Jurnal Kajian Veteriner
ISSN: 2356-4113
E-ISSN: 2528-6021
Vol. 13 No. 1: 40-46 (2025)
DOI: https://doi.org/10.35508/jkv.v13i1.19609

Molecular Identification of African Swine Fever Virus from an Outbreak in Kupang Region

(Identifikasi Molekuler Virus African Swine Fever yang Berasal dari Wabah di Daerah Kupang)

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ABSTRAK

African Swine Fever (ASF) adalah infeksi virus penting pada babi yang belakangan ini muncul kembali sebagai penyakit lintas batas yang menyebar dengan luas dan cepat. Konfirmasi pertama ASF tercatat di Indonesia pada akhir tahun 2019 dan kemudian meluas ke beberapa daerah termasuk Nusa Tenggara Timur (NTT). Informasi tentang virus ASF di NTT terbatas hingga saat ini. Studi ini bertujuan untuk mengidentifikasi keberadaan virus ASF pada kasus kematian massal babi di Kupang, Pulau Timor. Sampel ginjal dan limpa diambil dari delapan ekor babi yang mati mendadak di daerah Kupang. Teknik PCR konvensional dilakukan untuk mengidentifikasi daerah yang sangat terkonservasi dari genom virus ASF VP72. Hasil PCR menunjukkan bahwa semua sampel menampilkan panjang pita yang sesuai dengan pita target dari primer yang digunakan. Hasil ini menunjukkan bahwa virus ASF masih beredar di wilayah Kupang dan tetap menjadi ancaman bagi peternakan babi di daerah tersebut. Oleh karena itu, usaha untuk meningkatkan kesadaran publik terutama di kalangan produsen babi mengenai pentingnya penerapan biosekuriti harus dilakukan secara konsisten.

Kata Kunci : *African Swine Fever*; virus ASF; babi; Kupang; Nusa Tenggara Timur; PCR

INTRODUCTION

Recent outbreaks of African Swine Fever (ASF) in various regions have jeopardized the swine industry worldwide. The economic impact is significant, involving numerous stakeholders, despite its limited host range and lack of zoonotic transmission (Dixon et al., 2020). The continuing epidemic has significantly impacted backyard and smallholder pig farming in resource-constrained environments in the Caribbean, Asia, Eastern Europe, and the Pacific (Penrith et al., 2023).

ASF is a highly infectious and lethal viral hemorrhagic disease in pigs (Spickler, 2019). This disease is caused by the African Swine Fever Virus (ASFV), a double-stranded DNA (dsDNA) virus that belongs to the genus *Asfivirus*, family *Asfarviridae* (Alonso et al., 2018). This virus is resistant to environmental conditions, can remain infectious at low temperatures (Blome et al., 2020).

In Indonesia, mass pig deaths from backyard farms in Dairi and Humbang Hasundutan, North Sumatra Province, marked the emergence of ASF in September 2019. Since then, 521 ASF outbreaks have been reported from 21 North Sumatra districts, killing 40,000 pigs between July and December 2019 (Dharmayanti et al., 2021). By 2024, ASF has been documented in 26 out of 38 provinces in Indonesia, including Nusa Tenggara Timur (NTT) province (Kurniawan, 2024).

Timor Island in the NTT province reported an outbreak of ASF in February 2020, resulting in the death of 24,822 pigs throughout 12 districts and one city from January to June 2020 (DISNAKPROVNTT, 2020). In February 2021, WOAH reported that 2,432,501 pigs were at risk of contracting ASF in this region, and 62,961 pigs had died due to ASF in NTT out of 103 documented incidents. The ASF virus has been documented in nearly all districts of NTT (WOAH, 2021).

NTT Province has Indonesia's largest pig population (BPS 2020). Pig farming in NTT consists mainly of backyard and small-scale pig farms (Johns et al., 2009; Sawford et al., 2011). In NTT, pig farming provides extra income, emergency cash, animal protein, and social importance in traditional ceremonies for various ethnic groups (Leslie et al., 2015). The significant loss of pigs due to the ASF virus seriously impacts the economic and sociocultural components of NTT society.

Despite the prevalence of ASF in almost every region of NTT, data and information on the occurrence and development of ASF in NTT, particularly in the Kupang area, are limited to this day. Thus, the purpose of this study is to determine the presence of ASFV in cases of severe pig mortality in Kupang during the ASF outbreak. The finding will serve as a scientific basis for further ASFV molecular studies.

