The Occurrence and Anti-microbial Susceptibility of *Salmonellae* Isolated from Commercially Available Pig Ear Pet Treats

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**Impacts**

- Animal derived pet treats, including pig ear treats, are a popular gift item among dog owners.
- Animal-derived pet treats are not regulated in Canada, therefore they can carry bacteria that can be harmful to pets and their owners.
- People can get sick from exposure to pig ear treats, particularly if hands are not washed properly after handling the product.

**Keywords:**

Salmonella; pet treats; pet food; zoonoses; anti-microbial resistance

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**Summary**

In Canada, there have been reported outbreaks of human salmonellosis related to exposure to animal-derived pet treats, involving pig ear treats, beef steak patty dog treats and pet treats of seafood origin. As a follow-up to recommendations made to the pig ear treat industry in 1999, a total of 245 pig ear treats were purchased in two Canadian cities to provide evidence of adoption of the recommendations and to determine the current levels of *Salmonella* contamination of pig ear treats available at pet stores. An overall prevalence of 4% was observed, with isolates exhibiting resistance to up to seven anti-microbials. Serotypes recovered included *S. Bovismorbificans*, *S. Give*, *S. Derby* and *S. Typhimurium* var. Copenhagen. Although the prevalence observed during this study is lower than the prevalence observed in 1999, pig ear treats should still be considered as a possible source of *Salmonella* and anti-microbial resistant bacteria to humans and dogs in Canada.

**Introduction**

Recently introduced pet treats for dogs have included pig ears and other dried animal parts. Although the use of pig ears as dog treats began within the last decade and half, today over 200 million pig ear treats per year are produced for the North American pet treat market (P. Sockett, S. Isaacs, A. Ellis, C. Clark, C. Anand, F. Rodgers, J. MacDonald, J. Cunningham, K. Grimsrud, L. Stefaniw, L. Crowe, M. Welch, R. Ahmed, D. Woodward, unpublished data). The processing of these treats typically begins with their removal from pig carcasses at the processing plant where they are de-haired and frozen for transport to pet food production plants (Holland, 2003; White et al., 2003). Once at the pet food plant, the pig ears are thawed, dipped in sodium hypochlorite, dried, sprayed with flavouring, cooled and packaged (Holland, 2003).

In 1999, laboratory and epidemiological investigations identified pig ear dog treats as a source of *Salmonella enterica* subsp. *enterica* serovar Infantis infection in humans in Canada (Clark et al., 2001). During the course of the investigations, pig ears were obtained from several pet treat-producing plants and purchased from pet stores to assess the prevalence of *Salmonella* contaminated products in the market. Fifty-one per cent of pig ear treats and 38% of other types of animal derived pet treats (referred to as ‘pet treats’ in this study) were positive for *Salmonella*. Nineteen
different serotypes were found, including S. Infantis, S. Typhimurium and S. Derby.

As a result of the Canadian outbreak, the Food and Drug Administration (FDA) – Center for Veterinary Medicine (CVM) in the United States carried out a retail sampling study investigating the prevalence of *Salmonella* in pet treats available in US pet stores. A total of 158 pet treats were collected, of which 41% were contaminated with *Salmonella* (White et al., 2003). Twenty-four serotypes were identified, including S. Anatum, S. Typhimurium and S. Infantis. Thirty-six per cent of the isolates were resistant to at least one anti-microbial, whereas 13% were resistant to four or more anti-microbials.

Following the 1999 *Salmonella* outbreak, the pet food industry and federal and provincial public health agencies in Canada met to discuss the next steps for preventing future similar outbreaks from occurring. Several recommendations were made to the pig ear treat industry including the implementation of Good Manufacturing Practices (GMP), further public education, better product labelling, use of irradiation on finished products and a creation of an association or group to represent the pig ear treat industry (Anon, 1999).

As there is no routine surveillance of pig ear treats in Canada for *Salmonella* contamination, the primary objective of the study was to determine if there was evidence of industry uptake of the guidance provided in 1999 in the form of a decrease in *Salmonella* prevalence and to provide the current prevalence of *Salmonella* contamination in pig ear treats. The secondary objective was to assess the possibility of regional differences in *Salmonella* prevalence, and therefore risk to dogs and humans, by conducting the study in two similarly sized cities in different parts of the country. A third objective was to investigate the possible role of pig ear treats in the epidemiology of anti-microbial resistance.

