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The complete genome sequence of quail coronavirus identified in disease surveillance on quail farms in South Korea

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ABSTRACT

Avian carcasses collected from 103 flocks on 14 quail farms in Korea between 2022 and 2023 were diagnosed with viral diseases (22 flocks), bacterial disease (58 flocks), parasitic diseases (28 flocks) and non-infectious diseases (60 flocks). The only viral disease identified was viral enteritis in quails that showed pathological lesions in duodenum and appeared to be caused by quail coronavirus (QcoV) through viral metagenomics and RT-PCR assay. Two complete genomes of QCoV from samples diagnosed as viral enteritis were obtained using amplicon-based whole genome sequencing. The two QcoVs were gammacoronavirus, but were distinct from other avian coronaviruses. The spike genes of QCoV have 86.2 ~ 87.1 % identity with that of American turkey coronavirus, but other gene sequences of QcoV was found to be similar to those of Korean infectious bronchitis virus. Genetic analysis based on the complete genomic sequences found QCoVs had a genetic structure similar to avian coronaviruses, yet it seems to be a unique pathogen specific to quail. This is the first report about the complete genome and genetic analysis of QCoV and the result of disease surveillance in quail in South Korea.

Introduction

Quail (Coturnix) is a bird that lives mainly on the ground, belonging to the Galliformes and *Phasianidae* families. Taxonomically, 9 species of quail are registered (Shaw, 2004), and some of these species are used for various purposes, such as for meat, eggs, game, ornamental purpose, and research. The quail species most commonly used for research and industrial is the species known as Japanese quail (*Coturnix japonica*). This species is a migratory bird originally distributed in East Asia, and is presumed that Japanese quails were first domesticated in China, moved to Japan, raised and then spread around the world (Mizutani, 2003; Vali, 2008; Lansford and Cheng, 2024).

Some breeders even believe that Coturnix are resistant to disease, but infections with HPAI, Newcastle disease, Marek's disease, Infectious bursal disease, avian encephalitis virus, avian leukosis, *Avibacterium gallinarum*, quail bronchitis, ulcerative enteritis, fowl typhoid and coccidiosis have been known (Bigland et al., 1965; Singh et al., 2016; Nair et al., 2020; Lebdah et al., 2022).

Quail is classified as minor poultry along with pheasants, ostriches and geese in accordance with Korea's Animal Contagious Disease Prevention Act. The lack of disease surveillance to minor poultry species may pose a hazard to poultry industry because they may be reservoirs or transmitters of disease such as highly pathogenic avian influenza (HPAI) and fowl typhoid (Barrow and Neto, 2011; Bertran et al., 2013; Thanh et al., 2018). In Korea, surveillance for HPAI has been conducted thoroughly, since there have been 17 outbreaks of the disease on quail farms since 2006 (Jeong et al., 2009; Kim et al., 2012). According to statistics from the Ministry of Agriculture, Food and Rural Affairs of Korea, there were 107 quail farms nationwide in 2020, most of which raise quail for egg production, and among them, there were 85 commercial farms that raise more than 2,000 quails. However, no studies have been conducted on the other disease of quail, so it has not known which diseases affect the heath and productivity of quail industry.

In this study, quail disease surveillance to investigate possible diseases, diagnose carcasses, and identify pathogen characteristics of diseases was conducted on 14 quail farms (103 flocks) in South Korea from 2022 to 2023. Through this surveillance, QCoV was identified from 22 cases of viral enteritis in quail and the complete genome sequences of that were

Abbreviation: QCoV, quail coronavirus; HPAI, highly pathogenic avian influenza; IB, infectious bronchitis; TCoV, turkey coronavirus; IBV, infectious bronchitis virus; nt, nucleotide; aa, amino acid; WGS, whole genome sequencings.

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obtained and analyzed.

Materials and method

Samples

Quail carcasses and feces were collected from 103 flocks on 14 farms between 2022 and 2023. The 14 farms were selected proportionally by region from a total of 85 farms raising more than 2,000 quails in 9 provinces of South Korea. During the necropsy, samples for bacteriological culture, virus detection and histopathological examination were collected depending on the gross lesions. The surface of livers was seared with a hot spatula, and samples were obtained using a sterile swab for bacterial examination. Swap samples were subsequently cultured on blood agar and MacConkey agar plates. After incubation overnight at 37°C, single colony was selected, and species identity was determined using the Vitek 2 system (bioMérieux, France) according to the manufacturer's instructions. Tracheas, duodenums, livers, cecal tonsils and kidneys were obtained from five selected quail carcasses per flock and were pooled by organs. The collected organs for the virus detection were homogenized in 10 % phosphate-buffered saline and stored at −70 °C until processing. Sections of the organs with lesions including tracheas, duodenums, liver and kidneys, were collected and fixed in 5 % buffered formalin. The samples were embedded in paraffin blocks, and paraffin wax sections were cut (5 μm), dewaxed, stained with hematoxylin and eosin, and examined using light microscopy.

