Halal Analysis of An Aquatic Animal(*pangasius sutchii*) using RT-PCR For Detection of Porcine DNA

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Abstract— Pig (Sus sp.) and pig by products are considered as najasa (impurities) in Islam and forbidden in muslim consumer product. Animal fed on najasa are categorised as al-jāllalah (contaminated animals) which are allowed to be consumed as long as they have been quarantined for a certain period of time. During this quarantine period the animals will have undergone a natural purification process or istibra'. Patinfish (*pangasiussutchi*) are commonly consumed in Malaysia and maybe fed on najasa. This study was conducted to investigate the detection of pig DNA for patin fish(*pangasiussutchii*) after feeding with pig offal, based on the absence of the DNA in tank water, gut, skin and fish fillet by real time polymerase chain reaction (RT-PCR). A total of 3 fish samples (n=3*3) with three replicates for each samples were obtained from river located in Temerloh Pahang and fed with pig offal. Fish gut and the positive control was positive towards porcine DNA, however tank water, skin and fish fillet was negative towards porcine DNA.

Keywords—ḥalāl analysis; RT-PCR; al-istibra'; al-jāllalah

1. INTRODUCTION

In time that is full with economic rapidity science and technology, Muslim nor become increasingly difficult in choosing and determining whether the foods and drinks that used daily life are fulfil in Islamic requirement or not and legitimate eaten. One of the problems that Muslim facing today is when the fishes are feed with the najs to save the maintenance and fasten the growing of fishes . According to reference [19], the practice of feeding fish with pig intestine had been reported in Thailand. Furthermore, in early 2006, farmers at Batu Gajah, Tronoh and Papan Perak feed their fresh water fish(patin, and tilapia) with commercial feed made from animal protein and animal by products (blood, tissue or bone) which include that of pig origin had been reported in Malaysia [15].

Najs which refers to things that are themselves unacceptable, such as pork and all its derivatives, alcoholic drinks and ḥalāl food that is contaminated or comes into direct contact with things that are not permissible [11][12]. Pigs (Sus sp.) and any of their derivatives are considered as najasa (impurity) in Islam and totally haram (prohibited) in Muslim consumer products. There are, however, different opinions on the consumption of animals fed with najasa or known as al-jāllalah (contaminated animal). Fiqh (Islamic law) jurists from the Syafi'e and Ahmad school of thought regard it as prohibited to consume animals that are fed with najasacontinuously [6]. In Malaysia, the opinion of jurist from the Syafi'e school of though is applied for aquatic animals fed with najasa as indicated in section 3.4.1.1.2 of the Malaysian Standard for ḥalāl Food Production, Preparation, Handling and Storage- General Guideline (Second Revision) MS 1500:2009.

In al-jāllalah issues, there are several alternative purification of filth by Islamic Law. Some of that was based on method of al-Istibra'. Al Istibra' or al hans means a process of quarantine from filth to pure. For that reason, the contaminated animal will be quarantined and fed with uncontaminated feed in order that the animal until recoveries. The food can be pallet or others must be clear and clean from haram sources.

2. MATERIALS AND METHODS

A. Sample collection

A total of 3 fish samples (n=3*3) with three replicates for each samples were obtained from river located in Temerloh Pahang. The experimental fish were stocked in 3 tanks (1m x 0.5m) with density of 9 fish per tank. Another tank with 9 of fish for negative control. The tanks were fitted with a flow-through system and provided aeration. The fish were starved for a day in order to ensure complete emptying of the gut. The fish in the tank were fed with pig offal (mostly intestine and pig meat) which were bought from a market in Kuantan Pahang. The fish in the tank were fed daily at 9.00 am. Feeding was stop on the 4th day at 9.00 am. At day 5, 9.30 am, the fish were taken from first tank, slaughtered and dissected to observe and examine the gut, skin and fish fillet. Water from the first tank were also taken to analysis the contamination of porcine DNA. We used 3 different samples which were gut, skin and fish fillet in this study to present the outer part, middle part and inside part of the fish that exposed to contamination by pig product.

