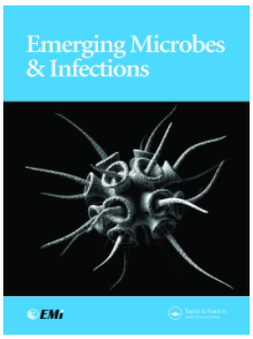

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Emerging and Re-Emerging Coronaviruses

Novel human coronavirus in an infant patient with pneumonia, Republic of Korea

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ABSTRACT

Coronaviruses (CoVs) pose a significant threat to public health, causing a wide spectrum of clinical manifestations and outcomes. Beyond precipitating global outbreaks, Human CoVs (HCoVs) are frequently found among patients with respiratory infections. To date, limited attention has been directed towards alphacoronaviruses due to their low prevalence and fatality rates. Nasal swab and serum samples were collected from a paediatric patient, and an epidemiological survey was conducted. Retrospective surveillance investigated the molecular prevalence of CoV in 880 rodents collected in the Republic of Korea (ROK) from 2018 to 2022. Next-generation sequencing (NGS) and phylogenetic analyses characterized the novel HCoV and closely related CoVs harboured by *Apodemus* spp. On 15 December 2022, a 103-day-old infant was admitted with fever, cough, sputum production, and rhinorrhea, diagnosed with human parainfluenza virus 1 (HPIV-1) and rhinovirus co-infection. Elevated AST/ALT levels indicated transient liver dysfunction on the fourth day of hospitalization. Metagenomic NGS (mNGS) identified a novel HCoV in nasal swab and serum samples. Retrospective rodent surveillance and phylogenetic analyses showed the novel HCoV was closely related to alphacoronaviruses carried by *Apodemus* spp. in the ROK and China. This case highlights the potential of mNGS to identify emerging pathogens and raises awareness of possible extra-respiratory manifestations, such as transient liver dysfunction, associated with novel HCoVs. While the liver injury in this case may be attributable to the novel HCoV, further research is necessary to elucidate its clinical significance, epidemiological prevalence, and zoonotic origins.

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KEYWORDS Coronavirus; pneumonia; retrospective surveillance; next-generation sequencing; phylogenetic analysis; genomic epidemiology; *Apodemus* species

Introduction

The *Coronaviridae* family comprises enveloped, positive-sense, single-stranded RNA viruses [1]. Coronaviruses (CoVs) infect a wide range of hosts, including humans and animals, posing a significant threat to public health. A notable recent human CoV (HCoV) infection is the CoV disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome CoV 2 (SARS-CoV-2) [2]. This virus presents a wide spectrum of clinical manifestations and outcomes, resulting in major disruptions of historical proportions in politics, economics, and healthcare [3].

In addition to causing global outbreaks, HCoVs are frequently endemic in patients with respiratory infections [4]. Seven CoVs from two distinct genera are recognized to infect humans. Common HCoV strains include 229E and NL63 from the genus *Alphacoronavirus* (α-CoV) and OC43 and HKU1 from the genus *Betacoronavirus* [5]. These strains primarily lead to seasonal outbreaks of upper respiratory tract infections, typically presenting with mild symptoms. In contrast, three other CoVs (severe acute respiratory syndrome CoV, Middle East respiratory syndrome CoV, and SARS-CoV-2), which have emerged through

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recent zoonotic transmission, are associated with lower respiratory tract disease, including acute respiratory distress syndrome [6–8]. In paediatric populations, the clinical spectrum of HCoV infections includes fever, upper respiratory disease, croup, asthma exacerbation, acute gastroenteritis, and febrile seizures [9].

Co-infection of HCoV with other respiratory viruses, such as human parainfluenza viruses (HPIV) 1–3, rhinoviruses, influenza A and B viruses, human adenoviruses, and human metapneumoviruses, has been observed in clinical settings [5,10]. Recently, a novel HCoV, belonging to canine CoV (CCoV), was isolated from a patient with pneumonia who was co-infected with a rhinovirus in Malaysia (CCoV-HuPn-2018) [11]. A novel recombinant CCoV strain was also identified and isolated from a patient with a travel history from Haiti who exhibited fever (HuCCoV Z19Haiti), supporting the zoonotic transmission [12]. Additionally, partial sequences of feline-like CoVs have been retrospectively detected in patients with influenza-like illnesses in the USA [13].

Rodents, as natural reservoirs of viral diversity, are crucial to the emergence of zoonotic pathogens [14,15]. Within the genus α-CoV, two notable rodent-derived strains, AcCoV-JC34 and Lucheng-19, exemplify the evolutionary complexity and genetic diversity of these viruses. AcCoV-JC34, identified in *Apodemus chevrieri* (Chevrier's field mouse) in Yunnan, China, is classified within the subgenus *Luchacovirus* [16]. This strain is particularly distinguished by the presence of a putative furin cleavage site in its spike (S) protein, a feature not commonly observed in other *Luchacovirus* members [17]. Meanwhile, Lucheng-19, discovered in *Rattus norvegicus* (Brown rat) in Lucheng, China, represents a unique lineage within α-CoV and is believed to have arisen through recombination events [18]. These findings underscore the rich genetic diversity of rodent-borne α-CoVs, highlighting the need for sustained surveillance to better understand their potential to emerge as zoonotic threats.

