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Research article

First detection and genetic characterization of a novel duck reovirus in Vietnam based on the S1 segment

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Abstract

Duck reovirus (DRV), a member of the *Orthoreovirus* genus within the *Reoviridae* family, is associated with significant economic losses in the duck farming industry. This study aimed to detect the presence of DRV and genetically characterize the S1 gene segment of DRV strains identified on duck farms in Tien Giang Province, Vietnam. A total of 110 liver and spleen samples were collected from ducks exhibiting clinical signs suggestive of DRV infection. Reverse transcription-polymerase chain reaction was used to detect DRV. Positive samples were subjectied to phylogenetic and pairwise distance analyses based on the S1 gene sequence to determine genetic relatedness. The overall incidence of DRV infection was 14.54% (16/110), with a statistically significant association observed between infection rate and duck age ($p < 0.05$). Notably, ducks aged less than 4 weeks were 7.4 times more likely to be infected with DRV compared with those older than 6 weeks (95% confidence interval, 1.49–36.68; $p = 0.01$). Phylogenetic analysis revealed that the detected strain, designated CTU/NDRV/TG2024 (GenBank Accession No. PV034365), is closely related to novel DRV (NDRV) strains, sharing 94.48%–96.69% nucleotide identity. These findings confirm the circulation of an NDRV strain in Tien Giang Province, representing the first documented occurrence of DRV in the Mekong Delta region of Vietnam.

Keywords: Novel duck reovirus, S1 segment, Vietnam.

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INTRODUCTION

Avian reoviruses (ARVs) belong to the genus *Orthoreovirus* within the family *Reoviridae*. These viruses are non-enveloped and possess a double-stranded RNA genome enclosed in a double-layered icosahedral capsid. The genome is segmented into 10 parts, categorized by size into large (L1–L3), medium (M1–M3), and small (S1–S4) groups, based on their electrophoretic mobility in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Benavente and Martínez-Costas, 2007). These segments encode proteins designated by the Greek letters λ (lambda), μ (mu), and σ (sigma), corresponding to the large, medium, and small segments, respectively. Among these, the S1 segment of duck reovirus (DRV) is particularly important because it encodes three proteins: p10, p18, and σC. These proteins play essential roles in viral replication, cell entry, host attachment, and overall pathogenicity (Du et al., 2020).

Historically, ARV strains were classified using conventional serological assays. However, advances in molecular biology have enabled more accurate and detailed characterization through the analysis of nucleotide sequences and predicted amino acid compositions. Notably, the novel DRV (NDRV) was first isolated from Ma ducks exhibiting hemorrhagic and necrotic hepatitis (Chen et al., 2012). In contrast, Muscovy DRV (MDRV) primarily affects Muscovy ducks and is a known cause of necrotic hepatitis in this species. Unlike MDRV, NDRV demonstrates broader host tropism, infecting various poultry species, including Muscovy, meat, hybrid, and geese.

According to Woźniakowski et al. (2014), NDRV infections are associated with high mortality rates, often exceeding 44%. Clinical signs include dehydration, enteritis, renal atrophy, and hemorrhagic lesions in the liver and spleen. A distinguishing molecular feature of NDRV is the presence of S1 segment's coding of a unique 162-amino-acid nonstructural protein, P18, which differentiates it from the P17 protein found in other avian *Orthoreoviruses* (Du et al., 2020).

Due to its high morbidity and mortality and its ability to infect multiple duck species, NDRV represents a significant threat to duck farming in Vietnam. The surveillance is necessary to detect circulating and emerging wild-type strains, which is critical for the development of effective prevention and control strategies. Furthermore, the genetic diversity and evolving virulence of NDRV pose a substantial risk to animal health. Therefore, this study aimed to detect the presence and genetic characteristics of circulating NDRV strains in duck farms in Vietnam, focusing on the analysis of the S1 segment. The findings are expected to contribute to improved disease control efforts and help reduce economic losses in the poultry industry.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were approved by the Institutional Animal Care and the Animal Ethics Committee of Can Tho University, Vietnam (Approval No. CTU-AEC24030, Approval Date: 26 December 2023)

Study period and location

This study was conducted from May 2023 to October 2024 in Tien Giang Province, Vietnam. Ducks showing clinical signs such as lethargy, stunted growth, hemorrhagic hepatitis, and splenic lesions were considered as suspected cases of DRV infection. A total of 110 liver and spleen samples were collected from affected ducks across five commercial duck farms in the region (Figure 1). All molecular diagnostics, including reverse transcription-polymerase chain reaction (RT-PCR), were carried out at the Laboratory of Advanced Veterinary Medicine, Faculty of Veterinary Medicine, College of Agriculture, Can Tho University.

