

## Animal issues associated with *Escherichia coli* O157:H7

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Since *Escherichia coli* O157:H7 was first recognized in 1982 as a human pathogen, considerable progress has been made in elucidating principal vehicles of transmission. Cattle have been identified as a major source of *E coli* O157:H7 infection of humans, with as many as 1 in 4 animals at slaughter shedding the pathogen in feces during the summer months.<sup>1</sup> Case-control studies of sporadic cases of *E coli* O157:H7 infection in the United States, Canada, and Europe have identified eating undercooked ground beef, visiting farms, and handling animals on the farm as principal risk factors for infection.<sup>2a</sup> Cattle manure, of which an estimated 1.2 billion tons are produced annually in the United States,<sup>3</sup> appears to be a principal source of the *E coli* O157:H7 problem. Animals, water, and food that contact cattle manure are potential vehicles of *E coli* O157:H7. An effective control program to substantially reduce *E coli* O157:H7 infections will require the implementation of intervention strategies throughout the food continuum, from farm to table. Promising intervention measures at the farm include competitive exclusion bacteria, bacteriophage, and targeted animal management practices addressing common points of contamination. Innovative intervention treatments are under development for use by food processors; however, most treatments have limitations that restrict their use to specific types of foods. For example, irradiation can create major off odors and flavors in foods that contain more than 10% fat. Consumers also have a role in implementing intervention controls in food handling and preparation. Unfortunately, many consumers eat high-risk foods, improperly handle and store foods, and ignore warnings regarding foods known to be unsafe. We all have a role in reducing the risk of foodborne ill-

ness, including *E coli* O157:H7 infections, but clearly more needs to be done on the farm, including validating proposed and developing innovative on-farm control measures.

### Public Health Concerns

Estimates by the Centers for Disease Control and Prevention (CDC) indicate that enterohemorrhagic *E coli* (EHEC) serotype O157:H7 is responsible for approximately 62,500 cases of foodborne infection annually in the United States.<sup>4</sup> These estimates include hospitalizations and 52 deaths, which are largely associated with cases of pediatric hemolytic uremic syndrome (HUS), a leading cause of renal failure in children. Sporadic cases in adults are less likely to be diagnosed and reported, because the gastrointestinal disease can be mild. The CDC, using the FoodNet surveillance system, reported an overall incidence of 2.1 to 2.8 cases/100,000 persons from 1996 to 1999.<sup>5</sup> Although enterohemorrhagic disease occurred in most of the confirmed cases, HUS was more likely to develop in female children (61% of cases) around 4 years of age. In the United States, the major serotype of EHEC is O157:H7, although other serotypes can be associated with enterohemorrhagic disease. *Escherichia coli* O157:H7 can be cultured from only about 40% of fecal samples of patients that have signs of enterohemorrhagic disease, although approximately 60% of HUS cases can be confirmed by bacteriologic culture. Toxin activity can be detected in 76% of fecal samples from HUS patients; however, < 25% of samples are usually tested.

Undercooked ground beef is the most commonly identified vehicle associated with outbreaks of *E coli* O157:H7 infection, and the bacteria have been cultured from 28% of cattle at slaughterhouses in the Midwest during the summer months.<sup>1</sup> However, some *E coli* O157 isolates may not contain the virulence factors necessary for human disease, and their importance on public health is not yet clear.<sup>6</sup> Although undercooked ground beef is the vehicle most often associated with human disease, other foods such as salami, sushi, ice cream, milk, cheese curds, unpasteurized apple cider and juice, lettuce, and alfalfa sprouts have also been implicated in outbreaks of illness. Contaminated water in lakes, ponds, and swimming pools has also been a major vehicle of outbreaks.<sup>7,8</sup> Several outbreaks involving children have been reported

