FULL PAPER *Public Health*

# Examination of Meat Components in Commercial Dog and Cat Feed by Using Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLPs) Technique

Hsien-Chi WANG[1)], Shu-Hwae LEE[2)], Tien-Jye CHANG[1)] and Min-Liang WONG[1)]*

[1)]*Department of Veterinary Medicine, College of Veterinary Medicine, National Chung-Hsing University, Taichung 402, and* [2)]*National Veterinary Research Institute, Council of Agriculture, 376 Chung-Cheng Rd. Tansui 251, Taiwan*

(Received 4 December 2003/Accepted 12 March 2004)

ABSTRACT. It has been shown that certain slow neurological diseases such as bovine spongiform encephalopathy (also known as "mad cow" disease) could be transmitted through contaminated food intake by animals; therefore, the examination of meat components in commercial feeds is important for the control of the disease in public health. The combination of polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs) technique was applied to examine the meat components in dog and cat commercial feeds. The partial nucleotide sequence (359 bp) of animal mitochondrial cytochrome *b* (cyt*b*, CYT) gene was amplified by PCR and then digested with restriction enzyme *Alu* I or *Mbo* I. In this work, eight brands of commercial dog and cat feeds available in Taiwan were examined. All brands of dog feeds that were tested contained meat from four different animals (cattle, pig, goat and chicken). In cat feeds, the chicken meat was found in five out of eight brands.

KEY WORDS: BSE, cyt *b* gene, dog and cat commercial diet, PCR, polymorphism.

*J. Vet. Med. Sci.* 66(7): 855–859, 2004

Transmissible diseases caused by abnormal prions can affect different species of animals by eating contaminated food [6]. The use of simple and sensitive techniques to examine food components can avoid the spread of some slow infectious diseases, such as scrapie, bovine spongiform encephalopathy (BSE) and feline spongiform encephalopathy (FSE) [6]. Several mitochondrial DNA genes, such as conserved sequence blocks (CSBs), 16S ribosomal RNA gene and cytochrome *b* (cyt*b*) gene, have been used to study animal evolution [3–5]. The cyt*b* gene is among the most extensively sequenced genes to date among vertebrates. By PCR, the cyt*b* gene was amplified and sequenced to compare the intra-species and inter-species differences of animals including amphibians, birds, fishes, and mammals [4, 5].

In this study, we used the PCR-RFLP technique to differentiate meat components among cattle, goat, pig, and chicken. The 359 bp fragments of cyt*b* gene were generated and distinct digestion patterns of these DNA fragment were observed after *Alu* I or *Mbo* I treatment. We investigated the components of commercial dog and cat feed in Taiwan. The results demonstrated that all tested dog feed was mixed food and the component in cat feed was relatively consistent with that on the label.

## MATERIALS AND METHODS

*Samples and DNA isolation*: All feeds for test were purchased from local supermarkets in Taichung, Taiwan and listed in Tables 1 and 2. DNA was isolated with a QIAamp® DNA mini kit (Qiagen, Valencia, CA, U.S.A.) and we followed the manual provided by the manufacturer to isolate DNA.

*PCR primer design and amplification of mitochondrial cytb gene*: The oligonucleotides for PCR were synthesized by Mission Biotech (Taipei, Taiwan). The partial sequence corresponding to the human mitochondrial DNA cyt*b* genes (14816–15714 nt) was amplified by forward primer CYTb 1 (5'-CCATCCAACATCTCAGCATGATGAAA-3') and reverse primer CYTb 2 (5'-GCCCCTCAGAAT-

Table 1. The canine commercial feed used in this work

| Canine | Brand | Component labeled |
|---|---|---|
| 1 | A | goat |
| 2 | B | chicken |
| 3 | C | beef |
| 4 | D | goat |
| 5 | E | chicken |
| 6 | F | beef |
| 7 | G | beef |
| 8 | H | goat |

Table 2. The feline commercial feed used in this work

| Feline | Brand | Component labeled |
|---|---|---|
| 1 | a | chicken |
| 2 | b | tuna, chicken |
| 3 | c | tuna, chicken |
| 4 | d | tuna, chicken |
| 5 | e | tuna |
| 6 | f | tuna, chicken |
| 7 | g | chicken |
| 8 | h | chicken |

* CORRESPONDENCE TO: WONG, M.-L., Department of Veterinary Medicine, College of Veterinary Medicine, National Chung-Hsing University, Taichung 402, Taiwan.

