In January, a 6-month-old boy was seen in the emergency department after 2 days of persistent watery diarrhea and vomiting accompanied by a low-grade fever and mild cough. The infant appeared dehydrated and required hospitalization. The patient attended a day-care center.

1. In addition to rotavirus, what other viral agents must be considered in the differential diagnosis of this infant’s disease? What agents would need consideration if the patient were a teenager or an adult?
2. How would the diagnosis of rotavirus have been confirmed?
3. How was the virus transmitted? How long was the patient contagious?
4. Who was at risk for serious disease?

Answers to these questions are available on StudentConsult.com.

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**SUMMARIES**

**Clinically Significant Organisms**

**Reoviruses**

**Trigger Words**

Fecal-oral, infantile diarrhea, double-double (capsid and double-stranded segmented RNA genome), oral vaccine

**Biology, Virulence, and Disease**

- Medium size, double capsid, double-stranded segmented RNA genome
- Capsid resistant to inactivation
- Encodes RNA-dependent RNA polymerase, replicates in cytoplasm
- Each segment encodes one or two proteins
- Mixed infection results in genetic mixing of segments: reassortment
- Rotavirus induces cholera-type diarrhea
- One of the most serious causes of diarrhea in young children
- Colorado tick fever, zoonosis, dengue-like disease with rash

**Epidemiology**

- Rotavirus
- Worldwide and ubiquitous, occurs year round

- Fecal-oral spread, very contagious, young children at risk for serious disease

**Diagnosis**

- ELISA for virus in stool

**Treatment, Prevention, and Control**

- Treatment: supportive rehydration
- Prevention: oral live vaccines administered 2, 4, 6 months of age
- Control: hand washing and good hygiene

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The Reoviridae consist of the orthoreoviruses, rotaviruses, orbiviruses, and coltiviruses (Table 51-1). The name reovirus was proposed in 1959 by Albert Sabin for a group of respiratory and enteric viruses that were not associated with any known disease (respiratory, enteric, orphan). The Reoviridae are nonenveloped viruses with double-layered protein capsids containing 10 to 12 segments of the double-stranded ribonucleic acid (dsRNA) genomes. These viruses are stable in detergents, over wide pH and temperature ranges, and in airborne aerosols. The orbiviruses and coltiviruses are spread by arthropods and are arboviruses.

The orthoreoviruses, also referred to as mammalian reoviruses or simply reoviruses, were first isolated in the 1950s from the stools of children. They are the prototype of this virus family, and the molecular basis of their pathogenesis has been studied extensively. In general, these viruses cause asymptomatic infections in humans.

Rotaviruses cause human infantile gastroenteritis, a very common disease. In fact, rotaviruses account for approximately 50% of all cases of diarrhea in children requiring hospitalization because of dehydration. Rotaviruses are even more of a problem in underdeveloped countries, where before the development of vaccines they were responsible for at least 1 million deaths each year from uncontrolled viral diarrhea in undernourished children. Fortunately, newer vaccines have lessened the incidence of this disease worldwide.
Answers

1. Since this is a watery diarrhea, norovirus, adenovirus, and bacterial agents such as cholera and toxigenic *Escherichia coli* must be considered. These agents would also cause diarrhea in adults.

2. Rotavirus can be detected in stool by enzyme-linked immunosorbent assay. Reverse transcriptase polymerase chain reaction can also be used.

3. The virus is transmitted by the fecal-oral route. The patient is contagious for 2 to 5 days after the onset of diarrhea.

4. The baby, because of his small size, is at high risk for dehydration.
that includes enzymes for RNA synthesis and 10 (reovirus) or 11 (rotavirus) different double-stranded RNA genomic segments. For rotavirus, the outer capsid has two layers, an intermediate layer consisting of the major capsid protein (VP6) and an outer layer that contains the viral attachment protein (VP4) and glycoprotein (VP7). Of interest, rotaviruses resemble enveloped viruses in that they (1) have glycoproteins (VP7, NSP4) that are on the outside of the virion, (2) acquire but then lose an envelope during assembly, and (3) appear to have a fusion protein activity that promotes direct penetration of the target cell membrane.

