**Enterohemorrhagic Escherichia coli Infections**

Verocytotoxin producing *Escherichia coli* (VTEC),
Shiga toxin producing *Escherichia coli* (STEC),
*Escherichia coli* O157:H7

**Importance**

Enterohemorrhagic *Escherichia coli* (EHEC) is a subset of pathogenic *E. coli* that can cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome (HUS), an important cause of acute renal failure in children and morbidity and mortality in adults. In the elderly, the case fatality rate for HUS can be as high as 50%. *E. coli* O157:H7 (EHEC O157:H7) has been recognized as a cause of this syndrome since the 1980s. The reservoirs for EHEC O157:H7 are ruminants, particularly cattle and sheep, which are infected asymptomatically and shed the organism in feces. Other animals such as rabbits and pigs can also carry this organism. Humans acquire EHEC O157:H7 by direct contact with animal carriers, their feces, and contaminated soil or water, or via the ingestion of underdone ground beef, other animal products, and contaminated vegetables and fruit. The infectious dose is very low, which increases the risk of disease.

Infections with EHEC in other serogroups, including members of O26, O91, O103, O104, O111, O113, O117, O118, O121, O128 and O145, are increasingly recognized as causes of hemorrhagic colitis and HUS. Some of these organisms may be as significant in human disease as EHEC O157:H7; however, they are not recognized on the media used to isolate this organism, and many laboratories do not routinely screen for other strains. Although many EHEC seem to be carried asymptomatically in animals, members of some non-O157 serogroups may cause enteric disease in young animals. In rabbits, EHEC O153 has been linked to a disease that resembles HUS.

**Etiology**

*Escherichia coli* is a Gram negative rod (bacillus) in the family Enterobacteriaceae. Most *E. coli* are normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins. The specific virulence factors can be used, together with the type of disease, to separate these organisms into pathotypes.

Verocytotoxigenic (or verotoxigenic) *E. coli* (VTEC) produce a toxin that is lethal to cultured African green monkey kidney cells (Vero cells) but not to some other cultured cell types. There are two major families of verocytotoxins, Vt1 and Vt2. A VTEC isolate may produce one or both toxins. Because verocytotoxin is homologous to the shiga toxins of *Shigella dysenteriae*, VTEC are also called shiga toxin-producing *E. coli* (STEC).

Enterohemorrhagic *E. coli* are VTEC that possess additional virulence factors, giving them the ability to cause hemorrhagic colitis and hemolytic uremic syndrome in humans. One key characteristic found in EHEC, but not exclusive to these organisms, is the ability to cause attaching and effacing (A/E) lesions on human intestinal epithelium. A/E lesions are characterized by close bacterial attachment to the epithelial cell membrane and the destruction of microvilli at the site of adherence. Some of the genes that are involved in producing A/E lesions can be used, together with the presence of the verocytotoxin, to help identify EHEC.

**Serotypes involved**

*E. coli* are serotyped based on the O (somatic lipopolysaccharide), H (flagellar) and K (capsular) antigens. Serotypes known to contain EHEC include *E. coli* O157:H7, the non-motile organism *E. coli* O157:H-, and members of other serogroups, particularly O26, O103, O111 and O145 but also O91, O104, O113, O117, O118, O121, O128 and others. *E. coli* O157:H- is closely related to *E. coli* O157:H7, but it not simply a nonmotile version of this organism; it also has a distinctive combination of phenotypic and virulence features.

Serotyping alone is not enough to identify an organism as an EHEC; virulence factors characteristic of these organisms must also be present. *E. coli* O157:H7 strains are relatively homogeneous, and nearly all of these organisms carry virulence factors associated with hemorrhagic colitis and HUS. Members of other serotypes can be
more heterogeneous. For example, *E. coli* in the serogroup O26 may have one, both or neither verocytotoxin genes, and only half of all *E. coli* O26 isolates in one study possessed *ehx*, a gene found on a plasmid associated with EHEC. Different organisms may carry distinct groups of virulence factors. For example, the virulence gene profiles of EHEC in serogroups O26 and O145 differ from each other, as well as from EHEC O157:H7. Because many virulence factors (including the verocytotoxin) are carried on plasmids or in bacteriophages, new *E. coli* strains that have novel disease patterns and/or are difficult to classify by the current systems can emerge.

**Geographic Distribution**

EHEC O157:H7 infections occur worldwide; infections have been reported on every continent except Antarctica. Other EHEC are probably also widely distributed. The importance of some serotypes may vary with the geographic area.

