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Molecular Characterization of Canine Coronavirus Strains Circulating in Baghdad and Wasit Provinces, Iraq

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Abstract

Canine enteric coronavirus (CECoV) is an enveloped RNA virus classified within the *Alphacoronavirus* genus. It contains a single-stranded, positive-sense RNA genome and is recognized as a common pathogen affecting the intestinal system of dogs, especially those that are young or not immunized. The present study was conducted to explore the molecular features of CECoV circulating among dogs in the Wasit and Baghdad provinces. For this purpose, genomic and phylogenetic analyses were performed using the Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) approach. A cross-sectional study was conducted by collecting rectal swabs from 170 dogs with gastrointestinal problems that presented to Baghdad and Wasit Veterinary private clinics from December 2023 to March 2025. The dogs were aged 2 months to 5 years. In each sample, we gathered clinical and demographic information, including household details and clinical history of diarrhea. The molecular testing results revealed that 23 positive samples (13.5%) out of 170 were examined. All the positive samples were classified as Canine enteric coronavirus type II based on molecular M and S genes analysis. The phylogenetic analysis results uncovered that the isolates of this paper were strictly linked to Canine enteric coronavirus. Reference isolates that had previously been isolated from dogs showed 94.59-96.23 identity with Brazilian and Iraqi strains for the M gene, and 90.91-93.65 identification with the Brazilian strain for the S gene. Given the significant diversity of Canine enteric coronavirus and its variable toxicity, the findings underscore the necessity of continuous monitoring research. This research was essential for enhancing understanding of the virus's epidemiology and development.

Keywords:

Canine enteric coronavirus (CECoV), m gene, molecular characterization, phylogenetic analysis, rt-pcr, s gene.

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Introduction

Viral animal enteritis caused by dogs can surely be observed in canine animals with a sudden deepening of the symptoms of nausea and diarrhea, especially in small puppy canine animals, and where the number of affected animals is staggering and simultaneously overwhelming. Four major viral agents are recognized as the principal causes of acute enteritis in dogs: *Canine parvovirus*, *Canine coronavirus*, *Canine rotavirus*, and *Canine distemper virus*. These pathogens have been reported worldwide, including in Iraq (Pollock & Carmichael, 1983; Al-Bayati et al., 2010; Al-Taee et al., 2023; Mansour & Hasso, 2021; Rosnelly et al., 2023).

Coronavirus infections are responsible for outbreaks that can result in high mortality rates, considerable economic loss, and cross-species transmission among a wide range of hosts, including humans as well as domestic and wild animals such as cattle, dogs, cats, chickens, rabbits, mice, rats, and pigs. Clinical presentations vary from subclinical cases to acute or mild disorders affecting several organ systems, particularly the respiratory and gastrointestinal tracts.

According to the International Committee on Taxonomy of Viruses (ICTV), coronaviruses are classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus (Dharangutti et al., 2025; Pratelli et al., 2001; Maclachlan & Dubovi, 2010; Pratelli, 2011; He et al., 2020). The genetic variability of the members of the order Nidovirales is quite substantial, and its main source is the high recombination rate of the RNA-dependent RNA polymerase (RdRp), which can be as much as 6% per generation year (Liu et al., 2014). The positive-sense single-stranded RNA virus (Singhal, 2020).

Canine coronavirus (CCoV) contains an RNA genome of approximately 30 kb. Its genome organization includes two partially overlapping open reading frames (ORF1a and ORF1b) that encode non-structural proteins, while the remaining regions code for four principal structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N). Among these, the spike glycoprotein is the most variable component and elicits the main virus-neutralizing antibody response. It is very important for the virus to get into the host cell, and it is involved in the process of viral infection (Pratelli, 2001; Matbou et al., 2017). Structurally, the S protein comprises a large ectodomain made up of two functional subunits: S1, responsible for receptor binding, and S2, which mediates membrane fusion (Li, 2016; Felsenstein, 1985). (CCoV) Canine coronavirus, which is an abbreviation for canine coronavirus, was first identified in 1971 and is the main disease that causes dogs to have diarrhea (Binn et al., 1974).

