Evaluation of the Risks of Shedding *Salmonellae* and Other Potential Pathogens by Therapy Dogs Fed Raw Diets in Ontario and Alberta

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Impacts

- Programmes that involve dogs visiting hospitalized people are popular; so are raw-meat diets for dogs.
- Consuming raw meat considerably increases a dog's risk of excreting harmful bacteria like *Salmonella*.
- Dog owners should avoid feeding raw meat to their pets if their animals interact with people whose immune systems may not be functioning optimally, such as hospital patients.

Keywords:
*Salmonella*; dog food; zoonoses; public health

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Summary

Dogs that participate in animal-assisted interventions (AAIs), often called 'therapy dogs', commonly interact with humans whose immune systems are not functioning optimally. The advisability of feeding raw meat (including poultry) to these animals remains a highly contentious issue, in spite of increasing evidence that raw meat is frequently contaminated with *Salmonella*. We set out to determine if consuming raw meat influences the risk of therapy dogs shedding *Salmonella* and other pathogens. Two hundred healthy therapy dogs from Ontario and Alberta were enrolled. Between May 2005 and November 2006, fecal specimens were collected from each dog every 2 months for 1 year, along with a log of places visited, antimicrobial use within the home, dog health status and diet. Specimens were cultured for *Salmonella*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum cephalosporinase (ESC) *Escherichia coli* and *Clostridium difficile*. Forty (20%) of the dogs were reported to have been fed raw meat at some point during the year. The incidence rate of *Salmonella* shedding in the raw meat-fed dogs was 0.61 cases/dog-year, compared with 0.08 cases/dog-year in dogs that did not eat raw meat (*P* < 0.001). Controlling for therapy dog group, the repeated measures, and pig ear consumption and diarrhea in the 2 months prior to specimen submission, dogs that consumed raw meat were significantly more likely to test positive for *Salmonella* at least once during the year than dogs that did not eat raw meat [odds ratio (OR) 22.7; 95% confidence interval (CI) 3.1–58.8; *P* < 0.001]. Specific *Salmonella* serovars were more common among dogs that consumed raw meat versus those that did not include *S. Typhimurium*, *S. Heidelberg* and *S. Kentucky*. Raw meat consumption was also significantly associated with shedding ESC *E. coli* (OR 17.2; 95% CI 9.4–32.3). No associations between *C. difficile*, MRSA or VRE and consumption of raw meat were detected. We recommend that dogs fed raw meat should be excluded from AAI programmes, particularly when the programmes involve interaction with humans at high risk of infection or adverse sequelae attributable to infection. Furthermore, although AAI dogs may not be representative...
of the general population of dogs, we also recommend that feeding of raw meat to dogs is to be avoided in homes where immunocompromised people live.

Introduction

Few issues in companion animal medicine spark as vehemently opposed views as does the feeding of raw meat (including poultry) to dogs. Diets containing raw meat can be purchased (commercial diets that are already prepared and usually frozen) or made at home using various recipes (homemade diets). Proponents of both types of raw-meat diet claim various health benefits for dogs, including healthier coats and skin, fresher breath, a longer life, alleviation of arthritis, and improved immune function (Billinghurst, 1993; Lonsdale, 2001). While none of the purported benefits have been objectively substantiated, owners of raw meat-fed animals stand by their convictions, their arguments strengthened by recent recalls of adulterated, processed pet foods (Burns, 2007).

On the other side of the argument stand those concerned with the implications of raw-meat diets for animal and public health. Although reports on pet dogs becoming ill from consuming raw meat are rare, they do exist (Fredriksson-Ahomaa et al., 2001), as do reports on cats becoming ill from similar diets (Stiver et al., 2003). Outbreaks of salmonellosis in groups of working and high performance dogs that consumed contaminated raw meat have also been documented (Caraway et al., 1959; Cantor et al., 1997; Morley et al., 2006). Furthermore, researchers have isolated Salmonella enterica subsp. enterica from 6% to 80% of a variety of raw-meat dog food samples (Joffe and Schlesinger, 2002; Weese et al., 2005; Strohmeyer et al., 2006). Several studies have demonstrated that dogs that consume contaminated raw meat can shed salmonellae in their feces (Joffe and Schlesinger, 2002; Morley et al., 2006; Finley et al., 2007). The overall contribution of dogs to the total proportion of cases of reported human salmonellosis requires further investigation, but some have estimated that contact with companion animals accounts for at least 1% of cases in the United States (Stehr-Green and Schantz, 1987; Sarwari et al., 2001). A few case reports have suggested that young children may be at particular risk of developing non-typhoidal salmonellosis through contact with infected or colonized dogs (Morse et al., 1976; Schutze et al., 1999; Sato et al., 2000).

