Bacteriological evaluation of commercial canine and feline raw diets

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Abstract — Twenty-five commercial raw diets for dogs and cats were evaluated bacteriologically. Coliforms were present in all diets, ranging from 3.5×10^3 to 9.4×10^6 CFU/g (mean 8.9×10^5; standard deviation 1.9×10^6). *Escherichia coli* was identified in 15/25 (64%) diets; however, *E. coli* O157 was not detected. *Salmonella* spp. were detected in 5/25 (20%) diets; 1 each of beef-, lamb-, quail-, chicken-, and ostrich-based diets. Sporeforming bacteria were identified from 4/25 (16%) samples on direct culture and 25/25 (100%) samples using enrichment culture. *Clostridium perfringens* was identified in 5/25 (20%) samples. A toxigenic strain of *C. difficile* was isolated from one turkey-based food. *Staphylococcus aureus* was isolated from 1/25 (4%) diets. *Campylobacter* spp. were not isolated from any of the diets.

Résumé — Évaluation bactériologique de rations alimentaires commerciales non cuites pour chiens et chats. Vingt-cinq rations commerciales non cuites pour chiens et chats ont été évaluées bactériologiquement. Des coliformes étaient présents dans toutes les rations dans une proportion allant de $3{,}5 \times 10^3$ à $9{,}4 \times 10^6$ UFC/g (moyenne $8{,}9 \times 10^5$; écart type $1{,}9 \times 10^6$). *Escherichia coli* a été identifié dans 15/25 (60 %) des rations alors que la souche O157 n'a pas été détectée. *Salmonella* spp. ont été détectées dans 5/25 des rations (20 %), une pour chaque type de ration — bœuf — agneau — caille — poulet — autruche. Des bactéries sporogènes ont été identifiées sur 4/25 (16 %) des échantillons par culture directe et 25/25 (100 %) par enrichissement de culture. Clostridium perfringens a été identifié dans 5/25 (20 %), des échantillons. Une souche toxicogène de *C. difficile* a été isolée d'une ration à base de dinde. *Staphylococcus aureus* a été isolé de 1 ration sur 25 (4 %). *Compylobacter* spp. n'ont été isolés d'aucune ration.

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Introduction

Feeding raw diets to cats and dogs is becoming increasingly popular. A variety of unsubstantiated benefits are used to support the feeding of raw diets, including beneficial effects on immune function, overall health, energy, skin and coat condition, chronic digestive, allergic and metabolic diseases, and provision of a 'more natural' diet (1,2). To meet the increasing demand, a number of companies are now marketing raw diets for cats and dogs, and these diets can be found at a variety of pet stores and a smaller number of veterinary clinics.

There is an inherent risk of bacterial contamination of raw meat for human or animal consumption, and a variety of bacterial pathogens are of concern (3–5). In animals fed raw meat diets, *Salmonella* spp. have received the most attention. One study reported isolation of *Salmonella* spp. from 80% of homemade raw diets for dogs, and 30% of fecal samples from dogs fed those diets (6), while another reported isolation of *Salmonella* spp. from 45% of raw meat samples used in diets of racing greyhounds (7). In addition to *Salmonella* spp., bacterial pathogens that could be of concern to animals or humans include *Campylobacter* spp., *Escherichia coli* (including *E. coli* O157), enterotoxigenic *Staphylococcus aureus, Clostridium perfringens,* and other pathogenic clostridia (3,5,8).

The sole reported study of commercial raw diets only evaluated 2 diets for a limited number of pathogens (9). Larger studies, both in sample size and number of pathogens, are required to make sound recommendations regarding the safety of these diets in terms of both animal and human health. Despite the lack of science, there is abundant conjecture about the feeding of raw diets, much of which may be misleading. On one Website supporting a commercial raw diet, it is claimed that "The FDA has stated that *Salmonella* is not harmful to dogs" (10), which is, to the authors' knowledge, an unfounded and misleading statement. One major proponent of raw diets has suggested that bacterial pathogens that may be found in raw foods are not able to cause disease in the dog because of the unique adaptation of its intestinal tract (2). There is no evidence to support this supposition and cases of salmonellosis in dogs and cats fed homemade raw diets have been reported (3,11,12).

Potential risks of feeding raw diets are multifaceted and need to be explored. In addition to animals being fed raw diets, there are potential concerns for people handling the food, people handling feces from animals fed raw diets, people handling food bowls, and animals

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exposed to animals that are fed raw diets. The potential increase in pathogen shedding by animals fed raw meat diets and its effects on high-risk populations, such as hospitalized animals, kenneled animals, and compromised humans (children, elderly, immunosuppressed, concurrently ill), are unknown.

