UDC 637

DETECTING BEEF MEATBALL CONTAMINATION WITH POLYMERASE CHAIN REACTION

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ABSTRACT
The objective of the study was to describe how much rat and swine primer developed from cytochrome b could detect rat and pork in processed beef products sold in North Maluku. The settings of the study were the traditional markets and supermarkets in several cities in North Maluku such as Ternate, Tidore Kepulauan, West Halmahera, North Halmahera, Central Halmahera, South Halmahera, East Halmahera, Sula Island and Morotai Island. The data collection lasted between May and June, 2015. The samples were analyzed in the Biotechnology Lab of Unkhair in July, 2015. To detect rat and swine DNA, the researchers used the PCR (Polymerase Chain Reaction) method with Top Taq master mix Kit kit (250) (Catalog no. 200403) Swine Primer: Forward: 5'CTA CAT AAG ATAT ATC CAC CAC A 3 'Reverse: 5' ACA TTG TGG GAT CTT CTA GGT 3 'Product size: 290 bp. Rat Primer: forward SIM (5'-GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA-3'). Reverse (5'GAATGGGATTTT GTTGGAGTTT-3 '). Out of 41 samples, sample 3, 4 and 5 taken in Jailolo contained rat DNA (positive); the samples were amplified with 499 base pair length (bp). In addition, sample 2, 7, 8 and 10 from Ternate as well as sample 4 from Morotai Island was also found positive (containing rat DNA). In terms of swine DNA, all of the samples came back negative. The amplification showed that none of the meatball samples contained pork. No pig DNA was amplified in the gel.

KEY WORDS
Meatball, beef, PCR, detection, primers.

People are adding foreign substance to processed beef products more particularly ones that do not look like fresh beef. Generally, people use their five senses to first identify physical characteristics of meat testing its quality. Therefore, foreign substance is commonly found in processed meet for instance meatball, beef jerky, burger patty, sausage, and shredded meat (abon). Besides adding foreign substance to the processed meat, some people also add it to certain sides such as broth for meatball, or mayonnaise for burger. Lawrie, R.A (2003) argued several approach can be used to detect and quantify swine DNA in food. The first approach is to determine ratio between several chemicals and assume that this ratio is fixed. Foreign substance will change this ratio or cause anomalies in its chemical composition. The second approach is to look for certain markers in food, either chemical or morphological, that can reveal that certain food contains swine DNA. The third approach is physico-chemical analysis. PCR becomes an important tool for identifying meat from various animal species. PCR is the multiplication of DNA molecules of a certain size in-vitro through temperature change mechanism. PCR reactions mimic DNA replication that occurs in living things. In short, PCR is replication of certain regions of printed DNA (temple) with the help of DNA polymerase enzymes.

DNA polymerase is an enzyme that catalyzes polymerization of DNA. Recently, Taq DNA polymerase enzyme is frequently since the enzyme reacts at high temperature so that enzyme addition in every cycle is not longer necessary and PCR process can be completed using one instrument (Gaffar, 2007). The taq DNA polymerase enzyme consists of two types, namely natural enzymes isolated from Thermus aquaticus bacterial cells and recombinant enzymes synthesized in Escherichia coli bacterial cell (Muladno, 2010).

METHODS OF RESEARCH

In order to detect and quantify pork derivative based on differences in characteristics and both minor as well as major components of the pork derivatives in food, their physical-chemical constants and ideal biological measurement, one should select quick, valid, reproducible, user-friendly and affordable method of analysis (Tamino, 1988). Some of the methods of analysis available to serve the purpose are Fourier Transform Infra Red (FTIR) Spectroscope, chromatograph, Differential Scanning Calometry (DSC), Electric Nose (EN), as well as DNA and ELISA (Enzyme-linked immunosorbent assay)-based methods. An example of the DNA-based methods is Polymerase Chain Reaction (PCR) (Soeparno, et.al, 2001).

We detected swine DNA content using primer Forward: 5'CTA CAT AAG AAT ATC CAC CAC A 3'. Reverse **:** 5' ACA TTG TGG GAT CTT CTA GGT 3' (Product size: 290 bp) and rat DNA content with primer *forward: 5'GACCTCCCAGCTCCAT-CAAACATCTTGATGAAA-3'. Reverse:* 5'-GAATGGGATTTTGTCTGCGTTGGAGTTT-3' was conducted in Biotechnology Laboratorium of Khairun University.

