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Preliminary PCR Screening and Optimization for Five Suspected Viruses in *Haemaphysalis bispinosa* Ticks from Moa Buffalo (*Bubalus bubalis*)

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Abstract: Tick-borne viruses are becoming a serious threat to livestock health, especially in tropical regions where monitoring systems are still limited. *H. bispinosa*, the main external parasite of water buffalo in Eastern Indonesia, is suspected of carrying several veterinarians' important viral pathogens. This study aims to detect five viruses consisting of DNA viruses (BuGyKV, BoHV-1, and OvHV-2) and RNA viruses (FMDV and TAMV). FMDV and TAMV, both RNA viruses, need to be converted into complementary DNA (cDNA) through reverse transcription, which is then used as a template for PCR amplification using virus-specific primers. All viruses were detected using specific primers and extensive annealing temperature optimization. The results showed weak and nonspecific amplification bands at one temperature for BuGyKV, BoHV-1, and OvHV-2, but these bands did not match the expected amplicon size. No amplification bands were observed for FMDV and TAMV under any conditions. These findings suggest that this amplification failure is likely due to primer mismatch with local virus variants resulting from geographical differences. Overall, it can be concluded that the five target viruses were not detected in the *H. bispinosa* samples examined. This preliminary study provides an initial virological overview of *H. bispinosa* ticks from Moa Island and emphasizes the need for more sensitive detection methods, such as nested PCR, qPCR, or metagenomic approaches, to improve the detection of low-titer viruses in ticks.

Keywords: BuGyKV; *Haemaphysalis bispinosa*; Moa buffalo; PCR; Tick-borne viruses

Introduction

Ticks are among the most medically and economically important arthropods because they act as vectors of a wide range of pathogens, including viruses, bacteria, and protozoa, affecting both livestock and humans. In tropical and subtropical regions, tick infestation causes direct losses through blood depletion and reduced body weight, while indirect impacts arise from the transmission of infectious agents that limit livestock productivity (Ali et al., 2019; Karim et al., 2017). Recent advances in molecular diagnostics, particularly PCR, have revealed a much higher level of viral diversity in ticks than previously recognized. Viruses from families such as Genomoviridae, Nairoviridae, and Phenuiviridae, as well as various novel circular DNA viruses, have been reported from ticks in different parts of the world (Ma et al., 2025; Moming et al., 2021; Zeng et al., 2025). These findings highlight the need to expand tick-borne virus surveillance, especially in island and tropical ecosystems where epidemiological information remains scarce.

In ruminant production systems, ticks represent one of the major biological constraints. Although the prevalence of tick infestation in buffalo is often reported to be lower than in cattle, its impact should not be underestimated (Nithikathkul et al., 2002). Parasitism has been described as the foremost inhibiting factor in

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the development of livestock populations, including buffalo (Abbasi et al., 2017). Tick-borne protozoan diseases such as babesiosis, theileriosis, and anaplasmosis continue to cause substantial economic losses worldwide (Abdallah et al., 2017; Fuente et al., 2017). Climate conditions strongly influence tick distribution and abundance, making island ecosystems particularly vulnerable to changes in tick ecology and pathogen transmission dynamics (Anderson et al., 2013). These global concerns are highly relevant to Indonesia, where buffalo remain an essential component of rural livelihoods.

Moa buffalo (*B. bubalis* Linnaeus, 1758) is a unique local genetic resource officially recognized by the Indonesian government and distributed exclusively on Moa Island, Southwest Maluku District (Minister of Agriculture Decree No. 2911/Kpts/OT.140/6/2011). This buffalo population exhibits genetic and phenotypic uniformity and shows remarkable adaptation to the island's harsh environment, characterized by short rainfall and prolonged dry seasons. Moa Buffalo have high socioeconomic and cultural value for the Moa community, yet the island's dependence on inter-island animal movement creates potential pathways for the introduction of infectious agents through livestock trade and ectoparasites attached to animals. Despite this strategic importance, Moa Island has not been widely investigated for the risk of tick-borne virus transmission.

One tick species closely associated with buffalo in South and Southeast Asia is *H. bispinosa*, a hematophagous ectoparasite capable of carrying various infectious agents (Utami et al., 2024; Utami & Kunda, 2023). However, information regarding the virome of this species in Indonesia, particularly in eastern regions such as Maluku, remains extremely limited. Several viruses of veterinary and zoonotic concern including Bovine herpesvirus-1 (BoHV-1), Ovine herpesvirus-2 (OvHV-2), Foot-and-mouth disease virus (FMDV), and Tamdy virus (TAMV) have been reported in other countries (Riaz et al., 2021; Zeng et al., 2025). In addition, circular DNA viruses such as Bubaline-associated gemykrogvirus (BuGyKV) have recently been detected in ruminants and arthropods elsewhere (König et al., 2023; Yan et al., 2024), yet their presence in *H. bispinosa* ticks from Indonesia has never been documented.

