BACTERIAL PATHOGENS IN RURAL WATER SUPPLIES IN SOUTHERN ALBERTA, CANADA

V. P. J. Gannon,¹ T. A. Graham,¹ Susan Read,² Kim Ziebell,² Ann Muckle,² J. Mori,³ J. Thomas,⁴ B. Selinger,⁴ I. Townshend,³ J. Byrne³

¹Laboratory for Foodborne Zoonoses, Population and Public Health Branch, Health Canada, Lethbridge, Alberta, Canada
²Laboratory for Foodborne Zoonoses, Population and Public Health Branch, Health Canada, Guelph, Ontario, Canada
³Department of Geography, University of Lethbridge, Lethbridge, Alberta, Canada
⁴Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada

Raw river and irrigation water in the Oldman River Basin in southern Alberta was tested for the presence of two bacterial pathogens, Escherichia coli O157:H7 and Salmonella spp., over the last 2 yr (2000–2001). The number of E. coli O157:H7 and Salmonella spp. isolated from raw water peaked during the summer months. While E. coli O157:H7 was only isolated from 11/802 (1.3%) of raw water samples over the entire sampling season in 2000 and from 16/806 (2.0%) of the samples in 2001, the pathogen was isolated one or more times from 10/35 (28.5%) sampling sites in 2000 and from 13/40 (32.5%) sampling sites in 2001. Salmonella was isolated from 44/802 (5.5%) of raw water samples in 2000 and from 122/822 (14.9%) of the samples in 2001; the pathogen was isolated one or more times from 25/35 (71.4%) sampling sites in 2000 and from 29/40 (72.5%) sampling sites in 2001. Certain sites had multiple pathogen isolations in the same year and from year to year. Salmonella Rublislaw was the most common Salmonella serovar isolated in both years, accounting for 52.4% of isolates.

Southern Alberta has the highest rate of gastrointestinal illness in the province (Waters et al., 1994). This area is known for its dry climate, extensive field crop irrigation system, and expanding animal agriculture industry. Recently,

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Address correspondence to V. P. J. Gannon, Laboratory for Foodborne Zoonoses, Population and Public Health Branch, Health Canada, PO Box 640, Lethbridge, Alberta T1J 3Z4, Canada. E-mail: gannonv@inspection.gc.ar
there has been much public concern about the safety and quality of water in this and other regions of the country. Outbreaks of waterborne illness with E. coli O157:H7 and Campylobacter spp. in Walkerton, Ontario, in 2000 (Kondro, 2000) and with Cryptosporidium in North Battleford, Saskatchewan, in 2001 have underlined the importance of adequate water treatment and adequate source water protection.

Epidemiological evidence also suggests that the risk to human health from enteric infections is greatest in rural areas where cattle densities are highest (Johnson et al., 1999). Concern for human health in regions of high livestock density not only stems from an increased probability of surface-water contamination with manure but extends also to drinking-water supplies for individual farm residences and rural communities where water treatment may be less than optimal.

In this study, river and irrigation water was tested in the Oldman River Basin in southern Alberta for the presence of two bacterial pathogens (E. coli O157:H7 and Salmonella spp.) over a 2-yr period (2000–2001). This was conducted to assess levels of contamination of rural surface drinking-water sources and possible risks to human health if this water is inadequately treated.

**MATERIALS AND METHODS**

**Study Area**

The Oldman River Basin is in a semi-arid zone located in the southern part of the province of Alberta. To the north of the city of Lethbridge, Alberta, irrigation is used for field crops and there is a highly developed cattle feedlot industry associated with the irrigated land of the Lethbridge Northern Irrigation District (LNID; Figure 1). According to the 2001 Alberta Agriculture and Rural Development Census (Alberta Agriculture Food and Rural Development, 2001), there are approximately 1.1 million head of cattle in the Census Division that includes this region, which is approximately twice that of any of the other 18 census divisions in the province. The Little Bow River passes along the northern border of the LNID and joins with the Oldman River approximately 40 km downstream of the city of Lethbridge, Alberta. The LNID consists of a vast network of irrigation canals and associated holding reservoirs that pass through this region and eventually also drain into the Oldman River. Although many large livestock feeding facilities are present in this region, sampling sites were selected based on surface water flow and not livestock density.

