Current Research and Development for Vaccination against E. coli O157:H7

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Background

Escherichia coli O157:H7 is a bacterium that has been associated with foodborne and waterborne disease in humans (Stewart 1999). Cattle have been identified as a major source of the bacteria to humans, although the bacteria do not appear to cause clinical disease in cattle (Stewart 1999, Sargeant 2003, Khaitsa 2003). Currently, there are no practical pre-harvest interventions that have been proven consistently effective in significantly reducing *E. coli* O157:H7 fecal shedding and hide contamination in cattle (NCBA 2004).

Escherichia coli O157:H7 is widespread and ubiquitous in cattle (Stewart 1999, Sargeant 2003, Khaitsa 2003). Fecal and hide prevalence has been significantly correlated with carcass contamination, indicating a role for pre-harvest interventions in live cattle (Elder 2000). Vaccination appears to be an attractive pre-slaughter intervention in cattle to possibly reduce the disease risk to humans.

Bovine intestinal colonization

Based on experimental studies, it has been demonstrated that the expression of intimin and Tir are required for *E. coli* O157:H7 intestinal colonization (Potter 2004). Tir is integrated into the host cell membrane where it serves as a receptor for the bacterial outer member protein intimin. Intimin is required for the development of attaching-effacing lesions in neonatal calves. Adult bovine colonic and rectal epithelia are susceptible to *E. coli* O157:H7 induced attaching-effacing lesions. A study in piglets showed the colostrum containing intimin (O157)-specific antibodies protected piglets from EHEC O157:H7 colonization and intestinal damage (Dean-Nystrom 2002). Oral administration of bovine colostrum inhibited bacterial attachment in a murine model (Funatogawa 2002). Mice either immunized intraperitoneally with intimin expressed from plant cells, fed transgenic plant cells or both and boostered orally exhibited a reduced duration of *E. coli* O157:H7 fecal shedding after challenge (Judge 2004). Mice immunized intranasally three times with outer member protein extracted from the whole cell of *E. coli* O157:H7 and orally challenged with live *E. coli* O157:H7 were significantly protected (Zhang Y 2004). These experimental findings suggest that secreted proteins are attractive targets for vaccine development in cattle.

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Experimental challenge studies in cattle

Cattle, either 6 month old calves or yearlings, were immunized either two or three times SC with a vaccine created with supernatant proteins containing type III secreted proteins (Esps and Tir) and formulated with VSA3 such that the protein concentration was 50 or 200 µg/dose. Cattle were challenged 2 wk later with 10^8 CFU of *E. coli* O157:H7 by oral-gastric intubation (Potter 2004). The vaccine induced specific antibody titres to type III secreted proteins (anti-EHEC, anti-Tir, Anti-EspA). Fewer vaccinated cattle shed bacteria in lower amounts (cfu) compared to controls.

Vaccine Field Trials in Cattle

A study was conducted in a Nebraska feedlot in 24 pens of 192 yearling steers (Potter 2004). The vaccine consisted of secreted proteins of *E. coli* O157:H7 and it was administered SC (50 µg/dose) on days 0, 21 and 42 post-arrival. Cattle were followed to slaughter at 104 days on feed. Vaccination did not affect average daily gain, dry matter intake, gain to feed, marbling score, fat thickness or yield grade. The pre-immunization prevalence of feedlot cattle shedding *E. coli* O157:H7 averaged 30%. The average proportion of cattle shedding *E. coli* O157:H7 was 8.8% in vaccinated pens and 21.3% in nonvaccinated pens (P = 0.04).

The efficacy of 1, 2 or 3 doses of the secreted protein vaccine (Potter 2004) was studied in a Nebraska feedlot (Smith 2003). Within each pen of cattle, 2 animals were either vaccinated 0, 1, 2 or 3 times. In total there were 60 pens of vaccinated cattle totaling 480 animals. There were also 12 external control pens containing 128 animals. Cattle were monitored 128 days. Vaccine efficacy for 1, 2 or 3

doses was 52%, 58% and 68% compared to external controls (P < 0.01). Cattle that were vaccinated 3 times were 35% less likely to shed *E. coli* O157:H7 than unvaccinated cattle within the same pen (P = 0.06).

