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Identification of Dog Meat Species by Polymerase Chain Reaction (PCR)

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ABSTRACT: Recently, consumption of animal protein, especially meat consumption in Indonesian society continues to have a tren a significant increase from year to year. As a country with Muslim majority, the provision of food of animal origin that are safe, healthy, whole and halal is a challenge that must be met in order to meet the demand for animal protein. The existence of a food safety issue, namely the phenomenon of adulteration of meat consumption is a priority to be anticipated. Meat adulterations have been reported mainly in processed meat products such as beef meatballs mixed with pork or chicken meat. Nevertheless, meat adulteration by adding dog meat into the beef products, is feared will happen too. This is because dog meat is also widely consumed by the public. The purpose of this study was to detect the DNA of dog meat and dog meat content in the beef meatballs with simplex polymerase chain reaction technique (PCR). The study was conducted using sample of fresh and cooked dog meat to determine DNA of dog species and determined dog meat content in beef meat balls using variation level of mixture at 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%, respectively. Furthermore, determination of species of dog meat is done by PCR. The results indicated that dog meat species were accurately determined in PCR. It is concluded that PCR can be useful for fast, easy, and reliable control of adulterated consumer meat products. These findings may contribute to a better awareness about food safety and concern of halal food.

Keywords: dog meat, adulteration, meat ball, PCR

INTRODUCTION

The level of consumption of dog meat in Indonesia tends to increase today. The consumption of dog meat is present in several regions in Indonesia, including Yogyakarta, Solo, Jakarta, Bandung, Bali, Medan, Manado and others (Prabowo, 2014). Dog meat consumption tends to increase is not in accordance with the rules of food of animal origin that are safe, healthy, whole, and halal (ASUH). Food safety of animal origin is food that does not contain ingredients that may interfere with or endanger human health. Food of animal origin is a healthy food derived from healthy animals and cut, or dealt with in ways determined. Food of animal origin is a whole food that does not deviate or reduced by a substance. Food of animal origin is a halal food derived from animals were handled in accordance with Islamic law. Dog meat, including meat that is forbidden in Islamic law is therefore is necessary to get control. Increased consumption of dog meat is high enough feared will lead to adulteration of the consumption of halal meat to dog meat. Adulteration of food of animal origin with the dog meat is quite profitable since stray dogs in some countries performed at a cheap price (Rahman *et al.*, 2014). Facts on society shows that lately people have noticed the authenticity of meat and processed meat products must halal for consumption (Nakyinsige, 2012). In Indonesia, the government has set rules regarding halal food contained in the Decree of the Ministry of Religion of the Republic of Indonesia Number 578 of 2001, " Halal food is food that does not contain elements or illicit material that is forbidden to be

consumed by Muslims and processing does not conflict with Islamic law". It is also listed in the Act no. 18 in 2009, in the Part of the Veterinary Public Health, Article 56. jo. Act no. 41 in 2014, "Veterinary Public Health is an organization of animal health in the form: b). Guarantee the safety, health, wholeness, and halal animal products".

One way of monitoring that can be done is by conducting laboratory tests on meat and food products of animal origin were falsified. Test against counterfeiting of food products of animal origin using Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) has been widely reported. The PCR technique were considered in this study to reveal a method of rapid detection, high sensitivity, inexpensive, and accurate of dog meat adulteration in Indonesia.

MATERIAL AND METHODS

Meat samples. Meat samples were in the form of fresh dog meat and processed dog meat sold in stalls and meatballs. Meat ball samples were made from mixture between beef and dog meat with some differences in concentration of 5 to 10%. The mixtures of meat were prepared in a total weight of 100 g. Meat samples were stored at –20 ± 1 °C until analyzed.

Genomic DNA extraction. Genomic DNA was extracted from each meat samples by using Genomic DNA mini kit (Geneaid, USA) and used for PCR analysis. The amount of 30 mg of flesh were cut into pieces and put in a 1.5 ml microcentrifuge tube. Meat samples were crushed using a micro pastel and 200 µl GT buffer were added for homogenized. The amount of 20 µl proteinase K (10 mg/ml) was added for cell lysis. Then DNA genomic extracted according to kit instructions. The purified DNA was eluted in elution buffer provided with kit and stored at –20 °C, and the extracted DNA was checked by Nanodrop Spectrophotometer.

Purity and concentration of DNA. DNA concentration was calculated at a wavelength of 260 nm, protein absorbance at a wavelength of 280 nm and purity of DNA was calculated by comparing the OD 260 and OD 280. The concentration of DNA (ug/ml) = A 260 x 50 x dilution factor (Sambrook and Russel, 2001).

