Are high-end cat and dog food less likely to be mislabeled than low-end pet foods?

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Abstract

With a pet food market value of 4.5 billion CAD in 2021 and over 16 million cats and dogs collectively in households across the nation, pets are clearly central to the lives of many Canadians. With this much monetary value and the clear compassion people have for their pets, people have been rightfully outraged when multiple studies showed high percentages of mislabeling of pet foods. While many studies have confirmed high rates of pet food fraud, less is known about how rates of mislabeling vary with perceived food quality. Thus, using DNA isolation, polymerase chain reaction (PCR), and gel electrophoresis techniques, this study compares the rate of pet food mislabelling in high-end pet foods and low-end pet foods. Four groups were tested with five samples each: High-end dog food, low-end dog food, high-end cat food, and low-end cat food. We hypothesized that the rate of mislabelling would be significantly lower in high-end pet food in comparison to low-end pet foods due to the perceived higher quality of ingredients used in production. Results showed that mislabeling was pervasive in both high and low-end foods, with more than 60% of foods mislabeled in each group. From lowest to highest rates of mislabeling, results showed high-end dog food was the least mislabeled (60%), followed by low-end dog food (80%), followed by high and low-end cat foods, which had the same rate of mislabeling (100%).

Are high-end cat and dog food less likely to be mislabeled than low-end pet foods?

As the population of household pets in Canada grows, the pet food industry has increased in value year after year, amounting to a market value of over 4.5 billion CAD in 2022 (Globe Newswire, 2022; Canine Animal Health Institute, 2022). As a result of this increase, the offered range of pet food has also grown, aiming to stand out from the competition with more unique blends of meats. Correct labelling of the contents of these meat blends is paramount when considering pet health. Pets have the possibility of food-related sensitivities and allergies which can cause adverse food reactions such as ear infections, skin lesions, and upset bowels. In both cases of cats and dogs, meat products are often the source of these sensitivities (Ricci, 2009). Therefore, pet owners have grown increasingly concerned with further control over the dietary ramifications of the products that they purchase and the standards associated with higher quality and prices.

Research has indicated that despite the increased demand for high-quality foods, mislabelling of pet food is a widespread occurrence (Wall, 2022). For example, one study found pet foods contained undeclared protein in as many as 85% of samples, and another found that about 70% of samples were contaminated with endangered fish species such as the shortfin Mako (Cardenosa, 2019; Olivry et Mueller, 2018). In these cases, mislabelling was defined as either undeclared proteins being present in the form of meats that were not on the label, or the labelled protein being absent itself in the product (Olivry et Mueller, 2018; Ricci, 2018).

Concerning this high rate of mislabeling in pet foods, this observational study was conducted to determine if the perceived quality and price of pet foods influenced the frequency of mislabeling. Specifically, high-end dog and cat foods were compared to corresponding low-end foods to examine if the perceived quality of foods based on the price had any impact on

rates of mislabelling. The rate of mislabelling was predicted to be significantly lower in high-end pet food in comparison to low-end pet foods due to the perceived higher quality of ingredients and quality of control used in production.

Methods

Sampling

We purchased most of our samples from PetSmart and used the filters on their website to differentiate between high-end and low-end. So, under the food category, we chose "Value Brands" for our low-end food and "Premium Natural" for our high-end. Then the 20 food packages were brought to the lab. A small piece of the samples (about 0.5*0.5*0.5 cm) was transferred into labelled 1.5 ml tubes and mashed using autoclaved toothpicks. For dry pet food, an extra step was taken to maximize their reaction with lysis reagent. Hence, they were soaked in autoclaved water and incubated at 65 °C for 30 minutes and spun down in the centrifuge at high speed for 10 minutes before extraction.

DNA Isolation

A DNA isolation kit was used that contained all the required reagents. First, 300 ul of Cell Lysis Solution with Proteinase K was added to each tube and incubated at 65°C for 15 minutes (vortex every 5 min). Immediately after incubation, the samples were placed on ice for 5 min. Then, 150 ul protein precipitate reagent was added to the samples. The mixture was vortexed for 10 seconds and centrifuged at maximum speed for 10 minutes. The supernatant under the fat layer was transferred to a new 1.5 mL tube and mixed with 500 ul ice-cold isopropanol. The tubes were inverted 40 times and centrifuged at maximum speed for another 10 minutes.

After centrifugation, a quick assessment of the pellet color was done since many of the samples contained spice and other impurities. The isopropanol was poured off and replaced with 500 ul ethanol in each sample without disturbing the pellet. The ethanol was poured off and this step was repeated with another 500 ul of ethanol. Then the tubes were left on a piece of Kim wipe upside down with the cap open overnight.