PCR remains a pragmatic and affordable surveillance method for specific target detection in areas with limited facilities, but amplification failure is frequently encountered due to the very low abundance of viral nucleic acids relative to host DNA, primer–template mismatches, and deterioration of nucleic acid quality (Kuleš et al., 2017; Lannutti et al., 2022). Optimization of annealing temperature and rigorous evaluation of primer performance are therefore essential to obtain reliable detection. Based on these considerations, the present study aims to detect BuGyKV, BoHV-1, OvHV-2, FMDV, and TAMV in *H. bispinosa* ticks collected from Moa buffalo. The findings are expected to provide baseline data for tick virological surveillance in the Maluku region and to support early disease mitigation strategies in island livestock systems.

Method

Ethical Approval

All research procedures involving sampling were non-invasive and did not cause physical stress to the animals, as ticks were only collected from the body surface. Animal handling followed animal welfare guidelines for invertebrate ectoparasitic research and was approved by the South Maluku Regency Animal Husbandry Office, including the collection of tick samples from Moa buffalo, to ensure no negative impacts on animal welfare during the sampling process. Figure 1 is the overall stage of the research which is shown in the following flowchart.

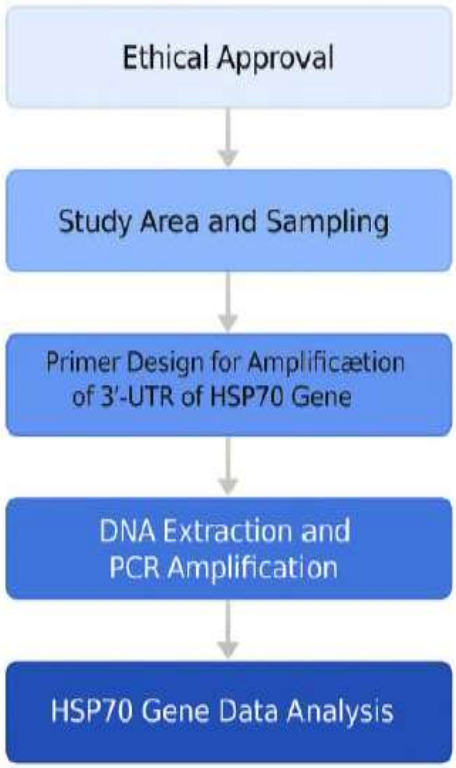


Figure 1. Research flow chart

Study Area and Tick Collection

This research was conducted on Moa Island, Southwest Maluku Regency, one of the centers of buffalo farming in the Maluku region. Sampling was conducted in four villages: Werwaru, Tounwawan, Klis, and Moain (Figure 2) from March to May 2025.

Figure 2 . Map of tick collection locations on Moa Island

A total of 85 individuals (45 males and 40 females) of local buffalo (*B. bubalis*) were visually examined on several body parts that are the predilection locations for ticks (lower abdomen, perineum, neck, and groin). A total of 100 adult ticks were collected, cleaned, labeled with special identification tags, and then recorded with information about the location and date of collection. All ticks obtained were divided into 5 pools for DNA extraction. Identification of *H. bispinosa* ticks was carried out using a special morphological key for the Ixodidae family and referring to the research findings of Utami et al. (2024) and confirmed with a taxonomic reference guide for ticks in the Australasia region (Durden et al., 2008; Kwak & Ng, 2022). Intact adult ticks were collected using sterile tweezers and placed into 1.5 mL microcentrifuge tubes containing 70% ethanol. All samples were coded, stored in a cooler, and then taken to the Biotechnology Laboratory for preparation and molecular analysis at the Biochemistry Laboratory of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta.

Primer Design for Amplification

This study used five specific primer sets to detect the presence of five target viruses: Bubaline-associated gemykrogvirus (BuGyKV), Bovine herpesvirus-1 (BoHV-1, gI gene), Ovine herpesvirus-2 (OvHV-2), Foot-and-mouth disease virus (FMDV), and Tamdy virus (TAMV). The primer sequences used to amplify the target genes of each viral DNA were obtained from previous publications with validated gene targets (Table 1). Prior to use, all primers were analyzed in silico using BLAST (NCBI) to ensure specificity, predict potential dimers/loops, and assess compatibility with reported viral sequences in Southeast Asia. The primers were then optimized through testing over a wide annealing temperature range.

