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Colonization and Transmission of *Escherichia coli* O157:H7 in Swine

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Introduction

Escherichia coli O157:H7 and other serogroups of Shiga toxin-producing *E. coli* (STEC) have emerged over the last several decades as a significant cause of foodborne illness in the United States. Approximately 5–10% of people clinically infected by these bacteria develop a systemic disease, hemolytic uremic syndrome, which has a fatality rate of approximately 5%. The Centers for Disease Control estimates that STEC cause some 110,000 illnesses and 90 deaths annually in the United States (Mead et al. 1999). In addition, the economic consequences of recalling large lots of food for public health reasons are significant. Cattle are considered to be the primary reservoir for STEC. Depending on the season, the methods used for bacterial culture and the age of the animals, the prevalence of *E. coli* O157:H7 in U.S. cattle ranges from 2–28% (Hancock et al. 1994; Elder et al. 2000). *E. coli* O157:H7 has also been recovered from other ruminants such as sheep (Kudva et al. 1996) and deer (Keene et al. 1997; Sargeant et al. 1999).

Epidemiology of *E. coli* O157:H7 in Swine

In contrast to ruminants, STEC are only occasionally recovered from nonruminant animals such as dogs, birds, and raccoons (Beutin et al. 1993; Wallace et al. 1997; Hancock et al. 1998). A 1995 USDA swine survey involving 4,200 head reported the prevalence of *E. coli* O157:H7 was <0.07% (Bush 1997) and the 2000 USDA survey did not recover any *E. coli* O157:H7 from 2,526 animals (APHIS 2001). However, *E. coli* O157:H7 has been recovered from the colon contents of

6/305 (2%) pigs at a U.S. slaughter facility (Feder et al. 2003) and from 13/1,102 swine fecal samples collected at agricultural fairs (Keen et al. 2006). The organism has also been recovered from healthy swine in Japan (3/221), The Netherlands (1/145), Norway (2/1,976), Chile (13/120), and Canada (40/660) (Heuvelink et al. 1999; Nakazawa et al. 1999; Johnsen et al. 2001; Gyles et al. 2002; Borie et al. 1997). During the summer of 2006, spinach potentially contaminated by both feral swine and cattle manure caused a large outbreak of human illness in the United States (Jay et al. 2007). In addition, a small family cluster of *E. coli* O157:H7 infections was traced back to dry pork salami (Conedera et al. 2007).

Experimental Infections

Experimentally, we have shown that *E. coli* O157:H7 can establish and maintain a population in the intestinal tract of some market-weight pigs for at least two months (Booher et al. 2002). In that experiment two different strains of *E. coli* O157:H7 were included in a cocktail inoculum along with three other pathogenic

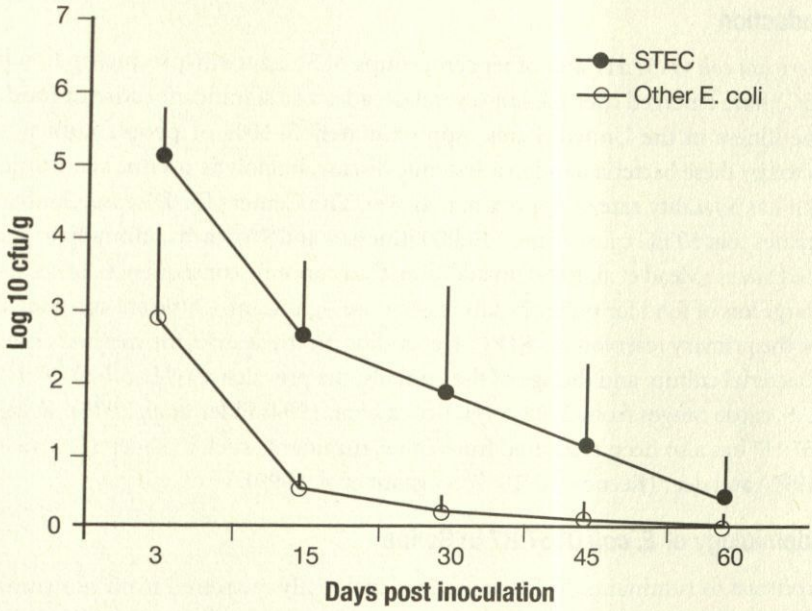

Figure 3.1. Mean fecal shedding of *E. coli* by pigs inoculated with 5 strain cocktail. The inoculum consisted of 2 *E. coli* O157:H7 strains (STEC), 2 enterotoxigenic *E. coli* strains and an enteropathogenic *E. coli* strain.