**Materials and Methods**

**Sample size**

A sample size was calculated based on a 50% prevalence observed by Clark et al. (2001) in tested pig ear treats. To determine the prevalence a sample size of 278 pig ear treats was calculated based on the formula \( n = Z^2 \times p \times (1-p) / L^2 \) with confidence level \( Z \) of 95% and an allowable error \( L \) of 6% (Martin et al., 1987). This was rounded up to 300 pig ear treats for the total sample size. To investigate possible geographical differences, the sample was divided between two sites. It was calculated that if one of the sites had samples with a *Salmonella* prevalence of 50%, then a difference of 16% could be detected between the study sites.

**Retail sampling**

The two study sites were selected based on similar population sizes and characteristics. Calgary, Alberta, was selected because it was the site of the 1999 *Salmonella* outbreak related to pig ear treats. Mississauga, Ontario, was chosen because its population size is similar to Calgary’s and is relatively close to the study laboratory, which simplified logistic considerations related to sample collection.

Names and contact information for pet stores were obtained by conducting Internet searches (Yellowpages.ca, Canada411.ca, and 411.ca) using the keywords: ‘pet stores’, ‘pet food’ and ‘pets’. A sampling frame was created from the resulting store list. Any additional pet stores found during the sampling period were added to the sampling frame. Each store in the sampling frame, whether part of a chain or an individual store, had an equivalent chance of being selected for sampling on a given sampling day.

Prior to each sampling day, six stores were selected randomly in the scheduled city. Four stores were visited, with the extra two serving as alternates in the event that a selected store was closed or did not have any pig ears in stock. The Canadian Integrated Program for Antimicrobial Resistance (CIPARS) retail sampling protocols (Government of Canada, 2006) were adapted to provide standardized instructions for field staff ensuring uniformity in data collection and sampling procedures. Labels with an ID number and the province of origin were affixed to each sample package.

When purchasing packaged pig ears, smaller packages with a minimum of 2 pig ear treats were purchased. If two stores from the same chain were visited on the same day, products were sampled from bulk bins at one store and pre-packaged products at the next store. When pig ear treats were obtained from bulk bins, two ears that had been in contact with each other in the bin were sampled. These were considered as one sample. All products, whether pre-packaged or bulk products, were placed in sealed Ziploc® (S. C. Johnson and Sons Ltd., Brantford, ON, Canada) bags to prevent cross-contamination.

Products purchased in Mississauga were taken directly to the study laboratory on the day of sampling. Products purchased in Calgary were sent to the laboratory within 4 days of purchase. The Ziploc® bags containing the samples were placed in a cooler for transportation. All samples were stored at room temperature until further processing.

**Salmonella isolation and identification**

Upon arrival at the Canadian Research Institute for Food Safety (CRIFS) laboratory, University of Guelph, samples...
were processed using aseptic techniques. Three methods were used in parallel for the isolation of *Salmonella* (Fig. 1). The first method followed the FDA Bacteriological Analytical Manual. The second and third methods were derived specifically for this study in consultation with *Salmonella* experts at the Ontario Veterinary College, University of Guelph and the Laboratory of Foodborne Zoonoses, Public Health Agency of Canada (LFZ-Guelph), Guelph, Ontario. All three methods were used, as there are no well-established methods for the recovery of *Salmonella* from these types of samples.

Twenty-five grams of each sample was mixed with 225 ml of buffered peptone water (BPW), soaked at room temperature for 60 min, massaged manually, with sterile gloves, in the BPW to provide thorough mixing, placed in a stomacher and blended for 1–2 min, and incubated at 35°C for 18–24 h.

Once incubated, the enriched solution was divided and transferred into three different broths for parallel testing. In method one, 0.1 ml of the enriched solution was added to 10 ml of Rappaport Vassiliadis (RV) broth; in method two, 1 ml of the enriched solution was added to 10 ml of tetrathionate (TT) broth; and in method three, 1 ml of BPW was added to 10 ml of RV broth. All three solutions were incubated at 42°C for 24 h. After incubation, a loopful of the solutions from each method was inoculated onto three selective media: XLT4 agar, brilliant green sulphate agar and bismuth-sulphite agar. These plates

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**Fig. 1.** Protocol for the isolation of *Salmonella* from pet treats.
Prevalence of *Salmonella* Contamination in Pig Ear Treats

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were incubated at 35°C for 24 h for methods 1 and 2, and at 37°C for method 3.

