ABSTRACT  The first major outbreaks caused by enterohemorrhagic *Escherichia coli* (EHEC) raised public and medical awareness of the risks associated with acquiring this potentially deadly infection. The widespread presence of these organisms in the environment, the severity of the clinical sequelae, and the lack of treatment options and effective preventive measures demand that we obtain a better understanding of how this group of organisms cause disease. Animal models allow study of the processes and factors that contribute to disease and, as such, form a valuable tool in the repertoire of infectious disease researchers. Yet despite more than 30 years of research, it seems that no single model host reproduces the full spectrum of clinical disease induced by EHEC in humans. In the first part of this review, a synopsis of what is known about EHEC infections is garnered from human outbreaks and biopsy specimens. The main features and limitations of EHEC infection models that are based on the three most commonly used species (pigs, rabbits, and mice) are described within a historical context. Recent advances are highlighted, and a brief overview of models based on other species is given. Finally, the impact of the host on moderating EHEC infection is considered in light of growing evidence for the need to consider the biology and virulence strategies of EHEC in the context of its niche within the intestine.

INTRODUCTION
Since the first recognized *Escherichia coli* O157:H7 outbreak over 3 decades ago (1), investigators have sought to identify suitable animal hosts that allow study of enterohemorrhagic *E. coli* (EHEC)-mediated disease. The value of any animal infection model ultimately relies on its ability to reproduce the human disease and enable the mechanistic processes that lead to clinical disease, pathogen carriage, and transmission to be examined. As yet, no single animal model mimics the full spectrum of disease caused by EHEC infection. However, since Moxley and Francis’s review in 1998 (2), several advances have been made in the field, including the generation of a Shiga toxin (Stx)-producing *Citrobacter rodentium*-murine model, a human intestine xenograft murine model, and a renewed interest in the use of rabbit models. This article reviews what is known about EHEC-mediated disease from human outbreaks and biopsy studies, and within a historical context, describes the features and limitations of EHEC infection models that are based on the three most commonly used species (pigs, rabbits, and mice). Recent new advances are highlighted and discussed in light of mounting evidence for the need to study the biology and virulence strategies of EHEC in the context of its niche within the intestine. The reader is directed elsewhere for excellent reviews on the environmental sources of EHEC infection (3), EHEC interactions with the intestinal epithelium (4), the molecular basis of pathogenicity (5–8), and the current status of treatment options (9).
OBSERVATIONS FROM HUMAN OUTBREAKS

Most of what is known about EHEC pathogenesis is based on outbreaks caused by *E. coli* O157:H7 (1, 5, 10). From these outbreaks, it is widely accepted that EHEC infection is acquired through the ingestion of feces-contaminated food or water or by hand-to-mouth transmission following contact with infected animals (including humans) or their surroundings. Whereas some individuals who ingest the organism remain asymptomatic, others develop severe abdominal pain and diarrhea typically within 3 to 4 days (11). This can progress to a bloody diarrhea (or hemorrhagic colitis), and in approximately 5 to 15% of infected individuals a potentially fatal hemolytic-uremic syndrome (HUS) develops (12). HUS is defined as a clinical syndrome comprising thrombotic microangiopathy, thrombocytopenia, and hemolytic anemia that leads to acute kidney injury, for which there is no effective treatment. The ubiquitously of the causative agent, the severity of the clinical sequelae, and the lack of treatment options and effective preventive measures demand a better understanding of how this group of organisms cause disease.

The pathophysiology of disease is largely attributed to the ability of EHEC to colonize the mammalian intestine and produce Shiga-like toxin, a family of potent cytotoxins that are able to cross the epithelial barrier and act at sites distal to the intestine, inhibiting protein synthesis in sensitive target endothelial cells. Consistent with this idea, Stx has been detected on the surface of polymorphonuclear leukocytes (PMNs) circulating in the blood of some infected individuals and in their feces (13, 14). In contrast, bacteremia has not been reported (1, 15), and the organism remains in the intestinal lumen where it is shed in feces. Exactly how Stx crosses the epithelial barrier remains poorly defined, but it is generally believed that distribution of the Stx receptor, globotriaosylceramide (Gb3), determines the site of tissue damage (16, 17). The presence of Gb3 in the endothelial cells that line the blood vessels (vasculature) and the subsequent host cell response to the cellular damage it causes explain the resulting clinical disease (18). Acute renal failure occurs because the highest concentrations of Gb3 are found in this organ (19); however, Gb3 is also detected in the microvasculature of the colon and the cerebellum (20–22). Conflicting views on whether Gb3 is also expressed on the surface of colonic epithelial cells exist in the literature (23–25).

