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Foot and Mouth Disease Virus Detection in Bali Cattle (*Bos sondaicus*) by RT-PCR in Lombok Island

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ABSTRACT

Aphthovirus is responsible for foot-and-mouth disease (FMD) in cloven-hoofed animals, a highly infectious disease that has significant economic repercussions in various countries, including Indonesia. The present study aimed to use reverse transcription polymerase chain reaction (RT-PCR) to detect FMD in suspected Bali cattle in West Lombok, Indonesia. The current study was an observational, descriptive investigation conducted from July to August 2025, collecting 15 swab samples from male Bali cattle with an average weight of 210 kilograms and an average age of 2 years old. The samples were collected via purposive sampling from cattle demonstrating clinical signs of FMD, specifically from oral vesicular fluid. The samples were sourced from two smallholder farms in West Lombok; farm one, with 12 samples, and farm two, with three samples. The FMD was identified with a prevalence rate of 20% (3 out of 15), and a 328 bp DNA fragment was detected during gel electrophoresis. The current result indicated that the virus pathogen was detected in 20% of samples, and RT-PCR can be used as a high-sensitivity diagnostic method for this disease.

Keywords: Bali cattle, Foot-and-Mouth disease, PCR, Reverse transcription

INTRODUCTION

Bali cattle (*Bos sondaicus*), known for their high productivity and genetic diversity, are among Indonesia's most valuable livestock (Saili, 2020; Hayanti et al., 2022). Bali cattle are essential to Indonesia's food security, offering villagers both income and a source of livestock protein. These cattle are distributed across Indonesia, notably on Lombok Island in West Nusa Tenggara. According to the Central Statistics Agency of West Nusa Tenggara Province, Indonesia, there were 1,219,784 Bali cattle recorded in the province in 2022 (BPS, 2020; 2024).

Lombok Island supplies beef to several regions in Indonesia through the 1,000 Cattle Village program. This program is a government policy to achieve food self-sufficiency, initiated by a pilot project in West Nusa Tenggara Province, which is one of the five largest beef cattle-producing provinces in Indonesia. Since 2013, Lombok Island has encompassed around 504 livestock farmer groups, of which 238 comprised novice and conventional farmers. Considering the potential of Bali cattle, which are beef-producing cattle in West Nusa Tenggara Province, the existence of Bali cattle farms is crucial for supporting the national food security (Ranta et al., 2022). Since 2022, Foot-and-Mouth Disease (FMD) has become a major problem on Lombok Island, especially in Central Lombok, where 28,612 cases have been reported out of a cattle population of 323,232 (Bani and Asruddin, 2022; Septiani et al., 2023).

Cattle farming is particularly economically affected by FMD. In non-endemic countries, the economic impact of FMD can exceed US$1.5 billion annually, while in endemic areas, losses can range from US$6.5 billion to US$21 billion (Knight-Jones and Rushton, 2013). There are seven serotypes of the RNA virus that cause FMD, including O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3, which belong to the genus Aphthovirus within the Picornaviridae family (Grubman and Baxt, 2004). Indicators of Foot-and-Mouth Disease (FMD) in cattle encompass fever (40°C), diminished appetite, decreased rumen activity, excessive salivation, lowered milk yield, difficulty breathing, rapid breathing, and groaning. Additionally, there may be vesicular lesions, erosions, and ulcers observed in the oral cavity, interdigital spaces, as well as on the muzzle and teats (Mohebbi et al., 2017). The FMD in Indonesia is a disease that has reemerged, with historical records indicating its presence as early as 1887 (Zainuddin et al., 2022). The FMD spreads rapidly and has caused outbreaks in several regions worldwide, including Indonesia. The FMD virus circulating in Southeast Asia is serotype O, topotype ME-SA, which has been widely reported in Myanmar and Thailand (Bo et al., 2019;

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Chanchaidechachai et al., 2021). The FMD outbreak in Indonesia occurred in May 2022 and was detected with serotype O/ME-SA/Ind-2001 (Susila et al., 2023).

