Virus Research 353 (2025) 199541

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Genetic characteristics and pathogenesis of clade 2.3.4.4b H5N1 high pathogenicity avian influenza virus isolated from poultry in South Korea, 2022–2023

Ra Mi Cha [a], Min-Ji Park [a], Yoon-Gi Baek [a], Yu-Na Lee [a], Yunyueng Jang [a], Yong-Myung Kang [a], Gyeong-Beom Heo [a], Se-Hee An [a], Kwang-Nyeong Lee [a], Jae-Kyeom Kim [b], Hye-Ryoung Kim [b], Youn-Jeong Lee [a], Eun-Kyoung Lee [a,*]

[a] Avian Influenza Research & Diagnostic Division, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do 39660, South Korea
[b] Avian Disease Research Division, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do 39660, South Korea

ARTICLE INFO

Keywords:
High pathogenicity avian influenza
H5N1
Pathogenesis
Genotype
Chicken
Duck

ABSTRACT

During the 2022–2023 winter season in South Korea, a novel clade 2.3.4.4b H5N1 HPAIV was first detected in wild birds, which then subsequently caused multiple outbreaks in poultry farms and wild birds. This study aimed to investigate the genetic characteristics of H5N1 HPAIVs isolated during the 2022–2023, along with their pathogenicity and transmissibility in chickens and ducks. The clade 2.3.4.4b H5N1 HPAIV viruses caused outbreaks in 75 poultry farms and detected in 174 wild bird cases. Phylogenetic analysis of hemagglutinin genes revealed that the South Korean H5N1 HPAIV isolates were closely related to Eurasian and American HPAIVs isolated between 2022 and 2023. In total, 21 diverse genotypes (22G0–22G20) were identified in virus isolates from poultry and wild birds, among which 22G7 was the dominant genotype. The 22G1 genotype (A/duck/Korea/H493/2022(H5N1)) caused high virulence and pathogenicity, with a 100 % mortality rate in specific-pathogen-free chickens. Ducks inoculated with genotypes 22G1 or 22G7 (A/duck/Korea/H537/2022(H5N1)) showed neurological signs, with 60 %–80 % mortality rate. In the contact groups of ducks, 100 % of transmissibility was observed. Notably, in the 22G7-inoculated group, viral shedding via the cloacal route was longer, and viral replication in the cecal tonsil was higher than that in the 22G1-inoculated group, which may have contributed to the dominancy of the 22G7 genotype. Therefore, better understanding of the genetic and pathogenic features of HPAI viruses is important for effective virus control in the field.

1. Introduction

H5 High Pathogenicity Avian Influenza viruses (HPAIVs) belong to the A/Goose/Guangdong/1/1996 (Gs/Gd) lineage consistently cause outbreaks in both domestic poultry and wild birds worldwide, causing huge economic losses to related industries and becoming a threat to public health [1–5]. Owing to diverse and extensive reassortment events in poultry and wild birds, Gs/Gd H5 HPAIVs have evolved into multiple hemagglutinin (HA) phylogenetic clades [6–8]. Notably, clade 2.3.4.4 viruses have been reported to be circulating globally since 2014. In 2020–2022, clade 2.3.4.4b H5N8 viruses caused outbreaks and subsequently H5N1 HPAIVs emerged as the most prevalent viruses worldwide, including in Europe, Asia, and America [3–5,8–10]. Reportedly, H5N1 HPAIVs caused one of the largest HPAIV epidemics in Europe, extensively affecting both poultry and wild birds in 2021–2022 [11–13]. The virus, thereafter spreading to North and South America, further causing multiple outbreaks in poultry and wild birds along with spillover to wild mammals [4,5,10].

From 2020 to 2022, clade 2.3.4.4b H5Nx HPAIVs were also introduced into South Korea. The H5N8 HPAIV isolate, which was genetically similar to the European H5 viruses isolated in 2020, caused outbreaks in poultry and wild birds in the 2020–2021 winter season and classified into two major genotypes (G1 and G2) [14]. During In the following winter season of 2021–2022, the novel clade 2.3.4.4b H5N1 HPAIV was first isolated from wild birds and poultry farms [15]. Phylogenetic analysis of HA genes revealed that the close association of H5N1 viruses

* Corresponding author at: Avian Influenza Research & Diagnostic Division, Animal and Plant Quarantine Agency, 177, Hyeoksin 8-ro, Gimcheon-si, Gyeongsangbuk-do 39660, South Korea.
E-mail address: ensenble@korea.kr (E.-K. Lee).

https://doi.org/10.1016/j.virusres.2025.199541
Received 3 December 2024; Received in revised form 31 January 2025; Accepted 31 January 2025

to the 2020–2021 Eurasian H5 and G2 genotypes of H5N8 HPAIVs. The South Korean H5N1 viruses presented four distinct genotypes, with an internal gene configuration similar to that of Eurasian low pathogenicity AI viruses (LPAIVs). Additionally, the H5N1 HPAIVs exhibited high virulence, pathogenicity, and transmissibility in specific-pathogen-free (SPF) chickens. In ducks, even with a longer period of viral shedding and higher transmission rates, the virus caused no mortality. These findings were similar to those reported for 2020–2021 H5N8 HPAIV isolates in South Korea [15,16].

During the 2022–2023 winter season, novel H5N1 HPAIVs belonging to clade 2.3.4.4b were identified in October 2022 [17], following which, H5N1 HPAIV caused outbreaks in multiple poultry farms and detected in many wild bird cases [18]. In this study, we investigated the genetic characteristics of South Korean clade 2.3.4.4b H5N1 HPAIVs isolated from poultry in 2022–2023 winter season and examined the pathogenicity and transmissibility of the virus in SPF chickens and ducks.

2. Materials and methods

2.1. Viruses

In the 2022–2023 winter season, H5N1 HPAIVs were isolated from 75 outbreaks in poultry farms and 23 wild bird cases by the Animal and Plant Quarantine Agency (APQA), South Korea (Table S1). Herein, 96 H5N1 HPAIV isolates (poultry: 75; wild birds: 21) were genetically characterized. Selected Korean H5N1 HPAIV isolates from wild birds identified by the National Institute of Wild Disease Control and Prevention (NIWDC) have been included for genetic analysis (https://me.go.kr/niwdc/web/index.do?menuId=50) [18]. Additionally, a DNA barcoding system utilizing mitochondrial DNA from feces was used to determine the species of wild birds, as previously described [19]. A/duck/Korea/H493/2022(H5N1) (H493/22), the first detected H5N1 HPAIV isolate (22G1 genotype), and A/duck/Korea/H537/2022(H5N1) (H537/22), the 22G7 genotype responsible for most outbreaks, were tested for pathogenicity and transmissibility in SPF chickens and ducks [17].