The next day, each sample was then dehydrated with a 30 uL TE buffer.

PCR

To fragment the isolated DNA samples based on their meat content, we ran a PCR reaction with seven primers, one forward and six reverse primers that were each specific to one type of meat (goat, chicken, cattle, sheep, pig, and horse). To reduce the risk of mislabeling and errors, a master mix was prepared using the recipe stated in Table 1 in the appendix

After the master mix was aliquoted to each tube, 1 ul of each sample was added (dH2O for the negative control) regardless of their DNA concentration. After all the samples were ready, the PCR instrument was set up to the following setting: initial denaturation at 95°C for 2 min, then 35 cycles of denaturation at 95°C for 0.5 min, annealing at 60°C for 0.5 min, and extension 72°C for 0.5 min; lastly, the final extension occurred at 72°C for 5 min.

Gel electrophoresis

To each sample, 4 ul of 6X dye was added and mixed with the pipette. Any bubbles formed from mixing the sample with the dye were shaken down manually and set aside to rest for five minutes.

In the meantime, 6 ul of ladder solution was pipetted into every fifth cell of the 4% NiSeieve GTG gel. Then, 8 ul of each sample was pipetted into individual gel cells. A diagram of their arrangement in the gel was made to track each sample’s location.

Each DNA fragment (DNA barcode) from all the samples was run on the 4% gel for 30 min at 100V in the TAE buffer. The gel was examined under a UV light, then run at 180V in the TAE buffer for an additional 30 minutes to further develop the bands. The gel was examined for a final time under the UV light and cover, and photos of the resulting bands were taken on an iPhone 12 for further analysis (shown in figure 1).

Results

The experiment has a total of twenty samples with five samples in each group: high-end dog (HD) food, low-end dog (LD) food, high-end cat (HC) food, and low-end cat (LC) food. Observed data is gathered from the gel electrophoresis results and shown in table 1 with categories high-end, low-end, mislabeled, and not mislabeled (mislabeled means either an undeclared meat present or the labelled protein being absent in the product). In the gel electrophoresis, samples HC 5, LC 1 and LC 2 didn't show any bands. Thus no conclusion should be drawn about whether they are mislabeled or not, and these three samples will not be considered as a part of the result. The analysis results are summarized in Table 4 in the appendix. However, HD1 has a unique situation. It was observed to have lamb, pig and horse meat which are red meat as labelled but since horse meat is not listed in the ingredients on the food package, this sample was considered mislabeled.

Table 1

Observed data from analyzing the agarose gel results shown in Figure 1

	Mislabeled	Not mislabeled	Total
High-end	7	2	9
Low-end	7	1	8
Total	14	3	17

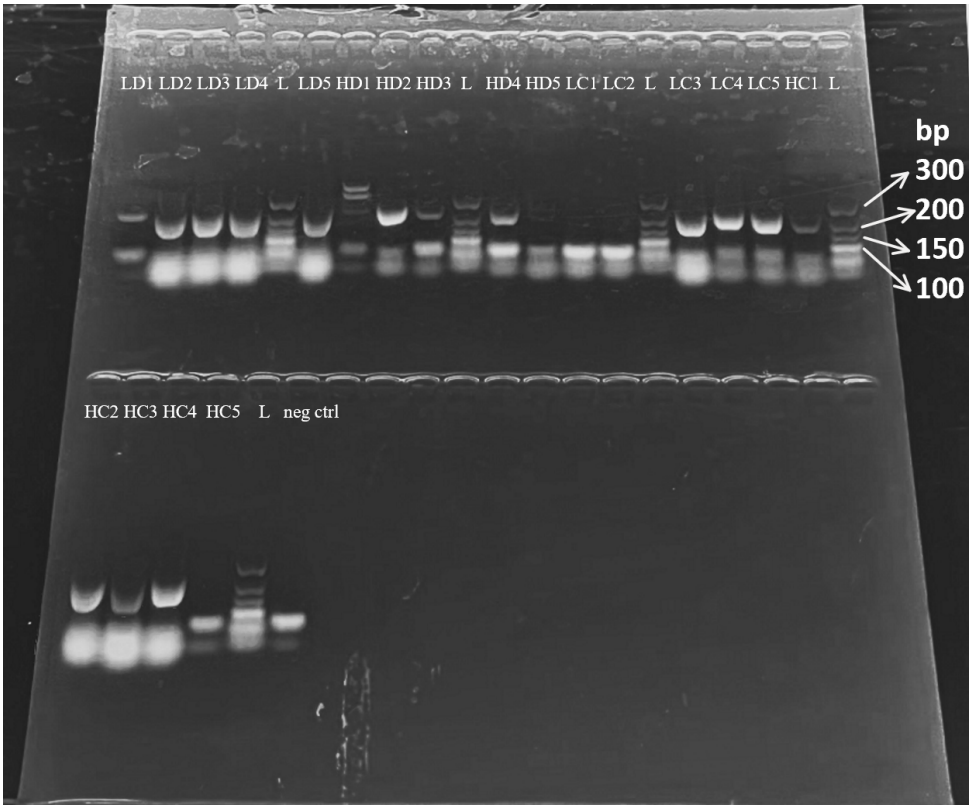
Table 2

Mislabeled rate of each 4 categories based on the observed data in table 4 in the appendix.