In a Canadian feedlot trial conducted during October 2001 to December 2002, a vaccine similar to that previously reported (Potter 2004) was administered to 218 pens of feedlot cattle (average: 250 animals per pen) in 9 feedlots in Alberta and Saskatchewan (Van Donkersgoed 2004). Cattle were immunized on arrival processing and again at re-implanting and followed to slaughter. The proportion of feedlot pens positive and mean within pen prevalence by *E. coli* O157:H7 vaccine and sampling time is shown in Table 1. The pen prevalence was highly variable, ranging from 0% to 80% at arrival processing, 0% to 87% at revaccination, and 0% to 90% pre-slaughter. The pen prevalence of *E. coli* O157:H7 in feces was significantly (P <0.001) different among feedlots at arrival, revaccination and pre-slaughter. Controlling for feedlot and age of cattle, no significant association (P > 0.25) was found between vaccine and pen prevalence at any time point. Within any one feedlot, there was no significant vaccine effect. Failure to see a vaccine benefit may be due to a poor serological response to the vaccine. In 2 feedlots, samples were collected to measure serological response to Tir and EHEC. In one feedlot, vaccinated cattle had a significantly higher antibody response (P < 0.0001) than nonvaccinates whereas in the other feedlot, there was no difference in serological response between vaccine groups. The vaccine used in this study was produced under scaled-up growth conditions rather than the laboratory-scale production used in the previous feedlot trials (Potter 2004, Smith 2003). Further investigation is ongoing to determine whether this production process affected antigen stability in the vaccine or the adjuvant affected the immunological response to the antigens.

Table 1. Proportion of feedlot pens positive and mean within pen prevalence by E. coli O157:H7 vaccine and sampling time.

	% positive pens		Mean (SE) within pen prevalence %	
	Control	Vaccine	Control	Vaccine
N pens	109	109	109	109
Arrival processing	34.9	30.3	4.0 (1.0)	2.2 (0.6)
Revaccination	41.3	35.8	5.5 (1.2)	4.9 (1.1)
Pre-slaughter	37.6	38.5	6.6 (1.3)	6.1 (1.4)

An *E. coli* O157:H7 bacterin (FDAH Vaccine, Fort Dodge Animal Health) was tested in a commercial feedlot in Colorado during March and May 2003 (Ranson 2003). Three pens of vaccinated cattle and three pens of controls containing 200 yearling cattle per pen were vaccinated twice, 30 days apart and fed 90 days to slaughter. Vaccination reduced hide prevalence from 40.3% to 20.0% and fecal prevalence from 45.8% to 14.7%.

Summary

Experimental challenge studies have shown the type III excreted proteins from *E. coli* O157:H7 can induce a protective immune response in cattle. Field studies conducted to date with either the secreted protein vaccine or a whole cell bacterin have shown some efficacy in reducing *E. coli* O157:H7 shedding in feces and contamination of hides, although results were not always consistent. Further research is required in commercial feedlots in both Canada and the United States under typical management systems to validate findings and determine impacts on foodborne disease risks and environmental contamination.

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References

Dean-Nystrom, E.A., Gansheroff, L.J., Mills, M., Moon, H.W., O'Brien, A.D. Vaccination of pregnant dams with intimin (O157) protects suckling piglets from Escherichia coli O157:H7 infection. Infect Immun 2002; 70: 2414-8.

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

Funatogawa, K., Ide, T., Kirikae, F., Saruta, K., Nakano, M., Kirkikae, T. Use of immunoglobulin enriched bovine colostrums against oral challenge with enterohaemorrhagic Escherichia coli O157:H7 in mice. Microbiol Immunol 2002; 46: 761-6.

Judge, N.A., Mason, H.S., O'Brien, A.D. Plant cell-based intimin vaccine given orally to mice primed with intimin reduces time of Escherichia coli O157:H7 shedding in feces. Infect Immun 2004: 72: 168-75.

Khaitsa, M.L., Smith, R., Stoner, J.A., *et al.* Incidence, duration and prevalence of Escherichia coli O157:H7 fecal shedding by feedlot cattle during the finishing period. J Food Prot 2003; 66: 1972-1977.

National Cattlemen's Beef Association. *E. coli* O157 Solutions: The Pre-Harvest Commitment. National Cattlemen's Beef Association, Colorado. 2004.

Potter, A.A., Klashinsky, S., Li, Y., *et al.* Decreased shedding of Escherichia coli O157:H7 by cattle following vaccination with type III secreted proteins. Vaccine 2004; 22: 362-269.

Ransom, J., Belk, K. Investigation of On-Farm Management Practices as Pre-Harvest Beef Microbiological Interventions. Project Summary, National Cattlemen's Beef Association. June 2003.

Sargeant, J.M., Sanderson, M.W., Smith, R.A., Griffin, D.D. Escherichia coli O157 in feedlot cattle feces and water in four major feeder-cattle states in the USA. Prev Vet Med 2003; 61: 127-135.

Smith, David., University of Nebraska, personal communication.

Stewart CD, Flint HJ. Escherichia coli O157 in Farm Animals. New York: CABI Publishing, 1999: 147-169.

Van Donkersgoed, J., Hancock, D., Potter, A.A., Rogan, D. Escherichia coli O157:H7 vaccine field trial in nine feedlots in Alberta and Saskatchewan. Can Vet J 2004: in progress.

Zhang, U., Lu, S.Q., Zhang, Y.Z., Zhao, B.X., Xu, J.G. The study for the immuno-protection of *E. coli* O157:H7 outer membrane protein. Zhonghus Yi Xue Za Zhi 2004: 84: 58-62.