Primers. Primers PCR primers for the amplification of dog meat were designed as described by Martin *et al.* (2007), forward primers were AATTGAATCGGGCCATGAA and reverse primers were CTCCTCTTGTGTTTTAGTTAAGTTAATCTG.

Polymerase Chain Reaction (PCR). The 25-µl reaction mixture was prepared in an Eppendorf tube containing 2 µl sample DNA, 1.25 µl forward primer, 1.25 µl reverse primer, 8 µl ddH2O and 12.5 µl Kappa. The thermocycler was programmed for 40-cycle PCR. The PCR program could be seen in the Table 1.

Table 1. PCR Program for analyze DNA dog meat

Step	Temperature (°C)	Time	Cycle (X)
Predenaturation	93	2 minutes	1
Denaturation	93	30 second	40
Annealing	50	30 second	40
Extension	72	15 second	40
Last extension	72	3 minutes	1

Electrophoresis was run on agarose gel (2%) at 50 V, 1 hour. The resulting gel was stained with FluoroSafe DNA Stain and DNA loading dye (Geneaid), visualized using a UV transilluminator, and photographed with digital camera.

RESULTS AND DISCUSSION

Results of calculation the purity and concentration of DNA meat samples shown in Table 3.

Table 3. Calculation of DNA purity and concentration

No.	Samples	OD 260	OD 280	Purity of DNA (µg/ml)	Concentration of DNA
1.	Fresh beef	0.1508	0.1915	2.262	0.787
2.	Bovine meat balls	0.2340	0.2642	3.510	0.886
3.	Fresh dog meat	0.1790	0.2155	2.685	0.830
4.	Processed dog meat	0.1733	0.2095	2.599	0.827
5.	Dog meat ball 5%	0.1519	0.1918	2.278	0.792
6.	Dog meat ball 10%	0.1559	0.1960	2.338	0.795
7.	Dog meat ball 20%	0.1461	0.1864	2.191	0.784
8.	Dog meat ball 30%	0.1504	0.1904	2.256	0.790
9.	Dog meat ball 40%	0.1478	0.1886	2.217	0.784
10.	Dog meat ball 50%	0.1403	0.1816	2.104	0.773
11.	Dog meat ball 60%	0.1471	0.1871	2.206	0.786
12.	Dog meat ball 70%	0.1561	0.1968	2.341	0.793
13.	Dog meat ball 80%	0.1434	0.1838	2.151	0.780
14.	Dog meat ball 90%	0.1425	0.1829	2.137	0.779
15.	Dog meat ball 100%	0.1447	0.1852	2.170	0.781

There were differences variation in the concentration of DNA that could be caused by physical treatment given as well as the ability to break down the cell extraction buffer (Mulyani et al., 2011). The purity of DNA obtained does not indicate the value range of 1.8 to 2. The purity of DNA that are less than 1.8 indicate contamination of protein and or phenol while DNA purity values greater than 2 indicates contamination of Ribo Nucleic Acid (RNA) (Clark and Christopher, 2000). Thenawijaya (1995) stated that the purity of DNA is affected by the presence of fat, proteins, polysaccharides and organic materials.

Furthermore, the results showed the DNA isolation band intensities varying obtained on the results of electrophoresis using a 2% agarose gel (Figure 1). Concentration and purity of DNA does not necessarily indicate a band with a thickness of high intensity.