The genomic segments of rotaviruses and reoviruses encode structural and nonstructural proteins. As for the influenza virus, reassortment of gene segments can occur and thus create hybrid viruses. The genomic segments of reovirus, the proteins they encode, and their functions are summarized in Table 51-2; those of rotavirus are summarized in Table 51-3. Core proteins include enzymatic activities required for the transcription of messenger RNA (mRNA). They include a 5′-methyl guanosine mRNA capping enzyme and an RNA polymerase. The σ1 protein (reovirus) and VP4 (rotavirus) are located at the vertices of the capsid and extend from the surface like spike proteins. They have several functions, including viral attachment and hemagglutination, and they elicit neutralizing antibodies. VP4 is activated by protease cleavage into VP5 and VP8 proteins, exposing a structure similar to that of the fusion proteins of paramyxoviruses. Its cleavage facilitates productive entry of the virus into cells.
Reoviruses and rotaviruses can also be taken up by receptor-mediated endocytosis. The ISVP releases the core into the cytoplasm, and the enzymes in the core initiate mRNA production. The dsRNA remains in the core. Transcription of the genome occurs in two phases, early and late. In a manner similar to a negative-sense RNA virus, each of the negative-sense (−) RNA strands is used as a template by virion core enzymes, which synthesize individual mRNAs. Virus-encoded enzymes within the core add a 5′-methyl guanosine cap and a 3′-polyadenylate tail. The 5′-methyl guanosine cap was first discovered for reovirus mRNA and then shown to occur for cellular mRNA. The mRNA then leaves the core and is translated. Later, virion proteins and positive-sense (+) RNA segments associate together into corelike structures that...

**Box 51-1 Unique Features of Reoviridae**

**Double-layered capsid** virion (60 to 80 nm) has icosahedral symmetry containing 10 to 12 (depending on the virus) unique double-stranded genomic segments (double:double virus). Virion is resistant to environmental and gastrointestinal conditions (e.g., detergents, acidic pH, drying). Rotavirus and orthoreovirus virions are activated by mild proteolysis to intermediate/infectious subviral particles, increasing their infectivity. Inner capsid contains a complete transcription system, including RNA-dependent RNA polymerase and enzymes for 5′ capping and polyadenylation addition. Viral replication occurs in the cytoplasm. Double-stranded RNA remains in the inner core. Inner capsid aggregates around (+) RNA and transcribes (−) RNA in the cytoplasm. Rotavirus-filled inner capsid buds into the endoplasmic reticulum, acquiring its outer capsid and a membrane, which is then lost. Virus is released by cell lysis.

**Replication**

Replication of reoviruses and rotaviruses starts with ingestion of the virus (Figure 51-5). The virion outer capsid protects the inner nucleocapsid and core from the environment, especially the acidic environment of the gastrointestinal tract. The complete virion is then partially digested in the gastrointestinal tract and activated by protease cleavage and loss of the external capsid proteins (σ3/VP7) and cleavage of the σ1/VP4 protein to produce the ISVP. The σ1/VP4 protein at the vertices of the ISVP binds to sialic acid–containing glycoproteins on epithelial and other cells. Additional receptors include the β-adrenergic receptor for reovirus and integrin molecules for rotavirus. The VP4 of rotavirus also promotes penetration of the virion into the cell. Whole virions of reovirus and rotavirus can also be taken up by receptor-mediated endocytosis.