As such, any Stx-producing strain capable of colonizing the mammalian intestine could potentially cause Stx-mediated disease in humans. In reality, *E. coli* O157:H7 strains are the predominant serotype associated with EHEC outbreaks in the United Kingdom and elsewhere (26–28). The reason behind this selection is not clear but may be related to serotype-specific differences in prevalence, persistence, or survival in the environment as well as their inherent virulence. *E. coli* O157:H7 strains usually contain the locus of enterocyte effacement (LEE) pathogenicity island, a virulence attribute deemed to play a critical role in mediating bacterial attachment to the intestinal epithelium (29–31). Among non-O157 strains, bacteria belonging to serogroups O26, O103, O111, O121, O45, and O145 are most commonly reported from infected individuals (28). Some serogroups, notably O113, lack the LEE pathogenicity island, and compared to the LEE-positive O157 strains, relatively little is known about the factors that are important in mediating their attachment to the epithelial surface (32, 33).

Epidemiological studies suggest that non-O157 serogroups cause a similar but somewhat less severe range of clinical manifestations than O157 strains (28, 34, 35). Retrospective analyses of patient clinical profiles from a U.S. hospital revealed similar incidences of bloody diarrhea and HUS in children presenting with either non-O157 (17 patients) or O157 (33 patients) infections (36). In contrast, a larger study using German national surveillance data concluded that other than for the recent O104 outbreak strains, patients presenting with non-O157 infections were half as likely to be hospitalized and one-tenth as likely to die compared to those infected with O157 (37). An unexpectedly high percentage of people developed HUS following the outbreak of Stx-producing enteraggregative *E. coli* O104:H4 that unfolded in northern Germany during the summer of 2011 (37, 38). In addition, the affected target population consisted of young, healthy females rather than individuals at the extremes of age, who are more likely to be sickened during an *E. coli* O157:H7 outbreak. Thus, clinical outcome may be dependent on the demographic of the affected population as well as the characteristics of the infectious organism. Predicting which strain and host factors led to the progression of a more severe clinical outcome is critical to the development and management of effective control strategies to prevent the loss of human life.

Volunteer Studies

Due to the potentially life-threatening sequelae associated with EHEC infections, volunteer studies using Stx-producing strains are deemed unethical. However, volunteer studies using the closely related diarrheal pathogen enteropathogenic *E. coli* (EPEC) have been performed and demonstrate a clear role in pathogenesis.
for some of the virulence attributes shared by the two pathotypes of *E. coli*. For example, studies reveal that bacterial adherence to the epithelial surface mediated by Tir-intimin interactions play a major role in the development of diarrhea. Deletion of EspB, a type III secreted protein and required for targeting of Tir to the host cell membrane (39), decreased the incidence and severity of diarrhea in volunteers who ingested the mutant (40). Similarly, deletion of intimin, a 94-kDa outer membrane protein encoded by the gene *eaeA*, also reduced the severity of infection in volunteers (41). In both studies, however, a few individuals who received the mutant strains still developed diarrhea, albeit producing stools of reduced volume and frequency. Moreover, the numbers of organisms recovered from their stools were similar to those receiving wild-type organisms, at least at around the time of peak organism excretion from the host (41). These results indicate that factors other than Tir and intimin contribute to EHEC colonization in the intestine, and in this regard, the biological significance of Tir-intimin-mediated attachment (and the resultant formation of the characteristic attaching and effacing [A/E] lesion) during disease is not really understood.

**Biopsies from Infected Individuals**

**Intestinal damage**

Relatively few studies report on the intestinal changes that occur in individuals infected with EHEC, probably due to the risk of worsening the underlying clinical condition. Colonoscopy examination of infected individuals, which allows the full length of the large intestine to be viewed, found that the most severe disease occurred in the cecum and ascending colon (42, 43). Here, tissue damage consisting of edema, erythema (redness), hemorrhage, and erosion was evident over a wide area and was associated with a marked narrowing of the luminal space. In some individuals, long ulcer-like lesions were also noted on the surface of the tissue. Patchy and less severe areas of inflammatory damage were observed in the transverse colon, descending colon, and sigmoid colon, consistent with descriptions from sigmoidoscopy examinations (44, 45).

Histologic examination of the damaged areas revealed destruction of the surface epithelium and involvement of the lamina propria, while the deeper colonic crypts remained largely intact (42, 43). Focal areas with acute inflammation were evident, with neutrophil infiltration of the lamina propria and, in some cases, the formation of crypt abscesses. Hemorrhage and edema were commonly observed in the lamina propria, as were apoptotic cells in the surface epithelium and colonic crypts. Small fibrin/platelet thrombi formed within the mucosal capillaries. It is interesting to note that adherent bacteria were not observed in the tissue sections, despite the samples being collected during the acute phase of the disease and only a few days after the onset of bloody diarrhea (42, 44). These findings indicate that during EHEC infection the integrity of the epithelial barrier is compromised; whether this occurs due to the direct action of the bacterium or Stx or due to the resulting host response to infection is not clear.

