Fraud Identification in Meatballs Product Using Porcine Detection KIT and Multiplex Polymerase Chain Reaction Methods

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ABSTRACT: Transparancy in meat label is very important. Accurate labeling is essential for protection of consumer health and religious credence, as well as to ensure the authentication of product before consumers decided to purchase. Pork adulteration and it's derivates in food product, without any clearly labeling is consider to be fraudulent in trade. The presence of pork in food products are serious concern for moslem as pork is prohibited by The Holy Qur'an. The aim of this research was to determine the presence of pork and to identify meat origins in ten meatballs, taken from northern Yogyakarta-Indonesia. Two methods based on protein and DNA were used for identification. They were Porcine Detection KIT and Multiplex Polymerase Chain Reaction. Porcine Detection KIT is based on principle of immunochromatographic rapid test. The target antigens are bound by highly specific antibodies attached to test line and colored microparticles. While, Multiplex Polymerase Chain Reaction is capable of amplifying few DNA target into milion copies of DNA, using multiple primers. The DNA was isolated from sample using Genomic mini KIT. Optimizing of PCR was conducted in advanced to obtain the most optimum annealing temperatures for DNA amplification. Three sets of primer used in this study for multiplex amplification: pig (*Sus scrofa*), cow (*Bos taurus*) and chicken (*Gallus gallus*). PCR products were analyzed by electrophoresis on 1.5% agarose gel run in TBE1x buffer at 100V. The results showed that Porcine Detection KIT and Multiplex Polymerase Chain Reaction can detect the presence of pork in samples. Porcine Detection KIT can detect one sample which contaminated with pork. Multiplex PCR not only can detect the presence of pork, but also beef and chicken. There were two samples contaminated with pork according to multiplex PCR detection

Keywords: Meatball, Porcine Detection KT, Multiplex Polymerase Chain Reaction

INTRODUCTION

Transparency in meat label is very important. Accurate labeling is essential for protection of consumer health and religious credence, as well as to ensure the authentication of product before consumers decided to purchase. Pork adulteration and it's derivates in food product, without any clearly labeling is consider to be fraud in trade. According to Indonesia law, UU No.18/2012, fraud in food products might be subjected to punishment. Besides it, most of Indonesian people are Muslim. Meatball is one of many popular food in Indonesia. In the recent year, there were some issues related to meatball fraudulent. In December 2012, the Department of Livestock and Fisheries of South Jakarta district found a stall selling meatball that contain pork in the Kebayoran Baru (Syailendra, 2012). At the same time, Assessment Body of Food, Drug and Cosmetic Majelis Ulama Indonesia, East Kalimantan, find meatball products in Samarinda and Kutai regency indicated mixed with pork (Amirullah, 2012). Pork protein, due to its being cheap and readily available, might be fraudulently used to substitute other animal proteins. Moslems are required to

eat halal food by ignoring food from pork origine. Halal is an Arabic term which means permitted, allowed, authorised, approved, santioned, lawful, legal, legimate or licit. Guidelines for halal are given by Allah in the Holy Qur'an. Halal meat must be obtained from halal animals and processing only. Over last decades, meat industry has enforced strong measures towards establishment of effective traceability systems to preserve food safety and quality from fram to fork (Shackel, 2008).

The meat chain conforming to all halal requirements is very complex and the risk of cross-contamination is substantial (Bonne *et.al*, 2008). During processing, food products might be subjected to thermal treatments (e.g. cooking, pasteurisation and sterilisation), high pressure, pH modification, irradiation and drying. Pork protein detection might be impossible, particularly if proteins are degraded or severely or altered during processing. In such case, DNA based methods like PCR can be employed to detect pork detection adulteration in meat products. Identification methods using highly degraded substrate should be based on the analysis of very short DNA fragments, preferebly 100-200 base pair. The objectives of this research was to detect commercial meat balls from fraudulent sources by using Porcine Detection KIT and Multiplex PCR. Porcine DetectionKIT is based on immunochromatography, antigen in the sample is bound by a very specific antibodies on the test strip form the antigen-antibody complex. Test strips also contain dyes for marking the anitigen microparticles that are bound by the antibody samples. Amplification of PCR is based on the hybridisation of specific oligonucleotides to a target DNA and synthesis. The amplification of DNA fragments, followed by agarose gel electrophoresis for fragment size verification.