The FMD virus particles can be detected using a sandwich enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR). The main advantage of RT-PCR over ELISA for detecting FMD in cattle is its significantly higher sensitivity (Paixão et al., 2008). The reported sandwich ELISA for FMD virus targeting the VP1 protein achieved 100% specificity and 80% sensitivity, though positive results require verification by a neutralization test. Conversely, a multiplex PCR test exhibited 100% specificity but only minimal sensitivity (Sharma et al., 2015). In 2023, a study indicated that 58% of cattle in Lomongan and Surabaya, East Java, were infected with FMD as detected by RT-PCR (Dinana et al., 2023). Another study on the diagnosis of FMD in field conditions in Brazil, using RT-PCR, identified three positive samples out of 260 oral swabs, which were collected from cattle in areas with FMD outbreaks (Paixão et al., 2008).

Several initiatives have been undertaken on Lombok Island to overcome FMD. These strategies include educating livestock farmers through participatory methods that emphasize early detection, implementing biosafety and biosecurity vaccination programs, and addressing the impact of livestock movement and regulations (Kholik et al., 2024). Government and community efforts to combat FMD have been inadequate, resulting in an FMD outbreak in Lombok Island, potentially allowing the virus to remain in the environment, infecting susceptible animals, and becoming a source of FMD transmission. The virus can only survive long-term when it is repeatedly brought in from outside the herd (McLachlan et al., 2019). Theoretically, an alternative mechanism for the presence of the FMD virus in a region could be the carrier's ability to support its persistence (Guyver-Fletcher et al., 2022). Accurate detection of FMD virus in susceptible animals is essential as an initial step, supported by surveillance of carriers and the environment, and accompanied by increased public knowledge to overcome FMD disease. Humphreys et al. (2025) stated that critical knowledge gaps in FMD epidemiology hinder effective control of FMD.

According to Ministry of Agriculture Decree No. 708 of 2024, all regencies in Bali and West Nusa Tenggara, Indonesia, are affected by FMD (Denpasar Veterinary Center, 2025). Molecular-based investigations on FMD virus in smallholder livestock are essential for early detection and accurate diagnosis using RT-PCR to prevent transmission. Several studies have used RT-PCR to detect and perform initial screening for FMD virus in cattle. Given the large number of smallholder farms, especially in West Lombok, early detection of the FMD virus is critically important. This study would be a novel approach for practical field application, enabling fast, accurate diagnosis and early warning detection to help eradicate FMD virus transmission. The current study aimed to detect FMD virus in Bali cattle on smallholder farms in West Lombok, Indonesia, using RT-PCR based on clinical signs.

MATERIALS AND METHODS

Ethical approval

This study was conducted according to the guidelines of Mandalika University, Mataram, Indonesia. The Samples of vesicular fluid were collected for FMD virus analysis under the supervision of a veterinarian from the Faculty of Veterinary Medicine, Mandalika University of Education, Mataram, Indonesia, with 129/C3/DT.05.00/PL/2025 contract number.

Study design

The present observational descriptive study was conducted from July to August 2025, focusing on Bali cattle on two smallholder farms on Lombok Island, West Nusa Tenggara Province, Indonesia, bordering Bali Island. This border area has the potential for FMD transmission between different regions due to the presence of Lembar Port (a trade center). These farms were suspected of FMD infection and exhibited FMD-like signs. Purposive sampling was used to select animals exhibiting signs of FMD. The male Bali cattle, around 2 years old with an average weight of 210 kg, were observed for clinical signs of FMD, including nasal discharge, oral lesions, and muscle weakness. The sample size for the Bali cattle nasal vesicular fluid swab was determined using the Thrusfield formula for detecting disease in the population (Thrusfield, 2007).

