Prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in surface waters of southern Alberta and its relation to manure sources

J.Y.M. Johnson, J.E. Thomas, T.A. Graham, I. Townshend, J. Byrne, L.B. Selinger, and V.P.J. Gannon

Abstract: The Oldman River watershed in southern Alberta, Canada, is an extensively irrigated region in which intensive agricultural practices have flourished. Concern over water quality in the basin has been expressed because of high levels of enteric disease indigenous to the region. To address these concerns, we conducted a 2-year study to estimate the prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in surface water within the basin. This study is the first of its kind to identify *E. coli* O157:H7 repeatedly in surface water collected from a Canadian watershed. Prevalence of *E. coli* O157:H7 and *Salmonella* spp. in water samples was 0.9% (*n* = 1483) and 6.2% (*n* = 1429), respectively. While data examined at a regional level show a relationship between high livestock density and high pathogen levels in southern Alberta, statistical analysis of point source data indicates that predicted manure output from bovine, swine, and poultry feeding operations was not directly associated with either *Salmonella* spp. or *E. coli* O157:H7 prevalence. However, geography and weather variables, which are likely to influence bacterial runoff, were not considered in this model. We also postulate that variations in time, amount, and frequency of manure application onto agricultural lands may have influenced levels of surface-water contamination with these bacterial pathogens.

Key words: water, *Salmonella*, *E. coli* O157:H7, manure output, agriculture.

Résumé : Le bassin versant de la rivière Oldman, dans le sud de l’Alberta, Canada, est une région fortement irriguée où des pratiques d’agriculture intensive se sont développées. Des inquiétudes quant à la qualité de l’eau dans le bassin ont été émises en raison des taux élevés de maladies entériques qui seinfissent dans la région. Pour répondre à ces inquiétudes, une étude d’une durée de deux ans a été accomplie afin d’évaluer la prévalence de *Escherichia coli* O157:H7 et de *Salmonella* spp. dans les eaux de surface du bassin. Cette étude est la première de ce type à identifier du *E. coli* O157:H7 à répétition dans de l’eau de surface d’un bassin versant canadien. La prévalence de *E. coli* O157:H7 et de *Salmonella* spp. dans les échantillons d’eau était de 0,9 % (*n* = 1483) et de 6,2 % (*n* = 1429), respectivement. Bien que les données au niveau régional qui ont été examinées révèlent un rapport entre la densité élevée du bétail et les niveaux élevés de pathogènes dans le sud de l’Alberta, l’analyse statistique des données des sources ponctuelles indique que la production projetée de fumier provenant de l’alimentation d’élevages bovins, porcins ou de volaille n’était pas directement associée à la prévalence de *Salmonella* spp. ou de *E. coli* O157:H7. Toutefois, les variables géographiques et climatiques, qui pourraient influencer le ruissellement de bactéries, n’ont pas été retenues dans ce modèle. Nous postulons également que les variations de temps, de quantité et de fréquence d’épandage de fumier sur les terres agricoles ont pu avoir un effet sur la contamination de l’eau de surface par ces bactéries pathogènes.


Introduction

Southern Alberta is a semi-arid region in which the Oldman River drainage basin is the principal source of water for agriculture, industry, and both drinking and recreational waters used by rural and urban centres. Approximately 10% of the Oldman River watershed is irrigated agricultural land. Irrigation waters are used for the production of feed needed to maintain intensive livestock operations, for livestock watering, as well as for recreation and residential purposes.
Since 1991, the livestock industry in Alberta has expanded significantly. Between 1991 and 2001, the number of cattle and calves in Alberta increased from approximately 4.7 to 6.6 million (Alberta Agriculture 1991; Alberta Agriculture Food and Rural Development 1996; Statistics Canada Online 2002). From 1996 to 2001, the total number of pigs on Alberta farms grew from 1.7 million to more than 2.0 million and the total number of poultry from 10.6 to 13.3 million (Statistics Canada Online 2002). Much of this activity has been centred in southern Alberta, a region that also has one of the highest levels of enteric disease in the province (Khakhria et al. 1996).