The procedures were as follow:

Preparation prior to PCR: Measuring composition to make PCR mix based on the number of samples; Preparing the control positive DNA (positive samples may be used as the control positive);

Making PCR Mix with the Top Taq master mix Kit (250) (Catalog no. 200403) with the procedure that adopted the food ingredient detection procedures (Genecraft Labs, 2014). The procedures were as follow: Melt the Top Taq master mix Kit (when stored under the temperature of–20°C), DNA template or cDNA, primer, and RNase-free water; Vortex and centrifuged for a few seconds; Make the PCR mix in 1.7 milliliter sterilized LifeTouch Microcentrifuge tube based on Table 1.

Table 1 – Master Mix Reaction Mixture

Component	1X (µl)	… X (µl)	Final Concentration
RNase-free water	8.5		
Top Taq master mix 2X	12.5 µl		1x
Primer F (10M)	1		0.4 µM
Primer R (10M)	1		0.4 µM
DNA Template or cDNA	2		≤500 ng/reaction
Total volume of reaction	25		

Mix the PCR mix based on the number of the samples but the template, and distributed them into 0.2 ml tube for about 23 µl each. Add 2 µl of the DNA template or cDNA (≤500 ng/reaction) into the PCR tube filled with the PCR Mix.

Table 2 – Master Mix Reaction Mixture

Component	1X (µl)	… X (µl)	Final Concentration
RNase-free water	8.5		
Top Taq master mix 2X	12.5 µl		1x
Primer F (10M)	1		0.4 µM
Primer R (10M)	1		0.4 µM
DNA Template or cDNA	2		≤500 ng/reaction
Total volume of reaction	25		

RESULTS AND DISCUSSION

Based on BLAST analysis, both the forward or reverse swine primer sequence that had undergone sequence matching was a really good instrument because its Query cover was 100% and the E-Value was really low. Figure 1 described the Query cover and e-value of the swine primer.

Double alignment aimed to align and match between the results of sequences obtained from research samples and the ones from Genbank data. The results (BLAST) provided

information on which animals having similarities with the DNA sample sequence. The information obtained from BLAST is in the form of Score, Query coverage, E-Value and maximum identity (Madem, 2013).

Query coverage refered to percentage of nucleotides length that matched the database contained in BLAST. E-Value score referred to estimated value that provided a statistically significant measure of both sequences. Higher E-value vindicated lower homology among sequences while lower E-Value indicated higher homologous level among sequences (Sagita, 2016).

Description	Max score	Total score	Query cover	E value	Ident	Accession
Sus scrofa domesticus breed Mangalica mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	KJ746686.1
Sus scrofa strain PB137 12S ribosomal RNA gene, partial sequence; mitochondrial	44.1	44.1	100%	0.011	100%	KF781323.1
Sus scrofa strain T04-57 12S ribosomal RNA gene, partial sequence; mitochondrial	44.1	44.1	100%	0.011	100%	KF781337.1
Sus scrofa breed DLY mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	KF569218.1
Sus scrofa isolate 3 breed Turopolje mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	JN601073.1
Sus scrofa isolate 2 breed Turopolje mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	JN601072.1
Sus scrofa isolate Mangalitsa swallow belly breed 2 mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	JN601069.1
Sus scrofa isolate Mangalitsa swallow belly breed 1 mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	JN601068.1
Sus scrofa isolate Mangalitsa Blonde breed 43-6 mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	JN601067.1
Sus scrofa isolate Mangalitsa Blonde breed 43-5 mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	JN601066.1
Sus scrofa domesticus breed pietrain mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	KC469587.1
Sus scrofa breed Large White mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	KC250275.1
Sus scrofa isolate Pig1 12S ribosomal RNA gene, partial sequence; mitochondrial	44.1	44.1	100%	0.011	100%	GQ926971.1
Sus scrofa domestica mitochondrial DNA, complete genome	44.1	44.1	100%	0.011	100%	AP003428.1
Sus scrofa breed European wild boar haplotype WB6 mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	FJ237003.1
Sus scrofa breed European wild boar haplotype WB5 mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	FJ237002.1
Sus scrofa breed European wild boar haplotype WB4 mitochondrion, complete genome	44.1	44.1	100%	0.011	100%	FJ237001.1