B. DNA extraction

Each 0.2g sample was ground using mortar and pestle. The samples were transferred to 50ml centrifuge tubes containing 15 -mL RY1 buffer, provided in the Tri Omic^{TM}, DNA, RNA& protein extraction kit (Biotech Company, Malaysia). A volume of 1 -mL 10 %(w/v) Sodium Dodecyl Sulphate (SDS) were added into the mixtures. The tubes were vortexed for 10 seconds, incubated at 65^{o}C for 1 hours. After that, an equal volume of chloroform was added into the mixtures and followed by vortexing for 5 seconds. The mixtures were then centrifuged at 6000rpm at 4^{o}C for 20 minutes. Supernatants were transferred into fresh 50-mL centrifuge tubes and equal volume of chloroform was added into the mixture. The mixture then centrifuge, 30 minutes at 6000rpm, 4^{o}C. Supernatants were transfer into fresh 50-mL centrifuge tube and 100 %(v/v) of ethanol, 2 volume and o.1 volume of sodium acetate were added. After that, the precipitate process was proceeded by stored the mixture at -20^{o}C, 30 minutes. After 30 minutes, the tubes were centrifuged, 45 minutes, at the same speed and temperature. The liquid was discarded and the remaining solid in the tube was added with 5-mL 80 %(v/v) of ethanol, centrifuge at 30 minutes at the same speed. Washing step was carried out by applying 5-mL 100 %(v/v) ethanol into the centrifuge tube, centrifuge at the same condition for 10 minutes to remove excess ethanol. The liquid was discarded and the remaining solid was dried at 65^{o}C, for 15 minutes. After that, the precipitate was dissolved in 1-mL distilled water and stored in chiller for 1 night. RNAse treatment was proceeded by added 500 µg/µL into the mixture and shacked for 1 night at room temperature. The mixture were transferred to 2 -mL microcentrifuge tube containing 1-mL RY3 buffer provided in Tri Omic^{TM} (Biotech company, Malaysia) and were shacked gently. A volume of 750 µL of the mixtures ware transferred to separate spin columns and centrifuge at 12000rpm at room temperature for 30 s. The pass through was discarded and the steps were repeated for the remaining mixture. After that, 700 µL PW buffer provided in Gene All Expin^{TM} (Geneall Biotechnology, Seoul, Korea) was transferred to each of the spin column and centrifuge at 12000 rpm at room temperature for 30s. The mixtures were centrifuge for an additional 2 minutes to remove residual wash buffer for 2 minutes at the same condition. Receiver tube of the spin column was then removed and the spin column was placed onto fresh cap-cut 1.5-mL microcentrifuge tubes. After that 50 µL distilled water was pipetted into the membrane of each spin column and allowed to stand for 5 minutes, followed by centrifuged at 12000 rpm for 1 minutes at room temperature. The genomic DNA recovered was then transferred to fresh 1.5 –mL microcentrifuge tubes and stored at -20^{o}C for further use.

C. DNA quantification and agarose gel electrophoresis

A set of primers were used to specifically amplify a pig DNA marker using RT-PCR. Briefly, amplification was achieved using the Mastercycler Gradient (EppendorfepRealplex, Germany). Each strips reaction tube were contained 10 µl 2 X SYBR® Green PCR mix, 1 µl forward primer, 1 µl reverse primer, 6 µl of distilled water and 2 µl of DNA template. The RT-PCR amplification was run under the following condition: an initial denaturation step of 95^{o}C for 3 min to completely denature the DNA template, followed by 50 cycles of denaturation at 94^{o}C for 40s, annealing at 58^{o}C for 40s and extension at 72^{o}C for 40s. Electrophoresis separation of 10µl of RT-PCR product was performed in 0.1% agarose gel in 1 x TAE buffer pH 8.0. Electrophoresis was perform at constant voltage (80V) at 30 minutes. A 100bp DNA ladder (Thermo Scientific, USA) was used as a size reference and DNA from pig tissue was used as a positive control. Gel images were captured using the Gel analysis system. Pig DNA was considered present when 300 bp amplicons were detected in the gut sample extract by gel electrophoresis.

3.RESULT AND DISCUSSION

The animal fed on impurities is can be considered as al-jāllalah (contaminated animals) as there is a noticeable change in the state (physical, protein, structure, odour, taste and colour). In this study verified present of DNA porcine in gut content of *pangasiussutchii* fed with pig offal. We used pig offal in this study to represent the worst case of contamination by pig products.