Detection of pathogens that can infect quail

Total nucleic acid from tissue samples was extracted using a QIAamp DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). Thirteen viruses and two bacteria that can infect quail were selected through a few references and inspected using the PCR/RT-PCR method suggested in related literatures as shown in Table 1 (Noteborn et al., 1992; Wang et al., 1997; Smith et al., 1998; Dhinakar Raj et al., 2001; Caterina et al., 2004; Xie et al., 2005, 2006; Kim et al., 2007; Merino et al., 2009; Woźniakowski et al., 2011; Singh et al., 2016; Fadhilah et al., 2020; Song et al., 2021). The PCR product was confirmed by direct sequencing.

Table 1
PCR/RT-PCR tests for 15 causative agents of disease that can infect farmed quails.

Target pathogen	PCR/RT-PCR (No. positive/No. total flock)	Reference
Avian Influenza virus	0/103	Xie et al., 2006
Newcastle Disease virus	0/103	Merino et al., 2009
Infectious bursal disease virus	0/103	Caterina et al., 2004
Avian Encephalomyelitis virus	0/103	Xie et al., 2005
Avian Leukosis virus	0/103	Smith et al., 1998.
Duck viral hepatitis virus	0/103	Kim et al., 2007
Marek's disease virus	0/103	Woźniakowski et al., 2011
Reticuloendotheliosis virus	0/103	Song et al., 2021
Chicken Infectious Anemia virus	0/103	Noteborn et al., 1992
Fowl pox virus	0/103	Song et al., 2021
Egg Drop syndrome virus	0/103	Dhinakar Raj et al., 2001
Mycoplasma gallinarum	0/103	Wang et al., 1997
Mycoplasma synoviae	0/103	Wang et al., 1997
Avian coronavirus	28/103(27.2 %)*	Fadhilah et al., 2020
fowl adenovirus	7/103(6.8 %)*	Singh et al., 2016.

* p-value calculated by Chi-square test was < 0.05, suggesting that the results are likely to vary depending on the target pathogen.

Viral metagenomics

To identify a potential etiologic pathogen, feces of the bird diagnosed with viral enteritis were collected after necropsy and promptly processed via blending into a 10 % homogenate in sterile phosphate-buffered saline containing 0.4 mg/mL gentamicin. Other feces of the birds diagnosed with non-infectious disease was used as negative control for the sample pretreatment and sequencing. The collected supernatant was subsequently filtered and was added to 8 % polyethylene glycolv6000 and 0.5 M NaCl as previously described (Yamamoto et al., 1970; Veronese and Mero, 2008). DNA/RNA extraction from the resuspended pellet, cDNA synthesis and viral DNA/RNA amplification were performed as previously described (Kim et al., 2023). The amplified PCR fragments were purified and were quantified. An Illumina library was prepared using a Nextera XT DNA Library Preparation kit (Illumina, San Diego, CA, USA) and quantified using a Qubit fluorometer (Invitrogen, Waltham, MA, USA) and 4150 TapeStation system (Agilent Technologies, Santa Clara, CA, USA). Libraries were denatured and sequenced via Illumina MiniSeq using a MiniSeq Reagent kit (Illumina, San Diego, CA, USA).

The extracted dataset of paired-end reads was processed using Cutadapt (v2.8) and Trimmomatic (v0.39) to remove adapter, primer, and homopolymer sequences and shorter reads than 100 bp (Bolger et al., 2014; Martin, 2011). The remaining qualified reads were assembled into contigs using metaSPAdes (v3.15.4) (Nurk et al., 2017). The contigs were used as an index to align the qualified reads and a magnitude dataset was created to count the number of reads for the contigs. Finally, the contigs were classified using the Kraken2 (v2.1.2) program (Wood et al., 2019) with the NCBI nt database and graphically represented using a Krona Chart.

Whole genome sequencing of quail coronavirus

Most of the sequences of the QCoV excluding the spike (S) gene were similar to those of chicken IBV as reported in previous studies (Kim et al., 2021). Therefore, amplicon-based whole genome sequencing (WGS) of IBV GI-19 lineage was applied to obtain complete genome of quail coronavirus according the previous study (Le et al., 2024). Briefly, avian coronavirus quantification in the RNA samples were performed using real-time RT-PCR targeting the 5′-UTR region of IBV, as previously described (Callison et al., 2006). Next, cDNA was synthesized for RNA samples showing low CT (< 22) values and PCR was performed using six primer sets that could amplify six amplicons covering the entire genome. The amplicons were pooled, libraries were prepared and then next-generation paired-end sequencing was performed. A new set of primers for S gene of QCoV was designed (F:5′-GGMG GAATWGTAAYTATTTAC-3′ and R: 5′-CATTAAACAGAYTTYTTA GGTC-3′), and it was supported by endpoint RT- PCR and Sanger sequencing.