**Extraintestinal damage**

Readers are referred to two reviews describing the detailed pathological manifestations of Stx-mediated kidney damage in humans (6, 12). Summarized below are the key pathological findings evident in patients who present with diarrhea and HUS. Inward and colleagues reported that the renal pathology consisted of endothelial swelling and glomerular thrombosis with congested rather than ischemic glomeruli (46). In addition, they noted the presence of significant numbers of neutrophils compared to control tissue. Glomerular capillaries appeared to be the main site of damage in the patients. As such, the site of damage mirrors the distribution of Gb3, the Stx receptor, in humans. The highest densities of Gb3 were detected on glomerular endothelial cells, although Gb3 has also been detected in renal proximal tubules (6). Consistent with these findings, Stx was found to bind to both kidney tubular and glomeruli of a pediatric patient with HUS, but only the tubules of a geriatric patient with HUS (16). Whether these findings reflect differences in Gb3 expression, the stage of HUS, or some other factor is not known.

**HISTORICAL PERSPECTIVE ON ANIMAL MODELS OF EHEC-MEDIATED DISEASE**

**Pigs**

Nearly 30 years ago, Tzipori and Francis described the first use of gnotobiotic piglets to study the newly emerging group of pathogenic *E. coli* strains that included *E. coli* O157:H7 (47, 48). Gnotobiotic piglets, which are delivered via cesarean section, maintained in germ-free incubators, and artificially reared (aka cesarean-derived colostrum-deprived [CDCD] piglets), had been used previously to study other human enteric pathogens, including cryptosporidium and pathogenic *E. coli* (49, 50). Oral infection of 1-day-old CDCD piglets with high numbers (10¹⁰ CFU) of *E. coli* O157:H7 caused watery but not bloody diarrhea in most infected animals by 2 to 4 days post inoculation. In these early studies, no other signs of
disease were reported. As was found in human infections, histologic abnormalities were detected in the cecum and colon, and primarily consisted of destruction of the mucosal brush border and inflammation (51). These first investigations recognized that E. coli O157:H7 caused intestinal lesions that were morphologically similar to those previously described for EPEC (52), and thus EHEC was considered a member of the same pathotype group (53). However, while EHEC and EPEC apparently shared one common mechanism of bacterial attachment, they adhered to different sites in the intestine. EPEC colonized both the small and large intestine, whereas only the large intestine was colonized by EHEC (54).

Neither the pO157 plasmid nor Stx appeared to play a role in lesion formation as mutants deleted for these factors still caused intestinal disease (54, 55). Instead, genes encoded in the now well-described LEE pathogenicity island proved to be critical for intimate bacterial attachment in vitro and intestinal lesion formation in vivo (29, 30). Consistent with findings from volunteer studies, deletion of eaeA prevented bacterial attachment and A/E lesion formation, yet allowed similar numbers of wild-type and mutant cells to be recovered in intestinal homogenates obtained from the infected animals (56). These findings suggest that the gnotobiotic piglet intestine, like the human intestine, is permissive for EHEC replication and the organism is capable of surviving in this niche.

In addition to intestinal disease, subsequent studies reported that some piglets exhibit signs of central nervous system (CNS) disease, including ataxia (55). Indeed, support for a role for Stx in extraintestinal disease initially came from a study where gnotobiotic piglets were given either orally administered bacteria or intraperitoneally administered polymyxin B-treated bacterial cell lysates (57). In both instances, piglets that exhibited CNS disease showed evidence of vascular damage in the cerebellum. The vascular damage bore similarities to lesions seen in the brain of a patient who died following E. coli O157:H7 infection and in piglets with naturally acquired edema disease (57, 58), infections that are both marked by the presence of Stx. While Stx was found to be important in the development of extraintestinal disease, intimate bacterial attachment to the epithelial surface was not required for this to occur (59).

While early studies focused on the effect of Stx on the CNS, a significant advance in the usefulness of this model was made when investigators reported that gnotobiotic piglets also developed renal lesions following oral EHEC infection (59–61). Gunzer and colleagues were the first to report that the kidneys of infected animals exhibited signs of endothelial cell damage (60). Damage was characterized by diffuse glomerular endothelial swelling and congestion and a narrowing of the capillary vessels. In most instances, morphological signs of thrombotic microangiopathy, which is a hallmark characteristic of HUS in humans, were also noted. Gunzer’s findings were confirmed following retrospective examination of kidney samples obtained from multiple EHEC-infected and control piglets (61). Despite kidney damage, unlike humans, the piglets do not exhibit clinical signs of kidney failure (e.g., elevated creatinine levels) (60). As such, while piglets are one of the few species that show both intestinal and systemic disease following EHEC infection, they do not fully recapitulate all facets of the human disease.

