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Prevalence of foodborne viruses and influenza A virus from poultry processing plants to retailed chickens

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Introduction: Foodborne viruses are a serious concern in public health. This study investigated the prevalence of eight foodborne viruses norovirus (NoV), adenovirus (AdV), sapovirus (SapoV), astrovirus, hepatitis A virus (HAV), hepatitis E virus (HEV), rotavirus, aichivirus, and influenza A virus (IAV).

Material and method: A total of 316 chicken samples were collected from three poultry processing plants to commercial markets (local and online). RT-qPCR- and PCR-positive amplicons obtained from monitoring were confirmed by sequence analysis.

Results: Foodborne viruses and IAV were not found in poultry processing plants. Of the 100 chickens purchased from the local and online markets, 19 (19.0%) AdV and 2 (2.0%) SapoV were detected. NoV, astrovirus, HAV, HEV, rotavirus, aichivirus, and IAV were not detected in the retailed chickens. Phylogenetic analysis identified 18 human AdV-41, one porcine AdV, and two SapoV-GI.1. It was the first case of the discovery of the SapoV gene in chicken. The average contamination level of detected AdV was 2.4 log DNA copies/g, but there were cases where the highest level was 5.35 log DNA copies/g.

Discussion: This study highlights the importance of chicken's contribution to the transmission of AdV with the possibility of annual variability with emerging symptoms. The prevention of AdV contamination in the food chain from slaughterhouses to retail markets should be monitored and controlled in further study.

KEYWORDS

foodborne virus, adenovirus, chicken, poultry processing plant, influenza A virus

1. Introduction

Meat consumption is increasing worldwide. Poultry consumption is expected to increase in high-income countries, driven by a growing preference for white meat, which is more convenient to prepare and perceived as a better food choice (OECD-FAO, 2017, 2022). Furthermore, consumption continues to increase in low- and middle-income countries as poultry is cheaper than other meats. According to the OECD and FAO reports in 2022, poultry meat is expected to account for 47% of the protein consumed from meat sources globally by 2031 due to a favorable meat-to-feed price ratio compared to other ruminants combined with short production cycles (OECD-FAO, 2022). Among the poultry, chicken dominates meat consumption as it is generally affordable, low in fat, and faces few religious and cultural barriers (OECD-FAO, 2017).

Foodborne illnesses from chicken consumption were often caused by *Campylobacter* and *Salmonella* spp. bacteria (Mwangi et al., 2019; Frosth et al., 2020; Golden and Mishra, 2020; Zhuge et al., 2021). There were fewer relations between foodborne viruses and chicken in the viral metagenomic research on chicken (Zhang et al., 2014). However, since the 2019 SARS-CoV-2 pandemic, there has been an ever-growing list of emerging viral pathogens that could threaten the food supply (Tetro, 2014; Ceylan et al., 2020). These include well-known foodborne viruses such as norovirus (NoV), adenovirus (AdV), sapovirus (SapoV), astrovirus, hepatitis A virus (HAV), hepatitis E virus (HEV), rotavirus, and avian influenza, as well as the less well-known aichivirus (Tetro, 2014).

Foodborne viruses were transmitted to humans primarily through the consumption of contaminated food or water (Tetro, 2014). Common symptoms of foodborne viruses are fever, vomiting, diarrhea, and headache (Pal and Ayele, 2020). Furthermore, depending on the severity of the infection, HAV causes jaundice and acute hepatitis (Gholizadeh et al., 2023). The symptoms of HEV vary from mild to severe, with jaundice appearing rapidly and acute liver failure occurring in <1% of patients (Webb et al., 2020). In South Korea, HAV and HEV were designated as class 2 infectious diseases, and NoV, AdV, SapoV, astrovirus, and rotavirus A were defined as class 4 intestinal infectious diseases according to the Waterborne and Foodborne Infectious Diseases Control Guidelines (KCDC, 2022). Symptomatic cases of HAV were reported: 17,598 cases in 2019 and 3,989 cases in 2020 (KCDC, 2022). One hundred and ninety-one HEV symptomatic cases have been reported since it was added to legal infectious diseases in 2020 (KCDC, 2022). Rotavirus A, astrovirus, AdV, NoV, and SapoV were reported in 2020 as 1,416, 201, 192, 3,219, and 70 cases, respectively (KCDC, 2022). Cases of outbreaks occurred in the order of NoV (121 cases, 27.0%), *Campylobacter* spp. (24 cases, 5.1%), and *Salmonella* spp. (22 cases, 4.7%) as the causative pathogens in 2021 (KCDC, 2022). Foodborne viral illness is primarily caused by NoV and HAV through the consumption of shellfish (oyster, clam) (Cho et al., 2021; Hyun et al., 2022; Yoo et al., 2022).