MATERIALS AND METHODS

Kidneys and spleens were collected from 8 dead pigs from Kupang backyard farms. Purposive sampling was used in this study, and organ samples were obtained from recently reported dead pigs (less than an hour).

The samples were tested at the Institute of Tropical Disease (ITD), Airlangga University in Surabaya. DNA was extracted using the QIAamp® DNA Mini Kit (QIAGEN). ASFV was identified using the conventional PCR (Polymerase Chain Reaction) method and a pair of primers (PPA-1 and PPA-2) that target a highly conserved region of the ASF virus genome (VP72). These primers amplify a segment of the VP72 gene that is 257 base pairs long (Aguero et al., 2003).

This study used Intron Biotechnology's 2X PCR Master Mix Solution (i-TaqTM). The PCR amplification products were then viewed using 2% agarose gel electrophoresis.

RESULTS AND DISCUSSION

The PCR showed 257 bp bands in all eight Kupang backyard pig farm samples (Figure 1). All samples fall within this PCR test's primer target band range. This primer has previously been used to detect ASFV in domestic pigs in Uganda, China, Vietnam, India, South Korea, and other countries (Zhou et al., 2018; Kim et al., 2020; Nguyen et al., 2021; Rajkhowa et al., 2022). According to WOAH (2021), ASF cannot be differentiated from Classical Swine Fever (CSF) or other hemorrhagic diseases in pigs based only on clinical or post-mortem examinations. Thus, laboratory tests are essential for distinguishing among these different diseases. Among the several laboratory tests available for detecting ASF infections, genomic detection by the PCR method is acknowledged as the gold standard for ASF diagnosis (WOAH, 2021). Identifying the ASF virus and its genetic variety in different locations is critical for conducting extensive investigations, understanding its dissemination and distribution patterns, and assisting in vaccine development and global eradication initiatives.

ASF virus genetic diversity and molecular epidemiology are often assessed by sequencing the 3' terminal end of the B646L gene, which encodes the major capsid protein p72. The gene's partial sequencing revealed 24 ASF genotypes (I-XXIV) (Quembo et al., 2018; Netherton et al., 2019; Urbano et al., 2021).

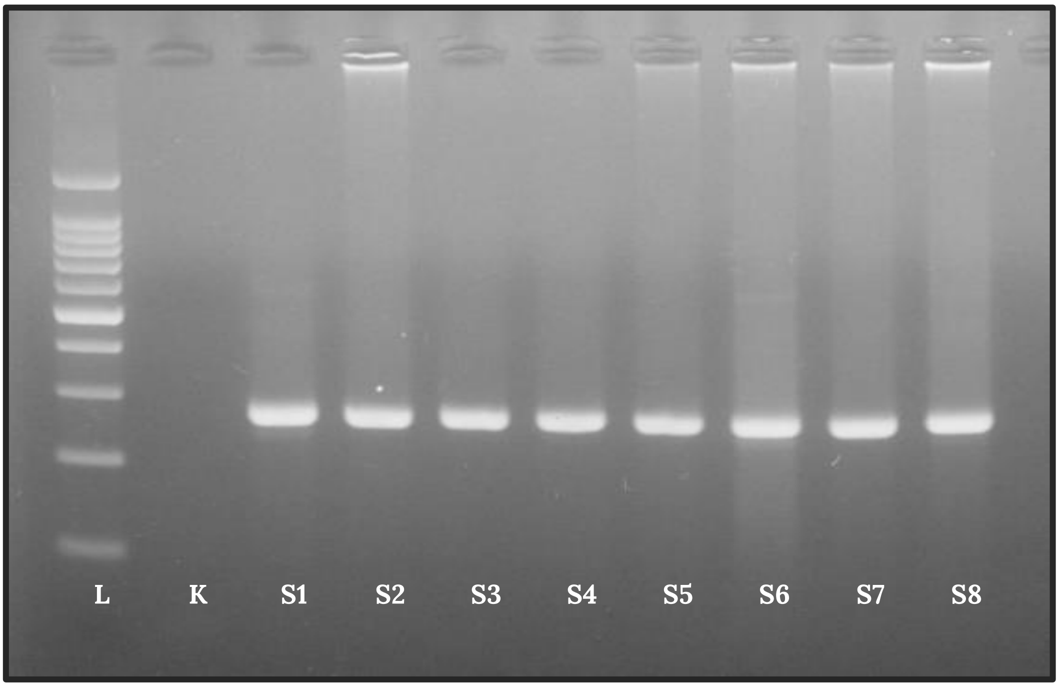

Figure 1. The visualization of the agarose gel electrophoresis results from 8 samples collected from the Kupang area.
L (Ladder); K- (Negative control); S1-S8 (Sample 1- 8)

