PALL

Food and Beverage

Easy and Effective Detection of *Salmonella spp.* and Pathogenic *E. coli* O157 in Pet Food

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Abstract

The use of the GeneDisc® System to detect pathogenic *E. coli* O157 and *Salmonella* spp. in twelve different pet food products was evaluated. Recall lists were consulted to identify brands that had been recently subject to product recalls due to potential pathogen contamination. The pet food evaluation included dry dog food, refrigerated dog food, frozen dog and cat food, dry cat food, pet treats such as jerky, and bird feeds. The evaluated dog and cat foods included ingredients such as chicken, turkey, salmon, fats and/or grains, in addition to various vitamins, supplements and preservatives. The evaluated bird feed included ingredients such as seeds, grains and/or vegetables in addition to various vitamins, supplements and preservatives.

Each pet food was processed by inoculating three samples with pathogenic *E. coli* O157, three samples with *Salmonella* spp. and one left non-inoculated as a control. During testing, it was discovered that the frozen pet food contained a wild type *Salmonella* spp. and *Proteus mirabilis* at the time of purchase. Wild type bacteria are unadulterated bacteria found in nature. All tested pet food products were determined to be compatible with the GeneDisc System for pathogen detection (pathogenic *E. coli* O157 and *Salmonella* spp.), including the frozen pet food with preexisting contamination. Both the laboratory test strain of *Salmonella* and the wild type strain were detected in the frozen food. The GeneDisc System exhibited its ability to detect lab strain and wild type *Salmonella* spp. in the presence of other targets (pathogenic *E. coli* O157) as well as non targets (wild type *P. mirabilis*) as seen with the frozen pet food samples.

Introduction

The Food Safety Modernization Act (FSMA) set forth by the Food and Drug Administration (FDA) requires pathogen detection and identification in pet food products to help ensure its safety. Pet food pathogens not only represent a threat to pets, but they also pose a potential threat to humans when handling pet food[1]. The FDA has zero tolerance for *Salmonella* spp. in pet food. In support of this safety goal, Pall Corporation has developed an effective method for detecting *Salmonella* spp. in dry dog food and has been granted AOAC PTM certification.

To expand upon the AOAC validation, a feasibility study was performed to assess the ability of the GeneDisc system to detect pathogenic *E. coli* O157 and *Salmonella* spp. in various pet food products. Pet food products are made from a variety of ingredients any of which could potentially interfere with bacterial detection and identification. This study was conducted to evaluate the use of the GeneDisc System's ability to detect *Salmonella* spp. and pathogenic *E. coli* O157 in various pet foods.

Recent recall lists were consulted to determine which pet food brands to test. Pet food brands not found in recent recalls were chosen as well brands found on the recall lists. These pet foods were chosen to broadly demonstrate their compatibility with the GeneDisc System. The pet food brands with recent recalls were also chosen to increase the probability of purchasing a pet food with contamination.

GeneDisc System

Test Methods and Materials

The twelve tested products consisted of:

- three dry dog foods
- one refrigerated dog food
- one frozen dog/cat food
- three dry cat foods
- two dog treats
- two bird feeds

All twelve products contained added vitamins and supplements. Eight of twelve products listed a meat as the first ingredient. Of these eight, four listed a grain as the second ingredient, three listed a vegetable and one listed vitamin E. Of the four food products that did not list meat as the first ingredient, two listed chicken meal followed by brown rice and the last two were bird feed which listed milo and millet as the primary ingredients.

The sample descriptions are found in Table 1.

Table 1: Sample Descriptions
Description of each sample including type of food and first two ingredients listed on product packaging.

Sample	Pet Food Type	Ingredient (First, Second)
Dry Dog Food 1	Dogs	Chicken, Corn Meal
Dry Dog Food 2	Dogs	Organic Chicken, Chicken Meal
Dry Dog Food 3	Dogs	Chicken, Chicken meal
Refrigerated	Dogs	Turkey, Eggs
Dog Treats 1 (Jerky)	Dogs	Chicken, Vitamin E
Dog Treats 2	Dogs	Wild caught salmon, Sweet potatoes
Frozen Pet Food I	Cats & Dogs	Turkey necks, Turkey organs (liver, gizzards, hearts)
Frozen Pet Food N	Cats & Dogs	Turkey necks, Turkey organs (liver, gizzards, hearts)
Dry Cat Food 1	Cats	Chicken meal, Brown rice
Dry Cat Food 2	Cats	Chicken, Chicken by-product meal
Dry Cat Food 3	Cats	Chicken meal, Brown rice
Bird Feed 1	For most wild birds	Milo, Cracked corn
Bird Feed 2	Birds	White Millet, Milo