Some public health officials have expressed concern regarding the suitability of raw meat for dogs that participate in animal-assisted interventions (AAIs) such as visiting with people in hospitals and long-term care facilities (Finley et al., 2006, 2007), where patients or residents are typically at higher risk of infection or damaging sequelae attributable to infection than are healthy people. Their concern is amplified by evidence that healthcare-associated pathogens, such as methicillin-resistant Staphylococcus aureus (MRSA; Kitai et al., 2005; Kwon et al., 2006), extended-spectrum beta-lactamase (ESBL) Escherichia coli (Jensen et al., 2006), vancomycin-resistant enterococci (VRE; Pavia et al., 2000; Wilson and McAfee, 2002) and toxigenic strains of Clostridium difficile (Rodriguez-Palacios et al., 2007) can be isolated from raw meat for human consumption in North America and/or other parts of the world. Extended-spectrum cephalosporinase (ESC) E. coli, another pathogen of possible concern in healthcare settings, has also been detected in retail meats (Zhao et al., 2001; Yan et al., 2004).

The main objective of this investigation was to test whether consumption of raw meat by AAI dogs is associated with an increased incidence of shedding Salmonella and other healthcare-associated pathogens.

Materials and Methods

Subjects

Dogs were originally recruited in order to compare pathogen acquisition rates in AAI dogs exposed to human healthcare facilities relative to AAI dogs with no exposure to healthcare settings. Dogs were qualified for inclusion in the healthcare-exposed group if they had recently been recruited to visit healthcare facilities, and were scheduled to make their first visit within 1 week of a baseline test. Those that had already visited a human healthcare facility were excluded. Dogs were eligible for the unexposed group if they had never visited a human healthcare facility in the past, and were recently recruited for or were already participating in, other AAIs such as child literacy programmes or visiting retirement homes or schools.

All canine AAI organizations in Ontario that had been previously identified by our research group (Lefebvre et al., 2006a) were invited to participate through a letter addressed to organization managers. The managers advertised the study to their members, who informed the managers of their interest in participating. A list of volunteers and their contact information was provided to the researchers. A sample size of 80 dogs/groups was deemed necessary to achieve at least 80% power (\( \alpha = 0.05 \),...
two-tailed test) and was calculated on the basis of the predicted prevalences of several pathogens within the two different healthcare-exposure groups (Lefebvre et al., 2006b). Alberta AAI groups identified through internet searches and/or contact with Ontario associations were also invited to join the study to facilitate attainment of the necessary sample size. Alberta was selected as opposed to other provinces because of the availability of several different, well-established AAI organizations from which to select.

As dog-handler teams volunteered, the same veterinarian (SL) visited each and collected baseline fecal specimens and nasal swabs from the dogs. Handlers were instructed on proper specimen collection techniques, and each was provided with a kit of supplies and instructions (verbal and written) for submitting nasal and fecal specimens by courier within 24 h of collection for every 2 months for 1 year. The 2-month interval was chosen based on available resources. To preserve anonymity, a code was assigned to each animal, and coded specimen labels were provided. At the time of recruitment, information was gathered on animal diet, breed, age, sex and health status and places recently visited in the role of AAI dog. Handlers were supplied with a logbook to accompany each set of specimens (for a total of six book/specimen sets) to record information on variables that may influence the likelihood of acquiring pathogens, or that may indicate infection because of acquisition. Data requested included antimicrobial use (for a total of six book/specimen sets) to record information on variables that may influence the likelihood of acquiring pathogens, or that may indicate infection because of acquisition. Data requested included antimicrobial use, antibiotic exposure, and biological responses of the animal (e.g., vomiting, diarrhea).

Informed consent was obtained from all owners of the participating dogs. Ethics approval was secured from the University of Guelph's Research Ethics Board and Animal Care Committee.

### Bacteriology

The viability of *Salmonella* in canine fecal specimens over a range of temperatures and delivery distances was tested by the veterinarian submitting specimens known to contain *Salmonella* to the laboratory by use of the same expedited delivery system that the participants would use, from each city or town of recruitment. These specimens were subjected to bacteriologic culture by use of the same methods that would be used to isolate *Salmonella* from specimens obtained from AAI dogs.

As fecal specimens were received by the laboratory, a portion of each was submitted for immediate bacteriologic culture for *C. difficile*, MRSA and VRE. *Clostridium difficile* was isolated by coating swabs with fecal material and placing the swabs into 8 ml of cycloserine-cefoxitin-fructose broth with 0.1% sodium taurocholate. Tubes were incubated aerobically at 37°C for 7 days, at which time 2 ml of broth from each tube was transferred into sterile tubes, mixed with an equal amount of absolute alcohol, and left at room temperature for 30 min. Samples were centrifuged at 8000 g for 10 min. The supernatant was discarded, and the pellet was plated on to blood agar and incubated at 37°C for 48 h in an anaerobic chamber. Negative specimens were retested by use of the same procedure after 14 days of incubation in an anaerobic chamber. Colonies of *C. difficile* were confirmed via characteristic colonial morphology and odour, colour change around colonies in CCF agar plates, Gram stain and detection of l-proline-aminopeptidase production. Isolates were characterized by their ability to produce toxins A, B and *C. difficile* toxin by PCR.

