

PRELIMINARY DETECTION OF *Gyrovirus galga 1* FROM DOMESTIC DOGS IN HANOI IN 2024-2025

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ABSTRACT

This study aimed to detect the presence of *Gyrovirus galga 1* (GyVg1) in domestic dogs from several districts in Hanoi, Vietnam. A total of 63 fecal samples were collected from dogs raised in the former Dong Anh, Long Bien, and Gia Lam districts between March 2024 and May 2025. Viral DNA was detected using polymerase chain reaction (PCR). The overall positive detection rate of GyVg1 was 12.7%, with rates of 14.29%, 12.12%, and 11.11% in Dong Anh, Long Bien, and Gia Lam districts, respectively. Partial sequences of the overlapping VP2 and VP3 genes from three representative strains were analyzed, revealing high nucleotide similarity rates ranging from 99.40% to 99.85%. Phylogenetic analysis demonstrated that these strains belong to *Gyrovirus galga 1* and they clustered closely with strains previously reported in dogs and cats from China in 2022. Additionally, several amino acid substitutions were identified in the VP2 and VP3 proteins of the Vietnamese strains. These findings provide the first evidence of GyVg1 circulation in domestic dogs in Hanoi and lay the groundwork for future studies on the virus's epidemiology, genetic diversity, and potential cross-species transmission.

Keywords: *Gyrovirus galga 1*, domestic dogs, Hanoi, PCR, phylogenetic analysis.

Phát hiện sơ bộ *Gyrovirus galga 1* lưu hành ở chó nuôi tại Hà Nội năm 2024-2025

TÓM TẮT

Nghiên cứu này được thực hiện nhằm phát hiện sự hiện diện của *Gyrovirus galga 1* (GyVg1) ở chó nuôi tại một số huyện ở Hà Nội. Tổng số 63 mẫu phân được thu thập từ chó nuôi tại các huyện Đông Anh, Long Biên và Gia Lâm từ tháng 3/2024 đến 5/2025. Kết quả phản ứng PCR cho thấy, tỷ lệ phát hiện vật liệu di truyền của GyVg1 là 12,7%, với tỷ lệ dương tính lần lượt là 14,29%; 12,12% và 11,11% tại các huyện Đông Anh, Long Biên và Gia Lâm. Kết quả phân tích trình tự một phần của gen VP2 và VP3 từ ba chủng đại diện cho thấy độ tương đồng nucleotide cao, dao động từ 99,40% đến 99,85%. Phân tích cây phát sinh chủng loài cho thấy các chủng này thuộc về *Gyrovirus galga 1* và có mối liên hệ chặt chẽ với các chủng đã được báo cáo trước đây ở chó và mèo tại Trung Quốc vào năm 2022. Ngoài ra, một số đột biến thay thế axit amin đã được xác định trong protein VP2 và VP3 của các chủng GyVg1 ở Việt Nam. Những phát hiện này sơ bộ cung cấp bằng chứng về sự lưu hành của GyVg1 ở chó nuôi tại Hà Nội và đặt nền tảng cho các nghiên cứu trong tương lai về dịch tễ học, đa dạng di truyền và khả năng lây truyền chéo giữa các loài của virus này.

Từ khóa: *Gyrovirus galga 1*, chó, Hà Nội, PCR, phân tích cây phát sinh chủng loài.

1. INTRODUCTION

In 2011, *Human Gyrovirus* (HGyV) was first reported in skin samples collected from healthy individuals. *Gyrovirus galga* (GyVg1), previously named *Avian Gyrovirus 2* (AGyV2), was discovered in sick chickens in Brazil and shared

a 96% genome sequence similarity with HGyV (Rijsewijk *et al.*, 2011). Although GyVg1 is primarily found in chickens, which are considered its main hosts, its DNA has also been detected in human blood samples, including those from HIV-infected individuals, organ transplant patients in Italy, and healthy blood

donors in France (Maggi *et al.*, 2012; Biagini *et al.*, 2013). Furthermore, GyVg1 DNA has been identified in various species such as humans (Sauvage *et al.*, 2011; Ye *et al.*, 2015), ferrets (Fehér *et al.*, 2015), snakes (Wu *et al.*, 2019), cats (Niu *et al.*, 2019), dogs (Liu *et al.*, 2022), and a range of zoo animals (Ji *et al.*, 2022).