2.2. Sequencing, phylogenetic analysis, and genome constellation

H5N1 HPAIV isolates were sequenced, as previously described [20,21]. Briefly, viral RNA was extracted using a Patho Gene-spin DNA/RNA Extraction Kit (iNtRON Biotechnology, Seoul, South Korea) and amplified via reverse transcription polymerase chain reaction (PCR). The PCR products were then purified and sequencing libraries were generated using the Nextera DNA Flex Library Prep Kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. Complete genome sequencing was performed using the MiSeq next-generation sequencing platform (Illumina, San Diego, CA, USA). Finally, genome sequences were assembled directly using the CLC Genomics Workbench software (Qiagen) and BioEdit software. The viral genome sequences were deposited in the Global Initiative on Sharing All Influenza Data (GISAID) database (http://platform.gisaid.org; accessed on May 8, 2023); the accession numbers of each virus are listed in Table S1.

Hemagglutinin (HA) and neuraminidase (NA) gene sequences from 96 H5N1 HPAIV isolates were subjected to phylogenetic analysis. The reference dataset used to construct phylogenetic trees based on HA and NA sequences was obtained from the EpiFlu database of the GISAID platform (https://www.gisaid.org/). This dataset included HA and NA gene sequences from H5 and H5N1 viruses detected in Eurasia and North America (2020–2022) and Eurasia (2017–2022), respectively (Table S2). Next, maximum-likelihood phylogenies were generated using MEGA 6.0, with 1000 bootstrap iterations, and a monophyletic cluster was defined by a high bootstrap support (>70 %). The genome constellation was assessed based on the genetic diversity of H5N1 HPAIVs, which was determined using RAxML version 8.2.12, with gamma distribution and a general time-reversible model [22]. A dataset including all AI virus sequences collected from January 1, 2012, to January 5, 2022 (accessed on 12 May 2022), was obtained from the GISAID and GenBank databases, and this dataset was used for the phylogenetic analysis of each internal gene (PB2, PB1, PA, NP, MP, NS) (Fig. S4). Additionally, genotypes were analysed according to tree topology, and a nucleotide sequence identity of >97 % was considered significant at the bootstrap support value of >70.

2.3. Bayesian phylodynamic analysis (maximum clade credibility (MCC) tree analysis

The transmission dynamics of South Korean HPAIVs were characterized by host types by constructing an MCC tree for the HA gene using Bayesian Evolutionary Analysis by Sampling Trees software(BEAST), version 1.10.4, as previously described [14]. The dataset included H5N1 HPAIVs isolated from poultry (chickens and ducks) farms and wild birds in 2022–2023 [18] and the sequences were reduced using the Cluster Database at High Identity with Tolerance (CD-HIT) Suit. The final data set contained 117 sequences, including 93 viruses (chickens: 34, ducks: 38, and wild birds: 21) selected from viruses isolated in this study by host type. An uncorrelated log-normal distribution relaxed clock method, the Hasegawa–Kishino–Yano (HKY) nucleotide substitution model, and the GMRF Bayesian Skyride coalescent prior. Three posterior trees with effective sample sizes of >200 in Tracer 1.7.1 (http://tree.bio.ed.ac.uk/software/tracer/) after 50 million runs were combined using LogCombiner v1.10.4 (https://www.beast2.org/programs/). Next, 10 % of each run was removed as a burn-in and MCC trees were generated using TreeAnnotator v1.8.1 (http://beast.bio.ed.ac.uk/TreeAnnotator/) and visualized using FigTree 1.4.4 (http://tree.bio.ed.ac.uk/ software/figtree/). The transition of viruses between host types was evaluated using the discrete ancestral state reconstruction method and asymmetric host transitions. The Bayesian stochastic search variable selection was employed to identify the best-supported host transitions using the Bayes factor (BF) test. The BF and posterior probabilities were calculated using SpreaD3 v0.9.6. and considered significant when the values were >4 and 0.5, respectively. The number of transitions between host types (Markov jump) and the time between host-type changes (Markov reward) in poultry were calculated.

2.4. Animal experiments

For animal experiments, 5-week-old SPF chickens (Gallus gallus domesticus) were obtained from CAVAC (ChunAn, South Korea), and 2-week-old ducks were obtained from a commercial duck farm. All birds were negative for avian influenza (AI) antibodies and were housed in negative pressure, high efficiency, air filtered isolation cabinets within a biosafety level 3 facility. Water and feed were provided ad libitum. An intravenous pathogenicity index (IVPI) test of SPF chickens was performed according to the World Organization of Animal Health (WOAH) guidelines [19]. SPF chickens were divided into four groups (n = 5/group) and intranasally inoculated with 0.1 mL of H493/22(H5N1) (genotype 22G1), which was serially diluted 10-fold ranging from 10^3 to 10^6 mean egg infectious dose (EID_{50}), to determine the mean chicken lethal dose (cLD_{50}) of the virus. Similarly, ducks (n = 5/group) were intranasally inoculated with 10^6 EID_{50}/0.1 mL of H493/22(H5N1) (22G1 genotype) and H537/22(H5N1)(22G7 genotype). After 8 h, three naïve chickens or ducks were cohoused with 10^6 EID_{50} virus-inoculated groups to determine viral transmissibility. Additionally, three birds were inoculated intranasally with 10^6 EID_{50}/0.1 mL of the virus to examine viral replication in the internal organs at 3 d post-inoculation (dpi). Clinical signs and Mean Death Time (MDT) were observed for 1–14dpi. Serum samples were collected from any survived birds at 14 dpi, and hemagglutination inhibition (HI) tests were performed. cLD_{50} were calculated using the method described by Reed and Muench [23]. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of APQA (approval no.: 2021–612,

Fig. 1. Maximum-likelihood phylogenetic tree of the hemagglutinin gene of South Korean H5N1 High Pathogenicity Avian Influenza Virus (HPAIV) isolates from 2022 to 2023. The scale bar represents the number of nucleotide substitutions per site. A monophyletic cluster was defined when bootstrap values (1000 replicates) were >70 %. Red dot: H5N1 HPAIVs isolated from poultry; Blue dot: H5N1 HPAIVs isolated from wild birds, Green dot: 2021–2022 H5N1 HPAIV isolates in Korea; Bold: H5N1 HPAIVs isolated from Animal and Plant Quarantine Agency.

2023–765).