MATERIALS AND METHODS

Fresh beef, pork and chicken samples which were used for positive samples were bought from supermarket in Yogyakarta, as well as ten different meat ball samples). Samples were stored -18℃ until used . These samples were grounded and then diluted in PBS solution into the concentration of 10% (0.2 g/2 ml), sentrifugated on 2.000 rpm for 20 minutes and immunochromatographic strip test were applied to the supernatan of the samples for regarding the solution migration using a porcine detection test-KIT XEMA.

DNA extraction was prepared using Genomic DNA Mini KIT Geneaid Germany with minor modification PCR amplification was performed in final volume of 25 µL containing 12.5 µL Master Mix PCR, 3 µL Primer, 4.5 µL H_2O-PCR, 1 µL $MgCl_2$ and 4 µL for eah template DNA of the samples. PCR was carried out in a INFINIGEN PCR Machine Thermocycler. The cycling conditions: after the initial heat denaturation for 5 min at 94 ℃ followed by 35 cycles at 94℃ for 30 sec, 35 cycles at 59 ℃ (for pork primers) and 57 ℃ (for beef and chicken primers) for 1 min, 35 cycles at 72 ℃ for 1 min and a final extension at 72 ℃ for 5 min. Multiplex PCR was developed using each of the primer sets previously designed for simplex PCR. As for simplex PCR, amplification was performed in a final volume of 25 µL containing 12.5 µL Master Mix PCR, 3 µL Primer, 4.5 µL H_2O-PCR, 1 µL $MgCl_2$ and 4 µL for eah template DNA of the samples. The choice of template concentration depend on the nature of sample. PCR products were analyze by electrophoresis on 1.5% agarose gel (Bioron) run in TBE 1X buffer for 55 min at 100 V.

RESULTS AND DISCUSSION

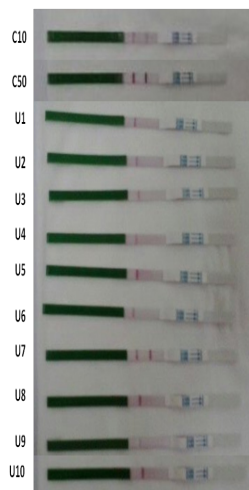**Porcine Detection KIT**

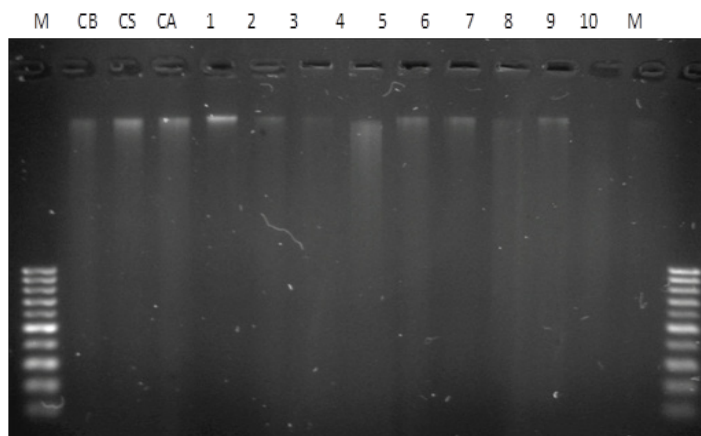Fig 1. Porcine Detection KIT test strip

Fig 2 The emergence of DNA in the positive control and the samples isolated
CB: Control Positive Pork; CS: Control Positive Beef CA: Control Positive Chicken and M: Marker

Quick qualitative results have found using Porcine Detection-KIT. Based on the Figure 1. has showed the red line on the test strip. The picture was taken 15 minutes after the test strip is dipped in the supernatant samples meatballs. Positive results of samples containing pork indicated by the appearance of two red lines, while one red line showed the negative results. Samples with ambiguous resuls were retested.

The principle of testing with porcine-KIT detection is based on immunochromatografi. Antigen in the sample is bound by a very specific antibodies on the test strip form the antigen-antibody complex. Test strips also contain dyes for marking the anitigen microparticles that are bound by the antibody samples.

The Immunochromatographic test has several advantages over traditional immunoassays, such as simplicity of procedure, rapid operation and immediate results, low cost, no requirements for skilled technicians or expensive equipment. This test also suitable for the on-site detection of antibodies (Shangjin Cui, 2008)

Polymerase Chain Reaction

In Figure 2. can be seen the emergence of DNA in the positive control and the samples were isolated. Based on Figure 2. can be seen that the DNA positive control in the pork, beef and chicken can be seen clearly, showing a lot of isolated DNA. In the sample No. 1, DNA samples were clearly visible, followed by samples number 5,6 and 8. While the sample number 2,3,4 and 7 were not so obvious, but still visible, whereas in sample numbers 9 and 10 is hardly noticeable. DNA samples isolated in lines number 2,3,4 and 7 are not so obvious. This is possibility that DNA was degraded during the cooking process of meatballs or meatballs are heated repeatedly. Bottero *et al.* (2003), DALMASSO *et al.* (2003) states that the DNA is degraded into the smallest fragment can still be done by PCR amplification. In the sample number 4 and 7, visualization under UV light visible generate smear. This can occur because on meatball dough contain contaminant

compounds such as oligopeptides, polysaccharides, proteins or other organic materials (Nuraini, 2004).

