protein subunits protrude from the capsid, forming viral receptors. These receptors bind neutralizing antibody and attach to specific host cell receptors. The initial step in norovirus infection is attachment of virus to cell receptors expressed on the surface of mucosal cells in the small intestine. These receptors are ABH blood group antigens. Approximately 20% of individuals lack the ABH blood group antigens on the surface of the intestinal mucosal layer. Such persons are called non-secretors and they exhibit a varying degree of resistance to infection with noroviruses.

Noroviruses play a major role in gastrointestinal illness. Outbreaks of norovirus gastroenteritis affect nursing homes, schools, cruise ships, camps, shelters, restaurants, and hospitals. These outbreaks are difficult to control and may involve several different norovirus strains. The noroviruses are frequent causative agents of sporadic cases of gastroenteritis that occur within communities and are also involved in travelers’ diarrhea (gastrointestinal illness common in travelers from industrialized countries to high-risk developing regions). The important role of noroviruses as human pathogens is facilitated by their high infectivity, ease of transmission of infection, resistance of noroviruses to adverse environmental conditions, and continual evolution of new virus strains. Immunity to norovirus is strain-specific and variation in strain specificity results in new outbreaks of disease.

Currently there is no specific treatment for norovirus gastroenteritis; a vaccine for norovirus infections is in the research stage of development.

Diagnosis of norovirus gastroenteritis relies on molecular methods. The real-time RT-PCR assay is the method of choice. Other diagnostic methods include electron microscopy and ELISA antibody assays.

REFERENCES

Norovirus

REVIEW QUESTIONS

Course #DL-996

Choose the one best answer:

1. The Norwalk agent is a
   a. fungus
   b. virus
   c. bacterium
   d. protozoan

2. Symptoms of norovirus gastroenteritis include
   a. vomiting
   b. sneezing
   c. coughing
   d. rash

3. Winter vomiting disease refers to
   a. rotavirus infections
   b. adenovirus infections
   c. typhoid fever
   d. norovirus gastroenteritis

4. The Norwalk agent is a member of
   a. influenza virus group
   b. family Caliciviridae
   c. genus Sapovirus
   d. genus Vesivirus

5. Caliciviruses infect
   a. only plants
   b. only humans
   c. many animal species
   d. only marine mammals

6. Noroviruses infect
   a. only humans
   b. plants
   c. several animal species
   d. bacteria

7. Noroviruses
   a. have a double-stranded DNA genome
   b. have a helical capsid
   c. are single-stranded RNA viruses
   d. do not have a protein capsid
8. The norovirus capsid
   a. consists of 180 protein subunits
   b. has a spiral shape
   c. is surrounded by a lipid envelope
   d. consists of glucose subunits

9. Noroviruses
   a. are highly infectious
   b. are easily inactivated by disinfectants
   c. cannot be transmitted person-to-person
   d. are unable to survive cold temperatures

10. Norovirus gastroenteritis is transmitted
    a. only by contaminated vegetables
    b. only by contaminated shellfish
    c. by oral-fecal route
    d. only by personal contact

11. Noroviruses play an important role in diseases such as
    a. pneumonia
    b. common cold
    c. arthritis
    d. travelers’ diarrhea

12. The epidemic strains of norovirus cited in discussions of the recent outbreaks belong to
    a. genogroup I
    b. genogroup II
    c. genogroup III
    d. genogroup IV

13. Noroviruses infect the
    a. respiratory tract
    b. skin
    c. genital tract
    d. intestinal mucosa

14. Histo-blood group antigens are
    a. used as host cell receptors by noroviruses
    b. identical in all individuals
    c. never present in body fluids
    d. expressed on mucosal surfaces of all individuals
15. Persons who are non-secretors are
   a. always blood type A
   b. identical in all individuals
   c. have increased resistance to norovirus infection
   d. are susceptible to all virus infections

16. Genetic susceptibility to norovirus infections is related to
   a. age
   b. gender
   c. eye pigmentation
   d. histo-blood group antigens

17. Control measures for norovirus gastroenteritis outbreaks include
   a. improved environmental disinfection
   b. extensive use of penicillin
   c. isolation of all patients for several months
   d. treatment of patients with broad-spectrum antibiotics

18. Immune response to norovirus
   a. has not been demonstrated
   b. is strain-specific
   c. lasts for entire lifetime
   d. occurs only in non-secretors

19. Laboratory identification of norovirus infections relies on
   a. culture of clinical samples
   b. inoculation of guinea pigs
   c. real-time RT-PCR
   d. gram stains of clinical specimens

20. Immunologic tests used in diagnosis of norovirus infections include
   a. ELISA assays
   b. complement fixation tests
   c. blood typing
   d. slide agglutination tests