The quality of the raw Illumina sequencing reads was assessed by fastp (Chen et al., 2018). Adapter trimming was performed using Cutadapt and Trimmomatic with the default parameters (Bolger et al., 2014). The filtered reads were then aligned to the reference genome (TCoV strain ATCC and MN/310/96, NCBI accession no. EU022526 and GQ427176) using BWA-MEM (Li and Durbin, 2010) with the default settings. Subsequently, the mapped reads were retrieved using the Bamtofastq tool and subjected to de novo assembly using MEGAHIT software (v. 1.2.9) (Li et al., 2015). Mapping of reads to the reference genome was performed using BWA-MEM and Samtools. Genome annotation and Pairwise sequence identity was conducted using the CLC Main Workbench software (v.20.0.4; Qiagen, Germany).

Genetic analysis and similarity analysis

Nucleotide and amino acid sequences were aligned and trimmed using CLC main workbench 7 (CLC bio, Aarhus, Denmark). Phylogenetic

trees were generated by the maximum-likelihood method with 1000 bootstrap replications using MEGA 11 (Tamura et al., 2021), with related species published in the DDBJ/EMBL/GenBank database. Sequences showing similarities were found using a GenBank nucleotide BLAST search, and nucleotide (nt) and amino acid (aa) identity was calculated using CLC workbench. Simplot v. 3.5.1 was used to detect potential recombination events (Lole et al., 1999). The full genome sequences were compared with those of QCoV, TCoV and IBV. Consecutive avian coronavirus nucleotide sequences based on the multiple alignment results were used for similarity plotting analysis with a window size of 200 bp and a step size of 20 bp.

Isolation of quail coronavirus

Virus isolation was attempted through two blind passages using 5 ~ 7 days old quail embryonated egg, 9 ~ 11 days old SPF chicken embryonated eggs, chicken embryo fibroblast cell and leghorn male hepatoma cell. RT-PCR to find virus replication was carried out with RNA extracted from allantoic fluids harvested and cell supernatant.

Statistical analysis

The results of PCR assay and disease diagnosis were analyzed using Chi-square test of Microsoft Excel software. The probability value (p) < 0.05 was considered to be statistically significant.

Result

Outline of disease surveillance on quail farms

Quail carcasses collected from 2022 to 2023 were diagnosed with various disease through necropsy, histopathology, bacterial isolation and virus detection. Viral enteritis, presumed to be caused by quail coronavirus, was diagnosed in 22 of 103 flocks (21.4 %). Colibacillosis, necrotic enteritis caused by *Clostridium perfringens*, staphylococcosis and avian botulism were diagnosed in 18.4 %, 12.6 %, 11.7 and 9.7 % of flock, respectively. Coccidiosis, being the most frequently identified disease at 26.2 %, and cryptosporidiosis were diagnosed among the parasitic diseases. As non-infectious disease, fatty liver syndrome and leg fracture were diagnosed in more than 10 % of the tested flocks, and pulmonary hemorrhage and renal lymphosarcoma were also confirmed in rare cases (Table 2). As a result of PCR testing to detect the presence of the pathogens of fifteen disease, only two viruses, avian coronavirus and fowl adenovirus were found from 28 cases (27.2 %) and 7 cases (6.8 %), respectively (Table 1). Fowl adenovirus was positive in seven flock but pathological lesions by adenovirus was not observed. Partial sequences of hexon gene were related with fowl adenovirus genotype C

Quail viral enteritis

Viral enteritis among viral disease was only diagnosed based on gross and histopathological lesions. Affected birds showed gross lesions of dilation, thinning and mucosal congestion of duodenum and microscopic lesions of decreased villous length, lymphocyte infiltration of the lamina propria, crypt epithelial cell death and exfoliated cells in the crypt. In some cases, focal necrosis of hepatocytes and periportal lymphocyte infiltration were observed along with duodenal lesions. It may be due to other complex factors, but no bacteria were isolated (Fig. 1.) Avian coronavirus was detected from 28 cases using RT-PCR and 22 cases of them were showed histopathological lesions on duodenum but six cases have no lesions (Tables 1 and 2).

Viral metagenomics analysis

To identify the potential viruses associated with enteritis, viral metagenomics using duodenum samples was performed to two cases with viral enteritis and one case without viral enteritis, yielding a total of 1,531,972 reads, 7,298,922 reads and 4,783,509 reads, respectively. Of these, 2.5 % (n = 38,029), 4.4 % (n = 323,928) and 1.6 % (n = 74,589) were assigned to viral reads. QCoV was commonly identified from two cases with viral enteritis. Calicivirus was also present in two cases of enteritis, but there were insufficient sequence reads for further analysis. Quail picornavirus and bacteriophage was present in all three cases, while retrovirus, *flaviviridae* and picobirnavirus were identified only in the third case, which was not enteritis (Table 3).

Table 2
Final diagnosis results of farmed quail carcasses in Korea from 2022 to 2023.