Sample collection

Sampling was conducted from July to August 2025 following the protocol established by Edwards et al. (2024). A total of 15 nasal swab samples were collected from Bali cattle showing clinical signs of FMD on two smallholder farms in West Lombok, Indonesia. These farms were the Gerung People's Farm, located at latitude -8.686738° and longitude 116.0941378 (8°41'12.3"S 116°05'38.9" E; Farm 1), and the Lembar People's Farm, located at latitude -8.767573 and longitude 116.074415 (8°46'03.3"S 116°04'27.9"E; Farm 2; Figure 1). From the first farm, with a population of 50 Bali

cattle, 12 samples were collected, and from the second farm, with 10 Bali cattle, three samples were collected. The samples were collected by swabbing vesicular lesions and oral discharge directly from the cattle using a sterile swab. The collected samples were stored at -80°C in the laboratory of the Faculty of Veterinary Medicine at Mandalika University of Education, Mataram, Indonesia, until further analysis (Edwards et al., 2024). The samples were then transferred to the diagnostic laboratory at the Professor Nidom Foundation, Indonesia, in an ice box.

Figure 1. Sampling locations. Location marked with an asterisk, number 1 is the Gerung smallholder farm, Gerung City, Indonesia. Location marked with an asterisk, number 2 is Lembar smallholder farm, Lembar City, Indonesia (Source: Google Maps)

Extraction of RNA virus

Following the manufacturer's instructions, FMD viral RNA was extracted from samples using the QiAamp viral RNA mini kit (Qiagen, Hilden, Germany) and eluted in 50 µL of nuclease (DNase)-free water (Edwards et al., 2024).

RT-PCR procedure

Viral RNA was amplified by RT-PCR using universal primers, including the forward primer 5′-GCCTGGTCTTTCCAGGTCT-3′ and the reverse primer 5′-CCAGTCCCCTTCTCAGATC-3′ (Reid et al., 2000), resulting in an amplicon of 328 bp.The PCR reaction mixture was prepared by combining 4 µl of 5× first-strand buffer, 2 µl of acetylated bovine serum albumin (1 mg/ml), 1 µl of dNTPs (10 mM), 0.2 µl of dithiothreitol (DTT; 1 M), and 1 µl of Moloney murine leukemia virus reverse transcriptase (200 U/µl). The amplification protocol consisted of an initial pre-denaturation at 94 °C for 5 min, followed by denaturation at 94 °C for 20 s, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. A final extension step was performed at 72 °C for 7 min. The PCR was conducted for a total of 35 cycles (WOAH, 2022). According to the study by Dinana et al. (2023), the results of FMD viral amplification by RT-PCR were visualized on an agarose gel 2%.

RESULTS AND DISCUSSION

Bali cattle examined on two farms exhibited FMD-like clinical signs, including lesions on the snout, hypersalivation, fever, and hoof lesions (Figure 2). The RT-PCR results of the vesicular fluid swabs yielded a 20% FMD detection rate (3/15). One positive FMD sample out of 12 samples collected from smallholder Farm 1, and two positive FMD samples out of three samples collected from smallholder Farm 2. Additionally, the presence of the 328 bp DNA band on gel electrophoresis indicated a positive result for FMD virus infection in Bali cattle (Figures 3 and 4).

These current results, demonstrating a 20% detection rate for FMD, are consistent with those of Dinana et al. (2023) in Lamongan and Surabaya, Indonesia, who used RT-PCR with universal primers and detected the 328 bp FMD virus segment in symptomatic cattle. In Al-Qadisiyah Province, Iraq, FMD has been detected in 73 samples (75.3%) using RT-PCR with universal primers at 330 bp (Mansour et al., 2018). The differences between the present results and other studies using RT-PCR are likely due to genetic variation in the virus. Although universal primers are designed to detect all strains of FMD virus, differences in viral genetic sequences can prevent efficient primer annealing. Meanwhile, Sharma et al. (2015) indicated that the detection of FMD virus using the VP1 target region protein in a sandwich ELISA should be confirmed by a neutralization test, which is more time-consuming. To confirm a suspected FMD diagnosis based on clinical signs, RT-PCR was performed on persistently infected cattle under field conditions on a dairy farm in India following the natural FMD outbreak (Biswal et al., 2019).