Here, we report the first case of pneumonia with concurrent temporary abnormal liver function in an infant co-infected with a novel HCoV, HPIV-1, and rhinovirus. The α-CoV carried by *Apodemus* spp., closely related to this novel HCoV, was identified and characterized through retrospective rodent surveillance conducted in the Republic of Korea (ROK) from 2018 to 2022.

Materials and methods

Ethics statement

Human specimens were collected with an exemption of consent (K2023-0634-1) from the Institutional Review Board of Korea University Medical Center (KUMC), Ansan, ROK. Wildlife was handled per the ethical guidelines (#2016–49, 2019–4, 2019–171, 2021–75, and 2022–34) of the Korea University Institutional Animal Care and Use Committee, Korea University, Seoul, ROK. The small mammals were transported to an animal biosafety level 3 facility at Korea University, where they were euthanised by cardiac puncture under alfaxalone-xylazine anaesthesia.

Patient specimen collection and epidemiological survey

Nasal swabs and serum samples were collected from a paediatric patient at the Korea University Ansan Hospital, Ansan, ROK. An epidemiological interview was conducted with the patient's parents. The epidemiological questionnaire included personal information, dates of symptom onset and hospitalization, diagnosis, clinical symptoms, field activities, exposure history to domestic or wild animals, travel history before infection, and vaccination history. Medical staff reviewed the charts.

Metagenomic next-generation sequencing (mNGS)

mNGS was performed on nasal swabs and serum specimens from a patient using an in-house modified sequence-independent single-primer amplification (SISPA) method. Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Host rRNA depletion was conducted to remove total rRNA using a NEBNext rRNA Depletion Kit (Human/Mouse/Rat) V2 (New England Biolabs, Ipswich, MA, UK). First-strand cDNA was synthesized from isolated RNA using the SuperScript™ IV First-Strand Synthesis System (Invitrogen) with FR26RV-N (5′-GCC GGA GCT CTG CAG ATA TCN NNN NN-3′). Double-stranded (ds) cDNA was generated using 5 units of Klenow 3′ → 5′ exo DNA polymerase (Enzynomics, Daejeon, ROK), 0.5 μL of dNTP mixtures (each 0.5 mM), and 1 μL of RNaseH (Invitrogen). The ds cDNA was purified using Agencourt AMPure XP beads (Beckman Coulter, CA, USA). Purified cDNA was enriched by random fragment amplification using MyTaq Red mix (Bioline, Taunton, MA, USA) and the FR20RV (5′-GCC GGA GCT CTG CAG ATA TC-3′). The first and second cycling conditions were: initial denaturation was at 98°C for 30 s, followed by 38 or 25 cycles of denaturation at 98°C for 10 s annealing at 54°C for 20 s, and elongation at 72°C for 45 s. Polymerase chain reaction (PCR) products were purified using Agencourt AMPure XP beads (Beckman Coulter). Amplicons were prepared using a Ligation Sequencing Kit (SQK-LSK109) according to the manufacturer's protocol (Oxford Nanopore Technologies, London, UK). Each library was sequenced using a singleplex

assay on an MK1C system (ONT) with an R9 flow cell without cross-contamination.

Retrospective rodent surveillance

The rodents were provided by the Hantavirus Surveillance Project of Korea University, Seoul, ROK [19–22]. Rodent trapping and taxonomic classification procedures have been described previously [23]. CoV infection was confirmed using in-house reverse-transcription PCR (RT–PCR). Total RNA was extracted from rodent faecal specimens using TRI Reagent LS Solution (Invitrogen, Waltham, MA, USA) following the manufacturer's instructions. Reverse transcription was performed from 1 μg of total RNA with the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA). Nested RT–PCR was conducted in a 25 μL reaction mixture containing 0.625 U of Ex Taq DNA polymerase (TaKaRa BIO, Shiga, Japan), 2.5 μL of 10 X Ex Taq buffer, 2 μL of dNTP mixture (each 2 mM), 1 μL of forward and reverse direction primers (final concentration: each 0.4 μM), and 1.5 μL of cDNA template. The cycling condition included an initial denaturation at 94°C for 5 min, followed by 6 cycles of denaturation at 94°C for 30 s, annealing at 37°C for 40 s, and elongation at 72°C for 1 min; and then by 32 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 40 s, and elongation at 72°C for 1 min; with a final extension at 72°C for 5 min (ProFlex PCR System, Life Technology, CA, USA). CoV-specific primer sequences were CoV-R-2F (outer): 5'-GGC ACT GTT GTA TCA AAT GCC ATG-3', CoV-R-2R (outer): 5'-CAG CAG TAA CAG CCA CAG CCA C-3', CoV-R-2NF (inner): 5'-GCT TAA GTG TGT GGC GCT CTG-3'and CoV-R-2R (inner): 5'-CAG CAG TAA CAG CCA CAG CCA C-3' for RNA-dependent RNA polymerase (RdRp) gene; CoV-S-2F (outer): 5'-GGT GGC ATA GGG CCA CTC AAG-3', CoV-S-2R (outer): 5'-CCT ACG CAG ACC ATA ATT GCC-3', CoV-S-2NF (inner): 5'-CAA CAG CCT GCT GTT GAT GTG C-3', CoV-S-2R (inner): 5'-CCT ACG CAG ACC ATA ATT GCC-3' for S gene.