Outbreaks of foodborne virus disease caused by chicken consumption resulted in 59 outbreaks, 1,548 illnesses, 28 hospitalizations, and one death from 2009 to 2020 in the USA (Dewey-Mattia et al., 2018; White et al., 2022). In the past 15 years, the highest number of reported illness was 337 cases, and in 2018, there were reports of NoV GII cases linked to consumption of chicken wraps (White et al., 2022). One death from NoV occurred after eating chicken dishes from a restaurant in California in 2014 (White et al., 2022). Other than NoV cases, one of them involved eating chicken coconut curry due to SapoV infection in 2015 (Dewey-Mattia et al., 2018). In addition, there were 108 cases of rotavirus A outbreak caused by eating chicken or tuna salad sandwiches in 2000 (CDC, 2000).

Since 2009, due to the swine flu pandemic (also known as the "novel flu virus") caused by highly pathogenic influenza A virus (IAV) H1N1 (strain pandemic H1N1/09 virus), there have been 700 million to 1.4 billion estimated suspension cases worldwide, and 18,449 people have died (WHO, 2009, 2011; CIDRAP, 2013). Since the large-scale pandemic, there has been a continuous awareness of IAV among consumers and farmers (Cui et al., 2022). In Taiwan, the IAV H5N1 virus was detected in live birds, and surface samples were collected from the food market in 2004–2005 (Amonsin et al., 2008). In 2003, the IAV H5N1 was isolated from imported poultry meat in Japan (Mase et al., 2005). Due to these risks and consumer awareness, IAV infection through food intake is also a concern in food safety.

Most foodborne diseases are under-reported or not reported at all, leaving many gaps in our understanding of how viruses affect food (Tetro, 2014). In this study, the foodborne viruses (NoV, AdV, SapoV, astrovirus, HAV, HEV, rotavirus, and aichivirus) and IAV prevalence from poultry processing plants to commercial markets (local and online) were studied through multilateral cooperation according to the food distribution channels. Phylogenetic analysis was used to investigate the relationship between the detected viruses.

2. Materials and methods

2.1. Ethical statement

This study only used chicken meat samples without animal or patient material and thus did not require approval from the ethics committee.

2.2. Sample collection from poultry processing plants and commercial markets (local and online)

We sampled 317 samples of chicken from poultry processing plants and commercial markets (local and online markets). Local markets are physical marketplaces for buying and selling goods and services in a specific area, while online markets are digital marketplaces that allow for buying and selling goods and services over the internet, from anywhere.

The schematic flow of the poultry processing line is shown in Supplementary Figure 1. Eight red arrows indicated the collection parts: post-pickers, pre-evisceration, post-evisceration, pre-washing, post-washing, post-spray chilling, post-air chilling, and selection. A total of 216 samples were collected by dividing three chickens into three parts (legs, wings, and breasts) at each of the eight stages in three units.

Chicken samples were collected depending on the commercial market type. A total of 57 and 43 chickens were purchased from the local and online markets, respectively. Among the 43 chickens from the online markets, 22 were domestic and 21 were imported. All the chickens purchased in local markets were domestic (Table 1).

2.3. Sample preparation and nucleic acid extraction

Nucleic acid extraction was used to prepare the sample after elution and concentration (Son et al., 2014). Chicken legs and wings were deboned using sterilized scissors and a knife, and only the meat parts were collected. Consequently, it was homogenized

TABLE 1 The type and number of samples collected from the market.

Sample Origin	Local market	Online market	Total
Domestic	57	22	79
Chicken meat	48	1	49
By-product	9	21	30
Imported	0	21	21
Chicken meat	0	21	21
By-product	0	0	0
Total	57	43	100

to take 10 g. For chicken breast and gizzard, 2 g was taken for each spot and an appropriate amount (over 10 g) was randomly sampled. After homogenization, 10 g was taken. The chicken meat homogenate was eluted in phosphate-buffered saline (PBS, pH 7.4) elution buffer at a ratio of 1:10. After elution, the elute was centrifuged at 8,000 × *g* for 30 min at 4°C. The first supernatant was collected in a new tube, while the remnant pellet was mixed with the same amount of elution buffer. After a second centrifugation using the same conditions, the second supernatant was pooled with the first supernatant (Son et al., 2014). The sample elution was used for concentration using vivaspin-20 ultrafiltration with a nominal molecular weight limit of 10 kDa (Sartorius, Göttingen, Germany). The tissue homogenate was ultra-filtered with centrifugation at 8,000 × *g* for 30 min at 4°C (Son et al., 2014). The final concentration was extracted nucleic acid using an RNeasy mini kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The final elution was performed once with a 50 μl elution buffer. The nucleic acid was stored at −80°C until analysis (Tian et al., 2010).

2.4. RT-qPCR or qPCR for virus monitoring

One-step RT-qPCR or qPCR was used for investigating eight foodborne viruses (NoV, AdV, SapoV, astrovirus, HAV, HEV, rotavirus, and aichivirus) and IAV. Primers and probes for one-step RT-qPCR or qPCR are presented in Table 2 (Spackman et al., 2002; Tian et al., 2010; Nielsen et al., 2013; Shin et al., 2019; Wang et al., 2020; Lee et al., 2021; Yeo et al., 2022). One-step RT-qPCR for each RNA virus was performed using a one-step RT-PCR kit (Qiagen, Hilden, Germany). AdV was identified by qPCR using Premix Ex Taq (2X)™ (Takara, Shiga, Japan) performed on the CFX96™ Real-Time PCR system (Bio-Rad, California, United States). Titration of AdV was used with quantitative genomic DNA of human AdV-41 (ATCC® VR-930DQ, Virginia, United States) (Yeo et al., 2022).