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following a visit to a county fair, farm, or petting zoo following contact with carrier animals. Handling calves is a common source of human infection, as calves are colonized by *E coli* O157:H7.<sup>9</sup> Although no cases have been definitively confirmed in humans, dogs have been implicated in a few sporadic infections in children.<sup>9</sup> Person-to-person transmission has also been reported as a source of infection in child-care centers.<sup>10</sup>

### Current Issues Associated with Animals

Cattle, including dairy animal production systems, are important sources of *E coli* O157:H7. *Escherichia coli* O157:H7 is a transient member of the normal flora of cows and has been only exceptionally associated with clinical disease in neonatal calves. The prevalence of *E coli* O157:H7 has been reported to be from < 1 to 5% in numerous studies<sup>11-16</sup> in the United States, Canada, Australia, Norway, and Finland; however, studies<sup>17-19</sup> using more sensitive methods for detection now report rates of *E coli* O157:H7 fecal contamination as high as 13 to 28% of animals. The mean duration that an individual animal has positive culture results is 30 days, but the range in duration can vary from a few days to a year.<sup>16,20</sup> This variation has been attributed to many factors such as diet, drinking water contamination, competing microbial flora, immune response, age, breed, *E coli* strain, housing conditions, and season. Warm weather (summer months) correlates with an increase in rates of *E coli* O157:H7 fecal shedding. *Escherichia coli* O157:H7 can be found in the feces of calves that are only 48 to 72 hours old. Stresses, such as pregnancy and calving, do not increase the shedding of *E coli* O157:H7 in cattle. Strains of *E coli* O157:H7 carried in the gastrointestinal tract of cattle are often being replaced by new and different strains, but herd infection is still maintained. Calves derive **Shiga toxin-producing *E coli* (STEC) isolates like *E coli* O157:H7 from the general herd and not solely from the dam.**<sup>11</sup> Many STEC strains cycle with a high turnover through individual cattle, though predominant strains exist collectively on a farm and can be specific to that farm.<sup>21</sup> Calves at weaning are of the age group that most frequently shed STEC. Goats, sheep, and swine can also be carriers of *E coli* O157:H7; however, swine do not appear to be a major source of the pathogen.

Horses and ponies also can be carriers of *E coli* O157:H7, as has been observed in 2 outbreak investigations and in studies<sup>22-24</sup> of sources of *E coli* O157:H7 in the farm environment.

Signs of disease have not been observed in deer that are carriers of *E coli* O157:H7. The prevalence of *E coli* O157:H7 in deer was 2.5% in north central Kansas, which is similar to the prevalence found in cattle in the same geographic area.<sup>20</sup> Cattle and deer that used the same pasture carried many of the same strains of *E coli* O157:H7 as determined by genetic subtyping. A more recent study<sup>25</sup> in the southeastern United States revealed that only a small number (0.63%) of deer fecal samples were positive during 1 year but not the next. A much higher percentage (4.3%) of fecal samples from cattle obtained from the same location a year later were positive, but the isolates were genetically distinct from the deer isolates as determined by **pulsed-field gel electrophoresis (PFGE)**. These differences in find-

ings may reflect regional variations in the epidemiologic characteristics of *E coli* O157:H7. It has not been elucidated whether deer are a source of infection to cattle or vice versa; however, it appears that deer in the southeast most likely acquire the pathogen from cattle.

