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# Prevalence of *Escherichia coli* O157:H7 in Surface Water Near Cattle Feedlots

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## Abstract

Between April 2009 and July 2011, 311 surface water samples in 48 cattle feedlots distributed in an area of about 67,000 km$^2$ were analyzed to examine the environmental dissemination of *Escherichia coli* O157:H7. Samples were taken inside and outside the pens, exposed and not exposed to runoff from corrals, near the feedlots. Two types of samples were defined: (1) exposed surface waters (ESW; $n = 251$), downstream from cattle pens; and (2) nonexposed surface waters (NESW; $n = 60$), upstream from cattle pens. By multiplex PCR, 177 (70.5%) ESW samples were $rfb_{O157}$-positive, and 62 (24.7%) *E. coli* O157, and 32 (12.7%) Shiga toxin-producing *E. coli* (STEC) O157:H7 strains were isolated. In the NESW samples, 36 (60.0%) were $rfb_{O157}$- positive, and 9 (15.0%) *E. coli* O157, and 6 (10.0%) STEC O157:H7 strains were isolated. These results showed that the environmental surface waters exposed to liquid discharges from intensive livestock operations tended to be contaminated with more STEC O157:H7 than NESW. However, no significant difference was found. This fact emphasizes the relevance of other horizontal routes of transmission, as the persistence of *E. coli* in the environment resulting from extensive livestock farming. By *Xba*I-PFGE, some patterns identified are included in the Argentine Database of *E. coli* O157, corresponding to strains isolated from hemolytic uremic syndrome and diarrhea cases, food, and animals, such as AREXHX01.0022, second prevalent pattern in Argentina, representing 5.5% of the total database. In the study area, characterized by the abundance of waterways, pathogens contained in feedlot runoff could reach recreational waters and also contaminate produce through irrigation, increasing the potential dissemination of STEC O157:H7 and the risk of human infections. The control of runoff systems from intensive livestock is necessary, but other alternatives should be explored to solve the problem of the presence of *E. coli* O157 in the aquatic rural environment.

## Introduction

Serotype O157:H7 is the predominant Shiga toxin–producing *Escherichia coli* (STEC) that causes severe foodborne illness, including hemolytic uremic syndrome (HUS), worldwide (Karmali *et al.,* 2003). The major animal carriers are healthy domesticated ruminants, primarily cattle (Gyles, 2007). Although undercooked beef and unpasteurized dairy products have been considered the main sources of human infection, it has become progressively clearer that routes other than foods of bovine origin could contribute to the epidemiology of human disease. Environmental contamination with this pathogen, such as vegetables that have been in contact with *E. coli* O157:H7–contaminated manure (fertilizer) or polluted irrigation water during stages of production and processing, has been pointed out as possible routes of transmission (Oliveira *et al*., 2012).

*Escherichia coli* O157:H7 can survive in water with low carbon source concentration and frequently has been isolated from streams and rivers (Wang *et al*., 1996; Vital *et al*., 2008; Tanaro *et al*., 2010). Runoff from livestock feeding operations could contaminate surface and groundwater, increasing the risk of foodborne illness mediated by water (Jackson *et al*., 1998; O'Connor, 2002; Johnson *et al*., 2003). Many water-related outbreaks associated with STEC, particularly *E. coli* O157:H7, have been described (Chalmers *et al*., 2000; Muniesa *et al*., 2006; Keene *et al*., 1994; Ackman *et al*., 1997; Verma *et al*., 2007; Jackson *et al*., 1998; Mannix *et al*., 2007).

Other routes of spread of *E. coli* O157:H7, as biological vectors, should be considered. However, the extent of STEC O157:H7 transmission mediated by animal vectors is not clear. Despite numerous studies on the reservoir and transmission routes, many questions remain unanswered, and new studies are needed to better understand the ecology of this pathogen.

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In Argentina, concentrated feeding operations have increased while extensive systems are reduced to provide additional cultivated land.

The objectives of this work were to examine the environmental dissemination of *E. coli* O157:H7 on surface waters of the cattle feedlots environment, and to establish the possible clonal relationships between environmental isolates and clinical or food strains included in the National Database of Argentina.