2.5. Sequencing analysis and phylogenetic tree

For genotype determination, we applied sequencing analysis, and nested RT-PCR or PCR was performed to obtain an amplicon. The AdV was amplified by nested PCR using primer: hex1deg (17,602–17,626: 5′-GCC SCA RTG GKC WTA CAT GCA CAT C-3′) and hex2deg (17,891–17,921: 5′-CAG CAC SCC ICG RAT GTC AAA-3′) for the amplification of the outer hexon gene. Nehex3deg (16,452–16,4796: 5′-GCC CHY GCM ACI GAI ACS TAC TTC-3′) and nehex4deg (16,594–16,623: 5′-CCY ACR GCC AGI GTR WAI CGM RCY TTG TA-3′) were used for inner gene amplification and direct sequencing (Lee et al., 2021).

SapoV was performed using the one-step RT-PCR kit (Bioneer, Daejeon, South Korea) using primers: SV-F11 (5,098–5,117: 5′-GCY TGG TTY ATA GGT GGT AC-3′), SV-R1 (5,878–5,857: 5′-CWG GTG AMA CMC CAT TKT CCA T-3′), SV-F21 (5,157–5,177: 5′- ANT AGT GTT TGA RAT GGA GGG-3′), and SV-R2 (5,591–5,572: 5′- GWG GGR TCA ACM CCW GGT GG-3′). SV-F21 and SV-R2 were used for direct sequencing primers (Okada et al., 2002).

Influenza A virus-positive samples were amplified by a one-step RT-PCR kit (Bioneer, Daejeon, South Korea) with two sets of pan-primers for the Haemagglutinin (HA) and Neuraminidase (NA) genes. For HA gene amplification, Bm-HA-1 (5′-TAT TCG TCT CAG GGA GCA AAA GCA GGG G-3′) and Bm-NS-890R (5′-ATT CGT CTC GTA TTA GTA GAA ACA AGG GTG TTT T-3′) were used as a primer to obtain a PCR product of 1,800 bp (Hoffmann et al., 2001). NA gene was amplified using primers Bm-NA-1 (5′-TAT TGG TCT CAG GGA GCA AAA GCA GGG G-3′) and Ba-NA-1413R (5′-ATT GGT CTC GTA TTA GTA GAA ACA AGG AGT TTT T-3′) as 1,500 bp (Hoffmann et al., 2001).

The PCR products were purified using ExoSAP-IT™ (Applied Biosystems, Waltham, United States). Purified samples were directly sequenced on a SeqStudio using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Waltham, United States). All nucleotide sequences were first edited with the SeqMan program (DNASTAR, Madison, WI, United States), and the sequences were analyzed with different viral genotype sequences using BLAST (Anaclerio et al., 2021). Multiple alignments of different nucleotide sequences of the AdV and SaopV reference sequences were performed using DNASTAR Lasergene MegAlign Pro (DNASTAR Inc., WI, United States). Phylogenetic analysis of aligned sequences was performed in DNASTAR Lasergene MegAlign Pro (DNASTAR Inc., WI, United States), using RAxML v8 under the GTR GAMMA model with 500 rapid bootstrapping replicates and a search for the best-scoring maximum likelihood tree (Stamatakis, 2014). The reference sequences to build the phylogenetic tree were based on BLAST results with a high E-value (0.001) depending on the genotype. The GenBank accession number was presented in Supplementary Table S1.

2.6. Statistical analysis

The differences in detection rates of foodborne viruses and IAV between the poultry processing plants and market samples were analyzed using one-way Student's *t*-tests. *P*-values of < 0.05 were considered statistically significant. All statistical analyses were performed using R version 4.1.0.

TABLE 2 Primers and probes for foodborne viruses and influenza A virus monitoring in RT-qPCR and qPCR.