	Mislabeled rate
High-end dog	60 %
High-end cat	100 %
Low-end dog	80 %
Low-end cat	100 %
Total	82.35 %

Figure 1

4% agarose gel containing all the PCR samples reacted with specific primers for 6 meat kinds: goat, chicken, cattle, sheep, pig and horse with an expected DNA fragment size of 157, 227, 274, 331, 398 and 439 bp (Matsunaga et al., 1999). Note that the abbreviations stated on the image for the order of loading, L, H, D, and C, stand for low-end, high-end, dog, and cat respectively. In addition, the sample numbers are each associated with one food brand shown in table 4 with an analysis of each band on the gel.



Discussion

In this observational study, groups of high and low-end dog and cat foods underwent DNA extraction, PCR, and gel electrophoresis. Our specific PCR primers for goat, chicken, cattle, sheep, pig and horse DNA allowed us to determine if any of this meat were present in the samples. The objective was to determine the frequency of undeclared DNA in all groups to examine any differences across perceived food quality. Five samples of each group: high-end dog food, low-end dog food, high-end cat food and low-end cat food, were prepared for a total of 20 samples. Of all samples, 17 produced gel bands indicating successful PCR and gel separation, while three samples were not successful. Of the successful samples, the majority (82.35%) were found to be mislabelled, meaning the DNA identified did not match the product label or unlisted DNA was identified in addition to the DNA indicated on the label. This is consistent with existing literature reviews that find high rates of mislabeling in a wide range of pet foods, including diets marketed as 'elimination diets' for diagnosing and treating pet food sensitivity (Olivry et Mueller, 2018; Ricci et al., 2018) . In other words, pet owners trying to identify pet food allergies may be paying for expensive elimination diet foods without getting accurate results, as the meat type on the label frequently does not match the DNA actually present.

From lowest to highest rates of mislabeling, our results showed high-end dog food was the least mislabeled (60%), followed by low-end dog food (80%), followed by high and low-end cat foods, which had equal rates of mislabeling (100%). These results show that while some dog foods were correctly labelled, all cat food samples regardless of perceived quality were mislabeled. While minimal literature compares the frequency of mislabeled foods between pet species, the reasoning for this may be that cat foods are predominantly protein-based, while dog foods are primarily carbohydrate-based, and contain less protein overall (Lewis, n.d; MeowMix,

n.d). In other words, there is more meat needed in the production of cat food than there is dog food, creating a larger window for contamination to occur. Comparatively, dog foods require less protein and thus may have a smaller window for contamination.

While both high-end and low-end dog foods showed lower rates of mislabelling compared to cat foods, it is important to note that the majority of both groups were mislabelled. As such, despite the difference seen in dog food quality supporting our initial prediction that high-end foods are less frequently mislabeled, the overall results suggest that for both cat and dog foods, the perceived quality of the food has minimal influence on the rate of mislabelling. Further studies should be done to determine if the difference in mislabelling between high-end and low-end dog foods (20%) is significant.

Looking at the types of proteins that most often contaminated pet foods, our data shows that goat meat was the most common contaminant, found in 71% of mislabeled samples. This is inconsistent with existing literature that commonly finds chicken as the main contaminant in cat and dog foods (Okuma & Hellberg, 2015). Explanations for the high rate of goat DNA contamination remain speculative, but because much of the meat used in pet foods come from livestock deemed unsafe for human consumption, it is possible that the meat supply is volatile, depending on the types of livestock most discarded at the time (Doyle, 2022; Hyde, 2021; Budd, 2022). Continued investigation regarding the origin of the pet food proteins and the product life cycle of these meats is essential to understand where and why high rates of mislabelling are occurring.

Overwhelmingly, our data show that both high and low-end pet foods are mislabelled, suggesting that the perceived quality of foods does not make a significant difference in the

likelihood of food contamination. In other words, mislabelling occurs at high rates regardless of the quality of pet food, which is not what we predicted at the outset of this study.