**Transmission**

EHEC are transmitted by the fecal–oral route. They can be spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources. Birds and flies are potential vectors. In one experiment, EHEC O157:H7 was transmitted in aerosols when the distance between pigs was at least 10 feet. The organism was thought to have become aerosolized during high pressure washing of pens, but normal feeding and rooting behavior may have also contributed.

The reservoir hosts and epidemiology may vary with the organism. Ruminants, particularly cattle and sheep, are the most important reservoir hosts for EHEC O157:H7. A small proportion of the cattle in a herd can be responsible for shedding more than 95% of the organisms. These animals, which are called super-shedders, are colonized at the terminal rectum, and can remain infected much longer than other cattle. Super-shedders might also occur among sheep. Animals that are not normal reservoir hosts for EHEC O157:H7 may serve as secondary reservoirs after contact with ruminants. EHEC O157:H7 is mainly transmitted to humans by the consumption of contaminated food and water, or by contact with animals, feces and contaminated soil. Person-to-person transmission can contribute to disease spread during outbreaks; however, humans do not appear to be a maintenance host for this organism. Most human cases have been linked to direct or indirect contact with cattle, but some have been associated with other species including sheep, goats (unpasteurized goat milk), pigs (dry fermented pork salami), deer (venison), horses, rabbits and birds. The infectious dose for humans is estimated to be under 100 organisms, and might be as few as 10.

Foodborne outbreaks with EHEC O157:H7 are often caused by eating undercooked or unpasteurized animal products, particularly ground beef but also other meats and sausages, and unpasteurized milk and cheese. Other outbreaks have been linked to alfalfa or radish sprouts, lettuce, spinach and other contaminated vegetables, as well as unpasteurized cider. Irrigation water contaminated with feces is an important source of EHEC O157:H7 on vegetables. This organism can attach to plants, and survives well on the surface of a variety of fruits, vegetables and fresh culinary herbs. Depending on the environmental conditions, small numbers of bacteria left on washed vegetables may multiply significantly over several days. EHEC O157:H7 can be internalized in the tissues of some plants including lettuce, where it may not be susceptible to washing. Fruit flies can transmit this organism to apples, where it can multiply in wounded tissues. EHEC O157:H7 can remain viable for long periods in many food products. It can survive for at least nine months in ground beef stored at -20°C (-4°F). It is tolerant of acidity, and remains infectious for weeks to months in acidic foods such as mayonnaise, sausage, apple cider and cheddar at refrigeration temperatures. It also resists drying.

Some human cases are caused by exposure to contaminated soil or water. EHEC are usually eliminated by municipal water treatment, but these organisms may occur in private water supplies such as wells. Swimming in contaminated water, especially lakes and streams, has been associated with some infections. Soil contamination has caused outbreaks at campgrounds and other sites, often when the site had been grazed earlier by livestock. The reported survival time for EHEC O157:H7 in contaminated soil varies from a month to more than 7 months. This organism can also survive for 2 months or longer in some freshwater sources, especially at cold temperatures, and it may remain viable for two weeks in marine water. One study indicated that EHEC O157:H7 is inactivated in slurry within two weeks; another suggested that it can survive up to three months.

The epidemiology of other serotypes of EHEC is poorly understood. The reservoirs for EHEC O26 may be animals. Members of this *E. coli* serogroup have been found in various species including cattle, pigs, sheep, goats, rabbits and chickens. They are common in healthy animals as well as animals with diarrhea. Although the source of the organism is not known in many human cases, some EHEC O26 outbreaks have been foodborne (beef products and unpasteurized milk), associated with animal contact, or linked to water contaminated with feces. Possible person-to-person transmission has also been reported. VTEC O103 has been found in cattle, sheep and goats, as well as healthy and sick humans. In contrast, EHEC O157:H- has rarely been isolated from cattle and horses, and it was absent in more than 1800 fecal samples from cattle, sheep, goats and deer in Germany and the Czech Republic. However, one outbreak was foodborne (sausages), and contact with an infected cow and horse were the probable sources of infection in another outbreak. It is possible that humans are a reservoir host for this organism.
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**Disinfection**

*E. coli* can be killed by numerous disinfectants including 1% sodium hypochlorite, 70% ethanol, phenolic or iodine-based disinfectants, glutaraldehyde and formaldehyde. This organism can also be inactivated by moist heat (121°C [250°F] for at least 15 min) or dry heat (160–170°C [320–338°F] for at least 1 hour). Foods can be made safe by cooking them to a minimum temperature of 160°F/71°C. Ionizing radiation or chemical treatment with a sodium hypochlorite solution may reduce or eliminate bacteria on produce.