2.5. Viral shedding, virus replication in organs, and HI test in chicken and ducks

Oropharyngeal (OP) and cloacal (CL) swabs were collected from 10^6 EID_{50}-inoculated and contact groups at 1, 2, 3, 4, 5, 6, 7, 10, and 14 dpi to monitor viral shedding. The virus was isolated from swab samples as previously described [16,20]. Briefly, OP and CL swab samples were suspended in 1 mL of Dulbecco's modified Eagle's medium (Gibco, Invitrogen, USA) containing antibiotics (Invitrogen), and each tissue sample was homogenized (10 % wt/vol). Homogenized samples were centrifuged, and the supernatant was titrated against chicken embryo fibroblasts (DF-1) to determine the 50 % tissue culture infective dose ($TCID_{50}$). The viral titers were calculated using the method described by Reed and Muench [20]. Area under the curve (AUC) analysis were performed using GraphPad Prism 5 (San Diego, CA, USA) to compare the virus shedding period between two different virus inoculated ducks. To detect viral replications in different organs, three birds were euthanized and necropsied, and 12 organs (trachea, thymus, heart, lung, kidney, brain, pancreas, cecal tonsil, liver, spleen, muscle, and proventriculus) were collected. and virus replication were measured in each organ by DF-1 cell titration. The limit of viral detection was <1 $\log_{10}$ $TCID_{50}$/0.1 mL. Statistical significance was determined by a Student's *t*-test for independent samples using GraphPad Prism 5 (San Diego, CA, USA). The HI test of serum samples from the remaining birds alive at 14 dpi was performed according to WOAH standard procedures [16].

2.6. Necropsy and histopathology in ducks

Necropsy and histopathological examinations were conducted in accordance with an APQA diagnostic protocol. Three euthanized ducks were necropsied at 3 dpi to examine gross lesions, and their tissues (trachea, lungs, heart, brain, cecal tonsil, pancreas, liver, spleen, thymus, kidneys, proventriculus, and skeletal muscle) were collected for histopathological examination. Collected tissues were fixed for 24 h in 10 % neutral-buffered formalin and embedded in paraffin blocks, which were then cut into 5-μm-thick sections, dewaxed, and stained with hematoxylin–eosin. Additionally, four ducks that died during the experiment were necropsied and sampled following the same procedure.

3. Results

3.1. Detection of H5N1 HPAIVs isolated in 2022–2023 in South Korea

Clade 2.3.4.4b H5N1 HPAIV was first detected in a wild mandarin duck in October 2022 [17]. From October 2022 to April 2023, 75 outbreaks in poultry farms (chickens ($n = 34$), ducks ($n = 38$), and minor poultry ($n = 3$)) were reported across the country (Fig. S1; Korean Animal Health Integrated System). In addition, 174 H5N1 HPAIV were detected in wild birds by APQA ($n = 23$) and NIWDC ($n = 151$) [18].

3.2. Genetic characterization of H5N1 HPAIV isolates

The genetic characteristics of isolated HPAIVs were investigated using a phylogenetic tree analysis of the HA and NA genes. In total, 96 South Korean H5N1 virus isolates from poultry ($n = 75$) and wild birds ($n = 21$) were analysed (Table S1). All H5N1 HPAIV isolates harboured multibasic amino acid sequences (PLRERRKR × GLF) at the HA cleavage site (Table S1). Analysis of the HA gene confirmed the clade 2.3.4.4b and a close relation of viruses with HPAIVs isolated from Eurasian and American countries in 2021–2023 (Fig. 1). Most South Korean H5N1 HPAIVs belonged to the G2c genotype group, with only one belonging to the G2d group. In addition, three H5N1 virus isolates (H625/22, WA537/22, and H633/22) clustered with North American H5N1 viruses isolated in 2021–2023 [17]. Phylogenetic analysis of the NA gene also showed that H5N1 viruses were closely related to Eurasian and American viruses isolated in 2021–2023, similar to HA gene analysis (Fig. S2). Bayesian phylodynamic analysis of the HA gene in selected South Korean H5N1 HPAI isolates was performed according to host type (chicken and ducks ($n = 72$); wild birds ($n = 45$)) [18]. Host transition analysis, including Markov jump and BF, and posterior probability analysis revealed significant values for wild birds-to-ducks, and wild birds-to-chickens virus transmission (Table 1). Moreover, MCC tree analysis indicated that the highest transmission of viruses was observed in wild birds-to-chickens or -ducks transmissions (Fig. S3).

Table 1
Virus transition between different host species (chicken, ducks and wild birds) in South Korea.

Transition From	Transition to	Mean actual migration rate[1] (95 % HPD)	Markov jump	Posterior Probability	Bayers factor
wild birds	duck	1.50 (0.14–3.20)	13.98 (9.00–19.00)	1.00	3682.16
wild birds	chicken	1.34 (0.13–2.93)	12.28 (7.00–17.00)	1.00	2629.76
Duck	chicken	0.80 (0.00–2.12)	3.69 (0.00–8.00)	0.96	29.62
Duck	wild birds	0.22 (0.00–2.69)	0.14 (0.00–1.00)	–	–
Chicken	duck	0.54 (0.00–2.32)	1.76 (0.00–6.00)	0.69	2.68
Chicken	wild birds	0.39 (0.00–2.32)	0.76 (0.00–3.00)	0.55	1.49

[1] Actual migration rate was calculated as the rate x indicator; HPD, highest probability density.

3.3. Genome constellation of H5N1 HPAIVs isolated in South Korea

Phylogenetic analysis of each of the eight gene segments revealed 10 distinct genotypes (22G0–22G9) in H5N1 HPAIV isolates from poultry outbreaks (Figs. 2B and S4). Additionally, 19 genotypes (namely 22G1–22G8 and G10–G20) were identified in H5N1 HPAIV isolated from wild birds (Figs. 2A and S4) [18]. The HA and NA genes in all genotypes belonged to the same cluster as the Eurasian/American HPAIVs isolated in 2021–2023. However, most genotypes presented a diverse combination of reassorted internal genes, which may have originated from Eurasian LPAIVs (Table 2). Specifically, the 22G4–22G11 genotypes comprised a combination of different internal genes, except HA, NP, NA, and M, which maintained the same cluster as that in the 22G3 genotype. Among all genotypes, the 22G7 genotype was identified as the dominant genotype in both poultry and wild birds and was isolated throughout the period of November 2022 to April 2023 (Figs. 2 and S4).