	Disease	No. positive flocks*	% of diagnosis
Viral disease	Viral enteritis	**22**	**21.4**
Bacterial disease	Colibacillosis	19	18.4
	Necrotic enteritis	13	12.6
	Staphylococcosis	12	11.7
	Botulism	10	9.7
	Enterococcosis	3	2.9
	Salmonellosis	1	1.0
Subtotal		**58**	**56.3**
Parasitic disease	Coccidiosis	27	26.2
	Cryptosporidiosis	1	1.0
Subtotal		**28**	**27.2**
Non-infectious disease	Fatty liver hemorrhagic syndrome	17	16.5
	Leg fracture	11	10.7
	Putrefaction	9	8.7
	Pulmonary hemorrhage	2	1.9
	Lymphosarcoma	1	1.0
	Tracheitis	1	1.0
	Pododermatitis	1	1.0
	Unknown	18	17.5
Subtotal		**60**	**58.3**

* There were complicated diseases in the same flock.
* p-value calculated by Chi-square test was < 0.05, suggesting that the results are likely to vary depending on the diseases.

Genetic analysis of quail coronavirus

The complete genome of QCoV, the putative pathogen causing quail viral enteritis, has been obtained from two cases, AD23/23 and AR19/23. These sequences of the AD23/23 and AR19/23 virus were assembled into one continuous sequence of 27,477 nt and 27,611 nt, respectively. The order, number and size of open reading frame (ORF)s were similar to avian gammacoronaviruses: following the 5′ untranslated region (UTR), ORF1ab, 1a, S, 3a, E, M, 4b, 4c, 5a, 5b, N, 6b and 3′ UTR. Sequences of complete genomes of two QCoVs were deposited into GenBank under accession numbers PQ570514 and PQ570515.

Similarity plot analysis showed ORF 1ab, 5a, 5b and 6b of both QCoVs were closed to those of Korean IBV KM91 and S, 3a, E, M, 4b, 4c and N gene of QCoVs were similar to those of TCoV-ATCC strain (Fig. 2). A GenBank nucleotide BLAST search found that the full-length sequence of AD23/23 strain and AR19/23 strain was 89.4 % and 89.6 % nt sequence identity with TCoV-ATCC strain, respectively. The S genes of QCoVs showed 87.1 % and 86.2 % nt identity with TCoV MN/310/96 as the closest relationship based on a BLASTn search, but the other 11 genes of QCoVs showed 87.8 to 93.4 % nt identity with those of IBVs. In addition, the two QCoVs did not have 3b gene unlike IBV or TCoV and only AD23/23 virus had traces of 3b gene of 15 nt starting with the universal initiation codon AUG (Table 4).

The phylogenetic analysis based on the complete genome sequence and the S genes revealed that two QCoVs were clustered with American TCoV, which was different from IBV. The 1ab genes of QCoVs were classified as gammacoronavirus and closely related to Korean IBV (Fig. 3). Two QCoVs in this study were in the same branch in the S gene phylogeny with Italy QCoV and Korean QCoV identified in 2013, but the partial S1 genes of four QCoVs showed 80.2 to 86.7 % identities at the nt