In addition to the inherent biological constraints associated with the use of gnotobiotic animals (e.g., poorly developed immune systems, a lack of normal microflora), most researchers lack the specialized facilities needed to rear animals under sterile conditions. To overcome some of these limitations, conventional piglets were assessed for their susceptibility to EHEC infection. Naturally born piglets, which feed directly from the sow and either remain with the sow for the duration of the study or are subsequently hand-reared and housed in individual microbiological isolators, offer an additional advantage as they enable passive immunization studies to be performed (62). Surprisingly, continuously suckling piglets were found to exhibit more severe neurological disease and more quickly succumbed to EHEC infection than traditional CDCD animals (63). The reason for this finding is not known, but it may be related to the amount of Stx produced under the different conditions in the intestine. Consistent with this idea, Shringi and colleagues found that strains that produced higher amounts of Stx caused earlier and more severe signs of CNS disease in conventionally born piglets (64). Somewhat surprising, no signs of kidney damage were found in these animals, perhaps due to the rapid onset of neurological symptoms. The relationships between animal husbandry practice, the amount of intestinal Stx, and the resulting pathology warrant rigorous investigation in this species to further define this model system.

**Rabbits**

Rabbits have been used both to study the toxic effects of Stx and the intestinal biology of the organism, and as such, the age, breed, and route of infection affect their response to infection. Suckling New Zealand White (NZW) rabbits are naturally susceptible to oral infection by EHEC and, like piglets, develop diarrhea. Farmer and
colleagues first reported that oral inoculation of high numbers (>10^9 CFU) of *E. coli* O157:H7 caused watery diarrhea in young (5 to 10 days old) but not older (20 days old) rabbits. In contrast, the ingestion of control (nonpathogenic) *E. coli* strains failed to cause any signs of morbidity in the animals (65, 66). Large numbers of the bacteria could be recovered from the intestine, suggesting that the organism is able to multiply in this environment (65). Subsequent studies identified the colon as the principal site of EHEC-mediated disease, where histological abnormalities, including edema, hemorrhage, the presence of an inflammatory infiltrate, and mucosal epithelial apoptosis, were observed (66, 67). In these young animals, the development of diarrhea appears to be driven by inflammation-mediated changes in colonic ion transport resulting in decreased Na absorption and increased Cl secretion (68, 69). Unlike humans, suckling rabbits do not develop signs of renal disease; however, the reproduction of diarrhea and colonic inflammation, two key features of EHEC infection in humans, indicates that they may be a useful model for studying the intestinal manifestations of the disease.

The first suckling rabbit studies suggested a role for Stx in the development of diarrhea because natural isolates that produced differing amounts of Stx gave rise to differing severities of disease in the animals (66, 67). We later confirmed a role for Stx in *E. coli* O157:H7 pathogenicity using genetically defined isogenic bacterial mutants (31). Three-day-old NZW rabbits infected with an isogenic mutant that lacked the genes coding for Stx2 developed a transient diarrhea and largely non-inflammatory intestinal pathology. Thus, Stx2 appeared to increase the severity and duration of EHEC-induced diarrhea and modulate the host response to infection. However, Stx2 did not appear to act as a colonization factor because similar numbers of wild-type and mutant organisms were recovered from the colon at various times postinfection. In contrast, deletion of the genes coding for the bacterial outer membrane adhesin, intimin (*eaeA*), and the translocated intimin receptor (*tir*) markedly reduced the ability of the organism to colonize the rabbit intestine (31). Thus, in contrast to piglets, EHEC attachment to the epithelial surface is required for the organism to persist and cause intestinal disease.

One of the strengths of the suckling rabbit model is that it allowed, for the first time, a means to study factors that were important in mediating intestinal colonization and disease. Thus, using suckling rabbits we found that factors other than Tir and intimin influenced the ability of EHEC to colonize the intestine and cause disease. For example, surface-exposed structures such as the long polar fimbriae (Lpf), curli, and the O-antigen capsule all contributed to the ability of EHEC to colonize and survive in the intestine (70–72). Indeed, our studies began to reveal the complexities of regulating colonization factor expression in the intestine. While expression of Lpf contributes to EHEC intestinal colonization, in the absence of these structures, the organism appears to overexpress curli, appendages more usually associated with adherence to abiotic surfaces (73). In addition, we found that bacterial factors (other than Tir), which are translocated into the host cell via the organism’s type III secretion system, also modulate intestinal colonization (71). For example, EspFu (also known as TccP) appears to promote the spread of EHEC to new sites in the intestine. Rabbits infected with the *espFu* mutant contained significantly fewer organisms in their ceca and colons at 7 but not 2 days postinfection. Moreover, this mutant covered a significantly smaller area of the intestine than the wild type in the gnotobiotic piglets (74). However, many of the LEE-encoded effector proteins caused only minor changes in the ability of the EHEC to colonize the intestine, most likely because of functional redundancy between the different proteins (71).

