EISSN: 2455-8931 doi: 10.14202/IJOH.2025.171-177 OPEN ACCESS

International Journal of One Health

RESEARCH ARTICLE

Comprehensive surveillance and molecular detection of the Nipah virus in fruit bats (*Pteropus vampyrus*) across Indonesia: Insights from 2023 to 2024

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ABSTRACT

Background and Aim: The Nipah virus (NiV), a highly pathogenic zoonotic paramyxovirus, presents a severe public health threat due to its high case fatality rate and potential for human-to-human transmission. Indonesia, with its extensive fruit bat habitats and dense human population, remains a crucial region for proactive NiV surveillance. This study represents the first national-scale molecular screening of NiV in *Pteropus vampyrus* populations across Indonesia, aimed at detecting viral circulation, identifying zoonotic hotspots, and establishing foundational data to inform public health preparedness through a One Health framework.

Materials and Methods: A cross-sectional surveillance study was carried out from June 2023 to January 2024 in 13 locations across Western, Central, and Eastern Indonesia. Fruit bats were sampled in both natural roosts and anthropogenic environments such as bat markets. A total of 305 biological samples (250 swabs and 55 organ tissues) were collected and processed using quantitative reverse transcription polymerase chain reaction targeting the NiV nucleocapsid (N) gene. Positive samples (cycle threshold [Ct] ≤38) were further analyzed through partial gene sequencing and phylogenetic comparison.

Results: Out of 305 bat samples, 4 (1.31%) tested positive for NiV RNA, all originating from Central and East Kalimantan. Ct values ranged from 31.0 to 34.0, indicating low viral loads. Sequence analysis of the partial N gene revealed a 95% similarity to Malaysian NiV strains, suggesting possible regional viral exchange. No positive cases were recorded in Sumatra or Java, despite extensive sampling. Ecological or host-specific resistance may explain this geographical disparity.

Conclusion: This pioneering surveillance study confirms the presence of NiV in specific Indonesian bat populations and identifies Central and East Kalimantan as potential spillover zones. The findings emphasize the urgent need for a continuous, nationally coordinated surveillance program integrating wildlife, livestock, and human health sectors. Future research should prioritize longitudinal studies, ecological modeling, and genomic analyses to refine risk assessments and enhance outbreak preparedness under the One Health paradigm.

Keywords: Indonesia, molecular surveillance, Nipah virus, One Health, *Pteropus vampyrus*, quantitative reverse transcription polymerase chain reaction, zoonosis.

INTRODUCTION

Since its emergence in 1998, the Nipah virus (NiV), a highly pathogenic paramyxovirus, has been recognized as a significant zoonotic threat, primarily maintained in natural reservoirs by fruit bats of the genus *Pteropus*. Its capacity to cause severe respiratory illness in animals and fatal encephalitis in humans, combined with high mortality rates, underscores the urgent need for robust surveillance, particularly in regions with dense *Pteropus* bat populations, such as Indonesia. NiV has been implicated in multiple outbreaks of respiratory disease in pigs and encephalitis in humans, often with high case fatality rates [1].

The transmission of NiV from bats to humans has been a central topic in zoonotic disease research.

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Received: 30-12-2024, **Accepted:** 21-04-2025, **Published Online:** 09-06-2025
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How to cite: Nidom RV, Indrasari S, Prakoso D, Nidom AN, and Afifah B (2025) Comprehensive surveillance and molecular detection of the Nipah virus in fruit bats (*Pteropus vampyrus*) across Indonesia: insights from 2023 to 2024, *Int. J. One Health*, 11(1): 171–177.

Epidemiological evidence suggests that the consumption of date palm sap contaminated with bat secretions plays a pivotal role in human infections [2]. This study represents the first large-scale molecular surveillance of NiV in fruit bat populations across Indonesia, employing advanced molecular techniques to detect viral RNA and evaluate zoonotic transmission risks [3].

De Wit and Munster [2] have established *Pteropus* fruit bats as the natural reservoir hosts of NiV, with zoonotic transmission occurring either directly or through intermediate hosts. The virus has been isolated from bat saliva and urine, indicating multiple potential routes of human exposure [4]. Employing a One Health approach, this study integrates ecological, animal, and human health data to provide a holistic understanding of NiV dynamics and to support proactive mitigation strategies.