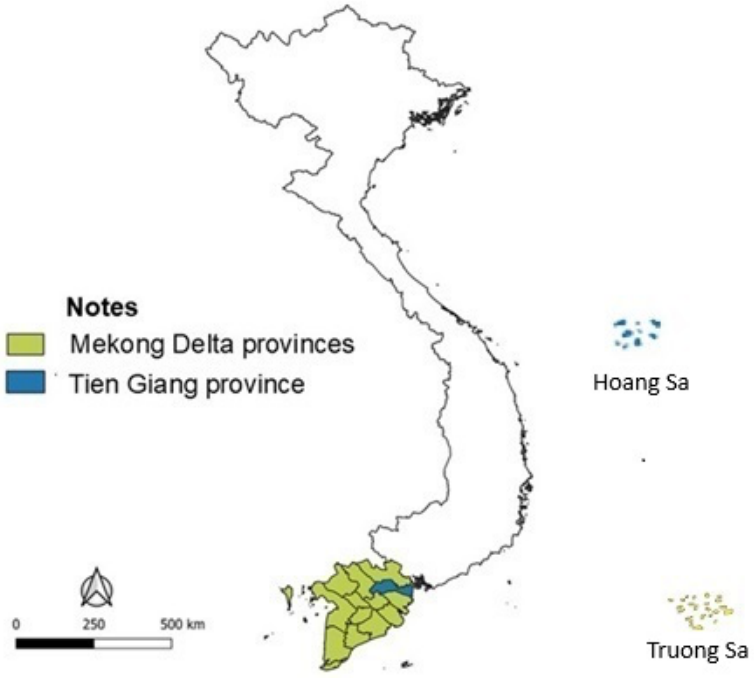

Figure 1 The Viet Nam map shows the area where samples were collected. Samples were collected from Tien Giang province (Coordinates: 10°25′N 106°10′E)

Sample collection

Liver and spleen samples were aseptically collected from ducks suspected of DRV infection. The samples were initially stored in sterile containers, maintained at 4°C during transport, and subsequently preserved at −70°C until further analysis.

RT-PCR for screening DRV

RNA extraction and cDNA synthesis

Total RNA was extracted from tissue samples using the NEXprep RNA extraction kit (South Korea), according to the manufacturer's protocol. Complementary DNA (cDNA) synthesis was conducted using the SensiFAST cDNA Synthesis Kit (Bioline, UK). Each 20 µL total reaction contained 10 µL of RNase-free water, 5 µL of extracted RNA, 1 µL of reverse transcriptase primer, and 4 µL of 5x TransAmp Buffer. The mixture was incubated on ice and subjected to the following thermal conditions: 25°C for 10 min, 45°C for 10 min, and 85°C for 5 min, followed by immediate cooling on ice for at least 1 min. The resulting cDNA was used immediately for PCR amplification.

Polymerase Chain Reaction (PCR)

Amplification of the S1 gene segment was performed using the MyTaq DNA Polymerase Kit (Bioline, UK) and primers reported by Liu et al. (2011): The forward primer sequence was 5′-CTT TCG GGA ATC GTG GTC-3′ and the reverse primer was 5′-CTG GAC TCA GGC AGC GTA-3′. A 25 µL total reaction volume was prepared by mixing 6.5 µL nuclease-free water, 12.5 µL MyTaq 2× Mix, 1 µL of each 20 µM primer, and 5 µL of cDNA template. PCR conditions were as follows: initial denaturation at 95°C for 15 s; 35 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s; and a final extension at 72°C for 3 min. Amplified products were visualized by 1.5% (*w/v*) agarose gel electrophoresis and imaged using a UV transilluminator (UVDI, USA).

Sequencing and phylogenetic analysis of the S1 gene

Sequencing of the amplified S1 segment was carried out in both directions using virus-specific primers. Raw chromatogram files were analyzed using GENETYX version 12 to resolve ambiguous nucleotide positions. Sequence alignment was performed using ClustalW algorithm implemented in BioEdit version 7.2.0 (Thompson et al., 1994; Alzohairy, 2011). Phylogenetic analyses were analyzed using MEGA version 6.06 (Kumar et al., 2016). Phylogenetic trees were constructed using the maximum likelihood method based on the General Time Reversible model with node support assessed by 1,000 bootstrap replicates. Reference strains used for tree construction are listed in Table 1.

