Detection of Porcine DNA in Burger Products Using Real Time-Polymerase Chain Reaction (RT-PCR)

Anna Priangani Roswiem[a], Dedy Suseno[a,1,*], Triayu Septiani[a]

[a] Halal Research Center, Lembaga Penelitian, Universitas YARSI, Jakarta, Indonesia
[1] dedy.suseno@yarsi.ac.id
* corresponding author

ARTICLE INFO

Article history
Received June 25, 2020
Revised August 10, 2020
Accepted November 13, 2020

Keywords:
Burger
pork DNA
halal logo / label
processed meat products
RT-PCR

ABSTRACT

Burgers are one of the processed meat products currently being traded in Indonesia to the suburbs and even to the villages. The raw materials for burgers are beef, chicken, fish, and pork. Until now, the price of beef is still costly for many customers. However, it turns out that many burger producers sell their products at very low prices. This condition raises suspicion from consumers that the producers mix or replace the beef with pork. Based on this issue, this study aims to examine the content of pork (porcine DNA) in burger products sold in traditional markets in Jakarta and frozen food outlets in the villages around Jakarta. Pig DNA testing used the Real-Time Polymerase Chain Reaction (RT-PCR) method. The results showed that porcine DNA was not detected in all the test samples (10 samples) from different brands, including those with the MUI halal logo.

1. Introduction

The demand for food from animal origin continues to increase from day to day, in line with increasing public awareness of the benefits of nutrition for human life. Along with this demand, food safety and halalness of animal origin are significant concerns for some consumers (Maryati, i2020; Nirwandar, 2020).

In Indonesia, processed meat products such as meatballs, sausages, and burgers are loved by many. In general, the raw materials for making meatballs, sausages, and burgers are beef or chicken. Until now, the price of beef and chicken is still very high. As a result, many producers/sellers of meatballs, sausages, and burgers mix or replace beef or chicken with other cheaper meat, such as pork, wild boar, or rat meat. Since most of the Indonesian population is Muslim, this issue could disturb Muslims' inner peace because they are forbidden to consume pork, wild boar, or rat meat. Mixing or replacing beef or chicken with other meat usually aims to reduce processed meat's production costs. Consequently, the sellers could sell their products at affordable prices to get more profit. The problem arises if the mixing or replacement process uses the types of meat that are not allowed to be consumed by specific communities related to religion or culture. For example, a study found that some sausage products sold in traditional and modern markets in Yogyakarta are made from a mixture of beef and pork. The issue raised from this study resulted in public concerns and unrest (primarily among Muslims) related to food safety and halal aspects (Datucali and Sali, 2020).

Many burger products are traded in traditional markets. Their sales have penetrated to the suburbs or villages, which offer lower prices than in big cities. Therefore, this research wanted to determine

whether beef burger products traded in traditional markets and frozen food outlets in Jakarta or its suburbs contain pork. The primary ingredient of a burger product is meat. One method for identifying animal meat species is the Real Time-Polymerase Chain Reaction (RT-PCR) technique. The RT-PCR technique was chosen as an identification tool because of its high accuracy in detecting pork in the processed meat product. (Cai, et al., 2012; Erwanto, et al., 2018; Janosi (2006); Soedjono (2004).

Therefore, this study aimed to examine the pork content (porcine DNA) in burger products sold in traditional markets in Jakarta and frozen food outlets in the villages around Jakarta, regardless they are halal-labeled or not.

2. Method

2.1 Materials

The samples included burger products sold at a low price, either with or without a halal logo. All samples were uncooked. The reagents for detecting porcine DNA included DN-easy Mericon Food, Mericon Pig Kit, agarose, DNA Ladder, loading dye, and TAE buffer.

The following equipment was used in this study: the micropipette (Eppendorf), Nanodrop Spectrophotometer (Tecan), 10 µL, 100 µL, 1000 µL tips, Real-Time-Polymerase Chain Reaction (Mygomini) instrument, electrophoresis device (Biorad), and gel documentation (GBox SensGene).

2.2 Detection of Porcine DNA using RT-PCR

Porcine DNA analysis on samples was carried out using the Mericon Pig Kit. We added 130 µl multiplex PCR master mix to the Mericon assay tube, followed by vortexing and centrifugation. We then added 200 µl nucleic acid dilution buffer into DNA positive, followed by vortexing and centrifuging. The sample components and reaction parameters are given in the following table.

