



SHORT COMMUNICATION

First Report of Zoonotic Hepatitis E Virus (HEV-3b) in Toraja Pigs, Indonesia, with a Novel Variant

Dimas Ahmad Rizaldi¹, Okti Nadia Poetri², Dimas Novianto^{3,4}, Leriarni Tumanan⁵ and Ekowati Handharyani^{6*}

¹Animal Biomedicine Program, School of Veterinary Medicine and Biomedical Sciences IPB University, Bogor, 16680, Indonesia; ²Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences IPB University, Bogor, 16680, Indonesia; ³Center of Excellence in Veterinary Parasitology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand; ⁴Research Center for Veterinary Science, National Research and Innovation Agency, Bogor, 16911, Indonesia; ⁵Department of Agriculture, Food Security, and Fisheries of Tana Toraja, Makale, 91811, Indonesia; ⁶Division of Pathology, School of Veterinary Medicine and Biomedical Sciences IPB University, Bogor, 16680, Indonesia

*Corresponding author: ekowatieko@apps.ipb.ac.id

ARTICLE HISTORY (25-525)

Received: June 06, 2025
Revised: October 04, 2025
Accepted: October 11, 2025
Published online: October 30, 2025

Key words:

Hepatitis E virus
Indonesia
Mutation
Pig farming
Tana Toraja
Zoonosis

ABSTRACT

Surveillance of swine hepatitis E virus (HEV) in Eastern Indonesia has not been previously conducted. Tana Toraja Regency, a major pig-farming area in Sulawesi, is characterized by close human-pig interactions that may facilitate zoonotic transmission. In January 2024, 100 pig fecal samples were collected from 14 smallholder farms and pooled by farm, resulting in 14 pooled samples. HEV detection was performed using reverse transcription-nested PCR targeting the ORF2 and ORF1 methyltransferase (MeT) regions. HEV was identified in two farms (14.3%). Phylogenetic and pairwise alignment analyses classified the Tana Toraja HEV strains as subtype 3b, showing the highest similarity to human-derived HEV isolates from Japan. Sequence analysis further revealed a novel single nucleotide variant leading to an A83T amino acid substitution in the ORF1 MeT gene, which was predicted to be deleterious. This study provides the first evidence of swine HEV occurrence in Eastern Indonesia and highlights its potential zoonotic risks.

To Cite This Article: Rizaldi DA, Poetri ON, Novianto D, Tumanan L and Handharyani E, 2025. First report of zoonotic hepatitis E virus (HEV-3b) in toraja pigs, Indonesia, with a novel variant. Pak Vet J. <http://dx.doi.org/10.29261/pakvetj/2025.287>

INTRODUCTION

Hepatitis E virus (HEV) is the only human hepatitis virus with a confirmed zoonotic nature (Brown *et al.*, 2025). Hepatitis E virus (HEV) is an RNA virus with a single-stranded positive-sense genome. It belongs to the *Hepeviridae* family and the genus *Orthohepevirus*. To date, eight distinct genotypes (HEV-1 to HEV-8) have been identified. Among these, HEV-3 and HEV-4 are recognized for their zoonotic capacity, with pigs and wild boars identified as the primary reservoir species (Ahmad *et al.*, 2022). In humans, the infection is generally self-limiting and manifests as acute hepatitis. However, in immunocompromised individuals, it may progress to a chronic infection and can be life-threatening (Luo *et al.*, 2024). In pigs, infection is usually subclinical but plays a significant role in viral transmission (Meester *et al.*, 2021). Zoonotic transmission from pigs to humans occurs via the fecal-oral route or consumption of infected pork products (Ahmad *et al.*, 2022).

Globally, human HEV infection is estimated to affect 20 million people annually, with only 3.3 million symptomatic cases (Liang *et al.*, 2022). In pigs, HEV antibody seroprevalence can reach up to 60%, and viral RNA has been detected in approximately 13% of pigs and pork products (Li *et al.*, 2021). Nevertheless, HEV remains under-recognized in several countries, including Indonesia. Historically, hepatitis E outbreaks were reported in West Kalimantan Province in 1987 and East Java Province in 1998. Since then, HEV surveys in Indonesia have been scarce and mostly limited to the western region (Widasari *et al.*, 2013; Raji *et al.*, 2021). The high global prevalence of HEV, combined with historical outbreaks in Indonesia, underscores its potential as a re-emerging zoonosis.

