A Prevalence Survey of *Salmonella* in Raw Milk in Ontario, 1986-87

SCOTT A. MCEWEN1, S. WAYNE MARTIN1, ROBERT C. CLARKE2 AND SUSAN E. TAMBLYN3

Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada. N1G 2W1; Animal Pathology Laboratory, Agriculture Canada, 110 Stone Road W., Guelph, Ontario, Canada. N1G 3W4; Perth District Health Unit, Stratford, Ontario, Canada. N5A 1L4

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ABSTRACT

In a survey to determine the incidence and prevalence of *Salmonella* in the bulk milk supply of dairy farms in southwestern Ontario 1986-87, milk filters from 813 farms were cultured over four sampling periods in six months. Prevalence rates during the four sampling periods were as follows: September 1986: 1.23%; October-November: 0.40%; December-January: 0.19%; and February 1987: 0%. Incidence rates increased from 0.15% per month in the first sampling period to 0.20% in the second and third period, then dropped to 0% in the fourth sampling period. Eight isolates of *S. muenster* and two of *S. mbundaka* were recovered from nine different farms. All of the isolates were sensitive to the commonly used antimicrobials tested. Owing to the steady decline in the prevalence over the study period, no seasonal patterns were apparent.

The results of this survey indicate that the presence of *Salmonella* in bulk milk supplies is dynamic.

Foodborne salmonellosis continues to be a significant public health problem in Canada, with approximately 7500 reported cases in 1985 alone (14). In recent years, salmonellosis in humans has been linked to consumption of raw or improperly pasteurized milk or milk products (4,8,12,19,20). Some of these outbreaks have involved large numbers of people. In 1984, approximately 2,700 human cases of salmonellosis were linked to the consumption of contaminated cheddar cheese from eastern Canada (8). In another outbreak that occurred in Illinois in 1985, approximately 16,000 people contracted salmonellosis from contaminated milk (12). Other incidents are smaller in scale, for example, in a recent case-control study conducted in Ontario it was shown that human infection with *Salmonella* was common on farms where the bulk milk was contaminated with these organisms (22). Among inhabitants of *Salmonella*-positive farms in that study, consumption of raw milk was strongly associated with human infection.

The majority of reports documenting the isolation of *Salmonella* from milk are descriptions of investigations of human disease caused by these organisms (4,19,20). Few of these studies provide estimates of the prevalence of the various serotypes of *Salmonella* that may be present in milk. A survey of *Salmonella* and other pathogens from bulk milk and swabs obtained at the transport truck level was recently reported from the midwestern U.S.A., where the prevalence of *Salmonella* contamination of truckloads of milk was 4.7%, and involved a total of 13 different serovars (15). In a survey of bulk milk in Ontario in 1986, the prevalence of *Salmonella* contamination at the farm level was approximately 2.8%, and the predominant isolate was *Salmonella muenster*, with occasional farms having *S. montevideo* or *S. newington* (13).

Isolations of *Salmonella* from humans in the United States have a distinct seasonal pattern, with peak numbers in the late summer and troughs in winter (3). Similar findings have been reported in hospitalized horses (2). In humans, it is assumed that the seasonal changes in the number of isolates reflect an underlying change in the prevalence (and/or incidence) rate. The temporal pattern of these organisms in food supplies or animal populations is unclear, although there is some evidence that their presence in animal populations is dynamic. Beginning in 1981, for example, the number of human and animal (predominantly bovine) isolates of *S. muenster* in Ontario increased dramatically over the number reported in previous years (19). This trend continued until the latter half of 1983, when the number of reported human and animal isolates of *S. muenster* began to decline. Despite this decline, however, this particular serotype was reported in bulk milk supplies and in association with human disease in 1986 (13,21).

The objective of this study was to determine the prevalence and incidence of *Salmonella* in the bulk milk supply of dairy farms in southwestern Ontario during the fall of 1986 and winter of 1987 and to note seasonal trends where present.

MATERIALS AND METHODS

The procedures used for the selection of farms, collection and culture of milk filters and characterization of *Salmonella*...
isolates used in this study were similar to those previously reported (13). In brief, dairy farms were randomly sampled using a two-stage sampling technique, with bulk milk transporters as the primary sampling units (n=60) and dairy farms served by these transport companies as the secondary sampling units (n=5221). The 554 farms that had participated in an earlier survey (December, 1985 to March, 1986 (13)) were again asked to participate in the present study. An additional 415 dairy farms that represented the geographical area served by the milk transport companies were selected.