Quantitative PCR (qPCR)

qPCR was conducted using the TaqMan Multiplex Master Mix (Applied Biosystems) on a QuantStudio 5 Flex Real-Time PCR System (Applied Biosystems). 25 μL of reaction mixture included 10 μL of TaqMan PCR Multiplex Master Mix (Applied Biosystems), 2 μL of cDNA template, 1 μL of TaqMan probe (10 nM), 0.5 μL of forward and reverse primers (each 36 nM), and 6 μL of Distilled water (D.W.). The PCR protocol consisted of an initial denaturation at 95°C for 20 s, followed by 40 cycles of 95°C for 1 s and 60°C for 20 s. Oligonucleotide primer and probe sequences were Apo-CoV-R-F (forward direction): 5'-GCC TAA TCC TGA TCC TAG CCG-3', Apo-CoV-R-R (reverse direction): 5'-AAG AGA CAC ATA GCG TTC AAG-3', and Apo-CoV-R-P (probe): 5'-TAMRA-CTG CTG GTG TTT TTG ATG-BHQ-3' for RdRp gene.

Multiplex PCR-based NGS

Multiplex PCR-based NGS was conducted to acquire the genomic sequences of CoV from rodent faecal specimens. cDNA was amplified with overlapped seven parts of HCoV-KUMC22-3-specific multiplex primer mixtures using Solg 2X Uh-Taq PCR Smart Mix (Solgent, Daejeon, ROK) following manufacturer's instructions. The enrichment was performed with the following composition: 12.5 μL 2X Uh premix, 2 μL of each primer mixture, 10.5 μL of D.W., and 1 μL of the DNA template in 25 μL final reaction mixture. The PCR conditions were as follows: an initial denaturation at 95°C for 15 min, followed by 40 cycles at 95°C for 20 s, 50°C for 40 s, and 72°C for 1 min, and a final elongation at 72°C for 3 min. DNA libraries were prepared and sequenced using a Ligation Sequencing Kit (SQK-LSK109) with a Native Barcoding Kit (EXP-NBD104 and NBD114), according to our previous standard protocols [20]. The multiplex primer sequences are listed in Supplementary Table 1.

NGS data analysis

Raw data underwent basecalling, demultiplexing, and trimming of adaptor sequences using Guppy version 3.0.3 in MinKNOW (ONT) from the MK1C system. Subsequently, the filtered reads were consolidated into a single FASTA file using Porechop version 9.0. Viral reads were mapped to the reference genomic sequence of the AcCoV-JC34 strain, and consensus sequences were extracted using CLC Genomics Workbench version 22 (Qiagen) [16]. Manual polishing was performed based on our error-correction criteria [20].

Phylogenetic analysis

The CoV genomic sequences were aligned using the Clustal W algorithm in Lasergene version 5 (DNASTAR, Madison, WI, USA). Phylogenies were reconstructed using the best-fit substitution model of evolution and the maximum likelihood method in MEGA 7 [24]. Topologies were evaluated using a bootstrap analysis with 1,000 iterations.

Virus isolation

Nasal swabs and serum samples from a patient were inoculated into MRC-5 cells (ATCC, #CCL-171). Following 1.5 h of adsorption, excess inoculum was

removed, and the viral suspension was replaced with 5.5 mL of Dulbecco's modified Eagle's medium supplemented with 2% fetal bovine serum (Lonza, Basel, Switzerland), 16 μg/mL of TPCK-Trypsin (Thermo Fisher Scientific, Waltham, MA, USA), and 0.1% gentamicin (Gibco, Life Technologies, Carlsbad, CA, USA). The cells were then cultured at 37°C in an incubator with 5% CO_2 and passaged every five days. Viral isolation was confirmed at each passage by conventional RT–PCR [25,26].

Results

Case description

On 15 December 2022, a 103-day-old infant was hospitalized with symptoms of fever, cough, sputum production, and rhinorrhoea. Physical examination revealed redness of the left tympanic membrane, indicative of acute otitis media. Laboratory tests confirmed the presence of HPIV-1 and rhinovirus through respiratory virus multiplex real-time PCR (Supplementary Table 2). In the subsequent days, the fever subsided, and the respiratory symptoms improved. However, on the fourth day of hospitalization, liver function tests showed significantly elevated AST/ALT levels (462/350 IU/L) (Table 1). Abdominal ultrasonography indicated mildly increased liver parenchymal echogenicity with subtle, coarse textures. Serological tests for viral hepatitis were negative. Chest radiography revealed air trapping in both lungs and peribronchial infiltration in the left lower lung (Figure 1). The detection of HCoV infection related to the AcCoV-JC34 strain was identified using mNGS in nasal swabs and serum specimens. Bacterial blood cultures were negative. Conservative treatment led to gradual improvements in liver function and respiratory symptoms, and the infant was discharged on the eighth day. Post-discharge, the infant developed a transient whole-body skin rash and itching, which resolved spontaneously. Further investigation showed elevated total IgE levels of 10.0 IU/L, with no specific allergens identified. Importantly, the infant had no history of drug use that could have potentially induced liver injury (Supplementary Table 3).