Table 1. Primer sequence for virus detection in Moa buffalo ticks (*B. bubalis*)

Virus	Primer Sequence (5'->3')	Lenght	Reference	Target Gen
Gemykrogvirus (BuGyKV)	F: GATCGTTCGCTTCTTTCGGTAT R: TGGCTAGGCGCACAAAAAC	60 bp	(Lechmann et al., 2021)	associated protein (Rep) gene
Bovine alphaherpesvirus 1 (BoAV-1)	F: TGTGGACCTAAACCTCACGGT R: GTAGTCGAGCAGACCCGTGTC	97 bp	(X. Ma et al., 2025)	glikoprotein I (gI) gene
Foot-and-mouth disease (FMD) virus	F: GCCTGGTCTTTCCAGGTCT R: CCAGTCCCCTTCTCAGATC	328 bp	(Tesfaye et al., 2020)	3Dpol gene
Ovine herpesvirus-2 (OvHV-2)	F: AGTCTGGGTATATGAATCCAGATGGCTCTC R: AAGATAAGCACCAGTTATGCATCTGATAAA	422 bp	(Riaz et al., 2021)	tegument protein (pORF48) gene
Tamdy virus (TAMV)	F: ACACGTTTCTTGGGAGATGC R: GAGCTTGCGCTGCTTTTATT	168 bp	(Cui et al., 2025)	small (S) gene segment

DNA Extraction and PCR Amplification

The collected ticks were cleaned and homogenized using 200 µL of 10x PBS solution. DNA extraction was performed using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol with some adjustments for arthropod samples. Prior to extraction, *H. bispinosa* ticks were washed three times with 1× PBS to remove surface contaminants, then crushed using a sterile homogenizer until the tissue was evenly disrupted. The lysis step was carried out using lysis buffer containing proteinase-K and incubated at 56°C for 3–12 hours until the tissue was completely degraded. DNA was then purified through a silica column and eluted in 50 µL of nuclease-free buffer. DNA concentration and purity were measured using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific) at 260/280 nm wavelengths to ensure a purity ratio within the range of 1.7–2.0.

Given the potentially low concentration of viral DNA in tick samples, concentration normalization was not performed to maintain the presence of viral DNA, and extensive annealing temperature optimization was

performed. Meanwhile detection of FMDV and TAMV, both RNA viruses, was performed using reverse transcription–polymerase chain reaction (RT-PCR). Total RNA extracted from tick samples was first reverse-transcribed into complementary DNA (cDNA), which subsequently served as a template for PCR amplification using virus-specific primers. This approach was employed to ensure sensitive and accurate detection of viral RNA, considering the inherent instability and low abundance of RNA viruses in arthropod samples (Dias et al., 2023).

PCR reactions were carried out in a total volume of 25 µL using a commercial PCR master mix containing Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer. The PCR program included initial denaturation at 95°C for 3 minutes, followed by 35 cycles consisting of denaturation (95°C, 30 seconds), annealing at the tested temperature (30 seconds), and extension (72°C, 30–60 seconds depending on the target size), and a final extension at 72°C for 5 minutes. PCR products were visualized by 1.5% agarose gel electrophoresis stained with DNA intercalating dye and evaluated using a UV transilluminator.

Bioinformatic Data Analysis

PCR results will be visualized through agarose gel electrophoresis to ensure the successful amplification of five viruses suspected of infesting *H. bispinosa* ticks from Moa buffalo. The presence of amplicons at basepair sizes corresponding to the target gene indicates a specific size, which is then purified and continued with sequencing to obtain accurate nucleotide data. Sequencing results are analyzed bioinformatically using MEGA 12 software (Kumar et al., 2024), and compared with the NCBI database via BLASTn to identify the level of similarity with the reference virus and ensure the specificity of the amplification followed by determination of genetic identity, multiple sequence alignment, and phylogenetic tree reconstruction.

Results and Discussion

PCR Screening and Phased Optimization for Detecting Five Target Viruses in H. bispinosa Ticks

This study conducted molecular screening of five viruses suspected to be associated with ticks.*H. bispinosa*, namely BuGyKV, OvHV-2, BoHV-1, FMDV, and TAMV, using a conventional PCR approach combined with stepwise annealing temperature optimization. All optimization stages were designed to address the main challenges in virus detection in ectoparasite samples, particularly low viral titers and host DNA dominance. Overall results showed no specific amplicons for all target genes, despite varying annealing temperatures and adjusting template concentrations. Gel electrophoresis visualization served as the main basis for interpreting PCR results at each optimization stage.

In the DNA virus group, i.e BuGyKV (bubaline-gemykrogvirus associated protein (Rep) gene), BoHV-1 (glycoprotein I (gI) gene), and OvHV-2 (tegument protein (pORF48) gene), the initial PCR optimization stage (PCR optimization stage 1) with 1 ng and 5 ng template DNA input using an annealing temperature of 60°C for BuGyKV and OvHV-2 and 62°C for BoHV-1 did not produce a specific DNA band that corresponds to the target size (Figure 3). Further optimization stage 2 with 5 ng template DNA input, with an annealing temperature range of (BuGyKV 58, 59, 61–63°C; OvHV-2 59, 61–64°C, and BoHV-1 (gI) at an annealing temperature range of 63–67°C) also showed a consistent pattern, i.e the absence of specific amplification, although the band Weak, smear, or non-specific multibands were observed under certain conditions (Figure 4). The third stage of optimization, carried out with successively wider annealing temperatures (BuGyKV 64–68°C; OvHV-2 65–69°C, and BoHV-1 (gI) with annealing temperatures of 68–72°C), also showed a consistent pattern.