*Escherichia coli* O157:H7 infection has been documented several times in dogs<sup>9,26</sup> but never in cats. All the reports are associated with the farm environment. One of the reports identified an outbreak of *E coli* O157:H7, where epidemiologically related strains of O157:H7 were isolated from a dog, a pony from the same farm, and a child that developed bloody diarrhea after the infection.<sup>9</sup> Although in this study the cattle and the goat had negative test results, the limited number of samples taken and the fact the agent is shed intermittently does not rule out cattle as the source of the pathogens. In a study of the sources of *E coli* O157:H7 in feedlots and farms in the northwestern United States, 65 dog samples and 33 cat samples were obtained and analyzed for the presence of *E coli* O157:H7. Results for all cat samples were negative, and 3.1% of the dog samples tested positive. The probable source of the bacterium for the dogs on these farms could have been the cattle directly or the water troughs.<sup>26</sup> In a comparison study<sup>27</sup> using a newly developed phage typing scheme for *E coli* O157:H7, it was determined that an STEC strain isolated from a dog was of the same phage type as *E coli* O157:H7 isolates obtained from humans. Two additional reports, both from the United Kingdom, associating *E coli* O157:H7 with dogs have been reported through Pro-Med<sup>28</sup> and the FS Net.<sup>29</sup> The Pro-Med report<sup>28</sup> describes a geriatric, double incontinent dog that shed *E coli* O157:H7 in its feces. The dog's diet consisted of dry food and occasional food scraps. The FS Net report<sup>29</sup> described results of an outbreak investigation in which 90 fecal samples from dogs were obtained from 4 different beaches, and 7.8% of the samples were contaminated with *E coli* O157:H7. The role of dogs in transmitting *E coli* O157:H7 to humans should be considered as a potential risk factor for infection.

### Antimicrobial Resistance

Compared with other foodborne pathogens, antimicrobial resistance in *E coli* O157 is generally low (0.8 to 8%) and limited to a few antimicrobials, tetracycline, streptomycin, sulfamethoxazole, and trimethoprim.<sup>30</sup> Antimicrobial resistance in *E coli* O157:H7 appears to be attributable to the acquisition of conjugative R plasmids,<sup>31</sup> possibly from the microflora of its ruminant host. Although epidemiologic studies<sup>32,33</sup> suggest that administering antimicrobials to animals is at least partially responsible for the emergence of antimicrobial resistance in important foodborne pathogens, there is little direct experimental evidence to indicate the frequency of these events. We do not know why antimicrobial resistance has been slow to develop, despite the existing reservoir for drug resistance genes in other *E coli* and *Salmonella* spp colonizing various animal species, including cattle.<sup>34</sup> Acquisition of antimicrobial resistant plasmids can result in an ecological disadvantage for the recipient of plasmids if it has to compete with the resident microflora. We do not know whether R plasmids might also present *E coli* O157:H7

with similar hardship in the rumen or elsewhere in the gastrointestinal tract. It is also possible that the O157 virulence plasmid prohibits the establishment of certain R plasmids that belong to the same incompatibility group as the virulence plasmid, preventing coexistence of both plasmids.<sup>35</sup> A limited repertoire of R plasmids belonging to incompatibility groups compatible with the O157 virulence plasmid may explain the slow and limited emergence of resistance in *E coli* O157:H7.

### Pathogenesis of EHEC

Enterohemorrhagic *E coli* are characterized by the presence of Shiga toxin (Stx) genes, locus for enterocyte effacement (LEE), and a large molecular weight plasmid that encodes for a hemolysin. These 3 virulence factors are present in most *E coli* associated with bloody diarrhea and HUS in humans.

The LEE is a large cluster of genes that collectively are responsible for the intimate attachment of the bacterium to the apical membrane of the enterocyte and subsequent destruction or effacement of the microvilli.<sup>36</sup> The intimate attachment of the bacterial cell to the epithelium is attributed to the adhesin, intimin, and Tir, a bacterial protein, which is inserted into the host membrane and serves as the receptor for intimin. Both factors are part of LEE in enteropathogenic *E coli* (EPEC) and EHEC. Intimin appears to be an essential component in initiating attachment, colonization, and the subsequent pathologic changes that follow infection with EPEC and EHEC.<sup>37</sup> Outside of its role in the disease process in human infections, it is uncertain whether intimin has a role in the colonization of healthy cattle.<sup>38</sup>

In addition to LEE, *E coli* O157:H7 possesses a large molecular weight plasmid that contains several putative virulence genes, including a pore-forming hemolysin.<sup>35</sup> Virulence plasmids are common features of pathogenic *E coli*, encoding toxins, adhesins, and other factors necessary for colonization, survival, and ability to cause disease in its animal host. The contribution of any of these plasmid-associated factors to disease is currently unknown.