The objective of this descriptive study was to evaluate commercial raw pet foods for the presence of a variety of bacterial pathogens.

Materials and methods

Commercial raw food diets were purchased from retailers in Guelph and Kitchener/Waterloo, Ontario. All diets were used before their expiry dates and stored as per label recommendations until evaluation. Frozen diets were thawed at room temperature prior to evaluation and were processed while still cool to prevent bacterial growth at room temperature. A freeze-dried diet was rehydrated as per label recommendations. Approximately 200 g of each diet was homogenized in a blender that was sterilized prior to each use. Samples were evaluated for the presence of total coliforms and *E. coli* in particular, *Salmonella* spp., *Campylobacter* spp., sporeformers, *Clostridium perfringens, C. difficile,* and *Staphylococcus aureus.*

Serial 10-fold dilutions of 1 g of homogenized food sample were performed in phosphate buffered saline (pH 7.4). One hundred microliter-aliquots were inoculated onto MacConkey's agar and incubated aerobically at 35ºC for 24 h. Total coliform count was determined on plates containing between 30 and 300 colonies; *E. coli* was identified on plates via colony morphology, Gram stain appearance (Gram negative rods), and biochemical characteristics, using a commercial biochemical identification system (BBL Enterotube II; Becton Dickinson Microbiology Systems, Sparks, Maryland, USA). Medium enriched for the culture of *E. coli* was also used: 500 mg of food sample was inoculated into 9 mL of a selective enrichment broth for *E. coli* (E. C. broth; Oxoid, Nepean, Ontario) and incubated aerobically at 35ºC for 24 h. One hundred microliters of broth was then inoculated onto MacConkey's agar and processed as described above.

Approximately 100 μg of each sample was inoculated onto xylose lysine tergitol 4 (XLT-4) agar (Becton-Dickinson, Sparks, Maryland, USA) for isolation of *Salmonella* spp., and incubated aerobically at 37ºC for 48 h. Medium enriched for the culture of *Salmonella* was also used: 500 mg of each sample was inoculated into 9 mL of buffered peptone water and incubated aerobically at 35ºC for 24 h. One milliliter was then inoculated into 9 mL of Mueller-Kaufmann tetrathionate broth (Oxoid) and incubated for 48 h at 37ºC. This culture was then subcultured onto XTL-4 agar and incubated as described above. Black colonies were subcultured onto blood agar and identified via a commercial biochemical identification system (BBL Enterotube II; Becton Dickinson Microbiology Systems). Isolates were submitted to the Health Canada Laboratory for Foodborne Zoonoses for confirmation.

Culture for *Campylobacter* spp. was done by inoculating 100 μg of food sample onto a medium containing trimethoprim, rifampin, polymixin B, and cycloheximide (Preston Selective Medium; Oxoid) and incubating at 37ºC in a microaerophilic environment for 48 h, and by inoculating 500 mg of food into 9 mL of a selective enrichment broth (Preston Selective Broth; Oxoid) and incubating in a microaerophilic environment at 37ºC for 48 h prior to inoculation of 100 μL onto the selective agar described above. *Campylobacter* spp. were identified through colony morphology, Gram stain appearance, catalase reaction, and oxidase reaction.

Spore selection was performed by heat shocking 500 mg of food in 1 mL of PBS at 70ºC for 1 h. A 50-μL aliquot was then inoculated onto blood agar and tryptose sulphite cycloserine and Shidi, Ferguson perfringens (TSC+SFP) agar (Oxoid). A 50-μL aliquot was also inoculated into 3 mL of tryptone soya broth and incubated at 35ºC for 24 h prior to inoculation onto blood agar and TSC + SFP agar. Colonies with a characteristic appearance of *C. difficile* on blood agar were subcultured onto cycloserine-cefoxitin fructose agar (*Clostridium difficile* selective medium; Oxoid) and identification was confirmed through colony morphology (roughened, 'ground-glass' nonhemolytic colonies), Gram stain appearance (long, thin gram-positive rods with terminal spores), and detection of L-proline-aminopeptidase production (ProDisc; Remel, Lenexa, Kansas, USA) (13). Determination of toxigenicity was based on identification of genes encoding toxins A and B (14). Ribotyping of *C. difficile* was performed as previously described (15). Isolates with characteristic colonial morphology of *C. perfringens* were subcultured and identified via Gram stain (short, thick gram-positive rods), colony morphology (round, black colonies with a zone of clearing indicating lecithinase reaction), and biochemical characteristics, using a commercial biochemical assay (API Rapid ID 32 A; BioMerieux, St. Laurent, Quebec).