The ISVP releases the core into the cytoplasm, and the enzymes in the core initiate mRNA production. The dsRNA always remains in the core. Transcription of the genome occurs in two phases, early and late. In a manner similar to a negative-sense RNA virus, each of the negative-sense (−) RNA strands is used as a template by virion core enzymes, which synthesize individual mRNAs. Virus-encoded enzymes within the core add a 5′-methyl guanosine cap and a 3′-polyadenylate tail. The 5′-methyl guanosine cap was first discovered for reovirus mRNA and then shown to occur for cellular mRNA. The mRNA then leaves the core and is translated. Later, virion proteins and positive-sense (+) RNA segments associate together into corelike structures that...
e virus loses the envelope and leaves the cell on cell lysis. The inner capsids aggregate and "dock" onto the NSP4 protein in the endoplasmic reticulum, acquiring VP7 and its outer capsid and an envelope. The mRNA is also enclosed into inner capsids as a template to replicate the double-stranded genome. VP7 and NSP4 are synthesized as RNA binding, important for genome replication and packaging.

<table>
<thead>
<tr>
<th>Genomic Segments (Molecular Weight, Da)</th>
<th>Protein</th>
<th>Function (If Known)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large Segments (2.8 × 10^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>λ3 (inner capsid)</td>
<td>Polymerase</td>
</tr>
<tr>
<td>2</td>
<td>λ2 (outer capsid)</td>
<td>Capping enzyme</td>
</tr>
<tr>
<td>3</td>
<td>λ1 (inner capsid)</td>
<td>Transcriptase component</td>
</tr>
<tr>
<td>Medium Segments (1.4 × 10^6)</td>
<td>μ2 (inner capsid)</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>μ1C (outer capsid)</td>
<td>Cleaved from μ1, complexes with σ3, promotes entry</td>
</tr>
<tr>
<td>3</td>
<td>μNS</td>
<td>Promotes viral assembly*</td>
</tr>
<tr>
<td>Small Segments (0.7 × 10^6)</td>
<td>σ1 (outer capsid)</td>
<td>Viral attachment protein, hemagglutinin, determines tissue tropism†</td>
</tr>
<tr>
<td>2</td>
<td>σ2 (inner capsid)</td>
<td>Facilitates viral RNA synthesis</td>
</tr>
<tr>
<td>3</td>
<td>σNS</td>
<td>Facilitates viral RNA synthesis</td>
</tr>
<tr>
<td>4</td>
<td>σ3 (outer capsid)</td>
<td>Major component of outer capsid with μ1C</td>
</tr>
</tbody>
</table>


*Proteins are not found in the virion.
†Target of neutralizing antibodies.

**FIGURE 51-5 Replication of rotavirus.** Rotavirus virions can be activated by protease (e.g., in the gastrointestinal tract) to produce an intermediate/infectious subviral particle (ISVP). The virion or ISVP binds, penetrates the cell, and loses its outer capsid. The virus loses the envelope and leaves the cell upon cell lysis. Centrioles aggregate into large cytoplasmic inclusions. The (+) RNA segments are copied to produce (−) RNAs in the new cores, replicating the double-stranded genome. The new cores either generate more (+) RNA or are assembled into virions.

The assembly processes for reovirus and rotavirus differ. In the assembly of reovirus, the outer capsid proteins associate with the core, and the virion leaves the cell upon cell lysis. Assembly of rotavirus resembles that of an enveloped virus in that the rotavirus cores associate with the NSP4 viral protein on the outside of the endoplasmic reticulum (ER); on budding into the ER, they acquire its VP7 outer capsid glycoprotein. The membrane is lost in the ER, and the virus leaves the cell during cell lysis. Cellular macromolecular synthesis is inhibited within 8 hours of infection.

### Orthoreoviruses (Mammalian Reoviruses)

The orthoreoviruses are ubiquitous. The virions are very stable and have been detected in sewage and river water. The mammalian reoviruses occur in three serotypes referred to as reovirus types 1, 2, and 3; these serotypes are based on neutralization and hemagglutination inhibition tests.
Pathogenesis and Immunity
Orthoreoviruses do not cause significant disease in humans. However, studies of reovirus disease in mice have advanced our understanding of the pathogenesis of viral infections in humans. Depending on the reovirus strain, the virus can be neurotropic or viscerotropic in mice. The functions and virulence properties of the reovirus proteins were identified through comparison of the activities of interstrain hybrid (reassortant) viruses that differ in only one genomic segment (encoding one protein). With this approach, the new activity is attributable to the genomic segment from the other virus strain.

