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Short communication

Prevalence of shiga toxin-encoding bacteria and shiga toxin-producing *Escherichia coli* isolates from dairy farms and county fairs

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Abstract

Shiga toxin-encoding bacteria (STB) and shiga toxin-producing *Escherichia coli* (STEC) were detected and isolated from dairy cattle and their farm environment and from manure piles at Minnesota (MN) county fairs from 2001 to 2002.

A total of 2540 samples were collected from 28 dairy cattle farms (8 organic and 20 conventional), 17 calf pens (5 organic and 12 conventional), and 12 county fairs. STB were detected from 71 (3.2%) of 2208 fecal samples with 20 (71.4%) of 28 dairy farms having at least one positive animal sample. In samples collected from conventional farms, 41 (2.3%) of 1750 fecal samples were STB-positive and 13 (65%) of 20 farms had at least one positive animal. Thirty (6.6%) of 458 fecal samples from organic farms were STB-positive and 7 (87.5%) of 8 farms had at least one positive animal. STB was detected from 31 (17.4%) of 178 samples and 7 (58.3%) out of 12 manure piles at county fairs. A total of 43 STEC isolates were recovered and belonged to 26 different serotypes (19 O and 18 H types). Among STEC, 60.5% possessed only *stx*1, 30.2% *stx*2, and 9.3% both *stx*1 and *stx*2. The genes *eae* and *hly*A were detected in more than 50% of the STEC isolates.

STB can be found on most dairy cattle farms including organic and conventional herds and county fairs. The presence of these potentially pathogenic bacteria in county fairs may pose a risk to the public who have contact with cattle or their environment.
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Keywords: Shiga toxin-encoding bacteria; Organic farms; Virulence; County fairs

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1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are a major cause of food-borne illness capable of causing hemorrhagic colitis and hemolytic uremic syndrome (HUS) in children (Tarr, 1995). STEC include serotype O157:H7 and more than 100 non-O157 serotypes such as O111 and O26. Recently, several of these non-O157 STEC serovars have been linked to an increasing number of gastroenteritis infections and HUS in people. According to the Centers for Disease Control and Prevention (CDC), it is estimated that *E. coli* O157 causes 73,000 cases and 61 deaths, and non-O157 STEC lead to 36,000 infections and 30 deaths annually in the US (Mead et al., 1999).

Non-O157 STEC infections are under-diagnosed and under-reported as human pathogens compared to O157 which is distinguished by its inability to ferment sorbitol. *E. coli* O111 and O26 are the most common etiological agents associated with non-O157 STEC infections in the US. In Europe and South America, non-O157 STEC are a major cause of human enteric illness. Non-O157 STEC infections in Germany account for half of all STEC-linked diseases (Gerber et al., 2002; Verweyen et al., 1999). Non-O157 STEC outbreaks are rarely reported and difficult to detect with current surveillance systems in the US. Previous non-O157 outbreaks include consumption of raw milk (CDC, 1995) and contaminated beef products (Werber et al., 2002), camping (CDC, 2000), and direct animal contact (Smith et al., 2004).

Recently, prevalence studies of STEC from farm environments have demonstrated a high herd prevalence and environmental contamination (Cobbold et al., 2004; Hancock et al., 1997). The estimated proportion of animals infected in each herd varied from 0 to 100% (Blanco et al., 1996, 1997; Wilson et al., 1996). Currently, little work has been done to describe occurrence of STEC on dairy farms in the US. Our objective was to describe the prevalence and distribution of shiga toxin-encoding bacteria, and the serotype and virulence factor gene characteristics of STEC detected and isolated from dairy cattle of organic and conventional Minnesota dairies and county fairs.

2. Materials and methods

2.1. Herd selection

Holstein dairy herds in Minnesota were selected on the basis of farm type (organic versus conventional) and herd size (based on the number of milking and dry cows: 30–49, 50–99, 100–199, and $\geq$200 cows) and sampled over an 8-month period as part of a multi-state project (Fossler et al., 2004). Herd selection criteria included: each farm had at least 30 milking cows, had at least 90% of cows of Holstein breed, and raised their own calves and heifers for replacement animals. Conventional farms within approximately 100 miles of the University of Minnesota were randomly selected within herd-size categories from those farms meeting herd inclusion criteria and with owners who said they were willing to participate. Organic farms certified by a recognized certification agency were included in this study based on selection criteria and their willingness to participate. A total of 28 enrolled farms (8 organic and 20 conventional) were sampled up to three times (mean: 2.0) from April to October in 2001. After the first visit, subsequent herd visits were conducted at approximately 2-month intervals. In addition, a willing subset of farms participating in 2001 was sampled during the summer of 2002 with 17 farms (5 organic and 12 conventional) participated. Samples were collected from calf pens (either group or individual housing).

2.2. Sample collection

In 2001, approximately 10 g fecal samples were collected directly from the rectum of individual animals using plastic sleeve gloves (or disposable gloves) and transported to the laboratory immediately for bacterial culture. Fecal samples were taken from healthy lactating cows, preweaned calves (heifer calves receiving milk or milk replacer), periparturient cows (cows due to calve within 14 days or cows within 14 days after calving), sick cows, and culled cows (cows to be culled within 14 days). Target numbers of samples were collected from each animal type based on herd size. There were 30, 40, 50, and 55 total cattle fecal samples per visit targeted from herds with 30–49, 50–99, 100–199, and $\geq$200 cows, respectively. To obtain demographic data from farms, a management questionnaire was administered to farm personnel at

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