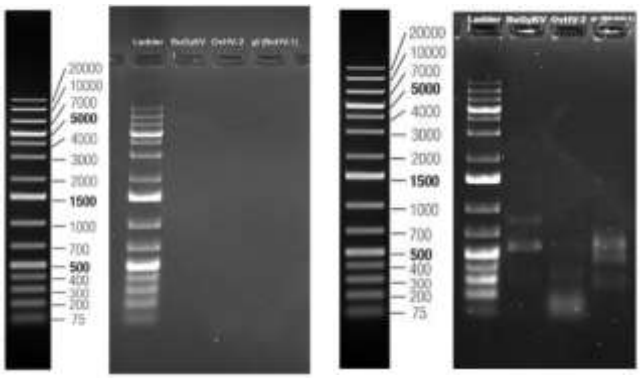


Figure 3. PCR optimization 1: Annealing temperatures of 60°C for BuGyKV and OvHV-2 and 62°C for BoHV-1: (a) 1 ng DNA input; and (b) 5 ng DNA input

These non-specific bands do not correspond to the target size, thus interpreted as mispriming or primer-dimer formation. Biologically, this finding is consistent with reports that viruses from the Genomoviridae family and ruminant herpesviruses are generally present at very low levels in ticks and do not actively replicate, so conventional PCR is often not sensitive enough to detect them (Ci-Xiu et al., 2015; Cui et al., 2025; R. Ma et al., 2022; Riaz et al., 2021; Tesfaye et al., 2020).

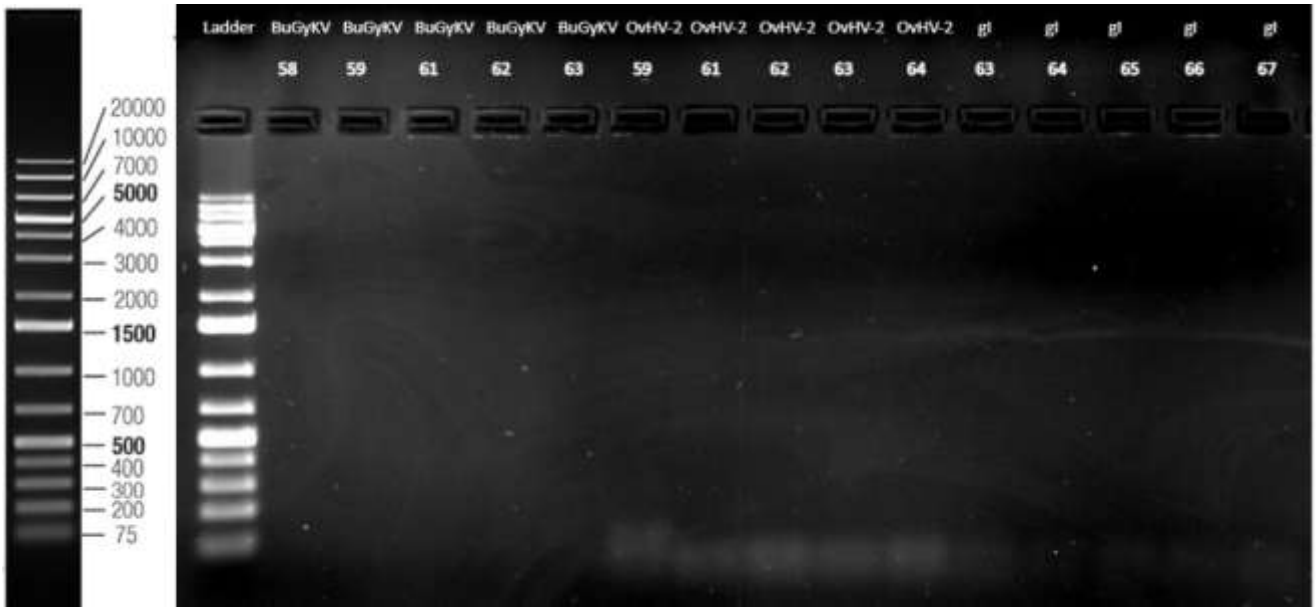


Figure 4. PCR optimization 2: Annealing temperatures (BuGyKV: 58-63°C); (OvHV-2: 59-64°C), and (BoHV-1 (gI): 63-67°C), with 5 ng of DNA input

FMDV detection targeting the conserved region of the 3Dpol gene was performed using five annealing temperature variations (53–57°C). All optimization steps showed no amplification bands at the target size (Figure 5). Given that the 3Dpol gene is a commonly used and relatively sensitive target for FMDV detection, these negative results indicate that viral RNA was likely absent from the tested tick samples or present at very low concentrations. This finding is consistent with epidemiological understanding that ticks are not the primary biological vector of FMDV (Yoo, 2011) and that the presence of the virus in ectoparasites, if present, is usually transient and dependent on the host's viremia phase (Byamukama et al., 2025; Elnekave et al., 2016).

