Oral Presentation (AQ-8)

Detection of the Foot and Mouth Disease in Buffalo Meat Originating From India with the Reverse-Transcription PCR Method

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INTRODUCTION

The foot and mouth disease (FMD) is still a major issue in the world of animal health. To date, not many countries are free from FMD; Indonesia is a country that has been FMD-free since 1986 and has been acknowledged by the World Animal Health Organization (OIE) from 1990. This success was not easily obtained, as a huge amount of energy, funds, and thoughts were expended over a period of nearly one hundred years. The regional situation in Asia at present is still a huge threat to the potential of FMD entry. Malaysia, Thailand, and India are risks for the re-entry of the disease to Indonesia.

There are constant efforts to smuggle meat in from regional Asian countries. The eastern coast of Sumatra is still a favorite area for importing meat illegally from countries not yet FMD-free. Moreover, the import of frozen boneless meat from FMD-free zones in India is still a threat and could potentially spread FMD to our country. Therefore, there need to be anticipation efforts and an early detection ability to identify the FMD virus which might be carried by imported meat. The meat that enters illegally and comes from FMD-free zones in India has the potential for bringing and spreading FMD; The lack of a specific method to be used as a tool for detection of FMD through imported or illegal meat.

The purpose of this method development activity is to acquire a suitable, quick, and highly accurate method in detection of FMD which might be carried by imported or illegal meat; one of these methods is assessment using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). This method is expected to become a standard method in FMD testing in the Agricultural Quarantine Agency.

MATERIAL AND METHODS

Sample Pretreatment: weigh 25 mg meat put in a 2 ml microcentrifuge tube, add one 1 stainless steel bead. Add 300 ul PBS or 0.9% NaCl to each tube. Put tube in tissue lyser adapter set. Operate tissuelyser II for 2 minutes at 25 Hz. Centrifuge the sample at 14,000 G for 2 minutes. Collect and use 200 ul supernatant as "starting material". Working Procedure based on the Qiamp cador Kit procedure.

RT- PCR Procedure For RNA detection
Preparation prior to PCR: Calculate the composition for making the PCR mix according to the number of samples to be used. Prepare a positive control (synthetic) :
Primer F 328 bp GCCTGGTCTTTCCAGGTCT
Primer R 328 bp CCAGTCCCCTTCTAGATC.
Preparation of the PCR Mix with One Step RT PCR Kit. Dissolve One step ahead RT - PCR Master mix, RNA template, primer, and RNAse-free water and Q-solution. Spin down for a few seconds. Make PCR Mix in a sterile 1.7 ml Life Touch Microcentrifuge tube. Make PCR mix according to the number of samples except for the template, then distribute to 0.2 ml test tubes each 23 µl (25 µl subtracted by the 2 µl template). Add 2 µl RNA template (≤ 500 ng/reaction) to the PCR tubes that have been filled with PCR mix. Program the PCR machine to target < 1kilo bp with annealing 55^0C and all the ampliflyng methods according to the kit that used.

Electrophoresis Procedure: The PCR results, 5µl each, were mixed with the 1 µl loading dye and 1µl mix (sybrgreen1 + TAE 1X/TBE1X). Pour into agarose 2% gel wells in the electrophoresis chamber. One of the wells is filled with 6 µl of ladder/marker 100 bp plus by also mixing 1µl mix (sybrgreen1 + TAE 1X/TBE1X). Provide a power supply at 100 volt for 40 minutes.

RESULTS AND DISCUSSION

From the Method Development activity, it was revealed that: The most suitable annealing temperature for the mastermix reagent and sample matrix is 55 ^{o}C. This is why this temperature was used for DNA amplification. Meat matrices can be used as sample matrices. Conventional PCR has demonstrated good results in FMD testing, thus it can be used as an assessment method in the laboratory.

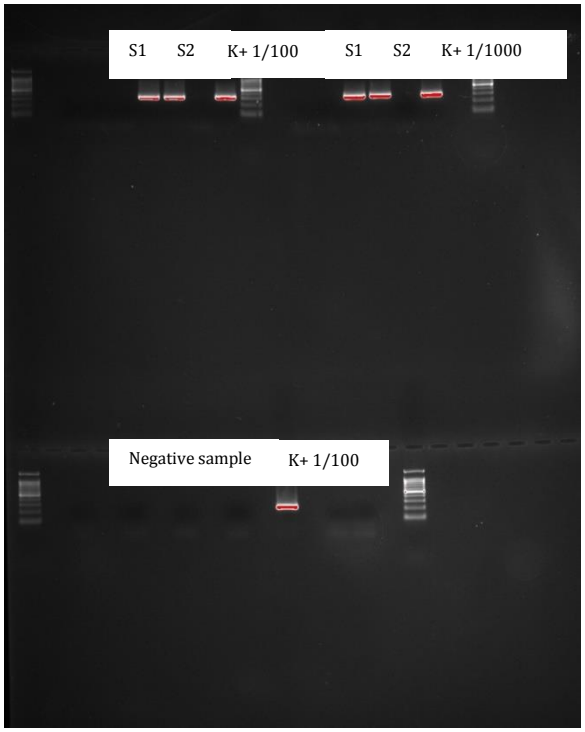

Figure 1. Electrophoresis result in buffalo meat with FMD synthetic positive control

CONCLUSION

There needs to be adjustments and improvements to real-time PCR; therefore, it could be continued in the following period.

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