Despite being sensitive to the intestinal manifestations of EHEC infection, suckling rabbits do not develop HUS or any other evidence of renal failure. The reasons for this are not clear but may be related to the species-specific distribution of Gb3. Zoja and colleagues reported that Gb3 is present in the tissues of the brain, lung, spinal cord, and intestine of weaned NZW rabbits but is missing from the heart, liver, and kidney (75). While Gb3 homologs have recently been detected in the kidney cortex of NZW and Dutch Belted (DB) rabbits, their role in disease and relative affinity for Stx remain unclear (76). In young rabbits (day 5 of life), Gb3 has been detected in lipid extracts obtained from the colon (77). Conversely, the appearance of Gb3 appears to be maturationally regulated in the small intestine, as jejunal microvillous membranes isolated from rabbits less than 16 days of age did not bind Stx (78, 79). Together, these observations may explain the sensitivity of the rabbit colon to the effects of luminal Stx in sucking animals, and the predominantly neurological manifestations of Stx when given intravenously to adult rabbits (see below).

Following a natural outbreak of bloody diarrhea and sudden death in a group of 10-week-old DB rabbits in which an Stx1-producing *E. coli* O153 isolate was recovered, the possibility was raised that this breed of rabbits may be more sensitive to the systemic effects of Stx than NZW rabbits (80). Experimental infection of 8-
week-old weaned DB rabbits with the outbreak strain confirmed that it was able to cause diarrhea and renal vascular and glomerular lesions in the animals (76). As part of this study, the authors also examined the response to infection by an E. coli O157:H7 clinical isolate. Rabbits infected with E. coli O157 lost less weight (mean ± SE: −4.6% ± 1.6 versus −18.1% ± 4.7) and shed fewer organisms (range 10^3 to 10^5 versus ≥10^5 to 10^9 CFU/g) than O153-infected rabbits, perhaps suggesting that the latter strain is better adapted to colonizing the rabbit intestine. Panda and colleagues observed no significant differences in the response of weaned NZW or DB rabbits to E. coli O157 infection (81). However, these authors noted that renal lesions did not appear to be a consistent feature in the infected rabbits. Renal pathology was detected in only 1 of 11 infected rabbits, and as such, the reproducibility of this clinical outcome would need to be improved to enable study of EHEC-induced renal disease or to allow novel therapeutics to be tested.

While suckling and young (10-week-old) rabbits are susceptible to oral administration of EHEC, older rabbits tend to be more recalcitrant to colonization by the organism. Two approaches have been used by investigators to circumvent this issue. The rabbit ligated ileal loop assay originally described by De and colleagues has been widely used to assess the enterotoxicity of pathogenic bacteria, their culture supernatants, or purified toxins (82). Likewise, both EHEC strains and purified Stx1 have been injected into ligated loops to reproduce the disease found in humans (2, 83). However, this “closed” loop model suffers from many limitations when intestinal pathogens such as EHEC are studied, including most notably an interruption to normal gut function (e.g., peristalsis).

The second approach involves the use of a natural rabbit diarrheal pathogen, E. coli strain RDEC-1, which, while naturally lacking Stx, contains the LEE pathogenicity island (84). The histological lesions caused by RDEC-1 appeared indistinguishable from those reported for other A/E pathogens, including EHEC (52, 85). To better reproduce the virulence repertoire of EHEC, Sjogren and colleagues generated an Stx-producing variant of RDEC-1 by infecting RDEC-1 with an Stx1-converting bacteriophage (86). By comparing the disease caused by the two strains, the authors observed that Stx-producing RDEC-1 induced a more severe infection than the parent strain did, causing earlier weight loss and higher rates of mortality in the animals. While these studies indicate a role for Stx in the development of severe disease, the usefulness of this model is somewhat limited by an inability to study whether additional EHEC virulence factors are important to infection.

Intravenous administration of purified Stx causes thrombotic microangiopathy in several organs in rabbits, consistent with the reported distribution of Gb3 (73, 87). The location of the resulting damage appears to depend on the type of Stx used because Stx1 primarily located to the microvasculature of the brain and spinal cord, whereas Stx2 localizes to the microvasculature of the cecum. These differences in tissue tropism also likely explain the greater lethality of Stx1 than Stx2 in rabbits, although this does not reflect the epidemiological data from human outbreaks.

**Mice**

Mice, the workhorse species of infectious disease research, are naturally resistant to colonization by EHEC and fail to develop signs of intestinal disease following oral infection. In contrast, systemic Stx causes acute kidney damage and death in the animals (88). However, key differences exist between Stx-mediated renal damage in mice and humans (12). Renal damage in mice consists of acute tubular necrosis, which is deemed characteristic of toxin insult, rather than the prothrombotic condition created following Stx-mediated endothelial injury in humans. HUS is viewed as a thrombotic microangiopathy of the glomerular blood vessels that develops with or without tubular cell death (12). This difference in pathology likely reflects species-specific differences in Gb3 distribution. In mice, Gb3 is detected in the microvascular endothelial cells of the brain cortex and pia mater (membranes surrounding the brain and spinal cord) and the capillary vascular endothelial cells surrounding renal tubules (17). As noted earlier, the highest concentrations of Gb3 expression in humans occur in the kidney, specifically on the glomerular endothelium (6).