*Corresponding Author

Reprinted from J. Vet. Med. Sci. 66(7)855-859. 2004

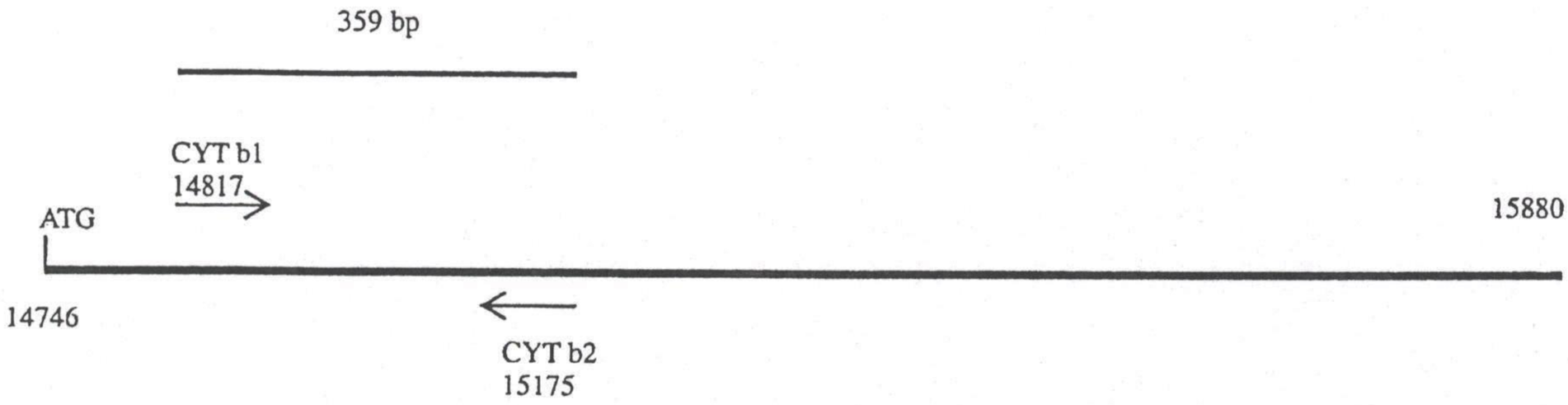


Fig. 1. Schematic representation of human cytochrome b gene from GenBank (accession no. AY275537). The primers used for PCR were CYTb 1 and CYTb 2 and the expected amplified product was 359 bp in size.

GATATTTGTCCTCA-3') [5]. The coding region of cyt*b* gene is 1135 bp [GenBank accession number AY275537], and the locations of our primers and the expected product are depicted in Fig. 1. Amplification of meat DNA for cyt*b* was carried out in a 50-$\mu l$ volume containing 1X reaction buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, TaKaRa), 20 $\mu$M each of dATP, dTTP, dCTP, and dGTP (TaKaRa), 2.5 units Taq enzyme (TaKaRa), 200 pM of each primer and 5 $\mu l$ DNA as template. After denaturation at 94°C for 5 min, the reaction was cycled 35 times at 94°C for 1s, 55°C for 1 min, 72°C for 1 min, and then final extension at 72°C for 7 min. The amplification was performed by a Gene Amp 2400 PCR system (Perkin Elmer, Wellesley, MA, U.S.A.).

*Restriction endonuclease digestion and DNA analysis by agarose gel electrophoresis*: PCR products were digested by restriction endonuclease *Alu* I or *Mbo* I. Two units of each enzyme were added to 7 $\mu l$ of PCR product in a final volume of 20 $\mu l$ digestion mixture at 37°C for 3 hr. The digested products were analyzed in 2% agarose gel in 1X TAE buffer (0.04 M Tris-acetate and 0.001 M EDTA). The sizes of products were estimated by comparison with a Bio-100 DNA Ladder (PROtech Technology Enterprise Co., Ltd., Taipei, Taiwan).