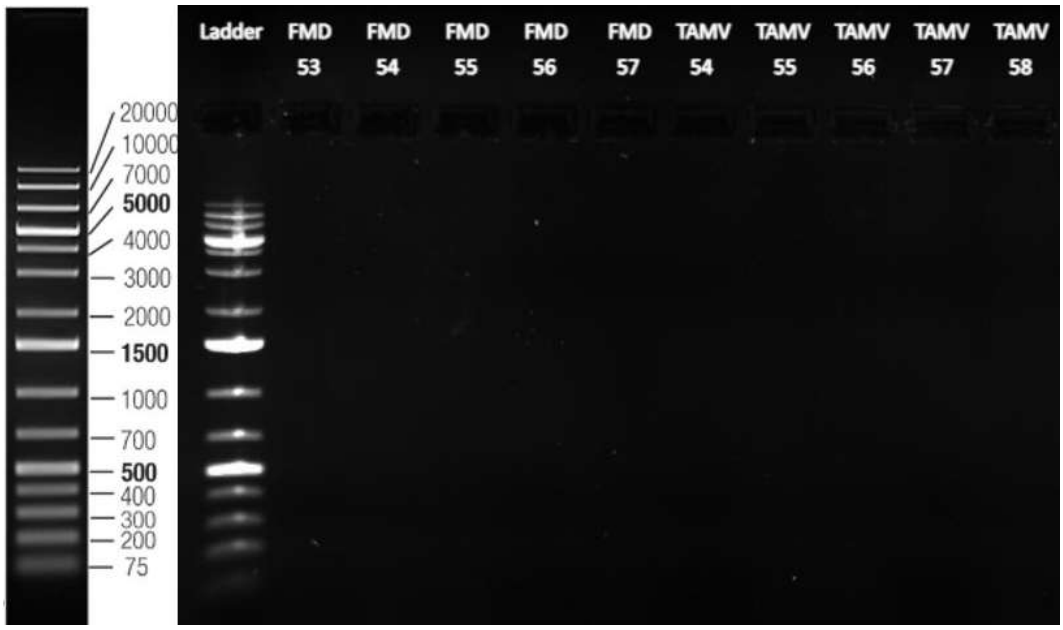
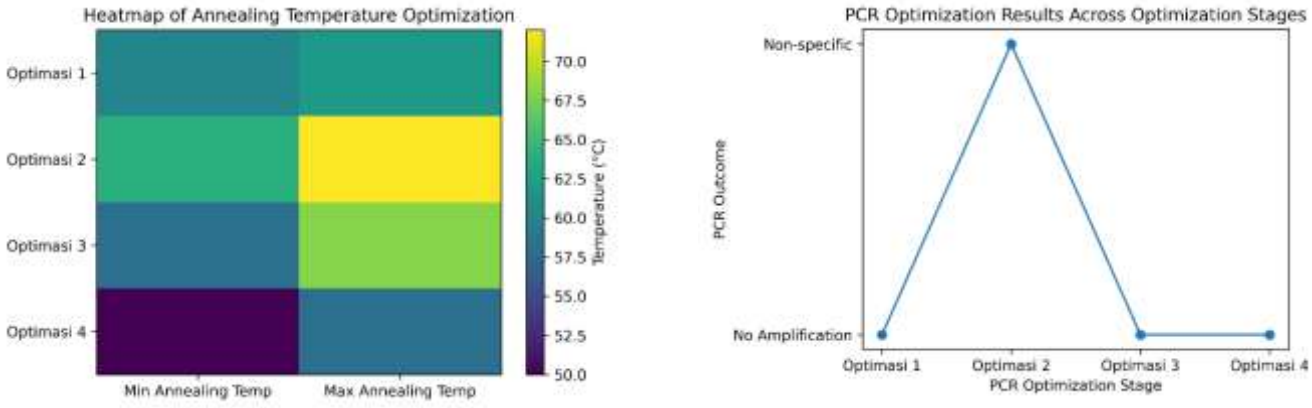


Figure 5. PCR amplification of FMDV (53-57°C), and TAMV (54-58°C), with 5 ng DNA input

Molecular screening of TAMV targeting the small (S) gene segment also failed to produce any amplification bands under all tested annealing conditions (54–58°C) (Figure 5). TAMV is an emerging RNA virus from the Nairoviridae family commonly found in certain genera of ticks in Central and East Asia (Moming et al., 2024; Wu et al., 2024). The absence of TAMV in ticks *H. bispinosa* from Moa Island may reflect

several possibilities, including low viral RNA titers, RNA degradation during the extraction process, or the absence of TAMV in the Moa tick-buffalo tick ecological system in this study area. This pattern of results is consistent with reports that nairovirus detection in arthropods often requires more sensitive methods, such as nested PCR, qPCR, or metagenomic approaches (Gao et al., 2025; Liu et al., 2022; Tokarz et al., 2014).



