Jurnal Medik Veteriner
DOI: 10.20473/jmv.vol6.iss2.2023.191–196
October 2023, Vol.6 No.2, 191–196
online at https://e-journal.unair.ac.id/JMV

Detection of Foot and Mouth Disease Virus in Cattle in Lamongan and Surabaya, Indonesia Using RT-PCR Method

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Abstract

Foot and mouth disease (FMD) virus was reported as an outbreak in Indonesia in April 2022 and belonged to serotype O/ME-SA/Ind-2001e is spread in the country. This study aimed to detect the causative agent based on clinical symptoms in cattle that have the vesicle in the mouth and hooves. A total of 25 samples were collected during August 2022 from Lamongan and Surabaya, Indonesia. FMD was identified in 58% (7/12) using reverse transcription polymerase chain reaction (RT-PCR), respectively. The samples were performed using universal primers with 328 bp length for primary diagnosis of FMD. These findings indicate that the spread of FMD viruses is highly contagious, so rapid and accurate diagnosis is needed as an effort to control and monitor FMD viruses.

Keywords: cattle, foot and mouth diseases, Reverse Transcription Polymerase Chain Reaction

Received: 11 January 2023 **Revised:** 23 January 2023 **Accepted:** 25 February 2023

INTRODUCTION

Foot and mouth disease (FMD) virus is one of the highly contagious diseases in cloven-hoofed animals worldwide, including cattle, buffalo, goats, sheep, and swine (Chen *et al*., 2020). The rapid spread of the FMD caused many cases of outbreaks to occur in several regions of the world, causing a decrease in productivity and livestock breeding (Zinnah *et al*., 2012). The FMD outbreaks occurred in Indonesia in April 2022 originating from Aceh province, and East Java province with a total of 3128 cases (Ditjenpkh, 2022). the results of the virus identification belong to serotype O topotype Middle East-South Asia (ME-SA) lineage Ind-2001 sub lineage e (Susila *et al*., 2022). The most prevalent causative agent of FMD in Southeast Asia is serotype O topotype ME-SA which has been widely reported in Cambodia, Myanmar, Bangladesh, and Thailand (Bo *et al*., 2019; Chanchaidechachai *et al*., 2021; Ryoo *et al*., 2021; Zhu *et al*., 2018).

Etiology of FMD is a single-stranded positive-sense RNA virus belonging to the genus Aphthovirus, family Picornaviridae with 8500 nucleotides (nt) in length surrounded by structural proteins (VP1, VP2, VP3, and VP4) and non-structural proteins (Lpro, 2A, 2B, 3A, 3B, 3Cpro, and 3Dpro) (Li *et al*., 2021). FMD was characterized by high genetic variability and there are seven different serotypes in the world, namely O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2, and SAT 3 (Di Nardo *et al*., 2014). The VP1 coding nucleotide sequence has been used for the genetic characterization of FMD strains due to its significance for viral attachment and entry into host cells, serotype specificity, and protective immunity (Ahmad *et al*., 2022).

FMD is a re-emerging disease because Indonesia experienced a widespread FMD outbreak in 1884 with O serotype topotype Indonesia-1 and Indonesia 2 and in 1990 Indonesia was declared free of FMD (Silitonga, 2017). In 2022, there was an outbreak of FMD disease that caused many losses in the livestock sector, especially beef cattle farming. This study

aimed to detect FMD from field cases in beef cattle using the reverse transcriptase-polymerase chain reaction (RT-PCR) method. The results of this study are expected to provide information to beef cattle farmers and can be used in policy and control of the virus to overcome losses due to FMD disease.

MATERIALS AND METHODS

Sample Collection

A total of 12 samples were obtained from Lamongan (n=5) and Surabaya (n=7). Samples were collected from cattle with diagnosed FMD in August 2022. Samples were taken from vesicle swabs of lesions in the mouth and feet of beef cattle from smallholder farms. Samples were collected with Phosphate Buffer Saline (PBS) with a 5% antibiotics transport medium. Samples were stored at -80°C freezer in the laboratory until analysis (Wulandari *et al*., 2021).

Extraction

FMD viral RNA was extracted from vesicle swab suspension using a QIAmp Viral RNA mini kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The RNA was eluted with 50 µl of RNase-, DNase-free water and stored at -80°C prior to use.