## RESULTS

To confirm the PCR-RFLPs technique that can amplify and differentiate DNA among four kinds of meat, we extracted DNA from cattle, pig, goat, and chicken meat. By use of the cyt*b* primers described above, DNA was subjected to PCR and yielded a 359 bp fragment. The PCR products were digested with *Alu* I or *Mbo* I. There is only one *Alu* I restriction enzyme site in beef and pork DNA; there is no *Alu* I cleavage site in goat or chicken DNA. To *Mbo* I restriction enzyme digestion, there is one cut site in pig and goat DNA, and no cleavage site in cattle or chicken DNA (Table 3). Using the PCR-RFLP obtained with *Alu* I and *Mbo* I, we were able to differentiate DNA among four kinds of meat. Although the two fragments digested by *Alu* I treatment of beef cyt*b* DNA could not be separated clearly in 2% agarose gel, the fragments (244 and 115 bp) digested by the *Alu* I treatment of pig DNA were well resolved (Fig. 2). Hence cattle DNA could be distinguished from pig DNA after *Alu* I digestion.

Table 3. Expected sizes of PCR-RFLPs for different choices of meat

| Meat species | *Alu* I cut in bp | *Mbo* I cut in bp |
|---|---|---|
| cattle | 190<br>169 | 359 |
| pig | 244<br>115 | 244<br>115 |
| goat | 359 | 213<br>115 |
| chicken | 359 | 359 |

In this study, we tested eight kinds of dog and cat feeds (Tables 1 and 2). Although all the canine feed labeled with only one kind of meat, our result of PCR-RFLP showed DNA fragments of at least three kinds of mixed meats of four kinds of animals (Fig. 3). Therefore, the misleading labels may be harmful to some food allergy dogs, because the owner may feed the allergic components not described in the nutritional directions. In the feline feed, five brands of feed were found to contain chicken meat (Fig. 4A and 4B). Because the primers CYTb 1 and CYTb 2 were not appropriate to amplify the n gene of fish, the negative results of the examination of chicken meat (samples 4, 5 and 6 in Table 2) suggested that perhaps only tuna meat was included in these samples.

## DISCUSSION

Identification of the meat components of feeds could avoid the disease developing and spreading, for example, food allergy and bovine spongiform encephalopathy. The mammalian and avian cyt*b* genes are highly similar, and phylogenetic relationships were used for investigating taxonomy [3–5]. There are several techniques of analysis of food component, including mid-infrared spectroscopy, enzyme-linked immunosorbent assay, and capillary electrophoresis. These techniques are expensive, time consuming, and/or of low specificity [1, 2, 8, 9]. Usually, DNA samples