The widespread occurrence of GyVg1 among chickens and other animals indicates it could have implications for human health. This virus, classified under the *Gyrovirus* genus within the *Anelloviridae* family, has a single-stranded, negative-sense circular DNA genome of 2375 or 2376 nucleotides. It contains three open reading frames (ORFs) that code for the proteins VP1 (capsid protein), VP2 (a nonstructural protein), and VP3 (a functional protein that triggers programmed cell death) (Yao *et al.*, 2017). In chickens, GyVg1 infection can lead to neurological damage, cognitive issues, and weight loss (dos Santos *et al.*, 2012). Although definitive evidence of GyVg1 pathogenicity is still lacking, necropsy reports in diseased chickens describe hemorrhaging, tissue edema, glandular stomach erosion, and craniofacial swelling. These pathological changes suggest that GyVg1 could pose a notable health threat to poultry (dos Santos *et al.*, 2012; Abolnik & Wandrag, 2014; Mase *et al.*, 2022).

Given its presence in chickens and its potential for transmission to humans, further investigation into GyVg1 in other animals like cats and dogs is essential to better understand its transmission pathways. Prior studies have detected GyVg1 in cat feces and dog blood (Niu *et al.*, 2019; Liu *et al.*, 2022). However, the virus has not been successfully isolated yet, and its infectivity in other species remains uncertain. Therefore, identifying circulating GyVg1 strains in different animals is critical for assessing their pathogenicity and potential risk to humans. In Vietnam, the information on GyVg1 is still limited. A report by Tran *et al.* (2004) indicated that GyVg1 was found in chickens in several northern provinces of Vietnam; but, no confirmed cases have been documented in other species to

date. Additionally, in recent years, as urbanization has expanded in Hanoi, more people are raising pets such as dogs and cats, leading to increased close contact between humans and their animals. Therefore, this study aimed to determine the presence of GyVg1 in dogs in Hanoi and to preliminarily analyze the genetic characteristics of the virus, providing baseline data for surveillance and disease control.

2. MATERIALS AND METHODS

2.1. Sample collection

Sixty-three fecal samples were collected from domestic dogs aged 2 to 36 months brought to several veterinary clinics in the the former Dong Anh, Long Bien, and Gia Lam districts of Hanoi from March 2024 through May 2025. Sampling was randomized among the domestic dogs; however, no dog showed diarrhea in the study. Then, samples were stored in separate containers and kept at -20°C until laboratory processing. A 10% (w/v) fecal of each sample was prepared in phosphate-buffered saline supplemented with gentamycin (10 mg/ml) for each sample. The homogenates were stored at -80°C at the Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Vietnam.

2.2. DNA extraction and polymerase chain reaction (PCR)

Total DNA was extracted from each homogenized samples using a Viral Gene-spin™ Viral DNA/RNA Extraction Kit (iNtRON Biotechnology, Seoul, Korea) according to the manufacturer's instructions.

For viral genome detection, the GyVg1-F1/R1 primers (Table 1) were used to amplify the target genomic region of 344 base pairs (bp) that encodes the overlapping VP2 and VP3 gene regions (Xu *et al.*, 2024). For sequencing, the primer pairs GyVg_F1/R1 and GyVg_QC2 F/R (Table 1) were employed to amplify the initial segment of the GyVg1 genome, specifically a 733 bp region from nucleotide 349 to 1082 (Yao *et al.*, 2017).