Feces and nasal swabs were tested for MRSA via direct inoculation onto mannitol salt agar with 2 µg/ml oxacillin, and following 24 h enrichment in a broth of tryptone, mannitol, yeast extract and 7.5% NaCl. Inoculated plates were incubated in 5% CO2 at 35°C for 48 h. Suspected MRSA isolates were identified via a positive coagulase reaction and positive latex agglutination test for *S. aureus* (Pastorex Staph-Plus kit; Bio-Rad, Hercules, CA, USA). Confirmation of methicillin resistance was performed by detection of growth on Mueller-Hinton agar with 6 mg/ml oxacillin (Oxoid, Nepean, ON, Canada) and detection of penicillin-binding protein 2a via latex-agglutination test (Oxoid).

Fecal specimens were cultured for VRE by use of direct and enrichment methods following the protocol provided by the manufacturer of the culture media (Oxoid). Identities of catalase-negative, Gram-positive cocci were confirmed as enterococci via the API Strep biochemical identification test (Oxoid), and were speciated following the recommendations of Chen et al. (2000). Minimum inhibitory concentration testing for susceptibility to vancomycin was assessed using E-test strips (BD Biodisk, Solna, Sweden) following international standards (NCCLS, 2002). The presence of the vanA gene in resistant enterococcal isolates was confirmed via real-time PCR using a commercial Light Cycler kit (Roche Diagnostics, Penzberg, Germany), following the manufacturer’s instructions.

To culture the remaining organisms of interest, a fecal slurry was prepared by aseptically homogenizing 10 g of the fresh fecal specimen with 10 ml 0.85% sterile saline. Selective isolation of ESBL *E. coli* was performed by inoculating ESBL selective agar (MacConkey with 2 µg/ml cefpodoxime; Oxoid) with a loopful of fecal slurry. Plates were incubated at 37°C for 24–48 h. Potential *E. coli* colonies were identified by gross morphology. Six distinct
colonies were subcultured onto MacConkey plates and incubated for 24–48 h at 37°C. The identity of each isolate was confirmed by positive indole, negative citrate utilization, and negative urea tests. Isolates were subsequently cryopreserved.

Three isolates per fecal specimen were inoculated onto tryptic soya agar (TSA) slants and submitted to the Laboratory for Foodborne Zoonoses (LFZ), Public Health Agency of Canada, for antimicrobial susceptibility testing using a broth microdilution method (SensiTest® automated system; Trek Diagnostics, Westlake, OH, USA). Susceptibility to the standard panel of antimicrobials utilized in the National Antimicrobial Monitoring System was assessed (including, among other antimicrobials, cefoxitin, ceftriaxone, and amoxicillin/clavulanic acid) using the breakpoints recommended by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute [CLSI], 2005). Isolates were assumed to be ESBL or ESC E. coli if they demonstrated susceptibility patterns consistent with laboratory standards for presumptive identification (Public Health Agency of Canada, Canadian External Quality Assessment Advisory Group for Antibiotic Resistance, 1998). Specifically, ESBL isolates were presumptively identified by reduced susceptibility to ceftriaxone, but not to cefoxitin or amoxicillin/clavulanic acid. Extended-spectrum cephalosporinase candidates were required to show reduced susceptibility to all three antimicrobials.

Isolates presumptively identified as ESC E. coli candidates were confirmed to carry the AmpC β-lactamase (cephamycinase) bla<sub>CMY-2</sub> gene via conventional PCR assay using an in-house protocol that had been cross-validated for specificity and sensitivity on a collection of positive and negative E. coli isolates with known β-lactam susceptibility phenotypes and genotypes. Multiple positive and negative controls were included in the PCR assays performed using a Beckman NX robotics instrument.

Isolation of Salmonella was achieved by transferring 0.5 ml of fecal slurry into a tube containing 4.5 ml of BPW. Tubes were incubated at 37°C for 18–24 h. Two parallel isolation methods were subsequently used. In the first, 0.1 ml of the BPW mixture was inoculated onto modified semi-solid Rappaport–Vassiliadis medium (Oxoid) containing 1 ml/l novobiocin, and incubated at 42°C for 24–72 h. Cultures were examined on a daily basis for evidence of migration during incubation. Those demonstrating migration were subcultured onto both MacConkey and XLT4 agar (Oxoid) and incubated at 37°C for 18–24 h. From each MacConkey plate, three isolated colonies that showed typical Salmonella morphology were streaked onto three separate TSA plates (Oxoid). The same was performed for two isolate colonies that demonstrated H<sub>2</sub>S production on XLT4 plates. TSA plates were incubated at 37°C for 18–24 h.