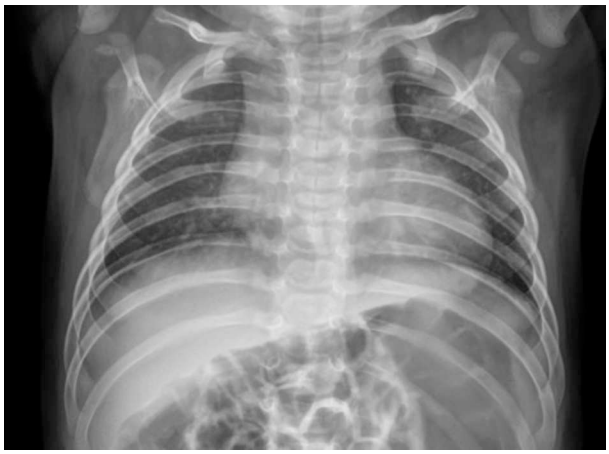

Figure 1. On the fourth day of admission, a chest x-ray revealed air trappings in both lungs and peribronchial infiltration in the left lower lung.

Table 1. Summary of complete blood count, liver function, serological, and microbiology tests for patient KUMC22-3 during hospitalization.

Observation	Days of hospitalization						Normal value or range
	Day 1 (Hospitalization)	Day 4	Day 5	Day 6	Day 8 (Discharge)	Day 14 (After discharge)	
Complete blood count							
WBC count (x10^3/μL)	8.43	11.04	N.D	N.D	N.D	N.D	6.0-14.0
PLT count (x10^3/μL)	387	473	N.D	N.D	N.D	N.D	150–400
Neutrophil (%)	23.2	21.3	N.D	N.D	N.D	N.D	57–67
Lymphocyte (%)	51.4	62.2	N.D	N.D	N.D	N.D	25–33
Monocyte (%)	19	8.4	N.D	N.D	N.D	N.D	3–7
Eosinophil (%)	5.9	7.7	N.D	N.D	N.D	N.D	1–3
Basophil (%)	0.5	0.4	N.D	N.D	N.D	N.D	0-0.75
CRP (mg/dL)	0.54	0.79	N.D	N.D	N.D	N.D	0.08-1.12
Liver function test							
AST (IU/L)	69	462	485	140	38	30	22–63
ALT (IU/L)	57	350	430	274	149	37	12–45
GGT (IU/L)	N.D	N.D	44	44	39	27	8–90
Total bilirubin (mg/dL)	< 0.15	N.D	<0.15	0.22	0.20	0.15	<1.0
Serological test							
Anti-HAV IgM, HBV (HBs Ag and Ab), HCV Ab	N.D	Negative	N.D	N.D	N.D	N.D	–
Anti-(CMV, EBV, and HSV) IgM	N.D	Negative	N.D	N.D	N.D	N.D	–
Microbiology test							
Virus diagnostic: molecular method	HPIV-1/ rhinovirus	–	HCoV	–	–	–	–
Bacterial diagnostic: blood culture	No growth after 5 days						

KUMC, Korea University Medical Center; WBC, white blood cell; PLT, platelet; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, Gamma-glutamyl transferase; HAV, hepatitis A virus; IgM, Immunoglobulin M; HBV, hepatitis B virus; HBs, hepatitis B surface; Ag, antigen; Ab, antibody; HCV, hepatitis C virus; CMV, cytomegalovirus; EBV, Epstein-barr virus; HSV, Herpes simplex virus; N.D, not determined; HPIV-1, human parainfluenza virus 1; HCoV, human coronavirus.

Epidemiological survey

Following the diagnosis of HCoV infection in the KUMC22-3 patient, an epidemiological investigation was conducted (Table 2). The infant was born vaginally at 39 weeks of gestation without perinatal complications, weighing 2.86 kg. Approximately 25 days after birth, the patient experienced asymptomatic COVID-19, which was independently identified through real-time PCR testing. This coincided with infections among all family members; however, the patient did not exhibit any clinical symptoms at the time of hospitalization. The patient's medical history was unremarkable. The infant received all vaccinations according to standard ROK guidelines and did not require medication. The patient demonstrated appropriate eye contact and babbling, indicating normal developmental progress. Both parents, of Korean ethnicity, were healthy and had no significant medical history. The patient and family resided in an urban apartment in Hwaseong-si, Gyeonggi Province, without any pets and had no reported contact with wild animals within the past six months.

Retrospective rodent surveillance for CoV

A total of 880 *Apodemus* spp. were collected from 19 areas in Gangwon, Gyeonggi, Chungcheongnam, Jeollanam Provinces, and Jeju Island, ROK, from 2018 to 2022 (Figure 2). The molecular prevalence of α-CoV RNA was 16/880 (1.8%), with 4/97 (4.1%) in Yanggu-gun, Gangwon Province, and 12/71 (16.9%) in Jeju-si, Jeju Island, ROK (Table 3). CoV RNA was undetectable in rodents captured from the Gyeonggi, Chungcheongnam, and Jeollanam Provinces. Quantification of α-CoV RNA was determined from faecal samples of the rodents (Supplementary Table 4).

Table 2. Epidemiological characteristics of patient KUMC22-3 with HCoV infection.