Following incubation, plates were examined for colonies suggestive of *Salmonella*. At least two typical colonies as well as two atypical colonies were transferred from each agar plate and inoculated into a MacConkey agar plate for purification. Plates were incubated at 35°C for 24 h for methods 1 and 2, and 37°C for method 3.

From each MacConkey plate, a sterile needle was used to inoculate suspect colonies into ¼ nutrient agar plates (NAP) and incubated for 18–24 h at 37°C. From these plates, growths were inoculated into a triple sugar iron (TSI) agar slant and a urea slant and incubated for 18–24 h at 37°C. If the TSI slant was positive and the urea slant negative, an indole test was conducted, followed by an agglutination test if the indole test was negative. If the results from agglutination were positive, a loopful of growth from the NAP was inoculated into a tryptic soy agar slant and submitted for serotyping.

All *Salmonella* isolates were submitted to the *Salmonella* serotyping laboratory LFZ-Guelph, for serotyping and phage typing, and to the CIPARS laboratory, LFZ-Guelph, for anti-microbial susceptibility testing. Anti-microbial susceptibility testing was conducted using broth microdilution (Sensititre™; Trek Diagnostics, Westlake, OH, USA) and 16 anti-microbials (aminoglycosides, clavulanic acid, amoxicillin/clavulanic acid, ampicillin, cefotaxime, ceftiofur, cephalothin, chloramphenicol, ciprofloxacin, gentamicine, kanamycin, nalidixic acid, streptomycin, sulphamethoxazole, tetracycline and trimethoprim-sulphamethoxazole). The resistance breakpoints were those used by the CIPARS and the US National Antimicrobial Resistance Monitoring System (NARMS), which are derived from Clinical Laboratory Standards Institute breakpoints.

Statistical analysis

The data were analysed with publicly available software (Epi Info version 6.04d; Centres for Disease Control and Prevention, Atlanta, GA, USA). Pig ear treats were considered to be positive if *Salmonella* was recovered from any of the three methods used. The *Salmonella* prevalence was compared using a two-tailed Fisher’s exact test (Petrie and Watson, 1999).

Results

Prevalence of *Salmonella* in pig ear treats

A total of 22 and 27 pet stores in Mississauga and Calgary, respectively, were included in the sampling frame. There was one chain with 11 outlets, one chain with two outlets and 13 individual stores in the Mississauga sampling frame. The Calgary sampling frame consisted of one chain with seven outlets, two with three, one with two and 12 individual stores. Sampling in Mississauga took place from November 14, 2003 to February 19, 2004, and in Calgary from April 6, 2004 to July 30, 2004. Of 145 pig ear treats purchased in Mississauga, 115 (79%) were sampled from bulk bins and 30 (21%) were pre-packaged (Table 1). It was not possible to determine the country of origin for bulk bin samples. For those products purchased with information on origin, six samples were from Canada, followed by the US (five), Germany (two) and Belgium (two). Five (17%) of pig ear treats were identified as having received irradiation treatment. Only one (0.7%) of 145 pig ear treats was positive for *Salmonella*. A total of 21 isolates were obtained from the positive pig ear using three different isolation methods in parallel. All were identified as the same strain of *Salmonella enterica* serovar Typhimurium var. Copenhagen phage type (PT) 109. This positive pig ear was pre-packaged, had not been irradiated and was imported from the US.

In Calgary, a total of 150 pig ear treats were purchased of which 122 (81%) were from pet store bulk bins and 28 (19%) were pre-packaged (Table 1). None of the samples collected were identified as having received irradiation treatment. For those products purchased with information on origin, 30 samples were from the US and 18 from Canada. Eleven samples (7%) were contaminated with *Salmonella*, a contamination rate that was significantly (P = 0.007) greater than the 0.7% found in Mississauga. Of the positive samples, 10 were purchased from bulk bins and three were pre-packaged, one was from Canada and two from the US.