Table 1. Primers used in this study

Name	Primer sequence (5' - 3')	PCR product (bp)	Reference	Purpose
GyVg1-F1	CGTGTCCGCCAGCAGAAAC	344	Xu <i>et al.</i> (2024)	Detection
GyVg1-R1	GGTAGAAGCCAAAGCGTCCAC			
GyVg_QC-2F	TCACAGCCAATCAGAATTGAGCACG	733	Yao <i>et al.</i> (2017)	Nucleotide sequencing
GyVg_QC-2R	TTCTACGCGCATATCGAAATTTACC			

PCR reactions were performed in a total volume of 25 µl containing 2 µl of DNA template, 12.5 µl of GoTaq Green Master Mix (Promega, USA), 1 µl of each primer (10 µM), and 8.5 µl of distilled water. The following thermal conditions were utilized according to previous studies (Ye *et al.*, 2015; Yao *et al.*, 2017). A brief initial denaturation was performed at 95°C for 5 minutes, followed by 40 amplification cycles consisting of 30 seconds at 95°C, 30 seconds at 60°C, and 40 seconds at 72°C. This was concluded with a final extension at 72°C for 10 minutes. The PCR products were then run on a 1.2% agarose gel in TAE 1X at 100V for 30 minutes. Afterward, the gels were stained with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Seoul, Korea) and the DNA bands were visualized under UV illumination.

The PCR amplification of a samples produced a single, specific 344 bp band, with no evidence of non-specific products. In contrast, the negative control, which contained only distilled water, showed no bands. A positive control was not available in the experiment. A suspected PCR amplicon was then confirmed by sequencing and used as a positive control in subsequent reactions if identified as GyVg1.

2.3. Nucleotide sequencing and phylogenetic analysis

The PCR amplicons were purified with GeneClean II Kits (MP Biomedicals, Santa Ana, CA, USA). The sequencing of the GyVg1 genomes was carried out by 1st BASE, Malaysia (<http://base-asia.com/>). For sequence analysis, a representative sample from each sampling area was randomly chosen and subjected to sequencing.

The obtained sequences were examined by GENETYX ver.10 software (GENETYX Corp., Tokyo, Japan) and compared to existing sequencing through BLAST homology searches. Predicted amino acid (aa) comparisons were aligned and analyzed using the Clustal W algorithm of BioEdit (version 7.2) (Thompson *et al.*, 1994; Hall, 1999). Evolutionary distances were estimated based on the aligned sequences and the Kimura 2-parameter model. A phylogenetic tree was constructed using 47 sequences - including the sequences obtained in this study and those retrieved from GenBank - based on the Maximum Likelihood approach with 1,000 bootstrap replicates, implemented in MEGA 6.0 software (Tamura *et al.*, 2013).

2.4. Data Analysis

The data in this study were analyzed using algorithms implemented in Microsoft Excel. The 95% confidence intervals (CI) were calculated using the UCSF CTSI Sample Size Calculator (Kohn & Senyak, 2025)