Pigs are a major livestock commodity in Eastern Indonesia, with one of the key production areas located in the Tana Toraja Regency of South Sulawesi Province. In this region, the local Toraja pig holds significant cultural value (Lase *et al.* 2024). Farming systems are typically smallholder-based, semi-intensive, and located near the

farmers' residences. The close interactions between local communities and pigs, which serve as potential reservoirs for HEV, indicate a tangible zoonotic risk. However, no HEV surveillance has been conducted in the Tana Toraja Regency. Therefore, this study aimed to (1) detect HEV in local Toraja pigs, (2) conduct molecular characterization and phylogenetic analysis, and (3) assess the genetic variations of the obtained HEV sequences. This study represents the first documented attempt to detect HEV in swine from the eastern region of Indonesia.

MATERIALS AND METHODS

Ethical clearance: This study was conducted with the permission and supervision of the SVMBS IPB University Animal Ethics Committee (certificate number 217/KEH/SKE/VI/2024).

Sampling: Sampling was conducted in January 2024, yielding 100 pig fecal samples from 14 smallholder farms. The number of samples collected per farm varied, and they were aggregated into farm-level composite pools, resulting in 14 pooled samples. Each composite was placed in sterile containers and obtained from pens housing local pigs of different ages reared in groups. Convenience sampling was applied during animal health service visits by the Agricultural Agency of Tana Toraja Regency.

RNA extraction: A 10% (w/v) fecal suspension was prepared in 1x phosphate-buffered saline (PBS) (HiMedia®, Pennsylvania, USA) for each pooled sample, vortexed for 30s, and centrifuged at 4500rpm for 10min. The supernatant was collected and used as the extraction material. Viral RNA was extracted by Viral Nucleic Acid Extraction Kit II (Geneaid®, New Taipei City, Taiwan) following the manufacturer's instructions. The quantity of the extracted genome was determined using a microspectrophotometer (Thermo Scientific™ NanoDrop™ 2000/2000c, Delaware, USA).

HEV RNA detection: HEV RNA detection was performed using reverse transcription nested polymerase chain reaction (RT-nested PCR), targeting a 348bp fragment of ORF2 and a 459bp fragment of ORF1 methyltransferase (MeT) genes, using primer sequences previously described by Batmagnai *et al.* (2023) and Widasari *et al.* (2013), respectively. The RNA extracts were used as templates for the initial amplification (RT-PCR), and the resulting amplicons were subsequently used as templates for the second-stage amplification (nested-PCR).

The RT-PCR was performed using the MyTaq™ One-Step RT-PCR Kit (Meridian Bioscience®, Tennessee, US) in a total reaction volume 25µL, consisted of 1µL forward primer, 1µL reverse primer, 2.5µL template RNA, 12.5µL 2x MyTaq One-Step Mix, 0.5µL reverse transcriptase, 0.5µL ribosafe RNase inhibitor, and 7µL DEPC-treated water for every tested sample. The thermal cycles consisted of reverse transcription (45°C, 20min), polymerase activation (95°C, 1min), followed by 40 cycles of denaturation (95°C, 10s), annealing (60°C, 10s), and elongation (72°C, 30s).

The PCR amplicons from the previous step were then used as templates in the second-stage amplification (nested

PCR). The amplification was conducted using MyTaq™ HS Red Mix Kit (Meridian Bioscience®, Tennessee, US) with a total reaction volume of 50µL consisted of 1µL forward primer, 1µL reverse primer, 1µL template, 25µL MyTaq HS Red Mix 2x, and 22µL DEPC-treated water for every tested sample. The thermal cycle consisted of initial denaturation (95°C, 1min), followed by 30 cycles of denaturation (95°C, 15s), annealing (55°C for ORF2/55,8°C for ORF1 for 15 s), and elongation (72°C, 10s). No template control (NTC) was used as a negative control.

The second stage PCR amplicons were electrophoresed in agarose gel 1,5% stained by ethidium bromide (Invitrogen™, California, USA). Electrophoresis was conducted in 1X TAE buffer at 100volts for 35min. The electropherogram was read using an ultraviolet transilluminator.