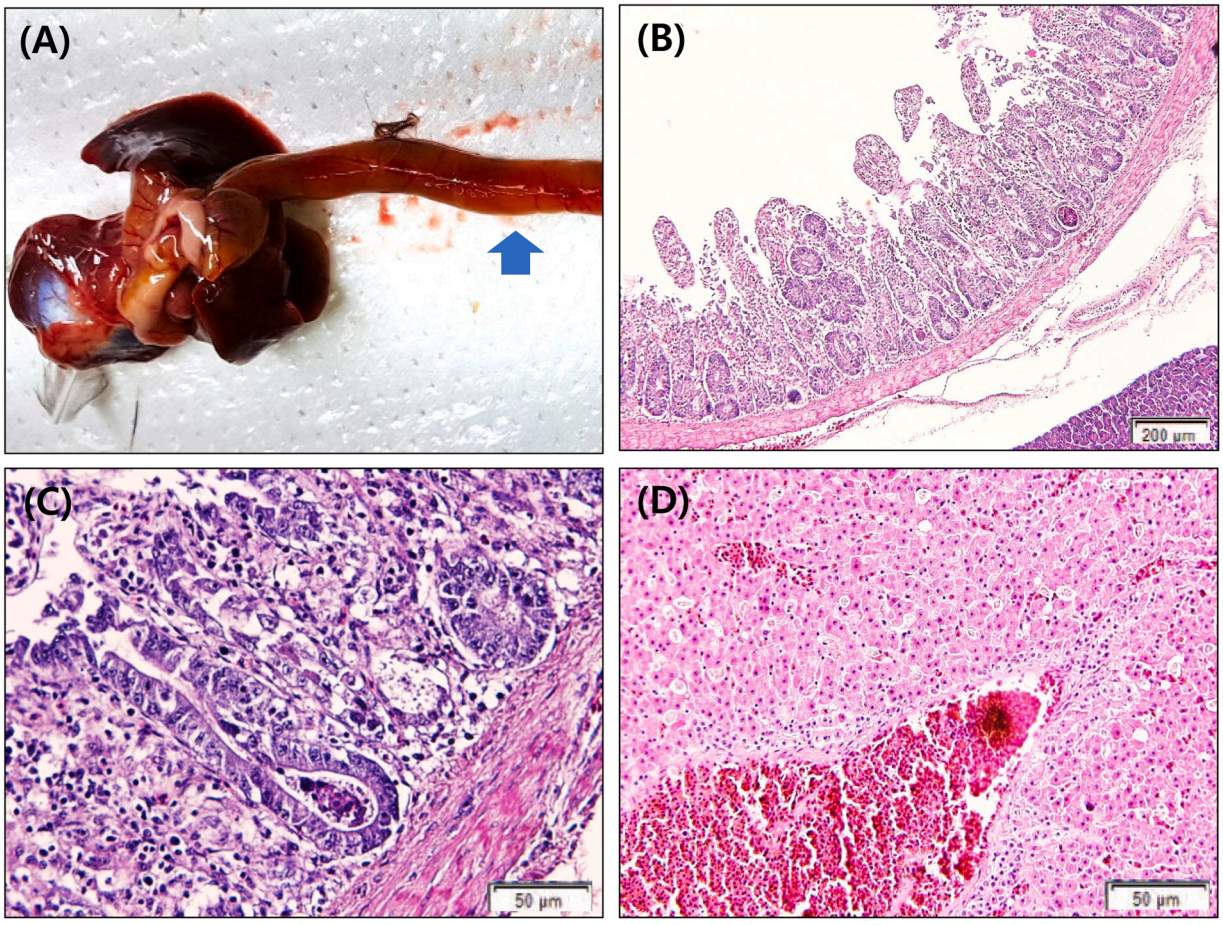

Fig. 1. Viral enteritis the by the putative pathogen quail coronavirus. (A) Gross lesion: dilation, thin wall (arrow) and congestion of duodenum. (B) histopathological lesion: shortening of the villi in the duodenum (X100), and (C) necrosis of crypt epithelium, presence of cellular debris within crypts, and lymphocytic infiltration in the submucosa of duodenum (X400). (D) diffuse focal necrosis of the liver and perivascular lymphocytic infiltration (X400).

Table 3
Viral metagenomics analysis in quails with viral enteritis.

		Case A		Case B		Case C	
Diagnosis based on traditional methods		Viral enteritis, Enterococcosis		Viral enteritis		Fatty liver syndrome, leg fracture	
Metagenomics	**Total read**	1,531,972	-	7,298,922	-	4,783,509	-
	Eukaryota	443,208	28.9 %	3,223,177	44.2 %	254,290	5.3 %
	Bacteria	343,980	22.5 %	2,435,298	33.4 %	3,799,828	79.4 %
	Virus	38,029	2.5 %	323,928	4.4 %	74,587	1.6 %
	picornavirus	17,554	(46.2 %)	25,722	(7.9 %)	65	(0.1 %)
	coronavirus	7,499	(19.7 %)	6,798	(2.1 %)	-	-
	calicivirus	1,315	(3.5 %)	2,332	(0.7 %)	-	-
	retroviridae	-	-	-	-	60	(0.1 %)
	flaviviridae	-	-	-	-	17	(<0.1 %)
	picobirnavirus	-	-	-	-	84	(0.1 %)
	bacteriophage	10,712	(28.2 %)	281,407	(86.9 %)	73,917	(99.1 %)
	others	949	(2.5 %)	7,669	(2.4 %)	444	(0.6 %)

level and 75.5 to 84.0 % identities at the aa levels, which reveal further diversity among QCoVs.

QCoV AD23/23 and AR19/23 had different length of S gene 1,207 and 1,213 aa, respectively. The consensus motif, SRRR/S and RRRR/S were found at the cleavage site of the S1 and S2 subunits in two strains. Secondary cleave site, NQGR/S and NHGR/S motif were detected in the S2 subunits. Hypervariable regions (HVR) in the S1 gene of QCoVs showed more similar patterns to TCoV than IBV, but QCoV strains showed low aa identity in the HVR2 (position 115-140) and HVR3 (275-292) region with other viruses, and the amino acid patterns unique to QCoV in the HVR2 were found (Table 5).

Isolation of virus

QCoV has not been cultured despite attempts to isolate the virus using cells, SPF chicken eggs and quail eggs. After several serial passages, there was no embryo death or cytopathic effect, and RT-PCR analysis showed that virus was not isolated.