Waterborne outbreaks of enteric disease have resulted either when public drinking water supplies were not adequately treated after contamination with surface water (Barwick et al. 2000; Jones and Roworth 1996; Kondro 2000; Swerdlow et al. 1992) or when surface waters contaminated with enteric pathogens have been used for recreational purposes (Keene et al. 1994; Kramer et al. 1996). While protozoa, such as Giardia and Cryptosporidium (Roach et al. 1993; Wallis et al. 1996), have been the focus of much concern in water contamination, enteric bacteria, such as Escherichia coli O157:H7 and Salmonella spp., which are leading causes of gastroenteritis in Canada and the United States (Baxter and Morton 1987; Kramer et al. 1996; Levy et al. 1998), can also be a significant source of water contamination. From 1993 to 1998, water contaminated by E. coli O157:H7 resulted in 454 cases of illness in the United States, while water contaminated with Salmonella species, such as Salmonella typhimurium, infected 625 individuals and caused seven deaths. Regions with high cattle density are prone to enteric disease (Johnson et al. 1999). Michel et al. (1999) performed epidemiological studies that showed the incidence of human infection with verotoxin-producing E. coli (VTEC) in Ontario between 1990 and 1995 was related to cattle density. In Canada, Alberta has the greatest population of cattle and one of the highest levels of gastroenteritis resulting from infection by Salmonella spp. and E. coli O157:H7 (Khakhria et al. 1996).

In this study, we examined the prevalence of E. coli O157:H7 and Salmonella spp. in surface waters within the Oldman River basin in southern Alberta and explored relationships between the prevalence of these pathogens and both animal agriculture and human population within the region.

Materials and methods

Study sites

The Oldman River basin is a semi-arid zone located in southern Alberta, Canada. Total precipitation in Lethbridge, the principal urban centre (1999 population 68 712), was 231.6 mm from May to October 1999 and 130.5 mm from May to October 2000 (information from the Agriculture and Agri-Food Canada Lethbridge Research Centre). Water samples were collected from surface-water drainage from irrigated agricultural regions, the Little Bow River, the Oldman River, sewage treatment plants, and the City of Lethbridge storm drains. Sampling sites were located east from the Rocky Mountains, along the Oldman River; north, along the Little Bow River and at inflow and outflow locations of the Lethbridge Northern Irrigation District; north of the Oldman River; and west of the Little Bow River (Figs. 1, 2, and 3), so that water samples were representative of the region. Although livestock feeding facilities were situated throughout the region with a nonhomogenous distribution, sampling sites were selected to reflect surface-water flow and not distribution of livestock density. In 1999, 522 samples were collected from 69 sites. During the following year, 84 sites were sampled and 961 samples were collected, including 38 samples from the City of Lethbridge storm drain system and six samples from six municipal sewage treatment plants within the Oldman River basin. Twenty-three sites were sampled regularly in both years. Sampling sites were selected to assess surface water at locations of interest (nonrandomized sampling).

Sample collection

Samples were taken either upstream from foothold or from water at approximately 30 cm below the surface, using a plastic container. Samples were collected in sterile, plastic bottles; placed on ice; and transported to the laboratory for testing.

Detection of E. coli O157:H7

Water samples were dispensed in 90-mL volumes into clean, sterile culture bottles. To each of these bottles, we added a 10-mL volume of 10× buffered peptone water (BPW) (Becton Dickinson, Sparks, Md., U.S.A.) and incubated the bottles at 37°C for approximately 6 h. At this point, 1 mL was withdrawn, added to 9 mL of trypicate soy broth supplemented with 20 µg/mL novobiocin (Difco, Detroit, Mich., U.S.A.), and held at 42°C for 6 ± 2 h. Immuno-magnetic separation using magnetic beads coated with E. coli O157 antibody was carried out, as described by the manufacturer (Dynal, Oslo, Norway). After the last wash step, the anti-E. coli O157 magnetic beads were resuspended in 50 µL of wash buffer and streaked onto sorbitol MacConkey agar containing cefixime (ctSMAC) (0.05 mg/L) and tellurite (2.5 mg/L) (Sanderson et al. 1995). The ctSMAC plates were incubated for 24 h at 42°C and translucent colonies tested by slide agglutination, using E. coli O157-specific antibody (Difco). Positive bacteria were inoculated into 5 mL brain heart infusion broth (Difco) and grown at 37°C overnight. Each of these bacterial cultures was tested by PCR using oligonucleotide primers that detect v1, v2, fliC, and eaeA0157 genes (Gannon et al. 1997). Putative E. coli O157:H7 cultures were sent to the Health Canada Verocytotoxigenic E. coli Reference Laboratory in Guelph, Ontario, for independent confirmation.