Understanding the circulation of NiV within bat populations is critical for anticipating and preventing spillover events. Lessons from outbreaks in countries such as Malaysia and Bangladesh emphasize the importance of recognizing various viral shedding mechanisms, including through bat urine, feces, saliva, and contaminated fruit [5]. Surveillance near human outbreak sites has proven effective in enhancing genomic sequencing efforts and in deepening our understanding of NiV ecology and evolution [6]. Furthermore, the detection of NiV RNA in bat blood reinforces the need for comprehensive surveillance strategies targeting multiple biological samples [7, 8].

Effective NiV surveillance must prioritize the identification of high-risk reservoirs, particularly within *Pteropus* species known to harbor the virus in Malaysia and other regions [9, 10]. Investigating the roosting behavior and habitat preferences of these bats can aid in delineating zones of elevated transmission risk [11]. The recent detection of the NiV genome in *Pteropus vampyrus* in Sumatra, Indonesia, further supports the necessity for focused monitoring within specific bat populations [12].

Despite multiple outbreaks of NiV in South and Southeast Asia, most molecular surveillance studies to date have focused on Bangladesh, India, and Malaysia, with comparatively limited research conducted in Indonesia – an archipelagic nation that harbors diverse *Pteropus* bat populations known to carry NiV. Although sporadic detections of NiV RNA in Indonesian fruit bats have been reported, large-scale, systematic surveillance efforts remain scarce. In addition, there is a paucity of integrated One Health approaches in the region that concurrently assess ecological, virological, and epidemiological data to evaluate the zoonotic spillover risk. This gap in surveillance is especially concerning given Indonesia's high human–bat interface, driven by agricultural expansion, deforestation, and the consumption of raw date palm products, all of which may facilitate viral spillover into human populations.

This study aims to conduct the first nationwide molecular surveillance of NiV in fruit bat populations across Indonesia, employing advanced viral RNA detection techniques. By integrating ecological data, host species behavior, and environmental factors through a One Health lens, the study seeks to identify hotspots of potential zoonotic transmission and assess the prevalence and distribution of NiV in bat reservoirs. The findings are expected to inform risk-based surveillance strategies and provide a scientific basis for early warning systems and public health interventions to prevent future outbreaks.

MATERIALS AND METHODS

Ethical approval

All procedures involving animals were conducted in accordance with the Institutional and National Guidelines for the ethical treatment of wildlife. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Professor Nidom Foundation (Approval No. 030623/IACUC/VII/2023). Stringent biosafety protocols were followed throughout the study to ensure the welfare of animals and the safety of personnel.

Study period and location

The study was conducted from June 2023 to February 2024. A nationwide cross-sectional study was conducted to evaluate the presence of NiV in fruit bat populations across Indonesia. The country was geographically divided into three macro regions: Western, Central, and Eastern Indonesia. From these, 13 representative ecological zones were selected using random stratified sampling techniques. Sampling locations included both natural bat roosts (e.g., caves) and human-associated interfaces such as bat trade markets. The distribution of these sampling sites is illustrated in Figure 1, and corresponding site codes are detailed in Table 1.

Sample collection

Samples were collected from June 2023 to January 2024. Fruit bats were sampled using simple random sampling. Oropharyngeal and rectal swabs were collected and stored in viral transport medium. Organ tissues were obtained from deceased bats and processed through mechanical homogenization. The resulting supernatant was preserved under cold chain conditions until further molecular analysis. All procedures were performed at the A-BSL3 Laboratory of the Professor Nidom Foundation, Surabaya, Indonesia.

RNA extraction

Total viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. All extractions were conducted in a Class II biosafety cabinet (Airtech, Japan) to prevent contamination. The extracted RNA was then stored at −80°C until use