*Escherichia coli* O157:H7 and other EHEC O serotypes produce a toxin similar in its amino acid sequence to Shigella toxin, hence the commonly used nomenclature for this *E coli* toxin in the literature, Shiga toxin or vero toxin. Among EHEC, there are 2 major immunologically distinct toxins, Stx1 and Stx2. Within the Stx2, there are additional antigenic variants. The Stx2v (variant)-producing *E coli* are associated with diseases in domestic animals, such as edema disease of swine. Enterohemorrhagic *E coli* that commonly cause human illnesses produce Stx1, Stx2, or both. The presence of the Stx2 in these EHEC has a profound influence on the progression of the disease from hemorrhagic colitis to HUS. As is common for many bacterial toxins, Stx consists of 2 subunits. The Stx-A subunit contains the enzymatic activity responsible for inhibiting protein synthesis, and the B-subunit acts as a lectin, binding the toxins to intestinal epithelial and kidney endothelial cells. The Stx is believed to be the major factor contributing to the lesions in HUS, although the O157 lipopolysaccharide may also contribute to this disease syndrome.<sup>39</sup>

The virulence and pathogenesis of EHEC are associated with the presence of  $\lambda$ -like phages harboring these

Stx genes.<sup>40</sup> Deoxyribonucleic acid damaging agents activate the dormant, toxigenic bacteriophages of *E coli* O157:H7 from its latent state. The virus particle may be activated from its latent state in its *E coli* host as a response to signals present in the gastrointestinal tract.<sup>41</sup> Once released, this virus can infect susceptible *E coli* hosts, creating new toxin-producing cells. In addition to releasing the virion from its quiescent state in *E coli*, DNA-damaging agents also can induce expression and release of toxin, consequences of which may be devastating to its animal host.<sup>42</sup> Therefore, antimicrobials that cause DNA damage in the bacterial cell (ie, quinolones) would be contraindicated for treatment of *E coli* infection.

### On-Farm Intervention Strategies

*Escherichia coli* O157:H7 is a transient inhabitant of the gastrointestinal tract of ruminants and other mammals. Cattle and sheep feces serve as sources for contamination of food products and water sources. *Escherichia coli* O157:H7 is widely distributed in cattle populations throughout the world. Prevalence of infection for individual cattle varies from 0 to 28% and herd incidence varies from 0 to 75%, with more recent surveys using more sensitive assays identifying<sup>1,17-19</sup> that the prevalence of *E coli* O157:H7 is considerably higher than reported previously. In a prevalence study of US dairies, *E coli* O157:H7-positive fecal samples were found in 75% of herds tested over a 6-month period.<sup>17</sup> In a survey of 120 fecal samples from each of 100 feedlots in 13 states, 63% of feedlots tested positive for *E coli* O157.<sup>18</sup> A large survey<sup>19</sup> of 10,415 fecal samples of US beef cattle at feedlots during October 1999 through September 2000 in 12 leading cattle feeding states revealed *E coli* O157 in 11% of samples, with the highest prevalence (19.9%) during September and the lowest (3.3%) during February.