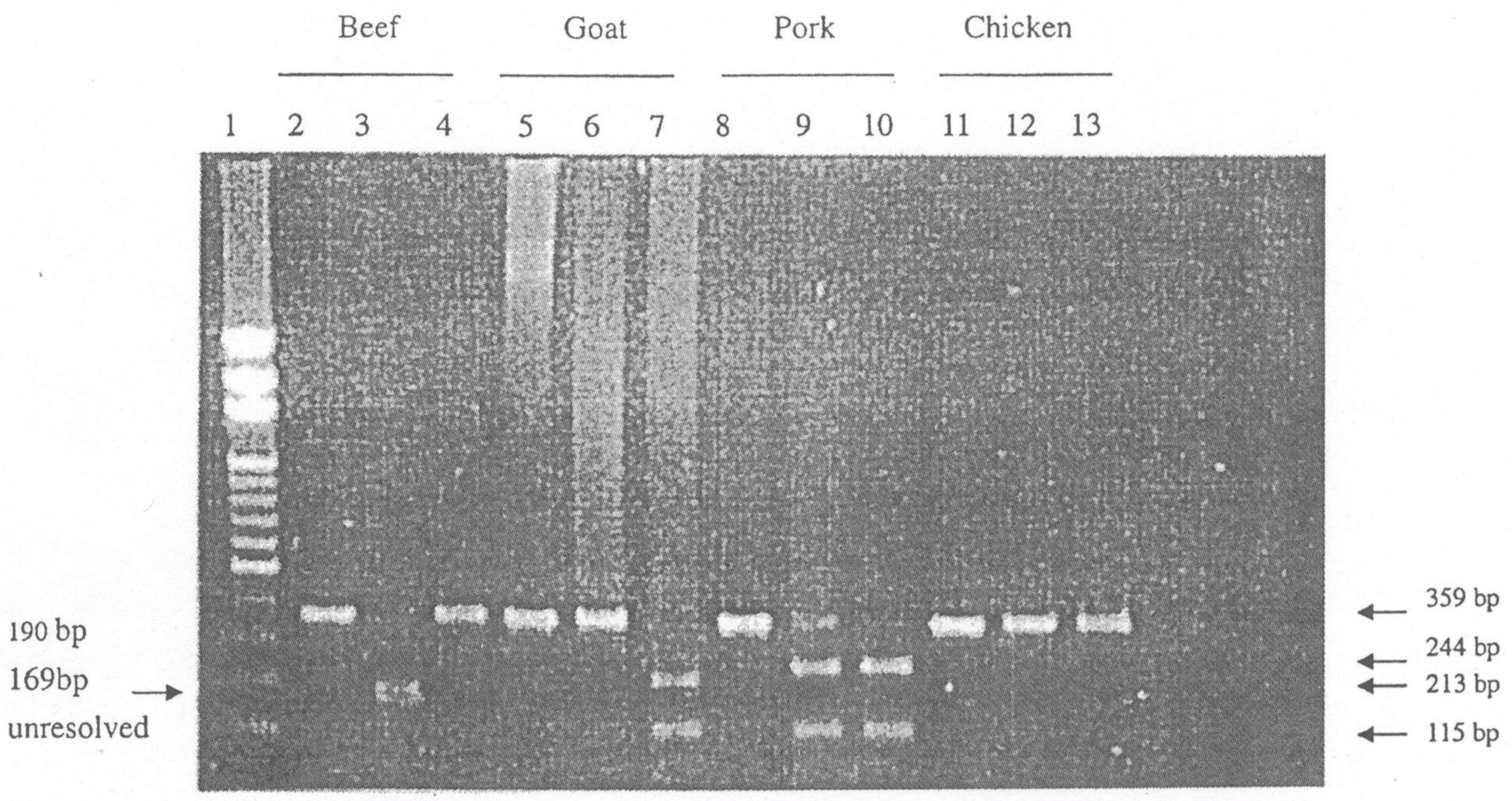


Fig. 2. Restriction enzyme patterns of cyt*b* PCR fragment with *Alu* I and *Mbo* I digestion. Lane 1: DNA markers; lanes 2–4: beef; lanes 5–7: goat; lanes 8–10: pork; lanes 11–13: chicken. Lanes 3, 6, 9, 12: PCR products digested by *Alu* I. Lanes 4, 7, 10, 13: the PCR products digested by *Mbo* I. Arrowheads on the right indicate the PCR products (359 bp) and digested bands (115 bp, 213 bp, 244 bp). Arrowhead on the left indicate the unresolved bands (169 bp and 190 bp) for beef. The DNA fragments (244 and 115 bp) obtained by the *Alu* I digestion of pork DNA were well separated.