**Infections in Humans**

**Incubation Period**

The incubation period for disease caused by EHEC O157:H7 ranges from one to 16 days. Most infections become apparent after 3-4 days; however, the median incubation period was 8 days in one outbreak at an institution.

**Clinical Signs**

Humans can be infected asymptomatically or they may develop watery diarrhea, hemorrhagic colitis and/or hemolytic uremic syndrome. Most symptomatic cases begin with diarrhea. Some cases resolve without treatment in approximately a week; others progress to hemorrhagic colitis within a few days. Hemorrhagic colitis is characterized by diarrhea with profuse, visible blood, accompanied by abdominal tenderness, and in many cases, by severe abdominal cramps. Some patients have a low-grade fever; in others, fever is absent. Nausea and vomiting may be seen, and dehydration is possible. Many cases of hemorrhagic colitis are self-limiting and resolve in approximately a week. Severe colitis may result in intestinal necrosis, perforation or the development of colonic strictures.

Hemolytic uremic syndrome occurs in up to 16% of patients with hemorrhagic colitis. This syndrome is most common in children, the elderly and those who are immunocompromised. It usually develops a week after the diarrhea begins, when the patient is improving. Occasionally, children develop HUS without prodromal diarrhea. HUS is characterized by kidney failure, hemolytic anemia and thrombocytopenia. The relative importance of these signs varies. Some patients with HUS have hemolytic anemia and/or thrombocytopenia with little or no renal disease, while others have significant kidney disease but no thrombocytopenia and/or minimal hemolysis. Extrarenal signs including CNS involvement with lethargy, irritability and seizures are common. In more severe cases, there may be paresis, stroke, cerebral edema or coma. Respiratory complications can include pleural effusion, fluid overload and adult respiratory distress syndrome. Elevation of pancreatic enzymes or pancreatitis may also be seen. Rhabdomyolysis and myocardial involvement are rare. The form of HUS usually seen in adults, particularly the elderly, is sometimes called thrombotic thrombocytopenic purpura (TTP). In TTP, there is typically less kidney damage than in children, but neurologic signs including stroke, seizures and CNS deterioration are more common. Death occurs most often in cases with serious extrarenal disease such as severe CNS signs. Approximately 65–85% of children recover from HUS without permanent damage; however, long-term renal complications including hypertension, renal insufficiency and end-stage renal failure also occur. Residual extrarenal problems such as transient or permanent insulin-dependent diabetes mellitus, pancreatic insufficiency, gastrointestinal complications or neurological defects such as poor fine-motor coordination are possible.

**Communicability**

Person-to-person transmission occurs by the fecal-oral route. Most people shed EHEC O157:H7 for approximately 7 to 9 days; a minority can excrete this organism for 3 weeks or longer after the onset of symptoms. In a few cases, shedding may continue for several months. Young children tend to shed the organism longer than adults. Transmission is particularly common among children still in diapers.

**Diagnostic Tests**

Because humans do not normally carry EHEC, clinical cases can be diagnosed by finding these organisms in fecal samples. Food and environmental samples may also be tested to determine the source of the infection. EHEC are sometimes difficult to identify. They are a minor population in the fecal flora or food. They also closely resemble commensal *E. coli* except in verocytotoxin production. However, the verocytotoxin alone does not necessarily identify an organism as EHEC; additional virulence factors must also be present. Many diagnostic laboratories can detect verocytotoxin-producing *E. coli* (VTEC) and identify EHEC O157:H7; non-O157 EHEC strains must often be sent to a reference laboratory for identification. There is no single technique that can be used to isolate all EHEC serotypes.