For the second Salmonella isolation method, 1 ml of BPW containing the fecal slurry was transferred to 9 ml of Rappaport–Vassiliadis broth (Oxoid) and incubated at 42°C for 18–24 h. After incubation, 0.1 ml iodine was added to 9 ml tetrahionate broth (Oxoid) and vortexed; then, 1 ml of Rappaport–Vassiliadis broth culture was transferred into the tetrahionate broth. The mixture was incubated at 37°C for 18–24 h. A loop of mixture was then inoculated onto an XLT4 plate and incubated at 37°C for 18–48 h. Two colonies that were identified as Salmonella by gross morphology and H<sub>2</sub>S production were subcultured onto separate MacConkey plates and incubated at 37°C for 18–24 h. One colony that demonstrated typical Salmonella morphology on each MacConkey plate was streaked onto a TSA plate and incubated at 37°C for 18–24 h.

For both methods, identities of Salmonella isolates were further screened by characteristic colour changes observed after inoculation into Triple Sugar Iron slants and incubation at 37°C for 18–24 h, and absence of colour change after inoculation of and incubation of urea slants. A commercial Salmonella O Poly A-I + Vi latex agglutination kit (Becton Dickinson and Co., Sparks, MD, USA) was used to confirm the presence of Salmonella. Confirmed isolates were streaked on TSA slants, incubated for 24 h at 37°C, and submitted to the LFZ for serotyping and antimicrobial susceptibility testing, as described above for ESBL and ESC E. coli.

Basic statistical analysis

Basic analyses were performed using Intercooled Stata 9.1 (StataCorp, College Station, TX, USA). Incidence rates (IRs) for the different diet groups with respect to pathogen culture results were calculated for each outcome using the following formula:

\[ IR = \frac{\text{Number of animals acquiring the pathogen for the first time}}{\text{Total animal - years at risk}} \]

Dogs whose baseline specimens tested positive for a pathogen were excluded from incidence rate calculations for that pathogen. Total animal-years at risk were determined by summing the amount of time that each dog in a given group (raw-meat-fed, non-raw-meat-fed or entire study sample) contributed to the study. Animals were censored from incidence calculations for a particular pathogen either at the first point of testing positive for that pathogen or at the end of their participation in the study, whichever occurred first. IR ratios (IRRs) and associated 95% confidence intervals (CIs) were calculated to compare rates between diet groups using a Fisher’s exact test for statistical significance, with non-raw meat-consuming dogs serving as the referent group.
Statistical modelling

Univariate linear and logistic regression modelling was used to compare the distribution of age, sex and breed by diet group. For simple comparisons of the risk of specific serovars of Salmonella enterica subsp. enterica based on diet, odds ratios (ORs) with 95% CIs and population attributable fractions (PAFs) were calculated to relate raw meat consumers to other dogs, using Fisher’s exact tests.

For other measures of association, the primary exposure of interest was defined as whether or not the handler reported feeding the dog raw meat (including beef and poultry) or pig ear treats during the 2 months prior to specimen submission. Putative risk factors examined for confounding influences on any observed association between raw meat consumption and pathogen shedding included stable factors (age, breed and sex) and experiences assumed to vary over time (diarrhoea, antimicrobial exposure, visiting healthcare facilities and exposure to children when performing AAIs).

In these analyses, clustering of the outcomes was assumed and confirmed to occur at two levels – AAI group and dog – in most comparisons. Generalized linear mixed models (GLMMs) were built to examine relations between the primary exposure account for that clustering, using the SAS GLIMMIX program (The SAS Institute, 2006). A random intercept was introduced to control for clustering by groups, when the influence of including that intercept was statistically significant (P < 0.05). A first-order autoregressive covariance matrix was judged to be the best structure for accounting for the repeated measures within dogs.

If GLMM analysis showed that the association between raw meat exposure and the outcome was of liberal significance (i.e. P < 0.2), other predictors were added to, and eliminated from, the primary predictor model, one-at-a-time, to examine whether they had a confounding influence that should be controlled for, or whether they demonstrated an independent association with the outcomes when added. Confounding was judged to be present when the addition of one factor to the model changed the coefficient for the other factor(s) present by >20%. Confounders were retained in the final models, even if their association with the outcome was not significant. Subsequently, terms for interactions that were biologically plausible were created and added to determine their effect on the final models. Significant interaction terms were retained, and their component factors were included even if those factors were not significant on their own. When multiple models were derived for single outcomes, the best were selected using pseudo-Akaike information criteria (AIC; Molenberghs and Verbeke, 2005). Illnesses such as diarrhoea or extra-intestinal infections were also explored for relations with raw meat consumption using a similar GLMM strategy.

Results

Subjects

Two hundred dogs were enrolled between May and November 2005. Thirty dogs were originated from Alberta; the rest (n = 170) were from Ontario. Six of the dogs from Ontario stopped participating after the baseline sample collection, and these dogs were eliminated from the study. The remaining 194 dogs belonged to 156 different people, 19 different AAI groups and 14 different urban centres. Thirty of the dogs were from Alberta; the other 164 were from Ontario. During the course of the study, 10 dogs dropped out before their year of surveillance was completed (all from Ontario). Three dogs died from non-infectious causes, one moved overseas and could no longer participate, and six ceased participating in AAI programmes. These dogs were censored following submission of their last set of specimens. In total, 116 (59.8%) of the 194 dogs were exposed to healthcare facilities at some point during the study; however, the amount of time that these dogs were exposed amounted to only 79.7 years (958 months) out of the total 188.2 years (2260 months) of participation time. On average, dogs participated in AAIs once/week (range: once/month to six times/week; median: twice/week). Ninety-six (82.8%) of the 116 exposed dogs visited at least two healthcare facilities in any given month. Thirty dogs (25.9%) that visited healthcare facilities also participated in AAIs with children in the community.