A. Detection of porcine DNA

Before the start of the feeding trial, the feed used in this study was tested to confirm it as being pig origin. Fig. 1 shows that the feed used in this experiment were pig origin. White spot shown porcine DNA in pig origin using 2X SYBR® Green B PCR mix

Figure 1: Detection of pig sample specific amplicons in representative feed sample by Rt-PCR. Lane M, molecular size marker (RT-PCR marker 100bp), Lane 5-6 pig sample using 2 X SYBR® Green B PCR mix (duplicate)

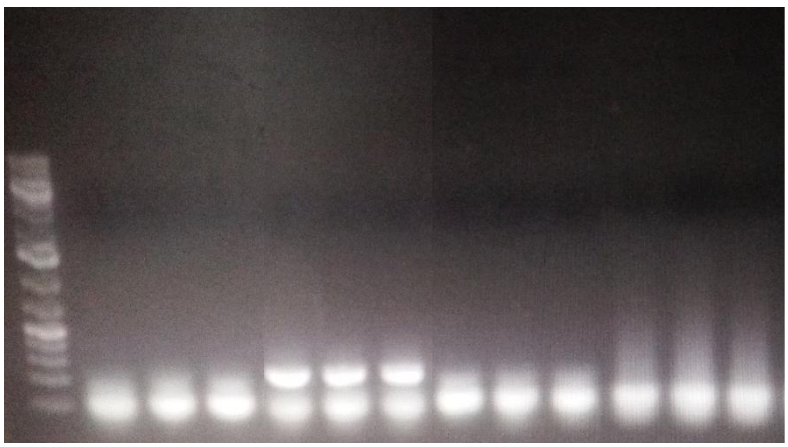

Figure 2: Detection of pig specific amplicons (300 bp) from fish. Lane M, molecular size marker (PCR marker 100bp). Lane 1-3: fish fillet, lane 4-6: fish gut, lane 7-9: fish skin, lane 10-12: water tank using 2X SYBR® Green B PCR mix

From Fig. 2, the positive detection of porcine DNA is shown by the two white spots in a lane while one white spot are negative detection. The detection of porcine DNA in gut, skin, fish fillet and tank water are shown in Table 1. The istibra' process entails an irreversible conversion of material to another totally different form or substance. In the case of *pangasius sutchii* being fed with pig offal the istibra' process could be achieved through digestion or hydrolysation of pig tissues into amino acids, simple sugar, fatty acid andglycerol are then absorbed through the *pangasius sutchii* gut wall into the blood stream. The digestion in fish is a process, beginning in the stomach and ending until food leaves the rectum as faeces.

Table 1: Summary of the porcine detection from fish, tank and pig origin

Sample	Source	Porcine detection
Fillet	Fish	-
Gut	Fish	+
Skin	Fish	-
Water	Tank	-
Pig	Pig origin	+

B.Observasion gut content

Most of the fish analysed immediately after feeding had their stomach full, the stomach was filled with pieces of pig tissue in its original form and appearance. At 24 h, the smell of the gut content was very pungent in *pangasius sutchii* with pig offal compared to the normal fishy smell in *pangasius sutchii* with no pig offal. The colour of the digested feed in the gut was initially whitish or greyishturned milky after 24h. Only clear liquid could liquid could be observe in the stomach after 48h.The stomach was completely empty from materials after 48h, however these observations were not consistent in across all the fish sample. One or two fish had empty stomachs even as early as 24h. The faster emptying of the stomach of certain fish could be due to the fact that these fish had not eaten during the last feeding time, ate less or were more active and thus digested the food at a faster rate. The observation of the gut content clearly indicated the irreversible morphological transformation of pig tissues into other materials which are different in form, appearance and colour. Protein properties of the digested materials were examined to confirm the chemical transformation of the digested feed using rapid pig test. In this study, pig was not detected in the gut of *pangasius sutchii* at 24h. This is not surprising because the pig tissue, would be digested to its simplest form of nutrient (amino acid, simple sugars, fatty acid and glycerol) before diffusing across the gut epithelium into blood stream to facilitate various life processes.[11]

4. CONCLUSION

Base on this study DNA porcine was detected in fish gut of fish that feeding on pig offal. There is a general guideline, set by jurists quarantine periods as follows: 40 days for camel, 30 days for cattle, 7 days for sheep and goat and three day for chicken. The period of quarantine based on the size and weight of the animal. For the next stage of the study, we will conduct a specific laboratory research to identify quarantine period and purification process for *pangasius sutchii*, focus on fish gut content

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