Figure 1 – Result of Blast Nucleotide Sequence of the Forward Swine Primer

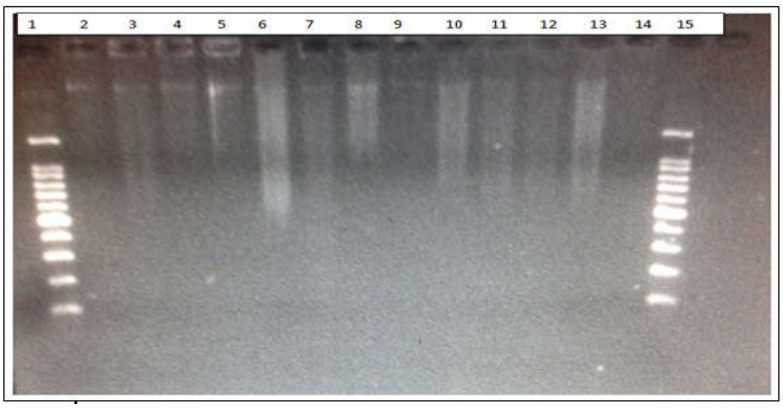

Figure 2 – The Amplification Result of the Meatball Samples containing Swine DNA Primer in Jailolo (sample 2-6), Ternate (sample 7-9 and 11), Morotai (12 and 13) and the control (sample 14)

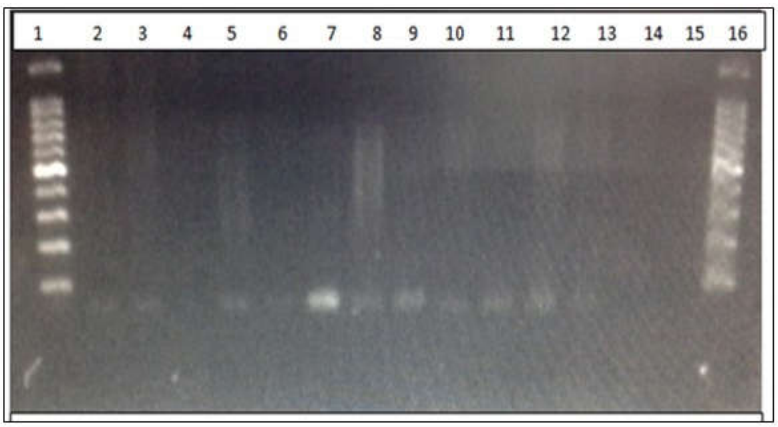

Figure 3 – The Amplification Result of the Meatball Samples containing Swine DNA Primer in Morotai (sample 2), Tobelo (sample 3-7), Morotai (8-11), Tidore (12-15) and DNA ladder 100bp* (sample 1,16)

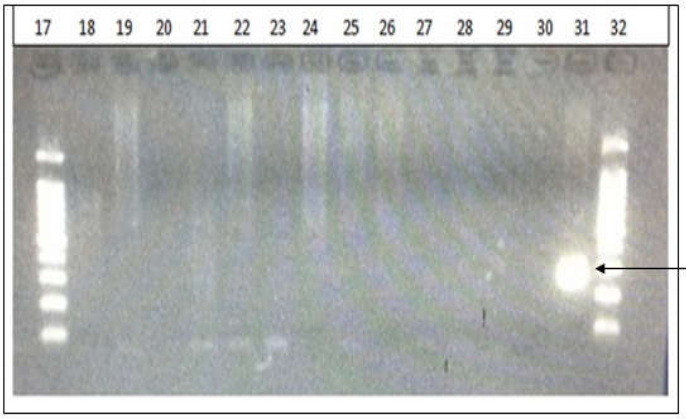

Figure 4 – The Amplification Result of the Meatball Samples containing Swine DNA Primer in East Halmahera (sample 18-22), Bacan (sample 23-27), Weda (sample 28, 29 and 30), swine control positive (sample 31) and DNA ladder 100bp* (sample 17 and 32)

Based on PCR analysis and the electrophoresis, none of the samples contained swine DNA (negative). The amplification results showed that the 41 meatball samples did not contain any pork. Not once was the swine DNA amplified in the gel. Figure 2-5 described the amplification results.

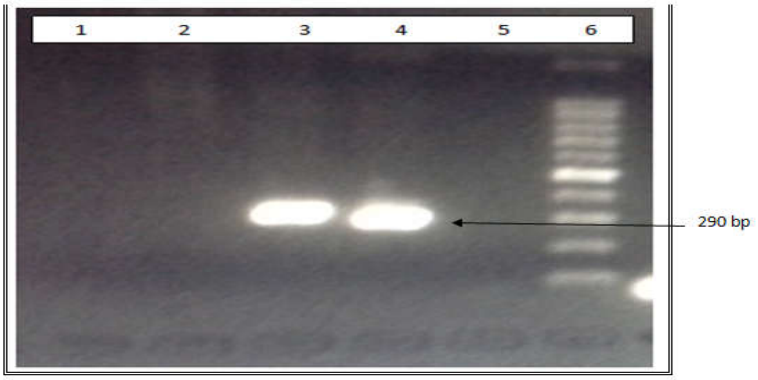

Figure 5 – The Amplification Result of the Meatball Samples containing Swine DNA Primer in Weda (sample 1 and 2), swine control positive (sample 3 and 4), control negative (sample 5), DNA ladder 100bp* (sample 6)