Forty (20.4%) dogs were reported to have consumed raw meat at some point during the study period. These animals contributed 32.7 years (392 months) of participation to the study, out of the total 188.2 years contributed by all participants. The dogs in the raw-meat-fed group did not differ significantly from other dogs with respect to age (P = 0.415), sex (P = 0.744) or breed (P = 0.577–0.952). Twenty-two (11.3%) of 194 were fed home-made raw-meat diets and 1 (0.5%) was fed a commercial raw meat diet for the full study period. Two dogs (1.0%) began a commercial raw-meat diet 2 months into the study. Four dogs (2.1%) were fed raw meat on just a few occasions within one 2-month interval. The remaining 11 raw-meat-fed dogs (5.7%) consumed diets of raw meat as their main food source for periods ranging between 2 and 10 months.

Bacteriology and basic analyses

A summary of culture results for the different pathogens by diet (raw-meat versus non-raw-meat) consumed
during the 2 months prior to specimen submission, and associated incidence rates are provided in Table 1.

*Salmonella* was successfully recovered from all specimens submitted in the viability trial, and from 47/1324 (3.5%) fecal specimens from 37/194 (19.1%) participating dogs over the study period. Five of the dogs were positive at baseline, and all the five of these had been fed raw meat in the 2 months before testing. Thirty-two others began to shed *Salmonella* during the study period: 12 of them (37.5%) had not been fed raw meat in the previous 2 months; the other 20 (62.5%) had. All of the dogs testing positive for *Salmonella* on multiple occasions were regular raw meat consumers. Seven (17.5%) of these raw meat-fed dogs tested positive for the same serovar on two consecutive occasions. Two others (5%) shed different serovars on three non-consecutive testing times. One dog (2.5%) tested positive for a different serovar four times in a row. The point prevalence of *Salmonella* at the various sampling times ranged from 2.5% to 25.0% in the raw-meat-consuming dogs, and 0 to 2.6% in the others.

Dogs from Alberta were significantly more likely to shed salmonellae than dogs from Ontario (OR 4.3; 95% CI 2.32–7.87; *P* < 0.001); however, this association did not achieve a significant relation with shedding *Salmonella* in the GLMMs. No other associations between pathogen shedding and province were detected.

The distribution of the different *Salmonella* serovars between the different diet groups is shown in Fig. 1. A Fisher’s exact test showed that *S.* Typhimurium was more common in raw meat-fed dogs over the year than in other dogs (95% CI, 4.90–1741.20; *P* < 0.001). The associated PAF was 0.85, meaning that 85% of *S.* Typhimurium in the study population was due to consumption of raw meat, assuming a causal relationship. A similar relation between raw meat consumption and *Salmonella* risk was found with *S.* Heidelberg (OR, 6543210

Table 1. Results for the isolation of selected pathogens from fecal specimens collected every 2 months for 1 year from dogs that participated in animal-assisted interventions in Ontario and Alberta (*n* = 1130 specimens from 194 dogs).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Total specimens positive after baseline (%)</th>
<th>Total dogs acquiring pathogen: n (IR)</th>
<th>IR in dogs consuming raw meat</th>
<th>IR in dogs consuming other foods</th>
<th>IRR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>42 (3.7)</td>
<td>32 (0.17)</td>
<td>0.612</td>
<td>0.077</td>
<td>7.94* 3.69–17.80</td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td>50 (4.4)</td>
<td>39 (0.207)</td>
<td>0.153</td>
<td>0.218</td>
<td>0.70 0.21–1.80</td>
</tr>
<tr>
<td><em>Methicillin-resistant S. aureus</em></td>
<td>9 (0.8)</td>
<td>9 (0.048)</td>
<td>0.061</td>
<td>0.045</td>
<td>1.36 0.14–7.14</td>
</tr>
<tr>
<td><em>Extended-spectrum cephalosporinase E. coli</em></td>
<td>84 (7.4)</td>
<td>37 (0.25)</td>
<td>0.857</td>
<td>0.058</td>
<td>14.82* 6.80–35.71</td>
</tr>
<tr>
<td><em>Vancomycin-resistant enterococci</em></td>
<td>1 (0.09)</td>
<td>1 (0.005)</td>
<td>0</td>
<td>0.006</td>
<td>n/a</td>
</tr>
</tbody>
</table>

IR, incidence rate = (no. of animals testing positive for the first time) / (total animal-years at risk) = cases/ dog-year; IRR, incidence rate ratio; n/a, outcome too rare to calculate valid incidence rate ratios. *Significant at *P* < 0.001.