doi: 10.14202/IJOH.2025.171-177

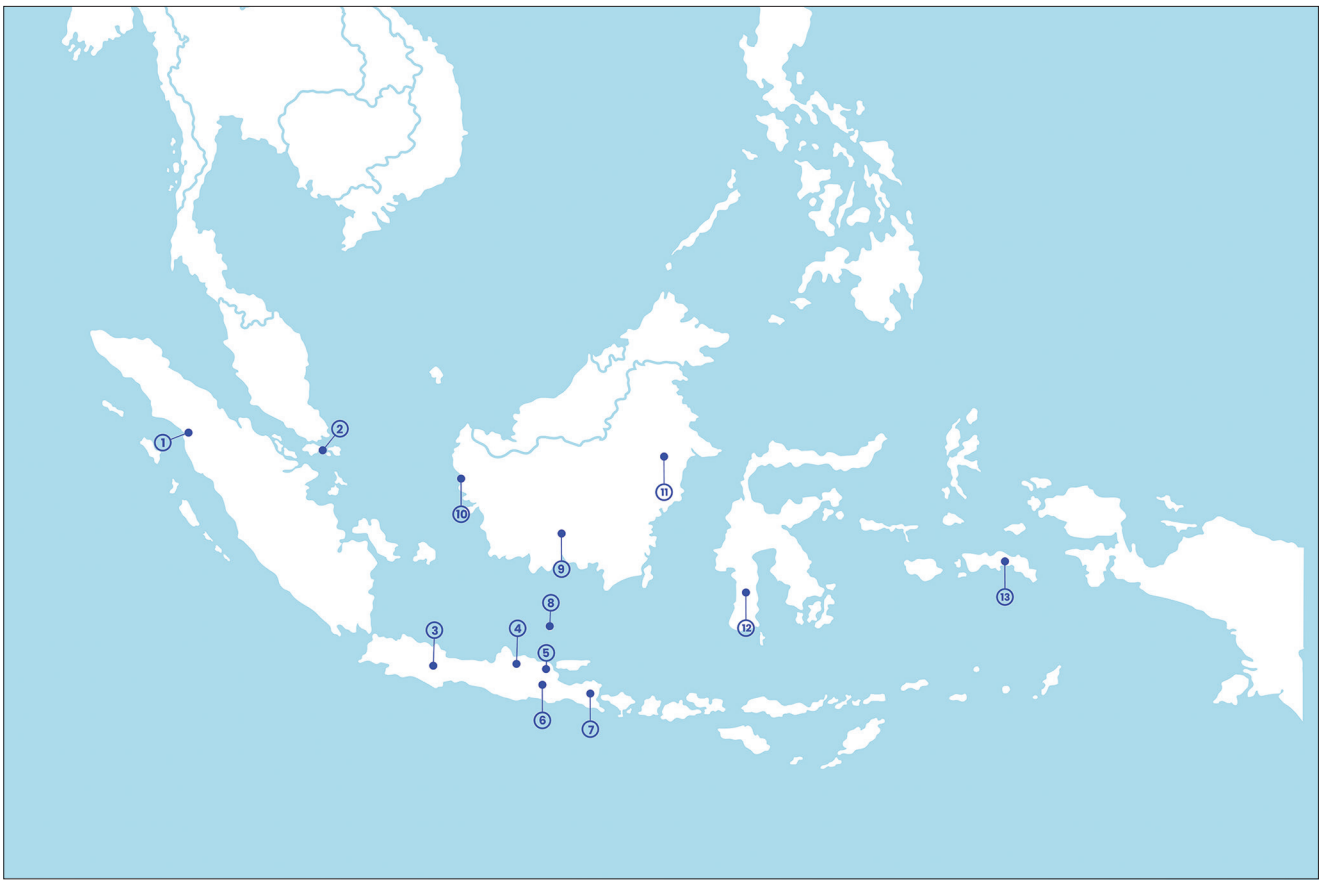

Figure 1: Mapping the location of caves or bat sales places in Indonesia. We took samples from 13 areas according to the numbered signs. The explanation of the sign number can be seen in Table 1 [The map was adopted from www.freepik.com. The map was edited using Adobe Illustrator ver.25.4.1 (64bit) 2021].

Table 1: Results of bat sample testing for Nipah virus N.

No.	Area	Origin	Total samples		Real-time PCR	
			Swabs	Organs	Positive	Negative
West part of Indonesia						
1.	Padang, West Sumatra	Caves and sellers	6	0	0	6
2.	Riau	Seller	63	20	0	83
3.	Garut, West Java	Seller	4	4	0	8
4.	Blora, Central Java	Seller and cave	37	10	0	47
5.	Lamongan, East Java	Seller and cave	10	2	0	12
6.	Kediri, East Java	Cave	5	0	0	5
7.	Jember, East Java	Caves and sellers	10	0	0	10
8.	Bawean island, East Java	Seller	27	7	0	34
Central Indonesia						
9.	Central Kalimantan	Cave and seller	18	2	1	19
10.	Pontianak, West Kalimantan	Cave	10	0	1	9
11.	East Kalimantan	Cave	20	0	2	18
12.	South Sulawesi	Cave	30	6	0	36
East Indonesia						
13.	Seram Island	Cave	10	4	0	14
Total samples			250	55	4	301

PCR=Polymerase chain reaction, N=Nucleocapsid

in reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays.