After ingestion and proteolytic production of the ISVP, the orthoreoviruses bind to M cells in the small intestine, which then transfer the virus to the lymphoid tissue of Peyer patches lining the intestines. The viruses then replicate and initiate a viremia. Although the virus is cytopathic in vitro, it causes few if any symptoms before entering the circulation and producing infection at a distant site. In the mouse model, the viral attachment protein (G1) facilitates viral spread to the mesenteric lymph nodes and determines whether the virus is neurotropic.

Mice, and presumably humans, mount protective humoral and cellular immune responses to outer capsid proteins. Although orthoreoviruses are normally lytic, they can also establish persistent infection in cell culture.

Epidemiology
The virus is primarily spread by the fecal-oral route and potentially in aerosols. As already mentioned, the orthoreoviruses have been found worldwide. Most people are infected during childhood.

Clinical Syndromes
Orthoreoviruses infect people of all ages; linking specific diseases to these agents has been difficult. Most infections are asymptomatic or so mild they go undetected. These viruses have been linked to common coldlike, mild upper respiratory tract illness (low-grade fever, rhinorrhea, and pharyngitis), gastrointestinal tract disease, and biliary atresia.

Laboratory Diagnosis
Human orthoreovirus infection can be detected through assay of the viral antigen or RNA in clinical material, virus isolation, or serologic assays for virus-specific antibody. Throat, nasopharyngeal, and stool specimens from patients with suspected upper respiratory tract or diarrheal disease are used as samples. Human orthoreoviruses can be isolated using mouse L-cell fibroblasts, primary monkey kidney cells, and HeLa cells. Serologic assays can be performed for epidemiologic purposes.

Treatment, Prevention, and Control
Orthoreovirus disease is mild and self-limited. For this reason, treatment has not been necessary, and prevention and control measures have not been developed.

• Rotaviruses
Rotaviruses are common agents of infantile diarrhea worldwide. The rotaviruses are a large group of gastroenteritis-causing viruses infecting many different mammals and birds.

Rotavirus virions are relatively stable to environmental abuse, including treatment with detergents, pH extremes of 3.5 to 10, and even repeated freezing and thawing. Within the intestine, proteolytic enzymes such as trypsin enhance infectivity.

Human and animal rotaviruses are divided into serotypes, groups, and subgroups. Serotypes are distinguished primarily by the VP7 (glycoprotein, G) and VP4 (protease-sensitive protein, P) outer capsid proteins. Groups are determined primarily on the basis of the antigenicity of VP6 and the electrophoretic mobility of the genomic segments. Seven groups (A to G) of human and animal rotaviruses have been identified on the basis of the VP6 inner capsid protein. Human disease is caused by group A rotavirus and occasionally group B and C rotaviruses.

Pathogenesis and Immunity
The rotavirus can survive the acidic environment in a buffered stomach or in a stomach after a meal and is converted to the ISVP by proteases (Box 51-2). Viral replication occurs after adsorption of the ISVP to columnar epithelial cells covering the villi of the small intestine. Approximately 8 hours after infection, cytoplasmic inclusions that contain newly synthesized proteins and RNA are seen. As many as 10^10 viral particles per gram of stool may be released during disease. Studies of the small intestine, either of experimentally infected animals or in biopsy specimens from infants, show shortening and blunting of the microvilli and mononuclear cell infiltration into the lamina propria.

Similar to cholera, rotavirus infection prevents absorption of water, causing a net secretion of water and loss of ions, which together result in a watery diarrhea. The NSP4 protein of rotavirus acts in a toxin-like manner to promote calcium ion influx into enterocytes which disrupts the cytoskeleton and the tight junctions to cause leakage and also the release of cytokines and neuronal activators which alter water absorption. The loss of fluids and electrolytes can lead to severe dehydration and even death if therapy does not include electrolyte replacement. Of interest, the diarrhea also promotes spread and transmission of the virus.