Farmers that agreed to participate in the present study were each asked to submit one used milk filter for culture in each of three sampling periods (Table 1). The filters were used during a single milking on the study farms and were collected and delivered under refrigeration to the laboratory within three to four days (13). The first sampling period corresponded to September 1986, the second, October and November and the third, December, 1986 and January 1987. As the prevalence of Salmonella decreased steadily during the initial three sampling periods, it was decided to culture a different group of dairy farms to be sure that our observations were correct. Thus, a new group of 482 dairy farms was asked to participate in a fourth sampling period in February 1987. In the latter, four milk transporters not previously enrolled in the study were randomly selected with probability proportional to the number of farms that they serviced (13). Milk filters were incubated overnight at 37°C in 40 ml of nutrient broth (Difco Laboratories, Detroit, Michigan). Ten ml of Rappaport-Vassiliadis (RV) enrichment broth (Oxoid Canada Inc., Nepean, Ontario) was inoculated with 0.1 ml of the nutrient broth culture and incubated for 24 h at 43°C. Loopsful of RV broth were streaked onto brilliant-green-sulfa (BGS) plates and incubated at 37°C for 24 h. The serotype, biotype and antimicrobial sensitivity patterns of Salmonella isolates were determined as previously reported (13).

The variability of the prevalence (the proportion of farms Salmonella-positive at a given point in time) and incidence (the proportion of farms that became Salmonella-positive per month) estimates was expressed as the standard error of the mean, and was calculated by considering transport company routes as clusters of unequal size (6). Seasonal patterns were evaluated by plotting prevalence rates by month of sampling. As the purpose of this report was primarily descriptive in nature, the data concerning seasonal patterns were not subjected to formal statistical analysis.

### RESULTS

Nine hundred and sixty-nine dairy farms in southwestern Ontario were initially asked to participate in this study and 759 (78.3%) agreed. During the first study period (September, 1986), 574 (75.6%) farms submitted milk filters. As in the previous study (13), all of the filters were moist and accompanied by 5-20 ml of milk in the plastic bag and most filters were partially covered by a variable amount of plant debris (straw and other bedding material) and occasionally milk or fibrin clots. Salmonella spp. were recovered from seven filters (1.23%, Table 1) in this study period: five were serotype S. muenster and two S. mbanda.ka. Five of these seven positive farms had previously yielded negative milk filters (13) and were thus considered new cases, therefore, the monthly incidence rate was 0.15%. Eleven farms that had been positive in March were negative in September.

In the second study period (October and November), 526 (53.8%) dairy farms submitted milk filters for culture and two of these were positive for Salmonella giving a prevalence of Salmonella infection of 0.40%. In both cases S. muenster was recovered from the milk filters. One case had been negative on previous culture; hence the monthly incidence rate was 0.20%. In the third study period (December, 1986 and January, 1987), 534 (54.7%) dairy farms submitted milk filters for culture. Only one filter in this study period was positive for Salmonella (S. muenster), therefore, the prevalence was 0.19%, and the incidence was 0.20%.

During the fourth study period (February, 1987), 239 filters from 278 (86%) participating farms were tested. None of these filters was positive for Salmonella, therefore the prevalence was 0.0%. Over the course of the entire four culture periods, milk filters from a total of 813 different farms were cultured.

There were 10 milk filter isolates of Salmonella in this study from nine different farms. All of the isolates were sensitive to the commonly used antimicrobials tested, and all shared the same biotype pattern.


<table>
<thead>
<tr>
<th>Study period</th>
<th>No. farms cultured</th>
<th>No. farms at risk</th>
<th>No. farms positive</th>
<th>New cases</th>
<th>Prevalence (SE)</th>
<th>Incidence per month (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>574</td>
<td>552b</td>
<td>7</td>
<td>5</td>
<td>1.23% (0.0071)</td>
<td>0.15% (0.0061)</td>
</tr>
<tr>
<td>2</td>
<td>526</td>
<td>499</td>
<td>2</td>
<td>1</td>
<td>0.40% (0.0035)</td>
<td>0.20% (0)</td>
</tr>
<tr>
<td>3</td>
<td>534</td>
<td>506</td>
<td>1</td>
<td>1</td>
<td>0.19% (0)</td>
<td>0.20% (0)</td>
</tr>
<tr>
<td>4</td>
<td>239</td>
<td>239</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>N/Ac</td>
</tr>
</tbody>
</table>

aNumber of farms Salmonella-negative on previous culture.

bStandard error of the mean.