Selective and differential media have been developed for EHEC O157:H7, based on its lack of β-glucuronidase activity and the inability of most strains to rapidly ferment sorbitol. MacConkey agar containing 1% sorbitol (SMAC), often with cefixime and either rhamnose or potassium tellurite, is frequently used. Hemorrhagic colitis agar can be used to isolate EHEC O157:H7 from foods. Other media, including commercial chromogenic agars (e.g., rainbow agar), are also available. Because other strains of *E. coli*, as well as other bacteria, can grow on these media, prior enrichment for *E. coli* O157 aids detection, particularly in samples from food and the environment. For enrichment, samples may be cultured in liquid enrichment medium, or immunomagnetic separation (IMS) can be used to...
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Concentrate the members of serogroup O157 before plating. In IMS, magnetic beads coated with an antibody to the O157 antigen are used to bind these organisms. Colonies suspected to be EHEC O157:H7 are confirmed to be *E. coli* with biochemical tests, and shown to have the O157 somatic antigen and H7 flagellar antigen with immunoassays. A variety of tests including enzyme-linked immunosorbent assays (ELISAs), agglutination, PCR, immunoblotting or Vero cell assay can be used to detect the verocytotoxin or its genes. Phage typing and pulsed field gel electrophoresis can subtype EHEC O157:H7 for epidemiology; these tests are generally done by reference laboratories. Subtyping is important in finding the source of an outbreak and tracing transmission.

The techniques used to identify EHEC O157:H7 can miss atypical strains of this organism, including rare sorbitol-fermenting isolates. They are also ineffective for detecting EHEC O157:H−, which ferments sorbitol and is beta-glucuronidase positive. Identification of EHEC O157:H− is laborious, but it can be done by IMS followed by plating samples onto SMAC and testing individual sorbitol-fermenting colonies to detect the O157 antigen, verocytotoxins or their genes, and/or other virulence factors.

Selective media and isolation techniques have been developed for few non-O157 EHEC. IMS beads are commercially available for concentrating some common EHEC serogroups including O26, O103, O111 and O145. A selective rhamnose MacConkey medium containing ceftaxime and tellurite (CT-RMAC) is used to isolate and identify EHEC O26. Isolation of most non-O157 EHEC relies on screening colonies for verocytotoxin, the genes that produce this toxin and/or other virulence genes associated with EHEC. MacConkey agar or other media normally used to culture *E. coli* can be used to grow these organisms. Some prescreening techniques target specific serogroups or serotypes known to be associated with human EHEC disease. Techniques to identify most non-O157 EHEC are very labor-intensive, and these tests are not available at most laboratories.

Immunological and nucleic acid-based tests that detect O and H antigens, verocytotoxin or various genes associated with EHEC can be used for presumptive diagnosis. These rapid tests can determine whether potential pathogens are present in samples before isolation. They include dipstick and membrane technologies, agglutination tests, microplate assays, colony immunoblotting, PCR, immunofluorescence and ELISAs. Although verocytotoxin production can aid identification, VTEC are not necessarily EHEC and additional virulence factors must usually be identified. Verocytotoxin-negative derivatives of the EHEC may occasionally be found by the time HUS develops. The results from rapid tests are confirmed by isolating the organism. In humans, EHEC may not be found in feces after one week.

Serology is also valuable in humans, particularly later in the course of the disease when EHEC are difficult to find. Indirect ELISAs can detect antibodies to EHEC O157:H7 for months after infection. Cross-reactions with other bacteria can be seen.

**Treatment**

Treatment of hemorrhagic colitis is supportive, and may include fluids and a bland diet. Antibiotics are controversial and are usually avoided: they do not seem to reduce symptoms, prevent complications or decrease shedding, and they may increase the risk of HUS. The use of antimotility (antidiarrheal) agents in hemorrhagic colitis also seems to increase the risk for developing HUS. Patients with complications may require intensive care including dialysis, transfusion and/or platelet infusion. Patients who develop irreversible kidney failure may need a kidney transplant.

**Prevention**

Frequent hand washing, especially before eating or preparing food, and good hygiene are important in preventing transmission from animals and their environment. Hand washing facilities should be available in petting zoos and other areas where the public may contact livestock, and eating and drinking should be discouraged at these sites. To protect children and other household members, people who work with animals should keep their work clothing, including shoes, away from the main living areas and launder these items separately. Two children apparently became infected with EHEC O157:H7 after contact with bird (rook) feces, possibly via their father’s soiled work shoes or contaminated overalls. After a number of outbreaks associated with camping in the U.K., the Scottish *E. coli* O157 Task Force has recommended that ruminants not be grazed on land for at least three weeks before camping begins.

Techniques to reduce microbial contamination during slaughter and meat processing can reduce the risk of EHEC from this source. Screening and control programs have been established for EHEC O157:H7 in meat. To prevent cross-contamination during food preparation, consumers should wash their hands, counters, cutting boards, and utensils thoroughly after they have been in contact with raw meat. Meat should be cooked thoroughly to kill *E. coli*. Unpasteurized milk or other dairy products and unpasteurized juices should be avoided.