4. Pathogenesis and transmissibility in SPF chickens

The pathogenicity and transmissibility of A/duck/Korea/H493/2022(H5N1)(H493/22), an H5N1 HPAIV isolate (genotype 22G1) first detected in 2022–2023, was evaluated in SPF chickens. The IVPI of 2.98 confirmed that the virus was an HPAIV, according to WOAH standards [19]. The virus-inoculated chickens presented clinical signs of depression, greenish diarrhea, and neurological signs. The mortality rate was 100 %, with an MDT of 2.8 days at a cLD_{50} value of $10^{5.0}$ EID_{50} (Table 3). All three birds in the contact group died, with an MDT of 4.3 days. Seroconversion was not observed in any of the surviving SPF chickens. Virus shedding by H493/22-exposed chickens via the OP and CL routes was detected for 1–4 dpi. The peak titre was observed at 2–3 dpi. The contact group also showed similar levels of virus shedding, which lasted for 3–5 dpi. Furthermore, virus replications were observed in all internal

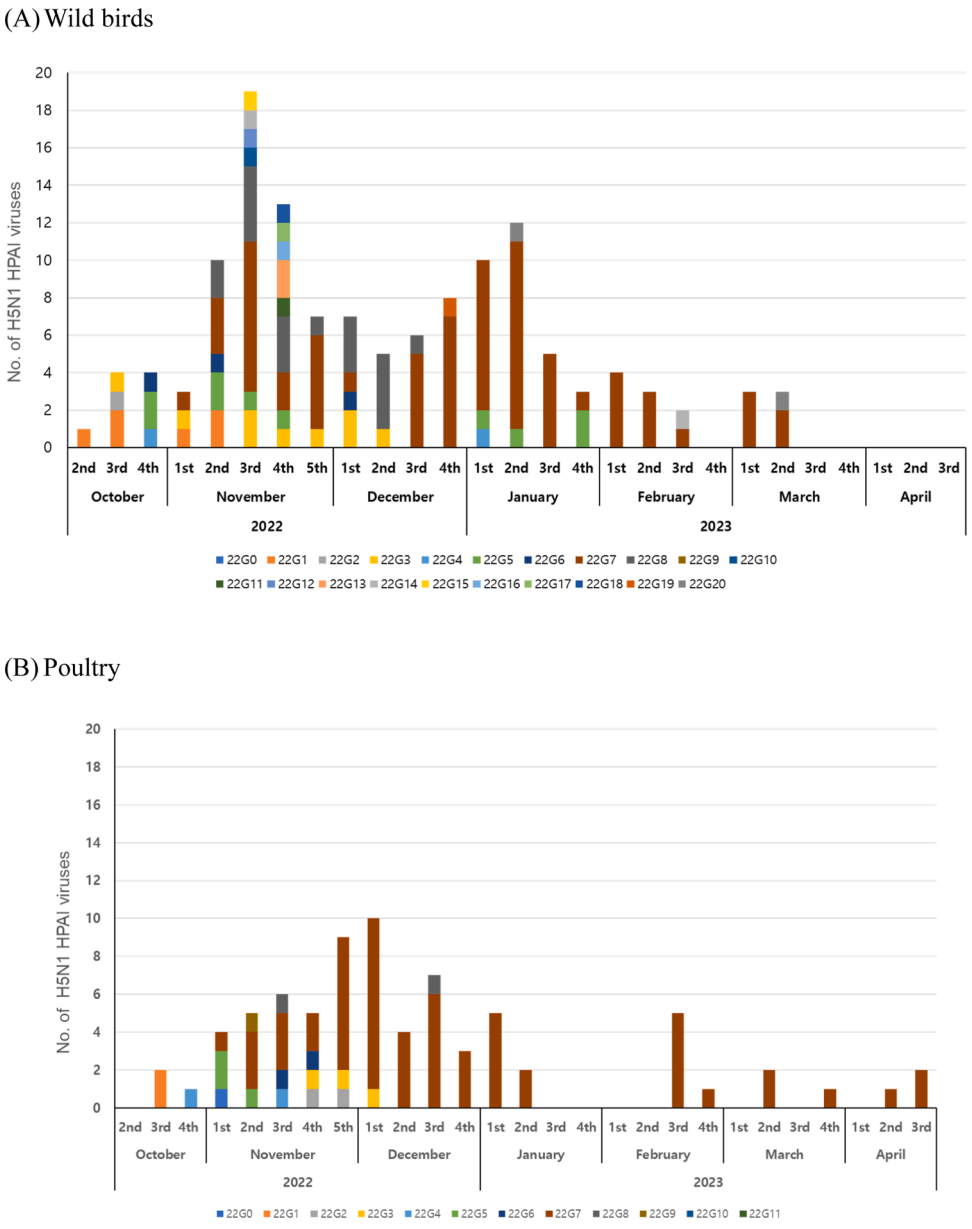

Fig. 2. Gene constellation of South Korean H5N1 high-pathogenicity avian influenza viruses (HPAIVs) isolated from poultry ($n = 75$) and wild birds ($n = 132$) in 2022–2023. Detailed gene constellation analysis of H5N1 HPAIVs was performed by phylogenetic analysis of all eight gene segments. The genotypes of the H5N1 HPAIVs isolates are shown according to isolation date (October 2022–April 2023).

organs at 3 dpi, with the highest viral titres was detected in the thymus (Fig. S5).

5. Pathogenesis and transmissibility in ducks

The pathogenicity and transmissibility of both H493/22(22G1) and A/duck/Korea/H537/2022(H5N1)(H537/22(22G7); responsible for most outbreaks in 2022–2023) were assessed in ducks. H493/22(22G1)-inoculated ducks presented clinical signs of depression and neurological signs, along with a mortality rate of 80 % (Table 3). The mortality rate in H537/22 (22G7)-inoculated ducks was slightly lower (60 %) than in the 22G1-inoculated group with a similar MDT (Table 3). Contact groups exposed to both viruses showed the same mortality rate (66.7 %) and had slightly different MDTs, demonstrating 100 % transmissibility. All surviving ducks in the inoculation and contact groups underwent seroconversion. In addition, extensive virus shedding via the OP and CL routes was observed in ducks inoculated with 22G1 and 22G7 at 1–7 dpi, and that in the contact group was observed at 2–10 dpi (Fig. 3). In particular, the 22G7-inoculated group exhibited virus shedding via the CL route for a longer period than the 22G1 group. We also compared the AUC values and found that 22G7 group has a larger area under the curve compared to 22G1 group (Fig. S9). At 3 dpi, viral replication was detected in all internal organs in both the inoculated groups; however, viral titers in the cecal tonsils of the 22G7-inoculated group were significantly higher compared to those of 22G1 group (Fig. 3).

Finally, gross and microscopic lesions were examined in the internal organs of 22G1-inoculated ducks at 3 dpi and deceased ducks to confirm HPAIV infection. Notably, no gross lesions were found in ducks euthanized at 3 dpi, whereas the deceased ducks presented opisthotonus, greenish diarrhea, gray–yellow necrosis in the liver, necrotic foci in the pancreas, and focal hyperemia and hemorrhage in the cerebrum (Fig. S6). Microscopic lesions were present in nine tissues from at least one duck at 3 dpi, with the trachea, lungs, and brain being heavily affected in all three euthanized ducks (Table S1; Fig. S7). In addition,

Table 2
Genome constellation of H5N1 (clade 2.3.4.4b) HPAI in South Korea in 2022–2023.