Immunity to infection depends upon antibody, primarily immunoglobulin (Ig)A, in the lumen of the gut. Antibodies to the VP7 and VP4 neutralize the virus. Actively or passively acquired antibody (including antibody in colostrum and mothers’ milk) can lessen the severity of disease but does not consistently prevent reinfection. In the absence of antibody, the inoculation of even small amounts of virus causes infection and diarrhea. Infection in infants and small children is generally symptomatic, whereas in adults it is usually asymptomatic.
Epidemiology
Rotaviruses are ubiquitous worldwide, with 95% of children infected by 3 to 5 years of age (Box 51-3). Rotaviruses are passed from person to person by the **fetal-oral route**. Maximal shedding of the virus occurs 2 to 5 days after the start of diarrhea but can occur without symptoms. The virus survives well on fomites (e.g., furniture and toys) and on hands because it can withstand drying. Outbreaks occur in preschools and day-care centers and among hospitalized infants.

Rotaviruses are **one of the most common causes of serious diarrhea in young children** worldwide. Prior to the vaccines, 4 out of 5 children would get rotavirus diarrhea and 1 out of 7 of them required medical help, with 20 to 50 deaths per year in the United States and as many as 500,000 deaths worldwide. In North America, outbreaks occur during the autumn, winter, and spring. More severe disease occurs in severely malnourished children. In developing countries, rotavirus diarrhea is a very contagious, severe, life-threatening disease for infants and occurs year round. Several outbreaks of group B rotavirus have occurred in China because of contaminated water supplies that affected millions of people.

Clinical Syndromes (Clinical Case 51-1; Box 51-4)
Rotavirus is a major cause of gastroenteritis. The incubation period for rotavirus diarrheal illness is estimated to be 48 hours. The major clinical findings in hospitalized patients are **vomiting, diarrhea, fever**, and **dehydration**. Neither fecal leukocytes nor blood occurs in stool for this form of diarrhea. Rotavirus gastroenteritis is a self-limited disease, and recovery is generally complete and without sequelae.

Box 51-3 Epidemiology of Rotavirus

**Disease/Viral Factors**
Capsid virus is resistant to environmental and gastrointestinal conditions. Large amounts of virus are released in fecal matter. Asymptomatic infection can result in release of virus.

**Transmission**
Virus is transmitted in fecal matter, especially in day-care settings. Respiratory transmission may be possible.

**Who Is at Risk?**
**Rotavirus Group A**
Infants < 24 months of age: at risk for infantile gastroenteritis with potential dehydration
Older children and adults: at risk for mild diarrhea
Undernourished people in underdeveloped countries: at risk for diarrhea, dehydration, and death

**Rotavirus Group B (Adult Diarrhea Rotavirus)**
Infants, older children, and adults in China: at risk for severe gastroenteritis

**Geography/Season**
Virus is found worldwide. Disease is more common in autumn, winter, and spring.

**Modes of Control**
Hand washing and isolation of known cases are modes of control. Live vaccines use attenuated human or bovine reassorted rotavirus. However, the infection may prove fatal in infants who are malnourished and dehydrated before the infection.

Laboratory Diagnosis
The clinical findings in patients with rotavirus infection resemble those of other viral diarrheas (e.g., Norwalk virus). Most patients have large quantities of virus in stool, making direct detection of viral antigen the method of choice for diagnosis. Enzyme-linked immunoassay and latex agglutination are quick, easy, and relatively inexpensive ways to detect rotavirus in stool. Viral particles in specimens can also be readily detected on electron microscopy or by immunoelectron microscopy. Reverse transcriptase polymerase chain reaction (RT-PCR) is useful to detect and distinguish the genotypes of rotavirus.

Cell culture of rotavirus requires pretreatment of the virus with trypsin to generate the ISVP for infection to occur but is not used for diagnostic purposes.

Treatment, Prevention, and Control
Rotaviruses are acquired very early in life. Their ubiquitous nature makes it difficult to limit the spread of the virus and infection. Hospitalized patients with disease must be isolated to limit spread of the infection to other susceptible patients.