Detection of Salmonella spp.

Water samples cultured in BPW, from the pre-enrichment medium described above, were incubated for 16–24 h at 37°C. The pre-enriched culture was inoculated into two separate enrichment broths, Rappaport Vassiliadis (RV) broth and tetrazionate brilliant green (TTBG) broth. For the RV broth, we inoculated 9.9 mL of the broth with 100 µL of the BPW culture. For the TTBG broth, we inoculated a 9.0-mL volume with 1.0 mL of the BPW culture. Both of the inoculated enrichment broths were incubated at 42°C for 24 h. Following enrichment, both the RV and the TTBG broth cultures were plated onto two different selective culture plates,
modified semi-solid Rappaport Vassiliadis (MSRV) agar (DeSmedt and Bolderdijk 1987) and brilliant green sulpha (BGS) agar (Becton Dickinson, Mississauga, Ont., Canada). For inoculation onto MSRV plates, a 20-µL aliquot of each culture was dispensed onto the outside edge of each individual plate. The plates were incubated at 42°C for 24 h. Motile bacteria producing halos greater than 20 mm in diameter in the MSRV medium were subcultured onto MacConkey (Difco) agar by taking an inoculation loop of cells from the leading edge of the halo and streaking onto MacConkey plates. Following inoculation, the MacConkey plates were then incubated at 37°C for 16–24 h. For testing on the BGS agar plates, both the RV and TTBG broths were gently mixed, and an inoculation loop of each of the cultures was plated onto BGS agar plates. The BGS agar plates were then incubated at 37°C for 16–24 h. Following incubation, three red colonies from each of the BGS plates were plated onto MacConkey agar plates. These MacConkey plates were then grown at 37°C for 16–24 h. For both the MSRV and BGS agar plating methods, suspect translucent colonies were picked from each of the MacConkey plates and tested for agglutination, using Salmonella O antiserum Poly A-I & Vi (Difco). Putative Salmonella-positive colonies were further tested using standard (Christensen’s) urea agar, triple sugar iron agar, lysine iron agar slants (D’Aoust and Purvis 1998), and sulphide indole motility (Difco) agar (Carter 1979). Those colonies exhibiting biochemical responses typical of Salmonella spp. were further tested using a PCR specific for the detection of the Salmonella invA gene (Chiu and Ou 1996). Salmonella culture isolates were confirmed at the Health Canada Salmonella Reference Laboratory in Guelph, Ontario.

**Prevalence**

The prevalence of both *E. coli O157:H7* and Salmonella spp. in water samples was calculated as

\[
\text{Prevalence} = \left( \frac{\text{No. samples from which pathogens were detected}}{\text{total no. samples tested}} \right) \times 100
\]