Host	Virus name	Subtype	Reassortant name	Genotype	Phylogenetic group within each gene segment							
					PB2	PB1	PA	HA	NP	NA	MP	NS
Wild Birds	WA496/22	H5N1	BDE5B1AB	22G1	B	D	E	5	B	1	A	B
	WA537/22	H5N1	FFE5D1AD	22G2	F	F	E	5	D	1	A	D
	H503/22	H5N1	BDE5B1AB	22G1	B	D	E	5	B	1	A	B
	H518/22	H5N1	ACF5A1AE	22G6	A	C	F	5	A	1	A	E
	H520/22	H5N1	BCC5A1AE	22G5	B	C	C	5	A	1	A	E
	H521/22	H5N1	DBD5A1AC	22G4	D	B	D	5	A	1	A	C
	WA608/22	H5N1	BCC5A1AE	22G5	B	C	C	5	A	1	A	E
	H536–4/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H547–2/22	H5N1	CCC5A1AE	22G8	C	C	C	5	A	1	A	E
	H571–3/22	H5N1	BCC5A1AE	22G5	B	C	C	5	A	1	A	E
	WA649/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	WB025/22	H5N1	ACF5A1AE	22G6	A	C	F	5	A	1	A	E
	WA709/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	WA737/22	H5N1	BCC5A1AE	22G5	B	C	C	5	A	1	A	E
	H586/22	H5N1	BCC5A1AE	22G5	B	C	C	5	A	1	A	E
	H599/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H588–4/22	H5N1	BEB5A1AE	22G3	B	E	B	5	A	1	A	E
	H593–4/22	H5N1	BAG5A1AE	22G10	B	A	G	5	A	1	A	E
	H600–1/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	WA766/22	H5N1	BCC5C1AE	22G11	B	C	C	5	C	1	A	E
	WA842/22	H5N1	BEB5A1AE	22G3	B	E	B	5	A	1	A	E
Poultry	H493/22	H5N1	BDE5B1AB	22G1	B	D	E	5	B	1	A	B
	H504/22	H5N1	BDE5B1AB	22G1	B	D	E	5	B	1	A	B
	H515/22	H5N1	DBD5A1AC	22G4	D	B	D	5	A	1	A	C
	H535/22	H5N1	BCC5A1AE	22G5	B	C	C	5	A	1	A	E
	H538/22	H5N1	BCC5A1AE	22G5	B	C	C	5	A	1	A	E
	H539/22	H5N1	EDE5B1AB	22G0	E	D	E	5	B	1	A	B
	H537/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H546/22	H5N1	BCC5A1AE	22G5	B	C	C	5	A	1	A	E
	H549/22	H5N1	CEH5A1AF	22G9	C	E	H	5	A	1	A	F
	H563/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H566/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H569/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H572/22	H5N1	CCC5A1AE	22G8	C	C	C	5	A	1	A	E
	H583/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H584/22	H5N1	DBD5A1AC	22G4	D	B	D	5	A	1	A	C
	H587/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H594/22	H5N1	ACF5A1AE	22G6	A	C	F	5	A	1	A	E
	H595/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H608/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H618/22	H5N1	ACF5A1AE	22G6	A	C	F	5	A	1	A	E
	H624/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H625/22	H5N1	FFE5D1AD	22G2	F	F	E	5	D	1	A	D
	H626/22	H5N1	BEB5A1AE	22G3	B	E	B	5	A	1	A	E
	H627/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H628/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H633/22	H5N1	FFE5D1AD	22G2	F	F	E	5	D	1	A	D
	H641/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H650/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H651/22	H5N1	BEB5A1AE	22G3	B	E	B	5	A	1	A	E
	H662/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H659/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H663/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H665/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H677/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H678/22	H5N1	BEB5A1AE	22G3	B	E	B	5	A	1	A	E
	H682/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H685/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H684/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H688/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H686/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H687/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H690/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H691/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H693/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H705/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H731/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H732/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H656/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H741/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H742/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H747/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H745/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A

(*continued on next page*)

Table 2 (*continued*)

Host	Virus name	Subtype	Reassortant name	Genotype	Phylogenetic group within each gene segment							
					PB2	PB1	PA	HA	NP	NA	MP	NS
	H751/22	H5N1	CCC5A1AE	22G8	C	C	C	5	A	1	A	E
	H754/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H758/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	H774/22	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C001/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C003/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C004/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	D013/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C015/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	D018/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C020/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C055/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C056/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	Q057/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C064/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C065/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C085/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	C112/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	D114/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	D169/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	D181/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	D194/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A
	D195/23	H5N1	AAA5A1AA	22G7	A	A	A	5	A	1	A	A

Table 3
Pathogenicity and transmissibility of H493/22 and H537/22 in SPF chickens and ducks.

Virus	Bird species	IVPI	Virus dose (EID_{50}/0.1 ml)	Mortality(%)	MDT (days)	HI titer[a] (log2, mean±SD)	cLD_{50} (EID_{50}/0.1 ml)
H493/22 (Genotype 22G1)	SPF chicken	2.98	$10^{6.4}$	5/5(100)	2.8	–	$10^{5.0}$
			$10^{5.4}$	5/5(100)	6.2	–	
			$10^{4.4}$	0/5(0.0)	–	0/5(0.0)	
			$10^{3.4}$	0/5(0.0)	–	0/5(0.0)	
			Contact	3/3(100)	4.3	–	
H493/22 (Genotype 22G1)	Duck	–	$10^{6.4}$	4/5(80)	5.3	1/1(3.0)	–
			Contact	2/3(66.7)	8.5	1/1(3.0)	
H537/22 (Genotype 22G7)	Duck	–	$10^{6.5}$	3/5(60)	5.7	2/2(5.0)	–
			Contact	2/3(66.7)	7	1/1(4.0)	–

lesions in the deceased ducks were more prominent in the brain, liver, pancreas, heart, and cloacal bursa than those in ducks euthanized at 3 dpi (Fig. S8).

6. Discussion

Clade 2.3.4.4b H5Nx HPAIVs have caused major outbreaks in poultry and wild birds globally. In general, wild birds play an important role in the worldwide dissemination of viruses. South Korea is one of the far-east countries affected by different H5Nx HPAIVs via migrating wild birds, specifically during the winter season. During 2022–2023, clade 2.3.4.4b H5N1 HPAIVs were responsible for major outbreaks in Eurasia and America, caused many outbreaks in poultry farms and wild birds across South Korea. In the present study, the genetic characteristics of this clade 2.3.4.4b H5N1 HPAIV isolated in South Korea were investigated, along with its pathogenicity and transmissibility in SPF chickens and ducks.