(a) (b)

Figure 6. (a) Heatmap of PCR annealing temperature optimization for five target viruses *H. bispinosa* ticks; (b)Summary of PCR optimization results for the detection of BuGyKV, OvHV-2, BoHV-1 (gI), FMDV, TAMV, and PCPV in H. bispinosa ticks

All PCR optimization steps in this study were grouped into four main stages based on the similarity of the experimental approach and the consistency of the amplification results, with the distribution of minimum and maximum annealing temperatures at each stage summarized in Figure 6a, and the PCR output and amplification specificity visualized in Figure 6b. Integration of PCR results and gel electrophoresis visualization showed that the lack of specific amplification of all target viruses occurred consistently across all optimization steps, making it unlikely to be due to a single technical failure but rather reflecting the limitations of conventional PCR in detecting low-titer viruses in tick samples. Although negative, these findings have important scientific value as baseline virological data, *H. bispinosa* from Moa Island and serve as a methodological basis for further research requiring molecular approaches with higher sensitivity.

Conclusion

Overall, the results of this study indicate that no specific amplicons were found for all target viruses, namely BuGyKV, BoHV-1, OvHV-2, FMDV, or TAMV, despite extensive annealing temperature optimization and variations in DNA input. This amplification failure is likely due to primer mismatches with local virus variants due to geographical differences. The results also indicate that ticks collected from Moa buffaloes during the sampling period likely did not carry the five viruses tested. These findings are important as a virological basis for the *H. bispinosa* population in Maluku and may serve as a foundation for recommendations for the use of more sensitive methods in the future, such as nested PCR, qPCR, or shotgun sequencing-based metagenomic approaches, which have been proven capable of detecting low-titer viral pathogens in arthropods.

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Author Contributions

Conceptualization, PU and RMK; methodology, RMK; validation, PU and RMK; formal analysis, PU and RMK; investigation, PU, and RMK; resources, RMK and PU; data curation, RMK; writing the original draft, PU, and RMK; writing the review and editing, RMK; visualization, RMK and PU. All authors have read and approved the published version of this manuscript.

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Conflicts of Interest

All authors declare that there is no conflict of interest in this article. The funding agency (LPPM UT) was not involved in the research design, data collection, analysis, interpretation, or writing of the publication from inception to completion of this

research.

References

Abbasi, F., Abbasi, I. H. R., Nissa, T. F., Bhutto, Z. A., Arain, M. A., Soomro, R. N., Siyal, F. A., & Fazlani, S. A. (2017). Epidemiological study of tick infestation in buffalo of various regions of district Khairpur, Pakistan. *Vet World*, *10*(6), 688–694. https://doi.org/10.14202/vetworld.2017.688-694

Abdallah, M. O., Niu, Q., Yang, J., Hassan, M. A., Yu, P., Guan, G., Chen, Z., Liu, G., Luo, J., & Yin, H. (2017). Identification of 12 piroplasms infecting ten tick species in China using reverse line blot hybridization. *Journal Parasitol*, *103*(3), 221–227. https://doi.org/10.1645/16-161

Ali, A., Khan, M. A., Zahid, H., Yaseen, P. M., Khan, M. Q., Nawab, J., Ur Rehman, Z., Ateeq, M., Khan, S., & Ibrahim, M. (2019). Seasonal dynamics, record of ticks infesting humans, wild and domestic animals, and molecular phylogeny of Rhipicephalus microplus in Khyber Pakhtunkhwa Pakistan. *Front Physiol*, *10*, 793. https://doi.org/10.3389/fphys.2019.00793

Byamukama, B., Amin, A., Mwiine, F. N., & Ekiri, A. B. (2025). Epidemiology and control strategies for foot-and-mouth disease in livestock and wildlife in Uganda: systematic review. *Vet Res Commun*, *49*(4). https://doi.org/10.1007/s11259-025-10791-z

Ci-Xiu, L., Mang, S., Jun-Hua, T., Xian-Dan, L., Yan-Jun, K., Liang-Jun, C., Xin-Cheng, Q., Jianguo, X., Holmes, E. C., & Zhang, Y.-Z. (2015). Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *Microbiology and Infectious Disease*. https://doi.org/10.7554/eLife.05378

