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Comparison of *E. coli* O157 and Shiga toxin-encoding genes (*stx*) prevalence between Ohio, USA and Norwegian dairy cattle

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Abstract

Environmental and food contamination with Shiga toxin-producing *Escherichia coli* (STEC) pose a threat to public health worldwide, with notable geographic differences in incidence of human disease caused by these organisms. The prevalence of *E. coli* O157 and total *stx*-positive specimens collected from mature dairy cattle in Ohio and Norwegian dairy farms was compared using identical laboratory methods in a cross-sectional survey. *E. coli* O157 was isolated from 5/750 (0.66%) of Ohio dairy cows from 4/50 (8%) different herds, whereas *E. coli* O157 was not isolated from any (0/680) cattle present in 50 Norwegian dairy herds. In contrast, at least one *stx*-positive faecal sample was identified by PCR on all (50/50) Norwegian farms but only on 70% (35/50) of Ohio farms. Average animal *stx* prevalence on Ohio farms was also lower; 14% vs. 61% in Ohio and Norwegian herds, respectively. Livestock feed contamination with generic *E. coli* was uncommon in Norway, 1/50 feeds testing positive, whereas 19/50 (38%) of feeds collected from Ohio farms were contaminated, some as high as 10^5 CFU/g. Despite extreme differences in on-farm management practices between countries, *stx* appear to be widely disseminated in cattle in both countries, while the human pathogenic O157 serotype is less widely disseminated in Norway than it is in Ohio. Geographic distribution differences of human pathogenic STEC serogroups in the bovine reservoir, as opposed to specific farm management practices affecting on farm STEC prevalence, may be an important defining factor influencing the incidence of human illnesses associated in different areas of the world.
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1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) includes a diverse group of bacteria, some of which are seldom associated with human disease while other strains, such as enterohemorrhagic *E. coli* serotype O157, are the cause of diarrhea, hemorrhagic colitis, and Hemolytic Uremic Syndrome (HUS) worldwide (Reilly, 1998). In the United States, the reported rate of human *E. coli* O157 infections, the most common serotype, averages 3 cases per 100,000 population per year and is as high as 4.6 cases per 100,000 population per year in some regions (Griffin and Tauxe, 1991; CDC, 2001). This level of disease incidence can be considered moderate when compared with other countries. For example, the incidence of HUS in children in Argentina is higher than that observed in the US (Gioffre et al., 2002; Padola et al., 2002) In stark contrast, between 1992 and 2003 only 96 human cases of disease caused by *E. coli* O157:H7 or other Shiga toxin-producing *E. coli* (STEC) were reported in all of Norway, giving incidence rates of 0–0.4 STEC infections per 100,000 inhabitants (Hofshagen et al., 2004). Likewise, *E. coli* O157 is infrequently reported as a cause of diarrhea or HUS in Australia (Elliott et al., 2001). Even when the large differences in population sizes between the countries are considered, it is clearly evident that both the incidence of HUS, and the fraction thereof attributable to serotype O157, differs significantly among countries.

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Most human STEC infections are thought to be acquired from the consumption of contaminated foods of bovine origin or from other foods (fruits and vegetables) and drinking water believed to be contaminated with bovine manure. Thus, it stands to reason that the incidence of human disease would be inherently lower in geographic areas where the incidence of water or food contamination with STEC is lower. Such would be the case for regions where the meat and milk supply are derived from a population of animals infrequently harbouring pathogenic STEC. Likewise, a lower prevalence of pathogenic STEC in cattle would also result in less environmental contamination with this organism and reduced risk of human exposure to this pathogen via environmental sources. Finally, management practices on farms prior to shipment of milk and cattle to slaughter are believed to impact the prevalence of foodborne pathogens in food-producing animals (LeJeune and Kauffman, 2005). In particular, livestock feed contamination with human pathogenic bacteria has been suggested as an important vehicle for the dissemination of foodborne pathogens to cattle (Hinton, 2000; Crump et al., 2002; Davis et al., 2003).