Partial sequencing and HEV identification: Samples that tested positive for HEV were sequenced using the Sanger method. Sequence chromatogram quality control was performed using MEGA11 (Tamura *et al.*, 2021). HEV genotype was identified using the Basic Local Alignment Search Tool (BLAST) in GenBank and HEV NET typing tool (<https://www.rivm.nl/mpf/typingtool/hev/>) (Ferri *et al.*, 2024). HEV subtypes were determined by aligning the samples' ORF2 and ORF1 partial sequences with reference isolates representing all subtypes (Smith *et al.*, 2020), followed by phylogenetic tree reconstruction using MEGA11 (Tamura *et al.*, 2021). Phylogenetic tree visualization was performed using the Interactive Tree of Life (iTOL) (Letunic and Bork, 2024). Subtype confirmation was performed using the pairwise nucleotide sequence alignment feature in EZBioCloud (Chalita *et al.*, 2024) and the HEV NET typing tool (Ferri *et al.*, 2024).

Genetic variation analysis: Nucleotide and amino acid variations were analyzed by visually inspecting the multiple sequence alignment of each HEV sequence and the 100 closest BLAST hits using MEGA11. The impact of the identified mutation on protein function was predicted *in silico* using the Protein Variation Effect Analyzer (PROVEAN) (Choi and Chan, 2015) and Sorting Intolerant From Tolerant (SIFT) (Sim *et al.*, 2012).

RESULTS AND DISCUSSION

Two of the 14 farms (14.3%) tested positive for HEV. One farm was positive for both the ORF2 (PQ513440) and ORF1 methyltransferase (MeT) genes (PQ513441), whereas the other was positive only for the ORF1 MeT gene (PV167195). All positive samples were obtained from pigs that exhibited no clinical signs, consistent with the generally asymptomatic nature of HEV infection in swine (Meester *et al.* 2021). The discrepancy between the ORF2 and ORF1 MeT detection results is likely due to the higher variability of the ORF2 gene (Muñoz-Chimeno *et al.*, 2022), which makes it more prone to primer-binding failure (Mesquita *et al.*, 2019; Bae *et al.*, 2022). Using two genetic targets reduced the risk of false-negative results, as demonstrated by the detection of HEV in one farm exclusively using the ORF1 MeT assay. Although HEV was detected in only two

farms, the possibility of previous exposure in other farms remains, given that fecal HEV shedding typically lasts only 1–7 weeks (Meester *et al.*, 2021).