**Mapping**

Prairie Farm Rehabilitation Administration (PFRA), Lethbridge, provided data of mapped manure output from confined animal agriculture operations in southern Alberta.
The numbers of animals for each confined livestock feeding operation in the region were estimated from information provided in field surveys, air surveillance photos, or expansion permits. Animal manure units (AMU) were estimated as the product of the number of animals by type for each location, the annual excreted nitrogen mass for animal types (Natural Resources Conservation Service 1999), and the inverse of the mass of nitrogen required to fertilize 1 acre of corn for 1 year in Alberta, 73 kg/year (PFRA). To obtain AMU estimates that correspond to water sampling sites, a 150-m² grid of AMU values over the study area was interpolated from the AMU point data for confined livestock operations (ArcView 3.0, ESRI, Redland, Fls., U.S.A.). Interpolated values for each cell of the surface were calculated as the sum of the products of the AMU operations within a 15-km radius and the inverse of the distance to those operations squared. In this study, we assume that transport of manure beyond a 15-km radius of an animal rearing facility would be prohibitively expensive and, consequently, unlikely. AMU surfaces were generated for bovine, poultry, swine, and combined animal feeding operations. Bovine operations included confined beef and dairy farms; swine operations included farrow-to-finish, weaner, and feeder hog farms; poultry operations included broiler, layer, turkey, and unknown poultry farms. Sampling sites from which more than two samples were collected and for which there were bovine, swine, and poultry operations within 15 km were used for AMU–pathogen prevalence analysis. AMU estimates at these sampling sites were calculated as the mean of triplicate measurements. To classify sampling sites outside the City of Lethbridge limits and provincial park boundaries by livestock agriculture activity, we made a 5-km² grid of mean AMU density over the study area. The AMU density of all grid cells were divided into four equal intervals and classified as low, medium, high, and very high AMU density.

### Statistical methods

The apparent prevalence of *E. coli* O157:H7 and *Salmonella* spp. associated with sampling sites was estimated as the percentage of pathogen-positive samples of all samples tested for both pathogens. The change in prevalence among sampling sites tested in both years was assessed using a paired sample *t* test. The association between the prevalence

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**Fig. 2.** Prevalence of *Salmonella* spp. in surface water and the animal manure output (AMU) in southern Alberta from 1999 to 2000; Universal Transverse Mercator (UTM) coordinates Zone 11, Datum, North American Datum (NAD) 27.
of *Salmonella* spp. and *Escherichia coli* O157:H7 and the AMU of sampling sites was assessed using generalized linear models, where inputs included AMU output from confined bovine, swine, and poultry livestock operations. The prevalence of *Salmonella* spp. among sampling sites followed a Poisson distribution; thus, a Poisson regression model was used (SAS, SAS Institute Inc., Cary, N.C., U.S.A.). The model was

$$\ln(\text{SALM}) - \ln(\text{SALMTEST}) = \alpha + \beta_1 \text{BOVAMU} + \beta_2 \text{SWNAMU} + \beta_3 \text{PLTAMU}$$

where SALM is the number of samples in which *Salmonella* spp. was detected from a given sampling site; SALMTEST is the number of samples tested from that site; $\alpha$ is the intercept; BOVAMU, SWNAMU, and PLTAMU are independent variables; and $\beta_1$, $\beta_2$, and $\beta_3$ are slopes (parameter estimates for bovine, swine, and poultry AMU, respectively). The prevalence of *E. coli* O157:H7 followed a binomial distribution, thus, the following logit regression model was used:

$$\logit(\text{ECOLI}) - \logit(\text{ECOLITEST}) = \alpha + \beta_1 \text{BOVAMU} + \beta_2 \text{SWNAMU} + \beta_3 \text{PLTAMU}$$

where ECOLI is the number of samples from a site positive for *E. coli* O157:H7, ECOLITEST is the number of samples tested from that site, and other variables are as defined previously. Type III likelihood ratio statistics were used to assess the strength of association for each animal type, and residual deviance statistics were used to assess goodness-of-fit. Logistic regression models were developed to describe the relationship between pathogen presence in water samples and AMU associated with the site from which the samples were collected, assuming the AMU at sites remained stable over the period of time in which samples were collected (SPSS 10.0.5, SPSS Inc., Chicago, Ill., U.S.A.). Model fit was assessed using the Hosmer and Lemeshow goodness-of-fit test.