Patient code	KUMC22-3
Age (days)	3 to <4 months (103)
Gender	Male
Onset date	15th December 2022
COVID-19 history	Asymptomatic COVID-19 infection around the 25 days after birth
Geographic region (Year)	Hwaseong-si, Gyeonggi Province, Republic of Korea (2022)
Living environment	Urban area
Exposure to domestic animals	No
Exposure to wild animals	No
Travel history (within 6 months)	No
Hospital admission	Yes
Intensive care unit admission	Yes
Other concomitant pathogens detected	Human parainfluenza virus 1 and rhinovirus

KUMC, Korea University Medical Center; HCoV, human coronavirus.

Complete-coding genome sequencing of CoV

The genomic sequences of HCoV were obtained from both the nasal swab and serum specimens of KUMC22-3, using SISPA-based NGS with coverage rates of 75.2% for the nasal swab and 71.1% for the serum samples (Supplementary Table 5). The mean number of viral reads mapped to the reference sequence (AcCoV-JC34 strain) was 30,524 (1.5%) for α-CoV genome, with an average depth of coverage of 603.9. Additionally, using multiplex PCR-based NGS, four complete-coding genomic sequences of α-CoVs (Ac18-16, Ac18-19, Ac20-8, and Aa22-10) were acquired from the faecal samples of rodents, with coverage rates ranging from 98.7% to 99.4% (Supplementary Table 6). The complete genomic sequences of the CoV coding region were recovered using conventional nested RT–PCR followed by Sanger sequencing.

Genomic characterization and phylogenetic analysis of CoV

HCoV KUMC22-3 exhibited a genomic structure characteristic of CoVs, arranged from 5′ to 3′ as follows: non-structural proteins in the ORF1ab region; structural proteins including S, envelope (E), membrane (M), and nucleocapsid (N); and accessory proteins (Supplementary Figure 1 and Supplementary Table 7). Amino acid identities of HCoV KUMC22-3 were 97.6%–98.6% for 3C-like protease, 96.2%–97.3% for RdRp, 94.7%–96.2% for helicase, 96.6%–97.4% for the S protein, 98.7%–100% for the E protein, 95.2%–96.8% for the M protein, and 94.9%–96.1% for the N protein, compared to those of α-CoVs carried by *Apodemus* spp. (Table 4). Phylogenetic analysis demonstrated that HCoV KUMC22-3 forms a homologous genetic lineage with the AcCoV, AaCoV, and AcCoV-JC34 strains (Figure 3 and Supplementary Figure 2).

Virus isolation

HPIV-1 was isolated from a nasal swab specimen of the KUMC22-3 patient using a cell culture-based method. The first isolate of HPIV-1 was confirmed at passage two (ten days post-inoculation) (Supplementary Figures 3–4). Attempts to culture HCoV and rhinovirus in MRC-5 cells were unsuccessful.

Discussion

We present the first documented case of pneumonia in an infant, complicated by co-infection involving a novel HCoV, HPIV-1, and rhinovirus. Co-infection of HCoV with other respiratory viruses, such as HPIV 1–3 and rhinoviruses, is frequently observed