Despite this fundamental difference in the pathological response to Stx in mice and humans, a rodent model of EHEC-mediated disease remains desirable. As such, many approaches have been taken to render mice more susceptible to oral infection or to present with a more accurate model of HUS. Because of the recent publication of an excellent review on murine models of EHEC and Stx infection by Mohawk and O’Brien (89) to which the readers are directed, this article summarizes only the main features and limitations of murine models before recent advances in the field are discussed.

The natural resistance to EHEC colonization in mice can be reduced by modifying the normal intestinal
microflora through pretreatment with antibiotics, use of germ-free animals, or via dietary-induced changes (see Table 1). All lead to increased numbers of EHEC in the intestine and the development of varying levels of renal toxic injury and death in the animals. In addition, the sensitivity of the host (e.g., “lipopolysaccharide [LPS]-responder” mice, MyD88 knockout mice) or the virulence of the bacterium (e.g., host-passaged organisms, treatment with mitomycin C) can be manipulated to further increase clinical severity. Moreover, to more accurately mimic the kidney disease seen during HUS, investigators have also tried coadministering endotoxin (LPS) with Stx. In this situation, LPS acts as an accessory proinflammatory mediator, which primes the host immune response and results in the induction of thrombocytopenia in the animals. However, the etiology of LPS plus Stx challenge differs from that of HUS and does not reflect what occurs during human infection (12).

An alternative approach that circumvents the problems of establishing EHEC colonization in mice is the use of the surrogate bacterium Citrobacter rodentium, a natural murine pathogen that induces colonic hyperplasia (90). Both EHEC and C. rodentium contain the LEE pathogenicity island that mediates Tir-intimin-based bacterial attachment to the epithelial surface (91). Because of this, C. rodentium has been used extensively to dissect the function and activity of all LEE-encoded proteins as well as many other non-LEE-encoded proteins that are translocated via the organism’s type III secretion system (92, 93). These studies have done much to advance our understanding of how translocated bacterial proteins interfere with host cell processes. However, while C. rodentium is useful for dissecting the role and function of type III secreted proteins, C. rodentium does not naturally contain the genes coding for Stx, the principal virulence factor of EHEC. To overcome this

### Table 1: Comparative murine models of EHEC-mediated disease

<table>
<thead>
<tr>
<th>Model</th>
<th>Host</th>
<th>Clinical disease</th>
<th>Key limitations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral infection</td>
<td>Streptomycin-treated CD-1 (5- to 8-week old-male mice)</td>
<td>Weight loss, renal tubular damage, mortality</td>
<td>Reduced microflora, artificial colonization, no intestinal disease</td>
<td>88, 122</td>
</tr>
<tr>
<td></td>
<td>Streptomycin + mitomycin C treatment Weaned ICR (3 weeks old)</td>
<td>Weight loss, renal tubular damage, mortality</td>
<td>Reduced microflora, no intestinal disease</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>BALB/c (17 to 20 days old); host-passaged bacteria</td>
<td>Mortality</td>
<td>No intestinal disease, rapid clearance of the pathogen, no glomerular disease</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>LPS responder C3H/HeN and C3H/HeJ (8 to 16 weeks old)</td>
<td>Anorexia, ruffled fur, neurological disease</td>
<td>No glomerular disease</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Intact microflora BALB/c (6 weeks old)</td>
<td>Weight loss, renal tubular damage, mortality</td>
<td>No glomerular disease</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>Streptomyces + MyD88−/− C57BL/6 (6 to 14 weeks old)</td>
<td>Weight loss, kidney toxic insult, mortality</td>
<td>No glomerular disease, host immune system abnormalities</td>
<td>127</td>
</tr>
<tr>
<td>Germ-free</td>
<td>Swiss Webster/3 days to 12 weeks old(IQI (8 weeks old)</td>
<td>Acute renal tubular necrosis, limited glomerular thrombosis, mortality</td>
<td>No natural microflora, poorly developed immune systems</td>
<td>128–130</td>
</tr>
<tr>
<td></td>
<td>C. rodentium C57BL/6 (5 to 8 weeks old)</td>
<td>Watery stools, weight loss, intestinal disease</td>
<td>Not EHEC</td>
<td>94</td>
</tr>
<tr>
<td>Injection</td>
<td>Conventional mice + IP injection of OMV* C57BL/6 (8 to 10 weeks old)</td>
<td>Acute renal tubular necrosis,</td>
<td>Nonbacterial, no thrombosis</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>Conventional mice + multiple IP Stx2 C57Bl/6J (8 to 10 weeks old)</td>
<td>Weight loss, signs of kidney failure, glomerular endothelial damage, neutrophilia</td>
<td>Nonbacterial</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>Conventional + IP or IV Stx1/2 CD-1 (6 to 8 weeks old)</td>
<td>Mild dehydration, renal tubular necrosis</td>
<td>No intestinal disease, no glomerular thrombosis</td>
<td>133</td>
</tr>
<tr>
<td>Other</td>
<td>Mice + human colonic xenograft model CB-17 SCID mice (6 to 8 weeks old) + 16 maturation</td>
<td>A/E lesion pathology, some mucosal damage</td>
<td>Incomplete immune system, no natural microflora, nonnatural organ environment, no systemic disease</td>
<td>96</td>
</tr>
</tbody>
</table>