*Escherichia coli* O157:H7 is not pathogenic in cattle and is shed in the feces of healthy cattle. *Escherichia coli* O157:H7 does not invade the gastrointestinal tract, and adherence to the mucosa does not appear to be a prerequisite for fecal shedding.<sup>37,43</sup> Fecal shedding is transient in cattle, often lasting 1 to 3 months or less, but *E coli* O157:H7 can persist on individual farms for up to 2 years.<sup>44</sup> *Escherichia coli* O157:H7 can be isolated from other sources on the farm, such as water, horses, sheep, milk filters, and stable flies. Fecal shedding is more prevalent in the United States and Canada during the summer months and is more prevalent in Britain in the spring and fall. Also, fecal shedding varies among different classes of animals. Weaned heifers between 3 months of age and breeding age are more likely to shed *E coli* O157:H7 in feces than adult cattle or younger calves.<sup>16,43</sup> Increased fecal shedding is associated with weaning and with the first month of lactation in dairy herds,<sup>44</sup> and culled dairy cattle have a higher prevalence than has been previously reported.<sup>45</sup> Contaminated water troughs, particularly those that are allowed to develop sediments, provide an environment for survival, proliferation, and horizontal spread of *E coli* O157:H7. Feces and *E coli* O157:H7 contaminated water can contaminate pastures and crops where it can survive and serve as a source for contamination of feed.<sup>46</sup> In addition, *E coli* O157:H7 proliferates to extremely high populations in moist silage.

Prevalence of *E coli* O157:H7 has been associated with some management practices on individual farms.<sup>18,47</sup> However, cause and effect relationships between specific management practice and increased fecal shedding have not been established.

The effects of different feeding practices were addressed in several prospective studies.<sup>43,48-52</sup> Growth of *E coli* and *Salmonella* spp increases in the rumen of sheep and cattle from which feed has been withheld. However, studies<sup>43,48</sup> in which calves were experimentally inoculated with *E coli* O157:H7 and subsequently had feed withheld failed to have an increase in fecal shedding. Results of studies<sup>49-52</sup> addressing the effects of feeding different amounts of concentrate and roughage, or feeds from different plant sources, are contradictory. Given that fecal shedding of *E coli* O157:H7 is transient in cattle, any feed change or feeding practice or stress that disturbs the normal flora of the intestine could promote transient colonization with *E coli* O157:H7 resulting in increased shedding. This may explain the association of increased shedding with the onset of lactation, weaning, and cattle recently placed on feed at feedlots.<sup>18,44</sup>

Undercooked beef products, unpasteurized milk and dairy products, and contaminated water are all potential vehicles for human infection with *E coli* O157:H7. Therefore, efforts to decrease fecal shedding of *E coli* O157:H7 in cattle must be considered as an important part in any plan to reduce the incidence of human infection. However, a transient, nonpathogenic, enteric bacterium that is not even host specific for cattle does not lend itself to conventional control measures. At present, there are few recommendations that can be made with confidence. The widespread prevalence of *E coli* O157:H7 on farms and the transient nature of shedding make preslaughter testing and trace back unpractical. Vaccination is not likely to succeed because mucosal colonization is not a requirement for shedding, and prior exposure does not prevent subsequent shedding.<sup>38,43</sup> Strategies to alter or stabilize the microflora to reduce shedding of *E coli* O157:H7 or coliforms in general are currently under investigation. Strategies to alter the rumen and intestinal microflora to exclude establishment of the transient *E coli*, even if only temporary, may prove to be beneficial if shedding were reduced prior to slaughter or if horizontal spread among cattle were reduced. Competitive exclusion using a mixture of *E coli* isolated from cattle feces reduces shedding in cattle experimentally inoculated with *E coli* O157:H7,<sup>53</sup> as does administration of bacteriophages that lyse *E coli* O157:H7.<sup>b</sup> A combination of substrates from feedstuffs, along with the proper indigenous microflora, can produce antimicrobial plant compounds that are toxic to coliforms. For example, *Prevotella* spp and *Bacteriodes* spp hydrolyze plant esculin to aglycones. These aglycones can inhibit growth of *E coli* O157:H7.<sup>54</sup> Improved overall sanitation on the farm to reduce fecal contamination of water troughs, other water supplies, and fecal soiling of hides should reduce environmental contamination and contamination of beef products at slaughter.