**Sampling**

Water samples were taken upstream from foothold using a pole, approximately 30 cm below the surface of the water, using sterile 250-ml plastic bottles. Samples were collected from the Little Bow River each week by Alberta Agriculture and Rural Development staff from 16 sites between 10 May and 16 October 2000 and from 21 sites between 9 May and 25 September 2001.
Water samples were taken each week from irrigation canals and reservoirs by staff of the LNID from 19 sites between 8 May and 2 October 2000 and between 4 June and 24 September 2001.

**Microbiology**

Ninety milliliters of each water sample was dispensed into glass culture bottles containing 10 ml of 10× buffered peptone water (BPW). These cultures were incubated at 37°C for approximately 6 h. For isolation of E. coli O157:H7, 1 ml of each BPW culture was removed and inoculated into 9 ml of modified trypticase soy broth containing 20 µg/ml of novobiocin (Padhye & Doyle, 1991), and this culture was incubated at 42°C for 18–24 h. Immunomagnetic capture of E. coli O157:H7 using paramagnetic beads coated with O157 antibody (Dynal, Oslo, Norway) was carried out using the manufacturer’s instructions. Following removal of the bead suspension from the culture, captured beads were spread onto sorbitol MacConkey agar containing 50 µg/ml cefixime and 2.5 mg/L tellurite (CT-SMAC; Chapman et al., 1994) and then incubated for 24 h at 42°C. Suspected colonies were tested by slide agglutination using O157 antiserum (Difco, Detroit, MI). If positive in the agglutination assay, bacterial colonies were tested using the E. coli O157:H7-specific polymerase chain reaction assay described by Gannon et al. (1997).

For the isolation of Salmonella, a modification of the methods of D’Aoust and Purvis (1998) and of DeSmedt and Bolderdijk (1987) was employed. Briefly, BPW–water culture described earlier was incubated for an additional...
18 h at 37°C. Following this, 1 ml of the BPW culture was inoculated into 9 ml of tetrathionate brilliant green broth (TBGB) and 0.1 ml of the BPW culture was inoculated into 9.9 ml of Rappaport Vassiliadis broth (RV). Each of these 2 broth cultures were incubated at 42°C for 24 h. Approximately 0.02 ml of each of these broth cultures was then inoculated onto the surfaces of modified semi-solid Rappaport Vassiliadis agar (MSRV) (DeSmedt & Bolderdijk, 1987) and brilliant green sulfa agar (BGSA; Becton Dickinson, Mississauga, ON, Canada) plates. For TBGB each plating medium was cultured at 37°C and 42°C for 24 h and for RVB cultures each plating medium was incubated at 42°C. Suspect bacterial colonies were subcultured onto MacConkey agar; from this medium, colonies were tested by slide agglutination with Salmonella O antiserum Poly A-1 and Vi (Difco) and inoculated onto Christensen’s urea agar, triple sugar iron agar, lysine iron agar slants and into SIM medium (Difco). Salmonella serotyping was performed by the OIE Salmonella Reference Laboratory of Health Canada in Guelph, Ontario.

RESULTS

Overall Prevalence

Escherichia coli O157:H7 was isolated from 1.7% (27/1608) of all surface water samples over the 2 yr of the study (Table 1). More specifically, it was isolated from 0.8% (4/384) of the sites from the Little Bow River in 2000 and from 2.5% (12/483) in 2001, and it was isolated from 1.7% (7/418) of LNID water samples in 2000 and 1.2% (4/323) in 2001.