Discussion

This study is about the first surveillance to quail disease in Korea. Surveillance results demonstrate that quail are susceptible to many

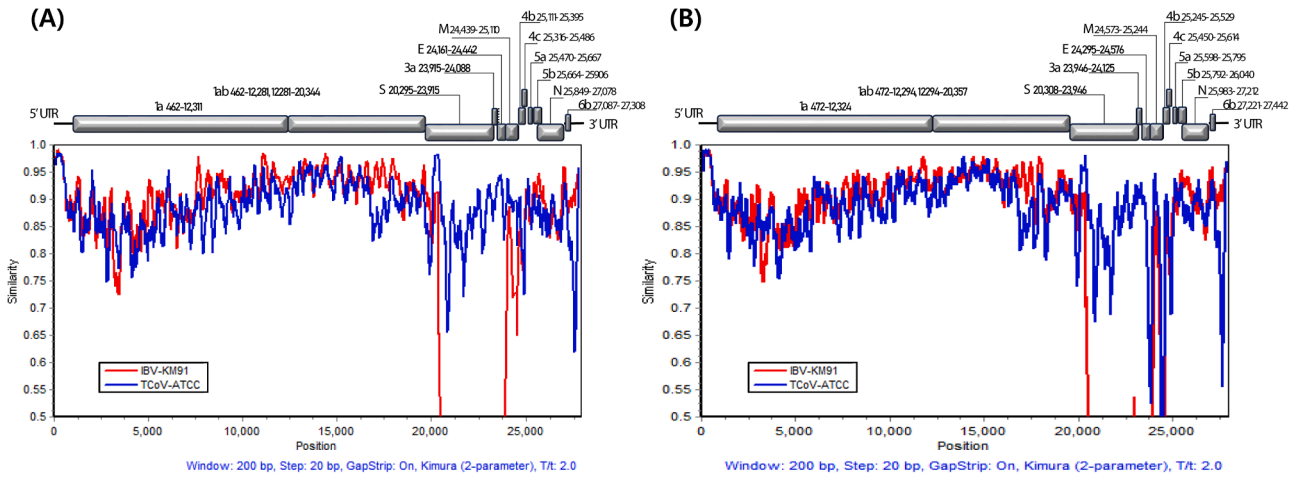

Fig. 2. Quail coronavirus genome organization and recombination analysis in the complete genome of quail coronavirus AD23/23 (A) and AR19/23 (B) and their putative parents (IBV-KM91(red) and TCoV-ATCC (blue)). The y-axis shows the percentage identity within a sliding window that is 200 bp wide and centered on the position plotted, with a step size between plots of 20 bp.

Table 4
Sequence identities of quail coronavirus compared with reference strains.

Genes	QCoV-AD23/23				QCoV-AR19/23			
	Size (nt)	nt identity with reference (%)	Reference	NCBI acc. No.	Size (nt)	nt identity with reference (%)	Reference	NCBI acc. No.
Full length	27,477	89.4	TCoV-ATCC	EU022526	27,611	89.6	TCoV-ATCC	EU022526
1ab	19,884	91.4	IBV-KM91	JQ977698	19,887	91.4	IBV-KM91	JQ977698
1a	11,850	93.4	IBV-KM91	JQ977698	11,853	93.4	IBV-KM91	JQ977698
S	3,621	87.1	TCoV-MN/310/96	KF652237	3,639	86.2	TCoV-MN/310/96	KF652237
3a	174	90.8	IBV-QIA/Q43	KU900744.	180	91.0	IBV-QIA/Q43	KU900744.
3b	15	-	-	-	-	-	-	-
E	282	91.3	IBV-Kr/109/2011	PQ178996	282	91.0	IBV-Kr/109/2011	PQ178996
M	672	91.3	IBV-D79/05	KU900740	672	92.0	IBV-CA1737	OQ095389
4b	285	91.9	IBV-KM91	JQ977698	285	92.6	IBV-KM91	JQ977698
4c	171	87.9	IBV-KM91	JQ977698	165	90.6	IBV-KM91	JQ977698
5a	198	92.9	IBV-QIA/03342	KU900743	198	91.4	IBV-QIA/03342	KU900743
5b	243	87.8	IBV-GX/YL130025	KR265092	249	93.0	IBV-GX/YL130025	KR265092
N	1,230	89.6	IBV-KM91	JQ977698	1,230	91.0	IBV-QIA/03342	KU900743
6b	222	91.9	IBV-QIA/03342	KU900743	222	92.3	IBV-QIA/03342	KU900743

diseases affecting galliform birds. In particular, infectious diseases such as colibacillosis, necrotic enteritis, staphylococcosis, botulism and coccidiosis have been frequent. Meanwhile, quail-specific diseases such as quail bronchitis and quail ulcerative enteritis were not identified, which may be due to the relatively low susceptibility to Japanese quails or the limited number of samples. Interestingly, viral enteritis was frequently observed, which appears to be caused by a coronavirus. Quail viral enteritis can cause damage to farm productivity by causing pathological lesions in the small intestine and diarrhea (Circella et al., 2007; Kim et al., 2021). In previous study and this study, an unbiased metagenomic analysis suggested that QCoVs are likely causative agents of enteritis. The QCoVs were also detected in RT-PCR assay using samples from birds diagnosed with viral enteritis. Unfortunately, the virus could not be isolated, which is probably due to the lack of a suitable cell line and further study, including in vivo models, will be required to isolated the QCoVs