## Materials and Methods

Between April 2009 and July 2011, 311 samples of surface water in 48 cattle feedlots were taken. The samples included those taken in and outside the pens, exposed and not exposed to runoff from corrals, near each feedlot setting. The farms were distributed in an area of about 67,000 $km^2$, in the province of Entre Rios. The region is located in Argentine Mesopotamia and is characterized by numerous watercourses and hills that do not exceed 100 m. It has a humid subtropical climate with an average rainfall of about 970 mm per year and 2 distinct seasons: a rainy, spring–summer, and another less rainy, autumn–winter. Much of the soil is rich in clay and its low permeability contributes to increase runoff.

Two types of samples were defined: (1) exposed surface waters (ESW; $n = 251$), to refer to samples exposed to runoff from cattle pens; and (2) nonexposed surface waters (NESW; $n = 60$), to refer to watercourses or lagoons near the feedlot but that do not receive runoff from the pens.

Two-liter samples were kept for 24 h at room temperature in bottles with Moore swabs (Barrett *et al*., 1980). The swabs were then placed in flasks with 100 mL of trypticase soy broth (TSB; Difco, Becton Dickinson, Franklin Lakes, NJ) for 5 h, and then exposed to acid shock (pH 4.0) for 30 min before neutralization with TSB-Tris (pH 8.7) and incubation at 42°C for 24 h (Grant *et al*., 2009). Immunomagnetic separation (Dynal, Compiàgne, France) for *E. coli* O157 was performed and the immunoconcentrate was streaked onto sorbitol MacConkey agar (Biokar Diagnostics, Beauvois, France), O157:H7 ID™ (bioMèrieux, Marcy l'Etoile, France) and Chromagar™ O157 (CHROMagar Microbiology, Paris, France). After incubation at 37°C for 24 h, the confluent growth zones and individual colonies were screened for $stx_1$, $stx_2$, and $rfb_{O157}$ genes by a multiplex PCR (mPCR) (Leotta *et al*., 2005). Confirmation of isolates as *E. coli* was performed through biochemical tests using API 20 E (bioMèrieux) and other supplementary tests (Ewing, 1986), and serotyping was conducted by standard procedure (Ørskov and Ørskov, 1984), with somatic O157 (Oxoid Basingstoke, UK) and flagellar H7 antisera (prepared at the Instituto Nacional de Producción de Biológicos–Administración Nacional de Laboratorios e Institutos de Salud ''Dr. Carlos G. Malbrán,'' Buenos Aires, Argentina). Genotyping of $stx_1$ and $stx_2$ variants was carried out according to Scheutz *et al*. (2012). Macrorestriction fragment analysis by pulsed-field gel electrophoresis (PFGE) was performed using the 24-h PulseNet standardized PFGE protocol for *E. coli* O157:H7 (Ribot *et al*., 2006). Restriction digestion of DNA was done with *Xba*I enzyme (Promega, Madison, WI). PFGE images of gels were obtained by Gel Doc 2000 (Bio-Rad, Hercules, CA). Analysis of TIFF images was carried out through the BioNumerics version 4.61 software package (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient and the unweighted-pair group with arithmetic mean to generate dendrograms with 1.5% tolerance values. The antimicrobial susceptibility was carried out by the Kirby–Bauer method following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2007).

### *Statistical analysis*

To establish the confidence interval to estimate the difference between two population proportions of STEC O157 strains isolated from NESW and ESW samples, we make *comparisons of two proportions* from *independent samples* using a free database program (EpiData).

## Results

By mPCR, 177 (70.5%) ESW samples were $rfb_{O157}$-positive, and 62 (24.7%) *E. coli* O157, and 32 (12.7%) STEC O157:H7 strains were isolated. In the NESW samples, 36 (60.0%) were $rfb_{O157}$-positive, and 9 (15.0%) *E. coli* O157, and 6 (10.0%) STEC O157:H7 strains were isolated (Table 1). All strains were $\beta$-D-glucuronidase negative, nonsorbitol fermenting, and susceptible to cephalothin, nalidixic acid, ciprofloxacin, trimethoprim–sulfamethoxazole, nitrofurantoin, gentamicin, and fosfomycin, while 16% were resistant to tetracycline and 9% to ampicillin and ampicillin–sulbactam.