3. RESULTS AND DISCUSSION

3.1. Detection of the GyVg1 genome in the field samples by the PCR method

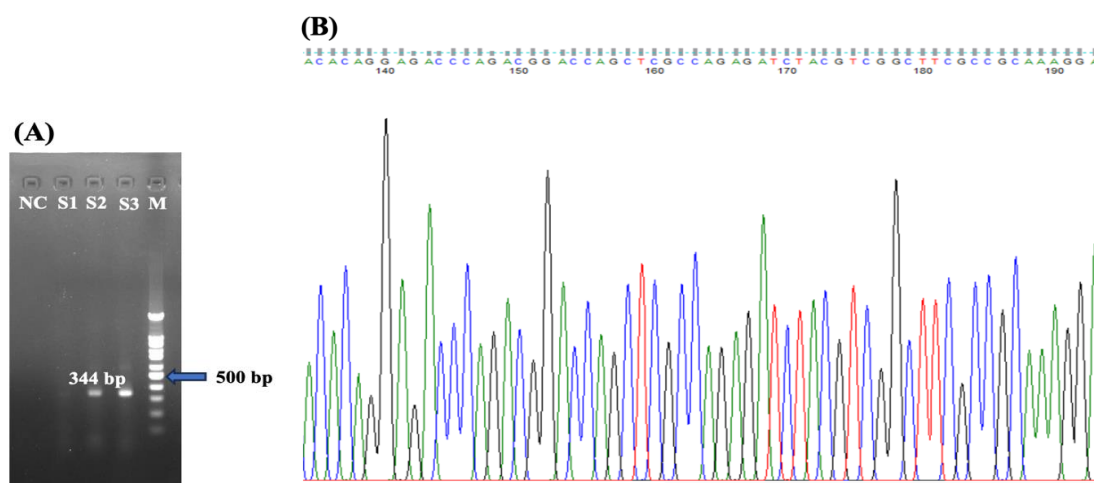
PCR amplification of the eight suspected positive field samples resulted in a single, specific band of 344 bp, without non-specific products observed, whereas the negative control containing only distilled water showed no amplification (Figure 1A). It has been strongly suggested that the previously published GyVg-F1/R1 primer pair could be used to diagnose the GyVg1 genome in field samples collected from dogs in Vietnam. In addition, there was no positive control sample used in the current study. As a result, the

suspected positive samples displaying a distinct 344 bp band were sequenced to confirm the presence of GyVg1 in the field samples (Figure 1B). The results of a sequence homology search from the GenBank database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (McGinnis & Madden, 2004) showed that the input sequence (310 nucleotides) was evaluated to have high similarity with *Gyrovirus* strains in chickens, humans, and dogs based on the gene sequence encoding the VP2 and VP3 proteins of GyVg1.

Diagnostic results revealed that eight out of a total of 63 samples (12.70%) were positive for the GyVg genome based on the PCR method. Specifically, the individual positivity rates in Dong Anh, Long Bien, and Gia Lam districts were 14.29%, 12.12%, and 11.11%, respectively (Table 2).

In this study, the GyVg1 genome was detected in domestic dogs raised in three districts in Hanoi. In a previous study, 12

GyVg1 strains were identified from 197 fecal samples collected from pet cats in China (Niu *et al.*, 2019). Another report showed the presence of GyVg1 in canine serum samples, with a positive rate of 0.28% (two out of 700 samples) using PCR (Liu *et al.*, 2022). Recently, GyVg1 was found in the serum of six dogs and three cats from Central and Eastern China in 2024. It has also been hypothesized that GyVg1 may be transmitted across species, with its occurrence suspected in infected chickens, humans, and other animal species (Rijsewijk *et al.*, 2011; Sauvage *et al.*, 2011; Fehér *et al.*, 2015; Niu *et al.*, 2019; Wu *et al.*, 2019). Combined with the current study, can be suggested that GyVg1 may have a wider geographic range than previously recognized. The results underscore the need for ongoing monitoring to give insights into the circulation and potential risk of GyVg1 infection in dogs and other species.