## Conclusion

Present estimates indicate more than 1.2 billion tons of cattle manure are produced annually in the

United States.<sup>3</sup> Cattle manure is an important source of *E coli* O157:H7 that is carried in the gastrointestinal tract of cattle and shed in their feces. Cattle feces can contaminate food when used as a soil amendment to fertilize crops, when introduced into water used to irrigate crops, when cattle graze near fields of food crops, when milk is obtained from dairy cows, and when intestinal contents or fecal-contaminated hides contact carcasses during slaughter operations. Because *E coli* O157:H7 is not an invasive pathogen, contamination of meat is principally from contact with feces from cattle. In addition, many recent outbreaks of *E coli* O157:H7 infections resulted from children handling cattle on a farm or contact with manure at a farm or fair.<sup>55,56</sup> Exposure to *E coli* O157:H7-contaminated food and water and direct contact with *E coli* O157:H7-contaminated manure are important public health problems. The veterinary community should play a leadership role in finding ways to reduce the risk of human exposure to *E coli* O157:H7 from animal sources.

<sup>a</sup>Kassenborg H, Hedberg C, Evans M, et al. Case-control study of sporadic *Escherichia coli* O157:H7 infections in 5 FoodNet sites (abstr) in *Proceedings. Int Conf Emerg Infect Dis* 1998;50.

<sup>b</sup>Waddell T, Mazzocco A, Johnson R, et al. Control of *Escherichia coli* O157:H7 infection of calves by bacteriophage (abstr), in *Proceedings. 4th Int Symp Workshop: Shiga Toxin (Verocytotoxin)-Producing Escherichia coli* Infections 2000;90.

## References

1. Elder RO, Keen JF, Siragusa GR, et al. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc Natl Acad Sci U S A* 2000;97:2999-3003.
2. Michel P, Wilson JB, Martin SW, et al. Temporal and geographic distributions of reported cases of *Escherichia coli* O157:H7 infection in Ontario. *Epidemiol Infect* 1999;122:193-200.
3. Minority Staff of the U.S. Senate Committee on Agriculture, Nutrition & Forestry. Animal waste pollution in America: an emerging national problem. 1997. Available at: <http://www.senate.gov/~agriculture/animalw.htm>. Accessed June 28, 2002.
4. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis* 1999;5:607-625.
5. Kennedy M, Angulo FJ, the FoodNet Working Group. Incidence of foodborne illness: 1999 data from FoodNet. *Irish J Agr Food Res* 2000;39:295-300.
6. Galland JC, Hyatt DR, Crupper SS, et al. Prevalence, antibiotic susceptibility, and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. *Appl Environ Microbiol* 2001;67:1619-1627.
7. Chalmers RM, Aird H, Bolton FJ. Waterborne *Escherichia coli* O157. *J Appl Microbiol* 2000;88:1245-1325.
8. Licence K, Oates KR, Syngue BA, et al. An outbreak of *E coli* O157 infection with evidence of spread from animals to man through contamination of a private water supply. *Epidemiol Infect* 2001;126:135-138.
9. Trevena WB, Hooper RS, Wray C, et al. Vero cytotoxin producing *Escherichia coli* O157 associated with companion animals. *Vet Rec* 1996;138:400.
10. Reida P, Wolff M, Pohls HW, et al. An outbreak due to enterohaemorrhagic *Escherichia coli* O157:H7 in a children day care center characterized by person-to-person transmission and environmental contamination. *Zentralbl Bacteriol* 1994;281:534-543.
11. Cobbold R, Desmarchelier P. A longitudinal study of Shiga-toxicogenic *Escherichia coli* (STEC) prevalence in three Australian dairy herds. *Vet Microbiol* 2000;71:3125-3137.
12. Hancock DD, Besser TE, Kinsel ML, et al. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. *Epidemiol Infect* 1994;113:199-207.