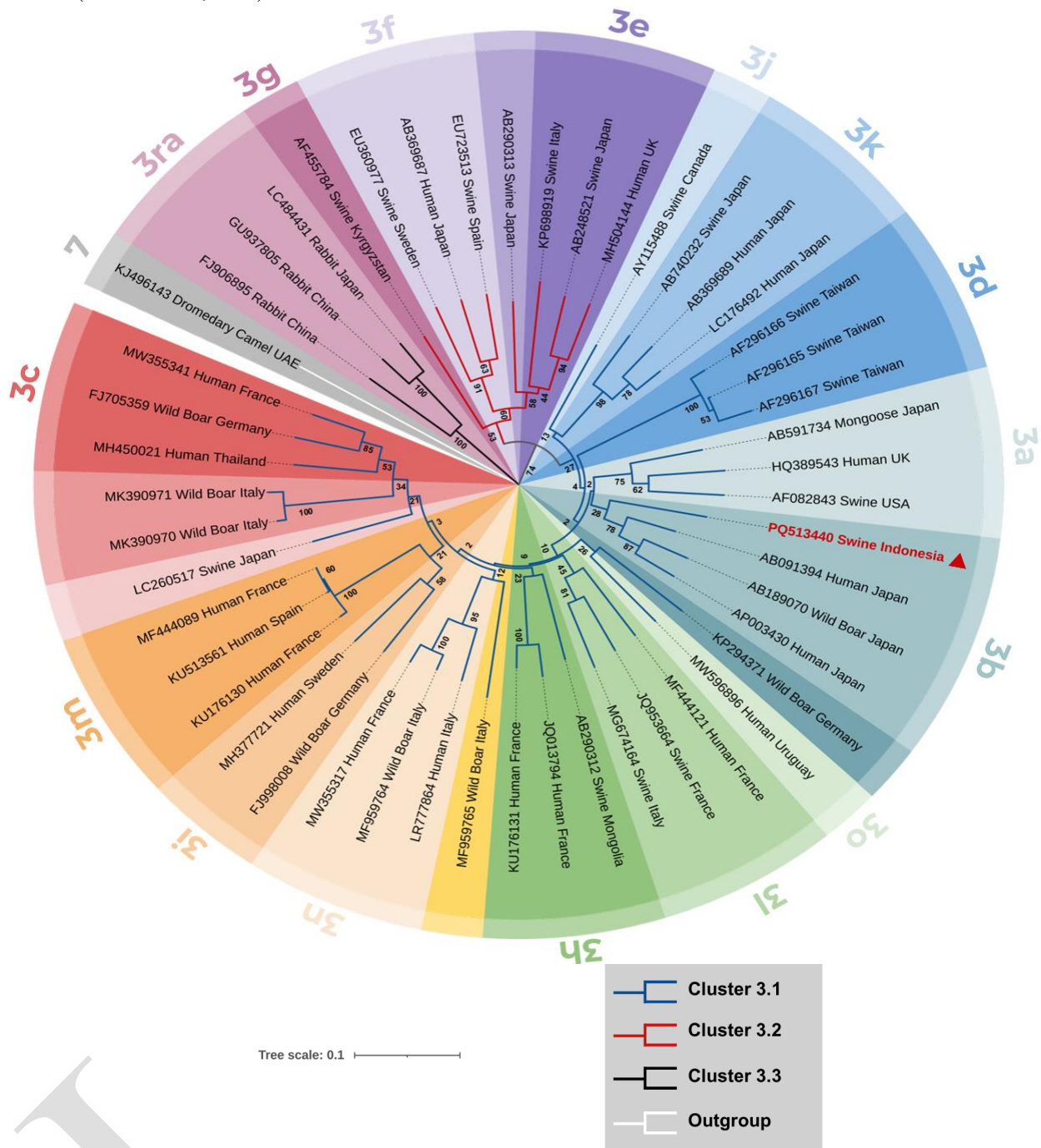


Fig. 1: Phylogenetic analysis of the partial ORF2 sequence (348 bp) of the sample PQ513440. The circular tree was reconstructed using the neighbor-joining method with the Tamura–Nei model and 1000 bootstrap replicates. Reference isolates representing each HEV-3 subtype were included, with an HEV-7 sequence (GenBank accession number KJ496143) used as an outgroup. Isolates without subtype labels represent uncategorized strains. PQ513440 is indicated by a red triangle and clusters within subtype 3b. The scale bar denotes the nucleotide substitution rate per site.

BLAST analysis revealed that the ORF2 sequence (PQ513440) shared 92.26% nucleotide identity with that of a human HEV strain from Japan (LC811153). Similarly, the ORF1 MeT sequences (PQ513441 and PV167195) exhibited 90.95% and 90.29% identity, respectively, with a human HEV strain from Japan (LC406629). No reports on HEV detection in humans from Tana Toraja are currently available, precluding local comparative analyses.

Phylogenetic analysis indicated that all HEV sequences from Tana Toraja clustered within the subtype

HEV-3b (Fig. 1 & 2). The ORF2 sequences showed greater divergence than the ORF1 MeT sequences, consistent with the higher genetic variability of ORF2 (Muñoz-Chimeno *et al.*, 2022). This difference reflects the distinct functions of these two genes. ORF2 encodes the HEV capsid protein, which plays a role in viral attachment and contains immunogenic epitopes, making it more susceptible to host immune pressure and structural variations. In contrast, the ORF1 MeT domain encodes an enzyme responsible for 5' capping of the HEV RNA

genome (Karpe, 2024), a process essential for viral replication, and is therefore more conserved. Pairwise alignment and validation using the HEV NET typing tool

confirmed that all Tana Toraja HEV sequences belonged to subtype HEV-3b, with greater divergence observed in ORF2 than in ORF1 MeT (Table 1).