Table 1 GenBank accession numbers and sources of isolated and reference reovirus strains

No.	Accession No.	Strain	Group	Country	Year	Host
1	PV034365	CTU/NDRV/TG2024	NDRV	Vietnam: Tien Giang	2023	Duck
2	MH510251	SH12	NDRV	China: Guangdong	2012	Muscovy Duck
3	MH510261	DH13	NDRV	China: Guangdong	2013	Muscovy Duck
4	KC312699	NP03/CHN/2009	NDRV	China	2009	Muscovy Duck
5	JX478266	J18	NDRV	China	2008	Muscovy Duck
6	KF154116	ZJ00M	NDRV	China	2000	Duck
7	MN747010	NDRV/GX-Y7/China/2018	NDRV	China	2018	Cherry Valley duck
8	MK955827	SY	NDRV	China	2018	Cherry Valley duck
9	MK749407	Duck/N-DRV-XT18/China/2018	NDRV	China	2018	Duck
10	PQ037221	WL01	NDRV	China	2023	Cherry Valley duck
11	KC493571	TH11	NDRV	China	2011	Duck
12	KJ879930	SD-12	NDRV	China	2012	Mallard wild duck
13	JX478256	091	NDRV	China	2009	Duck
14	KF741712	1733	ARV	China	2013	chicken
15	AF330703	S1133	ARV	Spain	2000	chicken
16	KF741772	Vaccine-S1133	ARV	China	2013	chicken
17	AF218359	138	ARV	Canada	1999	chicken
18	EF057398	T-98	ARV	China	2006	chicken
19	KJ871023	D1546	MDRV	France	2010	Muscovy Duck
20	KJ871013	D2044	MDRV	France	2012	Muscovy Duck
21	KF306088	ZJ2000M	MDRV	China	2011	Muscovy Duck
22	KC508653	815-12	MDRV	China	2010	Muscovy Duck

Recombination analysis

Recombination analysis of the CTU/NDRV/TG2024 strain was performed using SimPlot v3.5 and Recombination Detection Program (RDP version 4.70), following the method described by Khanh et al. (2017). SimPlot was used to identify potential recombination breakpoints based on a sliding window method (window size: 200 bp; step size: 20 bp), with nucleotide identity calculated using the Kimura 2-parameter model and a transition/transversion ratio of 2. Bootscan analysis was conducted using a 70% threshold for permuted tree signals. RDP4 analysis involved multiple detection methods, such as RDP, GENECONV, Bootscan, MaxChi, Chimera, SiScan, and 3Seq. Events with p values <0.05 were considered statistically significant.

Statistical Analysis

The association between DRV infection and age group was evaluated using the chi-square test and odds ratio (OR) analysis, performed in SPSS version 16.

The OR was calculated to assess the strength of association between exposure and infection, following the method described by Szumilas (2010). The formula used was:

$$OR = \frac{odd(D+/E-)}{odd(D-/E+)} = \frac{a/c}{b/d} = \frac{ad}{bc}$$

where *a* is the number of infected ducks with exposure, *b* is the number of uninfected ducks with exposure, *c* is the number of infected ducks without exposure, and *d* is the number of uninfected ducks without exposure.

RESULTS

The incidence of duck reovirus infection

Out of 110 samples tested using reverse transcription-polymerase chain reaction, 16 were positive for DRV, resulting in an overall infection rate of 14.54%. The age-wise distribution of DRV infection is presented in Table 2. Ducks younger than 4 weeks had the highest infection rate (28.57%), followed by those aged 4–6 weeks (11.11%) and those older than 6 weeks (5.13%; $p < 0.01$). Moreover, ducks under 4 weeks of age were significantly more likely to be infected with DRV, with an OR of 7.40 (95% confidence interval, 1.49–36.68; $p = 0.01$), compared with ducks older than 6 weeks. This finding aligns with previous studies reporting that younger birds are more susceptible to infection. This vulnerability is likely due to the immaturity of their immune systems and the reduction of maternal antibody protection during early development.