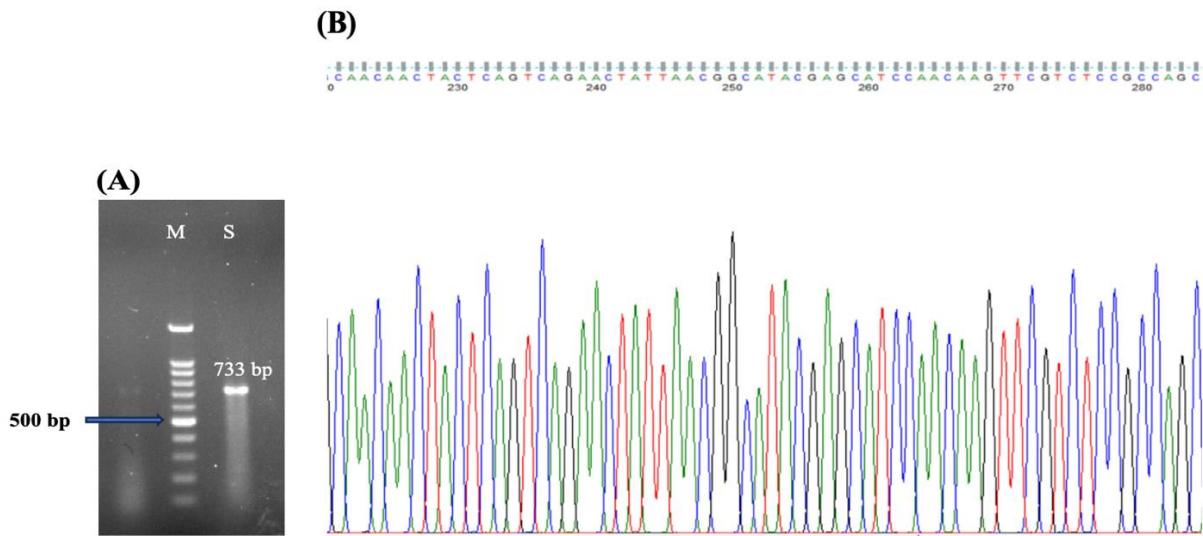


Note: M: DNA marker 100 bp (CSL-MDNA Cleaver Scientific, England); NC: negative control; S1, S2, S3: field samples.

Figure 1. Performance of GyVg1 genome detection: (A) PCR detection of GyVg1 genome; (B) Schematic diagram of PCR product sequencing result

Table 2. Detection of the GyVg1 genome in domestic dogs in Hanoi

District	Number of surveyed samples	Collection year	Number of positive samples	Positive rate (%)	95% CI
Dong Anh	21	2024	3	14.29	3.05-36.34
Long Bien	33	2025	4	12.12	3.40-28.20
Gia Lam	9	2025	1	11.11	0.28-48.25
Total	63		8	12.70	5.65-23.50



Note: M: DNA marker 100 bp (CSL-MDNA Cleaver Scientific, England); S: field sample.

Figure 2. Performance of GyVg1 genome sequencing:
(A) PCR amplification of the GyVg1 genome for sequencing (733 bp);
(B) Schematic diagram of a PCR product sequencing result

Table 3. Genome sequencing information of the three GyV1 strains circulating in Hanoi from 2024 through 2025

Virus strains	District	Year	Sequencing length (nucleotides)	Open reading frames
VNUA_DOG_DA1	Dong Anh	2024	699	2
VNUA_DOG_LB1	Long Bien	2025	699	2
VNUA_DOG_GL1	Gia Lam	2025	699	2

3.2. Genetic and phylogenetic analysis of the GyVg strains

Of the eight positive samples, three representative strains, labeled VNUA_DOG_DA1, VNUA_DOG_LB1, and VNUA_DOG_GL1 were selected for sequencing the partial VP2 and VP3 gene segments of GyVg1 using the QC2-F/R primer pair (Table 1). The PCR products exhibited a single, distinct band of 733 bp, with non-specific bands observed (Figure 2A). The products were then deemed suitable for genome sequencing of the GyVg1 strains. The resulting nucleotide sequences were clear, consistent, and free of background noise (Figure 2B). The VP2 and VP3 gene sequences of the three GyVg1 strains

were successfully obtained and were 699 bp in length (Table 3).