![Fig. 1. Distribution of serovars of *Salmonella enterica* subsp. enterica (percentage ± SEM) recovered over a 1-year period from the feces of dogs that participated in animal-assisted interventions in Ontario and Alberta, 2005–2006 (1130 total fecal specimens, 47 Salmonella isolates).](image-url)
A total of 106 fecal specimens from 59 dogs were identified as containing \( E. \ coli \) that possessed the AMR profile characteristic of ESC strains (reduced susceptibility to cefoxitin, ceftriaxone and amoxicillin/clavulanic acid); no isolates fit the ESBL \( E. \ coli \) AMR profile. All presumptive ESC strains were confirmed to carry the cephemycinase \( \text{bla}_{\text{CMY-2}} \) gene via PCR assay. No ESC \( E. \ coli \) isolates were recovered from 22 dogs at baseline, representing seven (20.6%) of the 34 dogs that were fed raw meat in the previous 2 months, and 15 (9.4%) of the 160 dogs that had not been fed raw meat. The remaining 84 isolates were recovered from fecal specimens from 37 other dogs over the following year. The point prevalence of these strains ranged from 15.0% to 45.0% in raw meat-fed dogs, and 0.6% to 9.7% in the others.

Methicillin-resistant \( \text{Staphylococcus aureus} \) was detected in the fecal specimens of six dogs and the nasal swabs of three. Two of the positive fecal specimens were from dogs that had consumed raw meat in the previous 2 months. One of the nasal swabs was obtained from a dog on a permanent raw-meat diet.

Vancomycin-resistant enterococci were isolated from only one fecal specimen, which belonged to a dog that had not consumed any raw meat during the study period.

\( \text{Clostridium difficile} \) was recovered from nine baseline specimens. Subsequently, 50 fecal specimens from 39 dogs were found to contain \( \text{C. difficile} \). Thirty-four of the dogs had not been exposed to raw meat; the rest had. Three of the dogs tested positive on three consecutive occasions: one had consumed raw meat, the other two had not. Four dogs tested positive twice in a row; none had been exposed to raw meat.

**Statistical modelling**

Generalized linear mixed model analysis showed that consumption of raw meat in the 2-month period preceding specimen submission was strongly associated with testing positive for \( \text{Salmonella} \) (OR, 26.32; 95% CI, 10.64–45.45; \( P < 0.001 \)). Adding a fixed effect for whether or not the dog experienced diarrhoea in the previous 2 months had no effect on the coefficient for raw meat consumption. In that model, diarrhoea was also statistically associated with \( \text{Salmonella} \) shedding (OR, 2.98; 95% CI, 1.31–6.77; \( P = 0.009 \)). Therefore, an interaction term was created for the two fixed effects. The resulting model is shown in Table 2. Table 2 also presents a second model that incorporated ‘diarrhoea’ and ‘pig ears’, and which has a lower AIC value, indicating it to be the superior model.

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw diet</td>
<td>27.03</td>
<td>10.42–71.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>83.33</td>
<td>0.02–1000.02</td>
<td>0.928</td>
</tr>
<tr>
<td>Raw diet and diarrhoea</td>
<td>56.21</td>
<td>8.40–376.16</td>
<td>0.05</td>
</tr>
<tr>
<td>Model 2 (AIC = 9086.57)</td>
<td>Raw diet</td>
<td>22.73</td>
<td>3.07–58.82</td>
</tr>
<tr>
<td>Pig ears</td>
<td>4.95</td>
<td>1.09–22.73</td>
<td>0.038</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>2.98</td>
<td>1.32–6.74</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Ten dogs were reported to have consumed pig ear treats during the study period. Adjusting for clustering within AAI groups and within dogs, those dogs were 12.2 times more likely to have shed \( \text{Salmonella} \) than dogs that had not consumed pig ear treats in the previous 2 months (95% CI, 3.23–45.45; \( P < 0.001 \)). When controlling for raw meat exposure, the OR for pig ear treats dropped to 5.03 (95% CI, 1.10–22.73; \( P = 0.04 \)). Consumption of pig ear chews continued to be significant when included in a model with the fixed effects of raw meat consumption and diarrhoea. Pig ear consumption was not significantly associated with the shedding of any other pathogen.

Generalized linear mixed model analyses showed that dogs that ate raw meat were 17.24 times more likely to have shed ESC \( E. \ coli \) than the other dogs (95% CI, 9.35–32.26; \( P < 0.001 \)). Adding prior antimicrobial treatment to the model as another fixed effect did not significantly influence this relation, and showed its own significant association with the outcome (OR, 2.82; 95% CI, 1.66–4.81; \( P < 0.001 \)). A term created for the interaction between raw meat consumption and prior antimicrobial treatment did not achieve a statistically significant association with shedding ESC \( E. \ coli \) when included in the model (\( P = 0.411 \)). Other variables, when added to the model, were not significant nor were they identified as confounders.