Figure 2. The clinical signs of foot and mouth disease in Bali cattle in West Lombok Regency of West Nusa Tenggara Province, Indonesia. **a**: The erosions on the nose (arrow), **b**: Hypersalivation (arrow), **c:** The ulcers in the mouth (arrow), **d:** The erosive lesions on the foot (arrow); Source: Authors of the current study.

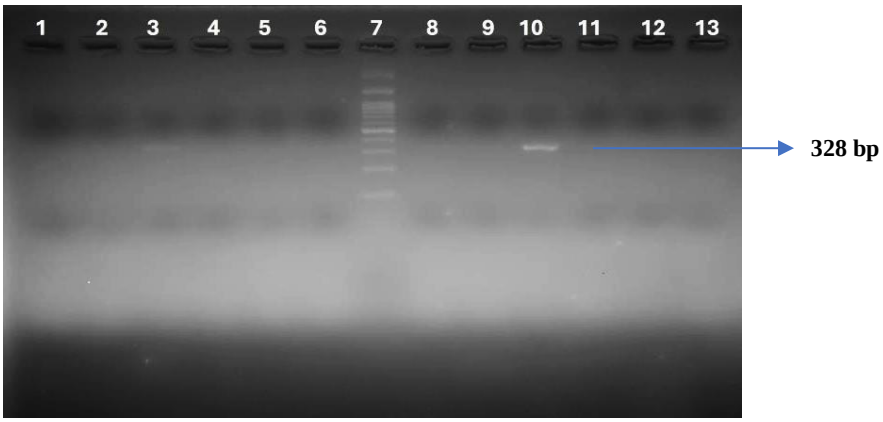

Figure 3. The RT-PCR product analysis using 2% agarose gel electrophoresis from Gerung smallholder farm in Gerung City, West Lombok Regency, West Nusa Tenggara, Indonesia (location 1). Lane 7: 100 bp marker ladder, lane 11: Positive bands of FMD at 328 bp, lanes 1,2,3,4,5,6,8,9,11,12,13: Negative bands of FMD.

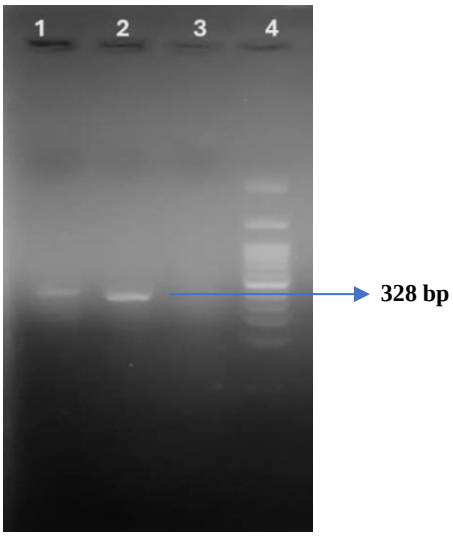

Figure 4. The RT-PCR product analysis using 2% agarose gel electrophoresis from Lembar smallholder farm in Lembar City, West Lombok Regency of West Nusa Tenggara Province, Indonesia (location 2). Lane 4: 100 bp marker ladder, lanes 1 and 2: Positive bands of FMD at 328 bp, lane 3: Negative bands of FMD