Water that may be contaminated should not be used to irrigate vegetable crops, and untreated manure/effluents should not be used on fruits or vegetables that will be eaten raw. Post-harvest measures include thorough washing of vegetables under running water to reduce bacterial numbers. Vegetables can also be disinfected with a dilute chlorine solution. It is safest to wash vegetables immediately before use; under some environmental conditions, populations of bacteria can build up again after
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a few days. EHEC carried internally in plant tissues are difficult to destroy except by irradiation or cooking.

Contamination of public water supplies is prevented by standard water treatment procedures. Livestock—should be kept away from private water supplies. Microbiological testing can also be considered. To the extent possible, people should avoid swallowing water when swimming or playing in lakes, ponds and streams.

Good hygiene, careful hand-washing and proper disposal of infectious feces can reduce person-to-person transmission. Thorough hand washing is especially important after changing diapers, after using the toilet, and before eating or preparing food. Bed linens, towels and soiled clothing from patients with hemorrhagic colitis should be washed separately, and toilet seats and flush handles should be cleaned appropriately. In some areas, regulations may prohibit infected children from attending daycare or school until they are no longer shedding organisms. Some authors suggest that isolating infected children from their young siblings or other young household members can significantly decrease the risk of secondary spread.

The risk to humans can also be reduced if EHEC carriage is decreased or eliminated in animals (see below).

**Morbidity and Mortality**

EHEC infections can occur as sporadic cases or in outbreaks. In North America, EHEC O157:H7 infections are most common from summer to autumn. In the U.K., they tend to occur from late spring to late summer. Seasonality might be caused by seasonal shedding patterns in animals, or it could be due to other factors such as eating undercooked meat at summer barbecues. In contrast, EHEC O157:H- infections, which are most common in children under 3 years of age, tend to be seen in the winter.

The incidence of EHEC in humans is difficult to determine, because cases of uncomplicated diarrhea may not be tested for these organisms. In 2004, the estimated annual incidence of EHEC O157:H7 reported in Scotland, the U.S., Germany, Australia, Japan and the Republic of Korea ranged from 0.08 to 4.1 per 100,000 population, with the highest incidence in Scotland. In the U.S., the Centers for Disease Control and Prevention (CDC) estimates that EHEC O157:H7 causes approximately 73,000 illnesses, 2,000 hospitalizations, and 50-60 deaths each year. The prevalence of non-O157 EHEC in human disease is probably underestimated, because most laboratories do not routinely screen for these organisms. In the U.S., the CDC estimates that the incidence of non-O157 EHEC is probably similar to O157 EHEC. In continental Europe, Latin America and Australia, some evidence suggests that non-O157 EHEC infections may be more common in humans than EHEC O157:H7.

In clinical cases, the mortality rate varies with the syndrome. Hemorrhagic colitis alone is usually self-limiting, but death is possible. The number of cases that progress to HUS varies with the organism and the outbreak. Approximately 5-10% of patients with hemorrhagic colitis from EHEC O157:H7 usually develop HUS; however, a 16% incidence was reported during a particularly virulent outbreak associated with spinach in the U.S. Complications and fatalities are particularly common among children, the elderly, and those who are immunosuppressed or have debilitating illnesses. HUS is fatal in 3–10% of children and TTP in up to 50% of the elderly.

**Infections in Animals**

**Species Affected**

Ruminants, especially cattle and sheep, are the major reservoirs for EHEC O157:H7. Bison and deer can be infected. This organism can sometimes be found in other mammals including pigs, rabbits, horses, dogs, raccoons and opossums, and in birds including chickens, turkeys, geese, pigeons, gulls, rooks and various other wild birds. In some instances, it is not known whether a species normally serves as a reservoir host or if it is only a temporary carrier. For example, rabbits shedding EHEC O157:H7 have caused outbreaks in humans, but most infected rabbits have been found near farms with infected cattle.