Shedding ESC \( E. \ coli \) was strongly associated with shedding \( \text{Salmonella} \), independent of an association with raw meat consumption (OR, 5.25; 95% CI, 3.42–8.06; \( P < 0.001 \)). A fecal specimen from one raw meat-consuming dog tested positive for strains of both \( \text{Salmonella} \) and \( E. \ coli \) that demonstrated reduced susceptibility to amoxicillin/clavulanic acid, ceftriaxone and cefoxitin.

The association between MRSA and consumption of raw meat was not statistically significant whether the outcome was MRSA cultured from both nasal and fecal specimens.
specimens (OR, 1.36; 95% CI, 0.28–6.58; P = 0.698) or from fecal specimens alone (OR, 0.97; 95% CI, 0.12–8.19; P = 0.978).

Generalized linear mixed model analysis showed that consumption of raw meat was not significantly associated with shedding C. difficile (OR, 0.59; 95% CI, 0.27–1.30; P = 0.193). Other variables (i.e. age, breed and exposure to healthcare facilities or children) were added to the model to see if there was any evidence of confounding; there was no such evidence.

In the logbooks, 21 dog owners recorded 25 incidents of diarrhoea in their dogs; four of the dogs experienced multiple incidents. However, there was no significant relation between dogs consuming raw meat or pig ear treats and their experiencing diarrhoea (P = 0.836 and 0.631, respectively). A review of the logs showed that 16/21 (76.2%) owners of dogs with diarrhoea continued to participate in AAI’s with their dogs the same week and, in one case, the same day as the illness was reported, in spite of their animal’s condition. Diarrhoeic dogs were also 14.92 times more likely to have been treated with antimicrobials in the same 2-month period than non-diarrhoeic dogs (95% CI 5.00–44.53; P < 0.001). Two of the dogs with both factors were treated with antimicrobials after the diarrhoea was documented; eight were treated in the week preceding the time that diarrhoea was recorded.

Over the study year, 38 separate incidents of extra-intestinal infections involving the skin, eyes, ears or urinary tract were reported by owners. One (2.6%), an ear infection, was reported in a dog that had consumed raw meat as part of its regular diet. The rest (97.4%) were reported in dogs that were never exposed to raw meat during the study period. The consumption of raw meat appeared was associated with reduced odds of extra-intestinal infections in dogs, compared with the odds in dogs that did not eat raw meat (OR, 0.06; 95% CI, 0.01–0.31; P < 0.001).

Discussion

Dogs that perform AAIs frequently interact with people whose immune systems are not functioning optimally, whether because of a person’s extreme age, stress level, physical condition, medication history or other factors. AAI programmes should, therefore, do everything possible to ensure that participating animals are at low risk of exposing participants to zoonotic pathogens. According to the results of this study, AAI dogs that consume raw meat or pig ear treats do not fit this crucial criterion. Further, the high proportion of AAI dogs currently being fed raw meat suggests that policies for selecting appropriate animals need to be re-examined in the interest of human health.

The pathogen of greatest concern with respect to feeding of raw meat to dogs is Salmonella. Sixty-one per cent of raw meat-consuming dogs were shedding salmonellae at some point during the year, compared with 8% of other dogs, an eight-fold difference. When adjusting for AAI group, repeated sampling, diarrhoea and the consumption of pig ear chews, the difference in the odds of shedding salmonellae exceeded 20-fold. These results support the findings of another study that observed that laboratory dogs fed one commercial raw meal experimentally contaminated with Salmonella were 11.4 times more likely to shed Salmonella within a week following consumption than dogs fed an uncontaminated raw meal (P = 0.01; Finley et al., 2007). Some of the dogs in our study shed salmonellae, albeit different serovars, for up to eight consecutive months.

Salmonella serovars Typhimurium, Kentucky and Heidelberg were the most common of the salmonellae recovered in this study. According to the 2005 Interim Report of the Canadian Integrated Programme for AMR Surveillance, S. Typhimurium and S. Heidelberg were among the top three serovars among salmonellae recovered from human stool specimens submitted to public health laboratories in Alberta and Ontario in 2005 (Public Health Agency of Canada, 2006). Among the canine isolates observed in our study, these are likely the serovars of greater risk to human health with respect to frequency of reporting. Could dogs be the source of some of these infections? The hypothesis is worth exploring, and has been proposed by others who have investigated discrepancies between the distributions of serovars found in food animals and those recovered from humans with salmonellosis (Sarwari et al., 2001).

The distribution of serovars detected in human specimens does not exactly parallel that of isolates from retail chicken in Canada. While S. Heidelberg and S. Kentucky were the serovars that were most frequently recovered from retail chicken in Ontario in 2005 (Public Health Agency of Canada, 2006), S. Typhimurium ranked among the lowest. S. Typhimurium is more often, albeit still rarely, recovered from other retail meats including retail ground beef in Alberta (Sorensen et al., 2002). In the United States, surveys report S. Typhimurium to rank high among the salmonellae recovered from various sources of ‘retail meat’ and ground beef (Zhao et al., 2002, 2006). Therefore, contaminated meat as a source of canine carriage and human infection with S. Typhimurium cannot be ruled out.