Of the total 38 STEC O157:H7 strains isolated, the prevalent genetic profile was $stx_{2a}$ (19, 50%) followed by $stx_{2a}$/$stx_{2c}$ (18, 47.4%), and only 1 (2.6%) strain was $stx_{1a}$/$stx_{2a}$. All strains harbored the *eae*, *ehxA*, and $fliC_{H7}$ genes. The *stx*-negative *E. coli* O157 strains were not analyzed. By *Xba*I-PFGE, 16 different patterns were established with 83.9% similarity, and 29 strains were grouped in 7 Clusters (I–VII) of 2–10 strains each one and 100% homology. Nine strains showed a unique pattern, and one of these strains harbored the $stx_{1a}$/$stx_{2a}$ genotype (Table 2 and Fig. 1).

Cluster I included 10 $stx_{2a}$ strains (AREXHX01.0489 pattern) isolated from 3 farms, each of them separated approximately for 300 km. Eight strains were isolated in farm A (August 2010), seven from ESW samples, and one from a

Table 1. Prevalence of *Escherichia coli* O157:H7 in Surface Environmental Water Near Feedlot Cattle

| *Sample category* | *No. of samples analyzed* | *No. (%) of positive samples* | *95% CI* | *No (%) of isolated* E. coli *O157 strains* | *95% CI* | *No. (%) of isolated STEC O157: H7 Strains* | *95% CI* |
|---|---|---|---|---|---|---|---|
| ESW | 251 | 177 (70.5) | 64.9–6.2 | 62 (24.7) | 19.4–30.0 | 32 (12.7) | 8.6–16.9 |
| NESW | 60 | 36 (60.0) | 47.6–72.4 | 9 (15.0) | 6.0–24.0 | 6 (10.0) | 2.4–17.6 |

ESW, exposed surface waters; NESW, nonexposed surface waters; 95% CI, 95% confidence interval.

Table 2. Distribution of Shiga Toxin–Producing *Escherichia coli* O157:H7 Strains in *Xba*I-Pulsed-Field Gel Electrophoresis (PFGE) Clusters

| *Cluster* | Xba*I-PFGE pattern* | *Isolation date* | *Type of sample* | *Farm* | *Geographical department* | *Genetic profile* |
|---|---|---|---|---|---|---|
| I | AREXHX01.0489 | 10/25/2010 | NESW | A | Gualeguay | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$ / *eae* / *ehx*A |
| | | 10/25/2010 | ESW | A | Gualeguay | |
| | | 10/25/2010 | ESW | A | Gualeguay | |
| | | 10/25/2010 | ESW | A | Gualeguay | |
| | | 10/25/2010 | ESW | A | Gualeguay | |
| | | 10/25/2010 | ESW | A | Gualeguay | |
| | | 10/25/2010 | ESW | A | Gualeguay | |
| | | 10/25/2010 | ESW | A | Gualeguay | |
| | | 08/30/2010 | ESW | B | Federación | |
| | | 11/24/2010 | ESW | C | La Paz | |
| | AREXHX01.0835 | 10/25/2010 | ESW | A | Gualeguay | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$ / *eae* / *ehx*A |
| | AREXHX01.0840 | 06/14/2010 | ESW | J | Colón | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{1a}$ / $stx_{2a}$ / *eae* / *ehx*A |
| II | AREXHX01.0837 | 08/30/2010 | ESW | C | La Paz | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$ / *eae* / *ehx*A |
| | | 08/30/2010 | ESW | C | La Paz | |
| | | 08/30/2010 | ESW | C | La Paz | |
| III | AREXHX01.0731 | 10/25/2010 | ESW | A | Gualeguay | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$ / *eae* / *ehx*A |
| | | 08/30/2010 | ESW | C | La Paz | |
| | | 08/30/2010 | NESW | D | La Paz | |
| | AREXHX01.0314 | 08/02/2010 | ESW | E | Gualeguaychú | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$ / *eae* / *ehx*A |
| | AREXHX01.0838 | 04/12/2010 | ESW | F | Islas del Ibicuy | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$ / *eae* / *ehx*A |
| IV | AREXHX01.0095 | 05/31/2010 | NESW | E | Gualeguaychú | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |
| | | 05/31/2010 | ESW | E | Gualeguaychú | |
| | AREXHX01.0834 | 11/24/2010 | ESW | B | Federación | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |
| | AREXHX01.0505 | 07/20/2010 | ESW | A | Gualeguay | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |
| | AREXHX01.0839 | 08/17/2010 | ESW | G | Nogoyá | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |
| V | AREXHX01.0832 | 06/21/2011 | NESW | A | Gualeguay | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |
| | | 07/04/2010 | ESW | A | Gualeguay | |
| VI | AREXHX01.0022 | 10/25/2010 | ESW | A | Gualeguay | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |
| | | 06/21/2011 | ESW | A | Gualeguay | |
| | | 07/04/2010 | ESW | A | Gualeguay | |
| | | 08/30/2010 | ESW | H | La Paz | |
| | | 11/24/2010 | ESW | B | Federación | |
| | | 08/30/2010 | NESW | H | La Paz | |
| | | 08/30/2010 | ESW | H | La Paz | |
| VII | AREXHX01.0093 | 08/30/2010 | ESW | C | La Paz | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |
| | | 06/21/2011 | ESW | A | Gualeguay | |
| | AREXHX01.0833 | 06/21/2011 | NESW | A | Gualeguay | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |
| | AREXHX01.0841 | 08/17/2010 | ESW | I | Nogoyá | $rfb_{O157}$ / $fliC_{H7}$ / $stx_{2a}$/$stx_{2c}$ / *eae* / *ehx*A |