The reservoir hosts for non-O157 EHEC are poorly understood. Members of serogroup O26 have been found in cattle, pigs, sheep, goats, rabbits and chickens; however, all of these organisms are not necessarily EHEC. VTEC O103 has been found in cattle, sheep and goats, as well as healthy and sick humans. EHEC O145 can occur in cattle, but less often than EHEC O26. One serotype, EHEC O145:H-, was isolated from a cat associated with a human case; whether the child infected the cat or the cat infected the child was uncertain. EHEC O157:H- has been found in a cow and a horse, but surveys in Germany and the Czech Republic suggest that this serotype is generally rare or absent in cattle, sheep, goats and deer. Domesticated rabbits appear to be reservoir hosts for EHEC O153:H- and O153:H7.

**Clinical Signs**

EHEC O157:H7 has not been associated with illness in naturally infected animals. In experimentally infected calves, this serotype does not seem to cause disease in animals older than one week of age. There is one report of bloody or mucoid diarrhea, with some deaths, after experimental infection of neonatal (less than 2-day-old) calves. Another study reported illness in gnotobiotic piglets. Dogs that were experimentally inoculated with EHEC O157:H7 developed transient acute diarrhea with decreased appetite and vomiting, but recovered spontaneously without complications in 1-2 days. In the same experiment, dogs inoculated with a non-O157 EHEC developed severe disease, with diarrhea and vomiting followed by lethargy.
and inappetence, dehydration and dramatic weight loss. These dogs also had neurological signs including seizures, cerebral infarction, blindness and coma, and died 5-6 days after the onset of clinical signs.

Members of some non-O157 EHEC serogroups including O26, O111, O118 and O103 may cause diarrhea and other gastrointestinal signs in young animals. EHEC O118:H16 is often associated with diarrhea in calves, and this has been confirmed by experimental challenge in newborn calves. However, epidemiological studies for most EHEC are suggestive rather than definitive. There are relatively few studies in animals using defined strains of *E. coli*, and most of these experiments were done before EHEC virulence determinants were well understood. VTEC strains that have been associated with diarrhea in experimentally infected calves include VTEC O5:H-, VTEC O8:H9, VTEC O26:H11, VTEC O26:H-, VTEC O26, VTEC O80:H- and VTEC O111:H-. In many but not all cases, the diarrhea was bloody and mucoid.

EHEC O153:H- has been linked to an outbreak of hemorrhagic diarrhea and an illness that resembled HUS in domesticated rabbits. Experimentally infected rabbits developed hemorrhagic diarrhea with lethargy, inappetence, dehydration and weight loss.

### Communicability

Subclinically infected animals can shed EHEC. Shedding may be transient or intermittent, and animals that have stopped excreting this organism can be recolonized. Calves are more likely to shed EHEC O157:H7 than adult cattle. Experimentally infected pigs could shed this organism for at least 2 months.

### Post Mortem Lesions

EHEC lesions in ruminants are usually characterized by inflammation of the intestinal mucosa, and are generally limited to the large intestine. In some cases, a fibrinohemorrhagic exudate is present.

In rabbits experimentally infected with EHEC O153, the cecum and/or proximal colon were edematous and thickened, and the serosal surfaces had petechial or ecchymotic hemorrhages. Pale kidneys were also reported.

Dogs infected with EHEC O157:H7 had no significant gross lesions. In dogs inoculated with a non-O157 EHEC strain, the primary cause of death was microvascular thrombosis leading to kidney failure and multiple organ failure. This syndrome resembled HUS. In these dogs, inflammation and edema occurred in the small and large intestines. The kidneys were pale, with a few petechiae on the serosal surface. The liver was enlarged, with inflammation and necrotic lesions.

### Diagnostic Tests

EHEC are zoonotic and the infectious dose is low. Caution should be used when handling samples; laboratory infections have been reported.

Carrier animals are usually detected by finding EHEC in fecal samples, which are either freshly voided or taken directly from the animal. Rectal swabs may also be used in some cases. Intestinal contents can be collected at slaughter. Repeated sampling, as well as sampling more animals, increases the chance of detection. In some studies, samples have been collected from hides in addition to feces. One study suggested that the EHEC O157:H7 status for a cattle farm or pen could be determined by walking around the pen in a pair of disposable, liquid absorbing overshoes. Samples should be kept cool (4°C/39°F) and cultured as soon as possible.

EHEC can be difficult to identify. They are a minor population in the fecal flora of animals. They also closely resemble commensal *E. coli* except in verocytotoxin production. However, the verocytotoxin alone does not necessarily identify an organism as EHEC; additional virulence factors must also be present. Many diagnostic laboratories can detect verocytotoxin-producing *E. coli* (VTEC) and identify EHEC O157:H7; non-O157 EHEC strains must often be sent to a reference laboratory for identification. There is no single technique that can be used to isolate all EHEC serotypes.