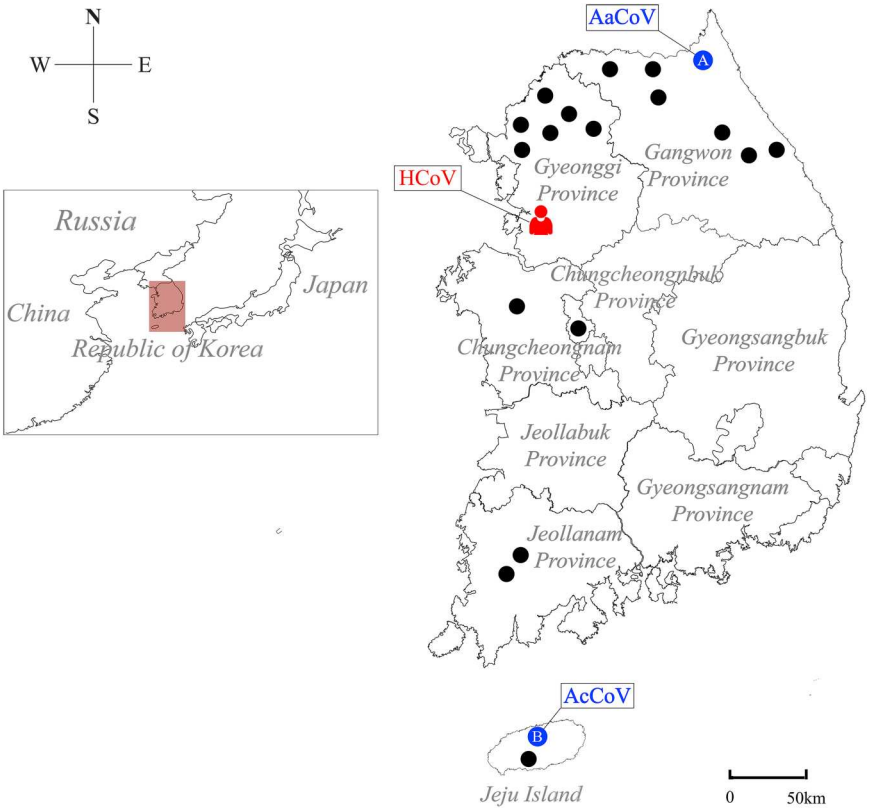

Figure 2. Geographical distribution of alphacoronavirus (α-CoV) strains from patient KUMC22-3 and rodents in the Republic of Korea (ROK), 2018-2022. The map illustrates the locations of HCoV KUMC22-3, identified in an urban area (red symbol; Hwaseong-si, Gyeonggi Province), and α-CoV RNA-positive *Apodemus* spp. (blue circles), collected from 19 regions across the ROK. These areas include Yanggu-gun, Cheorwon-gun, Chuncheon-si, Hongcheon-gun, Hwacheon-gun, Inje-gun, and Pyeongchang-gun in Gangwon Province; Gapyeong-si, Goyang-si, Paju-si, Pocheon-si, Yangju-si, and Yeoncheon-gun in Gyeonggi Province; Sejong-si and Yesan-gun in Chungcheongnam Province; Damyang-gun and Gwangju-si in Jeollanam Province; and Jeju-si and Seogwipo-si on Jeju Island. The red symbol marks the urban location where HCoV KUMC22-3 was detected, while blue circles denote sites where α-CoV RNA was identified in rodents, including (A) Yanggu-gun (for AaCoV Aa22-8, AaCoV Aa22-9, AaCoV Aa22-10, and AaCoV Aa22-12) and (B) Jeju-si (for AcCoV Ac18-6, AcCoV Ac18-14, AcCoV Ac18-15, AcCoV Ac18-16, AcCoV Ac18-18, AcCoV Ac18-19, AcCoV Ac20-5, AcCoV Ac20-8, AcCoV Ac20-12, AcCoV Ac20-17, AcCoV Ac20-19, and AcCoV Ac20-32). Black circles represent locations where α-CoV RNA was not detected in rodents during this study, as identified through retrospective surveillance. The map was created using a Quantum Geographical Information System (QGIS) 3.10 for Mac and modified using Adobe Illustrator CC 2019. Abbreviations: HCoV, human coronavirus; KUMC, Korea University Medical Center; AaCoV, *Apodemus agrarius* coronavirus; AcCoV, *Apodemus chejuensis* coronavirus.

in clinical practice [5,10,11]. The patient was admitted with symptoms of fever, cough, sputum production, and rhinorrhoea. While AST/ALT levels were temporarily elevated (462/350 IU/L) on the fourth day of admission, follow-up observations revealed rapid recovery, suggesting these abnormalities were transient. Although HPIV-1 was isolated from nasal swab specimens, its role in hepatic dysfunction remains uncertain. Rare instances of HPIV-related viraemia or systemic infection have been reported; however, a direct link to liver dysfunction is limited [27]. Liver impairment in humans due to HPIV is more likely associated with severe systemic infections rather than typical virus-specific manifestations [27,28]. Here, we identified and characterized the novel HCoV genome in both nasal swab and serum specimens, while HPIV-1 infection was detected only in nasal swab samples. These findings suggest that the reactive liver injury observed in this patient may be attributed to the novel HCoV infection. Notably, previous studies have linked COVID-19 to paediatric acute severe hepatitis and liver failure in cases with minimal or no respiratory symptoms [29]. Additionally, an HCoV HKU1-related paediatric acute liver failure case showed no significant respiratory involvement [30]. This highlights the need for further studies to determine whether abnormal liver function is a clinical feature of newly identified HCoV infections.

High-throughput sequencing technologies play an indispensable role in diagnosing infectious diseases, enabling precision management of patients, and controlling viral outbreaks [31–34]. The application of mNGS in this case allowed for the identification and genomic characterization of the novel pathogen, particularly for an illness with no clear etiology [35]. mNGS-based clinical sequencing, when integrated with epidemiological data, facilitates genomic surveillance of emerging viruses by elucidating their

Table 3. Molecular prevalence of alphacoronavirus (α-CoV) in Apodemus spp. from the Republic of Korea, 2018-2022.

Province	Trapping site	Number of *Apodemus* spp.	Positivity for α-CoV (%)
Gangwon	Yanggu-gun	97	4/97 (4.1)
	Cheorwon-gun	225	0/225
	Chuncheon-si	36	0/36
	Hongcheon-gun	42	0/42
	Hwacheon-gun	86	0/86
	Inje-gun	52	0/52
	Pyeongchang-gun	2	0/2
	Subtotal	**540**	**4/540 (0.7)**
Gyeonggi	Gapyeong-si	2	0/2
	Goyang-si	11	0/11
	Paju-si	118	0/118
	Pocheon-si	3	0/3
	Yangju-si	11	0/11
	Yeoncheon-gun	37	0/37
	Subtotal	**182**	**0/182**
Chungcheongnam	Sejong-si	27	0/27
	Yesan-gun	13	0/13
	Subtotal	**40**	**0/40**
Jeollanam	Damyang-gun	9	0/9
	Gwangju-si	23	0/23
	Subtotal	32	**0/32**
Jeju	Jeju-si	71	12/71 (16.9)
	Seogwipo-si	15	0/15
	Subtotal	**86**	**12/86 (14.0)**
Total		**880**	**16/880 (1.8)**

transmission dynamics and spread [36–38]. In this study, we obtained the complete coding genome sequence of HCoV KUMC22-3 from a nasal swab specimen of a patient using mNGS. Additionally, we recovered complete coding genome sequences of four rodent-borne α-CoVs using a newly developed amplicon-based NGS approach. Phylogenetic analyses revealed that HCoV KUMC22-3 was closely related to α-CoV strains and shared a common evolutionary ancestor with those carried by *Apodemus* spp. in ROK and China [16]. However, it is noteworthy that the patient was an infant residing in an urban apartment, with limited exposure to wild animals. Neither the patient nor their family-owned pets, and they had not travelled within the past six months before the HCoV infection. These findings underline the importance of further investigation to elucidate the origin, transmission, and pathogenicity of HCoV KUMC22-3 and related α-CoVs.