Cui, M., Zhu, H. C., Wang, X., Cao, Y., Liu, D., Carr, M. J., Guan, Y., Zhou, H., & Shi, W. (2025). Tamdy virus pathogenesis in immunocompetent and immunocompromised mouse models. *Virulence*, *16*(1). https://doi.org/10.1080/21505594.2025.2503457

Dias, B. . P., Barbosa, C. C., Ferreira, C. S., Santos, S., Arrieta, O. A. P., Malta, W. C., Gomes, M. L. M. D., Alves, Silva, M., Fonseca, J. . M., Borges, L. P., & Silva, B. . M. (2023). Challenges in Direct Detection of Flaviviruses: A Review. *Pathogens*, *12*(5), 643. https://doi.org/10.3390/pathogens12050643

Durden, L. A., Merker, S., & L, B. (2008). The tick fauna of Sulawesi, Indonesia (Acari: Ixodoidea: Argasidae and Ixodidae. *Exp Appl Acarol*, *45*(1–2), 85–110. https://doi.org/10.1007/s10493-008-9144-z

Elnekave, E., Maanen, K., Shilo, H., Gelman, B., Storm, N., Berdenstain, S., Berke, O., & Klement, E. (2016). Prevalence and risk factors for foot and mouth disease infection in small ruminants in Israel. *Prev Vet Med*, *125*, 82–8. https://doi.org/10.1016/j.prevetmed.2015.12.019

Fuente, J., Antunes, S., Bonnet, S., Cabezas-Cruz, A., Domingos, A. G., Estrada-Peña, A., Johnson, N., Kocan, K. M., Mansfield, K. L., Nijhof, A. M., Papa, A., Rudenko, N., Villar, M., Alberdi, P., Torina, A., Ayllón, N., Vancova, M., Golovchenko, M., Grubhoffer, L., … Rego, R. O. M. (2017). Tick-pathogen interactions and vector competence: Identification of molecular drivers for tick-borne diseases. *Front Cell Infect Microbiol*, *7*, 114. https://doi.org/10.3389/fcimb.2017.00114

Gao, W., Xia, X., Wang, T., Liu, Y., Wang, J., Li, Y., Xia, L., Jia, N., Jiang, J., Sun, Y., & Cui. (2025). Diverse virome and potential pathogens in five tick species from metropolis surroundings of Beijing and Tianjin, China. *NPJ Biofilms and Microbiomes*, 1–34. https://doi.org/10.1038/s41522-025-00878-5

Karim, S., Budachetri, K., Mukherjee, N., Williams, J., Kausar, A., Hassan, M. J., Adamson, S., Dowd, S. E., Apanskevich, D., Arijo, A., Sindhu, Z. U., Kakar, M. A., Khan, R. M. D., Ullah, S., Sajid, M. S., Ali, A., & Iqbal, Z. (2017). A study of ticks and tick-borne livestock pathogens in Pakistan. *PLoS Negl Trop Dis*, *11*(6), 5681. https://doi.org/10.1371/journal.pntd.0005681

König, M. T., Frölich, K., Jandowsky, A., Knauf-Witzens, T., Langner, C., Dietrich, R., Märtlbauer, E., & Didier, A. (2023). First insights into the occurrence of circular single-stranded DNA genomes in Asian and African cattle. *Animals (Basel)*, *13*(9). https://doi.org/10.3390/ani13091492

Kuleš, J., Potocnakova, L., & Bhide, K. (2017). The challenges and advances in diagnosis of vector-borne diseases: where do we stand? *Vector-Borne and Zoonotic Diseases*, *17*(5), 285–296. https://doi.org/10.1089/vbz.2016.2074

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. *Mol Biol Evol*, *41*(12), 263. https://doi.org/10.1093/molbev/msae263

Kwak, M. L., & Ng, A. (2022). The detection of three new Haemaphysalis ticks (Acari: Ixodidae) in Singapore and their potential threat to public health, companion animals, and wildlife. *Acarologia*, *62*(4), 927–940.