RT-PCR

RNA virus was amplified using a one-step RT-PCR (AMV) Takara Kit as previously described (Castells *et al*., 2020). The primers used for amplification were 5'GCCTGGTCTTTCCAGGTCT3' forward and 5'CCAGTCCCCTTCTCAGATC3' reverse (King and Henstock, 2016). The primers were synthesized by (Macrogen Co., Seoul, South Korea). Total 2,5 µl RNA sample eith 8 µl nuclease-free water, 2,5 µl, forward primer (4 pmol/ µl), 5 µl reverse primer (4 pmol/ µl), 4,2 µl, MgCl2 2,5 µl, dNTP Mix 1,25 µl, 10x one step buffer 1,25 µl, AMV r-tase 0,25 µl, AMV optimized Taq 0,25 µl, and RNAse inhibitor 0,25 µl were put into a PCR tube with the final mixture was 15 µl and then inserted into a conventional PCR machine. One-step RT-PCR cycle conditions were 30 minutes at 42°C followed by 5 minutes of 94°C inactivation, then at cycle 35 with conditions of denaturation 94°C for 60 seconds, anelling 55,5°C for 60 seconds, elongation 72°C for 60 seconds, and final extension 72°C for 10 minutes. Furthermore, PCR products were stored in a refrigerator before the electrophoresis process (Rahmahani *et al*., 2022).

Electrophoresis

The 2% concentration of gel electrophoresis was made using 3,2 g of agarose powder mixed with 160 ml of 1x TBE buffer, then heated and added 16 µl ethidium bromide, poured into a mold that had been given a tray and waited until it hardened. The gel that has hardened is inserted into an electrophoresis device that has been connected to a power supply and given 1x TBE buffer until it sinks. Samples that have been completed in PCR are taken as much as 5 µl and mixed with 1 µl Fluorescence DNA staining and a DNA Ladder of as much as 2 µl is also inserted into the first well to determine the size of the DNA results. The electrophoresis tank was closed, then the power supply was turned on at 100 volts for 30 minutes. Agarose was taken after the power supply was turned off and placed in the GelDoc device to determine the results of electrophoresis using UV light.

RESULTS AND DISCUSSION

Based on the result, the detection rate of FMD-positive samples using universal FMD primers was 58% (7/12), respectively. The positive result of FMD is indicated by the appearance of a 328 bp DNA band from the RT-PCR results in the well of the electrophoresis gel (Figures 1 and 2). The results of this study are consistent with the previous study that RT-PCR was used in the detection of FMD for primary diagnosis with a universal primer set 1F/1R with an expected band of 328 (Mansour *et al*., 2018). The universal primer pairs IF/IR-based RT-PCR along with sequence detection can be used in the primarily rapid detection of FMD in infected animals (Le *et al*., 2012; Mansour *et al*., 2018).

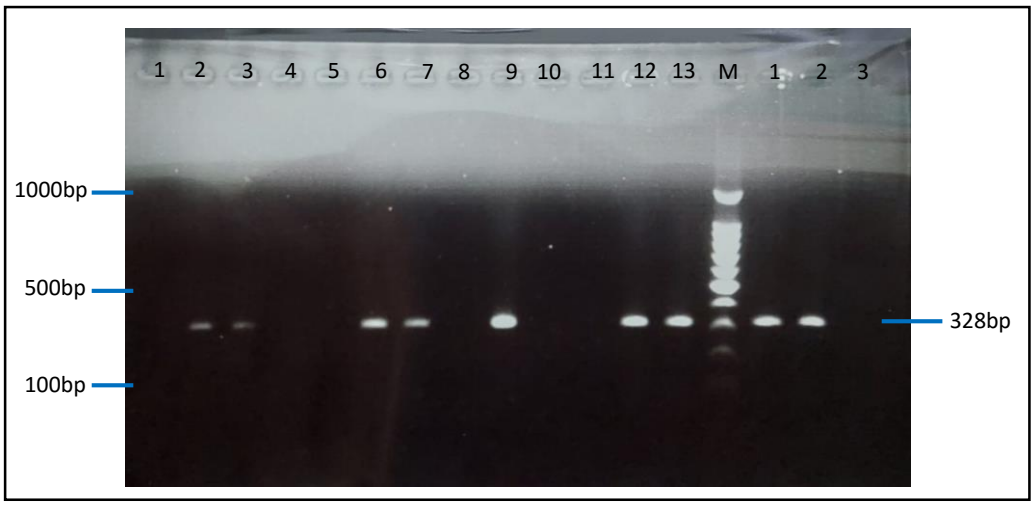

Figure 1. Agarose gel electrophoresis; M is 100 bp marker ladder DNA, Positive bands FMD in Banyuwangi samples No.2,3,6,7,9,12,13 and Surabaya samples No.1,2.