Direct culture for *Staphylococcus aureus* was performed by direct inoculation of 100 μg of food onto blood agar and aerobic incubation at 35ºC for 24 h. Culture for *S. aureus* using an enrichment medium was performed by inoculating 100 μg of food into 2 mL of enrichment broth (10 g/L tryptone, 75 g/L NaCl, 10 g/L mannitol, and 2.5 g/L yeast powder). The enrichment broth was then incubated aerobically at 35ºC for 24 h, after which 100 μL of it was inoculated onto blood agar and incubated at 35ºC for 24 h. *Staphylococcus aureus* was identified through colony morphology (large, round, beta-hemolytic), Gram stain appearance (gram-positive cocci), positive catalase reaction, positive coagulase reaction, and latex agglutination test (Pastorex Staph-Plus; Bio-Rad Laboratories, Mississauga, Ontario). Testing for all organisms was performed in triplicate.

Results

Twenty-five raw diets from 8 different manufacturers were evaluated. Thirteen diets were for dogs, 8 for cats, and 4 did not state the intended recipient. All but 1 diet was frozen; the remaining diet was in a freeze-dried preparation. Twenty-three diets had a single meat source listed on their label, while the remaining 2 contained 2 types of meat. Chicken was the most common meat source, being present in 7/25 (28%) diets. Other

meat sources included beef ($n = 5$), lamb ($n = 3$), ostrich ($n = 3$), rabbit ($n = 2$), salmon ($n = 2$), and 1 each of turkey, quail, goose, buffalo, and venison.

Coliforms were present in all diets, ranging from 3.5×10^3 to 9.4×10^6 CFU/g (mean value 8.9×10^5, $s = 1.9 \times 10^6$). The freeze-dried sample had the lowest coliform count, while coliform levels were in excess of 1×10^6 CFU/g in 4 diets. *Escherichia coli* was identified in 16 (64%) diets in both direct and enrichment cultures in all cases in which it was present. Monophasic *S.* Typhimurium were detected in 5 (20%) diets, all after enrichment culture. Meat sources from diets containing *Salmonella* spp. were beef, lamb, quail, chicken, and ostrich. *Campylobacter* spp. were not isolated from any sample. Sporeforming bacteria were identified from 4 (16%) samples on direct culture and from 25 (100%) samples using enriched medium. *Clostridium perfringens* was identified in 5 (20%) samples. *Clostridium difficile* was isolated on direct culture from 1 turkey-based food. Genes encoding for *C. difficile* toxins A and B were detected in vitro, and the ribotype of this isolate was indistinguishable from a type that has been identified in association with diarrheic disease in horses, dogs, and humans (L. Arroyo, unpublished data). *Staphylococcus aureus* was isolated from 1 (4%) diet when enrichment techniques were used.

Discussion

A variety of potential enteropathogens of both animals and humans were identified in the commercially available raw diets evaluated in this study. While adequate information regarding the health risks associated with feeding raw diets is currently lacking, scientific and anecdotal reports suggesting a risk are on the increase (12). Concerns regarding infectious disease associated with raw diets involve both animals and humans. For animals, the issue is exposure to enteropathogens with the possible development of disease, particularly salmonellosis and clostridial diarrhea. For humans, the risk of exposure to pathogens via direct or indirect contact with animal feces, or via contact with raw diets, must be considered, particularly with *Salmonella* spp., as fecal shedding of *Salmonella* spp. present in diets has been identified in dogs (6,11). Bacterial contamination of surfaces that have been in contact with raw diets has not been evaluated, but must be considered. Bacterial contamination of pet food bowls may be a potential source of infection for humans, particularly high-risk individuals, such as infants, elderly persons, and immunocompromised individuals. The effects of cleaning and disinfection protocols on the survival of zoonotic enteropathogens in food bowls have not been adequately evaluated to make informed recommendations.

Coliform numbers in food samples are used as an index of sanitation (16). The coliform level in all diets was in excess of the maximum allowable level of 1000 CFU/g for raw meat set by the Canadian Food Inspection Agency (17). The presence of *E. coli* and other coliforms in food samples most likely indicates fecal contamination (18,19); however, contaminated equipment or incorporation of meat from animals with *E. coli* bacteremia or septicemia could also be a cause. Enterohemorrhagic *E. coli* O157 is a cause of serious illness in humans, who can be infected with a dose of as few as 10 organisms (20). Specific culture for *E. coli* O157 was not performed in this study; however, the high *E. coli* levels identified here and identification of this organism in a homemade raw diet in a previous study (9) suggest that raw pet foods may present a risk to humans. The risk of disease in dogs and cats from exposure to *E. coli* in food is unclear; however certain strains of *E. coli* are recognized enteropathogens in these species (21).