Seven 375 gram samples were processed from each pet food product; three samples were inoculated with 5-10 CFU (colony forming units) of *Salmonella* spp., three were inoculated with 5-10 CFU of pathogenic *E. coli* O157, and one sample was left non-inoculated, as a negative control. Every sample was processed in three steps:

Step 1: Enrichment

- Each 375 g sample of pet food was added to 3375 mL of pre-warmed Buffered Peptone Water (BPW) in a blender bag, at 37 °C ± 1 °C.
- Each sample (except for the negative controls) was inoculated with 5-10 CFU of bacteria (either *E. coli* O157 or *Salmonella* spp.).
- All negative control samples were left non-inoculated.
- The contents of each blender bag were mixed by hand for 10 seconds.
- All the blender bags were incubated at 37 °C ± 1 °C for 16-18 hours.

Step 2: DNA Extraction

- One mL of each enriched sample was pipetted into a sterile 1.5 mL/2 mL micro centrifuge tube. This was performed in duplicate, leaving one tube available for repeatability testing.
- The samples were placed in a rack and left stationary for 2 minutes to allow settling of heavy debris.
- A 50 µL sample was taken from the top of the sample tube after settling and was transferred into the lysis tube for DNA extraction.
- The lysis tubes were incubated for 10 min @ 102 °C ± 2°C.

Step 3: Plate Loading and PCR Assay

GeneDisc STEC/*Salmonella* combo plates were loaded as per Pall Corporation's Standard Operating Procedure for PCR on the GeneDisc Cycler.

- Sample information was entered into the GeneDisc Cycler
- 36 uL of Master Mix was added to each sector of a GeneDisc Plate
- 36 uL of extracted samples was added to each sector
- Step by step instructions provided by the GeneDisc Cycler were followed
- GeneDisc Plate was loaded into the GeneDisc Cycler, PCR assay initiated
- Results were available within an hour and could be observed in real time

Repeatability

To ensure that results were repeatable, samples were chosen at random from the duplicate 1 mL enrichment samples, and a new extraction and PCR assay was performed on those samples. One *Salmonella* spp. sample and one *E. coli* O157 sample from each matrix were chosen as well as 3 negative controls. A total of twenty-seven samples were re-extracted and run on the GeneDisc Cycler for the repeatability study.

Results and Discussion

The results of the pet food bacterial detection tests using the GeneDisc System are found in Table 2. The presence of pathogenic *E. coli* O157 and *Salmonella* spp. was detected in each of the respective inoculated pet food samples, and no inhibition of the PCR assays was observed.

Table 2: Comparison of GeneDisc System results to inoculated samples
Comparison of the number of samples inoculated with pathogenic *E. coli* O157 or *Salmonella* spp to the number of samples where these bacteria were detected with the GeneDisc System. The GeneDisc System demonstrated 100% accuracy for the detection of pathogenic *E. coli* O157 and *Salmonella* spp in various pet foods. Identical results were obtained in a follow up study with 27 randomly selected pet food samples tested for repeatability.

Sample	Number of samples inoculated with *Salmonella* spp.	Number of samples inoculated with *Salmonella* spp. and detected with GeneDisc System	Percentage of samples inoculated with *Salmonella* spp. and detected with GeneDisc System	Number of samples inoculated with pathogenic *E. coli* 0157	Number of samples inoculated with pathogenic *E. coli* 0157 and detected with GeneDisc System	Percentage of samples inoculated with pathogenic *E. coli* 0157 and detected with GeneDisc System
Dry Dog Food 1	3	3	100	3	3	100
Dry Dog Food 2	3	3	100	3	3	100
Dry Dog Food 3	3	3	100	3	3	100
Refrigerated	3	3	100	3	3	100
Dog Treats 1 (Jerky)	3	3	100	3	3	100
Dog Treats 2	3	3	100	3	3	100
Frozen Pet Food I	3	3	100	3	3	100
Frozen Pet Food N	3	3	100	3	3	100
Dry Cat Food 1	3	3	100	3	3	100
Dry Cat Food 2	3	3	100	3	3	100
Dry Cat Food 3	3	3	100	3	3	100
Bird Feed 1	3	3	100	3	3	100
Bird Feed 2	3	3	100	3	3	100

The test design of targeting brands previously found on recall lists increased the probability of purchasing pet food with preexisting contamination. This was supported by the frozen pet food samples which were found to be positive for preexisting bacterial contamination. *Salmonella* spp. and other bacterial contaminants were detected in non-inoculated frozen food samples. The presence of preexisting bacterial contamination was confirmed both through repeat testing (Table 3) and with 16s RNA analysis of bacterial isolates by an independent external laboratory.

