

Investigation of *Listeria*, *Salmonella*, and Toxigenic *Escherichia coli* in Various Pet Foods

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Abstract

The Veterinary Laboratory Investigation and Response Network (Vet-LIRN), in collaboration with the Food Emergency Response Network (FERN) and its Microbiology Cooperative Agreement Program (MCAP) laboratories, conducted a study to evaluate the prevalence of selected microbial organisms in various types of pet foods. The goal of this blinded study was to help the Center for Veterinary Medicine prioritize potential future pet food-testing efforts. The study also increased the FERN laboratories' screening capabilities for foodborne pathogens in animal feed matrices, since such pathogens may also be a significant health risk to consumers who come into contact with pet foods. Six U.S. Food and Drug Administration FERN MCAP laboratories analyzed approximately 1056 samples over 2 years. Laboratories tested for *Salmonella*, *Listeria*, *Escherichia coli* O157:H7 enterohemorrhagic *E. coli*, and Shiga toxin-producing strains of *E. coli* (STEC). Dry and semimoist dog and cat foods purchased from local stores were tested during Phase 1. Raw dog and cat foods, exotic animal feed, and jerky-type treats purchased through the Internet were tested in Phase 2. Of the 480 dry and semimoist samples, only 2 tested positive: 1 for *Salmonella* and 1 for *Listeria greyii*. However, of the 576 samples analyzed during Phase 2, 66 samples were positive for *Listeria* (32 of those were *Listeria monocytogenes*) and 15 samples positive for *Salmonella*. These pathogens were isolated from raw foods and jerky-type treats, not the exotic animal dry feeds. This study showed that raw pet foods may harbor food safety pathogens, such as *Listeria monocytogenes* and *Salmonella*. Consumers should handle these products carefully, being mindful of the potential risks to human and animal health.

Introduction

NUMEROUS FOODBORNE PATHOGENS cause illness in the United States, including *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli*, including Shiga toxin-producing strains of *E. coli* (STEC). In 2012, FoodNet of the Centers for Disease Control and Prevention (CDC) reported a total of 33 deaths attributed to *Salmonella*, 13 deaths attributed to *Listeria*, and 2 deaths attributed to *E. coli* STEC (CDC, 2013). Overall, *Salmonella* (28%) and *Listeria monocytogenes* (19%) were listed as two of the leading causes of death re-

lated to foodborne illness in the United States in 2011 (Scallan *et al.*, 2011). Almost all cases of human listeriosis are related to foodborne contamination (Jackson *et al.*, 2010). Shiga toxin-producing strains of *E. coli* O157:H7 have also caused numerous deaths following consumption of contaminated foods (Chahed *et al.*, 2007). Infections may, however, also be caused by exposures other than consumption of contaminated food. *Salmonella* infections in people have also occurred from handling contaminated pet foods and pet treats (Adley *et al.*, 2011; Clark *et al.*, 2001; Finley *et al.*, 2006; Freeman *et al.*, 2013). A recent study showed that antibiotic-

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resistant *E. coli* could easily spread between humans and their pets (Martins *et al.*, 2013). Due to the serious nature of *Salmonella*, *Listeria*, and *E. coli* O157:H7, enterohemorrhagic *E. coli* (EHEC), and STEC-related illnesses, the Center for Veterinary Medicine's (CVM) Veterinary Laboratory Investigation and Response Network (Vet-LIRN) began working with the Food Emergency Response Network (FERN) and their Microbiology Cooperative Agreement Program (MCAP) laboratories to evaluate the prevalence of these microbial organisms in various types of pet foods and treats. Two phases of the study were conducted, with the first testing basic dry and semimoist dog and cat foods. This was followed by testing more unusual pet food products including raw foods, exotic animal feeds, and jerky-type treats. The goals of this collaborative study were to (1) increase the capability of FERN laboratories to work with animal feed matrices, and (2) provide CVM with data regarding the occurrence of food safety pathogens in various animal feeds to prioritize potential future pet-food testing efforts.

Materials and Methods

Sample collection

Phase 1 was from October 1, 2010 through September 30, 2011. Six laboratories purchased 20 samples each quarter comprising 5 samples from each of the follow feed types: dry dog food, semimoist dog food, dry cat food, and semimoist cat food, totaling 80 samples per laboratory. Dry food included pellet- or kibble-type food typically packaged in bags for retail sale. Semimoist foods were typically packaged in pouches for retail sale and included pouched dog and cat foods and food treats shaped as bacon, fish, pork chops, and burgers. Canned and wet pet foods were not collected as part of this project. Overall, 480 samples were analyzed in Phase 1.

