# Brief Communication Communication brève

## Survival of *Salmonella* Copenhagen in food bowls following contamination with experimentally inoculated raw meat: Effects of time, cleaning, and disinfection

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**Abstract —** There are concerns regarding the safety of feeding raw meat to household pets. This study demonstrated that *Salmonella* persists in food bowls that are inoculated with *Salmonella*-containing raw meat. Standard methods of cleaning and disinfection were minimally effective at eliminating *Salmonella* contamination.

**Résumé — Survivance de *Salmonella copenhagen* dans les bols de nourriture à la suite de contamination par de la viande crue inoculée expérimentalement : effets du temps, du nettoyage et de la désinfection.** La viande crue est une source de préoccupation pour la sécurité alimentaire des animaux de compagnie. Cette étude démontre que les bactéries persistent dans les bols de nourriture inoculés par de la viande crue contenant des salmonelles. Les méthodes courantes de nettoyage et de désinfection étaient minimalement efficaces pour éliminer la contamination aux salmonelles.

*(Traduit par Docteur André Blouin)*

*Can Vet J 2006;47:887–889*

The feeding of raw meat-based diets to dogs and cats has become increasingly popular. While initially used mainly for racing greyhounds and other performance dogs, raw food diets are now increasingly being fed to pets in households. A variety of concerns have been expressed regarding the feeding of raw meat, based mainly on the potential for contamination of foods with enteropathogens such as *Salmonella* spp. (1,2). Clinical disease and subclinical *Salmonella* spp. shedding have been reported in dogs and cats fed raw meat (1–4). A recent study identified *Salmonella* spp. in 20% of commercial raw pet foods purchased in Ontario (5).

Most of the attention to date regarding raw meat diets has been on evaluating diets for enteropathogen contamination and disease in animals from ingestion of *Salmonella*-contaminated meat. Little attention has been paid to indirect infection via handling of contaminated meat or items that have been in contact with contaminated food. Food bowls may be a potential source of infection for animals or humans in the household, if contaminated meat is fed and if the food bowl is not adequately disinfected. The objectives of this study were to evaluate persistence of *Salmonella* spp. and effects of different cleaning and disinfection regimens on experimentally inoculated food bowls.

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Reprints will not be available from the authors.

No external funding was provided for this study.

Commercial raw foods purchased for use in another study and determined to be free of *Salmonella* spp. were used as the base food. The meat sources of the diets were lamb ($n = 2$), chicken ($n = 1$), rabbit ($n = 1$), goose ($n = 1$), buffalo ($n = 1$), beef ($n = 1$), venison ($n = 1$), ostrich ($n = 1$), quail ($n = 1$), turkey ($n = 1$), and a combination of rabbit and salmon ($n = 1$). *Salmonella* Copenhagen that was previously isolated from a commercial raw pet food was grown in pure culture on blood agar. A MacFarland 0.5 dilution of the *Salmonella* sp. was prepared in phosphate buffered saline (PBS, pH 7.4). One milliliter of *Salmonella* suspension was used to inoculate 100 g of food that had been homogenized in a blender. Inoculated food samples were mixed thoroughly. Inoculated food was diluted serially 10-fold in PBS and 100-μL aliquots were pipetted onto XLT4 agar for quantification of the *Salmonella* sp.

Study 1 evaluated persistence of the *Salmonella* sp. over time in experimentally inoculated bowls stored at room temperature. Five 250-mL stainless steel and 5250-mL plastic pet food bowls were each contaminated with 2 g of inoculated food. The inoculated food was wiped over the bowl surface with a gloved hand, leaving a thin residue. After being allowed to dry for 1 h, 1 bowl of each type was tested (day 0). The remaining bowls were kept in a biosafety cabinet at room temperature, and 1 bowl of each type was tested on days 1, 2, 4, and 7. Sampling was performed by using an electrostatic dust collection cloth (Swiffer cloth; Proctor & Gamble, Toronto, Ontario) (6). The cloth was wiped over the entire interior surface of the bowl, then placed in 90 mL of buffered peptone water (BPW) and incubated at 35°C for 24 h. One milliliter of the incubated BPW was then inoculated into 9 mL of

Mueller-Kaufmann tetrathionate broth and incubated at 37°C for 24 h; 1 loopful of the broth was then inoculated onto XLT4 agar and incubated at 37°C for 48 h. Colonies were identified as *Salmonella* spp., based on colony morphology and color change of the agar surrounding the colonies, Gram staining morphology, and biochemical characteristics. Five replicates were made using beef- ($n = 1$), buffalo- ($n = 1$), venison- ($n = 1$), lamb- ($n = 1$), and ostrich-based ($n = 1$) diets.