We hypothesized that the lower reported incidence of human *E. coli* O157 infections in Norway compared to the US was a result of a lower prevalence of this pathogen in the bovine reservoir population in Norway. Because laboratory methods for the isolation of STEC and *E. coli* O157 from bovine feces are not standardized among research laboratories, it has not been appropriate to directly compare results between studies conducted by different laboratories. In this study we used identical sampling strategies and laboratory methods, to determine the prevalence of *stx*-positive bacteria and specifically *E. coli* O157, in dairy cow populations in two countries in which the epidemiology of human STEC infections differed substantially. As there is no well described method for detecting STEC in general, detection of Shiga toxin genes (*stx*) was used as an indicator of STEC. *stx* encodes the main virulence factors of STEC, the Shiga toxin. *stx* has reported in organisms other than *E. coli*, but this occurs infrequently. Cattle are not susceptible to colonization by *Shigella* spp., the prototypic *Stx*-encoding organism. Additionally, we explored farm management practices in the two countries, including feed hygiene, to identify possible explanatory factors that might contribute to differences in the bovine prevalence of *E. coli* O157 or *stx* carriage.

2. Materials and methods

Fifty dairy farms in each country were each visited on a single occasion during the months of June, July, and August 2002. Samples were collected from farms in southern Norway and within the state of Ohio, USA. To ensure these samples reflected typical herds of each country, the number of farms sampled were stratified based on the distribution of milk cows in various sized herds in each country (Anonymous, 2001; Anonymous, 2002). Herd size strata of farms visited in each country are listed in Table 1. During each farm visit, cotton tipped-swabs were rubbed along the recto-anal mucosal surface of 15 lactating cows, similar to the method described by Rice et al. (2003). If the farm had fewer than 15 lactating cows, all animals were sampled. On larger farms, the first 15 cattle restrained were sampled. Swabs were immediately inoculated into 3 ml of Buffered peptone water (BPW) and transported to participating research laboratory of each respective country (McDonough et al., 2000). In addition, a 10 g sample of feed concentrate was collected aseptically from the feed storage site (prior to livestock contact) on each farm after removing the surface 10–15 cm of feed present.

Table 1
Distribution (by number of lactating cows) of dairy herds in study

Number of cows/herd		Norway				Ohio			
		Herds		Animals		Herds		Animals	
Lower limit	Max	*n*	%	*n*	%	*n*	%	*n*	%
1	9	3	6	21	3.1				
10	19	24	48	322	47.3				
20	29	15	30	225	33.1	1	2	15	2
30	39	3	6	45	6.6	2	4	30	4
40	49	1	2	14	2.1	3	6	45	6
50	59	2	4	30	4.4	0	0	0	0
60	69	1	2	15	2.2	2	4	30	4
70	99					9	18	135	18
100	199					10	20	150	20
200	299					4	8	60	8
300	399					2	4	30	4
400	599					4	8	60	8
600	799					8	16	120	16
800	999					1	2	15	2
1000	3600					4	8	60	8
unknown		1	2	8	1.2				
Total		50	100	680		50	100	750	100

Swabs were vortexed to dislodge attached debris and subsequently incubated overnight at 42 °C. *E. coli* O157 present in 1 ml of each enrichment was concentrated using anti-O157 specific immunomagnetic beads and separated using automated methods following the manufacturer's instructions (AIMS, BeadRetreiver™, Dynal Biotech, Oslo, Norway). Beads were immediately plated on Sorbitol MacConkey agar containing cefixime (50 ng/ml) and tellurite (2.5 μg/ml) (Sanderson et al., 1995) and incubated overnight at 37 °C. Up to five suspect colonies from each plate were screened for biochemical and antigenic characteristics of *E. coli* O157 including metabolism of 4-methylumbelliferylbeta-D-glucuronide (MUG), lactose fermentation and reactivity with a commercially available latex agglutination kit specific for the O157 antigen (Oxoid). Suspect colonies were confirmed as *E. coli* O157 using a multiplex PCR reaction described by Hu et al. (1999).

One milliliter of the overnight BPW enrichments was mixed with 300 μl of glycerol and frozen at −70 °C for *stx* analysis at a later date. For the detection of *stx*, 100 μl of the previously frozen BPW enrichments was washed with an additional 900 μl of BPW and cells pelleted by centrifugation at 13,000 ×*g* for 2 min. Cells were washed again in 1 ml BPW and pelleted in the same manner. Cells were resuspended in BPW and boiled for 10 min to disrupt cells. Cells were cooled and 2 ng RNAse

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