Phase 2 was from October 1, 2011 through July 30, 2012. Six laboratories each received 30 samples purchased from the Internet during 3 time periods. Three feed types were tested including raw dog and cat foods, exotic animal food, and jerky-type treats. Laboratories received 30 samples each time period comprising 10 samples from each of the 3 feed types, totaling 90 samples per laboratory. Raw foods were usually frozen and comprised ground meat- or sausage-type tubes of products made from animals such as rabbits and cows. It is unknown how long frozen samples had been frozen prior to shipping. Dry foods excluded cat and dog foods, but included hamster, gerbil, rabbit, amphibian, or bird food and pellets. Jerky-type treats included chicken jerky products, pig ears, and bully stick-type products. All samples were tested within 4 months of receipt. Overall, 576 samples were analyzed in Phase 2.

The study was not a regulatory surveillance program, and manufacture information was blinded.

Microorganism isolation and identification

Laboratories analyzed for the presence of food safety pathogens including *Salmonella*, *Listeria*, *E. coli* O157:H7 (EHEC), and STEC. Analysis for the presence of *Salmonella* species in all food types was performed following AOAC Official Method of Analysis 2004.03 (AOAC, 2007), VIDAS[®] (BioMerieux, Inc., Hazelwood, MO), in conjunction with the Bacteriological Analytical Manual (BAM), Chapter 5, Online version, December 2007 (Andrews, 2007), with

some modifications. Laboratories performed biochemical identification from only 6 typical triple sugar iron agar (TSI)/purity plates instead of the BAM-required 12, and laboratories performed testing only on suspect isolates through serological somatic O tests. CVM enforces the *Salmonella* zero-tolerance policy in pet food, and any culture that is positive is considered contaminated; therefore enumeration was not conducted.

Analysis for the presence of *Listeria* species in all food types was performed following AOAC Official Method of Analysis 999.06 (AOAC, 2005), VIDAS[®] LIS (BioMerieux, Durham, NC), in conjunction with the BAM, Chapter 10, Online version, January 2003 (Hitchins, 2003), with some modifications. Laboratories performed biochemical identification from suspect isolates to the genus and species level, as required by BAM; however, laboratories did not perform any subtyping of isolates or enumeration tests.

Analysis for the presence of *E. coli* O157:H7 was performed following the U.S. Department of Agriculture Microbiological Laboratory Guidebook Chapters 5A.01 and 5.04 (USDA-FSIS 2008a, 2008b) using the BAX[®] MP (DuPont Qualicon, Wilmington, DE) screening assay kit in conjunction with immunomagnetic separation (IMS) assays on positive enrichments. Based upon their capabilities, laboratories had the option of performing IMS using Pathatrix[®] (Life Technologies, Carlsbad, CA), Bead Retriever[®] (Invitrogen, Carlsbad, CA), or OctoMACS[®] (Miltenyi Biotec Inc., Auburn, CA). Suspect isolates were biochemically identified and subjected to latex agglutination assays to confirm both O157 and H7 antigens using the RIM[®] kit (Remel, Lenexa, KS). Additionally, positive isolates were tested for toxins using the Meridian Premier[®] EHEC kit (Meridian Diagnostics Inc., Cincinnati, OH). During Phase 2, three laboratories performed additional testing for non-O157 STECs in Phase 2 matrices using the BAM, Chapter 4a, Online Version, July 2009, for both *E. coli* O157:H7 and non-O157 STECs (Feng *et al.*, 2009). The screening assay performed was the BAM real-time polymerase chain reaction (qPCR), followed by the BAM confirmation algorithm to the point of biochemical identification and latex agglutination.

Results

During Phase 1, a total of 480 samples of dry and semimoist dog and cat foods were analyzed. Only one sample (dry cat food) was confirmed positive for *Salmonella*. One sample (dry cat food) was confirmed positive for *L. greyii*. No positives were confirmed for *L. monocytogenes* or *E. coli* O157:H7.

During Phase 2, 576 samples including raw dog and cat foods, exotic animal food, and jerky-type treats were analyzed. These products were chosen for the second phase to widen the scope of the investigation beyond the routine animal food types which were tested in the past and during Phase 1. A total of 15 samples (8%) were confirmed positive for *Salmonella*, all positives were found in raw food products. A total of 32 samples (16%) were confirmed positive for *L. monocytogenes*, again, all in raw food products. Additionally, 34 samples (14%) were confirmed positive for other *Listeria* species in raw food products and jerky-type treats. In Phase 2, no *E. coli* O157:H7 was isolated from any products, but a total of 10 samples were confirmed positive for non-O157 STECs. Table 1 provides results of samples analyzed during Phase 2.

TABLE 1. PHASE 2 RESULTS BY TRIMESTER AND FOOD TYPE

Commodity name	Trimester 1			Trimester 2			Trimester 3			Total analyzed
	Raw foods	Dry (exotic foods)	Jerky type treat	Raw foods	Dry (exotic foods)	Jerky type treat	Raw foods	Dry (exotic foods)	Jerky type treat	
No. analyzed	57	60	65	74	70	66	65	50	59	576
No. SLM positive	5	0	0	9	0	0	1	0	0	15
No. LIST mono positive	5	0	0	17	0	0	10	0	0	32
No. other LIST positive	8	0	0	19	0	1	6	0	0	34
No. EHEC positive	0	0	0	0	0	0	0	0	0	0
No. STEC positive (non-O157)	2	0	1	4	0	1	2	0	0	10

SLM, *Salmonella*; LIST, *Listeria*; EHEC, enterohemorrhagic *Escherichia coli*; STEC, Shiga toxin-producing strains of *Escherichia coli*.