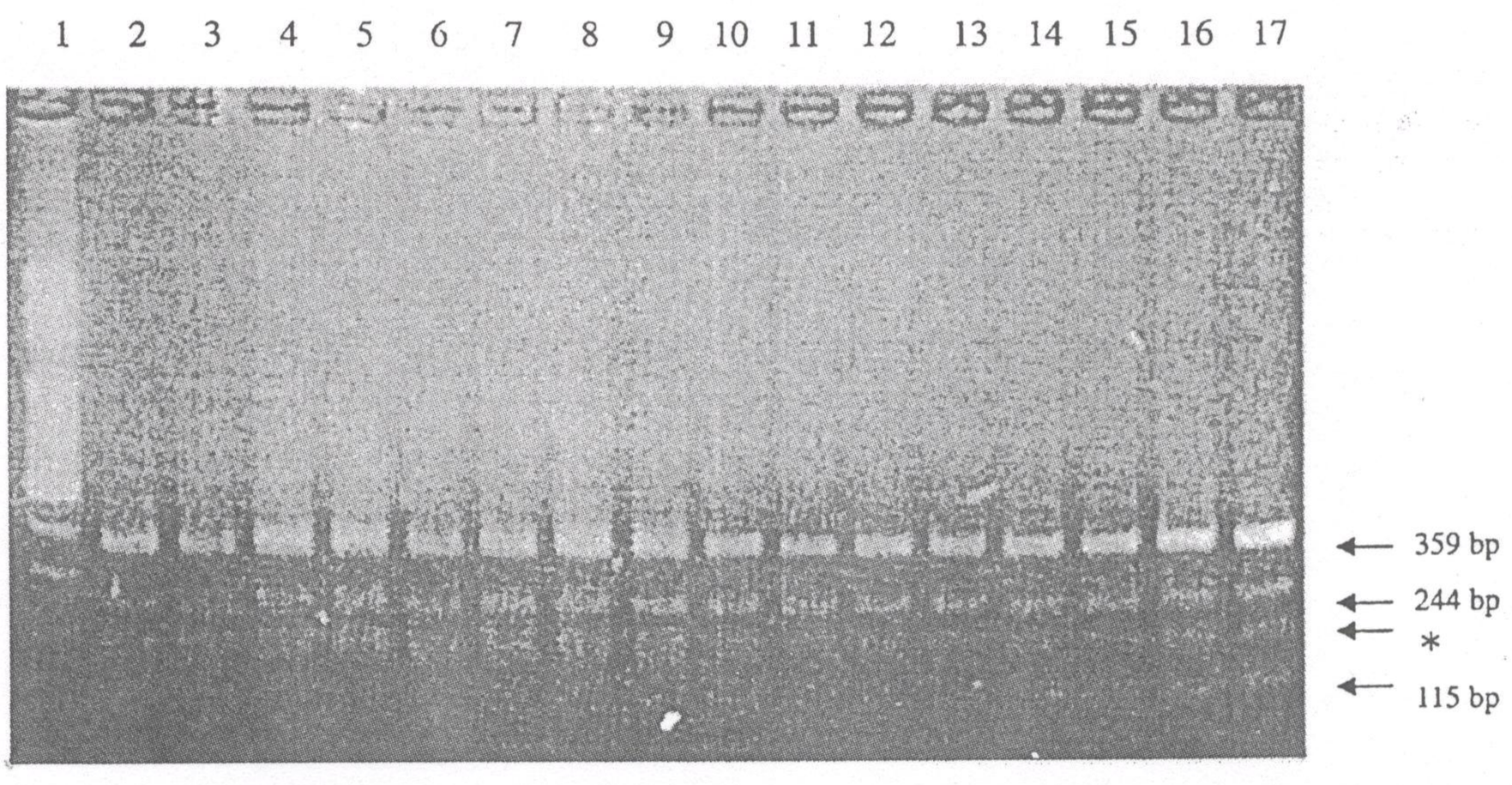


Fig. 3. Restriction enzyme profiles of cyt*b* PCR fragment in eight brands of canine commercial foods. The order of sample loading was the same as Table 1. Lanes 2–9: PCR products digested by *Alu* I. Lanes 10–17: PCR products digested by *Mbo* I. Arrowheads indicate bands which were essentially the same as those in Fig. 1. *: Lanes 2–9: unresolved 190 bp and 169 bp; lanes 10–17: 213 bp. The enzyme digestion pattern revealed DNA fragments of four kinds of mixed meats.

are more stable than proteins during thermal treatment. Application of PCR-RFLP to food analysis has shown to be useful because this technique is accurate, simple and moderate cost [5]. DNA polymorphism is useful for identifying the source of meat, because we can easily isolate DNA and conduct PCR with milk, blood, bone and meat [3]. Our primary goal was to differentiate the source meat among cattle, pig, goat, and chicken. To distinguish the four kinds of