Set the PCR program and insert the tube containing the sample and control into the machine. The RT-PCR program run according to the following details:

Table 1. Master mix kit RT - PCR

Componet	Sample	Positive PCR Control	Negative PCR Control
Reconstituted *mericon* Assay	10 µl	10 µl	10 µl
Sample DNA	10 µl	-	-
Dissolved Positive Control DNA	-	10 µl	-
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	-	-	10 µl
Total volume	20 µl	20 µl	20 µl

Table 2. Instrument setting of RT - PCR

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95ºC	Activation of HotStarTaq *Plus* DNA polymerase
3-step cycling:			
Denaturation	15s	95ºC	Data colletion at 60ºC
Annealing	15s	60ºC	
Extension	10s	72ºC	
Number of cycles	45		
Detection	**Reporter**	**Excitation/emission**	**Channel**
Target	FAM	495/520 nm	Green
Internal control	VIC	524/557 nm	Yellow

2.3 Sample Preparation

2.3.1 DNA Isolation

The DNAs were extracted using Qiagen DNeasy Mericon Food kit (Qiagen, USA). 200 mg of sample was taken and added with 1 mL of food lysis buffer (either 1 mL directly or 500 µL and 500 µL gradually, depending on the sample type). The purification process was performed by taking 1 mL of sample and adding 2.5 µL of proteinase-K, vortex and incubation at 60 °C for 30 minutes. The samples were centrifuged at 2500 x g for 5 minutes. The clear layer was transferred from the lysis tube (without touching the sediment at the bottom of the tube) to a new microtube containing 500 µm of chloroform. The tube was vortexed for 15 seconds and centrifuged at 14000 x g for 15 minutes. We took the clear layer and measured the volume. Then we added 1: 1 volume of phosphate buffer and vortexed the tube for 15 seconds. We placed all of the liquid into the Qiaquick spin column and centrifuged it at 17900 x g for 1 minute. The collected liquid in the collection tube was discarded. We added 500 µL Buffer AW2, centrifuged at 17900 x g for 1 minute, and discarded the supernatant. The Qiaquick spin column was placed in a new 2 mL collection tube and re-centrifuged at 17900 x g for 1 minute on a dry membrane. The collection tube was removed and the Qiaquick spin column was placed in a new 1.5 mL tube. We added 150 µL of EB buffer and let it stand for 1 minute at room temperature before centrifuging it for 1 minute. The eluted DNA can be used directly for PCR or stored at -20 °C before use.

2.3.2 DNA Purity Analysis

DNA purity analysis was carried out using the Tecan Nanodrop Spectrophotometer instrument. We placed Elusion Buffer (EB) as the blank on the plate, followed by placing 2 µL of extracted DNA sample on the Tecan plate. We then put the plate back into the instrument and performed the DNA purity reading using EB as the blank. The DNA purity reading generated a table that included the ratio of protein concentration to DNA concentration.

2.3.3 Data Analysis

The data generated from the reading were DNA purity and graphs showing the content of porcine DNA in burger samples using RT-PCR.

3. Result and Discussion

3.1 DNA Isolation and Purity

The burger samples used in this study were obtained from traditional markets in South, West, and Central Jakarta and several frozen food shops in the suburbs of Jakarta. The DNA purity and concentration are shown in Table 3.

Table 3. DNA Concentration and Purity Data of Burger Samples and Positive Control

Sample Number	Sample ID	Ratio A260/280	DNA Concentration ng/µL	Information
1	A	1.82	49	Halal-labeled product
2	B	1.83	47.1	Halal-labeled product
3	C	1.87	49.8	No halal logo
4	D	1.89	46.4	Halal-labeled product
5	E	1.84	71.6	No halal logo
6	F	1.86	84.2	Halal-labeled product
7	G	1.89	65.6	Halal-labeled product
8	BPC 1	1.83	75.6	Halal-labeled product
9	BPC 2	1.85	67.2	Halal-labeled product
10	BPL	1.9	29.4	Halal-labeled product
11	BPC 3	1.86	29.8	Halal-labeled product
12	BK +	1.83	63.8	Pork burger

All samples were packaged meat burgers; some have a halal logo in their packaging, and some do not have (Table 3). DNA concentration varies among samples; the largest concentration was 84.2 ng

/ µL. All samples showed a ratio value at A 260/280 nm between the range of 1.8 to 2.0. The value of the ratio range indicated that the isolated DNA is pure. If the mean value is less than 1.8 or more than 2.0, the total DNA extraction process does not go well. This situation is due to RNA or protein impurities, thus affecting the purity of DNA. In addition to RNA and protein, the presence of reagent components used during the isolation process, such as phenol, alcohol, and chloroform, can also affect the total purity of the isolated DNA (Widayat et al., 2019)

3.2 The Presence of Porcine DNA in samples

Mericon pig kit was used to detect the presence of porcine DNA in the samples. There are two dyes to observe the amplification curve, namely FAM and VIC. FAM dye is used to determine the presence of porcine DNA amplification curves in samples and positive controls. Meanwhile, VIC dye is used to see the internal control, where the Cq value on the VIC dye indicates the success of the RT-PCR process. All samples and positive controls must have an amplification curve and a Cq value below 33 on the VIC dye.