Avian coronavirus is highly relevant in the poultry industry. It causes various diseases depending on the species: infectious bronchitis (IB) in chicken (Jackwood and de Wit, 2020); enteritis and death in turkey (Maurel et al., 2011; Saif, 2020); respiratory, urinary, and reproductive syndromes in pheasant (Pennycott, 2000; Spackman and Cameron, 1983). Quail coronavirus (QCoV) was associated with an enteric syndrome in Italy in 2005 and Korea in 2013 and genetically related to turkey coronavirus (TCoV) and IBV (Circella et al., 2007; Kim et al., 2021). However, the full-length genome of QCoV has not yet been identified, and the pathogenicity and productivity loss caused by this virus are unknown. Recently, deltacoronavirus in quail was identified, suggesting that quail may serve as a mixing vessel for mammalian and avian viruses (Torres et al., 2017; Domańska-Blicharz et al., 2019). There are concerns that this species may accelerate mutations of coronavirus, which have the characteristic of evolving rapidly.

We successfully obtained the two full genome sequences of the QCoV believed to cause quail enteritis, although the virus was not isolated. Amplicon-based WGS used in this study was developed not only to obtain full genome sequences from clinical samples infected with IBV belonging to the GI-19 lineage, but also to be applied to various IBV lineages (Le et al., 2024). According to previous study (Kim et al., 2021), other genes except the S gene of QCoV were similar to those of Korean IBV, so amplicon-based WGS was applied to the QCoV and the whole genome could be obtained.

Avian coronaviruses have similar phylogenetic relationships and genomic structures. Their genome consists of four structural proteins: S,

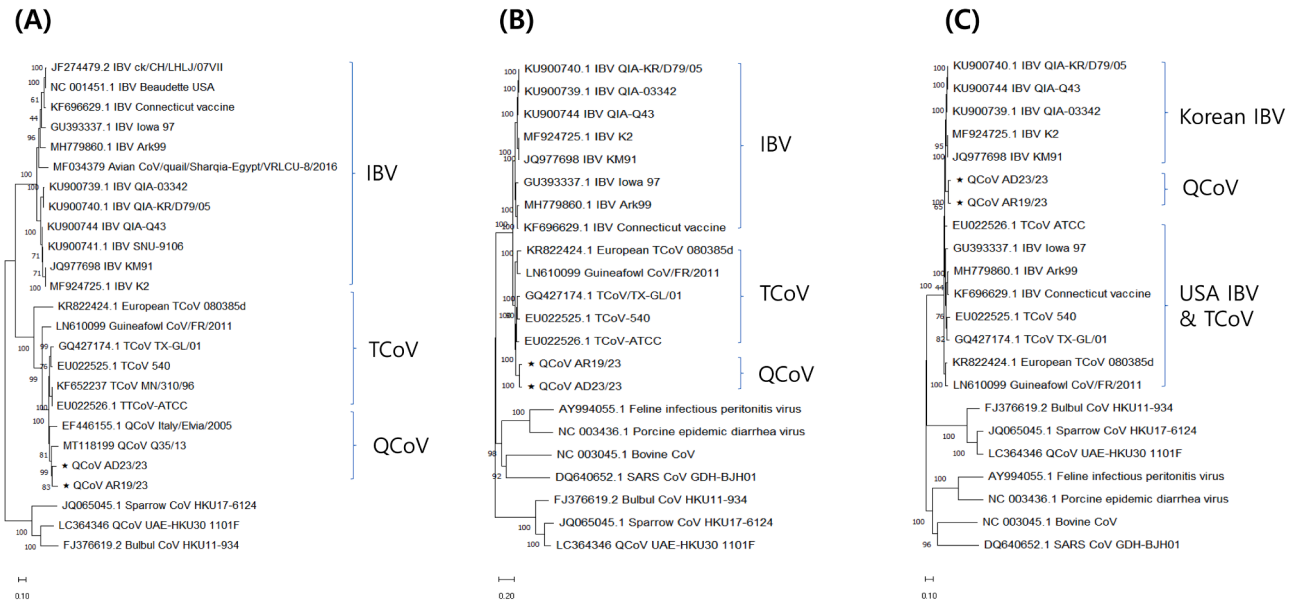

Fig. 3. Phylogenetic analysis of (A) the S genes, (B) the complete genomes and (C) the 1ab genes of quail coronavirus. The phylogenetic trees were generated using the maximum-likelihood method. Bootstrap values were deduced from 1,000. Two QCoVs identified in this study were marked with an asterisk.

Table 5
Sequence comparison of S gene of avian coronavirus.