Table 2 The incidence of duck reovirus infection by age group

Age Group (weeks)	Tested Samples	Positive Samples	Positive rates (%)	OR; 95% CI	P-value
<4	35	10	28.57[a]	-	-
4-6	36	4	11.11[ab]	3.2; 0.90 – 11.42	0.07
>6	39	2	5.13[b]	7.4; 1.49 – 36.68	0.01
Total	110	16	14.54		

Value with different superscript letters (a, b) in a column are significantly different (P<0.05)

Phylogenetic analysis and pairwise sequence comparison of S1 segment

Among the 16 DRV-positive samples, those with high-quality sequencing results were selected for genetic analysis. All sequenced samples yielded identical nucleotide sequences for the S1 segment and were designated CTU/NDRV/TG2024 (GenBank Accession No. PV034365). Phylogenetic analysis comparing this strain with 21 reference sequences is shown in Figure 2.

Figure 2 illustrates that all strains clustered into three main groups: MDRV, NDRV, and ARV. The CTU/NDRV/TG2024 strain grouped closely with NDRV strains previously isolated in China and was distinct from the MDRV and ARV clusters, suggesting that CTU/NDRV/TG2024 belongs to the NDRV group.

Pairwise nucleotide sequence comparisons further confirmed this classification. The S1 segment showed that CTU/NDRV/TG2024 shared high nucleotide similarity with NDRV reference strains (95.26%–96.69%) but low similarity with MDRV (36.46%–36.78%) and ARV (37.81%–39.07%) strains (Figure 3).

In summary, phylogenetic and sequence similarity analyses indicate that the CTU/NDRV/TG2024 strain circulating in Tien Giang Province, Vietnam, belongs to the NDRV group.