The number of isolates recovered from pig ears ranged from 1 to 25 isolates. A total of eight different serotypes were recovered from pig ear treats purchased. Thirty-six per cent (4/11) of the samples were contaminated with *S. Bovismorbificans*, followed by *S. Give* (3/11, 27%), *S. Derby* (2/11, 18%), *S. Manhattan* (1/11, 9%), *S. Worthington* (1/11, 9%), *S. Agona* (1/11, 9%), *S. London*.

| Table 1. Description of pig ear treats purchased from pet stores in two Canadian cities and *Salmonella* prevalence |
|-----------------|-----------------|
| Mississauga | Calgary |
| Total pig ears purchased | 145 | 150 |
| Stores from one chain | 44% | 25% |
| No. from bulk bins | 115 (79%) | 122 (81%) |
| No. pre-packaged | 30 (21%) | 28 (19%) |
| No. irradiated | 5 (17%) | 0 (0%) |
| Product appearance | Oily, smooth, cooked | Porous, spongy |
| Samples *Salmonella* positive | 1 (0.7%)* | 11 (7%)* |

*Significantly different prevalence at P < 0.007.
Three positive samples were contaminated with two different serotypes: S. Manhattan and S. Worthington, S. Bovismorbificans and S. London, and S. Agona and S. Give. The overall prevalence for the two sites combined was 4.1% (95% CI: 2.24–7.22).

**Anti-microbial susceptibility in pig ear treats**

Only one serotype-resistance pattern combination was observed in the samples purchased in Mississauga, whereas 19 different serotype-resistance pattern combinations were observed in Calgary, which likely represents the number of different strains. The isolate obtained from the Mississauga sample was resistant to tetracycline only. Fifty-eight per cent of all isolates recovered from Calgary samples were susceptible to all anti-microbials tested for, 11% were resistant to one anti-microbial, 5% to three anti-microbials and 26% were resistant to four or more anti-microbials. Thirty-seven per cent of isolates were resistant to sulphamethoxazole, with the two most common resistance profiles observed being AMP-STR-SMX-TCY (ampicillin, streptomycin, sulphamethoxazole, tetracycline) and AMP-CEP-STR-SMX-TCY (ampicillin, cefepimycyn, streptomycin, sulphamethoxazole, tetracycline) (Fig. 2).

**Discussion**

This study was conducted as a follow-up to recommendations made by federal, provincial and local health authorities to the pet treat industry after a number of Canadians became infected in 1999 with *S. Infantis*, apparently as a result of exposure to pig ear treats. At the time, 50% of pet treats, including pig ears, obtained through a Canadian retail survey, were contaminated with *Salmonella* (Clark et al., 2001). The prevalence for pig ear treats in 1999 was much higher than the prevalences we found in Mississauga and Calgary, suggesting an overall decline in public health risk from 1999 to 2004. In 2005, the Public Health Agency of Canada, the Pet Industry Joint Advisory Council and representatives of the pet treat industry, met to discuss the results from this retail survey. Pet treat producers showed that they have taken steps to reduce the level of contamination present in their products, which could explain the observed decrease in *Salmonella* prevalence between the two retail surveys.

The observed difference in *Salmonella* prevalence between the two cities could be the result of the higher proportion of irradiated product in the Mississauga (17%) samples compared with the Calgary (0%) samples. Although most of the Mississauga samples were from bulk bins, pre-packaged irradiated product could have been used to fill up the bins, contributing to the lower prevalence. Product appearance differed between the two cities. Pig ear treats sampled in Mississauga had an oily, smooth and cooked appearance, while pig ears purchased in Calgary were porous and spongy. The differences in appearance are an indication that different methods were used in processing, such as longer heat treatment and use of different chemicals or additional ingredients on the final product. Another difference between the two cities was the variety of pet store chains. In Mississauga, 44% of the stores surveyed were part of one pet store chain, compared with 25% of pet stores surveyed in Calgary that were part of one pet store chain. Thus, a higher proportion of the product sampled in Mississauga was produced or imported by the same company than in Calgary,

![Fig. 2. Anti-microbial resistance of *Salmonella* serotypes isolated from pig ear pet treats purchased in Calgary, Alberta (n = 19) and Mississauga, Ontario (n = 1). AMC, amoxicillin–clavulanic acid; AMK, amikacin; AMP, ampicillin; CEP, cefepimycyn; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; FOX, cefoxitin; GEN, gentamicine; KAN, kanamycin; NAL, nalidixic acid; SMX, sulphamethoxazole; STR, streptomycin; SXT, trimethoprim-sulphamethoxazole; TCY, tetracycline; TIO, cefitiofur.](image-url)
possibly decreasing the number of sources of the pig ear treat samples. If that source had good manufacturing practices it would have a major impact on the Mississauga results.