Strain	S full length (aa)	Hypervariable region of S1 gene			S1/S2 cleavage (536)	S2 2nd cleavage (698)
		HVR*1 (60~88)	HVR2 (115-140)†	HVR3 (275-292)		
TCoV-ATCC	1204	DVMRPPDGAYIQSGYYEPLFTGCFNQTYQ	YDAIGLMFWWGLSMGNSTPLFNLTWG——FF	LKGDAVSYNQSCVDSKYSF	RRRR/A	NQGR/S
IBV-KM91	1164	GGASECTVGVIKDVYNQSAASIAMTAPLQ	YSSGRGSCPITGLIPQNHIRISAMKN——S	HNETNASPNSGGVNSISTY	RFRR/S	PSGR/S
QCoV-Italy/Elvia/05	-	DVMRPPDGVYTQSGYYEPLFTGCFNQTNQ	YDA**F**G**I**MFWWGLN**TN**FDRSYD**PT**–NLTWGNFF	LKGDPVLYNRSCVDPKFSF	-	-
QCoV-Q35/13	-	DVMRPPDGAYIQSGYYEPIFTGCFNQTNQ	YDA**F**G**I**MFWWGLV**TN**EGNKTGE**PT**FNLTWGNFF	LKGEPVGYNQSCVDPKHPF	-	-
QCoV-AD23/23	1207	DVMRPPDGAYIQSGYYEPIFTGCFNQTNQ	CDA**F**G**I**MFWWGLR**TN**SNKTGGD**PT**FNLTLGNFL	LKGESVSYNQSCVGSNYSF	SRRR/S	NQGR/S
QCoV-AR19/23	1213	DVMRPPDGAYIQSGYYEPIFTGCFNQTNK	YDA**F**G**I**MFWWGLR**TN**TGSNQYIE**PT**FNLRLGDF	LKGEPVVYNQSCVDPKYPF	RRRR/S	NHGR/S

* HVR: hypervariable region of S1 gene.
† The amino acid variations unique to quail coronavirus were highlighted in bold.

envelope, membrane, and nucleocapsid. It also has a number of non-structural proteins that help the virus replicate and cause disease (Ducatez et al., 2015; Brown et al., 2016; Jackwood and de Wit, 2020). These proteins would be 3a, 3b, 4b, 4c, 5a and 5b whose number and nature vary depending on the species and even the avian coronavirus (Domanska-Blicharz and Sajewicz-Krukowska, 2021). The overall length and structure of QCoV genomes were similar to those of TCoV or IBV. However, the QCoV lacked the 3b gene, and the S gene of that was 3 to 49 amino acids longer. Variations in the HVR of the S1 gene resembled those seen in TCoV, but also showed diverse patterns even among different QCoV strains.

In phylogenetic analyses of the S and 1ab genes, QCoV appeared similar to TCoV or IBV, but in the whole-genome phylogeny, QCoVs formed its own distinct branch. Recombination analysis showed that the S gene of QCoV was similar to TCoV and other genes resembled IBV, overall homology was low at less than 91 %. The recombination events between QCoV, TCoV and IBV implicate avian coronaviruses with zoonotic potential have adapted to their hosts. S gene of avian coronavirus encodes the S glycoprotein, a critical structural protein that plays a key role in host cell infection and in determining the virus's host range and pathogenicity by its contribution to viral entry through mediating the attachment of the virus particle to the plasma membrane of the host cell (Cavanagh, 2007; Belouzard et al., 2012; van Beurden et al., 2017; Bakhshandeh et al., 2021). The similarity between the S genes of QCoV and TCoV appears to be associated with their pathogenicity, specifically in causing enteritis in quail and turkey infection. Therefore, our study demonstrates that QCoV is a species-specific virus with frequent mutation and it induces enteritis in the small intestine, similar to TCoV, and shows a moderate level of prevalence among quail in Korea.

In conclusion, we used an unbiased metagenomic approach for pathogen discovery from quail diagnosed viral enteritis and an amplicon-based WGS for obtaining the complete genome of the QCoV. The novel QCoV represents a new species in the gammacoronavirus of the *coronaviridae*, which is expected to be the cause of contagious enteric disease in quails. The complete genome sequences of two QCoV strains will aid our understanding of avian coronavirus in terms of molecular evolution and provide a basis for the diagnostics, control and prevention of QCoV infection in quail flocks. In addition, our surveillance results of the quail farms help increase understanding of naturally occurring diseases of quail, despite the potential biases in sample collection and the limitation of PCR-based pathogen detection and suggest that the need for biosecurity measures and the prevention of infectious diseases in quail farms.

Ethics approval and consent to participate

Not applicable for this study due to the carcasses were collected post-mortem for disease diagnostics.

Consent for publication

Not applicable

Availability of data and materials

The data generated or analyzed during this study are available from the corresponding author on reasonable request.

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Declaration of competing interest

The authors declare that they have no competing interests

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