E. coli strains (two enterotoxigenic porcine pathogens and one enteropathogenic human pathogen). All pigs were fed a commercially available antibiotic-free diet for two weeks prior to inoculation. The geometric mean fecal shedding of the *E. coli* O157:H7 strains was of a higher magnitude and occurred over a longer time period than shedding of the other *E. coli* strains (Figure 3.1). At necropsy, *E. coli* O157:H7 was recovered primarily from tissues of the lower gastrointestinal tract, as it is from experimentally inoculated ruminants (Cornick et al. 2000; Booher et al. 2002; Cornick and Helgerson 2004). In addition, significant numbers of *E. coli* O157:H7 may be recovered from the tonsils of some pigs (Booher et al. 2002; Cornick and Helgerson 2004). This is in contrast to sheep in which the organism is rarely, if ever recovered from the tonsils. Taken together these studies demonstrate that the magnitude and persistence of fecal shedding of *E. coli* O157:H7 by experimentally inoculated swine is similar to that which occurs in ruminants (Figure 3.2) and suggests that there is not an absolute biological barrier to the colonization of swine by *E. coli* O157:H7.

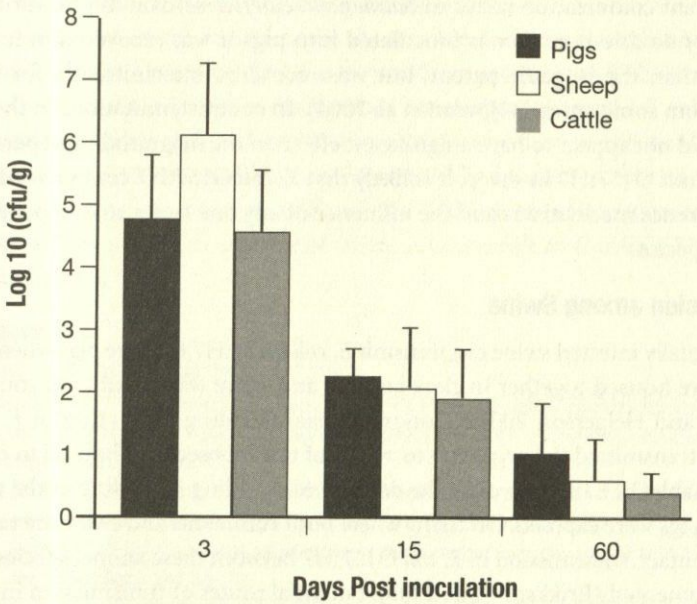

Figure 3.2. Mean fecal shedding of *E. coli* O157:H7 by pigs (n = 15), sheep (n = 39), and cattle (n = 8) inoculated with 10^{10} cfu.

Colonization Mechanisms

The mechanisms utilized by *E. coli* O157:H7 to colonize swine and ruminants are not completely understood. However, some of our work with isogenic mutants suggests that there are differences in how the organism interacts with the gastrointestinal epithelium in these two animal species. Intimin, an outer membrane protein that is required for colonization in some animal models of pathogenesis, and the intimin receptor, Tir, have been shown to be important in the colonization of both sheep and cattle by *E. coli* O157:H7 (Dean-Nystrom et al. 1998; Cornick et al. 2002; Woodward et al. 2003; Sheng et al. 2006; Vlisidou et al. 2006). However, when 12-week old pigs were dually inoculated with the wild-type strain and an isogenic Δ*eae* (intimin) mutant, similar numbers of both strains were recovered from fecal samples and from tissues throughout the alimentary tract for up to five weeks post inoculation (Jordan et al. 2005). This was confirmed by the work of Best et al. (2006) using a Shiga toxin negative parent strain and an isogenic intimin mutant. In addition, their work demonstrated that an aflagellar mutant also colonized pigs to a similar extent as the wild-type parent. The *E. coli* O157:H7 genome contains two homologous operons of long polar fimbriae (*lpf*), an important colonization factor of *Salmonella enterica* serovar Typhimurium. When a *lpf* double mutant was inoculated into pigs it was recovered in lower numbers than the isogenic parent, but was recovered intermittently for two months from some animals (Jordan et al. 2004). In contrast, mutations in the *lpf* operons did not appear to have a significant effect on the magnitude and persistence of *E. coli* O157:H7 in sheep. It is likely that *E. coli* O157:H7 contains redundant adherence mechanisms and the influence of any one factor may depend on the host species.