When mutant or virulent viruses infect several organs, they can cause mortality. The signs of the clinical features are usually intermediate and enhanced with the co-infection of the virus-linked illness with a number of disease-causing agents (pantropic canine coronavirus) (Zandi & Pourtaghi, 2023). Dog's pulmonary coronavirus is detected in doggies presenting with pulmonary signs and is an etiological agent of the infectious pulmonary illness complex of doggies (Decaro & Buonavoglia, 2008). Although dogs of all ages can develop coronavirus enteritis, pups are most frequently affected. Weight loss, diarrhea, nausea, dryness, and bleeding are noted, especially in infections with additional pathogens (Decaro & Lorusso, 2020). Due to acute dryness, deaths typically occur 24–36 hours after infection thresholding (Buonavoglia et al., 2006). It is undeniable that the canine coronavirus mainly reveals intestinal tropism and appears to induce gastroenteritis in young dogs (Pratelli, 2006). However, it was discovered that the new strain, CCoV CB/05, caused a fatal disease that was typified by the contagion spreading throughout the body (Decaro et al., 2008; Geetha & Gowsikraja, 2025).

This Article stresses the molecular screening and description of (CCoV) in home doggies in Baghdad and Wasit provinces of Iraq between 2023 and 2025. Canine coronavirus (CCoV) is a major etiological agent

of gastroenteritis in dogs, although certain strains are capable of causing more severe, systemic, and occasionally fatal infections (Baggyalakshmi et al., 2024).

Materials and Methods

Specimens Collection

The current cross-sectional study involved analyzing 170 fecal samples or swabs taken from domestic dogs showing signs of enteritis, aged between 2 months and 5 years, mainly Common breeds. These dogs were brought to private veterinary clinics in Baghdad and Wasit provinces, Iraq, during the period from December 2023 to March 2025. For molecular Detection, fecal samples were homogenized in phosphate-buffered saline (PBS) to a final concentration of 10% (w/v) and centrifuged at 1500 rpm for 10 minutes at room temperature. Noteworthy is that all the doggies participating in the study had not received any kind of CCV vaccination before, and all specimens were kept at -70°C. To concentrate on the specific effects of this virus, any mixed or other enteric viral infections were deliberately excluded.

RNA extraction and PCR amplification

The clarified supernatant was used for RNA isolation. Total viral RNA was extracted using a commercial viral nucleic acid extraction kit (Geneaid, Taiwan) following the manufacturer's guidelines. The complementary DNA (cDNA) was made with the help of the reverse transcription kit from Bioneer (Korea) and kept at -20 °C before the analysis. Canine enteric coronavirus (CECoV) detection was done by RT-PCR amplification of the M gene region of 409 bp, in agreement with the method of (Pratelli et al., 1999). The primers used were: CCV1 for the forward primer (5′-TCCAGATATGTAATGTTCGG-3′) and CCV2 for the reverse primer (5′-TCTGTTGAGTAATCACCAGCT-3′). For the amplification of the S gene, the primers SII-F (5′-TGCATTTGTGTCTCAGACTT-3′) and SII-R (5′-CCAAGGCCATTTTACATAAG-3′) were used. The product of the reaction was seen as a band of 694 bp (Pratelli et al., 2004).

The PCR reaction started with a 10-minute denaturation at 94ºC, followed by 34 cycles of denaturation at 94ºC for 1 minute, annealing at 55ºC for 1 minute, and extension at 72ºC for 3 minutes. Finally, the last extension was at 72ºC for 5 minutes. Amplification of the S gene was performed under the following thermocycling conditions: initial denaturation at 94 °C for 10 minutes, followed by 33 cycles of denaturation at 94 °C for 25 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 2 minutes, with a final extension step of 5 minutes at 72 °C. PCR amplicons were visualized on a 2% agarose gel stained with ethidium bromide. In summary, a prepared gel was loaded into wells with 10 µl of products blended with the buffering load. The gel was then electrophoresed for 90 minutes at 100 volts in analogy with a DNA 100 bp molecular weight ladder in an electrophoresis apparatus by employing Tris-acetate-EDTA buffer. Ultra-Violet light was utilized to view the product bands, and their size was evaluated using the previously described DNA molecular weight ladder standard (Sambrook et al., 1989).