Fig. 3. Prevalence of *Salmonella* spp. and *Escherichia coli* O157:H7 in surface water in southern Alberta and bovine manure output (AMU) surface estimated using inverse distance weighting from confined beef and dairy feeding operations; Universal Transverse Mercator (UTM) coordinates Zone 11, Datum, North American Datum (NAD) 27.
Results

Prevalence of Salmonella spp. and E. coli O157:H7

In 1999, water was collected every 2 weeks throughout the Oldman River basin from May through October. Salmonella spp. were isolated from 14 of 468 water samples, for a prevalence of 3.0%. In 2000, water was collected every week from May through September. Salmonella spp. were isolated from 74 of 961 samples, for a prevalence of 7.7%. This represents a 2.6-fold increase in the prevalence of Salmonella spp. within the Oldman River basin from 1999 to 2000. During this period, the basin received less than average rainfall for the region and was suffering from drought conditions. Consequently, because of lack of water, not all sites could be sampled consistently both in 1999 and in 2000; therefore, only bacterial prevalence at sites that were consistently sampled in both 1999 and 2000 was compared. No significant annual differences were observed in the prevalence of Salmonella spp. (t = −1.15; one-tailed p = 0.1319) at these sites (data not shown). On average, for 1999 and 2000, Salmonella spp. were detected in 88 of 1429 samples, with a prevalence (± SE) of 6.2% ± 0.6%. A high proportion (26.3%) of the 38 samples from municipal storm drain sites contained Salmonella spp., and one of these had the greatest Salmonella spp. prevalence (80.0% ± 20.0%; Table 1) observed in this study.

In 1999 and 2000, E. coli O157:H7 was isolated from 13 of 1483 water samples for a prevalence (mean ± SE) of 0.9% ± 0.2%. As with the Salmonella spp. data, no significant annual differences existed in the prevalence of E. coli O157:H7 (t = −1.451; one-tailed p = 0.0811) at sites that were consistently sampled both years (data not shown). On more than one occasion, E. coli O157:H7 was detected at an irrigation outflow within the City of Lethbridge (Table 1). However, most sites contaminated with E. coli O157:H7 were located near some form of agricultural activity (Fig. 1). Further examination of these sites showed that they were located (i) in areas with low to moderate livestock density (Table 1) or (ii) downstream of high-density livestock operations. In contrast, water obtained from regions with high AMU were, generally, not contaminated with E. coli O157:H7, although prevalence of Salmonella spp. was high in some cases (Table 1); animals in these regions were normally confined and did not have direct access to surface water. No samples positive for E. coli O157:H7 were found within urban storm drain sites.

Manure production

Mean AMU values at sampling sites were 1655, 240, and 394 for bovine, swine, and poultry operations, respectively. Figures 1, 2, and 3, respectively, are maps detailing E. coli O157:H7 prevalence, Salmonella spp. prevalence, and interpolated surface of AMU for bovine operations, using inverse distance weighting. Of 84 sampling sites, 10 were not within 15 km of a confined feeding operation and had no interpolated AMU value; i.e., sites without bovine, swine, or poultry AMU values were not included in the AMU–pathogen prevalence analysis, while the remaining 74 sites were included. Locations of high bacterial prevalence did not appear to be situated near large manure output sources (Figs. 1 and 2). Levels of aggregated manure output from local bovine (Fig. 3), swine, and poultry feeding operations were not high at sampling sites that had a high bacterial prevalence (interpolated swine and poultry AMU maps not shown).

Consequently, no direct correlation between bacterial prevalence and manure production from confined livestock feeding operations was identified. Bovine, poultry, and swine manure output associated with sampling sites was not a significant predictor of Salmonella spp. or E. coli O157:H7 prevalence at sampling sites (Table 2) or among individual samples (Table 3).

Discussion

This study is the first of its kind to isolate E. coli O157:H7

Table 1. Percentage bacterial prevalence ± standard error (SE) at 10 different sampling sites within the Oldman River Basin in 1999 and 2000, containing zero, low, medium, and high animal manure units (AMU); in storm drains; and in urban settings.