ESW, exposed surface waters; NESW, nonexposed surface waters.

NESW sample. The other two strains were recovered from ESW samples in farm B (August 2010) and farm C (November 2010). Cluster II (AREXHX001.0837) grouped three $stx_{2a}$ strains, isolated from different points of the effluents from farm C during the same sampling date (August 2010). Cluster III (AREXHX01.0731) included three $stx_{2a}$ strains isolated in three different farms (A, C, D), located in two departments of the province. One of the strains was obtained (October 2010) 300 km away from the others that were found in the same department (August 2010). These last two strains were isolated from NESW and ESW samples. Cluster IV (AREXHX01 .0095) included two $stx_{2a}$/$stx_{2c}$ strains isolated from the same farm E in May 2010. Both samples were taken in the same stream before (NESW) and after (ESW) receiving runoff from the feedlot pens. Cluster V (AREXHX01.0832) included two $stx_{2a}$/$stx_{2c}$ strains isolated from a NESW and ESW samples in the same farm A in June and July 2010. Cluster VI (AREXHX01.0022) included seven $stx_{2a}$/$stx_{2c}$ strains. They were isolated in three different locations (farms A, B, and H) separated by 150–250 km, in July (1), August (3), September (1), November (1) of 2010, and June (1) of 2011. Cluster VII (AREXHX01.0093) included 2 $stx_{2a}$/$stx_{2c}$ strains isolated in 2 feedlots (C, A) approximately 300 km distant, in August 2010 and June 2011, respectively.

## Discussion

In the present study, the environmental surface waters exposed to liquid discharges from intensive livestock operations showed high contamination (12.7%) with STEC O157:H7. Nevertheless, the presence of toxigenic *E. coli* O157 strains in NESW (10.0%) is also remarkable. A statistically significant difference between the two types of samples was not found ($p = 0.7153$).