Detecting FMD virus from nasal vesicular swabs in Bali cattle with RT-PCR was quick and precise, and it was a non-invasive method that simplified data collection for field veterinary officers. This enabled a timely response, thereby helping to control the transmission of FMD. The RT-PCR method for early detection of FMD in cattle provides benefits over ELISA, as it is more precise in identifying small quantities of viral genetic sequences, such as RNA. The ELISA typically detects viral antibodies, which do not necessarily indicate active infection (Brahma et al., 2024). The laboratory

results indicated that RT-PCR can provide a definitive diagnosis in supernatant fluid from cell cultures inoculated at the first passage, and its sensitivity is greater than that of ELISA in vesicular epithelial suspensions and at least equivalent to virus isolation in cell cultures in FMD examinations (Reid et al., 2023). The RT-PCR is an effective method for detecting FMD in the field compared to virus isolation and ELISA (Paixão et al., 2008). In Brazil, RT-PCR was used as a diagnostic tool to verify FMD in the field. Out of 460 oral swabs taken from cattle in both affected and FMD-free regions, three samples from areas experiencing FMD outbreaks tested positive via RT-PCR, whereas only two samples were confirmed positive through virus isolation and ELISA (Paixão et al., 2008). In Iraq, RT-PCR was conducted using primers for VP1 serotype O as the initial diagnostic examination for FMD in cattle from local farms in Sulaimani province. Phylogenetic analysis indicated that the resulting isolates were Pakistani (KU365843) and Iranian (KY091283) strains, with sequence identities of 96.00% and 95.00%, respectively (Baba Sheikh et al., 2021).

In a study conducted by Reid et al. (2000), RT-PCR detected the virus in 85% of 28 epithelial suspension samples, whereas ELISA yielded a lower detection rate of 62%. A previous study in Punjab, Pakistan, employing RT-PCR with the universal VP1 primer pair P1/P2 on 250 tissue and 175 secretion samples, detected FMD in 72.8% of tissue samples and 52.6% of secretion samples from cattle, buffalo, and goats (Saeed et al., 2011).

A research was carried out in West Lombok Regency, located in the West Nusa Tenggara Province of Indonesia, which shares a border with Bali. Consequently, this closeness presented a risk of transmission, given that the FMD virus has demonstrated the ability to easily cross-national boundaries and trigger epidemics in regions that are free from the disease (Mansour et al., 2018). Additionally, the FMD virus can spread rapidly without proper and immediate control measures. This risk was heightened on Lombok Island, where smallholder farms used conventional practices with minimal biosecurity and inadequate disinfection. Furthermore, the presence of animals such as goats, sheep, and buffalo around the farm could pose a risk, as they may carry the FMD virus. A study on FMD risk factors in East Java, Indonesia, found that the absence of routine disinfection (OR = 3.98) and the sharing of equipment with infected animals (OR = 3.39) were considerably associated with disease outbreaks (Rehman et al., 2025). Detecting FMD virus in Bali cattle using RT-PCR facilitated accurate diagnosis from molecular-based field isolates, enabling identification of the virus type and informing vaccination strategies. Moreover, RT-PCR helped trace transmission sources and supported control of FMD outbreaks in regions.

CONCLUSION

The RT-PCR method determined a prevalence rate of 20% for the FMD virus in the 15 collected samples (3 positive out of 15), with a 328 bp DNA fragment. The current results provided a deeper understanding of FMD virus distribution in the study area, serving as critical information for mapping viral distribution and informing effective FMD control strategies on Lombok Island. Further analysis of PCR product sequencing is essential to identify FMD viruses associated with the Indonesian government's vaccination program. In addition, FMD virus surveillance and monitoring of vaccination results are very necessary to anticipate the emergence of new strains of the FMD virus.

DECLARATIONS

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Authors' contributions

Kholik Kholik conceptualized and designed the study, collected samples, and prepared the manuscript. Akhmad Sukri designed the methodology and directed the study. Ieke Wulan Ayu assisted in the research process, edited, and checked the writing of the manuscript. Reviany Vibrianita Nidom and Setyarina Indrasari conducted sample examinations in the laboratory and data analysis. All authors read and approved the final edition of the manuscript for processing and publication in the present journal.

Availability of data and materials
All data and materials are available upon reasonable request from the corresponding author.

Competing interests
The authors declare no conflict of interest.

Ethical considerations
Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. The authors confirmed that AI tools has not been used during this research.

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