Sequence analysis

After purification of PCR products, they were unswervingly relying on Sanger sequencing, and every single sample was sequenced forward and reverse (Macrogen, Korea). M and S gene sequences were entered into NCBI GenBank only partially, and bioinformatics research was conducted to determine the genetic composition of the virus. CA: Applied Biosystems. The decision tree that has the most probable (-9,721.97) is shown. Phylogenetic relationships were reconstructed using the Maximum Likelihood (ML) approach under the Tamura–Nei (1993) nucleotide substitution model.

The initial heuristic search tree was determined by comparing the log-likelihood scores of the Maximum Parsimony (MP) and Neighbor-Joining (NJ) methods, following the procedure of (Saitou & Nei, 1987). Among ten MP searches initiated with randomly generated topologies, the shortest tree was retained as the most parsimonious. The (Tamura & Nei, 1993) model was employed to generate a pairwise distance matrix for the estimation of the NJ tree. The analysis incorporated 21 nucleotide sequences, covering a total of 732 aligned positions in the final dataset. Evolutionary analyses were performed using MEGA version 12 (Kumar et al., 2024), employing four parallel computational threads to improve processing efficiency. Based on the partial M and S gene sequences, the resulting phylogeny revealed patterns of genetic divergence and taxonomic clustering among multiple coronavirus strains, in agreement with the evolutionary framework described by (Tamura et al., 2007). The sequences made in this experiment related to nucleotide were seemingly placed at the GenBank with the accession numbers provided

Analytical Statistics

SPSS software, version 27, was used for all analyses. In order to investigate the possible correlation between the data gathered and the results of the study, the Chi-squared statistical test was used. The significance criterion for each test was established at $p<0.05$.

Results

Distribution of samples according to age and residence

The study in hand was important in the molecular Detection of CECoV in dogs in Baghdad and Wasit Provinces, Iraq. CECoV was investigated via RT-PCR in 23 out of 170 (13.5%) dogs as follows: 13 in Baghdad city and 10 in Wasit city, with no significant differences between them. The myriad of positive samples were from puppy animals less than 6 months with significant differences at the level of Significance, $P<0$, as shown in Table 1.

Table 1. Number of samples with positive results, cases with CECoV, according to age group

Age group	Number of samples of Baghdad Province / Positivity			Number of samples of Wasit Province / Positivity		
1-6 Months	37	6	16.2 **	26	5	19.2**
6-12 Months	28	3	10.7*	20	3	15*
1-5 years	20	2	10**	14	2	14.2**
5-10 years	15	1	6.6*	10	0	0
Total	100	13	13	70	10	14.2

*** Significance level P<0.05 , * P≥0.05*

Molecular Characterization of the M and S Genes of the Virus

The M (membrane protein) and S (spike protein) genes, which were chosen from the Canine Coronavirus (CCoV), were satisfactorily characterized in two Iraqi governorates.

Conventional PCR was performed on all samples in order to molecularly characterize the S and M genes. The lengths of the M gene (409 bp) and S gene (694 bp) were successfully amplified in CECoV-positive samples, as indicated in Figure 1A, B. Based on the examination of the two genes, all of the positive samples were categorized as CECoV type II (Figure 1).