H5N1 HPAIV caused 75 outbreaks in poultry farms and was identified in 174 wild bird cases during the 2022–2023 winter season (October 2022–April 2023), which was more than double the outbreaks reported in previous seasons [20]. Notably, most outbreaks occurred in the western regions of South Korea, which is a well-known habitat for migratory birds and harbours a high poultry population (Fig. S1, Table S1) [20,24]. The neighbouring country, Japan, also experienced one of the largest H5N1 HPAI outbreaks in the 2022–2023 season [25,26]. In Europe, a major HPAI epidemic in poultry and wild birds was reported from October 2021 to September 2022, which included many rare mass mortality events in seabird breeding colonies during the summer months [13]. It is important to understand the HPAIV situation during the early months of the year in Eurasia and other countries, as East-Asian countries are likely to affected by HPAIV during the winter. The results of this study suggest that the increased outbreaks of H5N1 HPAIV in poultry and wild birds in South Korea were a continuation of the global 2.3.4.4b H5 HPAIV panzootic.

The phylogenetic analyses of HA and NA genes of the H5N1 HPAIV isolates revealed the clade of the isolated virus (clade 2.3.4.4b; based on HA gene analysis) and its close relation to HPAIVs isolated in Eurasian and American countries in 2021–2023. Notably, the HA genes from most of the 2022–2023 HPAIV isolates belonged to a different cluster from that of the 2021–2022 H5N1 HPAIV isolates. Moreover, the major HA gene group detected in South Korea was related to the G2c HA subgroup, which is one of the three HA gene subgroups of Japanese HPAIVs detected in 2022–2023 [27]. Unlike the South Korean HPAIVs, most of the Japanese 2022–2023 HPAIV isolates belonged to the G2d group but not to the G2c group. Overall, these data suggest that H5N1 HPAIVs belonging to different H5 gene subgroups were introduced into South Korea and Japan, and the difference in major HPAIV outbreaks in poultry farms was probably attributable to the discrepancies in migrating wild bird flyways and local environmental factors such as geographic feature, location of farm and weather. Moreover, three H5N1 viruses isolates were detected during October–November 2022 that were genetically related to H5N1 HPAIVs isolated in North America. We previously reported, for the first time, that an American origin HPAI virus isolated from wild bird in South Korea [17], which is similar

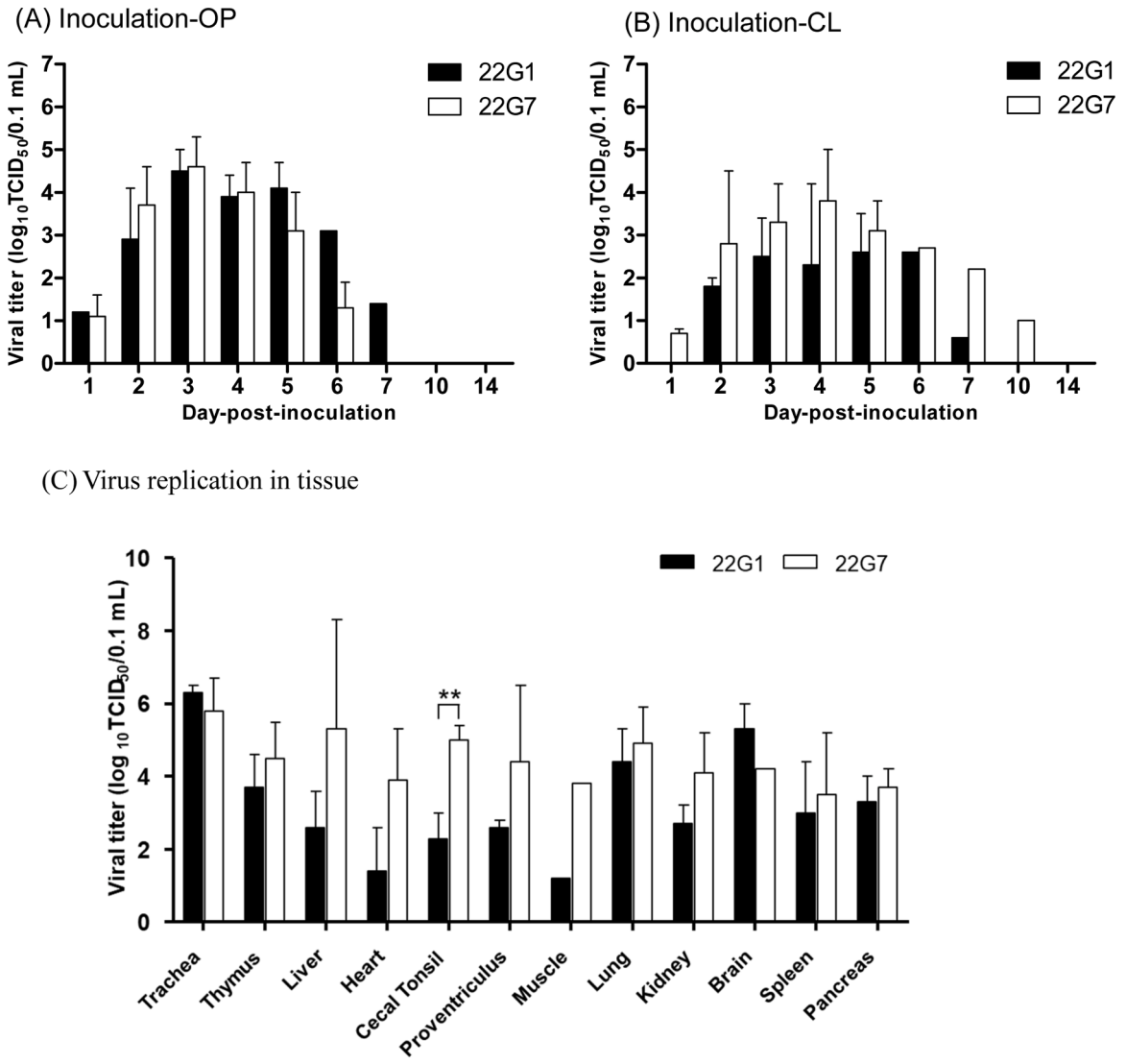

Fig. 3. Isolation of virus from oropharyngeal (OP) (A) and cloacal (CL) (B) swab samples. (C) Measurement of virus replication in various organs collected from 22G1 (H493/22(H5N1)) and 22G7(H537/22(H5N1))-inoculated ducks at 3 d post-inoculation (dpi). Birds were inoculated intranasally with 10^6 mean egg infectious dose/0.1 mL of virus, and internal organs from three birds were collected at 3 dpi. Viral titers were determined in DF-1 cells. Data are presented as the mean ± standard deviation. Each organ viral titer from 22G1 and 22G7 group were compared and performed statistical analysis (Student's t-test); **; $p < 0.01$.

to a HPAI virus detected in USA at April 2022. In this study, we identified two more similar H5N1 viruses isolated from poultry. These findings indicate the circulation of H5N1 HPAIVs across continents through the migration of wild birds [5,17]. Bayesian phylodynamic and host transition analyses of the 2022–2023 South Korean H5N1 HPAIV isolates from poultry and wild birds revealed that the 2022–2023 HPAIVs were most likely transmitted from wild birds to poultry (ducks or chickens). Overall, these data suggest that farm-to-farm transmission (such as poultry-to-poultry) was less in the 2022–2023 winter season, highlighting the role of migrating wild birds in introducing HPAIVs into South Korea [14].