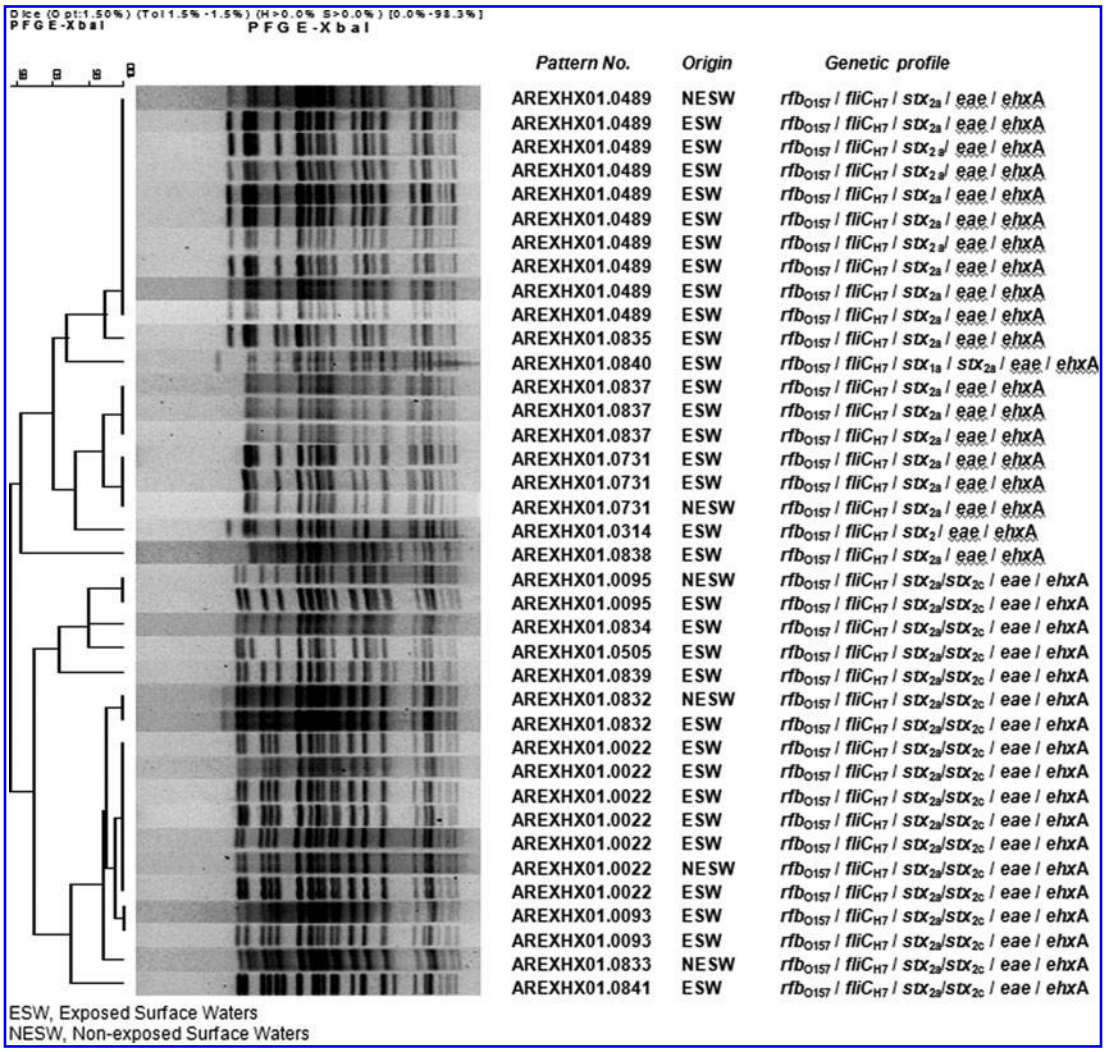


**FIG. 1.** Genotypic characterization and clonal relatedness of 38 Shiga toxin–producing *Escherichia coli* O157:H7 strains isolated from water in beef cattle farms, Entre Ríos Province, Argentina, 2010–2011.

The fact that indistinguishable strains have been isolated from samples taken at long distances, as seen in Clusters I, III, and VI, encourages further research about the role of birds as vectors (Nielsen *et al*., 2004). This assumption could be related to the observation of large flocks of birds eating semi-digested grains in manure of the cattle fed in the open pens at the sampling time.

Indistinguishable strains recovered from a stream of gentle current, before and after receiving feedlot effluent (Cluster IV), also suggest that the aquatic fauna could act as a vector. Brown *et al*. (2002) have shown that *E. coli* O157 can survive and replicate in a common environmental protozoan, *Acanthamoeba polyphaga*. As protozoa are widely distributed in water, soils, and especially effluents, they are likely to constitute an important environmental reservoir for transmission of *E. coli* O157 and other pathogens.