Several limitations in this study warrant consideration: (1) as this study is based on a single case, larger-scale and longitudinal cohort studies are essential to characterize the epidemiological prevalence, clinical significance, and disease severity associated with this novel α-CoV in humans and wild animals. (2) This study did not fulfil established causality standards, such as Koch's postulates or the Bradford Hill criteria, emphasizing the need for caution in interpreting our findings. Further research should aim to establish a causal relationship between HCoV KUMC22-3 infection and clinical outcomes.

Table 4. Sequence identities of complete genes between HCoV KUMC22-3 and representative alphacoronaviruses.

				Pairwise nucleotide and amino acid sequence similarity between HCoV KUMC22-3 and representative α-CoV genomic sequences (%)						
Alphacoronavirus (α-CoV)	Genome Size (nt)	GC content (%)	Accession No.	Entire genome (nt)	3CL nt (aa)	RdRp nt (aa)	Hel nt (aa)	S nt (aa)	E nt (aa)	M nt (aa)	N nt (aa)
HCoV KUMC22-3#	27,682	39.9	PP868300	–	–	–	–	–	–	–	–
AaCoV Aa22-10#	27,501	39.9	PP868301	93.0	93.5 (97.6)	93.7 (96.2)	95 (95.6)	93.3 (96.6)	97 (100)	92.9 (95.2)	93.6 (94.9)
AcCoV Ac18-16#	27,696	39.8	PP868302	96.8	98.1 (98.6)	97 (97.3)	97.1 (96.2)	96.5 (97.4)	97.9 (98.7)	96.5 (96.8)	94.8 (95.9)
AcCoV Ac18-19#	27,499	39.8	PP868303	96.8	98.1 (98.6)	96.9 (97.2)	97 (96.2)	96.7 (97.4)	97.9 (98.7)	96.5 (96.8)	94.6 (95.4)
AcCoV Ac20-8#	27,499	39.9	PP868304	96.7	98 (98.6)	96.9 (97.1)	96.8 (96.2)	96.5 (97.2)	97.9 (98.7)	96.5 (96.4)	95 (96.1)
AcCoV-JC34	27,682	40.1	NC_034972	95.0	95.4 (98)	94 (97.1)	95 (94.7)	97.5 (96.6)	97.5 (100)	95.4 (96)	96 (95.9)
RnCoV Lucheng-19	28,763	40.2	NC_032730	77.1	78.8 (86.5)	85 (93.4)	84.8 (92.1)	84.8 (68.8)	84.8 (93.6)	82.1 (91.5)	74.3 (77)
BtCoV HKU2	27,165	39.3	NC_009988	47.6	64.1 (58.4)	71.1 (74)	66.7 (72.1)	54.1 (40.8)	52.9 (34.6)	56.5 (52.2)	48.5 (32.3)
HCoV NL63	27,553	34.5	NC_005831	36.2	60.1 (54.1)	71.3 (72.5)	69.2 (71.5)	45.5 (23.1)	55.7 (33.8)	54.2 (48.2)	46.5 (30.7)
HCoV 229E	27,317	38.3	NC_002645	36.2	59.3 (54.1)	69.7 (71.6)	68.7 (72.9)	46.1 (25.4)	51 (35)	51.8 (45.4)	44 (29.8)
CCoV HuPn-2018	29,088	37.6	MW_591993	33.0	59.4 (56.8)	70.4 (74.7)	66.9 (68.5)	43.3 (21.7)	51.6 (39)	57.3 (53.3)	48.3 (29.4)
PEDV	28,033	42.0	NC_003436	35.4	58.6 (54.7)	68.6 (73)	65.4 (69.4)	44.4 (22.4)	47.3 (32.5)	53.9 (51.4)	44.9 (24.4)
TGEV	28,586	37.6	NC_038861	35.7	59.6 (57.1)	71.2 (74.6)	67.1 (69.4)	43.5 (22.4)	51.4 (36.6)	58.4 (53.6)	49.1 (30.7)
FCoV	29,355	38.1	NC_002306	35.5	61.3 (56.1)	70.4 (74.2)	66.6 (69.1)	42.4 (22.3)	56 (39.8)	58.6 (51.3)	47.5 (29.4)

#The complete coding sequence of CoV was determined in this study. These similarities were identified through the alignment of CoV genomes using the MUSCLE algorithm.
HCoV, human coronavirus; KUMC, Korea University Medical Center; AaCoV, *Apodemus agrarius* coronavirus; AcCoV, *Apodemus chejuensis* coronavirus; AcCoV-JC34, *Apodemus chevrieri* CoV-JC34; RnCoV, rat CoV; BtCoV, bat CoV; CCoV, canine CoV; PEDV, porcine epidemic diarrhoea virus; TGEV, transmissible gastroenteritis virus; FCoV, feline CoV; 3CL, 3C-like protease; RdRp, RNA-dependent RNA polymerase; Hel, helicase; S, spike; E, envelope; M, membrane; N, nucleocapsid.