Discussion

Overall, this project achieved both goals of increasing capability of laboratories to work with animal feed matrices and providing CVM with data regarding the occurrence of food safety pathogens in various animal feeds. The collaboration helped to enhance communication between networks and laboratories and increase the participating laboratory capability to handle human pathogenic bacteria in animal feed. Additionally, this study provided CVM with valuable information. This project was not a regulatory surveillance program, and no regulatory action occurred based on positive results; however, the results will be used to help CVM assess future testing needs and provide warnings to consumers.

This study showed that raw pet foods and jerky-type treats may harbor food safety pathogens, such as *L. monocytogenes*, *Salmonella*, and non-O157 STECs, and consumers should take appropriate precautions when handling these products. Numerous cases of human salmonellosis have been linked to contaminated dry dog and cat foods. From 2006 to 2008, an outbreak of *Salmonella Schwarzengrund*, which included 79 illnesses over 21 states, resulted in the recall of 105 brands of dry pet food and the permanent closure of the manufacturing plant (Behravesh *et al.*, 2010). In 2012, there was an outbreak of human *Salmonella enterica* serotype Infantis infections related to exposure to dry dog food (CDC, 2012; Imanishi *et al.*, 2014). With the dog and cat population in the United States estimated at 65 million and 78 million animals, respectively, according to the 2002 American Pet Product Manufacturers Association National Pet Survey (Finley *et al.*, 2006), a significant human population is exposed to pet food and treats. Surveys, between 2002 and 2009, for the presence of *Salmonella* in animal feeds, feed ingredients, pet foods, treats, and supplements showed that *Salmonella* prevalence decreased (especially in feed ingredients and pet foods and treats); however, outbreaks continue (Li *et al.*, 2012). Our study supports the conclusion that *Salmonella* prevalence in dry feeds has decreased, since we found only one *Salmonella* positive out of 480 dry and semimoist cat and dog food samples tested. Pet owners still need to take appropriate hygiene precautions, such as thorough hand washing, after handling pet food and treats.

In recent years, various groups have advocated feeding raw food diets to companion animals. This practice poses health risks to both animals and owners. A limited study, completed in Canada, showed that dogs fed a BARF (bones and raw

food) diet were more likely to shed *Salmonella* in their stool than dogs fed commercial diets (Joffe and Schlesinger, 2002). There is an increasing trend of feeding raw meat diets in the United States, and these raw diets pose a risk to pet owners due to an increased risk of bacterial contamination from handling these products (Freeman and Michel, 2001). The results from our investigation show that these raw pet food products can contain pathogenic bacteria. In 2011, a study in California evaluated raw horsemeat diets in zoo settings and screened for *Salmonella* and *E. coli*, but not *Listeria* (Singleton *et al.*, 2012). This study found one sample positive for *Salmonella* out of 54 samples that were screened using a sandwich ELISA test. The data show fewer positives than previous studies from zoos, which reported up to 60% positive findings for raw diets (Richter and al-Sheddy, 1990; Singleton *et al.*, 2012). The Singleton study tested a small number of samples (54) and did not use standard microbial culture methods, which may have resulted in underreporting of positives from the raw meat diet. Our study used standard culture methods, tested more samples, and screened for a wider range of bacteria, including *Listeria*. We found that the raw pet food products could be contaminated with either *Salmonella*, *Listeria monocytogenes*, or both pathogens.

Ours is the first report of *Listeria monocytogenes* contamination of commercial pet foods. There is one case report of an abortion in a dog consuming a raw food diet, although the source of the infection was not confirmed (LeJeune and Hancock, 2001). *L. monocytogenes* was isolated from dog and cat fecal samples in a study that looked at the occurrence of the pathogen in domestic and companion animals (Weber *et al.*, 1995). Thus, dogs and cats harbor, and sometimes can be adversely affected by, this pathogen. Due to the serious health consequences of *L. monocytogenes* infections, especially in pregnant women (Mylonakis *et al.*, 2002; Jackson *et al.*, 2010), it is important for veterinarians, public health experts, and consumers to be made aware of the potential presence of *L. monocytogenes* in raw pet foods. Owners who decide to feed these products should take strict precautions to avoid infection by thoroughly washing hands and disinfecting all surfaces and objects that come in contact with raw pet foods. Public health experts also need to consider the potential for exposure from raw pet foods when trying to determine the source of an infection. Finally, producers of these products should take steps to reduce the potential for contamination with food safety pathogens.

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