Furthermore, it should be noted that in the province of Entre Ríos the pastoral livestock system is also widespread and the ability of *E. coli* to remain viable in the environment for a long time should not be underestimated (Kudva *et al*., 1998; LeJeune *et al*., 2001). In *E. coli*, $\sigma^S$ factors transcribe more than 100 genes involved in cell survival and protection against various stress conditions, such as ultraviolet radiation (Gruber and Gross, 2003). This assumption could be supported by indistinguishable STEC O157 strains that have been recovered in the same place after 12 months (Table 2, Clusters V and VI).

In geographical areas as those that were studied, characterized by a profusion of waterways, pathogens present in feedlot runoff could reach recreational waters and also contaminate products through irrigation. In previous studies, we have confirmed the presence of *E. coli* O157:H7 in waters of the River Gualeguaychú in areas for recreational activities, such as clubs, beaches, and camping areas (Tanaro *et al*., 2002).

Some *Xba*I-PFGE patterns identified in the present study are also included in the Argentinean Database of *E. coli* O157, corresponding to strains isolated from HUS and diarrhea cases, food, and animals. Among them, AREXHX01 .0022 is the second prevalent pattern in Argentina, representing 5.5% of the total database. The postenteric HUS, mainly associated with *E. coli* O157:H7 infection, is a significant public health issue in Argentina, and the most prevalent *stx* subtypes associated with severe disease were those encoded by $stx_{2a}/stx_{2c}$, coincidentally one of the most

prevalent subtypes of the isolated strains from surface waters (Rivas, 2012).

## Conclusions

In this study, *E. coli* O157:H7 strains, related to those isolated from HUS cases and food, were recovered from surface water samples taken in areas near cattle feedlots.

In addition to runoff from feedlot pens, other sources or vectors must be considered as probable causes of this contamination.

Unlike extensive feeding systems, in intensive livestock operations the manure and slurry feedlot pens are concentrated in a small area, which may prevent the pathogen spreading to the environment, if appropriate treatments are applied. The efficiency of the control runoff systems to avoid the dissemination of pathogens should be specifically included in the regulations.

## Disclosure Statement

No competing financial interests exist.

## References


Ackman D, Marks S, Mack P, Caldwell M, Root T, Birkhead G. Swimming-associated haemorrhagic colitis due to *Escherichia coli* O157:H7 infection: Evidence of prolonged contamination of a fresh water lake. Epidemiol Infect 1997;119:1–8.

Barrett TJ, Blake PA, Morris GK, Puhr ND, Bradford HB, Wells JG. Use of Moore swabs for isolating *Vibrio cholerae* from sewage. J Clin Microbiol 1980;11:385–388.

Brown MRW, Smith AW, Barker J, Humphrey TJ, Dixon B, Reilly WJ. *Escherichia coli* O157 persistence in the environment. Microbiology 2002;148:1–2.

[CLSI] Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Information Supplement.* CLSI document M100-S17. Wayne, PA: CLSI, 2007.

Chalmers RM, Aird H, Bolton FJ. Waterborne *Escherichia coli* O157. Symp Ser Soc Appl Microbiol 2000;29:124S–132S.

Ewing WH. *Edwards and Ewing's Identification of Enterobacteriaceae*, 4th ed. New York: Elsevier Science Publishing Co., Inc., 1986.

Grant MA, Mogler MA, Harris DL. Comparison of enrichment procedures for Shiga toxin-producing *Escherichia coli* in waste from commercial swine farms. J Food Prot 2009; 72:1982–1986.

Gruber TM, Gross CA. Multiple sigma subunits and the partitioning of bacterial transcription space. Annu Rev Microbiol 2003;57:441–466.

Gyles CL. Shiga toxin-producing *Escherichia coli*: An overview. J Anim Sci 2007;85:E45–E62.

Jackson SG, Goodbrand RB, Johnson RP, Odorico VG, Alves D, Rahn K, Wilson JB, Welch MK, Khakhria R. *Escherichia coli* O157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm. Epidemiol Infect 1998;120:17–20.

Johnson JYM, Thomas JE, Graham TA, Townshend I, Byrne J, Selinger LB, Gannon VPJ. Prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in surface waters of southern Alberta and its relation to manure sources. Can J Microbiol 2003;49:326–335.

Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, Isaac-Renton J, Clark C, Rahn K, Kaper JB. Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. J Clin Microbiol 2003;41:4930–4940.