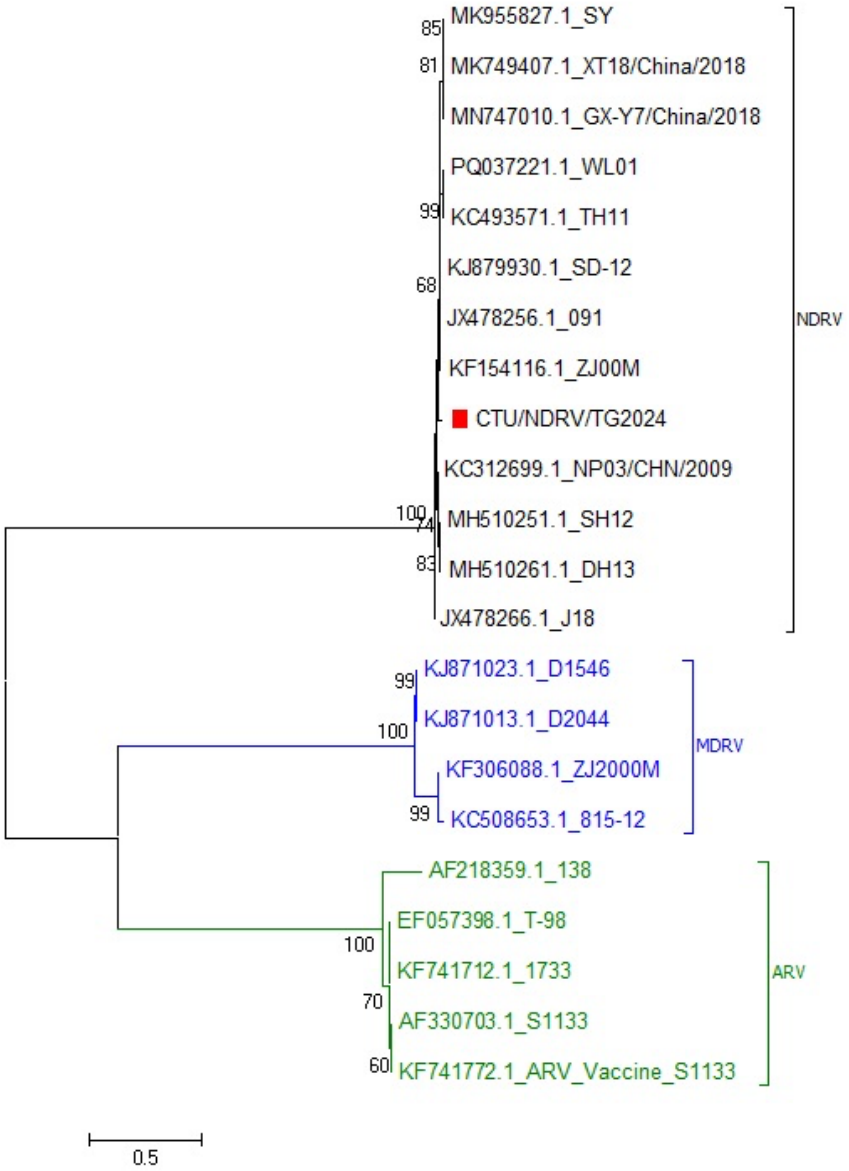

Figure 2 Phylogenetic relationships of CTU/NDRV/TG2024 strain and published reovirus strains based on nucleotide sequences of the S1 segment determined using MEGA 6 with the Clustal W method

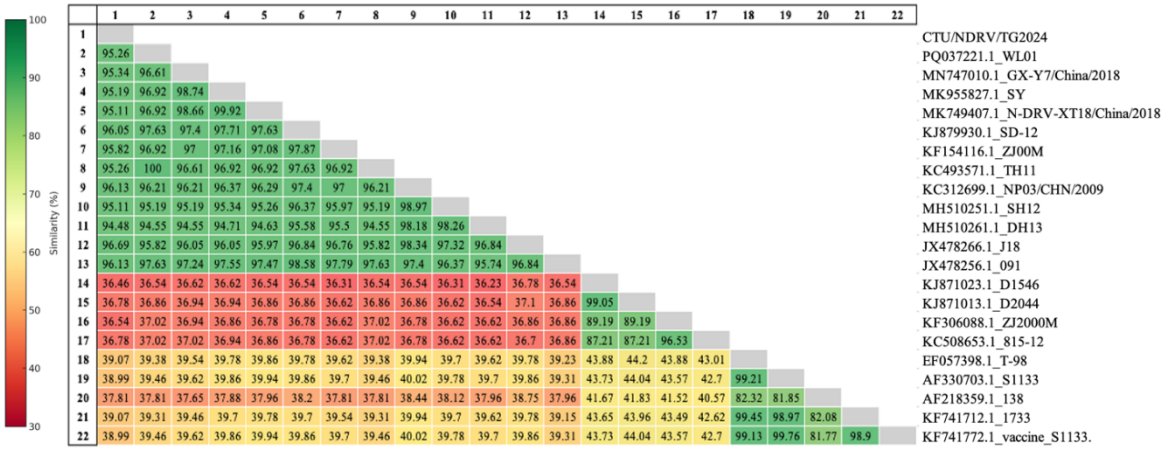

Figure 3 Nucleotide similarities of the S1 sequence between CTU/NDRV/TG2024 and reference DRV strains

Recombination analysis

Recombination analysis of the S1 gene segment of the CTU/NDRV/TG2024 strain was conducted using the SimPlot program and the RDP. No recombination events were detected.

DISCUSSION

Reovirus disease is an infectious condition affecting poultry and waterfowl, contributing to significant economic losses in the global livestock industry due to its widespread distribution and high pathogenicity. Avian reovirus, which predominantly affects poultry, especially chickens, was first documented in the United States in 1954 and is known to cause arthritis and growth retardation (Fahey and Crawley, 1954). MDRV, a pathogen that specifically affects Muscovy ducks, was initially described in South Africa in 1950 and later isolated in France in 1972 (Gaudry et al., 1972). To date, no confirmed cases of ARV or MDRV have been reported in ducks in Vietnam, making this study the first to genetically identify and characterize NDRV in the country.

NDRV was first isolated in 2005 from ducklings and classified as “novel” due to its broader host range, distinct clinical presentation, and unique cytopathic effects compared with ARV and MDRV (Chen et al., 2012). Malkinson et al. (1981) previously reported that DRV infection caused 30% morbidity and 20% mortality in Israeli poultry farms, typically affecting ducklings from 10 days old up to 6 weeks. Young ducks are especially vulnerable because their immune systems are still developing and their capacity to mount an effective immune response is limited. According to Li et al. (2016) and Zhang et al. (2016), NDRV infection most commonly occurs between 5 and 10 days of age, with reported infection rates of 10%–40% and mortality rates of 15%–50%.

