Sanger sequencing-based DNA barcoding: Detection of Commercial Dog Food Mislabeling

Author: *NI JIAMING (Jiangsu liangfeng high school international college), Shi Yanxuan(Ulink high school of SIP), He Zixuan (Soochow University High School Sino-Canada Program), Wu Zhifan（Kangchiao international school）*
Mentor: *Xinyue Wang*

Abstract

Studies consistently report mislabeling in commercial dog foods. This study aims to find out whether there are mismatched ingredients by extracting and comparing animal cytochrome c oxidase I (COI) DNA through sanger sequencing-based DNA barcoding technique. The comparison of authentic fresh raw meat (chicken, beef) purchased from local retailers with commercial dog snacks (four single-protein types: duck, chicken, rabbit rib, beef; and two mixed-protein products) enables clear and accurate detection of mislabeling issue. We isolated DNA from all samples using silica-based extraction, amplified the cytochrome c oxidase I (COI) gene via PCR, and obtained DNA barcodes using Sanger sequencing. Comparing the BLAST(Nucleotide Basic Local Alignment Search Tool) databases, barcodes from fresh raw meat with the barcodes of dog foods enabled mislabeling detection. Consequently, at least one of the species listed in the ingredients was not detected in three dog food samples, while three products showed supplementary species addition to those stated on the labels.

Introduction

The recent rise in pet ownership is attributed to the pandemic as well as an increase in human–pet bonding, and both have driven growth in the pet food industry. However, the concerns of the authenticity is intensifying. Mislabeling of dog food—where ingredients listed on the label do not match the actual contents—is a well-documented and ongoing issue. DNA barcoding has emerged as a powerful molecular tool for species identification, utilizing short, standardized genetic markers to authenticate biological materials. This technique holds particular significance in the pet food industry, where it serves as a critical safeguard against fraudulent labeling practices. Recent studies have revealed alarming rates of misrepresentation in commercial pet products, with approximately 30% containing undeclared animal components. A common form of adulteration involves substituting high-value animal species with lower-value alternatives. Such adulteration not only constitutes economic fraud but may also introduce potential health risks when inferior or allergenic ingredients are substituted for premium components.

Materials & Methods

Fresh raw chicken and beef were obtained from Chinese retailers. Commercial dog snacks (four single-protein types: duck, chicken, rabbit rib, beef; two mixed-protein products) were purchased from specialized pet stores in China, selecting prevalent market products. We store dog food in the refrigerator for freshness. DNA was extracted from all samples using silica-based isolation which is a high-accuracy DNA extraction approach. PCR amplification employed a vertebrate (non-fish) primer cocktail. CO1 primer mix contains Taq polymerase, CO1 primer, and ddH2O. Add 2 microliters DNA to 23 microliters primer mix and mix them together for the PCR reaction. Thermal cycling conditions were: Denaturation: 94°C for 15 s. Annealing: 54°C for 15 s. Extension: 72°C for 30 s (35 cycles). Amplification success was verified by 2% agarose gel electrophoresis (Fig 1), and DNA sequences were determined via Sanger sequencing by professional institution : Suzhou Genewiz Biotechnology Co., Ltd. (Sales. Genomics. CN@Azenta. com GENEWIZ).

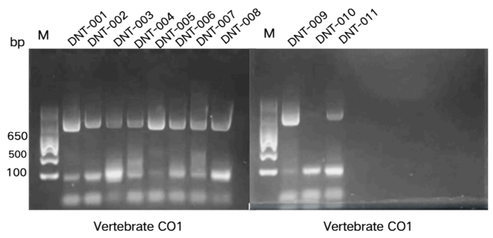

Fig.1 Gel electrophoresis analysis of DNA extracted from commercial dog food and raw fresh meat samples using silica DNA isolation methods.

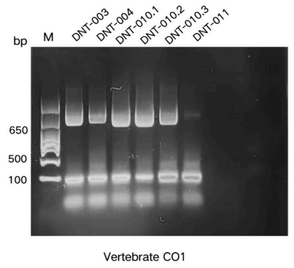

Fig.2 Re-analysis of gel electrophoresis presented clear and accurate bands. It has been confirmed that all the DNA samples were successfully extracted.

Table 1: Species composition testing results of commercial dog food reference samples.

Reference Sample	Composition		actual results	whether mislabeled	*notes
	Species	Ratio(%)			
DNT-003	chicken	100	Gallus gallus		
DNT-004	rabbit	100	Oryctolagus cuniculus		
DNT-005	chicken	52.3	Gallus gallus&Anser canagicus	mislabeled	*contain Anser canagicus (a type of goose) without labeling
	duck	13.2			
	quail	6			
DNT-006	duck	100	Anas platyrhynchos		
DNT-007	chicken	83.5	Gallus gallus&Sepiella maindroni&Anser canagicus	mislabeled	* contain Sepiella maindroni (a type of inkfish which without labeling) and Anser canagicus (a type of goose) without labeling
	krill	5.5			
	oyster	1.5			
	flying fish	1			
DNT-008	duck	100	Anas platyrhynchos		
DNT-009	duck	100	Anas platyrhynchos		
DNT-010	duck	100	Anas platyrhynchos		
DNT-011	beef	100	Gallus gallus&Anser canagicus	mislabeled	* only contain Gallus gallus (a type of chicken) and Anser canagicus (a type of goose) but without cattle species