Keene WE, McAnulty JM, Hoesly FC, Williams LP Jr, Hedberg K, Oxman GL, Barrett TJ, Pfaller MA, Fleming DW. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. N Engl J Med 1994;331:579–584.

Kudva IT, Blanch K, Hovde CJ. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Appl Environ Microbiol 1998;64:3166–3174.

LeJeune JT, Besser TE, Hancock DD. Cattle water troughs as reservoirs of *Escherichia coli* O157. Appl Environ Microbiol 2001;67:3053–3057.

Leotta GA, Chinen I, Epszteyn S, Miliwebsky E, Melamed IC, Motter M, Ferrer M, Marey E, Rivas M. Validación de una técnica de PCR múltiple para la detección de *Escherichia coli* productor de toxina Shiga. Rev Arg Microbiol 2005;37:1–11. (In Spanish.)

Mannix M, Whyte D, McNamara E, O'Connell N, Fitzgerald R, Mahony M, Prendiville T, Norris T, Curtin A, Carroll A, Whelan E, Buckley J, McCarthy J, Murphy M, Greally T. Large outbreak of *E. coli* O157 in 2005, Ireland. Euro Surveill 2007;12:54–56.

Muniesa M, Jofre J, Garcia-Aljaro C, Blanch AR. Occurrence of *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli* in the environment. Environ Sci Technol 2006;40:7141–7149.

Nielsen EM, Skov MN, Madsen JJ, Lodal J, Jespersen JB, Baggesen DL. Verocytotoxin-producing *Escherichia coli* in wild birds and rodents in close proximity to farms. Appl Environ Microbiol 2004;70:6944–6947.

O'Connor DR. *Report of the Walkerton Inquiry: The Events of May 2000 and Related Issues. Part 1.* Toronto: Ontario Ministry of the Attorney General, Queen's Printer for Ontario, 2002.

Oliveira M, Viñas I, Usall J, Anguera M, Abadias M. Presence and survival of *Escherichia coli* O157:H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. Int J Food Microbiol 2012;156:133–140.

Ørskov F, Ørskov I. Serotyping of *Escherichia coli*. In: *Methods in Microbiology, Vol. 14.* Bergan T (ed.). London: Academic Press, 1984, pp. 43–112.

Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 2006;3:59–67.

Rivas M. Vigilancia Epidemiológica y Molecular del Síndrome Urémico Hemolítico Asociado a STEC. 5° Congreso Argentino de Nefrología Pediátrica. Organizado por la Sociedad Argentina de Pediatría. Buenos Aires 23 de Junio de 2012. Available at: http://www.sap.org.ar/docs/congresos/2012/nefrologia/ppt/ rivas .pdf, accessed September 11, 2014. (In Spanish.)

Scheutz F, Teel LD, Beutin L, Pierard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sánchez M, Persson S, O'Brien AD. A multi-center evaluation of a sequence-based protocol to subtype Shiga toxins and standardize Stx nomenclature. J Clin Microbiol 2012;50:2951–2963.

Tanaro JD, Leotta GA, Lound LH, Galli L, Piaggio MC, Carbonari CC, Araujo S, Rivas M. *Escherichia coli* O157 in bovine feces and surface water streams in a beef cattle farm of Argentina. Foodborne Pathog Dis 2010;7:475–477.

Tanaro JD, Lound LH, Ledri S, Scarpin M, Schimpf MC, Casse JS, Larrivey MA, Rivas M. Desarrollo y aplicación de una metodología de aislamiento de *Escherichia coli* O157:H7 en el Municipio de Gualeguaychú. Rev Arg Microbiol 2002; 34:205–212. (In Spanish.)

Verma A, Bolton FJ, Fiefield D, Lamb P, Woloschin E, Smith N, McCann R. An outbreak of *E. coli* O157 associated with a swimming pool: An unusual vehicle of transmission. Epidemiol Infect 2007;135:989–992.

Vital M, Hammes F, Egli T. *Escherichia coli* O157 can grow in natural fresh water at low carbon concentrations. Environ Microbiol 2008;10:2387–2396.

Wang G, Zhao GT, Doyle MP. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. Appl Environ Microbiol 1996;62:2567–2570.

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