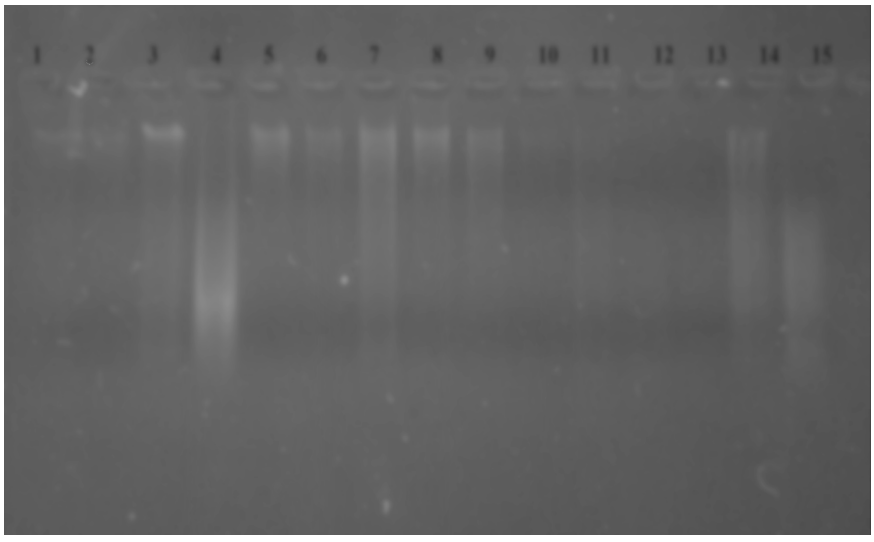

Figure 1. Agarose gel analysis from (1) fresh beef, (2) bovine meat balls, (3) fresh meat, (4) processed dog meat, (5) dog meat ball 5%, (6) dog meat ball 10%, (7) dog meat ball 20%, (8) dog meat ball 30%, (9) dog meat ball 40%, (10) dog meat ball 50%, (11) dog meat ball 60%, (12) dog meat ball 70%, (13) dog meat ball 80%,(14) dog meat ball 90%, (15) dog meat ball 100%

The quality of the isolated DNA can be influenced by the heating process and physical treatments on dogs and processed meat meatball dog. It is also influenced by the addition of spices or other ingredients such as flour (Andree, et al., 2004, Fibriana, 2001).

Figure 2 showed the result of PCR product electrophoresis of dog meats. In this study, primer used for PCR was 12S ribosomal RNA gene. The result of PCR product electrophoresis dog meats were specific using 12S ribosomal RNA gene residing on the size of 101 bp. (Martin, 2006). In this figure, fresh dog meat (no 3), processed dog meat (no. 4) and dog meatballs with various concentrations (no. 5-15) showed a fluorescent band and parallel to the size of 100 bp. Fresh beef (no 1) and bovine meat ball (no 2) were used as negative controls and showed no fluorescent band.

Ali *et al.* (2014) detected until 0.1% dog meat in beef sausage using PCR technique. Besides, Ilhak and Arslan, 2007 by using PCR technique also could detect the mixture of dog meat 0.1% in beef, goat and lamb. Matsunaga *et al.* (1999) also reported the PCR poducts of the various processed meat, such as beef, goat, lamb, chicken, horse and pork with temperature 100 oC or 120 oC, 120 min. Their results revealed that PCR was the method of choice for identifying meat species in muscle foods.

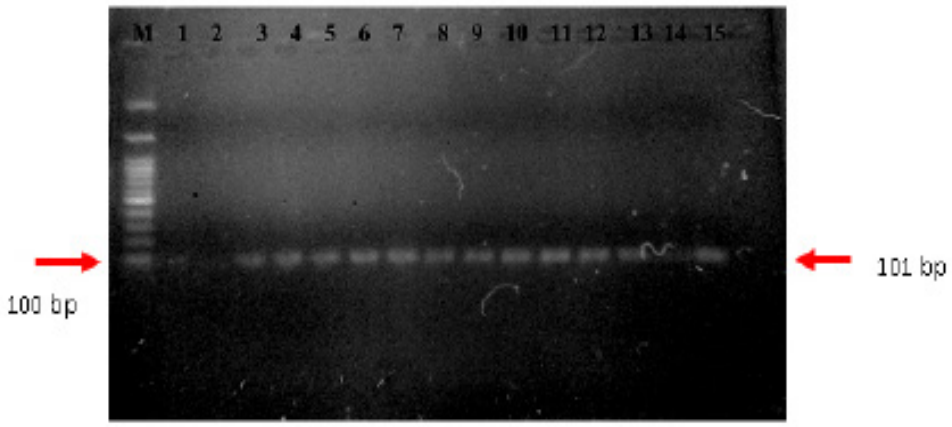

Figure 2. Agarose gel analysis of PCR products. (M) marker DNA ladder 100 bp,
(1) fresh beef, (2) bovine meat balls, (3) fresh meat, (4) processed dog meat, (5) dog meat ball 5%, (6) dog meat ball 10%, (7) dog meat ball 20%, (8) dog meat ball 30%, (9) dog meat ball 40%, (10) dog meat ball 50%, (11) dog meat ball 60%, (12) dog meat ball 70%, (13) dog meat ball 80%, (14) dog meat ball 90%, (15) dog meat ball 100%

CONCLUSION

The PCR techniques can be used to determine the DNA of fresh dog meat and processed so that halal food security can be realized. The PCR technique can be useful for fast, easy, and reliable control of adulterated consumer meat products. These findings may contribute to a better awareness about food safety and concern of halal food.

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