Multiplex PCR
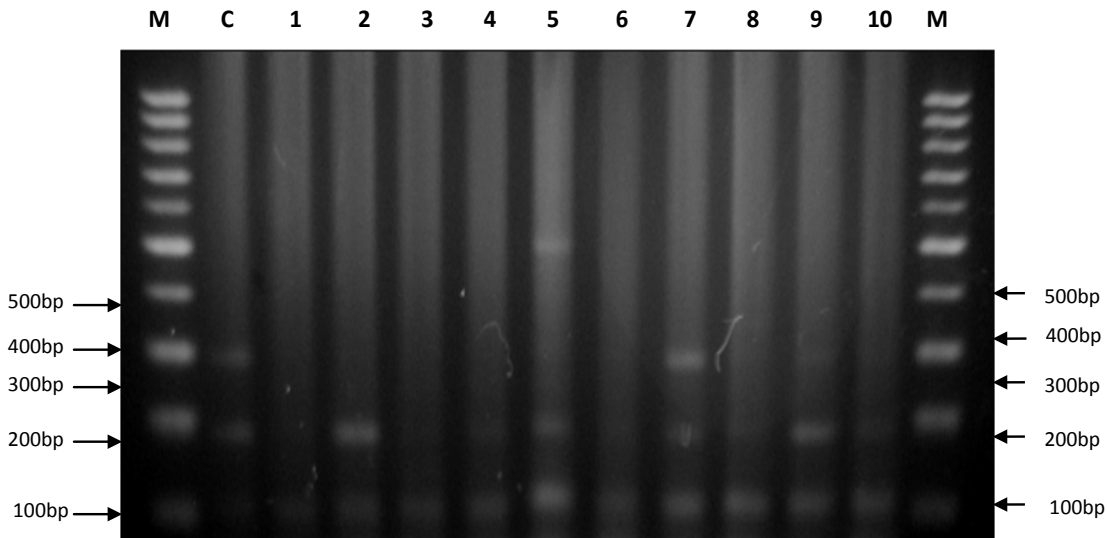
Multiplex PCR was performed using three primer types: pork, beef and chicken in a single reaction. Positive control used were fresh meat pork, beef and chicken in a single reaction. Figure 3. shows the results of multiplex PCR products electrophoresis with 1.5% agarose gel at a voltage of 100 volts for 60 minutes. It showed that the amplification of DNA in all samples with DNA fragments that appeared 290 bp for the pork (*Sus scrofa*), 104 bp for the species of beef (Bos taurus) and 183 bp for the species chicken (*Gallus gallus*).

Samples containing pork has indicated by the appearance of DNA at 290bp on the sample number 7 and number 9. The DNA appears on the sample number 9 is much thinner than the number 7 which is thick and clearly visible. Samples containing beef has shown by the appearance of DNA with 104 bp length. It were visible clearly on the sample numbers 1 to 10. Samples containing chicken has indicated at 183bp that can be seen in the sample numbers 2-10, but only the sample numbers 2,5,7 and 9 were visible, the rest has formed very thin. PCR is capable of amplifying very few copies of DNA and its detection limit is much lower than what is observed with protein based assays. PCR amplification is based on hybridization of specific oligonucleotides to a target DNA and synthesis of million copies flanked by these primers. The simplest PCR strategy applied to evaluate presence of any species in a meat product is the amplifiation of DNA fragment, followed by agarose gel electrophoresis for fragment size verification.

The main difficulty in the development of a multiplex PCR is the difference in length of amplified fragments, they should differ in length by 40-50 bp to permit adequate resolution of fragments by agarose gel electrophoresis. Other difficulties can arise when samples are subjected to severe heat treatments. In These cases, DNA fragmentation reduces the number of species that can be identified at the same time to four or five (Bottero, M. T. And Dalmasso,A, 2011)

CONCLUSION

Fraudulent meat balls in commercial market could be detected by Porcine Detection KIT and Multiplex Polymerase Chain Reaction.

ACKNOWLEDGEMENTS

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