*Outer membrane vesicles.
limitation, Mallick and colleagues recently developed an Stx-expressing *C. rodentium* strain by lysogenizing the bacterium with an Stx-containing phage (94). The phage taken up by *C. rodentium* was found to carry Stx genes belonging to an Stx2 variant called mucus-activatable Stx2d. Mucus-activatable Stx2d is a particularly toxic subtype of Stx2 that causes high mortality in mice but is usually found in non-LEE-encoding EHEC strains that are less commonly isolated in human outbreaks than EHEC containing Stx2 or Stx2c (95). Compared to wild-type *C. rodentium*, Stx-producing *C. rodentium* causes increased weight loss, severe destruction of the intestinal mucosa, and renal tubular damage. The development of this model allows study of robust intestinal colonization together with Stx-mediated disease, although it still does not overcome the different pathophysiological response to Stx seen in mice and humans. In addition, it does not easily allow study of other EHEC factors that are important to infection.

Another recent advance in the development of murine models has been the construction of human and bovine intestinal xenograft models (96). Immature germ-free human and bovine small intestine or colonic fetal tissues were subcutaneously transferred into SCID mice and the tissue left to develop for up to 16 weeks. The authors demonstrate that after maturation, the xenografted tissue displays appropriate organ-specific tissue architecture, complete with a range of cell types including goblet cells and microvilli. Thereafter, various EHEC strains were injected into the “intestinal lumen” of each organ. Compared to the small intestine xenografts, EHEC strains grew poorly in the lumen of the colonic xenografts. Despite this difference, distinct A/E lesions and mucosal damage were found only on tissue derived from the human colon, and not the human or bovine small intestine segments. Furthermore, A/E lesion formation was shown to be dependent on the expression of a functional type III secretion system, consistent with what has been reported in other model hosts (see above). While xenograft models allow an opportunity to study EHEC interactions with the human intestine without endangering human life, this approach faces several challenges. The “closed” nature of the segment, which does not allow any normal intestinal activity (e.g., peristalsis, food passage) to occur, the lack of a natural microflora, and the incomplete immune system of the host animal may prove to be significant limitations to the acceptance of this model system.

### Other Species

Other than pigs, rabbits, and mice, a plethora of animal species have been considered as model hosts of EHEC-induced disease (see Table 2). Like mice, ferrets are naturally resistant to oral infection by EHEC and require pretreatment with antibiotics to establish moderate numbers of the organism in the intestine (97). Despite significant weight loss following infection, none of the animals showed any signs of intestinal disease, and only around 20% showed signs of renal glomerular disease and/or thrombocytopenia. Greyhounds naturally develop cutaneous and renal glomerular vasculopathy, a disease that results in the formation of renal glomerular lesions, which are similar to those seen in human HUS (98, 99). While this model has not been used extensively since its first description, Raife and colleagues have used it to demonstrate that thrombin-dependent mechanisms