Virus	Target region	Primer	Sequence (5′-3′)	References
Norovirus GI	Rdrp/ORF2	COG1F	CGY TGG ATG CGN TTY CAT GA	Tian et al., 2010
		COG1R	CTT AGA CGC ATC ATC ATT YAC	
		RING1	FAM-AGA TYG CGA TCY CCT GTC CA-TAMRA	
Norovirus GII	Rdrp/ORF2	BPO-13	AIC CIA TGT TYA GIT GGA TGA G	Lee al., 2021
		BPO-13N	AGT CAA TGT TTA GGT GGA TGA G	
		BPO-14	TCG ACG CCA TCT TCA TTC ACA	
		BPO-18	FAM-CAC RTG GGA GGG CGA TCG CAA TC-TAMRA	
AdV	Fiber	JTVFF	AAC TTT CTC TCT TAA TAG ACG CC	Yeo et al., 2022
		JTVFR	AGG GGG CTA GAA AAC AAA A	
		JTVFP	HEX-CTG ACA CGG GCA CTC TTC GC-BHQ	
Astrovirus	Rdrp/ORF2	AstVF	CCD GCC AGR CTC ACA GAA GAG	Wang et al., 2020
		AstVR	GAC TTG CTA GCC ATC ACA CTY C	
		Probe	FAM-ACT CCA TCG CAT TTG GAG GGG AGG ACC-TAMRA	
HEV	ORF2/3	JHEV-F	GGT GGT TTC TGG GGT GAC	Yeo et al., 2022
		JVHEV-R	CGA AGG GGT TGG TTG GAT G	
		JHEV-P	FAM-ATT CTC AGC CCT TCG CAA TCC CCT-TAMRA	
HAV	5'UTR	Forward	GCG GCG GAT ATT GGT GAG	Yeo et al., 2022
		Reverse	CAA TGC ATC CAC TGG ATG AGA	
		Probe	FAM-TTA AGA CAA AAA CCA TTC AAC GCC GGA G-TAMRA	
Rotavirus	NSP3	NVP3-FDeg	ACC ATC TWC ACR TRA CCC TC	Shin et al., 2019
		NVP3-R1	GGT CAC ATA ACG CCC CTA TA	
		NVP3-Probe	FAM-ATG AGC ACA ATA GTT AAA AGC TAA CAC TGT CAA-TAMRA	
Aichivirus	5'UTR	Forward	CCC AGT GTG CGT AAC CTT CT	Nielsen et al., 2013
		Reverse	GTA CCT GCC TGG CAT YCC TA	
		Probe	HEX-ACG CCC TGT GCG GGA TGA AA-BHQ	
IAV	Matrix	M+25	AGA TGA GTC TTC TAA CCG AGG TCG	Spackman et al., 2002
		M-124	TGC AAA AAC ATC TTC AAG TCT CTG	
		M+64	FAM-TCA GGC CCC CTC AAA GCC GA-TAMRA	

3. Results

3.1. Prevalence of foodborne viruses and IAV from poultry processing plants to retailed chickens

As a result of the investigation, there were no foodborne viruses, and IAVs were identified through sequencing analysis in each processing unit of the three poultry processing plants (A, B, and C). In each step of the processing unit, the IAV was detected by RT-qPCR. IAV-suspected samples were identified in each step of sampling (Table 3). However, none of the IAVs were confirmed by sequencing.

Of the 100 chickens from the purchased local and online markets, 19 (19.0%) of AdV and 2 (2.0%) of SapoV were positive for foodborne viruses (Table 4). NoV GI, NoV GII, astrovirus, HAV, HEV, rotavirus, aichivirus, and IAV were not identified in the retailed chickens. Among 18 human AdV-41 positive samples, six were identified in imported chicken legs purchased from the online market, and 12 were domestic chicken purchased from the local market. Human AdV-41 was identified in two, one, eight, and one cases of chicken breast, wing, leg, and gizzard from the chicken meat purchased from the local market. One porcine AdV was identified from an imported chicken leg purchased from the online market. All samples contaminated with SapoV were domestic gizzards purchased from the online market (Supplementary Table S1). In this group, detection rates of foodborne viruses in the local and online markets were similar at 21.1% (12/57) and 20.9% (9/43) which was not a statistically significant difference ($p = 0.563$). The detection rate of the foodborne virus by country of origin was 33.3% (7/21) for imported chicken and 17.7% (14/79) for domestic chicken. Based on the

statistical analysis, the detection rate of foodborne viruses from the imported chicken was significantly higher than that from domestic chicken ($p = 0.045$).

The average AdV viral copy number was similar to 2.43 log DNA copies/g at the local market and 2.40 log RNA copies/g at the online market, as shown in Figure 1. There was no statistically significant difference in the average AdV viral copy number ($p = 0.941$). There were distinct differences in AdV titer (genome copy number) for each sampling, with a maximum of 3.24 log DNA copies/g and a minimum of 0.54 log DNA copies/g when purchased from the online market. For chickens purchased from the local market, the highest AdV contamination level was 5.35 log DNA copies/g and the minimum value was 1.24 log DNA copies/g. The information on virus-detected samples is presented in Supplementary Table S1.

3.2. Phylogenetic analysis of AdV in chicken samples

The AdV-positive samples were analyzed for the hexon gene using a phylogenetic tree, which was shown in Figure 2. Eleven AdV-41, twelve AdV-40, and eleven porcine AdV strain sequences were used as reference strains. Nineteen AdV genotypes were detected for the hexon gene, 18 were human AdV-41, and one was porcine AdV.

One human AdV-41 clade was formed with accession numbers MH289566–MK354237. The 18 human AdV-41 sequences detected had a nucleotide sequence identity of 98.54% with the reference AB330122 (strain Tak). Furthermore, the nucleotide sequence difference among the 18 human AdV-41 sequences was only 0.14%, indicating a high level of similarity between these sequences.