Selective and differential media have been developed for EHEC O157:H7, based on its lack of β-glucuronidase activity and the inability of most strains to rapidly ferment sorbitol. MacConkey agar containing 1% sorbitol (SMAC), often with cefixime and either rhamnose or potassium tellurite, is frequently used. Hemorrhagic colitis agar can be used to isolate EHEC O157:H7 from foods. Other media, including commercial chromogenic agars (e.g., rainbow agar), are also available. Because other strains of *E. coli*, as well as other bacteria, can grow on these media, prior enrichment for *E. coli* O157 aids detection. For enrichment, samples may be cultured in liquid enrichment medium, or immunomagnetic separation (IMS) can be used to concentrate the members of serogroup O157 before plating. In IMS, magnetic beads coated with an antibody to the O157 antigen are used to bind these organisms.

Colonies suspected to be EHEC O157:H7 are confirmed to be *E. coli* with biochemical tests, and shown to have the O157 somatic antigen and H7 flagellar antigen with immunoassays. A variety of tests including enzyme-linked immunosorbent assays, agglutination, PCR, immunoblotting or Vero cell assay can be used to detect the verocytotoxin or its genes. Phage typing and pulsed field gel electrophoresis can subtype EHEC O157:H7 for epidemiology; these tests are generally done by reference laboratories.

The techniques used to identify EHEC O157:H7 can miss atypical strains of this organism, including rare sorbitol-fermenting isolates. They are also ineffective for detecting EHEC O157:H-, which ferments sorbitol and is beta-glucuronidase positive. Identification of EHEC O157:H- is laborious, but can be done by IMS followed by
plating samples onto SMAC and testing individual sorbitol-fermenting colonies to detect the O157 antigen, verocytotoxins, and/or other virulence factors.

Selective media and isolation techniques have been developed for few non-O157 EHEC. IMS beads are commercially available for concentrating some common EHEC serogroups including O26, O103, O111 and O145. A selective rhamnose MacConkey medium containing cefixime and tellurite (CT-RMAC) is used to isolate and identify EHEC O26. Isolation of most non-O157 EHEC relies on screening colonies for verocytotoxin, the genes that produce this toxin and/or other virulence genes associated with EHEC. MacConkey agar or other media normally used to culture E. coli can be used to grow these organisms. Some prescreening techniques target specific serogroups or serotypes known to be associated with human EHEC disease. Techniques to identify most non-O157 EHEC are very labor-intensive, and these tests are not available at most laboratories.

Immunological and nucleic acid-based tests that detect O and H antigens, verocytotoxin or various genes associated with EHEC can be used for presumptive diagnosis. These rapid tests can determine whether potential pathogens are present in samples before isolation. They include dipstick and membrane technologies, agglutination tests, microplate assays, colony immunoblotting, PCR, immunofluorescence and ELISAs. Although verocytotoxin production can aid identification, VTEC are common in animals, and these organisms are not necessarily EHEC; additional virulence factors must also be identified. Verocytotoxin-negative derivatives of EHEC can occur. The results from rapid tests are confirmed by isolating the organism. Some kits validated for food and meat samples and kits for human clinical samples may lack sensitivity when testing fecal samples from animals.

Although cattle can produce antibodies to O157, serology is not used routinely in animals to diagnose infections with VTEC or EHEC.

Prevention

Prevention of shedding in domesticated animals, particularly ruminants, is expected to decrease the number of human infections. These techniques are still in development. Identifying and targeting super-shedders should be particularly effective. The removal of super-shedders from the herd might be helpful. In one study, allowing a pasture to lie fallow for the winter prevented the transmission of EHEC O157:H7 to susceptible animals the following spring. Other proposed interventions include vaccination; the application of disinfectants (e.g., chlorhexidine), various antimicrobial chemicals or bacteriophages to the terminal rectum; and the use of probiotics that would preferentially colonize the gastrointestinal tract. Dietary manipulations have also been proposed. These interventions are still in the research stage.