Eighty per cent of all the pig ear treats purchased for this study were obtained from bulk bins. This is of public health importance as there is no guarantee that the products found in these bins are from the same manufacturer, which increases the likelihood of cross-contamination. Conversations with pet store employees revealed that some of the products they receive are multiple pig ear packages, which are opened and emptied into bulk bins for individual sale. This compromises the ability of public health authorities to conduct a thorough recall or product trace-back, as the information on brand, lot number and other company-specific information would not be as readily available in case of an outbreak or emergency as would be the case for well-labelled packaged product. Bulk bins are also a source of infection for customers, particularly children reaching in to handle the ears because there is no packaging to prevent direct contact with contaminated surfaces. Furthermore, bulk bins are a potential source of cross-contamination for other products. This is of particular concern with regards to bulk bins in non-pet stores that sell bulk pig ears such as supermarkets and bulk food stores. Consumers handling both pet treats and ready-to-eat foods without using proper handling procedures could contaminate ready-to-eat food items such as fruits, vegetables and bulk snack items. One major North American pet store chain has attempted to reduce cross-contamination and possible human infection by changing their format for selling pig ears at their stores (Wright, 2002). The chain no longer sells individual, unwrapped pig ears at their checkout stations; instead, they are pre-wrapped in cellophane and have labels advising customers to wash their hands after the handling of such products. Other pet stores and non-pet store outlets should consider following this practice as an alternative to bulk bins.

All isolates obtained from Calgary and Mississauga samples were serotyped and tested for anti-microbial resistance. Therefore, it was possible to determine the range of Salmonella serotypes to which dogs and humans may be exposed to after eating or handling these products. In Mississauga, the isolate obtained from the positive pig ear was found to be S. Typhimurium var. Copenhagen (Government of Canada, 2006). Among all swine isolates recovered from cecal sampling in abattoir testing and all swine clinical isolates during 2004, 42% and 75% were resistant to tetracycline, respectively.

Among isolates recovered from samples purchased in Calgary, S. Bovismorbificans was the most prevalent, followed by S. Give and S. Derby. During the 1999 Canadian outbreak pig ear retail investigation, S. Bovismorbificans was recovered from 2.5% of pig ear treat samples and S. Derby from 9.8% of samples (Clark et al., 2001). No S. Give was recovered from the pig ear treat samples at that point in time. These three Salmonella serotypes are not commonly isolated from humans in Canada. However, through abattoir surveillance, S. Derby, S. Bovismorbificans and S. Give were recovered from 21%, 4.4% and 2.2% of swine cecal samples, respectively (Government of Canada, 2006).

Resistance observed in samples from both cities is of major concern, as it represents another route of exposure to anti-microbial resistant bacteria to both humans and animals coming into contact with these products. In the comparison analysis conducted by Pitout et al. (2003) of human and pet treat strains isolated during an outbreak of S. Newport PT 14 in Canada, they found that the phenotypic and genotypic similarities in both sources suggested the possibility of a transfer of multidrug-resistant Salmonella to humans via the handling of pet treats. This is important as complications can arise if anti-microbial treatment is required and sick individuals or pets are infected with anti-microbial-resistant pathogens.

Irradiation of pig ear and other pet treats could reduce contamination present in these products. In April 2001, the US FDA approved irradiation for dog chews, including pig ears treats (Market Research Centre, 2001). To date, there have been no published studies specifically conducted on the effects of irradiation on Salmonella contamination in pig ear treats. While most irradiation studies in pork have examined its effect on Trichinella, there have, however, also been studies on the reduction of Salmonella in poultry meat. A radiation dose of 1.5–3.0 kGy on poultry meat destroys between 99.9 and 99.999% of Salmonella (Olson, 1998). Further studies should be conducted on the effects of irradiation on Salmonella in many food products including animal part pet treats.

Although dogs eat pig ears as a treat, they are not considered fit for human consumption and as such are not subjected to the same regulations as food produced for human consumption. Such gaps in the regulatory system allow for exposure of pets and their owners to products highly contaminated with various bacteria, including Salmonella, and for the use of animal origin raw materials obtained from non-regulated sources. In the US, the
American Pet Products Manufacturers Association (APPMA) has developed Guidelines for the Manufacturing of Natural Pet Treats for Pets, which have been reviewed by the APPMA membership and the FDA. These guidelines are voluntary but are intended to encourage the production of safe uncontaminated pet treat products (APPMA, 2003). Regulations should be implemented in Canada for the production and importation of animal-derived pet treats to reduce the contamination levels found in such products and thereby reduce the risk to human and canine health. Better labelling should be implemented to state whether irradiation treatments have been used on the final products and to warn consumers of the possibility of the product being contaminated with harmful bacteria, which could not only cause disease in dogs but also in people in the immediate household.

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