Newly hatched ducklings rely on maternally derived antibodies passed through the egg for early protection against infectious diseases. However, these antibodies are short-lived and may wane before the duckling's immune system becomes fully functional. The findings of this study support earlier reports that younger ducks are more susceptible to reovirus infection. Specifically, ducks under 4 weeks of age were 7.4 times more likely to be infected than ducks older than 6 weeks, a statistically significant association. This aligns with existing literature indicating that immature immune function and the decline of maternal antibodies contribute to increased susceptibility in early life stages. Islam et al. (2020) also found that while factors such as breed, origin, and overall health had no significant

impact on ARV occurrence, variables such as age, sex, hygiene, and housing conditions were significantly associated with infection risk.

Novel DRV is a double-stranded RNA virus belonging to the *Reoviridae* family, genus *Orthoreovirus*. Its genome consists of 10 dsRNA segments: three large (L1, L2, and L3), three medium (M1, M2, and M3), and four small (S1, S2, S3, and S4) segments. Among these, the S1 segment plays a critical role by encoding the σC protein, which is involved in viral attachment, cell fusion, viral entry, induction of neutralizing antibodies, and pathogenicity (Benavente and Martínez-Costas, 2007; Du et al., 2020). Notably, the S1 segment is the most variable genomic region in ARVs and is widely used for classification purposes (Benavente and Martínez-Costas, 2007; Palomino-Tapia et al., 2018). In this study, phylogenetic analysis and pairwise nucleotide comparison of the S1 segment revealed that the CTU/NDRV/TG2024 strain isolated in Tien Giang Province, Vietnam, is genetically related to NDRV strains isolated in China. This close relationship suggests potential cross-border transmission, likely facilitated by poultry trade or migratory birds. Tien Giang lies along the Mekong Delta flyway, which intersects with migratory routes originating from southern China, reinforcing the plausibility of transboundary spread. The emergence of NDRV in southeast China in 2002 affected Muscovy ducks, Pekin ducks, and other species (Chen et al., 2012; Yun et al., 2014). Reported clinical symptoms include immunosuppression, growth retardation, severe diarrhea, and hemorrhagic or necrotic lesions in the liver and spleen (Li et al., 2016). In the present study, affected ducks displayed similar pathological signs, particularly hepatic hemorrhage and splenic necrosis observed during necropsy. Although mortality rates were not formally recorded, local farmers reported increased duckling mortality, particularly in those younger than 4 weeks, during the outbreak. NDRV infection may clinically resemble other duck diseases, such as duck hepatitis virus and duck Tembusu virus. The use of RT-PCR targeting the S1 segment enabled specific identification of NDRV in this study. Previous research has shown that NDRV exhibits a broader host range and higher pathogenicity than MDRV, infecting a wider variety of duck species (Yun et al., 2013; Wang et al., 2020). These characteristics make NDRV a significant threat to waterfowl health and productivity.

In summary, this study presents the first genetic characterization of NDRV in Vietnam. The results highlight the importance of continuous surveillance, particularly in border regions and along migratory routes, to monitor potential cross-border transmission. Genetic diversity in reovirus strains is critical to understanding viral evolution, epidemiology, and pathogenicity. This information also serves as a valuable resource for the development of targeted prevention and control strategies in veterinary medicine.

CONCLUSIONS

This study confirms the presence of NDRV, specifically strain CTU/NDRV/TG2024, in Tien Giang Province, Vietnam. The findings contribute valuable data to the understanding of NDRV epidemiology and highlight the need for enhanced surveillance and targeted vaccine development to mitigate mortality and economic losses in duck farming, particularly in the Mekong Delta region. Further research, including complete genome sequencing and pathogenicity assessment, is warranted to characterize this emerging strain better and inform effective control strategies.

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AUTHOR CONTRIBUTIONS

Nguyen Phuc Khanh: Conceptualization (supporting); Project administration (lead); Writing – review and editing (equal).
Phan Nhan: Conceptualization (lead); Writing – original draft (lead); Formal analysis (lead); Writing – review and editing (equal).
Nguyen Tran Phuoc Chien: Methodology (lead); Data curation (lead); Writing – review and editing (equal).
Chau Thi Huyen Trang: Visualization (lead); Writing – review and editing (equal).
Nguyen Thanh Lam: Investigation (lead); Data curation (supporting); Writing – review and editing (equal).
Tran Duy Khang: Software (lead); Formal analysis (supporting); Writing – review and editing (equal).
Truong Quynh Nhu: Methodology (supporting); Writing – review and editing (equal).
Nguyen Trong Ngu: Resources (lead); Supervision (lead); Writing – review and editing (equal).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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