RT-qPCR assay

Quantitative detection of NiV RNA was performed through real-time RT-qPCR using the QuantStudio 5 real-time PCR System (Applied Biosystems, USA). The amplification was carried out with AgPath-ID™ One-Step RT-PCR reagents (Applied Biosystems). Each 20 µL reaction mixture contained 2 µL of primer–probe mix, 11 µL of One-Step RT-PCR buffer, and 5 µL of template

RNA. The following primer and probe sequences targeting the N gene were employed [13, 14]:

- Forward primer (NiV_N_1198F): TCA-GCA-GGA-AGG-CAA-GAG -AGT-AA
- Reverse primer (NiV_N_1297R): CCC-CTT-CAT-CGA-TAT-CTT -GAT-CA
- Probe (NiV_N_1247probe): FAM-CCT-CCA-ATG-AGC-ACA-CCT-CCT-GCA-G-TAMRA.

Samples yielding cycle threshold (Ct) values >38 were considered negative for NiV RNA.

RESULTS

Regional sampling and site stratification

Indonesia is an archipelago with numerous bat caves, and in some areas, bats are a primary food source for rice. In this study, we sampled 13 regions by recording sellers and bat caves in each region. The division of regions is based on three regions in Indonesia: Western Indonesia, Central Indonesia, and Eastern Indonesia. Most sampling locations were on Java Island, considering that the island has the largest population in Indonesia. Therefore, in the event of an outbreak of the NiV, the risk of transmission between humans is the greatest.

Sample yield and positive detection rate

The total number of samples obtained was 250 in the form of swabs and 55 in the form of organs. Of the 305 samples tested, 4 (1.31%) tested positive for NiV RNA, with all positive samples originating from bat populations in Central and East Kalimantan, highlighting these regions as potential spillover hotspots. This finding not only confirms the presence of NiV in these previously unexplored regions but also suggests that the ecological conditions in Borneo may support the persistence and potential transmission of the virus. The overall test results are shown in Table 1.

Molecular characterization and regional comparison

Quantitative RT-qPCR targeting the highly conserved N gene of NiV was combined with next-generation sequencing (NGS) to provide a deeper genetic characterization of the detected viral strains. Data were analyzed using QuantStudio software (Design and Analysis software v 2.6.0, Applied Biosystem), and Ct values >38 were considered negative. The statistical analyses included regional positivity rate comparisons using Chi-square tests to identify significant hotspots of NiV presence. The chart of percentages is shown in Figure 2.

Sequence homology to Malaysian strains

Furthermore, although full genome isolation was not achieved, partial N-gene sequences were obtained, showing 95% similarity to NiV strains previously identified in Malaysia, suggesting potential cross-border viral dynamics. It is necessary to optimize the NGS method to establish the optimal pipeline for characterizing NiV samples.

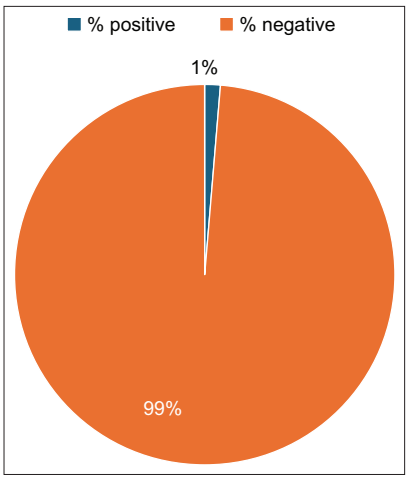

Figure 2: Pie chart of sample result distribution.

DISCUSSION

Need for zoonotic and human-to-human transmission preparedness

Efforts to prevent NiV outbreaks should also consider the potential for zoonotic and human-to-human transmission. While previous studies have documented the role of intermediate hosts (e.g., pigs and horses) in NiV outbreaks in Malaysia and Bangladesh, this study uniquely contributes to the literature by exploring the direct transmission risks in Indonesia [15].