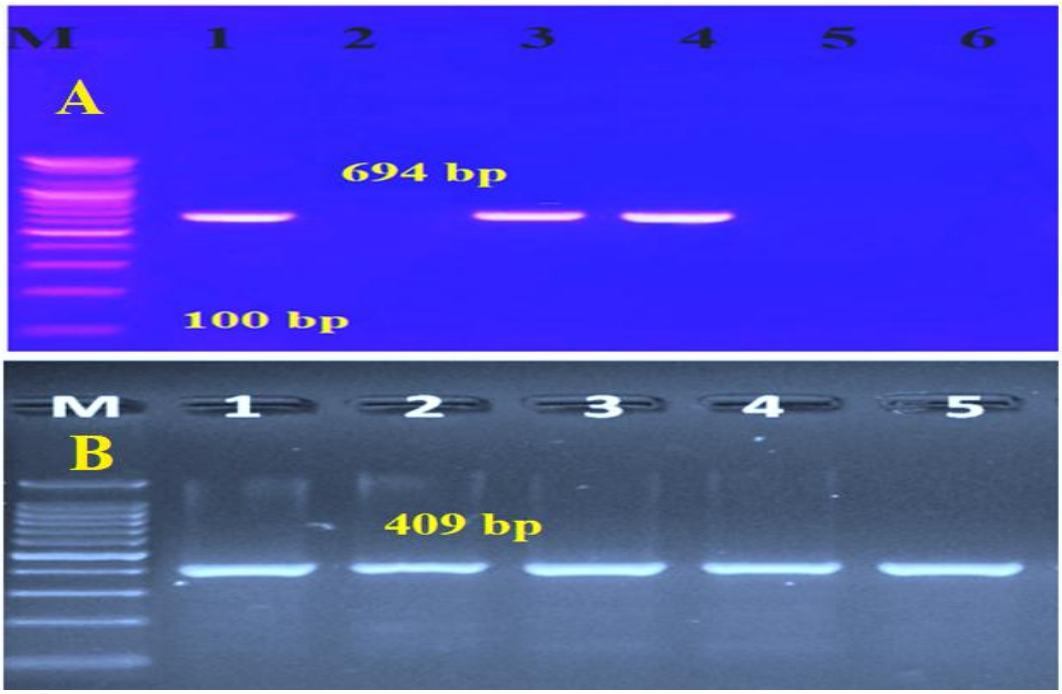

Figure 1. Conventional PCR amplification of (A) spike gene of CECoV. lane M: marker (100bp), lanes 1-3-4 positive result for spike gene (~694bp). (B) M gene of CECoV. Lane M: marker (100bp), lanes 1-5 positive result for M gene (~ 409 bp).

The virus M gene successful sequences (Canine coronavirus isolate S1 to S7). The nucleotide strings elucidated in current research were recorded in the Gene Bank with the accession No PV798545, PV798546, PV798547, PV799987, PV799988, and PV799989. They displayed high genetic identity 100% with the global strain, and 94.59-96.23% study strain identity, as shown in Table 2. Four of the positive CECoV samples were successfully sequenced for the S gene, confirming genetic identity and close relationships with both global and reference strains available in GenBank.

Table 2. M and S genes sequence identity of iraqi canine coronavirus isolates with reference and global strains

N Gene					
Sample ID	**Reference Strain Identity (%)**	**Reference Strain Name**	**Global Strain Identity (%)**	**Global Strain Name**	**Study Strains Identity (%)**
Canine coronavirus isolate S1 (PV798545)	95.68	**Canine coronavirus (DQ112226.1)**	100	**Canine coronavirus isolate Iraq/2023/MAH/2/1 (OR421198.1)**	
Canine coronavirus isolate S2 (PV798546)	95.33			Canine coronavirus isolate Iraq/2023/MAH/2/2. (**OR421199.1**)	
Canine coronavirus isolate S3 (PV798547)	95.96			**Canine coronavirus isolate Iraq/2023/MAH/2/3. (OR421200.1)**	
Canine coronavirus isolate S4 (PV799987)	96.77			**Canine coronavirus isolate Iraq/2023/MAH/1/ (OR147197.1)**	
Canine coronavirus isolate S5	96.08				

(PV799988)				Canine coronavirus isolate CAN2003/Dog/BRA/2019/CCoV-II (**OQ744008.1**)	94.59-96.23
Canine coronavirus isolate S6 (PV799989) Canine coronavirus isolate Baghdad, Iraq/2024/HU7 membrane protein gene	96.10				
S Gene					
Sample ID	**Reference Strain Identity (%)**	**Reference Strain Name**	**Global Strain Identity (%)**	**Global Strain Name**	**Study Strains Identity (%)**
SI Canine coronavirus isolate Wasit, Iraq/2024/HUI/S protein gene	94.08	**Canine coronavirus (DQ112226.1)**	100	Canine coronavirus strain 859-IIa S protein gene (**KF312729.1**)	90.91-93.65
SII Canine coronavirus isolate Wasit, Iraq /2024/ HUII /S protein gene	93.94				
SIII Canine coronavirus isolate Baghdad, Iraq/2024/HUIII/ S protein gene	93.95				
SIV Canine coronavirus isolate Baghdad, Iraq/2024/HUIV/S protein gene	90.35				