Based on its geographical distribution, it is known that all 24 genotypes of the ASFV have been identified from various regions in Africa (Penrith et al., 2023). Genotypes I and II are the most frequently reported ASF virus genotypes, accountable for numerous acute and sub-acute cases globally (Gallardo et al., 2021). Sardinia Island in Italy is the sole location outside Africa identified as endemic to ASFV genotype I (Torresi et al., 2020). In contrast, ASF outbreaks outside Africa, especially in Europe and the Asia-Pacific, are predominantly associated with genotype II (Kedkovid et al., 2020).

Dharmayanti's (2021) prior phylogenetic study determined that the ASFV samples from North Sumatra and West Java were similar, suggesting a shared source of infection, and classified them under the p72 genotype II and serogroup 8. The sequences from the Indonesian ASFVs were identical to those of other genotype II ASFVs from domestic pigs in Vietnam, China, and Russia. Moreover, the CD2v (EP402R) gene sequencing study revealed that the two ASFV isolates from North Sumatra and West Java are classified under serogroup 8 (SG8), the same group with ASFV from Georgia, Russia, Vietnam, and China (Dharmayanti et al., 2021). The ASFV strains obtained in Southeast Asian countries, specifically Vietnam, Indonesia, and Malaysia, exhibit high homology with one another and show genetic similarity to the genotype II ASFV isolated in China (Dharmayanti et al., 2021; Khoo et al., 2021; Nguyen et al., 2022).

Sandow et al. (2020) list several potential ASF virus entry routes into Indonesia, including swill feeding on commercial flights from infected countries or contaminated luggage, swill feeding of leftover hotel food from tourists from ASF-affected regions, and fomites carried by travelers from ASF-affected countries. A report from the WOAH (2021) suggests that transporting live pigs across regions and islands and fomites from farm workers, vehicles, and animals are the main risk factors for ASF in Indonesia. Arimurti et al. (2021) also found that food waste from foreign ships may serve as a route for ASF to reach Indonesia.

The province of NTT has the largest pig population in Indonesia, and ASF disease threatens the sustainability of the pig industry, notably in Kupang, which has the most pigs. Tenaya et al. (2023) reported that ASFV in East Nusa Tenggara and Bali devastated pig breeders, mainly small-scale farmers. The ASFV endemic phase and uncontrolled spread to non-infected areas caused significant damage. Several ASFV-infected pigs survive while maintaining the virus in their tissues and circulation. These carrier pigs provide a possible route for viral transmission (Tenaya et al., 2023). The extensive ASF infections adversely impacted farmers and significantly increased pork prices, severely affecting consumers' livelihoods. As of the end of June 2023, Indonesia has registered a modest total of 43 notifications, yet the outbreak has been confirmed in 10 out of 34 provinces within the swine sector, resulting in the death of almost 3.5 million pigs (Ito et al., 2023).

Given the importance of pigs to the local economy and socio-culture, disease management and eradication activities must be prioritized. Early detection programs, routine surveillance and reporting, epidemiological studies, and preventive measures, including biosecurity, livestock and product transportation monitoring, and stamping out, are used to control and eradicate the disease (Beltrán-Alcrudo et al., 2017).

CONCLUSION

ASF continues to represent a significant threat to NTT's pig farming sector. This is shown by the ongoing pig mortality connected to this virus, particularly in the Kupang region. This disease is a significant threat to animal health. It impacts the socioeconomic and cultural aspects of pig farmers, who are mostly small-scale and rely on their animals for traditional rituals and savings. Early detection, financial support, and biosecurity measures pose challenges in preventing and eradicating Kupang's ASF outbreaks. As a result, all stakeholders must actively contribute to raising farmers' awareness regarding biosecurity and ASF prevention.

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