Survey on *Salmonella* prevalence in slaughter pigs from Saskatchewan

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Abstract — A study on slaughter pigs from Saskatchewan detected *Salmonella* organisms in 12.5% and 5.2% of cecal content and ileocaecal lymph node samples, respectively. Cecal content prevalence was associated with larger farms and longer lairage periods. Antimicrobial resistance was detected in 41.5% of the isolates. *Salmonella* Enteritidis was the second most prevalent serotype.

Résumé — Enquête sur la prévalence de *Salmonella* chez les porcs d'abattage en Saskatchewan. Une étude sur les porcs d'abattage en Saskatchewan a détecté des organismes de type Salmonella dans 12,5 % des contenus caecaux et 5,2 % des nœuds lymphatiques iléocæcaux. La prévalence du contenu caecal a été associée à de plus grandes porcheries et à de plus longues périodes d'hébergement transitoire. La résistance aux antimicrobiens a été détectée dans 41,5 % des isolats. *Salmonella* enteretidis constituait le 2ième sérotype le plus prévalent.

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Salmonellosis is considered the leading cause of death due to foodborne bacterial pathogens in developed countries. In Canada, it ranks 6th among all notifiable diseases, and 2nd in bacterial foodborne illness, after campylobacteriosis (1).

In Europe, it has been estimated that the consumption of contaminated pork and its products may account for 10% to 23% of the total number of cases of human salmonellosis (2). There are no such studies in Canada, but it is assumed that decreasing the level of *Salmonella* infection in finishing pigs will have a positive effect on reducing the level of human infection.

Asymptomatic *Salmonella*-infected pigs are considered the major source of infection. They harbor *Salmonella* organisms in tonsils, the intestinal tract, and mesenteric lymph nodes that cannot be detected by traditional meat inspection methods. Stressful situations (transport, lairage, etc.) can trigger the shedding of salmonellae, which, in turn, will contribute to the contamination of carcasses and the environment at the slaughterhouse (3). Thus, testing pigs for *Salmonella* infection at slaughter can be considered a good indicator of the risk of pork contamination faced by plant managers.

At present, major pork exporting countries are implementing slaughter-based *Salmonella* surveillance programs to increase the safety of pork and pork products. Most European countries are about to establish such programs in response to a new European Union Zoonosis Directive (4). Canada exports about 55% to 60% of the pork produced in the country (5), but Quebec is the only province that has a control program for pig salmonellosis.

Salmonella control programs rely on the definition of appropriate diagnostic tests for surveillance, a prior knowledge on *Salmonella* prevalence and serotypes present, and additional information on the major on-farm risk factors. In this paper, we report the results of a pilot study carried out in Saskatchewan to estimate the extent of *Salmonella* carriage among a group of slaughtered pigs and to identify serotypes present in the province and their pattern of antimicrobial resistance (AR). The relationship between *Salmonella* carriage and some potential risk factors are also discussed.

From September 2005 to March 2006, 232 slaughtered pigs were sampled from 3 abattoirs (A, B, and C) conveniently selected on the basis of their closeness to the University of Saskatchewan. Abattoir A was a relatively large federally inspected plant that slaughters ~540 pigs/h. Slaughterhouses B and C were small plants with a very slow slaughter speed (≤ 10 pigs/h), inspected by provincial veterinarians.

On different week days, a maximum of 10 pigs entering the slaughter chain were chosen from a single producer (except for

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Table 1. Number of sampled pigs by farm size and results from bacteriologic culture on cecal content (CC) and ileocecal lymph node (ICLN) samples in a survey on *Salmonella* carriage in slaughter pigs in Saskatchewan

Number of marketed pigs/year	Number of producers	Number of pigs	Number of positive CC samples (%)	Number of positive ICLN samples (%)
< 500	5	56	3 (5.3)	3 (5.3)
500–2000	5	50	3 (6)	2 (4)
2000–5000	3	26	3 (11.5)	2 (7.7)
> 5000	8	100	20 (20)	5 (5)
Total	21	232	29 (12.5)	12 (5.2)

Table 2. *Salmonella* serotypes isolated from cecal contents and ileocaecal lymph nodes associated with antimicrobial resistance[a] in a survey on *Salmonella* infection in slaughter pigs in Saskatchewan[b]