13. Richards MS, Corkish JD, Sayers AR, et al. Studies on the presence of verocytotoxic *Escherichia coli* O157 in bovine faeces submitted for diagnostic purposes in England and Wales and on beef carcasses in abattoirs in the United Kingdom. *Epidemiol Infect* 1998;120:187–192.
14. Vold L, Klungseth JB, Kruse H, et al. Occurrence of Shiga-toxinogenic *Escherichia coli* O157 in Norwegian cattle herds. *Epidemiol Infect* 1998;120:21–28.
15. Wells JG, Shipman LD, Greene KD, et al. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. *J Clin Microbiol* 1991;29:985–989.
16. Zhao T, Doyle MP, Shere J, et al. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Appl Environ Microbiol* 1995;61:1290–1293.
17. Hancock DD, Besser TE, Rice DH, et al. Longitudinal study of *Escherichia coli* O157:H7 in fourteen cattle herds. *Epidemiol Infect* 1997;118:193–195.
18. Dargatz DA, Wells SJ, Thomas LA, et al. Factors associated with the presence of *Escherichia coli* O157:H7 in feces of feedlot cattle. *J Food Prot* 1997;60:466–470.
19. USDA-APHIS. *Escherichia coli* O157 in United States feed lots. Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, Colo: USDA, 2001;1–2.
20. Sargeant JM, Hafer DJ, Gillespie JR, et al. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle. *J Am Vet Med Assoc* 1999;215:792–794.
21. Cobbold R, Desmarchelier P. Characterization and clonal relationships of shiga-toxinogenic *Escherichia coli* (STEC) isolated from Australian dairy cattle. *Vet Microbiol* 2001;79:323–335.
22. Chalmers RM, Salmon RL, Wilshaw GA, et al. Verocytotoxin-producing *Escherichia coli* O157 in a farmer handling horses. *Lancet* 1997;349:1816.
23. Hancock DD, Besser TE, Rice DH. Ecology of *Escherichia coli* O157:H7 in cattle and impact of management practices. In: Kaper JB, O'Brien AD, eds. *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* strains. Washington, DC: ASM Press, 1998; 85–91.
24. Trevena WB, Hooper RS, Wray C, et al. Vero cytotoxin-producing *Escherichia coli* O157 associated with companion animals. *Vet Rec* 1996;138:400.
25. Fisher JR, Zhao T, Doyle MP, et al. Experimental and field studies of *Escherichia coli* O157:H7 in white-tailed deer. *Appl Environ Microbiol* 2001;67:1218–1224.
26. Hancock DD, Besser TE, Rice DH, et al. Multiple sources of *Escherichia coli* in feedlots and dairy farms in the Northwestern USA. *Prev Vet Med* 1998;35:11–19.
27. Khakhria R, Duck D, Lior H. Extended phage typing scheme for *Escherichia coli* O157:H7. *Epidemiol Infect* 1990;105:511–520.
28. ProMed. *E. coli* O157, dog, human-UK. 20010510.0899. Available at: <http://www.promedmail.org>. Accessed May 10, 2001.
29. Food Safety Network (FSNet), *E. coli* linked to dog feces. Available at: <http://www.plant.uoguelph.ca/safefood/archives/fsnet/2000/6-2000/fs-06-01-00-02.txt>. Accessed June 9, 2000.
30. NARMS. National Antimicrobial Resistance Monitoring Program: enteric pathogens. Food and Drug Administration, Centers for Disease Control and Prevention. Rockville, Md: USDA 1999;1–115.
31. Zhao S, White DG, Ge B, et al. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. *Appl Environ Microbiol* 2001;67:1558–1564.
32. Chaslus-Dancla E, Pohl P, Meurisse M, et al. High genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides gentamicin and apramycin. *Antimicrob Agents Chemother* 1991;35:590–593.
33. Cohen ML, Tauxe RV. Drug-resistant *Salmonella* in the United States: an epidemiological perspective. *Science* 1986;234:964–969.
34. Goldstein C, Lee MD, Sanchez S, et al. Incidence of class 1 and 2 integrases in clinical and normal flora bacteria from livestock, companion animals, and exotics. *Antimicrob Agents Chemother* 2001;45:723–726.