The positive association between consumption of pig ear treats and Salmonella shedding was expected because these items are prone to contamination with Salmonella, as reported by Finley et al. (2008), and elsewhere (Clark et al., 2001). Also not surprising was the finding that Sal-
monella-positive dogs that consumed raw meat and had been diarrhoeic during the previous 2 months were more likely to shed salmonellae than other dogs. Whether the Salmonella caused the diarrhoea, or the diarrhoea increased the likelihood of Salmonella being detected could not be determined. It is a cause for concern that, in spite of their dogs having diarrhoea, owners continued to have their dogs participate in AAI.s. This demonstrates a lack of understanding of, or regard for, the risks of zoonoses associated with AAI.s, a deficit that was characterized in a previous study of dog-owner teams with a similar demographic background. In that study, 40% of 90 owners of AAI dog were unable to name one pathogen transmissible from dogs to people (Lefebvre et al., 2006a).

To make record-keeping as simple as possible for participants, dog owners were not asked to record information on other potential dietary sources of Salmonella such as raw eggs, raw hides or other pet chews (Anonymous, 2000; Pitout et al., 2003). This may have led to an overestimation of the effect of pig ear treats and raw meat on shedding salmonellae and ESC E. coli; however, given the strong associations observed, the bias of failing to control for these other sources would not likely change the observed results significantly. In addition, owners were also not asked to identify specific types of meat fed to their animals. It is possible that particular meats such as poultry are riskier than others with respect to shedding salmonellae or the other pathogens studied here; however, that assessment could not be made here. Not enough dogs were fed raw-meat commercial diets here to draw a conclusion about the safety of commercial versus home-made raw-meat diets for AAI dogs.

Future research should examine the risk of commercial and/or home-made raw meat diets being contaminated with ESC E. coli. At present, the implications for human and canine health are unknown, but the data reported here indicated that raw meat-fed dogs in particular may be a major reservoir of ESC E. coli. Although transmission of ESC E. coli from dogs to humans has not been documented, which is possible. Extended-spectrum cephalosporinase E. coli infections are an emerging problem in North American healthcare settings (Friedland et al., 2003). Similar infections are also emerging in dogs (Sanchez et al., 2002).

The link between the co-carriage of Salmonella and ESC E. coli was unexpected due to prior reports of co-contamination of retail poultry with these bacteria (Zhao et al., 2002). This pattern is a cause for concern given the potential for E. coli to share the blaCMY-2 gene that encodes AmpC β-lactamase (cephamycinase) production in ESC strains (Winokur et al., 2001). Such genes are uncommon among Salmonella isolates from retail meat (Zhao et al., 2002; Chen et al., 2004). In fact, a potential ESC strain of Salmonella was detected in one dog in this study. As these strains are resistant or have reduced susceptibility to ceftriaxone, the drug of choice in treating severe paediatric cases of salmonellosis, one concern is that an increase in the prevalence of salmonellae harbouring the AmpC genetic profile will lead to higher frequencies of hard-to-treat infections (Allen and Poppe, 2002).

None of the data here suggested that dogs consuming raw meat were sicker than the other dogs. In fact, as far as the authors are aware, this is the first time a scientific study has revealed that consumption of raw meat was associated with a reduced risk of some common canine infections, such as UTIs and pyoderma. Given that raw meat-fed dogs were at a similar risk of being diagnosed with other, less commonly reported ailments such as cancer or hypothyroidism relative to other dogs (data not shown), this finding is unlikely to be due to owners of raw meat-fed dogs seeking veterinary care for their dogs less often, or their overlooking these infections more frequently. Furthermore, because owners of raw meat-fed dogs were unaware that data from their logbooks would be analyzed in this manner, it is unlikely that owners intentionally underreported instances of certain infections among their dogs.

Although significant associations between shedding of MRSA or C. difficile and raw meat consumption were not detected in the study reported here, some of the AAI dogs were shedding these organisms. The implications of these findings with respect to the health of human and canine participants in AAI.s is unknown and requires additional research. Transmission of C. difficile from dogs to humans has not yet been reported, although bidirectional dog–human transmission of MRSA appears to occur (Weese et al., 2006; Leonard and Markey, 2008).

In conclusion, AAI dogs that consume raw meat are at an increased risk for shedding both Salmonella and ESC E. coli. Those that eat pig ear chews are also at increased risk of shedding Salmonella. Dog owners who choose to feed these foods to their animals may be willing to dismiss the risks to their own health in light of the putative benefits to their dogs; however, they need to consider the potential risk to the health of others who will interact with their animals, particularly when those individuals may have immune systems that are not functioning optimally. The findings of this study and others support prohibiting the feeding of raw or unprocessed animal products to dogs that participate in AAI.s, particularly when the activities involve people that are highly susceptible to infections or their adverse sequelae. Furthermore, although the dogs in our study were likely not representative of the general canine population because of their activities and the care that they received, the evidence suggests that raw-meat diets for dogs should be avoided in any home in which immunocompromised people reside.
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