Unexpected negative results in high-risk areas

The absence of positive samples from regions with high human-bat interaction, such as Sumatra, suggests that ecological factors and bat species composition may play critical roles in influencing viral transmission dynamics, a finding that challenges current assumptions and opens new avenues for research [16].

Foundation for national NiV surveillance

This pioneering study establishes a foundation for a national NiV surveillance program advocating for continuous monitoring, improved molecular diagnostics, and the adoption of One Health strategies to prevent potential outbreaks. The One Health approach integrates human, animal, and environmental health, emphasizing the interconnectedness of these domains in preventing and controlling infectious diseases.

Comparative insights from Japanese encephalitis surveillance

In Indonesia, the implementation of the One Health principles has been demonstrated through studies focusing on the surveillance of Japanese encephalitis virus (JEV) in bats and mosquitoes. Diptyanusa *et al*. [16] highlighted the importance of understanding the role of bats as reservoir hosts for JEV, indicating that such surveillance can inform public health interventions. This study illustrates how bat population monitoring can provide insights into the dynamics of viral transmission and the potential risks to human health, aligning with

the One Health concept of addressing health threats at the animal-human-environment interface.

Supporting evidence for One Health approach

Moreover, the emergence of the NiV in various regions underscores the need for a One Health approach. Subhan [17] discussed the critical need for diagnostic and control measures for NiV within a One Health framework, emphasizing that effective management of zoonotic diseases requires collaboration across multiple sectors. Aditi and Shariff [18] echoed this perspective, arguing that given the transmission pathways from bats to humans, a One Health approach is essential for the prevention and control of NiV infections.

Strengthening surveillance networks

The role of bats in the transmission of zoonotic diseases was further supported by research that identified the need for enhanced surveillance efforts. For instance, Yadav *et al.* [19] advocated collaborative activities between animal and human health sectors to improve the surveillance of coronaviruses in bats, which aligns with the One Health approach. This collaboration is crucial for understanding the epidemiology of bat-borne viruses and mitigating potential spillover events.

Comparison with existing surveillance data

A significant study by Sendow *et al.* [9] focused on screening for NiV infection in West Kalimantan Province, Indonesia. In line with the research of Sendow *et al.* [9], we also found 1 positive sample in West Kalimantan. This research emphasized the necessity of sentinel surveillance to understand the epidemiology of henipavirus in bats, particularly given the historical context of NiV transmission from bats to humans in other regions, such as Bangladesh and Malaysia. The findi-ngs of this study underscore the potential for similar transmission dynamics in Indonesia, necessitating further investigation into local bat populations.

Sampling limitations in Sumatra

In a subsequent study, Sendow *et al.* [12] investigated the presence of NiV in the fruit bat species *P. vampyrus* in Sumatera, Indonesia. They employed real-time PCR techniques to detect viral RNA, revealing that the assays targeting the highly conserved N and M genes of henipavirus were effective in identifying infections in field samples. We did not find any positive samples of the NiV in bats collected in the Sumatra area. A wider sampling is needed to determine whether there are no positive samples from that area. This study not only confirmed the presence of NiV in Indonesian bats and highlighted the lack of BSL-4 laboratory facilities in the country, which poses challenges for comprehensive viral detection and research.

Diversity of paramyxoviruses in Indonesia

Further research by Sasaki *et al.* [20] expanded on the detection of paramyxovirus RNA in fruit bats from Indonesia and identified previously unidentified paramyxoviruses. This study involved screening RNA samples from bat spleens collected from various locations, reinforcing the notion that Indonesia is a significant area for the study of henipavirus. The detection of multiple paramyxoviruses in bats indicates a diverse viral landscape that poses risks for zoonotic transmission.

Geographical spread and ecological risk

In addition, Breed *et al*. [21] examined the distribution of henipavirus in Southeast Asia, including Indonesia. Their research suggested that the presence of the NiV is not restricted by geographical barriers, as evidence of the virus was found in bat populations across various regions, including Indonesia. These findings are crucial for understanding the epidemiological patterns of NiV and the potential for outbreaks.

Recent outbreak reflections

Recent outbreaks, particularly the one reported in Kerala, India, in 2023, have brought renewed attention to the NiV. This outbreak was traced back to contaminated palm sap, a common local delicacy, highlighting the direct link between bat activity and human infection [18, 22]. The rapid response to this outbreak included contact tracing and public health education, which are critical for controlling the spread of the virus [22, 23]. Furthermore, the ongoing surveillance of bat populations for NiV shedding is essential for the early detection of potential spillover events, as demonstrated by Mckee *et al.* [6], which focused on bat roosts after previous outbreaks.