Through phylogenetic analysis, CAU180379 was found to be related to porcine AdV. There was a high nucleotide sequence similarity of 99.27% with reference strain MT895403. The detected CAU180379 sequence had the same clade with reference strain LC70231 identified as porcine AdV type 5, but a different root from reference strain MK420451 identified as porcine AdV type 3. Phylogenetic analyses suggest that the CAU180379 strain sequences detected in this study could be of porcine AdV type 5 origin.

TABLE 3 Influenza A virus detection results by step of poultry processing plants.

Sample collection stage	RT-qPCR	Sequencing
Post-pickers	1/27 (3.70%)	0/27
Pre-evisceration	2/27 (7.40%)	0/27
Post-evisceration	4/27 (14.8%)	0/27
Pre-washing	1/27 (3.70%)	0/27
Post-washing	2/27 (7.40%)	0/27
Post-spray chilling	2/27 (7.40%)	0/27
Post-air chilling	4/27 (14.8%)	0/27
Selection	1/27 (3.70%)	0/27
Total	**17/216 (7.87%)**	**0/216**

3.3. Phylogenetic analysis of SapoV in chicken samples

We analyzed the SapoV VP1 capsid region, as presented in Figure 3. As reference strains—15, 5, 3, 2, 3, and 2—SapoV-GI.1, SapoV-GI.2, SapoV-GI.3, SapoV-GII, SapoV-GV, and SapoV-GIII were used, respectively. As a result of phylogenetic analysis, two detected SapoV were of the GI.1 type. A single clade composed of SapoV-GI has three sub-genotypes. Both detected SapoV-GI (CAU180457 and CAU180464) have high identities with MK450330 (99.5% nucleotide sequence identity). In addition, the CAU180457 and CAU180464 detected were 100% identical. The nucleotide sequence identity with the SapoV-GI.1, KP298674 reported from South Korea strain (Hu/GI.1/Seoul/ROK62/2013/KOR), was 96.1%. Both discovered viruses were found in samples of chicken gizzards, however, the samples were obtained from separate companies.

TABLE 4 The detection rate of foodborne viruses and influenza A virus in chicken samples.

Virus	Local market ($n = 57$)		Online market ($n = 43$)		Total ($n = 100$)
	Domestic ($n = 57$)	Imported ($n = 0$)	Domestic ($n = 22$)	Imported ($n = 21$)	
Adenovirus	12 (21.1%)	0	0	7 (33.3%)	19 (19.0%)
Norovirus	0	0	0	0	0
Sapovirus	0	0	2 (9.1%)	0	2 (2.0%)
Astrovirus	0	0	0	0	0
Hepatitis A virus	0	0	0	0	0
Hepatitis E virus	0	0	0	0	0
Rotavirus	0	0	0	0	0
Aichivirus	0	0	0	0	0
Influenza A virus	0	0	0	0	0
Total	12/57 (21.1%)		9/43 (20.9 %)		21 (21.0%)

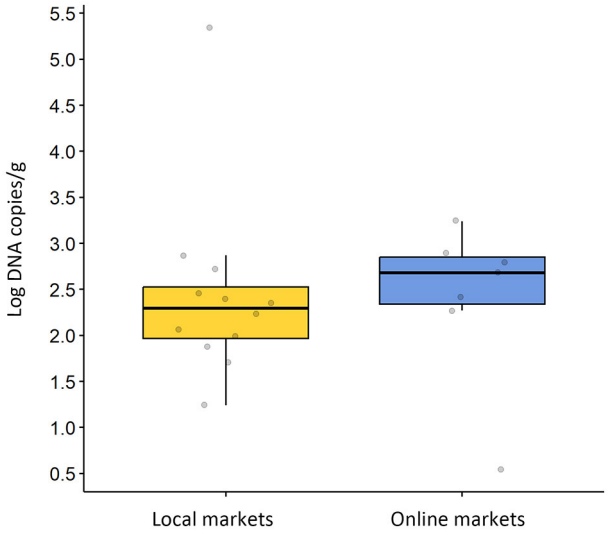

FIGURE 1
The detected levels of AdV genome copy number depending on the market type.

4. Discussion

This study investigated foodborne viruses and IAV in chickens from poultry processing plants to commercial markets (local and online markets). In order to identify the source of contamination, we analyzed the sequences of the detected viruses. However, AdV and SapoV were discovered, but other viruses including NoV, astrovirus, HAV, HEV, rotavirus, aichivirus, and IAV were not discovered from poultry processing plants to commercial markets. Through phylogenetic analysis, 18 human AdV-41, 1 porcine AdV, and 2 SapoV-GI.1 were identified.