The nucleotide identity among the three GyVg1 strains obtained in this study ranged from 99.40% to 99.85%. Comparison with *Gyrovirus* gene sequences from humans and various animals in the Genbank database revealed that the three GyVg1 strains found in this study in dogs circulating in three districts in Hanoi, Vietnam shared 97.30% to 100% nucleotide similarity with *Gyrovirus* strains in humans; 96.40% to 100% with *Gyrovirus* strains in poultry; and 96.41% to 99.26% with strains from other species (Table 4). Specifically, when compared to the *Gyrovirus* gene sequences previously isolated in dogs and cats, the nucleotide similarity ranged from 96.41% to 99.86%.

Table 4. Nucleotide sequence identities (%) of the partial VP2 and VP3 genes of Vietnamese dog GyVg1 strains with the presentative GyVg strains

Virus strains	Nucleotide identity (%)		
	VNUA_DOG_DA1	VNUA_DOG_LB1	VNUA_DOG_GL1
VNUA_DOG_DA1			
VNUA_DOG_LB1	99.55		
VNUA_DOG_GL1	99.40	99.85	
Human Gyrovirus strains ^a	97.90–99.55	97.45–100	97.30–99.85
Dog Gyrovirus strains ^b	97.90–99.86	96.41–99.55	96.26–96.56
Cat Gyrovirus strains ^c	99.86	96.41	96.41–99.55
Avian Gyrovirus strains ^d	97.01–99.55	96.40–100	96.41–99.25
Gyrovirus strains in other species (silver pheasant, lion, egret, tiger, sika deer, peafowl, hippopotamus) ^e	96.86	96.41	99.26

Note: a: Human Gyrovirus type 1/G13 (KJ452214); Human Gyrovirus type 1/915F 06 007 FD (FR823283); b: DOG01 (OR9221199); DOG02 (OR921200); DOG03 (OR921201); DOG04 (OR921202); DOG05 (OR921204); DOG06 (OR921205); GXBS-26 (OK245348); GXHG-32 (OK245349); c: CAT01 (OR921197); CAT02 (OR921199); CAT03 (OR921203); d: 33-GD201908 (OQ116645); GX2 (OR355450); 5 Vietnamese GyVg strains (PQ154631-PQ154635); e: HN2019-S1 (OK540280); HN2019-T1 (OK540282); HN2019-SD1 (OK540284); HN2019-P1 (OK540286); HN2019-PF1(OK540283); HN2019-H1(OK540281); HN2019-E1 (OK540279); 139 Anello-1 (OM892265).

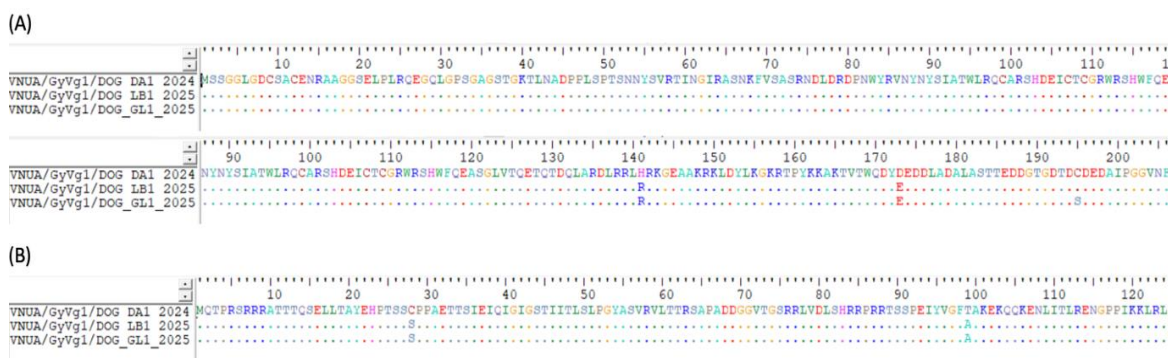


Figure 3. Analysis of amino acid substitutions of the three Vietnamese GyVg1 strains: (A) VP2 protein of the GyVg1 strain; (B) VP3 protein of the GyV1 strain