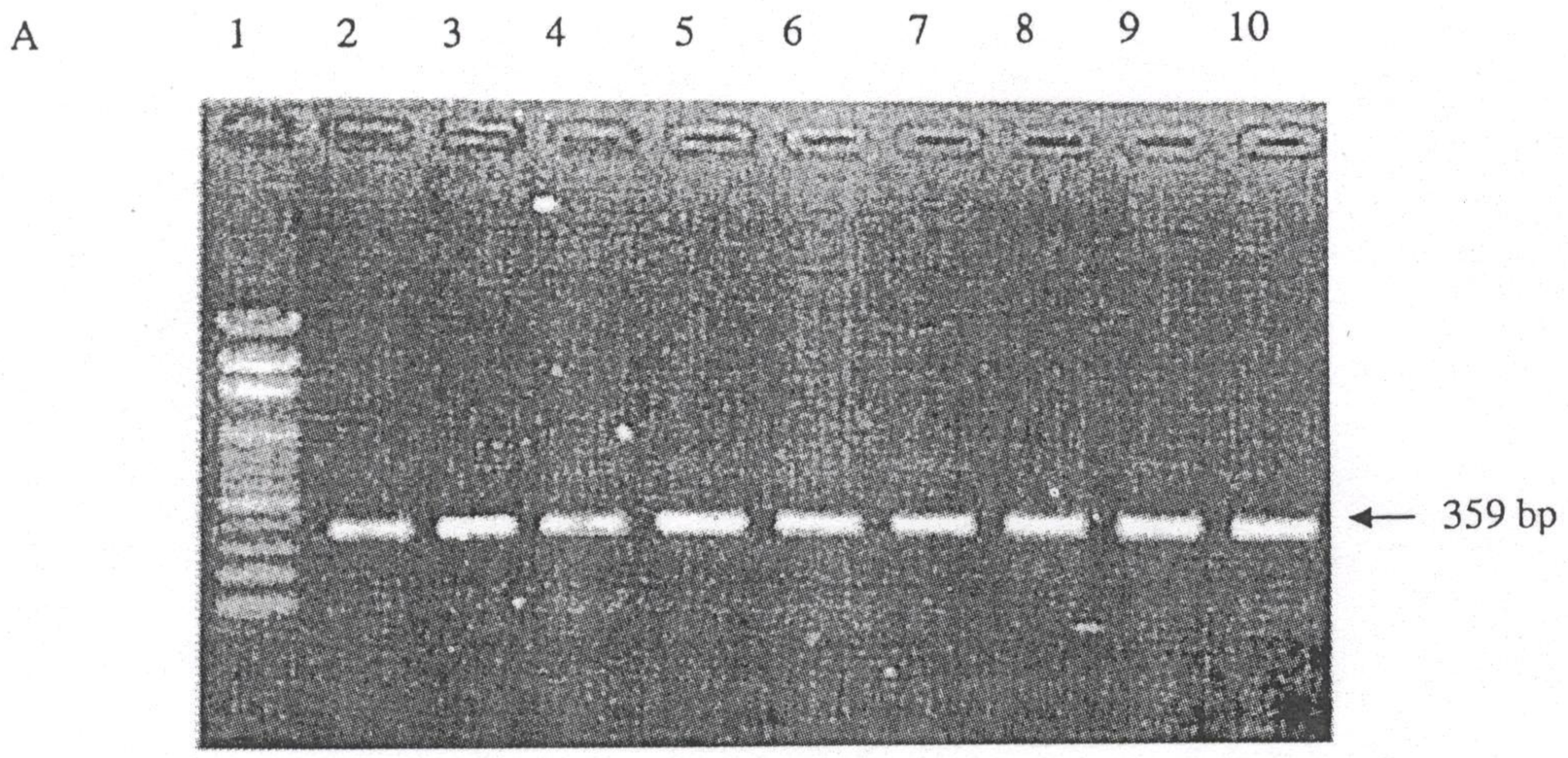


Fig. 4. Profiles of restriction enzyme cleavage of cyt*b* PCR fragment (arrowhead) in eight kinds of feline commercial foods. The order of sample loading was in accordance with Table 3. Lanes 1 and 11: DNA markers. Lanes 2, 5, 8 in Fig 3A and lanes 12, 15, 18, 21, 24 in Fig 3B were the cyt*b* PCR product. Lanes 3, 6, 9 in Fig. 3A and lanes 13, 16, 19, 22, 25 in Fig 3B were restriction profiles of cyt*b* PCR fragment obtained by *Alu* I digestion. Lanes 4, 7, 10 in Fig. 3A and lanes 14, 18, 20, 23, 26 in Fig. 3B were restriction profiles of cyt*b* PCR fragment obtained with *Mbo* I. The result showed that five brands of feline feed contained chicken meat.

meat, we amplified part of cyt*b* gene that is a highly conserved region [3, 4]. By combination of the *Alu* I and *Mbo* I restriction fragment length polymorphism, we can easily distinguish four kinds of meat (Table 3). In regular agarose gel, 190 bp and 169 bp DNA of beef could not be separated clearly after *Alu* I digestion (Fig. 2). Alternatively, we can also count the bands on the gel to differentiate beef from pork (one band for beef at the position of 150–200 bp and two bands for pork at 115 and 244 bp). The results of PCR-RFLP by *Mbo* I digestion could distinguish goat and chicken DNA. We could not detect the tuna component by using primers CYTb 1 or CYTb 2; this probably was due to the low base pairing of our primers to the tuna DNA sequences (84.6% between CYTb1 and tuna DNA, and 58.3% between CYTb 2 and tuna DNA). In summary, the results of this study were reliable and provide a method for rapid analysis of dog and cat feed.