Bat immunology and virus persistence

The immune response of bats to viral infections is another area of interest, as it may play a role in the persistence of viruses like NiV within bat populations. Edward *et al.* [13] discussed the unique immune systems of bats, which may allow them to coexist with pathogens that are lethal to other species. This aspect is crucial for understanding how NiV can remain endemic to bat populations and pose a significant threat to human health.

Surveillance prioritization and habitat mapping

By prioritizing surveillance in bat species most likely to serve as reservoir hosts for the NiV, surveillance systems can be optimized to maximize sampling efforts and increase the impact of field studies by Malabadi [22]. Furthermore, Epstein *et al*. [24] examined fruit bats as potential reservoirs for the NiV during outbreaks, indicating the importance of studying bat populations in surveillance efforts. Understanding bat roosting behavior and habitat selection, as highlighted by Hahn *et al*. [11], can aid in identifying potential outbreak locations and improving NiV surveillance strategies.

CONCLUSION

This nationwide surveillance study provides the first molecular evidence of NiV circulation in *Pteropus*

fruit bat populations across multiple ecological zones in Indonesia. Among the 305 bat samples analyzed, 1.31% tested positive for NiV RNA, with all positive samples originating from Central and East Kalimantan. These findings underscore Borneo as a previously underrecognized region with heightened zoonotic spillover potential. Notably, partial N-gene sequences showed 95% similarity to Malaysian NiV strains, suggesting potential cross-border viral flow and regional ecological connectivity.

The study's practical implications are substantial. The identification of NiV RNA in oropharyngeal swabs reinforces the potential for oral transmission through contaminated surfaces or food sources, such as date palm sap, thus highlighting the necessity for public health interventions that reduce human exposure to bat secretions. Furthermore, the absence of positive cases in Sumatra, despite high human–bat interaction, indicates that viral shedding dynamics may be shaped by complex ecological factors, including bat species composition and habitat conditions that warrant deeper investigation.

A key strength of this study is its application of a One Health surveillance framework that integrates environmental mapping, bat ecology, and molecular diagnostics. This holistic approach enhances the early detection of zoonotic pathogens and supports risk-based surveillance policies.

However, several limitations must be acknowledged. First, the inability to recover full viral genomes due to current NGS pipeline constraints limits comprehensive phylogenetic characterization. Second, the cross-sectional design precludes insights into temporal viral dynamics and seasonality. Third, the lack of serological data restricts conclusions on previous NiV exposure in bat populations.

Future research should focus on longitudinal sampling across seasons and ecological zones, incorporate seroepidemiological assays to detect past exposures, and expand sequencing capabilities to capture complete viral genomes. Establishing in-country BSL-4 facilities or regional partnerships for high-containment virology is also critical for advancing NiV research capacity in Indonesia.

In conclusion, this study provides foundational evidence supporting the implementation of a national NiV surveillance program in Indonesia, guided by One Health principles. Strengthening cross-sectoral collaboration and investing in diagnostic infrastructure are essential for mitigating the risk of future NiV outbreaks and protecting public health at the animal–human–environment interface.

AUTHORS' CONTRIBUTIONS

RVN: Designed the study, performed data analysis, and drafted and revised the manuscript. SI, DP, and BA: Designed the study and collected the samples. RVN, SI, DP, and ANN: PCR and molecular detection. RVN and SI: Interpretation and analysis of data. SI, ANN, and BA: Data analysis and revised the manuscript. All authors have read and approved the final manuscript.

ACKNOWLEDGEMENTS

We would like to thank BRIN Riset dan Inovasi untuk Indonesia Maju (RIIM). This research was supported by the RIIM LPDP and BRIN (grant no. 12/II.7/HK/2023). We would also like to thank Professor Nidom Foundation and Mr. Ilsan Arvan Nurgas and his teams in Sadita for helping us perform surveillance.

COMPETING INTERESTS

The authors declare that they have no competing interests.

PUBLISHER'S NOTE

Veterinary World (Publisher of International Journal of One Health) remains neutral with regard to jurisdictional claims in published map and institutional affiliation.

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