Further studies should be done to examine a larger sample size of pet foods, including both high and low-end foods, and should focus on foods marketed as 'elimination diets' for pets, as the mislabelling of these products may pose higher health risks to pets in the long term. Keep in mind that PCR is not quantitative, only detecting the presence of DNA or lack of.

Limitations to Consider

Potential limitations of this study are that we were unable to carry out a Chi-square test, which would have predicted the significance of the relationships seen in the data. A sample size of 50 is required for a Chi-square test, which is more than our sample size of 17. While a statistical analysis would benefit in supporting our discussion of the data, our results are well supported by existing literature and lead us to a confident, non-ambiguous conclusion on the influence of perceived pet food quality on food contamination. That being said, future studies should increase the sample size to 50 when possible to allow for further analysis of results.

As mentioned, three of the samples did not yield any bands in the gel electrophoresis, indicating that the DNA extraction was unsuccessful or the PCR was unsuccessful. This could be due to experimenter error in sample collection, handling samples, adding primers incorrectly, or mis-loading samples into gel wells.

Specifically, a possible source of error to consider in our sample collection is that three of the foods (HC 4; HD 1,2) were originally dried, in contrast to the remaining samples which were wet. To use the dried samples, an extra step was taken to extract DNA: We mashed the dry food into pieces and soaked them with deionized water before incubating the samples for 30 minutes to allow the dried food to soften. We then centrifuged the dry samples to remove excess water

before continuing with DNA extraction. While all of the dry samples were successful in PCR and gel electrophoresis, this extra step should be taken into consideration in future studies.

Looking at possible sources of error in the gel electrophoresis, the gel was run twice: first for 30 minutes at 100V and then again at 180V for 30 minutes. This was done because the initial run did not move the bands far enough. The second run produced the resulting bands, but it was very fast, and as a result gave the bands a more smeared appearance, which made the bands more difficult to correctly label. Future studies should consider running the gel at an intermediate voltage for a longer period of time to optimize band readability.

Conclusion

Pet food mislabeling is not a scandal of the past. This study found that while both the dog food groups observed contained a portion of foods that were found to contain the expected protein, the majority of each group was mislabeled. The high-end dog food group had a lower rate of mislabeling than the low-end dog food, which agrees with our initial hypothesis. However, both cat food groups were 100% mislabeled, meaning no difference in rates of mislabeling between high and low-end foods was seen. Considering the value placed on the health of household pets, further action should be taken to regulate pet food ingredients. Future research could take larger samples from a wider region to gain a better understanding of the pet food mislabeled on a national scale.

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Appendix

Table 3

PCR master mix preparation for the 20 samples and one negative control.

Component	Initial Concentration	Final Concentration
PCR buffer	10X	1X
dNTPs	10 mM	0.2 mM
MgCl2	25 mM	1.5 mM
5’ Primer Meat forward (SIM)	10 µM	0.4 µM
3’ Primer Goat “G”	10 µM	0.08 µM
3’ Primer Chicken “C”	10 µM	1.2 µM
3’ Primer Cattle “B”	10 µM	0.24 µM
3’ Primer Sheep “S”	10 µM	1.2 µM
3’ Primer Pig “P”	10 µM	0.24 µM
3’ Primer Horse “H”	10 µM	0.8 µM
Taq polymerase	1000U/200 µl	0.1 U/µl
Glycerol	50 %	10 %
dH2O	3.6 ul	N/A
Sample DNA or Sterile dH2O	1.0 ul	N/A
Final Volume	25 ul	N/A

Tabel 4

Indicates brand and their sample Ids.

Sample ID	Brand	Moisture	Observed Bands
HD 1	Acana - Red Meat Recipe	Dry	lamb, pig, horse
HD 2	Royal Canine Senior Large Dog - Chicken	Dry	chicken
HD 3	Simply Nourish - Chicken	Wet	chicken
HD 4	Blue Wilderness - Beef		chicken
HD 5	Lil' Plates - Lamb	Wet	chicken

LD 1	Blue delight - Turkey	wet	Chicken
LD 2	Cesar - Pork	wet	goat
LD 3	Cesar - Beef	wet	goat
LD 4	Cesar - Lamb	wet	goat
LD 5	Caesar - Chicken	wet	goat
HC 1	Premium Cat Food - White Fish & Salmon	dry	goat
HC 2	Instinct - Chicken	wet	goat
HC 3	Weruva - Chicken	wet	goat
HC 4	Temptations- Chicken	dry	goat
HC 5	Weruva - Tuna & Lamb	wet	none
LC 1	Fancy feast - Chicken & Beef	wet	none
LC 2	Fancy feast - Chicken	wet	none
LC 3	IAMS - Chicken	wet	goat
LC 4	IAMS - Chicken & Beef	wet	chicken
LC 5	IAMS - Turkey	wet	goat