<table>
<thead>
<tr>
<th>Site</th>
<th>Site classification*</th>
<th>n</th>
<th>Salmonella spp. %</th>
<th>SE</th>
<th>Escherichia coli O157:H7 %</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zero AMU density</td>
<td>21</td>
<td>19.0</td>
<td>8.8</td>
<td>4.8</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>Zero AMU density</td>
<td>24</td>
<td>13.0</td>
<td>6.9</td>
<td>8.3</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>Zero AMU density</td>
<td>17</td>
<td>18.0</td>
<td>9.5</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>Low AMU density</td>
<td>25</td>
<td>20.0</td>
<td>8.2</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>Medium AMU density</td>
<td>26</td>
<td>7.7</td>
<td>5.3</td>
<td>3.6</td>
<td>3.9</td>
</tr>
<tr>
<td>6</td>
<td>Medium AMU density</td>
<td>27</td>
<td>15.0</td>
<td>7.0</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td>High AMU density</td>
<td>6</td>
<td>33.0</td>
<td>21.0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>SD</td>
<td>4</td>
<td>25.0</td>
<td>25.0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>SD</td>
<td>5</td>
<td>80.0</td>
<td>20.0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>CL</td>
<td>22</td>
<td>4.6</td>
<td>4.6</td>
<td>9.1</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Note: SD, storm drain samples; CL, sites within the City of Lethbridge that were not storm drain samples.

*AMU density — zero, no confined livestock operations within a 5-km grid; low, mean AMU density is between 17 and 166 within a 5-km grid; medium, AMU density between 166 and 315 within a 5-km grid; and high, AMU density between 315 and 464 within a 5-km grid.
in surface waters collected from a Canadian watershed. Systematic sampling of surface water within the Oldman River basin in southern Alberta shows that it is often contaminated with *E. coli* O157:H7 and *Salmonella* spp. The presence of *E. coli* O157:H7 and *Salmonella* spp. in water presents a risk to human health when the untreated water is consumed directly or indirectly. Our results identify *E. coli* O157:H7 and *Salmonella* spp. contamination in water from surface drainage channels, some of which flows directly into the Oldman and Little Bow Rivers and from storm drains and sewage treatment plants within the Oldman River basin.

While much of the public concern over these pathogens is related to the consumption of contaminated food, concern has also been expressed over the contamination of water used for both drinking and recreational activities (Arvanitidou et al. 1997; Polo et al. 1998; Sargeant et al. 2000).

Michel et al. (1999) have shown that the risk of human infection by VTEC can be related to cattle density. In Canada, Alberta has the greatest population of cattle and one of the highest levels of gastroenteritis resulting from infection by *Salmonella* spp. and *E. coli* O157:H7 (Khakhria et al. 1996). Between 1991 and 1994, up to 77% of 205 children less than 15 years of age from Alberta that were diagnosed with hemolytic uremic syndrome (HUS) were likely infected with *E. coli* O157:H7 (Rowe et al. 1998). From 1987 to 1991, the mean annual rate of VTEC infection for the province was 12.1 cases per 100,000 compared with 2.0 per 100,000 in Scotland during the same period (Waters et al. 1994). Some of these cases might be the result of water contamination. In our study, we repeatedly isolated *E. coli* O157:H7 from surface waters within the Oldman River basin. Individuals who consumed this untreated water or used it for recreational purposes would be expected to be at risk of infection (Payne et al. 1997; O’Connor 2002).

Khaleel et al. (1980) have suggested that a high density of intensive animal rearing facilities could facilitate the contamination of surface water, resulting in large reservoirs for pathogenic bacteria. In 1992 in Swaziland, *E. coli* O157:H7 caused one of the largest outbreaks of bloody diarrhoea in the world (Effler et al. 2001). At the end of a 3-month drought, more than 40,000 people distributed over several hundred kilometres downstream of an agricultural district were reported ill after runoff from heavy rains contaminated food and water.

### Table 2. Associations between bacterial presence in surface water from sampling sites in southern Alberta and manure output.