Isolation of *Salmonella* spp. from 20% of raw diets was of concern, but it was not surprising, based on earlier reports. *Salmonella* sp. is a recognized pathogen of a variety of species, and salmonellosis has been reported in dogs and cats fed raw food contaminated with *Salmonella* spp. (7,11,12). Subclinical fecal shedding of *Salmonella* spp. by dogs fed raw diets has also been reported (6,11), creating the possibility of zoonotic transmission of disease via direct contact or through environmental contamination within households. *Salmonella* spp. can also be isolated from certain raw meat products intended for human consumption. In one study, *Salmonella* spp. were identified from 7.5% of ground beef, 44.6% of ground chicken, and 49.9% of ground turkey samples (4). The high prevalence of *Salmonella* spp.contamination of meat intended for human consumption should not be used as a reason to dismiss the significance of its prevalence in raw pet diets, because meat for humans is cooked prior to feeding.

Identification of sporeforming bacteria was not unexpected. Sporeformers in raw meat samples were likely of fecal or environmental origin, and bacterial spores are able to persist in food because they are highly resistant to environmental stressors. More important was the identification of a variety of potentially pathogenic sporeforming bacteria. *Clostridium perfringens* is a recognized cause of enteric disease in dogs (22,23) and is an important cause of food poisoning and sporadic diarrhea in humans (24,25). The potential for foodborne transmission of *C. perfringens*-associated disease in dogs is unclear at this point. The isolation of a toxigenic strain of *C. difficile* from 1 diet was surprising and has not been reported previously. *Clostridium difficile* is a recognized cause of diarrhea in dogs (22), cats (26), humans (24), and other species. The pathogenesis of *C. difficile*-associated disease in dogs and cats is poorly understood and the relevance of this finding is unclear. Contamination of food with *C. difficile* is more likely to be clinically relevant in humans or animals that are being treated with antimicrobials or chemotherapeutics; 2 important risk factors for the development of *C. difficile*-associated disease (27).

Campylobacter spp. are recognized enteropathogens in dogs and cats, and an important cause of foodborne diarrhea in humans. Additionally, contact with dogs has been reported as a risk factor for *Campylobacter*-associated disease in humans (28). Concern has been expressed about the potential for infection of dogs with this bacterium via raw meat (3); however, *Campylobacter* spp. were not isolated in this study. The failure to isolate *Campylobacter* spp. may relate to lack of contamination with this organism, storage of food samples prior to

purchase, or lack of sensitivity of the assay. All products, except the freeze-dried product, were stored in conventional freezers. *Campylobacter* spp. do not survive well at conventional freezer temperatures (29,30).

Food samples were processed while still cool to reduce the risk of bacterial growth during thawing. However, bacterial growth during thawing or growth in unconsumed food in bowls may be a concern. Growth during storage or in food residue may be particularly relevant for pathogens such as *St. aureus* that are likely of minimal concern at low levels but can cause serious disease if allowed to grow and produce toxins (8). This requires further evaluation to define the risks and create proper infection control recommendations.

There is currently inadequate information regarding the safety of raw diets in terms of both animal and human disease. However, considering the variety of infectious and potentially zoonotic pathogens identified here and in other studies, the potential risks must be taken seriously. Given these safety concerns, the absence of any scientific data indicating beneficial health effects of raw diets, and nutritional deficiencies that have been reported with such diets, it is difficult to recommend their use at this point. Veterinarians should discuss the risks of raw diets to pet owners who have decided to feed these diets. As reports of real and potential problems with raw diets increase, liability issues could arise if veterinarians do not discuss the potential risks, particularly if they are recommending or selling raw diets. Factors that should be discussed include safe handling of raw diets, disinfection of food and water bowls, proper handling of feces, personal hygiene (particularly hand hygiene) following contact with animals, raw foods, and food or water bowls, or feces. Since many potential pathogens can grow in raw meat at room temperature (8), unconsumed raw food should be promptly discarded and not be allowed to sit in bowls. Pathogen survival in food and water bowls has not been adequately evaluated; however, in the absence of other information, daily disinfection, ideally with 10% bleach solution, should be considered to reduce the potential burden of bacterial vegetative cells and spores. Further, it should be recommended that raw diets not be fed in households with young children, elderly persons, or immunosuppressed individuals. CVJ

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