Transmission among Swine

Experimentally infected swine can transmit *E. coli* O157:H7 to naïve pigs when the animals are housed together in close contact and share water and food sources (Cornick and Helgerson 2004). Donor animals shedding $<10^4$ cfu/g of *E. coli* O157:H7 transmitted the organism to >50% of the exposed pigs housed in close contact (Table 3.1). In some cases the donor was shedding $<10^2$ cfu/g at the time the naïve pigs were exposed. On farms where both ruminants and swine are raised in close contact, transmission of *E. coli* O157:H7 between these animal species has been documented (Erikkson et al. 2003). Potential routes of transmission in this study were traced back to animal management practices on these farms. Transmission of STEC experimentally has been reported in both calves (Besser et al. 2001; Cobbold and Desmarchelier 2002) and sheep (Kudva et al. 1997; Cornick et al. 2000). When the naïve animals shared a pen with the inoculated donor, the

Table 3.1. Transmission of E. coli O157:H7 from Inoculated Donor Pigs to Naïve Pen Mates

Time post exposure	Number of pigs shedding/number exposed	
	$\leq 10^{4a}$	$\geq 10^5$
3 days	10/17	5/5
2 weeks	12/17	5/5

[a]cfu/g of *E. coli* O157:H7 shed by the donor at the time it was moved in with the naïve pigs

transmission of STEC between calves was more efficient than it was when calves were confined in individual pens (Cobbold and Desmarchelier 2002). However, transmission has been documented between calves that did not have nose-to-nose contact with the inoculated donor (Besser et al. 2001). Horizontal transmission between individuals within a herd is likely to be an important component in the establishment and maintenance of an animal reservoir.

While much is known regarding the pathogenesis and virulence of STEC in human disease, much less is known about when and how animals become colonized by STEC. Understanding the factors that contribute to STEC colonization of animals and resolving the discrepancy between experimental *E. coli* O157:H7 infections in swine and the low prevalence of the organism in U.S. herds may suggest management strategies that would potentially decrease or eliminate the colonization and/or transmission of *E. coli* O157:H7. Such information may also be useful to prevent the emergence of swine as a reservoir of *E. coli* O157:H7 in the United States.

References

APHIS. 2001. Swine 2000. 2001.

Besser, T. E., B. L. Richards, D. H. Rice, and D. D. Hancock. 2001. *Escherichia coli* O157:H7 infection of calves: infectious dose and direct contact transmission. Epidemiol. Infect. 127:555–560.

Best, A., R. M. La Ragione, D. Clifford, W. A. Cooley, A. R. Sayers, and M. J. Woodward. 2006. A comparison of Shiga-toxin negative *Escherichia coli* O157 aflagellate and intimin deficient mutants in porcine in vitro and in vivo models of infection. Vet. Microbiol. 113:63–72.

Beutin, L., D. Geier, H. Steinruck, S. Zimmermann, and F. Scheutz. 1993. Prevalence and some properties of verotoxin (shiga-like toxin) producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. 31:2483–2488.

Booher, S., N. A. Cornick, and H. W. Moon. 2002. Persistence of *Escherichia coli* O157:H7 in experimentally infected swine. Vet. Microbiol. 89:69–81.

Borie, C., Z. Monreal, P. Guerrero, M. L. Sanchez, J. Martinez, C. Arellano, and V. Prado. 1997. Prevalencia y caracterizacion de *Escherichia coli* enterohemorragica aisladas de bovinos y cerdos sanos faenados en Santiago, Chile. Archives Med. Vet. 29:205–212.

Bush, E. 1997. US swine herd appears free of *Escherichia coli* O157:H7. Food Safety Digest: 4.

Cobbold, R., and P. Desmarchelier. 2002. Horizontal transmission of shiga toxin-producing *Escherichia coli* within groups of dairy calves. Appl. Environ. Microbiol. 68:4148–4152.

Conedera, G., E. Mattiazzi, F. Russo, E. Chiesa, I. Scorzato, S. Grandesso, A. Bessegato, A. Fioravanti, and A. Caprioli. 2007. A family outbreak of *Escherichia coli* O157 haemorrhagic colitis caused by pork meat salami. Epidemiol. Infect. 135:311–314.

Cornick, N. A., S. Booher, and H. W. Moon. 2002. Intimin facilitates colonization by *Escherichia coli* O157:H7 in ruminants. Infect. Immun. 70:2704–2707.

Cornick, N. A., S. L. Booher, T. A. Casey, and H. W. Moon. 2000. Persistent colonization of sheep by *E. coli* O157:H7 and other pathotypes of *E. coli.* Appl. Environ. Microbiol. 66:4926–4934.

Cornick, N. A., and A. F. Helgerson. 2004. Transmission and infectious dose of *Escherichia coli* O157:H7 in swine. Appl. Environ. Microbiol. 70:5331–5335.

Dean-Nystrom, E. A., B. T. Bosworth, H. W. Moon, and A. D. O'Brien. 1998. *Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves. Infect. Immun. 66:4560–4563.

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. USA 97:2999–3003.

Erikkson, E., E. Nerbrink, E. Borch, A. Aspan, and A. Gunnarsson. 2003. Verocytotoxin-producing *Escherichia coli* O157:H7 in the Swedish pig population. Vet. Rec. 152:712–717.