By fecal–oral transmission, human AdV-41 mainly caused gastroenteritis and diarrhea (Shieh, 2021). Although there were no specific studies on the infectious dose of AdV, enteric viruses were known to be infected with 10–100 viral particles (Prevost et al., 2016). An equivalent comparison was difficult because the particle number of AdV was not confirmed by protein assay, but we observed an average of 2.41 log DNA copies/g of AdV in this study. Among the AdV-positive samples, a high titer of 5.35 log DNA copies/g was detected in CAU180436 which was closed with MH289553 and MK420403, as shown in Figure 3. Reference strain MH289553 and MK420403, both discovered in 2018, were in different regions of Brazil and South Korea, respectively (Portal et al., 2019; Yeo et al., 2022). As shown in Figure 3, human AdV-41 strains discovered in 2018 constitute one clade with MH289566∼MK354237. In each respective node, there were construction clades depending on the collection year. Therefore, it could be inferred that the year-specific factor has a greater effect on the evolution of the human AdV-41 sequence than the regional factor, despite the short AdV sequence region. Moreover, in late 2021 and early 2022, cases of human AdV-41 caused hepatitis in children in the UK and the USA (Gutierrez Sanchez et al., 2022; Kelgeri et al., 2022). Therefore, as the yearly AdV-41 sequence mutations identified in this study have increased and new cases of AdV hepatitis have recently been reported, research on the association between AdV sequence mutations and the symptoms is needed.

This study was the first report to detect SapoV in chickens. In particular, cases found in gizzards were not the main subject of research in the field of food safety, thus, it was assumed that no reports have been made. SapoV was only detected in chicken gizzards purchased from the online market. Human SaVs were currently divided into 18 genotypes, 7 GI (GI.1–GI.7), 8 GII (GII.1–GII.8), a single GIV, and 2 GV (GV.1 and GV.2) (Okitsu et al., 2021). All SapoVs detected in this study were of the GI.1 type. The nucleotide sequence identities between the detected SapoV and the reference strains MK450330, KP298674, and LT841189 were 100.0, 96.1, and 99.8%, respectively. MK450330 and MK450331 having the same node were detected in Korean porcine stools in 2018 (Yeo et al., 2022). SapoV (CAU180457, CAU180464) detected in SapoV-GI.1 and KP298674 with high sequence identity was isolated from human stool in 2013 in South Korea (Choi et al., 2015). In previous studies, genotype GI was consistently dominant at over 70.0% during 2013–2019 in South Korea (Cho et al., 2021). SapoV detected in the feces of Korean patients in 2018 confirmed 19 cases of GI genotype and one case of GII genotype (Cho et al., 2021). In the GI group, the GI.1 genotype revealed the highest detection rate of 60.0% (Cho et al., 2021). SapoV-GI.1 (CAU180464 and CAU180457) detected in this study also had a very low homology with the GII strain (accession number: MH922771), with a nucleotide sequence identity of 55.7%.

In poultry processing plants, the IAV gene was detected as 17/216 (7.87%) by RT-qPCR. On the other hand, in the commercial market, only 1/100 (1.0%) was detected in chicken legs purchased from the online market. However, the sequence was not confirmed in all samples. In poultry processing plants, the IAV was detected by RT-qPCR at eight sample collection steps. Among them, the most frequently detected stages were post-evisceration and post-air chilling. In general, IAV was found in the caudal and cranial parts of the chicken lung (Rebel et al., 2011). Consistent with these reports, the highest detection rate in the post-evisceration stage could be attributed to cross-contamination during the extraction process. Our sample collection plant used the chilling system with chlorinated water spray cooling and air cooling. In the previous study of microbial cross-contamination experiments with chillers, in the water chillers, *Escherichia coli* K12 was dispersed in all directions from a single inoculated carcass, and transmission was increased by the use of chlorinated water sprays (Mead et al., 2000). Influenza viruses were highly stable and contagious in aerosols over a wide range of relative humidity (Kormuth et al., 2018). Due to these factors, it was probable that the IAV gene was detected in the chilling step.

The identified viruses (human AdV-41, porcine AdV, and SapoV-GI.1) do not use chicken as a host. Chicken contamination was not caused by an infection in the chicken itself. NoV, astrovirus, HAV, HEV, rotavirus, and aichivirus were not identified in chickens. These viruses were transmitted by the fecal–oral route, and the main infectious agent was human feces (Li et al., 2021). In particular, AdV was not only an indicator of fecal contamination but also an indicator of viral water quality (Rames et al., 2016). AdV was the only DNA virus among foodborne