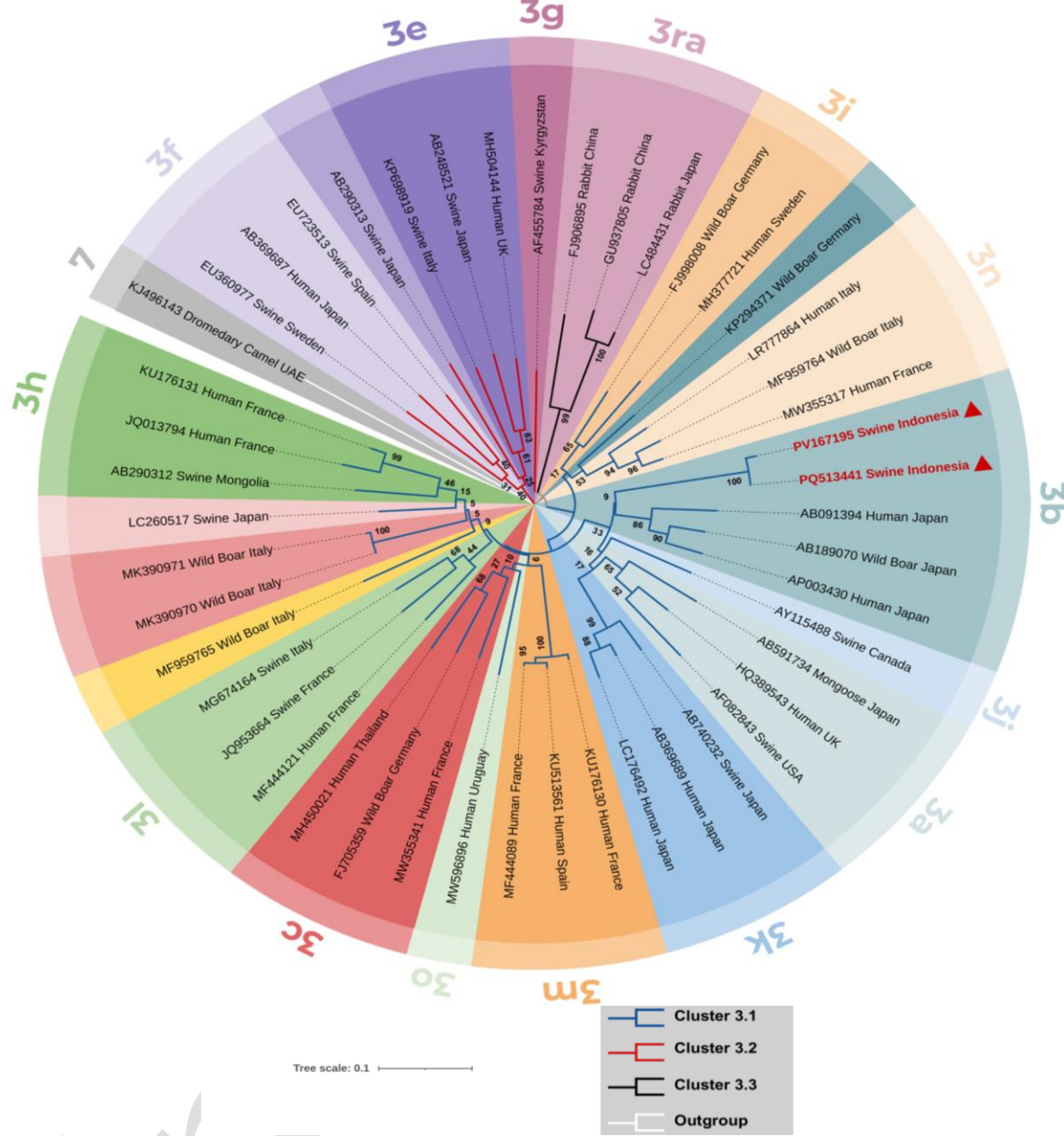


Fig. 2: Phylogenetic analysis of partial ORF1 sequences (459 bp) of HEV samples PQ513441 and PV167195. The circular tree was reconstructed using the neighbour-joining method with the Tamura–Nei model and 1000 bootstrap replicates. Reference isolates representing each HEV-3 subtype were included, with an HEV-7 sequence (GenBank accession number KJ496143) used as the outgroup. Isolates without subtype labels represent uncategorized strains. The PQ513441 and PV167195 are indicated by red triangles and clustered within subtype 3b. The scale bar denotes the nucleotide substitution rate per site.