Feder, I. E., M. Wallace, J. T. Gray, P. Fratamico, P. J. Fedorka-Cray, R. Pearce, J. E. Call, R. Perrine, and J. B. Luchansky. 2003. Isolation of *Escherichia coli* O157:H7 from intact colon fecal samples of swine. Emerg. Infect. Dis. 9:380–383.

Gyles, C. L., R. Friendship, K. Ziebell, S. Johnson, I. Yong, and R. Amezcua. 2002. *Escherichia coli* O157:H7 in pigs. Proc. Inter. Pig Vet. Soc. Abstract 191.

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice, and M. G. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington state. Epidemiol. Infect. 113:199–207.

Hancock, D. D., T. E. Besser, D. H. Rice, E. D. Ebel, D. E. Herriot, and L. V. Carpender. 1998. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA. Prev. Vet. Med. 35:11–19.

Heuvelink, A. E., J. T. M. Zwartkruis-Nahuis, F. L. A. M. Van den Biggelaar, W. J. Leeuwen, and E. de Boer. 1999. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from slaughter pigs and poultry. Int. J. Food Microbiol. 52:67–75.

Jay, M. T., M. Cooley, D. Carychao, G. W. Wiscomb, R. A. Sweitzer, L. Crawford-Miksza, J. A. Farrar, D. K. Lau, J. O. O'Connell, A. Millington, R. V. Asmundson, E. R. Atwill, and R. E. Mandrell. 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields

and cattle, central California coast. Emerg. Infect. Dis. 13:1908–1911.

Johnsen, G., Y. Wasteson, E. Heir, O. I. Berget, and H. Herikstad. 2001. *Escherichia coli* O157:H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. Int. J. Food Microbiol. 65:193–200.

Jordan, D. M., S. L. Booher, and H. W. Moon. 2005. *Escherichia coli* O157:H7 does not require intimin to persist in pigs. Infect. Immun. 73:1865–1867.

Jordan, D. M., N. A. Cornick, A. G. Torres, E. A. Dean-Nystrom, J. B. Kaper, and H. W. Moon. 2004. Long polar fimbriae contribute to colonization by *Escherichia coli* O157:H7 in vivo. Infect. Immun. 72:6168–6171.

Keen, J. E., T. E. Wittum, J. R. Dunn, J. L. Bono, and L. M. Durso. 2006. Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. Emerg. Infect. Dis. 12:780–786.

Keene, W. E., E. Sazie, J. Kok, D. H. Rice, D. D. Hancock, V. K. Balan, T. Zhao, and M. P. Doyle. 1997. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. JAMA 277:1229–1231.

Kudva, I. T., P. G. Hatfield, and C. J. Hovde. 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. J. Clin. Microbiol. 34:431–433.

Kudva, I. T., C. W. Hunt, C. J. Williams, U. M. Nance, and C. J. Hovde. 1997. Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. Appl. Environ. Microbiol. 63:3878–3886.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCraig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5: Sept.–Oct.: [on line] http://www.cdc.org.

Nakazawa, M., M. Akiba, and T. Sameshima. 1999. Swine as a potential reservoir of Shiga toxin-producing *Escherichia coli* O157:H7 in Japan. Emerg. Infect. Dis. Nov.–Dec.: [on line] http://www.cdc.org.

Sargeant, J. M., D. J. Hafer, J. R. Gillespie, R. D. Oberst, and S. J. A. Flood. 1999. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer sharing rangeland with cattle. J. Am. Vet. Med. Assoc. 215:792–794.

Sheng, H., J. Y. Lim, H. J. Knecht, J. Li, and C. J. Hovde. 2006. Role of *Escherichia coli* O157:H7 virulence factors in colonization at the bovine terminal rectal mucosa. Infect. Immun. 74:4685–4693.

Vlisidou, I., F. Dziva, R. M. LaRagione, A. Best, J. Garmendia, P. Hawes, P. Monaghan, S. A. Cawthraw, G. Frankel, M. J. Woodward, and M. P. Stevens. 2006. Role of intimin-tir and the tir-cytoskelton coupling protein in the colonization of calves and lambs by *Escherichia coli* O157:H7. Infect. Immun. 74:758–764.

Wallace, J. S., T. Cheasty, and K. Jones. 1997. Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds. J. Appl. Microbiol. 82:399–404.

Woodward, M. J., A. Best, K. A. Sprigings, G. R. Pearson, A. M. Skuse, A. Wales, C. M. Hayes, J. M. Roe, C. Low, and R. M. La Ragione. 2003. Non-toxigenic *Escherichia coli* O157:H7 strain NCTC12900 causes attaching-effacing lesions and eae-dependent persistence in weaned sheep. Int. J. Med. Microbiol. 293:299–308.