https://doi.org/10.24349/fz2l-kg9r
Lannutti, L., Gonzales, F. N., Dus Santos, M. J., Florin-Christensen, M., & Schnittger, L. (2022). Molecular detection and differentiation of arthropod, fungal, protozoan, bacterial and viral pathogens of honeybees. *Veterinary Sciences*, *9*(5), 221. https://doi.org/10.3390/vetsci9050221
Lechmann, J., Ackermann, M., Kaiser, V., & Bachofen, C. (2021). Viral infections shared between water buffaloes and small ruminants in Switzerland. *J Vet Diagn Invest*, *33*(5), 894–905. https://doi.org/10.1177/10406387211027131
Liu, Z., Li, L., Xu, W., Yuan, Y., & Liang. (2022). Extensive diversity of RNA viruses in ticks revealed by metagenomics in northeastern China. *PLOS Neglected Tropical Diseases*, *16*(12), 11017. https://doi.org/10.1371/journal.pntd.0011017
Ma, R., Zhao, M., Wang, H., Hou, R., Qin, K., Qian, Y., Zhang, H., Zhou, Y., Wu, W., Gu, J., Wang, X., Shen, Q., Liu, S., Liu, J., Bi, W., Yu, Y., Chong, H., Hong, Y., Ji, L., … W, Z. (2022). Virus of giant Panda-infesting ticks teveals novel Bunyaviruses and other viruses that are genetically close to those from giant Pandas. *Microbiol Spectr*, *10:e02034-*. https://doi.org/10.1128/spectrum.02034-22
Ma, X., Gu, W., Li, X., Ding, X., & Zhu, L. (2025). Fatty acid synthase may facilitate the trafficking of bovine alpha herpesvirus 1 out of the Golgi apparatus, potentially promoting viral infection. *Microbiol Spectr*, *13:e01388-*. https://doi.org/10.1128/spectrum.01388-25
Moming, A., Bai, Y., Wang, J., Zhang, Y., Tang, S., Fan, Z., Deng, F., & Shen, S. (2024). The known and unknown of global tick-borne viruses. *Viruses*, *16*(12), 1807. https://doi.org/10.3390/v16121807
Moming, A., Shen, S., Fang, Y., Zhang, J., Zhang, Y., Tang, S., Li, T., Hu, Z., Wang, H., Zhang, Y., Sun, S., Wang, L. F., & Deng, F. (2021). Evidence of human exposure to Tamdy Virus, Northwest China. *Emerg Infect Dis*, *27*(12), 3166–3170. https://doi.org/10.3201/eid2712.203532
Nithikathkul, C., Polseela, P., Changsap, B., & Leemingsawat, S. (2002). Ixodid ticks on domestic animals in Samut Prakan Province, Thailand. *Southeast Asian J Trop Med Public Health*, *33*(S3), 41–44. https://europepmc.org/article/med/12971472
Riaz, A., Dry, I., Dalziel, R., Rehman, S. U., Shah, M. A., Akhtar, H. M., Yousaf, A., & Baig, R. (2021). Molecular detection and characterization of ovine herpesvirus-2 using heminested PCR in Pakistan. *J Vet Sci*, *4*(51). https://doi.org/10.4142/jvs.2021.22.e51
Tesfaye, Y., Khan, F., & Gelaye, E. (2020). Molecular characterization of foot-and-mouth disease viruses collected from Northern and Central Ethiopia during the 2018 outbreak. *Vet World*, *13*(3), 542–548. https://doi.org/10.14202/vetworld.2020.542-548
Tokarz, R., Williams, S. H., Sameroff, S., Sanchez Leon, M., Jain, K., & Lipkin, W. I. (2014). Virome analysis of Amblyomma americanum, Dermacentor variabilis, and Ixodes scapularis ticks reveals novel highly divergent vertebrate and invertebrate viruses. *J Virol*, *88*(19), 11480–92. https://doi.org/10.1128/JVI.01858-14
Utami, P., & Kunda, R. M. (2023). Surface ultrastructure of ticks (Acari: Ixodidae) on Moa buffalo from Southwest Maluku District, Indonesia. *Biodiversitas*, *24*(6), 3230–3235. https://doi.org/10.13057/biodiv/d240617
Utami, P., Kunda, R. M., & Anaktototy, Y. (2024). First metagenome report of Haemaphysalis bispinosa ticks of Moa buffalo from Southwest Maluku District, Indonesia. *Nusantara Bioscience*, *16*(1), 130–138. https://doi.org/10.13057/nusbiosci/n160116
Wu, Y., Zhou, Q., Mao, M., Chen, H., & Qi, R. (2024). Diversity of species and geographic distribution of tick-borne viruses in China. *Front Microbiol*, *15*(1309698). https://doi.org/10.3389/fmicb.2024.1309698
Yan, T., Wang, Z., Li, R., Zhang, D., Song, Y., & Cheng, Z. (2024). Gyroviruses: current status and challenges. *Front Microbiol*, *15*(1449814). https://doi.org/10.3389/fmicb.2024.1449814
Yoo, H. S. (2011). Foot and mouth disease: etiology, epidemiology and control measures. *Infect Chemother*, *43*(2), 178–185. https://doi.org/10.3947/ic.2011.43.2.178
Zeng, W., Yang, L., Cui, L., Liang, C., Zhu, D., Fang, Y., Zhang, Y., & Liu, H. (2025). Virome analysis and detection of ticks and tick-borne viruses in Shanghai, China. *Front Microbiol*, *16*(1699705). https://doi.org/10.3389/fmicb.2025.1699705