In addition, the VP2 protein, consisting of a 231 amino acid sequence, has been regarded as a highly conserved protein of GyVg1 (Yao *et al.*, 2017). VP3, a non-structural protein with 123 amino acid residues, has been considered to induce apoptosis in tumor cells (Bullenkamp *et al.*, 2012). The VP2 and VP3 protein amino acid sequences of the three GyVg1 strains were analyzed and compared. Preliminary findings indicated that several amino acid substitutions were observed in the sequences of both the VP2

and VP3 proteins (Figure 3). As reported for GyVCA, the conserved motif ‘WLRQCARSHDEICTCGRWRSH’ within amino acid residues 95–115 was proposed to serve as a target for VP2 site-directed mutagenesis and to contribute to impaired viral particle replication (Peters *et al.*, 2002). This same motif has also been detected in GyVg1 VP2 sequences. However, further experimental evidence is needed to confirm whether these substitutions affect the functions of the GyVg VP2 and VP3 proteins.

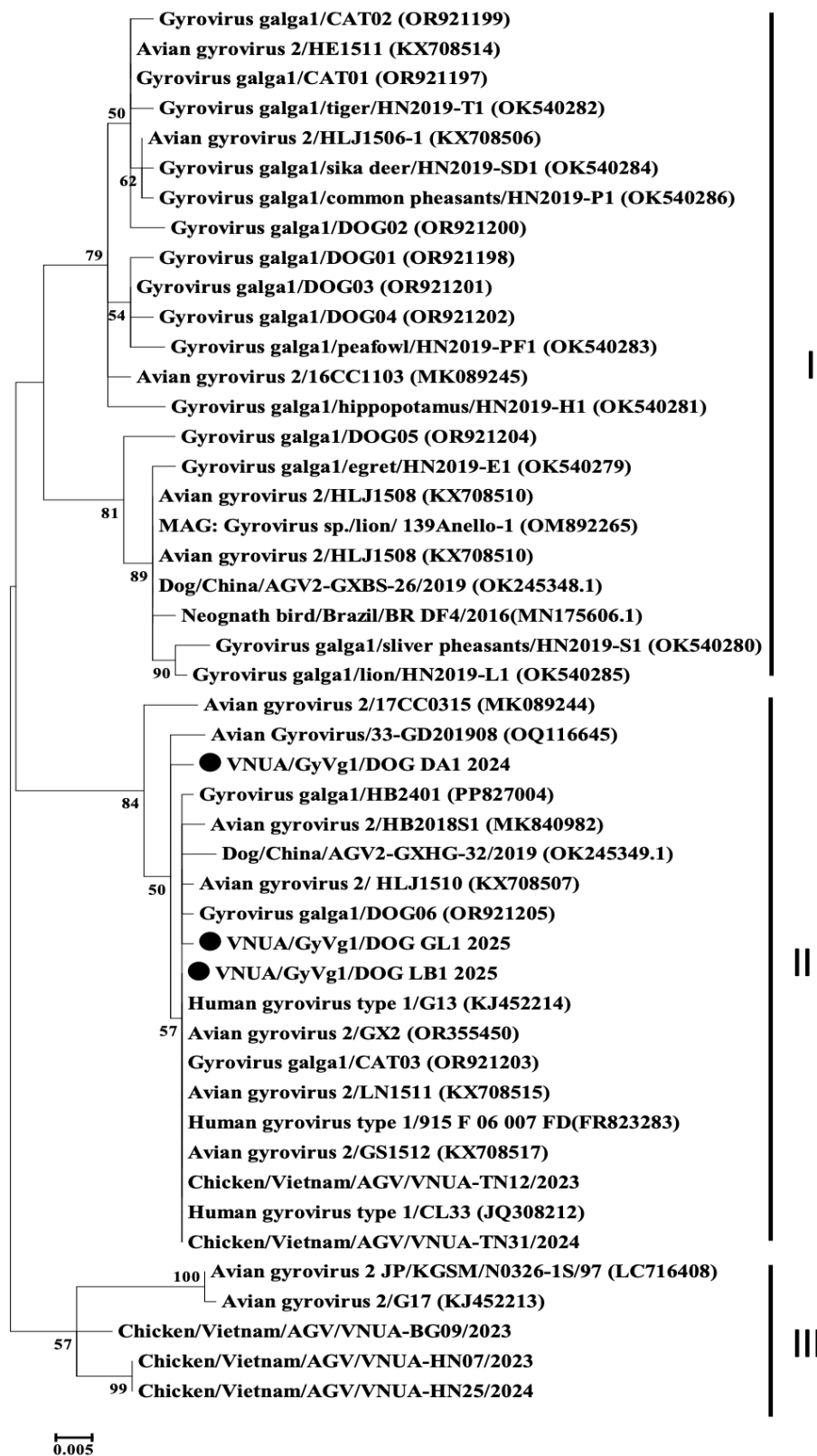


Figure 4. Phylogenetic tree of GyVg based on partial overlapping VP2 and VP3 gene sequences (699 nucleotides). The three GyVg1 strains in this study are marked with black circles on the phylogenetic tree

Phylogenetic analysis based on the partial VP2 and VP3 gene sequences of the three representative GyVg1 strains and the 44 *Gyrovirus* reference strains reported in various species from the NCBI database, including human, chickens, dogs, cats, tigers, neognath birds, lions, silver pheasants, egrets, peafowl, hippopotamuses, sika deer, and common pheasants, showed that VNUA_DOG_GL1_2025 and VNUA_DOG_LB1_2025 were clustered together in the same branch. These two strains were closely related to the *Human Gyrovirus* type 1/G13 strain, *Avian Gyrovirus* 2/GX2 strain, *Cat Gyrovirus*/CAT03 strain, and *Dog Gyrovirus* /AGV2-GXHG-32/2019 strain reported in China in 2022. In particular, VNUA_DOG_DA1_2024 formed a separate branch, closely related to *Avian Gyrovirus* /33-GD201908 (OQ116645) (Figure 4). Notably, all three