Study 2 evaluated survival of the *Salmonella* sp. in food bowls following cleaning and disinfection. Seven stainless steel and 7 plastic food bowls were contaminated, as described above. Six stainless steel and 6 plastic bowls were randomly assigned to 1 of 6 treatment groups: 1) warm water rinse, where the bowl was held under running for approximately 15 s; 2) warm water rinse and scrub, where the bowl was held under running water for approximately 15 s and then scrubbed with a sterile paper towel; 3) warm water rinse and scrub with nondish soap (Sunlight; Unilever Canada, Toronto, Ontario); 4) 5 min immersion in 10% bleach solution; 5) washing in a dishwasher at 85°C; and 6) warm water rinse and scrub with dish soap, followed by 5 min immersion in 10% bleach solution. One stainless steel and 1 plastic bowl received no cleaning (control). Following cleaning and drying, bowls were tested as described above. Twelve replicates were performed using venison- ($n = 1$), buffalo- ($n = 1$), beef- ($n = 1$), rabbit- ($n = 1$), lamb- ($n = 2$), quail ($n = 1$), rabbit and salmon- ($n = 1$), chicken- ($n = 1$), goose- ($n = 1$), rabbit- ($n = 1$), and turkey-based ($n = 1$) diets.

Kruskal-Wallis test with Dunn's Multiple Comparison test was used to compare the persistence of the *Salmonella* sp. between different treatment groups. Mann-Whitney signed-ranks test was used to compare the overall recovery of the *Salmonella* sp. from stainless steel versus plastic bowls, including all treatment groups. A $P$-value of $< 0.05$ was considered significant.

The mean concentration of the *Salmonella* sp. in inoculated food in study 1 was $5.4 \times 10^5$ CFU/g (range $6.2 \times 10^4$ to $9.6 \times 10^5$). The *Salmonella* sp. was isolated from all bowls throughout the entire study period of 7 d.

The mean concentration of the *Salmonella* sp. in inoculated food in study 2 was $5.6 \times 10^5$ CFU/g (range $1.3 \times 10^4$ to $9.6 \times 10^5$). Persistence of the *Salmonella* sp. in food bowls following cleaning or disinfection is presented in Table 1. In the stainless steel group, scrubbing with dish soap following by soaking in bleach was significantly more effective for the elimination of the *Salmonella* sp. than were no cleaning, warm water rinse, and warm water rinse and scrubbing ($P < 0.05$). There was no significant difference between other methods. There were no significant differences between any of the cleaning methods for plastic bowls ($P = 0.051$). When stainless steel and plastic bowls were analyzed together, scrubbing with dish soap followed by soaking in bleach was more effective than were no cleaning, warm water rinse, warm water rinse and scrub ($P < 0.001$ for each), and scrubbing with dish soap ($P < 0.01$). The difference between scrubbing followed by soaking in bleach and dishwasher cleaning was not significant. There was also no difference in overall persistence of the *Salmonella* sp. in stainless steel versus plastic bowls ($P = 0.99$).

**Table 1. Recovery of a *Salmonella* spp. from pet food bowls experimentally inoculated with *Salmonella* bacteria and subjected to different cleaning or disinfection protocols, expressed as the percentage of bowls from which *Salmonella* bacteria were recovered after cleaning or disinfection.**

| | Stainless steel | Plastic | Total |
|---|---|---|---|
| No cleaning | 12/12 (100%)[a] | 12/12 (100%)[a] | 24/24 (100%)[a] |
| Warm water rinse | 12/12 (100%)[a] | 11/12 (92%)[a] | 23/24 (96%)[a] |
| Rinse and scrub | 12/12 (100%)[a] | 11/12 (92%)[a] | 23/24 (96%)[a] |
| Scrub with soap | 10/12 (83%)[a] | 9/12 (75%)[a] | 19/24 (79%)[a] |
| Soak in 10% bleach | 8/12 (67%)[a,b] | 9/12 (75%)[a] | 17/24 (71%)[a,b] |
| Dishwasher | 8/12 (67%)[a,b] | 8/12 (67%)[a] | 16/24 (67%)[a,b] |
| Scrub/bleach soak | 4/12 (33%)[b] | 6/12 (50%)[a] | 10/24 (42%)[b] |

Different superscripts indicate significant differences between treatment groups ($P < 0.05$)

Persistence of the *Salmonella* sp. in food bowls at room temperature was not surprising, because the *Salmonella* spp. have been shown to be able to survive in the household environment (7,8). However, this study does highlight the concern that *Salmonella* spp. could persist for long periods in food bowls, if contaminated meat is fed and bowls are not properly disinfected. An additional factor to consider would be the potential for biofilm formation over time, which would further hamper disinfection.