The intensity of DNA ribbons of the meatball samples was varied based on amount of isolated DNA (meat) taken from the samples. It was also related to the fact that producers used very small amount of beef to make their meatballs. The more beef meatball producers used, the more DNA isolated from the meatball. More isolated beef DNA meant the higher the intensity of the DNA ribbons appeared in the gel.

Cytochrome b gene functions as a carrier of genetic code as do genes contained in the nucleus. The rat primer developed from the cytochrome b gene, has been shown to amplify DNA derived from the genus Rattus (Sulistyaningsih, 2007).

The cytochrome b gene is part of the cytochrome in the electron transport that was located in the mitochondrial respiration chain. The cytochrome b consists of eight helical transmembranes connected by an intra-membrane region or an extra membrane and encoded by mitochondrial DNA (Espoti et.al., 1993).

A description of the DNA amplification results from the meatball samples in which the rat DNA was found using the forward rat primer; 5 'GAC CTC CCA GCT CCA TCA AAA CTC ATC TTG ATG AAA-3' and the reverse rat primer; 5'-GAA TGG GAT GTG GTT GTGT GTTT-3 'TTT GTC GTT GTGT GTTT-3.' Based on BLAST analysis and sequence matching, both forward and reverse rat primer sequence was an excellent detector its Query cover was 100% and very low E-Value as Figure 6.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Ovis aries breed Garsi cytochrome b (cytb) gene, partial cds; mitochondrial	75.8	75.8	100%	2e-11	100%	KF420282.1
Cardinalis phoeniceus cytochrome b (cytb) gene, complete cds; mitochondrial	75.8	75.8	100%	2e-11	100%	EF530008.1
Cardinalis sinuatus isolate CONA250 cytochrome b (cytb) gene, complete cds; mitochondrial	69.9	69.9	92%	1e-09	100%	KC213567.1
Cardinalis sinuatus cytochrome b (cytb) gene, complete cds; mitochondrial	69.9	69.9	92%	1e-09	100%	EF530009.1
Microtus fortis isolate Fuyuan-ME270 cytochrome b gene, partial cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ857281.1
Rupicapra rupicapra carburiana mitochondrion, complete genome	67.9	67.9	100%	4e-09	97%	KJ184175.1
Rupicapra pyrenaica ornata mitochondrion, complete genome	67.9	67.9	100%	4e-09	97%	KJ184173.1
Microtus fortis isolate JL_2 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991954.1
Microtus fortis isolate JL_1 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991953.1
Microtus fortis isolate HLJ_3 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991952.1
Microtus fortis isolate HLJ_2 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991951.1
Microtus fortis isolate HLJ_1 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991950.1
Microtus fortis isolate FJ_17 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991946.1
Microtus fortis isolate FJ_14 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991943.1
Microtus fortis isolate FJ_9 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991937.1
Microtus fortis isolate FJ_4 cytochrome b (cytb) gene, complete cds; mitochondrial	67.9	67.9	100%	4e-09	97%	KJ991933.1

Figure 6 – Result of Blast Nucleutide Sequence of the Forward Rat Primer

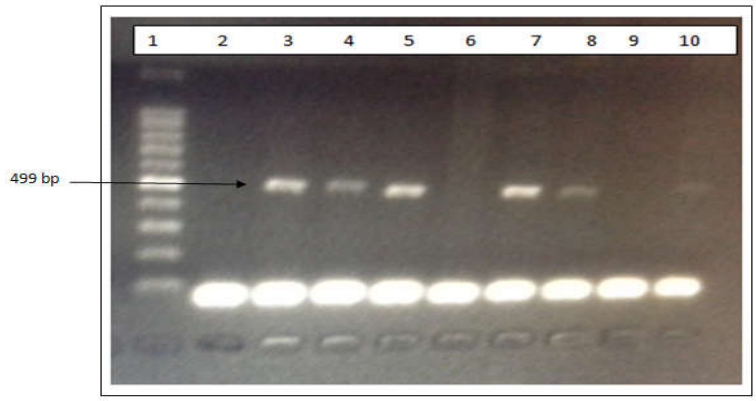

Figure 7 – The Amplification Result of the Meatball Samples containing Rat DNA Primer in Jailolo (sample 2-6), Ternate (sample 7-10) and DNA ladder 100bp* (sample 1)