ACKNOWLEDGMENT. This work was financially supported by a grant from the Council of Agriculture, Taiwan.

REFERENCES

1. Al-Jowder, O., Kemsley, E. K. and Wilson, R.H. 2002. Detection of adulteration in cooked meat products by mid-infrared spectroscopy. *J. Agric. Food. Chem.* **50**: 1325–1329.
2. Asensio, L., Gonzalez, I., Rodriguez M. A., Mayoral, B., Lopez-Calleja, I., Hernandez, P. E., Garcia, T. and Martin, R. 2003. Identification of grouper (*Epinephelus guaza*), wreck fish (*Polyprion americanus*), and Nile perch (*Lates niloticus*) fillets by polyclonal antibody-based enzyme-linked immunosorbent assay. *J. Agric. Food. Chem.* **51**: 1169–1172.
3. Desjardins, P. and Morais, R. 1990. Sequence and gene organi-

zation of the chicken mitochondrial genome. A novel gene order in higher vertebrates. *J. Mol. Biol.* **212**: 599–634.

4. Irwin, D. M. , Kocher, T. D. and Wilson, A. C. 1991. Evolution of the cytochrome *b* gene of mammals. *J. Mol. Evol.* **32**: 128–144.
5. Meyer, R., Hofelein, C., Luthy, J. and Candrian, U. 1995. Polymerase chain reaction-restriction fragment length polymorphism analysis: a simple method for species identification in food. *J. AOAC. Internat.* **78**: 1542–1551.
6. Prusiner, S. B., Telling, G. and Cohen, F.E. 1996. DeArmond SJ. Prion diseases of humans and animals. *Semin. Virol.* **7**: 159–173.
7. Skarpeid, H.J., Kvaal, K. and Hildrum, K.I. 1998. Identification of animal species in ground meat mixtures by multivariate analysis of isoelectric focusing protein profiles. *Electrophoresis* **19**: 3103–3109.
8. Vallejo-Cordoba, B. and Cota-Rivas, M. 1998. Meat species identification by linear discriminant analysis of capillary electrophoresis protein profiles. *J. Capillary. Electrophor.* **5**: 171–175.
9. Yamada, S., Nolte, H. and Zychlinsky, E. 1999. Identification and characterization of allergens in two species of tuna fish. *Ann. Allergy. Asthma. Immunol.* **82**: 395–400.

# 應用聚合酶連鎖反應－限制酵素切割圖譜(PCR-RFLPs)檢測市面販售犬、貓飼料中肉品種類來源

王咸祺[1]、李淑慧[2]、張天傑[1]、王孟亮[1]

1.國立中興大學獸醫學系

2.行政院農業委員會家畜衛生試驗所

**摘要** 本實驗目的爲利用聚合酶連鎖反應－限製酵素圖譜（PCR-RFLPs）檢測犬貓飼料中肉品種類來源。由於牛海綿狀腦病（俗稱狂牛症）之類的神經性疾病可能經由被污染的食物所引起，因此檢測動物飼料中肉品來源，對於獸醫公共衛生而言是極爲重要的。本實驗的結果利用聚合酶連鎖反應技術將動物細胞內粒線體的部分細胞素（cytochrome）b基因之片段增幅，之後再以限制酵素Alu 1及Mbo 1切割這些PCR產物，進而達到區分不同種類動物肉品來源之目的。本實驗共檢測八種市面上所販售的犬飼料以及貓飼料，結果顯示所有市售之犬飼料皆含有四種動物來源之肉類（牛、羊、豬、雞）；而在市售貓飼料部分所檢查之結果，有八分之五的品牌中有雞肉來源的成分。

---

本文轉載自 J. Vet. Med. Sci, 66(7): 855～859.2004