Management practices to decrease EHEC in the environment include the storage of effluents on a cement floor for 3 months or longer before discharge, and the collection of all liquids in a trap to minimize leaching of liquid manure into groundwater. Some EHEC may remain after long-term storage, and untreated manure and effluents should not be used on fruit and vegetable crops that will be eaten raw. To prevent transmission to animals, they should not be allowed to graze pastures for a period after effluent has been applied. Composting or treatment of manure before use as a fertilizer may reduce the risks from this source. During composting, the survival of the organism varies with the size of the compost heap, the temperature attained, and the dose of the organism. Other biological processes (aerobic and anaerobic digestion), heat drying, and/or chemical treatments have been proposed to sanitize farm effluents before discharge into the environment; the effect of some chemical treatments on the environment has not yet been determined.

Methods to eliminate EHEC from infected companion animals have not been established. However, oral autovaccination with a heat-inactivated EHEC strain (O145:H–) stopped shedding of the organism in a persistently infected cat.

Morbidity and Mortality

Surveys suggest that EHEC O157:H7 is widespread in cattle herds, but the prevalence in individual animals is low. Some studies have found that this organism is more common in cattle during the summer and early autumn. One study reported that the prevalence was higher when it was cooler, but more bacteria were shed in the summer. Other studies have not found seasonal patterns of shedding.

Prevalence rates for EHEC O157:H7 among cattle vary from less than 1% to 36%, depending on the country, type of herd studied and other conditions. One study suggested that 28% of the cattle at U.S. slaughterhouses carried EHEC O157:H7 during the summer. In a recent survey, 97% of U.S. agricultural fairs had at least one animal infected with EHEC O157:H7. Overall, this organism was isolated from 11.4% of the cattle, 1.2% of the pigs, 3.6% of the sheep and goats, and 5.2% of the fly pools at these fairs. In the U.K., estimates of the prevalence of EHEC O157:H7 in sheep range from 2.2 to 7.4%. In one study, the transient prevalence of this organism in sheep was 31%. A recent study from the U.S. found that many bison also carry EHEC O157:H7: the overall prevalence was 47%, and fecal prevalence on different sampling days varied from 17% to 83%. The prevalence in deer is reported to be less than 3%, and some surveys estimate that it is less than 0.5%. Sampling of wild rabbits associated with infected cattle farms found that 9.5% to 40% of the rabbits also carried this organism. One study reported that 2% of swine colon samples collected at slaughter contained EHEC O157:H7. Recent studies that use sensitive methods for detection report a higher prevalence than early surveys. However,
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highly sensitive techniques may also overestimate prevalence, as some animals shedding the organism may not be colonized, but only transiently infected by transmission from super-shedders or the environment.

Little is known about the prevalence of most non-O157 EHEC. One study reported that the mean prevalence for VTEC (not EHEC) was 31% in cattle and 42% in sheep. However, a gene associated with EHEC (eaeA) was more likely to be found in cattle VTEC isolates (16.5%) than sheep VTEC (7.5%).

Potential EHEC seem to be uncommon in most non-ruminant species. The prevalence of VTEC in dogs, cats, chickens and pigs appears to be much lower than in ruminants. EHEC O153 may be relatively common in rabbits. In one study, 25% of Dutch belted rabbits and 9% of the New Zealand white rabbits from one commercialsource had EHEC O153:H- or O153:H7 in their feces.

Morbidity in adult ruminants appears to be negligible or absent. Young animals may be affected by some serogroups of EHEC. Deaths have been reported in some experimentally infected animals including some calves, dogs inoculated with a non-O157 EHEC, and rabbits inoculated with EHEC O153. The morbidity and mortality rates are currently unknown.

### Internet Resources

- Centers for Disease Control and Prevention (CDC)  
- Medical Microbiology  
- MicroBioNet. Serotypes of VTEC  
- Public Health Agency of Canada. Material Safety Data Sheets  
- The Institute of Food Technologists  
  [http://www.ift.org](http://www.ift.org)
- The Merck Manual of Diagnosis and Therapy  
- U.S. FDA Foodborne Pathogenic Microorganisms and Natural Toxins Handbook (Bad Bug Book)  
  [http://vm.cfsan.fda.gov/~mow/intro.html](http://vm.cfsan.fda.gov/~mow/intro.html)
- USDA. FSIS. *E coli* O157:H7  
- World Organization for Animal Health (OIE)  
  [http://www.oie.int](http://www.oie.int)
- OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals  
  [http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/](http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/)
- OIE Terrestrial Animal Health Code  
  [http://www.oie.int/international-standard-setting/terrestrial-code/access-online/](http://www.oie.int/international-standard-setting/terrestrial-code/access-online/)

### References


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*Link defunct as of 2009