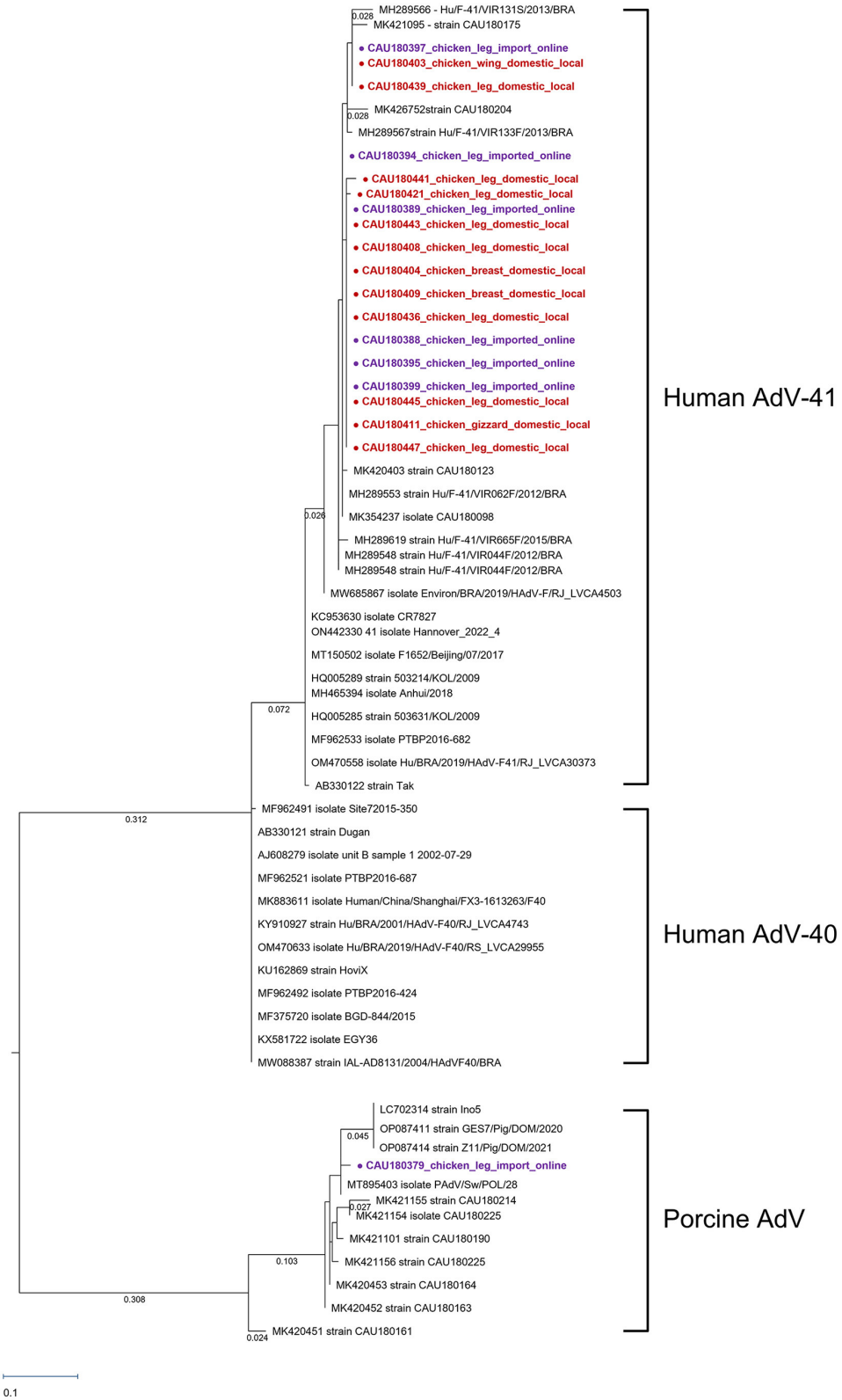

FIGURE 2
Phylogenetic tree of AdV detected in chickens. Sequences detected in this study are represented as circles (●). The red circles (●) were AdV samples purchased in local markets, and the purple circles (●) were AdV samples purchased in online markets. Phylogenetic tree analysis for the AdV hexon gene using the maximum likelihood method and based on the 171-bp sequence. The numbers of compressed sequence distances were shown in parentheses.