<table>
<thead>
<tr>
<th>Date</th>
<th>Little Bow River (%)</th>
<th>LNID (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>0/64 (0)</td>
<td>0/76 (0)</td>
<td>0/140 (0)</td>
</tr>
<tr>
<td>June</td>
<td>2/64 (3.2)</td>
<td>0/76 (0)</td>
<td>2/140 (1.4)</td>
</tr>
<tr>
<td>July</td>
<td>0/64 (0)</td>
<td>5/95 (5.3)</td>
<td>5/159 (3.1)</td>
</tr>
<tr>
<td>August</td>
<td>2/80 (2.5)</td>
<td>0/76 (0)</td>
<td>2/156 (1.3)</td>
</tr>
<tr>
<td>September</td>
<td>0/64 (0)</td>
<td>2/76 (2.6)</td>
<td>2/140 (1.4)</td>
</tr>
<tr>
<td>October</td>
<td>0/48 (0)</td>
<td>0/19 (0)</td>
<td>0/76 (0)</td>
</tr>
<tr>
<td>Year total</td>
<td>4/384 (0.8)</td>
<td>7/418 (1.7)</td>
<td>11/802 (1.4)</td>
</tr>
<tr>
<td>2001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>0/84 (0)</td>
<td>ND</td>
<td>0/84 (0)</td>
</tr>
<tr>
<td>June</td>
<td>3/126 (2.4)</td>
<td>1/76 (1.3)</td>
<td>4/202 (2.0)</td>
</tr>
<tr>
<td>July</td>
<td>5/84 (6.0)</td>
<td>1/95 (1.1)</td>
<td>6/179 (3.4)</td>
</tr>
<tr>
<td>August</td>
<td>3/105 (2.9)</td>
<td>2/76 (2.6)</td>
<td>5/181 (2.8)</td>
</tr>
<tr>
<td>September</td>
<td>1/84 (1.2)</td>
<td>0/76 (0)</td>
<td>1/160 (0.6)</td>
</tr>
<tr>
<td>Year total</td>
<td>12/483 (2.5)</td>
<td>4/323 (1.2)</td>
<td>16/806 (2.0)</td>
</tr>
<tr>
<td>Total both years</td>
<td>16/867 (1.8)</td>
<td>11/741 (1.6)</td>
<td>27/1608 (1.7)</td>
</tr>
</tbody>
</table>
Salmonella spp. were isolated much more frequently from both the Little Bow River and LNID irrigation canals than E. coli O157:H7 (Table 2). Overall, Salmonella was isolated from 10.3% (166/1629) of all water samples. In 2000, Salmonella spp. were isolated from 4.7% (18/384) of the water samples from the Little Bow River and in 2001 they were isolated from 16.7% (84/504) of the water samples from this system. The organism was isolated from 6.2% (26/418) of water samples from the LNID in 2000 and from 11.8% (38/326) in 2001.

**Monthly Incidence**

Escherichia coli O157:H7 and Salmonella spp. isolations peaked in July of both years (Tables 1 and 2). In July 2000 E. coli O157:H7 was isolated from 3.1% (5/159) of all water samples, and in July 2001 the organism was isolated from 3.4% (6/179) of all water samples. Similarly, Salmonella was isolated from 16.4% (26/159) and 30.2% (54/179) of all water samples in the months of July 2000 and July 2001, respectively. The month of May had the lowest isolation rates for E. coli O157:H7 in both years (0%); isolations increased for this organism during the summer months and declined again in October 2000 to 0% (0/76) and in September 2001 to 0.6% (1/160). Isolation rates for Salmonella remained below 4.7% during May, June, September, and October 2000 and below 6.7% during May, June, and September 2001.

**Sites Incidence**

Escherichia coli O157:H7 was isolated one or more times from 10/35 (28.5%) sampling sites in 2000 and from 13/40 (32.5%) sampling sites in 2001.
The organism was isolated in each of the 2 yr from 5 of 35 sampling sites (14.3%). The organism was isolated from 9/35 sites only once (4 from Little Bow River and 5 from LNID) and from one site 2 times (LNID) in 2000. This pathogen was isolated from 10 sites once (6 from Little Bow River and 4 from LNID) and from 3 sites twice (all from the Little Bow River) in 2001. Escherichia coli O157:H7 was not isolated from the same site from consecutive samples in either year (data not shown).

Salmonella was isolated one or more times from 22/35 (62.9%) sampling sites in 2000 and from 36/40 (90%) of sampling sites in 2001 (Figure 2B). The organism was not isolated in either 2000 or 2001 from three sites in the LNID. However, the organism was isolated from all other sites in the Little Bow River and LNID from one to six times during 2000 and from one to seven times in 2001.