Table 1: Pairwise nucleotide sequence alignment of Tana Toraja swine HEV samples against representative HEV genotype 3 subtypes.

No.	Subtype	NCBI number	accession	Similarity (%)		
				ORF2 PQ513440	ORF1 PQ513441	ORF1 PV167195
1	3a		AF082843	86,57	87,35	86,57
2	3b		AP003430	89,55	88,99	88,25
3	3c		FJ705359	87,16	85,71	85,13
4	3d		AF296165	85,63	NA	NA
5	3e		AB248521	84,78	83,61	83,45
6	3f		AB369687	84,18	84,54	84,41
7	3g		AF455784	87,16	81,97	81,29
8	3h		JQ013794	85,97	85,50	85,38
9	3i		FJ998008	85,97	85,71	85,61
10	3j		AY115488	87,76	88,29	87,53
11	3k		AB369689	86,27	88,76	88,01
12	3l		JQ953664	88,36	85,71	85,37

13	3m	KU513561	87,16	86,42	85,61
14	3n	MF959764	86,27	86,18	85,37
15	3o	MW596896	87,46	83,84	82,97
16	3ra	FJ906895	78,21	82,44	82,01

This study is the first to report HEV-3b in Indonesia. Until 2025, only three studies documented HEV detection and characterization in Indonesia, all of which reported HEV-3a or HEV-4 in Java and Bali (Widasari *et al.*, 2013; Raji *et al.*, 2021). The identification of a different subtype in Tana Toraja suggests the potential endemicity of HEV-3b in this area.

The ORF1 MeT sequence PV167195 harbors a novel single nucleotide variant that results in an A83T amino acid substitution. The ORF1 MeT domain of HEV-3 is

considered the most conserved region after the hypervariable region (HVR). Although the 83rd residue is not part of the major conserved motifs, it is not among the positions most prone to mutation (Muñoz-Chimeno *et al.*, 2022). Previously reported MeT mutations, such as H105R, have been associated with reduced viremia, whereas D29N and V27A have been linked to increased viral load, prolonged prothrombin time, and higher mortality in patients with HEV-1-associated liver failure in India (Borkakoti *et al.*, 2017).

Functional prediction analyses using PROVEAN and SIFT indicated that the A83T mutation was deleterious, as shown by a PROVEAN score below -2.5 (-3.239) and a SIFT score below 0.05 (0.00). A deleterious prediction suggests potential alterations in the function of the expressed protein, which could either reduce or enhance virulence (Sandell and Sharp, 2022). However, these predictions remain computational and require experimental validation.

Our findings represent the first record of swine HEV in Eastern Indonesia. As a zoonotic pathogen, the detection of HEV-3b in local pigs from Tana Toraja indicates a potential risk of transmission to humans, particularly among vulnerable populations. The present findings emphasize the importance of subsequent research to estimate the prevalence of HEV, explore the genetic heterogeneity of circulating strains, and establish whether transmission from pigs to humans occurs in Tana Toraja, Indonesia.

Acknowledgments: This study was funded by the PMDSU Research Grant Batch VII for the 2023-2024 scheme from the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia (No. 22195/IT3.D10/PT.01.03/P/B/2024).

Conflict of interest: The authors declare no conflicts of interests.

Authors contribution: DAR, ONP, DN, and EH conceived the research idea, designed the study, and developed the sampling strategy. DAR and LT carried out field sampling and collected descriptive data. DAR performed the sample testing. All authors contributed to data analysis and interpretation, manuscript writing, and figure preparation. All authors have read and approved the final version of the manuscript for publication.

REFERENCES

Ahmad T, Jin H, Dhama K, *et al.*, 2022. Hepatitis E virus in pigs and the environment: an updated review of public health concerns. *Narra J* 2(2):1-14.