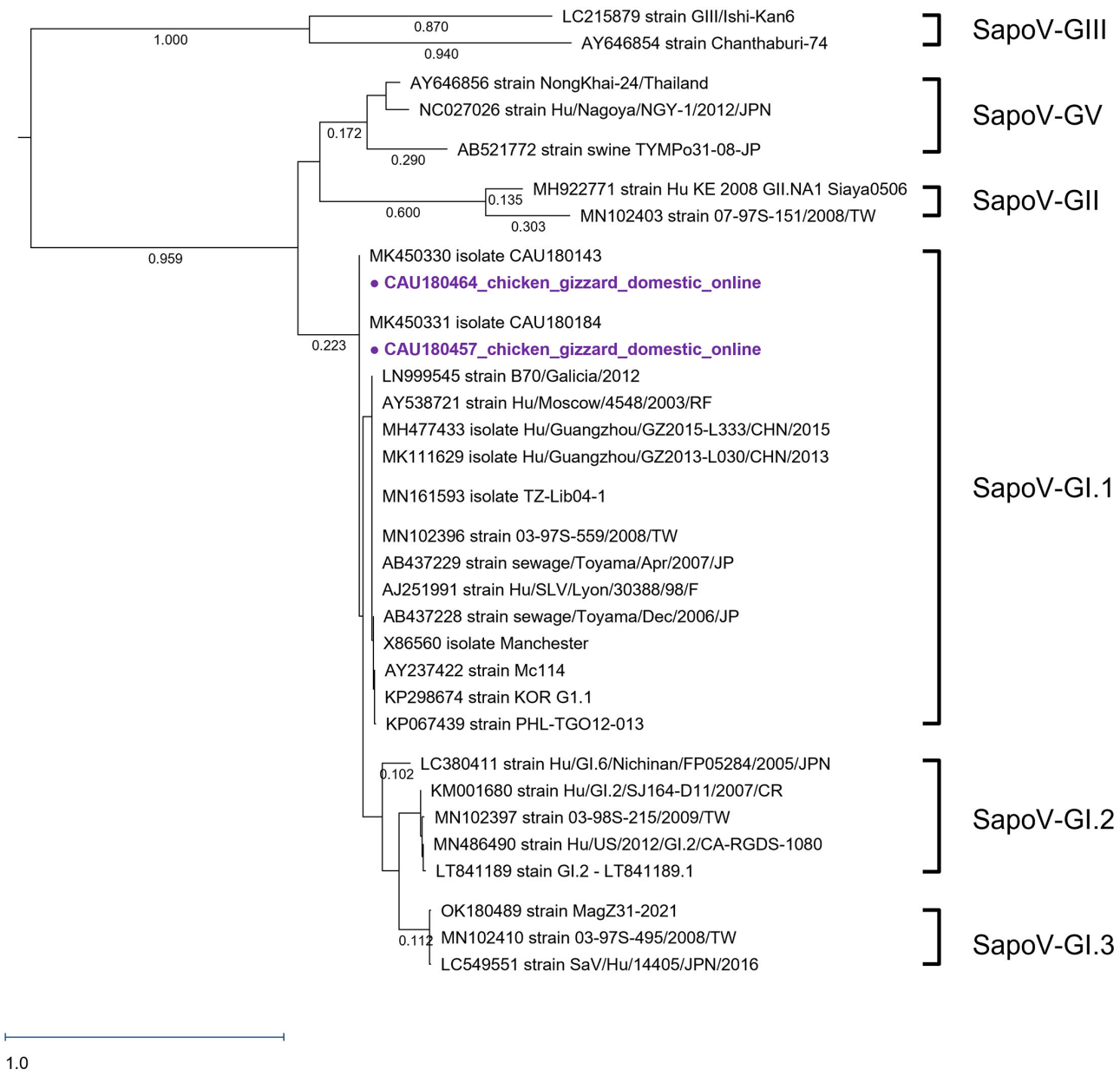

FIGURE 3
Phylogenetic analysis of SapoV detected in chickens. The SapoV phylogenetic tree was constructed following the maximum likelihood method and based on 416-bp sequences of the VP1 region. The purple circles (●) were AdV samples purchased in online markets. The numbers at the nodes of the tree indicate sequence distance.

viruses (Sarantis et al., 2004). As DNA virus genes were more stable compared to RNA viruses (Sarantis et al., 2004), AdV had a higher probability of being detected than RNA viruses.

Although nine viruses were searched, the sample number was not enough to investigate the epidemiology. Looking at the results of the AdV phylogenetic tree, it was detected without distinction according to origin and place of purchase, but imported chicken cannot be purchased from the local market, thus, there was a limitation in that the equivalent detection rate was not compared. In addition, though the hexon gene contained a hypervariable region in which the detected virus genotype was analyzed (Sarantis et al., 2004), it was compared to a relatively short sequence of 171 bp. Further research is needed on methods of physical and chemical reduction of chicken meat that can prevent AdV contamination derived from environmental and epidemiological traces through large-scale monitoring and whole-genome analysis.

5. Conclusion

Foodborne viruses and IAV were investigated in poultry processing plants to commercial markets. The detection rate of foodborne viruses from imported chicken was significantly higher than that from domestic chicken. Poultry processing plants had no relationship to commercial market contamination

viral sources, regardless of region. In the poultry processing plant, we could not identify any viruses. Contrary to the high concern of consumers against IAV contamination, IAV was not detected in any commercial chickens (local and online). As chickens are well cooked at high temperatures, the risk of IAV contamination in chickens was very low. However, 18 human AdV-41, 1 porcine AdV, and 2 SapoV-GI were identified in distributed chicken products in the local and online markets. Especially, AdV has the possibility for annual variability with emerging symptoms, thus, continuous follow-up from slaughterhouses to retail markets should be monitored and controlled.

Data availability statement

The data presented in the study are deposited in the GenBank repository, accession numbers OP846433-OP846451 and OP889258-OP889259.

Author contributions

DS, MR, and CC: conceptualization. DY, MH, and SJ: validation and visualization. DY and SJ: formal analysis, data curation, methodology, and software. DY and MS: investigation. MR and CC: supervision and funding acquisition. DY, MR, and CC: project administration. CC: resources. DY: writing—original draft preparation. DY, ZW, MH, SJ, and CC: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1113743/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The schematic flow of poultry processing plants sampled in this study. Red arrows (→) indicate each sampled step. In each sampling step, 27 (3×9) samples were sampled for each step.

SUPPLEMENTARY TABLE S1
Information on the samples which was detected by the foodborne virus.

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