		Number of resistant isolates									
		Amp		Cm		Tet		Tms		Total	
Serotype	Number of isolates	≥ 16 (μg/mL)	≥ 32 (μg/mL)	≥ 16 (μg/mL)	≥ 32 (μg/mL)	≥ 8 (μg/mL)	≥ 16 (μg/mL)	≥ 2/38 (μg/mL)	≥ 4/76 (μg/mL)	number of resistances[c]	PR[d] (%)
Agona	2	—	—	—	1	—	—	—	1	2	5.9
California	3	—	—	—	1	3	—	—	—	4	7.8
Derby	9	—	4	5	—	3	—	—	4	16	10.4
Enteritidis	8	—	—	1	—	—	—	—	1	2	1.5
Give	3	—	—	—	—	—	—	—	2	2	3.9
Schwarzengrund	2	—	—	—	—	2	—	—	—	2	5.9
Total	27	—	4	6	2	8	—	—	8	28	6.1

Amp — ampicillin; Cm — chloramphenicol; Tet — tetracycline; Tms — trimethoprim/sulfamethoxazole

[a] A total of 17 antibiotics were tested

[b] The following *Salmonella* serotypes were not related with antimicrobial resistance but were isolated in the study: *Salmonella* Anatum (1 isolate), *Salmonella* Litchfield (2 isolates), *Salmonella* Mbandaka (2 isolates), *Salmonella* Putten (1 isolate), *Salmonella* Typhimurium var. Copenhagen (7 isolates), *Salmonella* untypable (1 isolate).

[c] Intermediate resistance + resistant. Following the CLSI guidelines (6)

[d] PR — percent of resistance, calculated as summation of all measures of resistance for a given isolate to any of the antimicrobial agents in the test panel divided by the number of antimicrobials tested and further divided by the number of isolates examined (see ref. 8)

abattoir B where all pigs slaughtered that day were selected). A sampling protocol that avoided any interference with plant operations was used, thus strict random sampling was not applied, but there was not a purposive selection of animals either. A chain of ileocecal lymph nodes (ICLN) and 25 g of cecal content (CC) were collected from each animal.

Data regarding the farm of origin of the animals (location, size) and the potential risk factor for *Salmonella* excretion (transport and lairage times, and mixing with pigs from other farms before slaughter) were collected from the abattoir.

Ten grams of CC and 2 g of ICLN were pounded through a stomacher, inoculated into 90 and 18 mL of buffered peptone water (BPW), respectively, and incubated overnight at 37°C (pre-enrichment phase). One milliliter of the pre-enrichment broth was then subcultured in 9 mL of tetrathionate (TT) and selenite broth, and incubated at 37°C for 24 h. Another 0.1 mL was subcultured in 9.9 mL of Rappaport-Vassiliadis (RV) broth at 42°C for 24 h.

Each enrichment broth was then plated onto 4 selective solid media [Xylose-Lysine-Tergitol-4 (XLT-4), *Salmonella/Shigella* (SS), Hektoen-Enteric (HE), and MacConkey] and incubated at 37°C for 24 h. Suspected *Salmonella* colonies were subcultured onto blood agar and MacConkey agar and incubated at 37°C for another 24 h. Presumptive *Salmonella* isolates were confirmed by triple sugar indol (TSI) and urea biochemical tests (API 20E system; Biomerieux Canada, St. Laurent, Quebec), and serological agglutination (*Salmonella* poly-A-I antisera; Becton Dickinson, Sparks, Maryland, USA). All isolates determined to be *Salmonella* were submitted to the Enteric Reference Laboratory (ERL), National Laboratory for Bacteriology and Enteric Pathogens in Ottawa for further serotyping.