The ability of *Salmonella* spp. to persist in food bowls following cleaning and disinfection was somewhat surprising, particularly their survival following soaking in bleach and washing in a dishwasher at 85°C. Bleach is typically a highly effective disinfectant that would be expected to kill *Salmonella* spp. However, bleach is less effective in the presence of organic debris, so possibly the small amount of food residue in the bowl was enough to permit survival of the *Salmonella* sp. in some cases. This likely accounts for the finding that only scrubbing followed by soaking in bleach was effective at reducing the *Salmonella* sp. contamination. However, even this method of disinfection did not completely eliminate *Salmonella* bacteria in all bowls. The frequent recovery of the *Salmonella* sp. following dishwasher cleaning was somewhat surprising, considering the high water temperature. *Salmonella* spp. are not considered to be thermotolerant (9) and washing at 85°C would have been expected to kill *Salmonella* spp. Presumably, the *Salmonella* sp. was also able to persist within food residue in the bowl and the direct contact with hot water was limited. The persistence of the *Salmonella* sp. with the other cleaning protocols was not surprising.

The *Salmonella* sp. in food bowls was not quantified in this study and the culture technique that was used would detect very low numbers of *Salmonella* bacteria. It is likely that most, if not all, of the cleaning techniques resulted in some reduction of *Salmonella* bacteria. The clinical relevance of *Salmonella* spp. contamination of food bowls, at any level, in terms of animal and human health is unclear, and it is possible that reduction in numbers, not complete elimination, is the most important factor in most households. High numbers of *Salmonella* bacteria are generally required to cause disease in healthy individuals; therefore, the risk of contracting salmonellosis from handling contaminated bowls is likely low for most people. However, certain individuals are much more prone

to developing clinical salmonellosis, even when exposed to low levels of bacteria. Included in this group would be infants, elderly persons, immunocompromised individuals, and people treated with antimicrobials (10). In this group, persistence of *Salmonella* bacteria in food bowls, even at low levels, might be of concern. The frequent occurrence of these high-risk individuals in households and the ability of *Salmonella* spp. to replicate at room temperature (11) suggest that a qualitative assessment of *Salmonella* bacteria survival, such as was performed here, is relevant. Further, while this study only evaluated a *Salmonella* sp., results can be loosely extrapolated for other nonsporeforming enteropathogens. In particular, *Escherichia coli* O157, a potential cause of severe disease in humans, has been reported to have an infective dose of as few as 10 organisms (12). *Escherichia coli* O157 has been identified in raw pet food (1); therefore, the survival of even low numbers of *Salmonella* bacteria is of concern, because of the potential for the similar survival of *E. coli.*

This study does not confirm the risk of transmission of *Salmonella* spp. in households where raw diets are fed; however, it does highlight some concerns that should be considered when raw diets are fed. Further study is required to better evaluate the real risks to humans and animals from the feeding of raw diets to pets. However, in the absence of objective information, precautions should be taken to reduce the risk of human and animal disease because of the potential severity and transmissibility of this infectious agent. Avoidance of feeding raw meat diets would be prudent because of the risk of enteropathogen contamination, reports of animal disease, and the persistence of *Salmonella* spp. in food bowls that is reported here. If raw meat diets are fed, care should be taken in handling the raw foods and any in-contact items, including food bowls. Unconsumed raw meat should be not be left in bowls because of the potential for growth of enteropathogens at room temperature and for inadvertent contact by members of the household, especially children. Bowls should be disinfected shortly after feeding, and should be scrubbed to remove any food residue prior to disinfection. Soaking in a 10% bleach solution following removal of residue is reasonably effective; however, soaking in other disinfectants or hot water could also be effective. It is important to remember that disinfectant efficacy is also dependent on contact time and disinfectant dilution. CVJ

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