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>$\chi^2$</th>
<th>p</th>
<th>df</th>
<th>Error deviance/df</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALM</td>
<td>Intercept</td>
<td>-2.7698</td>
<td>0.5373</td>
<td>1.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BOVAMU</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.2491</td>
<td>0.6177</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWNAMU</td>
<td>-0.0005</td>
<td>0.0011</td>
<td>0.2267</td>
<td>0.6340</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PLTAMU</td>
<td>-0.0001</td>
<td>0.0008</td>
<td>0.0064</td>
<td>0.9365</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOLI</td>
<td>Intercept</td>
<td>-4.8072</td>
<td>1.7548</td>
<td>0.6412</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BOVAMU</td>
<td>0.0002</td>
<td>0.0003</td>
<td>0.2126</td>
<td>0.6448</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SWNAMU</td>
<td>-0.0036</td>
<td>0.0048</td>
<td>0.7043</td>
<td>0.4013</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PLTAMU</td>
<td>0.0014</td>
<td>0.0022</td>
<td>0.4125</td>
<td>0.5207</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Poisson and binomial distribution models of *Salmonella* spp. and *Escherichia coli* O157:H7 prevalence, respectively, as functions of inverse distance weighting of regional manure output from confined livestock feeding operations. SALM and ECOLI are the models of prevalence of *Salmonella* spp. and *E. coli* O157:H7, respectively. BOVAMU, PLTAMU, SWNAMU are independent variables. $\beta$ is the slope or parameter estimate for bovine, poultry, and swine animal manure units, respectively. df, degrees of freedom; SE, standard error.

### Table 3. Associations between bacterial presence and manure output (animal manure units) associated with surface-water samples of southern Alberta.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Manure type</th>
<th>B</th>
<th>Wald</th>
<th>$p$ (df = 1)</th>
<th>$\chi^2$</th>
<th>p (df = 3)</th>
<th>$\chi^2$</th>
<th>p (df = 8)</th>
<th>Model likelihood ratio test</th>
<th>Hosmer and Lemeshow goodness-of-fit test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Bovine</td>
<td>0.000</td>
<td>0.270</td>
<td>0.603</td>
<td>0.724</td>
<td>0.868</td>
<td>29.589</td>
<td>&lt;0.001</td>
<td>1</td>
<td>0.169</td>
</tr>
<tr>
<td></td>
<td>Swine</td>
<td>-0.001</td>
<td>0.231</td>
<td>0.630</td>
<td>0.724</td>
<td>0.868</td>
<td>29.589</td>
<td>&lt;0.001</td>
<td>1</td>
<td>0.169</td>
</tr>
<tr>
<td></td>
<td>Poultry</td>
<td>0.000</td>
<td>0.007</td>
<td>0.934</td>
<td>0.724</td>
<td>0.868</td>
<td>29.589</td>
<td>&lt;0.001</td>
<td>1</td>
<td>0.169</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>Bovine</td>
<td>0.000</td>
<td>0.008</td>
<td>0.928</td>
<td>2.358</td>
<td>0.501</td>
<td>8.085</td>
<td>0.425</td>
<td>2</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>Swine</td>
<td>-0.005</td>
<td>1.333</td>
<td>0.248</td>
<td>2.358</td>
<td>0.501</td>
<td>8.085</td>
<td>0.425</td>
<td>2</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>Poultry</td>
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<td>0.002</td>
<td>0.922</td>
<td>2.358</td>
<td>0.501</td>
<td>8.085</td>
<td>0.425</td>
<td>2</td>
<td>0.172</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>All types</td>
<td>0.000</td>
<td>0.169</td>
<td>0.681</td>
<td>0.172</td>
<td>0.678</td>
<td>14.165</td>
<td>0.078</td>
<td>2</td>
<td>0.172</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>All types</td>
<td>0.000</td>
<td>0.627</td>
<td>0.428</td>
<td>0.581</td>
<td>0.446</td>
<td>17.030</td>
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<td>2</td>
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**Note:** df, degrees of freedom; B, logistic regression coefficient; Wald, statistic to test significance of B.
Michel et al. (1999) have demonstrated an association between human VTEC infections and cattle density in Ontario, Canada. They suggested that regional manure spreading and water contamination might link animal density, a group-level risk factor, and human infection, an individual-level outcome (Valcour et al. 2002). Livestock manure is a reservoir for both Salmonella spp. and *E. coli* O157:H7 and is an important source of surface-water contamination. Cattle are recognized as reservoirs for *E. coli* O157:H7 (Dean-Nystrom et al. 1999), and cattle manure has been implicated as the source of contamination in the Walkerton outbreak that occurred in Ontario, Canada (Kondro 2000).