GyVg1 strains were located in a distinct branch apart from the cluster containing the three GgVg1 strains previously identified in chickens in Vietnam (VNUA_BG09/2023, VNUA_HN07/2023, and VNUA_HN25/2024) (Figure 4). With the growing complexity of urban life, pets, including cats and dogs, are becoming increasingly embedded in daily human activities. In addition, GyVg1 and other *Gyrovirus* strains were detected in pet cats in northeastern China in 2019 (Niu *et al.*, 2019). Later, in 2022, GyVg1 was also found in serum samples from pet dogs in additional Chinese provinces (Liu *et al.*, 2022). Therefore, taken together with the previous research, the current findings indicate that urban transmission routes of GyVg1 may exist. This trend warrants further epidemiological assessments to better understand the risks linked to emerging GyVg1 strains. Long-term monitoring and in-depth molecular investigations are needed to clarify the virus's circulation dynamics and its role in animal and human health. Acknowledging the current study's limitations, these results lay the groundwork for future efforts to develop effective control and prevention strategies against GyVg1 infection across diverse hosts

4. CONCLUSIONS

This study is the first to report the detection of *Gyrovirus galga 1* (GyVg1) infection in field samples collected from domestic dogs in several old districts of Hanoi between 2024 and 2025, with an overall positivity rate of 12.7%. Genetic analysis revealed that the three Vietnamese GyVg1 strains are closely related to strains previously identified in dogs and cats in China in 2022, as well as to *Human* and *Avian Gyrovirus* strains. These findings underscore the potential for cross-species transmission and highlight the importance of comprehensive surveillance. Given the preliminary nature of this study, further research involving larger sample sizes and broader geographic coverage is necessary to better understand the epidemiology, health impacts, and zoonotic risk of GyVg1.

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