Salmonella isolates were tested for susceptibility to 17 antibiotics. Antimicrobial susceptibility was determined on Mueller Hinton agar (Difco Laboratories, Detroit, Michigan, USA) by agar dilution tests in agreement with the Clinical and Laboratory Standards Institute (CLSI) guidelines (6). The antibiotics tested were as follows: neomycin at 4, 8, 16, 32 μg/mL; ticarcillin at 16, 32, 64, 128 μg/mL; kanamycin at 16, 32, 64 μg/mL; tetracycline at 4, 8, 16 μg/mL; ampicillin at 8, 16, 32 μg/mL; amoxicillin-clavulanic acid at 8/4, 16/8, 42/64 μg/mL; ceftiofur at 2, 4, 8 μg/mL; ceftazolin at 8, 16, 32 μg/mL; ceftriaxone at 8, 16, 32, 64 μg/mL; gentamicin at 4, 8, 16 μg/mL; enrofloxacin at 0.5, 1, 2, 4 μg/mL; trimethoprim-sulfamethoxazole at 2/38, 4/76 μg/mL; ciprofloxacine at 0.5, 1, 2, 4 μg/mL; amikacin at 16, 32, 64 μg/mL; chloramphenicol at 8, 16, 32 μg/mL; cephalothin at 8, 16, 32 μg/mL; and aztreonam at 8, 16, 32 μg/mL. All plates included a strain of *Escherichia coli, ATCC* 25922. A plate with no antimicrobials was included with each set of strains. Antimicrobial susceptibility for each isolate tested was interpreted, according to CLSI approved standards for humans (6) or animals (7), as susceptible, intermediate resistance, or resistant.

To compare the observed results with past trends in AR for *Salmonella* isolates from pigs in Canada, a summary measure describing the percentage of resistance (PR) to all antimicrobial agents was calculated, as described by Poppe et al (8).

Prevalences of positive CC and ICLN samples and their 95% confidence intervals (95% CI) were calculated. Chi-squared analyses were used to compare culture results and the factors studied (Epi Info, Centers for Disease Control, Atlanta, Georgia, USA). The transport and lairage times were categorized, based on their 33 and 66 percentiles before the analysis.

The number of pigs sampled was 160, 40, and 32 for slaughterhouses A, B, and C, respectively. Pigs belonged to 21 different producers, who were classified according to the number of pigs they marketed per year (Table 1). In 12.5% (95% CI = 8.3, 16.7) of the pigs, a *Salmonella* sp. was isolated from CC. Prevalence of positive CC samples was higher in pigs coming from larger farms (χ^2 for trends = 9.4; 3 df, $P = 0.002$) (Table 1). The average lairage time was 11.7 h (95% CI = 8.6, 14.9). The prevalence of positive CC samples was also higher for pigs spending longer periods in lairage (χ^2 for trends = 4.1; 2 df, $P = 0.043$) and for those slaughtered in abattoir A compared with B and C combined (16.2% vs 4.2%; $P = 0.01$). Bacteriological culture of ICLN yielded a prevalence of 5.2% (95% CI = 2.4, 8), but no relationships were observed with the size of the pig farm, lairage time, or slaughter plant.

The most prevalent *Salmonella* serotypes in CC were *Salmonella* Derby (25%), *Salmonella* Enteritidis (21.4%), and *Salmonella* California (10.7%). *Salmonella* Derby and *Salmonella* Enteritidis were also the most common serotypes after *Salmonella* Typhimurium var. Copenhagen in sampled ICLN. Overall, 11 (52.4%) of the farms provided at least 1 positive sample, with 2 of them providing more than 50% of all the positive CC samples. All farms, except for one, showing positive ICLN animals provided at least 1 animal with a positive CC.

Eighteen (43.9%; 95% CI = 28.7, 59.1) out of 41 *Salmonella* isolates (29 from CC and 12 from ICLN) had some degree of AR to 4 antibiotics (ampicillin, chloramphenicol, tetracycline, and trimethoprim/sulfamethoxazole). Four isolates (9.8%) were resistant to ampicillin. Two (4.9%) had AR to chloramphenicol and another 6 (14.6%) were classified as having intermediate resistance to this antibiotic (9). Seven (17.1%) isolates showed resistance to trimethoprim/sulfamethoxazole, and another 8 (19.5%) had intermediate resistance to tetracycline. Overall, 7 (17.1%) isolates had some level of resistance to at least 2 antibiotics, and 3 (7.3%) isolates to 3 antibiotics (Table 2).

The serotypes associated with AR are also shown in Table 2. Serotypes *Salmonella* Derby and *Salmonella* California showed the highest PR among all the serotypes (10.4% and 7.8%, respectively). The average PR for all *Salmonella* serotypes showing some degree of AR was 6.1%, this figure was somewhat lower than that observed in Canada between 1994 and 1997 (6.6–11.5) (8).

Information regarding the prevalence of *Salmonella* carriage in pigs is scarce in Saskatchewan. Although the small sample size and the selection of the pigs prevented the results being considered representative of the situation of pig salmonellosis in the province, the study added useful information for national and provincial animal health authorities.