The analysis of genetic constellations of the 2022–2023 South Korean H5N1 HPAIV isolates revealed 10 distinct genotypes of HPAIVs isolated from poultry farms, which is two–three times greater than that identified in the previous season. Additionally, 19 different viral genotypes were identified in wild birds. During the early winter season (October–November 2022), multiple genotypes were introduced in South Korea, and genotype 22G7 was the most detected genotype in wild birds after December 2022, causing large outbreaks on poultry farms. In addition, the findings of diverse H5N1 genotypes in South Korea show consistency with those in Europe; 30 different genotypes of H5N1 HPAIVs were identified in 2021–2022 [13].

The pathogenicity and transmissibility of H493/22(22G1) and H537/22(22G7) were examined in SPF chickens and ducks. H493/22 (22G1) was highly virulent in SPF chickens, with high mortality and transmission rate. These results are consistent with previous findings of South Korean HPAIV isolates [16,20]. A recent study reported that the Japanese H5N1 HPAIV isolated in November 2022 demonstrated high virulence and pathogenicity in chickens [27]. In ducks, H493/22(22G1) and H537/22(22G7) H5N1 viruses caused clinical signs and 60–80 % of mortality with high rates of transmissibility and viral shedding. The gross and microscopic lesions detected in 22G1-inoculated groups to further confirmed the increased pathogenicity by HPAI in ducks, since the ducks infected with HPAIV generally known to show mild clinical signs and low or no mortality. Notably, the 22G7-inoculated group showed more prolonged virus shedding via the CL route and a higher viral replication rate in the cecal tonsils than that in the 22G1-inoculated group. These factors may increase chances of virus exposure, which are important for longer virus transmission in the fields and contributed to the dominance of the 22G7 genotype among all H5N1 viruses isolated in the 2022–2023 winter season. Taken together, in the early period of the winter season, the most diverse heterogenic genotypes were introduced

from wild birds, and poultry outbreaks reflect the prevailing strains. Thereafter, 22G7 naturally became the dominant strain may be due to virus traits associated with transmissibility and pathogenesis.

The findings of this study regarding duck pathogenesis contrast with most previous reports in South Korea, which state that ducks only presented mild clinical signs and no mortality after HPAIV inoculation [16, 20]. In China, mallards inoculated with clade 2.3.4.4b H5N1 HPAIV isolated from a wild bird in February 2023, showed clinical signs of varying severity, but no mortality [28]. However, in the 2016–2017 winter season, South Korean H5N6 HPAIV isolates (clade 2.3.4.4C) showed notable pathogenicity and transmissibility in ducks, similar to those of 2022–2023 H5N1 HPAIVs [29]. Moreover, these previous duck studies were conducted with 2–3 weeks old SPF or domestic ducks, which are of a similar age to those used in this study. The age of ducks at challenge is one of important factors in the HPAI pathogenesis because older ducks are known to show fewer clinical signs and less severe pathogenicity. These data indicate that the pathogenicity of HPAI in ducks may vary depending on the HPAIV.

In this study, the genetic characteristics, pathogenicity, and transmissibility of H5N1 HPAIVs isolated in South Korea during the 2022–2023 winter season were investigated. Notably, the South Korean clade 2.3.4.4b H5N1 HPAIVs were closely related to Eurasian and American H5 HPAIVs isolated in 2021–2023, and multiple genotypes of viruses were identified in wild birds and poultry. Additionally, a phylodynamic host transition analysis of the HA gene revealed that viral transmission likely occurred from wild birds to poultry. These data suggest that migrating wild birds introduced the most diverse reassorted clade 2.3.4.4b H5N1 HPAIVs into the Korean Peninsula. Among these, the dominant genotype (22G7) in wild birds caused most outbreaks in poultry farms. Moreover, 22G7 virus-inoculated ducks showed longer virus shedding via the CL route and higher viral replication in the cecal tonsils than those in the 22G1 group, highlighting the dominant characteristics of the 22G7 genotype. Altogether, the findings of this study underscore the importance of elucidating genetic and pathogenic characteristics of H5N1 HPAIVs to establish biosecurity measures and surveillance programs for effective disease control in the field.

Funding

This work was supported by the Animal and Plant Quarantine Agency (APQA), Ministry of Agriculture, Food, and Rural Affairs of South Korea (B-1543418-2024-26-01).

Author statement

All authors have read and agreed to the revised version of the manuscript.

CRediT authorship contribution statement

Ra Mi Cha: Writing – review & editing, Writing – original draft, Project administration, Conceptualization. **Min-Ji Park:** Visualization, Investigation, Data curation. **Yoon-Gi Baek:** Visualization, Investigation, Formal analysis. **Yu-Na Lee:** Resources, Investigation. **Yunyueng Jang:** Validation, Data curation. **Yong-Myung Kang:** Resources, Methodology. **Gyeong-Beom Heo:** Validation, Software, Resources. **Se-Hee An:** Visualization, Resources. **Kwang-Nyeong Lee:** Supervision, Conceptualization. **Jae-Kyeom Kim:** Writing – original draft, Visualization, Investigation. **Hye-Ryoung Kim:** Visualization, Methodology, Conceptualization. **Youn-Jeong Lee:** Supervision, Project administration. **Eun-Kyoung Lee:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Jae-In Shin, Hae Ran Kang, Seo Yoon Hong, and Yeon Chan Choi for their help with animal experiments. All animal experiments complied with APQA IACUC regulations (approval no.: 2021–612, 2023–765).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2025.199541.

Data availability

The sequence data in this study are available at the GISAID database (https://platform.gisaid.org).