The Lethbridge region of southern Alberta is noted for high cattle density (Fig. 3) as well as for one of the highest levels of gastroenteritis in Canada, resulting from infection by *Salmonella* spp. and *E. coli* O157:H7 (Khakhria et al. 1996). This observation is similar to that of Michel et al. (1999), whose work was done using geographical scaling at the census consolidated subdivision level. In our study, both *Salmonella* spp. and *E. coli* O157:H7 were identified in surface-water samples throughout the Lethbridge region, and it is likely that cattle contributed to this contamination. However, when data from our study were examined using a smaller geographical scale capable of identifying potential point sources, we found that surface water obtained close to large-scale animal feeding operations was not necessarily more contaminated with zoonotic bacteria than were samples obtained far away from these operations; i.e., pathogen prevalence of surface water, not including groundwater, was not necessarily related to animal density when potential point sources of contamination were considered.

We note that producers in southern Alberta have been working to implement best management practices that minimize the risk of water contamination within the region. Animals often are confined and do not have direct access to surface water. Our data suggest that these practices may have a positive effect on water quality; i.e., no direct association of concentrated livestock facilities with pathogen prevalence was detected in this study, suggesting that good agricultural management practices can mitigate surface-water contamination within agricultural regions. These observations, however, do not preclude that the spreading of manure without sufficient treatment (e.g., composting) on fields may have contributed to the water contamination observed. Cows on pasture, wild deer, reptiles, and birds are reservoirs of pathogens, such as *Salmonella* spp. and *E. coli* O157:H7, and may also have contributed to water contamination (Bradley et al. 2001; Cizek et al. 1994; Fischer et al. 2001; Sargeant et al. 2000).

In our study, *Salmonella* spp. were more prevalent than *E. coli* O157:H7 in water samples. Because *Salmonella* spp. are present at a low level in beef and dairy herds from western Canada (Sorenson 2000; Van Donkersgoed et al. 1999), it is likely that other sources also contributed to the water contamination that we observed. Van Donzel and Geldreich (1971) have identified pigs as reservoirs for *Salmonella* spp. and note that they may contribute to surface-water contamination when runoff occurs. Domestic and wild birds may also have been the source of some of the bacterial isolates obtained, e.g., 42% of *Salmonella* spp. submitted to the Health Canada Salmonella Reference Laboratory in 1999 were isolated from poultry (Health Canada 2000).

In our study, a high number of the City of Lethbridge storm drains were contaminated with *Salmonella* spp. (Table 1). We note that much of the industry and a part of the population within the City of Lethbridge are engaged in agricultural or related activities. It is not clear what contribution agriculture surrounding the city made to the observed contamination. Further studies are needed to identify sources of contamination and to determine how city management practices within agricultural regions might differ from those in large urban centres.

The regional prevalence of *E. coli* O157:H7 was similar in our study to that reported by McGowan et al. (1989) in their study of surface water; however, the numbers of *Salmonella* spp. we detected were similar to that seen in some studies but were lower than that seen in others (Anonymous 1992; Efstratiou et al. 1998; Polo et al. 1998). We note that the prevalence of *E. coli* O157:H7 and *Salmonella* spp. throughout the Oldman River basin was not uniform (Figs. 1, 2, and 3). While this variation in prevalence may, in part, be the result of various land management practices, other factors, such as time of sampling, geography, and weather, could also influence these findings and other factors in any model developed.

We attempted to model the effect of local AMU on water contamination. However, the predictive strength of our model was limited. Incorporation of other variables, such as bacterial transport and survival, into the model might improve it. The velocity and direction of surface-water drainage, the irrigation water flow, and the surface wind at time of sample collection were not acknowledged within the model but could strengthen it by providing a link between the effect of local confined livestock operations and local water contamination (these data were not available for this study). Environmental conditions at time of sampling, such as water temperature, pH, and nutrient concentration, could also be used to assess the potential for bacterial survival in water. These variables could influence bacterial concentration in water and may have influenced the ability of our model to identify an association between local manure load and water contamination. In this study, we also assumed that transport of manure beyond a 15-km radius of an animal rearing facility would be prohibitively expensive and consequently, unlikely; this however, may not be the case.

**Acknowledgements**

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