35. Burland V, Shao Y, Perna NT, et al. The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. *Nucleic Acids Res* 1998;26:4196–4204.
36. Perna NT, Mayhew GF, Posfai G, et al. Molecular evolution of a pathogenicity island from enterohemorrhagic *Escherichia coli* O157:H7. *Infect Immun* 1998;66:3810–3817.
37. Dean-Nystrom B, Bosworth T, Moon HW, et al. *Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves. *Infect Immun* 1998;66:4560–4563.
38. Brown CA, Harmon BG, Zhao T, et al. Experimental *Escherichia coli* O157:H7 carriage in calves. *Appl Environ Microbiol* 1997;63:27–32.
39. Karpman D, Connell H, Svensson M, et al. The role of lipopolysaccharide and Shiga-like toxin in a mouse model of *Escherichia coli* O157:H7 infection. *J Infect Dis* 1997;175:611–620.
40. O'Brien AD, Newland JW, Miller SE, et al. Shiga-like toxin-converting phage from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science* 1984;226:694–696.
41. Acheson DW, Reidl J, Zhang X, et al. In vivo transduction with Shiga toxin 1-encoding phage. *Infect Immun* 1998;66:4496–4498.
42. Zhang X, McDaniel AD, Wolf LE, et al. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J Infect Dis* 2000;181:664–670.
43. Cray WC, Moon HW. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl Environ Microbiol* 1995;61:1586–1590.
44. Mechie SC, Chapman PA, Siddons CA. A fifteen-month study of *Escherichia coli* O157:H7 in a dairy herd. *Epidemiol Infect* 1997;118:17–25.
45. Rice DH, Ebel ED, Hancock DD, et al. *Escherichia coli* O157:H7 in cull dairy cows on farm and at slaughter. *J Food Prot* 1997;60:1386–1387.
46. Maule A. Survival of vero cytotoxigenic *Escherichia coli* O157:H7 in soil, water and on surfaces. *J Appl Microbiol* 2000;88:715–785.
47. Garber LP, Wells SJ, Hancock DD, et al. Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. *J Am Vet Med Assoc* 1995;207:46–49.
48. Harmon BG, Brown CA, Tkalcic S, et al. Fecal shedding and rumen growth of *Escherichia coli* O157:H7 in fasted calves. *J Food Prot* 1999;62:574–579.
49. Diez-Gonzalez F, Callaway TR, Russell JB. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. *Science* 1998;281:1666–1668.
50. Hovde CJ, Austin PR, Cloud KA, et al. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. *Appl Environ Microbiol* 1999;65:3233–3235.
51. Jordan D, McEwen SA. Effects of duration of fasting and short-term, high-roughage rations on the concentration of *Escherichia coli* biotype 1 in cattle feces. *J Food Prot* 1998;61:531–534.
52. Tkalcic S, Brown CA, Harmon BG, et al. Effects of diet on rumen proliferation and fecal shedding of *Escherichia coli* O157:H7 in calves. *J Food Prot* 2000;63:1630–1636.
53. Zhao T, Doyle MP, Harmon BG, et al. Reduction of carriage of enterohemorrhagic *Escherichia coli* O157:H7 in cattle by inoculation with probiotic bacteria. *J Clin Microbiol* 1998;36:641–647.
54. Duncan SH, Flint HJ, Stewart CS. Inhibitory activity of gut bacteria against *Escherichia coli* O157:H7 mediated by dietary plant metabolites. *Fed Eur Microbiol Soc Microbiol Lett* 1998;164:283–288.
55. Centers for Disease Control and Prevention. Public health dispatch: outbreak of *Escherichia coli* O157:H7 and *Campylobacter* among attendees of the Washington County Fair—New York, 1999. *MMWR Morbid Mortal Wkly Rep* 1999;48:803–804.
56. Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infections among children associated with farm visits—Pennsylvania and Washington, 2000. *MMWR Morbid Mortal Wkly Rep* 2001;50:293–297.