- Bae KS, Lee S, Lee JY, *et al.*, 2022. Development of diagnostic systems for wide range and highly sensitive detection of two waterborne hepatitis viruses from groundwater using the conventional reverse transcription nested PCR assay. *J Virol Methods* 299:1-7.
- Batmagnai E, Boldbaatar B, Sodbayasgalan A, *et al.*, 2023. Hepatitis E virus (HEV) spreads from pigs and sheep in Mongolia. *Animals* 13(5):1-11.
- Borkakoti J, Ahmed G, Kar P, 2017. Report of novel H105R, D29N, V27A mutations in the methyltransferase region of the HEV genome in patients with acute liver failure. *J Clin Virol* 91:1-4.
- Brown HM, Marlet J, León-Janampa N, *et al.*, 2025. Enhanced hepatitis E virus infection of polarized hepatocytes in vitro. *Sci Rep* 15:1-16.
- Chalita M, Kim YO, Park S, *et al.*, 2024. EzBioCloud: a genome-driven database and platform for microbiome identification and discovery. *Int J Syst Evol Microbiol* 74(6):1-6.
- Choi Y and Chan AP, 2015. PROVEAN web server: a tool to predict the functional effect of amino acid substitution and indels. *Bioinformatics* 31(16):2745-2747.
- Ferri G, Giantomassi G, Tognetti D, *et al.*, 2024. Hepatitis E virus RNA detection in liver and muscle tissues sampled from home slaughtered domestic pigs in Central Italy. *Food Environ Virol* 16(4):438-448.
- Karpe YA, 2024. Processing of the hepatitis E virus ORF1 nonstructural polyprotein. *Front Virol* 3:1-7.
- Lase JA, Surya and Da Costa MA, 2024. Germplasm of Indonesia local pig: potential, threat of extinction, and conservation strategy. *IOP Conf Ser: Earth Environ Sci* 1292:1-12.
- Letunic I and Bork P, 2024. Interactive tree of life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Res* 52:78-82.
- Li P, Ji Y, Li Y, *et al.*, 2021. Estimating the global prevalence of hepatitis E virus in swine and pork products. *One Health* 14:1-5.
- Liang Z, Wang L, Wang L, 2022. Updates on hepatitis E virus. *Chinese Med J* 135(10):1231-1233.
- Luo Q, Chen J, Zhang Y, *et al.*, 2024. Viral hepatitis E: clinical manifestation, treatment, and prevention. *Liver Res* 8(1):11-21.
- Meester M, Tobias TJ, Bouwknegt M, *et al.*, 2021. Infection dynamics and persistence of hepatitis E virus on pig farms – a review. *Porcine Health Manag* 7(1):1-16.
- Mesquita JR, Istrate C, Santos-Ferreira NLS, *et al.*, 2019. Short communication: detection and molecular characterization of hepatitis E virus in domestic animals of São Tomé and Príncipe. *Trop Anim Health Prod* 51(2):481-485.
- Muñoz-Chimeno M, Rodríguez-Paredes V, García-Lugo MA, *et al.*, 2022. Hepatitis E genotype 3 genome: a comprehensive analysis of entropy, motif conservation, relevant mutations, and clade-associated polymorphisms. *Front Microbiol* 13:1-12.
- Raji YE, Toung OP, Taib NM, *et al.*, 2021. A systematic review of the epidemiology of hepatitis E virus infection in South – Eastern Asia. *Virulence* 12(1):114-129.
- Sandell L and Sharp NP, 2022. Fitness effects of mutations: an assessment of PROVEAN predictions using mutation accumulation data. *Genome Biol Evol* 14(1):1-15.
- Sim NL, Kumar P, Hu J, *et al.*, 2012. SIFT web server: predicting effects of amino acid substitution on proteins. *Nucleic Acids Res* 40:452-457.
- Smith DB, Izopet J, Nicot F, *et al.*, 2020. Update: proposed reference sequences for subtypes of hepatitis E virus (species *Orthohepevirus A*). *J Gen Virol* 101(7):692-698.
- Tamura K, Stecher G and Kumar S, 2021. MEGA11: molecular evolutionary genetics analysis version 1.1. *Mol Biol Evol* 38:3022-27.
- Widasari DI, Yano Y, Utsumi T, *et al.*, 2013. Hepatitis E virus infection in two different regions of Indonesia with identification of swine HEV genotype 3. *Microbiol Immunol* 57(10):692-703.