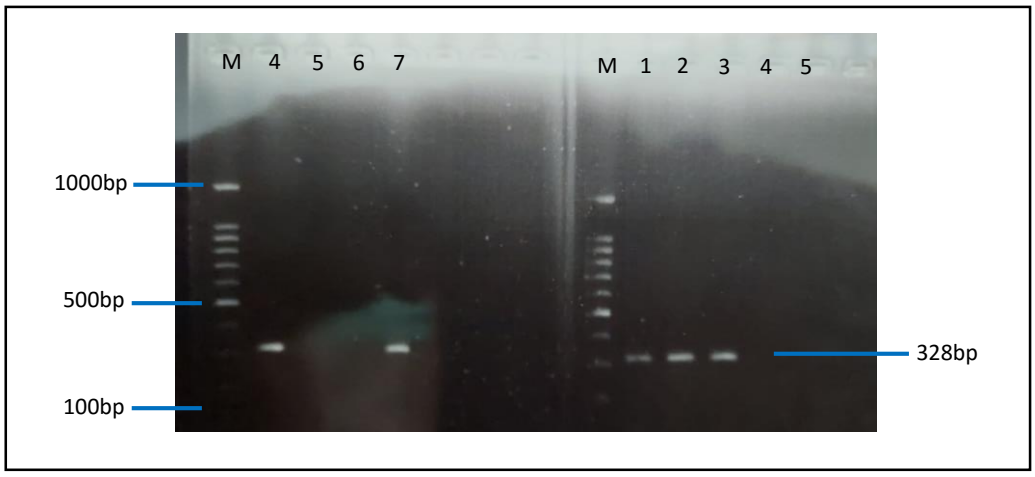

Figure 2. Agarose gel electrophoresis; M is 100 bp marker ladder DNA, Positive bands FMD in Surabaya samples No.4,7 and Lamongan samples No.1,2,3.

The RT-PCR test on the samples was conducted to confirm the diagnosis of suspected FMD infection that caused a decrease in livestock productivity referring to the findings of clinical symptoms such as an increase in body temperature of 40°C, excessive saliva secretion, loss of appetite and tasty, depression, and decreased milk product (Biswal *et al*., 2019). The most dominant in FMD infection is the discovery of vesicles on the tongue, gums, lips, and nose, between the hooves, so an RT-PCR examination was carried out for further examination (Sheikh *et al*., 2021). RT-PCR is a highly sensitive and specific test that can be used in first detecting FMD outbreaks worldwide (Madhanmohan *et al*., 2013; Sheikh *et al*., 2021; Wong *et al*., 2020). The FMD outbreak by December 2022 had spread to 16 provinces in Indonesia with 595,785 cases and 10,801 deaths (Ditjenpkh, 2022). Economic losses are not only experienced by beef cattle farmers but also by dairy cattle, sheep, and goat farmers. FMD can occur due to illegal trade of FMD-contaminated meat, illegal entry of FMD-infected livestock or carriers, and contaminated feed equipment (Silitonga, 2017).

FMD viruses have a low molecular agent weight ranging from 7,2 to 8,4 kb and diameter sizes ranging from 25 to 30 nm (Chanchaidechachai *et al*., 2021). The simple structure and small size of the virus facilitate the rapid transmission of the virus through the air, allowing it to spread over long distances in a very short time by following the wind speed (Puckette *et al*., 2017). Fast and accurate FMD diagnosis is

needed for the formulation and development of effective and targeted outbreak control policies so that FMD disease management is immediately under control. In this study, we investigated the FMD infection after an outbreak in Indonesia in April 2022 among cattle in East Java, Indonesia. Vaccination has been promoted in Indonesia since confirmed cases of FMD, surveillance must be carried out continuously to monitor virus serotypes and evaluate the emergence of new FMD strains.

CONCLUSION

It can be concluded that the band marker form RT-PCR Technique can accurately detect the presence of FMD Virus infection in beef cattle in smallholder farms in Lamongan and Surabaya.

ACKNOWLEDGEMENTS

The authors would like to thank the cattle farmers in Lamongan and Surabaya, and the staff in the Viral Diarrhea Laboratory for their contribution during the study.

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