**FIGURE 2.** Frequency histogram of the number of times E. coli O157:H7 (A) and Salmonella (B) were isolated at each of the LNID and Little Bow River sampling sites during 2000 and 2001.
Salmonellae were isolated from 8.3% (1/12) samples with E. coli O157 from the LNID during 2000 and 2001 and from 31.5% (5/16) of samples from the Little Bow River during these two years. This is similar to the overall isolation rate for Salmonella from samples from the LNID (8.6%; see Table 2) but is almost three times the Little Bow River Salmonella isolation rate (11.5%).

**Salmonella Serovars**

Salmonella enterica serovar Rubislaw was the most common serovar isolated from these surface waters (Figure 3). It accounted for 52.4% of all the Salmonella isolates. This Salmonella serovar ranged from 42.3% of isolates from the LNID in 2000 to 81.6% in 2001 and from 43.9% of Salmonella in the Little Bow River in 2000 to 66.7% in 2001 (data not shown). Other serovars isolated from LNID and Little Bow River during 2001 and 2000 included: Give (9.1%), I:11:r− (6.7%), IIIa:42:z51:− (3.7%), Typhimurium var Copenhagen (3.0%), Heidelberg (3.0%), I:rough-O:r−:enx (1.8%), IIIb:16:z10:enxz15 (1.8%), Infantis (1.8%), Mbandaka (1.8%), Montevideo (1.8%), Typhimurium (1.8%), Newport (1.2%), Agona (0.6%), Enteritis (0.6%), Hadar (0.6%), I:4,12:I:− (0.6%), I:6,8:eh:− (0.6%), IIIa:41:z4,z23:− (0.6%), IIIb:60:r:enxz15 (0.6%), Muenster (0.6%), and Sandiego (0.6%).

**DISCUSSION**

McGowan et al. (1989) first reported isolation of E. coli O157:H7 from 2 of 30 water samples from a reservoir near Philadelphia. Isaacson et al. (1993) reported isolation of E. coli O157:H7 from 18.4% of the water samples from a river in Swaziland following an outbreak of infection with the organism that affected more than 40,000 individuals. The organism has also been isolated from well water and other rural water systems following outbreaks of human
infection with E. coli O157:H7 in Japan (Akashi et al., 1994), the United Kingdom (License et al., 2001), the United States (Centers for Disease Control and Prevention, 1999; Olsen et al., 2002), and Canada (Jackson et al., 1998; Kondro, 2000). However, isolation of E. coli O157:H7 from waterborne outbreaks of infection with this pathogen has not always been successful, even where there has been strong epidemiological evidence pointing to drinking or recreational water as the source of the outbreak. Chalmers et al. (2000) noted that E. coli O157:H7 was only isolated from water samples in 2 out of 14 suspected waterborne outbreaks of human infection with this enteric pathogen, based on reports in the literature from 1985 to 1999. This may be explained to some extent by the use of inappropriate methods for isolation of the organism. In addition, the infectious dose of this pathogen for humans may be as few as 100 bacteria (Griffin & Tauxe, 1991) and certain methods may have too low a level of sensitivity or lack specificity in the detection of E. coli O157:H7 in water samples, particularly if methods are not sufficiently selective and there are high levels of background microflora in the sample. Failure to isolate the organism from water following human infection may also be explained by the transitory nature of the organism in surface waters. In this study, none of the sites had water samples positive for E. coli O157:H7 on two successive occasions.