### Table 2

<table>
<thead>
<tr>
<th>Model</th>
<th>Host</th>
<th>Clinical disease</th>
<th>Key limitations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrets</td>
<td>Campylobacter-free female (6 weeks old)</td>
<td>Weight loss, limited renal glomerular pathology</td>
<td>Limited natural microflora, no intestinal disease</td>
<td>97</td>
</tr>
<tr>
<td>Greyhound</td>
<td></td>
<td>Bloody diarrhea, microvascular thrombosis, HUS</td>
<td>Nonbacterial</td>
<td>99, 100</td>
</tr>
<tr>
<td>Monkeys</td>
<td>Macaca radiata (wild-caught adults)</td>
<td>Enteritis with neutrophil infiltration, A/E lesions seen, moderate tubular blood vessel disruption</td>
<td>High dose required to establish infection, no glomerular disease</td>
<td>134</td>
</tr>
<tr>
<td>Caenorhabditis elegans fed on EHEC strains</td>
<td>N2</td>
<td>Intestinal colonization, A/E lesion formation, mortality</td>
<td>Not a model of EHEC-mediated disease</td>
<td>106</td>
</tr>
<tr>
<td>Rats + IP injection of Stx</td>
<td>Sprague-Dawley (adult male)</td>
<td>Watery diarrhea, kidney failure, thrombocytopenia, hemolytic anemia, leukocytosis</td>
<td>Nonbacterial, crude bacterial supernatants were used</td>
<td>104</td>
</tr>
<tr>
<td>Baboons + Stx injection into cephalic vein</td>
<td></td>
<td>Renal failure, thrombocytopenia, glomerular endothelial injury, inflammation, mortality</td>
<td>Nonbacterial, not the natural route of infection</td>
<td>101–103</td>
</tr>
<tr>
<td>Chickens + crop cannulation of EHEC</td>
<td>1 day old</td>
<td>Persistent shedding of the organism, A/E lesions observed</td>
<td>No clinical signs</td>
<td>135</td>
</tr>
</tbody>
</table>
are important in Stx2-mediated pathogenesis. Treatment of greyhounds with lepirudin, a thrombin inhibitor, reduced HUS-like pathology and enabled the animals to survive beyond the normal course of the experiment \(^{100}\). Baboons appear to best mimic human HUS symptoms following intravenous injection of Stx \(^{101, 102}\). In this model, both Stx1 and Stx2 were able to induce HUS in the animals, although the dose, timing, and magnitude of the response differed \(^{103}\). Rats also develop HUS-like symptoms in response to intraperitoneal challenge of filtered EHEC culture supernatants \(^{104}\). However, since purified Stx preparations were not used in this model, further investigation is required to dissect the contribution of Stx versus other soluble factors. Finally, the nematode Caenorhabditis elegans has been put forward as a naturally infected and genetic tractable animal host in which to study EHEC infection. As found previously for other enteric pathogens (e.g., EPEC) \(^{105}\), E. coli O157:H7 infects and kills C. elegans \(^{106}\). The bacterium is able to multiply and produce A/E lesions in the digestive tract of the nematodes, and killing appears to be at least partly dependent on Stx2 (Stx2 was not important in this model). It remains to be seen whether this model will lead to new information on the pathogenesis and host immune response to infection; however, the availability of powerful genetic analysis methods for C. elegans may prove invaluable.

**ROLE OF THE HOST IN MODULATING EHEC VIRULENCE**

Like other enteric pathogens, EHEC uses many host- and microbiota-derived chemicals to regulate virulence gene expression when in the host \(^{107}\). To date, only a few of these have been described. For example, it is known that the two-component system encoded by QseBC acts to recognize and respond to the presence of the host hormones epinephrine and norepinephrine or to the microbiota-derived quorum-sensing molecule AI-3 to activate flagella expression and motility \(^{108}\). It is thought that signaling through this pathway may alert EHEC to its arrival in the colon, a key site of bacterial attachment. Consistent with this idea, QseC mutants exhibit a reduced ability to colonize the colon of infant rabbits \(^{109}\). EHEC also senses fucose, a microbiota-derived sugar, whose presence at high concentrations in the intestinal lumen inhibits LEE gene expression until the bacterium reaches the epithelial surface \(^{110}\). Deletion of FusKR, the fucose-sensing two-component system, reduced EHEC’s ability to colonize the rabbit intestine compared to the parental strain. In addition to sugars, EHEC also appears to sense xanthine oxidase, a host defense molecule that is constitutively expressed by intestinal epithelial cells. Physiological concentrations of xanthine oxidase upregulate EHEC virulence factor production only as the bacterium comes into close proximity to the epithelial surface \(^{111, 112}\). Finally, diet-induced changes to butyrate concentrations in the mouse intestine were found to cause increased mortality in animals following EHEC infection \(^{113}\). The mechanistic basis for this finding is hypothesized to be due to increased levels of Gb3 expression in the kidneys and intestines of mice fed high-fiber diets. However, mice fed high-fiber diets also contained significantly fewer commensal Escherichia spp. As such, this study highlights some of the complexities faced when dealing with intact biological systems, where the host and its associated microbiota are intimately linked.

Over the past decade, there has been a growing realization that the host and its associated microbiota profoundly influence the pathologies caused by noninfectious and infectious disease agents (e.g., see references \(^{114}\) to \(^{117}\)). Many factors including diet, stress, or the environment alter the physiochemical conditions in the gut and thereby influence the composition and activities of the intestinal microbiota \(^{118}\). In turn, the intestinal microbiota contributes to numerous biological processes in the host, including metabolic, nutritional, physiological, and immunological function. As such, individual differences in host microbiota characteristics may explain some of the variation in an individual’s response to EHEC during an outbreak.

Finally, a recent report suggests that uptake of Stx-encoding genetic material by host cells, rather than the toxic protein, may contribute to Stx-induced pathology in mice \(^{119, 120}\). These studies raise the question of whether Stx-coding bacteriophages, which are released along with Stx following bacterial lysis in the intestine, play a role in Stx dissemination \(^{121}\).

**CONCLUSIONS**

Pigs, rabbits, and mice are the three most common animal species used to study EHEC-mediated disease. Despite recent advances in the field, none reproduce the full clinical spectrum of illness seen during human infection. The choice of which animal model to use must be viewed within the context of the scientific question being asked. However, studies focused on understanding the biology of the organism need to be considered within a model system that reproduces as much as possible the natural state of the intestine.
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Animal Models of Enterohemorrhagic *Escherichia coli* Infection