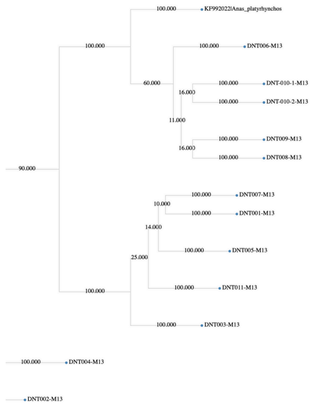

Fig.3 The phylogenetic tree of all the samples, with the reference goat-capara_hircus from database and Anas-platyrhynchos from genbank.

Results

The sequencing results revealed that most samples passed the quality control checks, although some required troubleshooting due to suboptimal sequencing quality. Specifically, samples DNT-003-M13F and DNT-003-M13R showed reduced read lengths, with the reverse primer exhibiting signs of insufficient priming. Similarly, DNT-007-M13R yielded a short read with priming issues, while DNT-007-M13F produced a readable but lower-quality sequence. For DNT-010, reverse primer displays Poor Quality. In contrast, the majority of samples, including DNT-001, DNT-002, DNT-004, DNT-005, DNT-006, DNT-008, DNT-009, DNT-011 and wild-type controls, produced high-quality sequences with read lengths, confirming successful amplification and sequencing.The DNA sequencing result of the samples in the DNA subway reflects that most of the dog food have matched ingredients, because there is a highly close DNA sequencing between them and the DNA extracted from the corresponding theoretical ingredients.

Discussion

Our study demonstrates that PCR-based DNA analysis is an effective tool for detecting meat ingredients in commercial dog food. This technology not only provides a reliable method for verifying pet food quality but also offers consumers a means to ensure the health and safety of their pets by identifying potential mislabeling or adulteration. However, several technical challenges emerged during the experiment. . Additionally, the first-generation sequencing method might have some limitations. For example, it relies on fluorescent labeling, and requires a large number of electrophoresis steps, so the cost of a single sample is relatively high. Furthermore, the sequence needs to be read one by one through gel electrophoresis, and the speed will be relatively slow.
Notably, our analysis revealed discrepancies between the labeled ingredients and the actual composition of certain products. The present of Sepiella maindroni (a type of inkfish) and Anser canagicus (goose) in the barcodes result of DNT-007 shows discrapencies with their lists of ingredients. DNT-011, labeled as beef, contained only goose and chicken DNA, raising concerns about either sample mislabeling during our processing or potential inaccuracies in the manufacturer's claims. These findings underscore the need for stricter regulatory oversight and more transparent labeling practices in the pet food industry. Moving forward, we aim to refine our methodology to quantify the relative proportions of different meat species in dog food. Future studies will focus on optimizing DNA extraction protocols for low-abundance targets and developing quantitative assays to provide more detailed compositional analysis. Such advancements could further enhance the reliability of this approach for ensuring product authenticity and safeguarding animal health.

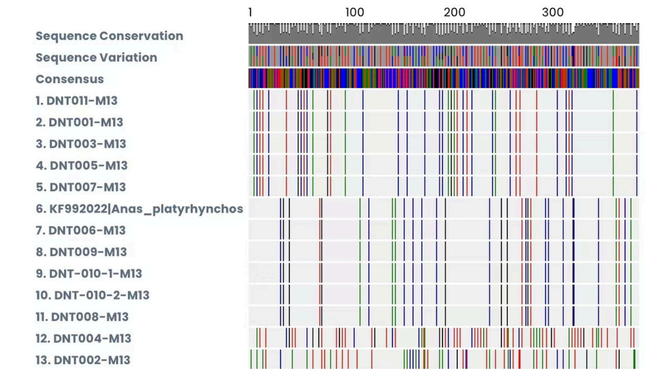

Fig.4 The muscle result. More similar DNA sequence reflect common species.

Acknowledgements

Thanks to our project mentor Xinyue Wang and the supportive institution: Cold Spring Harbor Asia DNA learning center.

References

Palumbo, F., Scariolo, F., Vannozzi, A., & Barcaccia, G. (2020). NGS-based barcoding with mini-COI gene target is useful for pet food market surveys aimed at mislabelling detectio Scientific Reports, 10. https://doi.org/10.1038/s41598-020-74918-9.
Will, K., Mishler, B., & Wheeler, Q. (2005). The perils of DNA barcoding and the need for integrative taxonomy.. Systematic biology, 54 5, 844-51 . https://doi.org/10.1080/10635150500354878.
Palumbo, F., Scariolo, F., Vannozzi, A., & Barcaccia, G. (2020). NGS-based barcoding with mini-COI gene target is useful for pet food market surveys aimed at mislabelling detectio Scientific Reports, 10. https://doi.org/10.1038/s41598-020-74918-9.
Crossley, B. M., Bai, J., Glaser, A., Maes, R., Porter, E.,