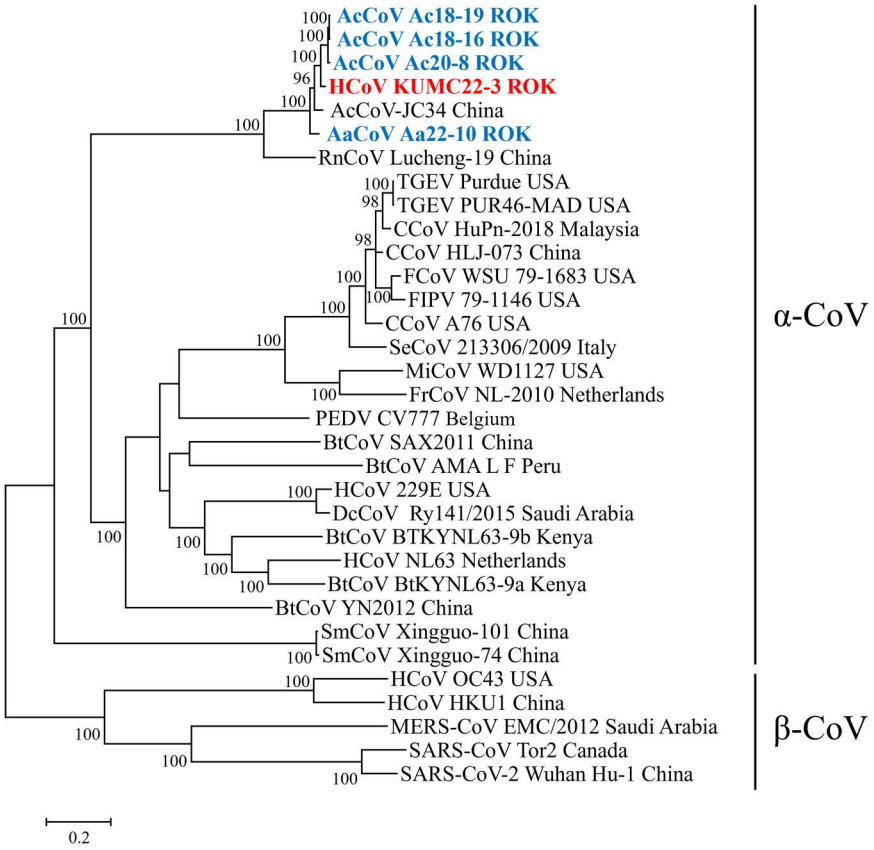

Figure 3. Phylogenetic analysis of HCoV KUMC22-3 in retrospective rodent surveillance. Complete-coding genomic sequences of alphacoronaviruses (α-CoVs) from the KUMC22-3 patient and faecal samples of *Apodemus* spp. were aligned with other representative CoVs. The phylogeny was generated using the maximum likelihood method with the GTR + G + I of the evolutionary model in MEGA 7. The numbers at each node represent bootstrap values determined from 1,000 iterations. The scale bar indicates the number of nucleotide substitutions per site. Bold red letter indicates the α-CoV strain identified from the KUMC22-3 patient in the ROK. Bold blue letters represent α-CoV variants detected from *Apodemus* spp. collected in the ROK. The accession numbers of the CoV genomic sequences in this study are shown in Supplementary table 8. Abbreviations: HCoV, human coronavirus; KUMC, Korea University Medical Center; AcCoV, *Apodemus chejuensis* coronavirus; ROK, Republic of Korea; CoV, coronavirus; AaCoV, *Apodemus agrarius* CoV; AcCoV-JC34, *Apodemus chevrieri* CoV JC34; RnCoV, Rat CoV; TGEV, transmissible gastroenteritis virus; CCoV, Canine CoV; FCoV, feline CoV; FIPV, feline infectious peritonitis virus; SeCoV, Swine enteric CoV; MiCoV, mink CoV; FrCoV, ferret CoV; PEDV, porcine epidemic diarrhoea virus; BtCoV, bat CoV; DcCoV, dromedary camel CoV; SmCoV, Suncus murinus CoV; MERS-CoV, Middle East respiratory syndrome CoV; SARS-CoV, Severe acute respiratory syndrome CoV.

In conclusion, we report the first case of pneumonia accompanied by transient liver dysfunction in an infant co-infected with the novel HCoV, HPIV-1, and rhinovirus. Phylogenetic analyses indicate that HCoV KUMC22-3 shared evolutionary ancestry with rodent-derived α-CoVs. Our study highlights the value of mNGS in identifying and characterizing novel pathogens in clinical practice. This report underscores the importance of heightened awareness among physicians regarding the potential clinical manifestations of newly identified HCoV.

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Author contributions

JWS designed and supervised this study. KP, MS, AN, and WKK wrote the initial version of the manuscript, which was revised by all other authors. KP, AN, JK, JN, SGK, BK, JP, YS, and HKC performed the experiments and analyzed the results. MS investigated a patient. MS, KSB, JHK, YSL, and JOS reviewed the medical charts of the infant patient. All authors have read and agreed to the published version of the manuscript.

Potential conflicts of interest

All authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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