It is known that cattle as well as other ruminants are reservoirs for E. coli O157 (Chapman et al., 1994; Van Donkersgoed et al., 1999; Elder et al., 2000). Recent studies have reported that this human pathogen can be isolated from cattle water troughs and that water probably plays an important role in the transmission of the organism within groups of cattle (Shere et al., 1998). Fortunately, the organism is sensitive to chlorine and does not appear to pose an adverse risk to human health if the water is properly treated (Kaneko, 1998). However, problems may arise if (1) there is a failure in water treatment, (2) water such as well water becomes contaminated and consumed, and (3) recreational use of untreated surface water leads to accidental intake. Therefore, the E. coli O157:H7 prevalence data presented in this study suggest that surface waters in this region of Alberta represent a risk for human infection with this pathogen if consumed untreated under the circumstances described above. This risk is further supported by laboratory studies conducted on the E. coli O157:H7 water isolates that show that they carry the same virulence attributes as clinical strains of human origin (e.g., toxin production) and are of the same phage types as E. coli O157:H7 strains associated with human illness (data not shown). It is also interesting to note that the peak prevalence of E. coli O157:H7 in the water in the summer months corresponds to the time when the organism is most common in the feces of cattle (Van Donkersgoed et al., 1999; Elder et al., 2000) and when there is a peak in human illness associated with this pathogen (Griffin & Tauxe, 1991). It is also noted that use of surface water for recreational purposes also peaks at this time.

In the present study, both E. coli O157:H7 and Salmonella were prevalent to a similar extent in irrigation and river water. Interestingly, certain sampling sites in both systems remained relatively free of pathogens while other sites
had these pathogens isolated from them on a number of occasions in the same year and from one year to the next. However, E. coli O157:H7 and Salmonella isolations were not always closely associated with one another. This suggests that the sources (host reservoirs) of these organisms may differ. It is interesting to note that in contrast to the present study, Van Donkersgoed et al. (1999) reported a much lower prevalence of Salmonella than that of E. coli O157:H7 in the feces of cattle at slaughter in southern Alberta. Further, Salmonella serovar Rubislaw was not among the serovars of isolates reported by those researchers. Among the 23 different serovars of Salmonella isolated from surface waters in our two-year study, more than half were S. enterica serovar Rubislaw. Interestingly, this serovar is rarely associated with human or animal disease in Alberta or in Canada for that matter (Khakhria et al., 1996). Human infection by this serovar reported in the literature includes infections associated with captive reptiles in Canada (Khakhria et al., 1996) and an outbreak in Germany associated with paprika (Lehmacher et al., 1995). Baudart et al. (2000) noted that Salmonella serovars isolated from river water in France represent a much greater number of Salmonella serovars than are encountered among human clinical isolates or from point sources of fecal pollution. They suggest that this greater diversity of Salmonella serovars in river water is a result of the large number of host species that act as reservoirs of the organism and may excrete their own range of specific Salmonella serovars, which find their way into rivers from nonpoint sources. Further, as in the study of Baudart et al. (2000), many of the Salmonella serovars (other than Rubislaw) isolated in this study suggest an avian origin—for example, serovars Mbandaka, Montevideo, Agona, Enteritis, and Hadar. Certain of these Salmonella serovars are also commonly associated with human clinical disease, for example, Typhimurium var Copenhagen, Typhimurium, Give, Infantis, Mbandaka, Newport, Agona, Enteritis, and Hadar (Khakhria et al., 1996), and therefore likely present a risk for human infection.

As noted earlier, there are many large livestock feeding facilities located near waterways in southern Alberta, and manure from these feeding operations is routinely spread onto adjacent fields. It is easy to understand how bacteria from this manure could enter surface waters with the correct combination of topography and precipitation. In addition, there is also pasture land where free-ranging domestic animal and wildlife species can excrete feces in or near these water systems. However, further research work will have to be conducted to establish the host species origins of these pathogenic bacteria. Studies are presently evaluating a number of other bacterial typing methods for use in tracing the sources of fecal pollution in water. In addition, the survival of these bacterial pathogens in raw and treated water is being studied and an analytical tool kit to detect a wider range of enteric pathogens in water is being developed.

**CONCLUSIONS**

Two human pathogens, E. coli O157:H7 and Salmonella spp., were isolated frequently from surface water of the Oldman River Basin during the summer
months. Consumption of inadequately treated water, recreational activities such as swimming, and the use of this water for irrigation and processing of raw edible plants represent a risk for human infections with these pathogens. Consumption of untreated waters by animals is likely to maintain these bacteria in the region. The information generated in this study should be useful in designing optimal public health intervention strategies such as public education campaigns, control of these pathogens in specific animal reservoirs, improved manure management systems for livestock operations, and development of effective water treatment procedures for rural communities.

REFERENCES


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