Furthermore, multiple sequence alignment, as illustrated in Figures 2 and 3, enabled genome annotation of the CECoV strains and revealed both conserved regions and distinct sequence variations. Comparative alignment across the M and S genes demonstrated notable genetic variability, as detailed in Figures 2,3.

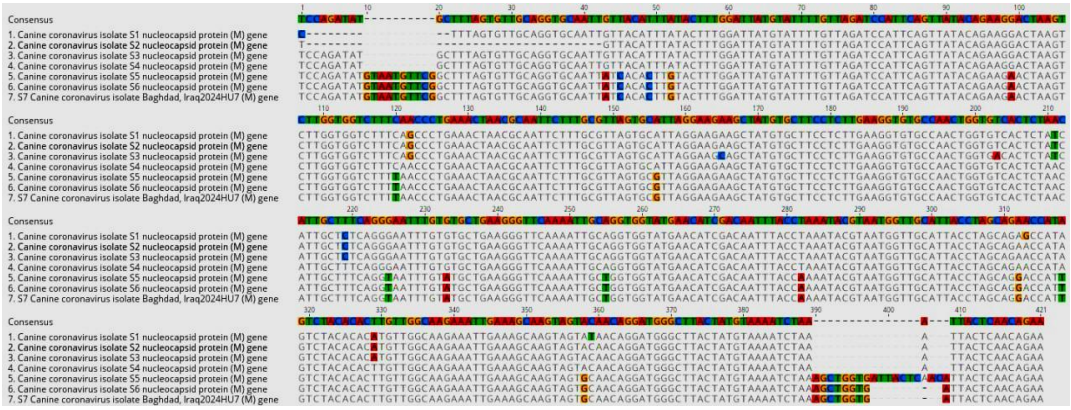

Figure 2. Multiple sequence alignment of CCoV M gene samples. Comparative analysis of the partial M gene sequences generated in this study and previously reported reference sequences was also conducted to identify non-synonymous substitutions. (–) unveiling undistinguishable amino acids