No specific antiviral therapy is available for a rotavirus infection. The morbidity and mortality associated with rotavirus diarrhea result from dehydration and electrolyte imbalances. Similar to the therapy for cholera, rehydration therapy is necessary to replace fluids so that blood volume and electrolyte and acid-base imbalances are corrected.

Development of a safe rotavirus vaccine was a high priority for protecting children, especially those in underdeveloped countries, from potentially fatal disease. Animal rotaviruses, such as the rhesus monkey rotavirus and the...
Colorado tick fever occurs in western and northwestern areas of the United States and western Canada at elevations of 4000 to 10,000 feet, the habitat of the wood tick Derma-centor andersoni (Figure 51-6). Ticks acquire the virus by feeding on a viremic host and subsequently transmit the virus in saliva when feeding on a new host. Natural hosts of this virus include many mammals, including squirrels, chipmunks, rabbits, and deer. Human disease is observed during the spring, summer, and autumn, seasons when humans are more likely to invade the habitat of the tick.

Clinical Syndromes

Colorado tick fever virus generally causes mild or subclinical infection. The symptoms of the acute disease resemble those of dengue fever. After a 3- to 6-day incubation period, symptomatic infections start with the sudden onset of fever, chills, headache, photophobia, myalgia, arthralgia, and lethargy (Figure 51-7). Characteristics of the infection include a biphasic fever, conjunctivitis, and possibly lymphadenopathy, hepatosplenomegaly, and a maculopapular or petechial rash. A leukopenia involving both neutrophils and lymphocytes is an important hallmark of the disease. Children occasionally have a more severe hemorrhagic disease. Colorado tick fever must be differentiated from Rocky Mountain spotted fever, a tick-borne rickettsial infection characterized by a rash, because the latter disease may require antibiotic treatment.

Laboratory Diagnosis

A diagnosis of Colorado tick fever can be established through direct detection of viral antigens, virus isolation, or serologic
tests. Viral antigen can be detected on the surfaces of erythrocytes in a blood smear through the use of immunofluorescence, and viral genomes can be detected with RT-PCR. Laboratory tests may be available through state public health departments or the Centers for Disease Control and Prevention. Serology can be performed for epidemiologic purposes.

**Treatment, Prevention, and Control**

No specific treatment is available for Colorado tick fever. The disease is generally self-limited, indicating that supportive care is sufficient. The viremia is long lasting, implying that infected patients should not donate blood soon after recovery. Prevention consists of (1) avoiding tick-infested areas, (2) using protective clothing and tick repellents, and (3) removing ticks before they bite. Unlike tick-borne rickettsial disease, in which prolonged feeding is required for the bacteria to be transmitted, the coltivirus from the tick’s saliva can enter the bloodstream rapidly.

**Bibliography**


**Websites**


Case Study and Questions
A 10-month-old Pakistani infant has watery diarrhea, vomiting, and fever for 4 days. The baby becomes very dehydrated and dies.

1. How could a diagnosis of rotavirus be confirmed?
2. How does this agent cause diarrhea?
3. What is the treatment?
4. How can the disease be prevented?
5. Why was this baby at such high risk for mortality?
6. Why is it important to immunize with the rotavirus vaccines so early in life and with a live attenuated oral vaccine?

Answers
1. Commercially available enzyme-linked immunosorbent assays detect rotavirus in stool.
2. The NSP4 protein of rotavirus has a toxin-like (e.g., cholera) activity to promote secretory diarrhea.
3. Treatment is fluid replacement.
4. There are two commercially available vaccines administered as early as possible during the first year of life.
5. Dehydration occurs very rapidly in babies because of their small size and the rapid fluid loss. Lack of access to a hospital and ability to rapidly rehydrate put this baby at greater risk.
6. Protection from rotavirus disease requires the continued presence of virus-specific secretory IgA in the intestine and IgG in the tissue. Infection of the mucosa is the only mechanism to elicit this response. The protection must be generated as early as possible because babies are exposed and at highest risk for serious disease.