*Not applicable.
The seasonal pattern of the prevalence of *Salmonella* infection was assessed visually by tabulating the culture results of the present and previous surveys (13) by month of sampling period. Given the rapid decline in the prevalence, no seasonal pattern was apparent.

**DISCUSSION**

The prevalence of *Salmonella* in the bulk milk of dairy farms in southwestern Ontario decreased from approximately 2.9% in the winter of 1985-1986 (13) to less than one percent only one year later. Most of the *Salmonella*-positive farms that had participated in the previous study (13) were *Salmonella*-negative in this study and fewer farms became positive during the course of this project, as evidenced by the low incidence rates in the second, third and fourth study periods. The rapid decline in the prevalence of *Salmonella* in the bulk milk made the identification of seasonal trends difficult. Seasonal variation in the number of *Salmonella* isolates from cases of human (3) and animal (9) disease have been described. It is not known, however, if these patterns in disease occurrence are due to seasonal differences in the levels of contamination of *Salmonella* in food, or due to host and environmental factors, or to a combination of these. Longer term studies that monitor farm bulk milk supplies over a number of years are required to more clearly identify temporal trends in prevalence of *Salmonella* in bulk milk supplies.

The observed decrease in prevalence appears to be due largely to the reduction in numbers of *S. muenster*. In the previous (1985-86) prevalence survey, 24 of 28 milk filter isolates were *S. muenster*. This particular serotype was infrequently isolated in Ontario prior to 1980, although it was recovered in increasing numbers from human and animal sources, including raw milk, in 1981 through 1983 (19). Since then, fewer isolations from humans, animals and milk (1) suggest that *S. muenster* is gradually disappearing from southwestern Ontario. The reasons for this apparent departure are elusive and speculative. Perhaps the bovine population in the area - which may have been the reservoir of the infection - has developed a degree of herd immunity and the infection is ceasing to disseminate between farms and animals.

In a recent report from Wisconsin, Michigan and Illinois (15), 32 of 678 (4.7%) bulk milk truck samples were *Salmonella*-positive. The unit of analysis in that study, however, was truckloads of milk, not farms. The authors state that milk from approximately 2,700 producers was included in the truckloads. If the assumption is made that a truckload of milk is contaminated by only one farm, then the prevalence of *Salmonella* contamination at the farm level in the American study is about 1.19%; very similar to the findings of the present survey.

A variety of serotypes of *Salmonella* have been isolated from raw milk (8,12,13,15,20,21). Several serotypes, notably *S. typhimurium* and *S. dublin* have been shown to possess plasmid-associated virulence factors that result in increased pathogenicity for animals and possibly man (5,10). This may explain why some serotypes of *Salmonella* are associated with milkborne outbreaks more than others. Most reports of *Salmonella* in milk are the result of investigations of outbreaks of human disease, which gives the impression that milkborne *Salmonella* tend to be virulent pathogens for humans. While this may be true in some cases, the results from this and previous surveys (13,15) suggest that the *Salmonella* serotypes often associated with outbreaks of severe disease in humans, such as *S. typhimurium*, may only be present sporadically in raw bulk milk supplies. Even sporadic occurrence of these particularly virulent serotypes, however, may lead to large scale outbreaks of human disease (8,12). Because raw milk is often collected and mixed together in large volumes prior to processing and wide distribution, *Salmonella* or other pathogens from only one farm may contaminate the food supply of large numbers of individuals if a critical control point, such as pasteurization, is somehow circumvented.

All of the *Salmonella* strains isolated from milk filters in this study were sensitive to the commonly used antimicrobials tested. These findings are consistent with those of a previous study in Ontario (13). In some reports of outbreaks of human salmonellosis caused by multiply resistant strains of *Salmonella*, it is conjectured that antimicrobial use in food animals, especially the practice of prolonged exposure of animals to subtherapeutic levels of antimicrobials for the purposes of growth promotion, is responsible for the development of these resistant strains (7,11,18,20). In some cases of human illness, the sources of these resistant strains appear to be dairy farm animals (18). As most of the antimicrobial use on dairy farms is therapeutic, rather than in prolonged subtherapeutic levels (16), the source of these strains is uncertain. The findings of the present and previous studies (13) that *Salmonella* strains isolated from bulk milk supplies in Ontario are uniformly sensitive to commonly used antimicrobials suggest that the occurrence of drug-resistant *Salmonella* in bulk milk may only be a transient event, or related to a particular organism that is itself short-lived.

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**REFERENCES**

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