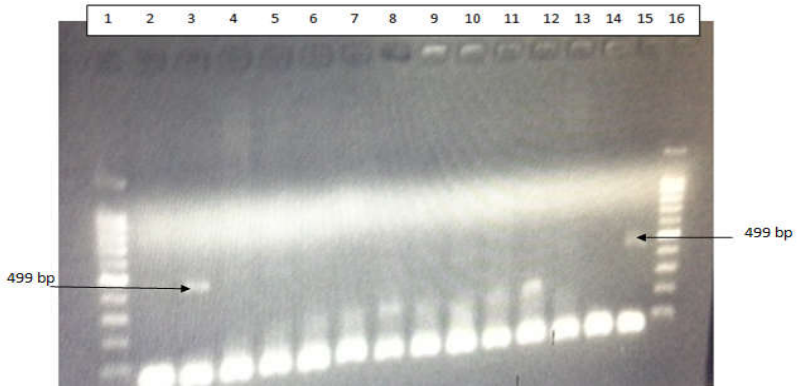

Figure 8 – The Amplification Result of the Meatball Samples containing Rat DNA Primer in Ternate (sample 2), DNA ladder 100bp, Morotai (sample 3-5), Tobelo (sample 6-10), Bacan (sample 11-14) and Tidore (sample 15)

The PCR process was carried out at the temperature of 94ºC for denaturation, 55ºC for annealing, 72ºC for extension for 1 minute and the final extension at the temperature of 72º for 10 minutes. Amplicone PCR was identified using electrophoresis gel using 2% agarose gel dissolved in a TAE buffer for 39 minutes using a 100 volt voltage (Sulandari and Zein, 2003).

Such result occurred due to the ingredients of the meatballs as presented above. The smaller proportion of beef the meatball had, the smaller the chances of the swine or rat DNA

to be isolated and therefore, there was lesser chance for their DNA ribbon intensity to capture in photos with ultraviolet light.

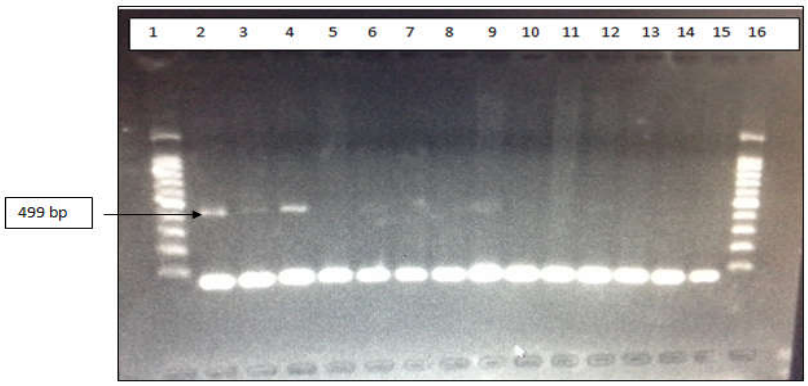

Figure 9 – The Amplification Result of the Meatball Samples containing Rat DNA Primer in Tidore (sample 2-4), Buli (sample 5-9), Bacan (sample 10-14), Weda (sample 15); DNA ladder 100bp* (sample 1 and 16). The meatball samples containing rat were sample 2 and 4 (Tidore)

Irmawati (2003) as cited in Ludyasari (2014) stated that thick and concentrated (not spread out) DNA ribbon showed high concentration and total DNA was extracted as a whole, while spread-out DNA ribbon showed bind between DNA molecules was detached during extraction process. Out of the 41 meatball samples analyzed in Figure 7, the meatball samples contaminated with rat meat were the samples from Jailolo (sample 3,4 and 5) that were amplified with 499 base pair (bp) length and the samples from Ternate (sample 7, 8 and 10). Based on Figure 8, the samples positively contaminated with rat meat were sample 2 from Ternate and sample 4 from Morotai Island. The amplified rat DNA (499 bp) was found in the meatball samples from Ternate (three samples), Jailolo (three samples), Tidore (four samples). Figure 7 described the results of the DNA amplification.

CONCLUSION

Based on the findings and discussions, the conclusions are as follows:

From 41 meatball samples obtained from several places in North Maluku, none of them contain pork or swine DNA;

Based on PCR Visualization, from 41 samples, 11 of them contained rat meat. Three samples from Ternate, three samples from Jailolo, one sample from Morotai and four samples from Tidore come back positive containing rat DNA.

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