In this study, we used a complex culture technique to enhance sensitivity that would not be suitable for routine surveillance. With this method, the percentage of positive CC samples detected (12.5%) was significantly higher than that reported in a previous study in Canadian abattoirs (5.2%; 95% CI = 4, 6.4) (10). The difference observed could be, in great part, due to the superior sensitivity of our technique, as a larger number of enrichment and selective media and a greater amount of feces (10 g *vs* 1 g, respectively) was used (10).

It has been postulated that culturing from ICLN would improve the sensitivity of the technique, because of the absence of competitive flora, and reflect more accurately the true infection status of the pigs sent to slaughter (11). Bacteriologic culture from ICLN yielded a much lower prevalence (5.2%) than that from CC, which suggests that many of the positive CC pigs were infected during transport and lairage. The association observed between the prevalence of positive CC samples and lairage time, and the lack of association between prevalence of positive ICLN samples and this variable, support this hypothesis. Indeed, environmental contamination with *Salmonella* organisms of slaughter premises and trailers is a common source of *Salmonella* infection (12); because of this, monitoring programs at farm level that rely exclusively on serological testing will likely miss a significant number of *Salmonella* carriers and thereby underestimate the potential for product contamination at slaughter.

The higher prevalence of *Salmonella* carriers found in pigs slaughtered in the largest abattoir would probably reveal the higher level of contamination of its holding pens due to the large number of animals slaughtered every day. The positive relationship between herd size and prevalence of positive CC samples might be due to higher levels of farm contamination in larger farms or simply a reflection of the fact that most of the pigs coming from these farms were slaughtered in the largest abattoir.

Salmonella Derby appeared in this survey as the most common as well as one of the most widespread serovars (4 farms), followed by *Salmonella* Enteritidis (4 farms), *Salmonella* Typhimurium (var. Copenhagen), and *Salmonella* California. *Salmonella* Derby, *Salmonella* Typhimurium (var. Copenhagen), and *Salmonella* California were among the serotypes most frequently isolated from pigs in Canada in 2004 (20.7%, 6.3%, and 3.3%, respectively) (13). *Salmonella* Enteritidis was the second most frequently isolated serotype from human samples (14), and the first in Saskatchewan, where 22% of the isolates belonged to this serotype (13). Although results from this survey would follow more or less those obtained from the national survey, the presence of *S.* Enteritidis near the top of the list was unexpected, as this serotype is common in poultry but not in pigs.

Interestingly, in the neighboring province of Alberta, *Salmonella* Enteritidis was found in 5% of the *Salmonella* isolates collected from 60 pig farms (15). In our study, this serotype was recovered from 4 farms located in distant locations. These findings might suggest some spread over the western provinces or simply cross contamination from neighboring avian farms. Given the importance of this serotype, its presence in pigs should be investigated and adequately monitored to assess the potential role that pork and pork products may have as a source of *Salmonella* infection in humans in the province.

The prevalence of AR to at least 1 antimicrobial (41.5%) did not significantly differ from that observed for Canada in 2004 (48%; 95% CI = 42.1, 53.9) (13). The prevalence of AR to antibiotics of importance in human medicine (aztreonam, ceftazolin, ceftriaxone) was null, similar to what was reported in the national survey. The mean PR (6.1%) was somewhat lower than the mean PR observed among *Salmonella* isolates from pigs in Canada between 1994 and 1997 (8). However, we calculated the PR as the sum of all measures of resistance (including both levels of resistance, intermediate and resistant), while Poppe et al (8) only included isolates that were considered resistant. The PR estimate would have been much lower (≈3%) had it been based only on the number of resistant isolates. Overall, the AR patterns observed did not differ from those in Canada, with ampicillin, chloramphenicol, tetracycline, and trimethoprim/sulfamethoxazole as the main antimicrobials involved in AR. Interestingly, while in the national survey, *Salmonella* Typhimurium var. Copenhagen was resistant to the largest number of antimicrobials (13), in this study, it was *Salmonella* Derby. Further studies regarding this issue seem to be guaranteed in Saskatchewan.

Authors' contributions

Dr. Mainar-Jaime was the principle investigator for the study and wrote the manuscript. Dr. Atashparvar, a graduate student, carried out most of the laboratory analyses (culture, PCR). Dr. Chirino-Trejo supervised Dr. Atashparvar's microbiological work and developed the antimicrobial resistance tests. Dr. Rahn was responsible for serotyping all the *Salmonella* isolates.

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