The results showed that all samples, positive control, and negative control showed amplification curves on VIC dye. All Cq values were below 33 (Figure 1). These two conditions showed that the amplification process on RT-PCR went well.

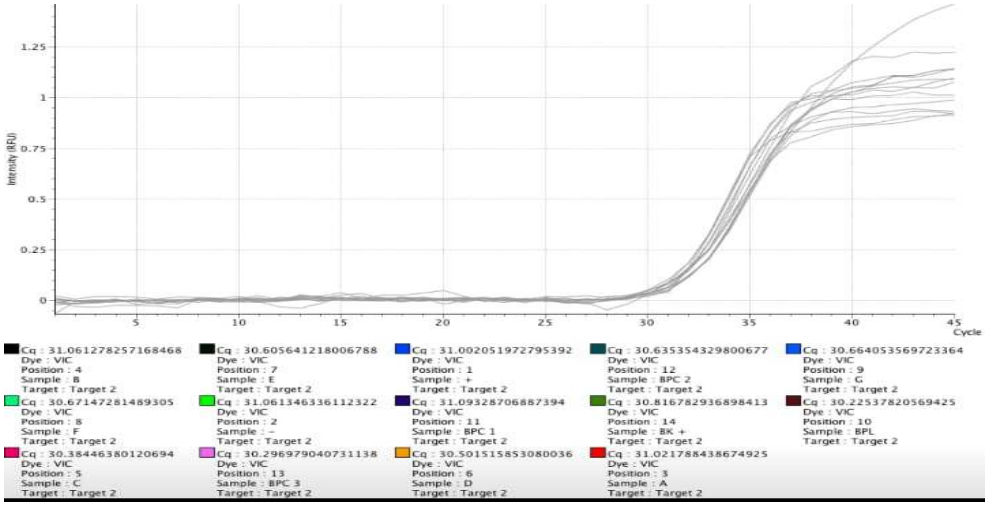

Fig 1. Amplification Graph and Cq Value of Sample, Positive and negative control on VIC dye

The amplification graph on FAM dye showed only positive controls, and BK + show porcine DNA amplification curves.

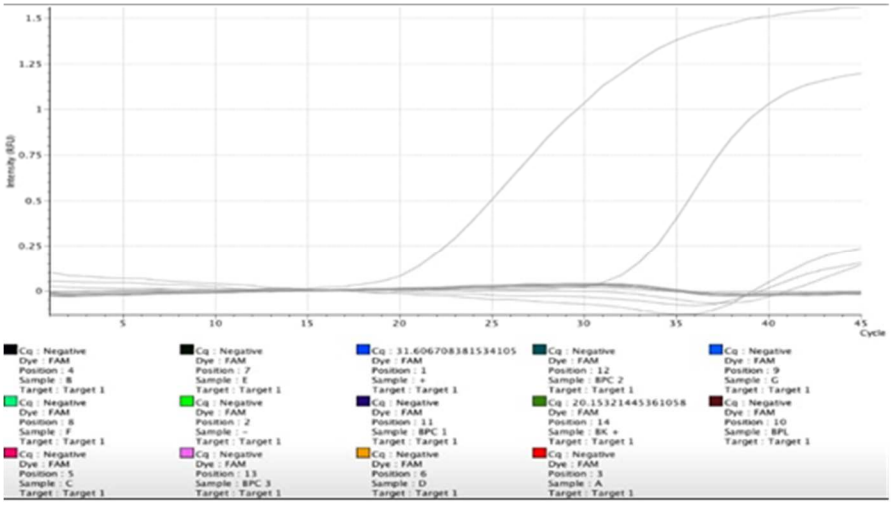

Fig 2. Amplification Graph of The Test Samples and Positive Controls on FAM dye

This was clearly because the positive controls and BK + did contain porcine DNA. Meanwhile, the other burger meat samples did not contain porcine DNA (the Cq value was "negative"). The Cq value of the BK + is 20.15, while the positive control is 31.60. The difference in these values might be that the porcine DNA concentration in BK + is higher than the porcine DNA positive control of the kit. Therefore, the BK + is amplified earlier than the kit's positive control

4. Conclusion

The samples of burger products used in this study, which were purchased from traditional markets in South, West, and Central Jakarta, and several frozen food shops in Jakarta's suburbs did not contain pork.

Acknowledgment

We would like to thank YARSI Foundation for providing Research Grant for this study

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