References

[1] Group WOFHNEW, 2008 Jul. Toward a unified nomenclature system for highly pathogenic avian influenza virus (H5N1). Emerg. Infect. Dis. 14 (7), e1.
[2] Poovorawan, Y., Pyungporn, S., Prachayangprecha, S., et al., 2013 Jul. Global alert to avian influenza virus infection: from H5N1 to H7N9. Pathog. Glob. Health 107 (5), 217–223.
[3] Lee, D.H., Criado, M.F., Swayne, D.E., 2021 Feb 1. Pathobiological origins and evolutionary history of highly pathogenic avian influenza viruses. Cold Spring Harb. Perspect. Med. 11 (2).
[4] Caliendo, V., Lewis, N.S., Pohlmann, A., et al., 2022 Jul 11. Transatlantic spread of highly pathogenic avian influenza H5N1 by wild birds from Europe to North America in 2021. Sci. Rep. 12 (1), 11729.
[5] Alkie, T.N., Lopes, S., Hisanaga, T., et al., 2022. A threat from both sides: multiple introductions of genetically distinct H5 HPAI viruses into Canada via both East Asia-Australasia/Pacific and Atlantic flyways. Virus Evol. 8 (2), veac077.
[6] Cui, P., Shi, J., Wang, C., et al., 2022 Dec. Global dissemination of H5N1 influenza viruses bearing the clade 2.3.4.4b HA gene and biologic analysis of the ones detected in China. Emerg. Microb. Infect. 11 (1), 1693–1704.
[7] Neumann, G., Chen, H., Gao, G.F., et al., 2010 Jan. H5N1 influenza viruses: outbreaks and biological properties. Cell. Res. 20 (1), 51–61.
[8] Lewis, N.S., Banyard, A.C., Whittard, E., et al., 2021 Dec. Emergence and spread of novel H5N8, H5N5 and H5N1 clade 2.3.4.4 highly pathogenic avian influenza in 2020. Emerg. Microb. Infect. 10 (1), 148–151.
[9] Pohlmann, A., King, J., Fusaro, A., et al., 2022 Aug 30. Has Epizootic become enzootic? Evidence for a fundamental change in the infection dynamics of highly pathogenic avian influenza in Europe, 2021. mBio 13 (4), e0060922.
[10] Castro-Sanguinetti, G.R., Gonzalez-Veliz, R., Callupe-Leyva, A., et al., 2024 Feb 13. Highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b from Peru forms a monophyletic group with Chilean isolates in South America. Sci. Rep. 14 (1), 3635.
[11] European Food Safety A, European Centre for Disease P, Control, 2022 Oct. Avian influenza overview June - September 2022. EFSA J. 20 (10), e07597.
[12] European Food Safety Authority ECfDP EURLfAI, Control, Adlhoch, C., et al., 2023 Jan. Avian influenza overview September - December 2022. EFSA J. 21 (1), e07786.
[13] Fusaro, A., Zecchin, B., Giussani, E., et al., 2024. High pathogenic avian influenza A(H5) viruses of clade 2.3.4.4b in Europe-why trends of virus evolution are more difficult to predict. Virus. Evol. 10 (1), veae027.
[14] Baek, Y.G., Lee, Y.N., Lee, D.H., et al., 2021 Mar 16. Multiple reassortants of H5N8 clade 2.3.4.4b highly pathogenic avian influenza viruses detected in South Korea during the winter of 2020-2021. Viruses 13 (3).
[15] Sagong, M., Lee, Y.N., Song, S., et al., 2022 Sep. Emergence of clade 2.3.4.4b novel reassortant H5N1 high pathogenicity avian influenza virus in South Korea during late 2021. Transbound. Emerg. Dis. 69 (5), e3255–e3260.
[16] Park, M.J., Cha, R.M., Kye, S.J., et al., 2021 Sep 23. Pathogenicity of H5N8 high Pathogenicity Avian influenza virus in chickens and ducks from South Korea in 2020-2021. Viruses 13 (10).
[17] Kang, Y.-M., Heo, G.-B., An, S.-H., et al., 2023. Introduction of multiple novel high pathogenicity avian influenza (H5N1) virus of clade 2.3. 4.4 b into South Korea in 2022. Transbound. Emerg. Dis. 2023.
[18] Seo, Y.R., Cho, A.Y., Si, Y.J., et al., 2024 Feb. Evolution and spread of highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b virus in wild birds, South Korea, 2022-2023. Emerg. Infect. Dis. 30 (2), 299–309.
[19] Lee, D.H., Lee, H.J., Lee, Y.J., et al., 2010 Apr. DNA barcoding techniques for avian influenza virus surveillance in migratory bird habitats. J. Wildl. Dis. 46 (2), 649–654.
[20] Cha, R.M., Lee, Y.N., Park, M.J., et al., 2023 Jun 20. Genetic characterization and pathogenesis of H5N1 high pathogenicity Avian influenza virus isolated in South Korea during 2021-2022. Viruses 15 (6).

[21] Lee, E.K., Lee, Y.N., Kye, S.J., et al., 2018 Jun 13. Characterization of a novel reassortant H5N6 highly pathogenic avian influenza virus clade 2.3.4.4 in Korea, 2017. Emerg. Microb. Infect. 7 (1), 103.
[22] Stamatakis, A., 2014 May 1. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30 (9), 1312–1313.
[23] Reed, L.J., Muench, H., 1938. A simpe method of estimating fifity percent endpoints. Am. J. Epidemiol. 27, 493–497.
[24] Baek, Y.G., Lee, Y.N., Park, Y.R., et al., 2022. Evolution, transmission, and pathogenicity of high pathogenicity Avian influenza virus A (H5N8) clade 2.3.4.4, South Korea, 2014-2016. Front. Vet. Sci. 9, 906944.
[25] Takadate, Y., Mine, J., Tsunekuni, R., et al., 2024 Sep. Genetic diversity of H5N1 and H5N2 high pathogenicity avian influenza viruses isolated from poultry in Japan during the winter of 2022-2023. Virus. Res. 347, 199425.
[26] Japan Ministry of Agriculture FaF. The largest highly pathogenic avian influenza epidemic in Japan. 2023.
[27] Hew, L.Y., Isoda, N., Takaya, F., et al., 2024. Continuous introduction of H5 high pathogenicity avian influenza viruses in Hokkaido, Japan: characterization of viruses isolated in winter 2022–2023 and early winter 2023–2024. Transbound. Emerg. Dis. 2024 (1), 1199876.
[28] Tian, J., Bai, X., Li, M., et al., 2023 Jul. Highly Pathogenic Avian Influenza Virus (H5N1) Clade 2.3.4.4b Introduced by Wild Birds, China, 2021, 29. Emerg Infect Dis, pp. 1367–1375.
[29] Kwon, J.H., Noh, J.Y., Jeong, J.H., et al., 2019 Apr. Different pathogenicity of two strains of clade 2.3.4.4c H5N6 highly pathogenic avian influenza viruses bearing different PA and NS gene in domestic ducks. Virology 530, 11–18.