Table 3: Bacterial detection in uninoculated frozen food contaminated with preexisting bacteria.
Salmonella spp. was detected in the Frozen Pet Food contaminated with preexisting bacteria. All six samples were enriched without inoculation.

Non-Inoculated Frozen Pet Food	Bacterial Detection Results: presence / absence (+ / –)	
	Pathogenic *E.coli* 0157	*Salmonella* spp.
1A	–	+
1B	–	+
2A	–	+
2B	–	+
3A	–	+
3B	–	+

The preexisting *Salmonella* spp. was detected at consistent cycle threshold values (Ct) in the frozen pet food samples inoculated with pathogenic *E. coli* O157 and the non-inoculated frozen food samples (Table 4). If an organism is present in a sample, the cycle threshold is the point at which the replicated DNA is first detected by the GeneDisc System. The Ct value is indirectly related to the starting concentration of the target organism. A higher starting bacterial concentration will have an earlier (smaller) Ct value because it will take less time for enough DNA to replicate and be detected, whereas if the starting concentration is low, the Ct value will occur later (be larger) due to more replications required in order to reach a detectable level of DNA. Consistent Ct values indicate that the pre-enrichment concentration of the preexisting *Salmonella* spp. was equivalent between these samples, and that contamination did not transpire post enrichment nor post extraction. Additionally, the uniformity of the Ct values indicates that the GeneDisc System is able to detect multiple targets simultaneously due to the presence of multiple organisms in these samples.

Table 4: *Salmonella* spp. Cycle Threshold Comparison in inoculated and uninoculated frozen food contaminated with preexisting bacteria.
The samples which were not inoculated with *Salmonella* spp., but were inoculated with *E. coli* O157, show similar Ct values for *Salmonella* spp. due to the presence of a wild-type contaminant. The samples which were inoculated with a laboratory strain of *Salmonella* spp. show a smaller Ct value than the others, and this is attributed to the increased *Salmonella* spp. concentration due to the presence of both the laboratory strain and the wild type strain.

Inoculated with:	*Salmonella* spp. Ct Value
E. coli O157 (1)	32.2
E. coli O157 (2)	33.2
E. coli O157 (3)	33
Salmonella spp. (1)	26.8
Salmonella spp. (2)	26.7
Salmonella spp. (3)	26.6
Non-inoculated	32.7

The frozen pet food samples that were inoculated with *Salmonella* spp. showed smaller Ct values (indicating earlier detection) than those not inoculated with *Salmonella* spp. An earlier Ct value of these samples indicates a higher starting concentration than those samples not inoculated with *Salmonella* spp. This was confirmed with additional tests indicating that all of the frozen pet food samples contained preexisting *Salmonella* spp. contamination.

Proteus mirabilis was also present in the frozen pet food as a preexisting contaminant. *P. mirabilis* was identified by an independent external laboratory along with the *Salmonella* spp. contaminant. *P. mirabilis* is not a target on the GeneDisc System, and it does not cross react with any of the targets. This was demonstrated by extracting the DNA of a *P. mirabilis* single colony and testing it on the GeneDisc System which resulted in absence for *Salmonella* spp. and pathogenic *E. coli* O157.

Repeatability testing was completed on 27 selected samples: including one *Salmonella* spp. sample and one pathogenic *E. coli* O157 sample from each matrix at random as well as 3 negative controls. All repeatability testing yielded results identical to the original testing.

Conclusions

All twelve pet food products were compatible with the GeneDisc System for detecting *Salmonella* spp. and pathogenic *E. coli* O157 at a concentration of 5-10 CFU/375 g of different types of pet food product, pre-enrichment. This includes the frozen pet food with preexisting contamination in which the laboratory test strain of *Salmonella* and the wild type strain were detected. The absence of inhibition of the PCR assays is attributed to the compatibility of the pet food products with the GeneDisc System.

The GeneDisc System demonstrated its ability to detect multiple targets simultaneously as well as distinguish targets from non targets as observed in the frozen pet food samples. The GeneDisc System method for detecting *Salmonella* spp. in dry dog food, which was granted AOAC PTM certification, is also applicable to other pet food products for detection of *Salmonella* spp. and pathogenic *E. coli* O157.

References

(1) Food and Drug Administration; Food Safety Modernization Act, http://www.fda.gov/Food/FoodSafety/FSMA/ucm298665.html

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