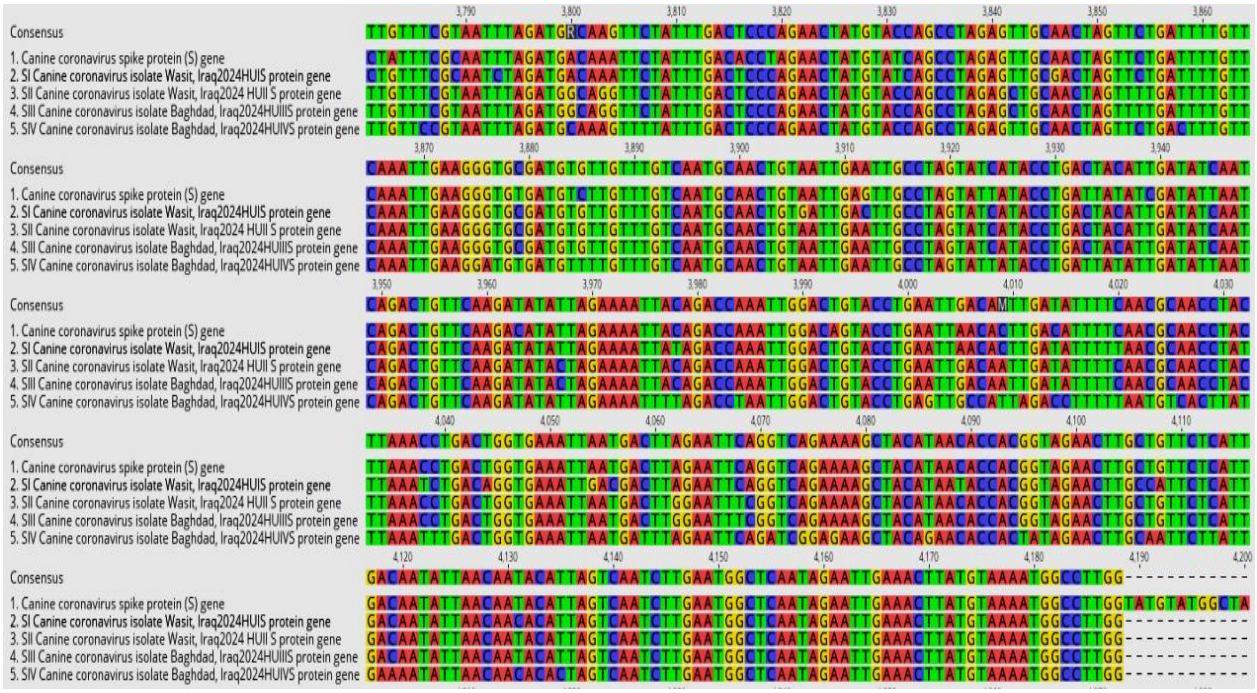

Figure 3. Multiple sequence alignment of CCoV S gene samples. These variations were noted between the sequences obtained here and those retrieved from GenBank, suggesting potential molecular divergence in circulating CCoV strains. (–) uncovering similar amino acids

The phylogenetic analysis of the botgenes sequences of CC,oV, graphically represented in Figures 4 and 5, uncovered four separate phylogenetic branches that matched the established coronavirus genera: *Alphacoronavirus, Betacoronavirus, Gammacoronavirus,* and *Deltacoronavirus.* Importantly, all the samples from the study were classified under the *Alphacoronavirus* genus, and they exhibited very close phylogenetic relationships with the reference strains.

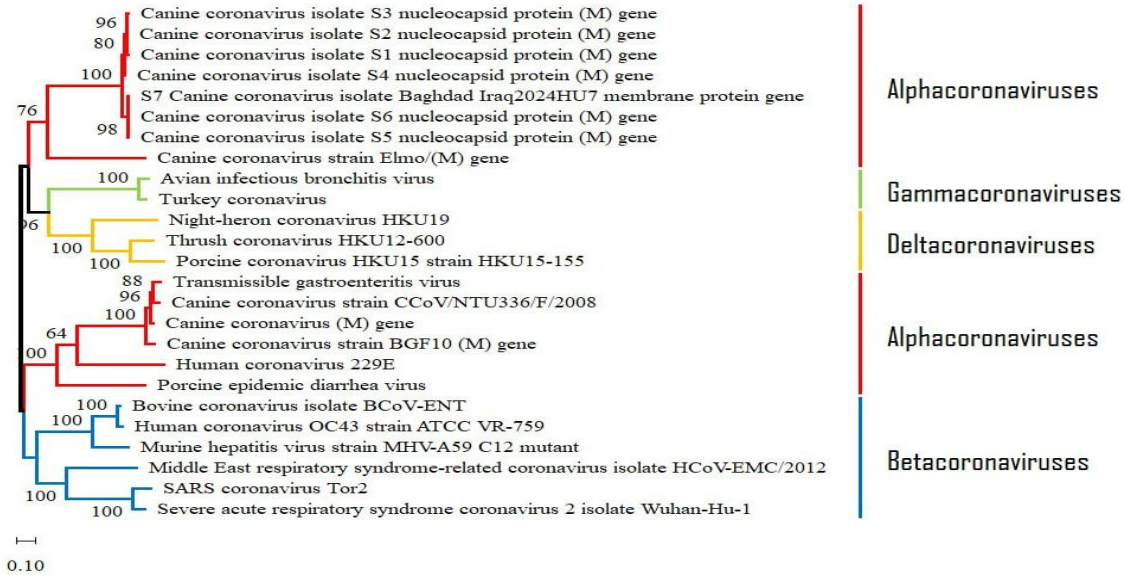

Figure 4. Phylogenetic tree and taxonomic diversity of coronaviruses: representative strains and genera regarding the M gene

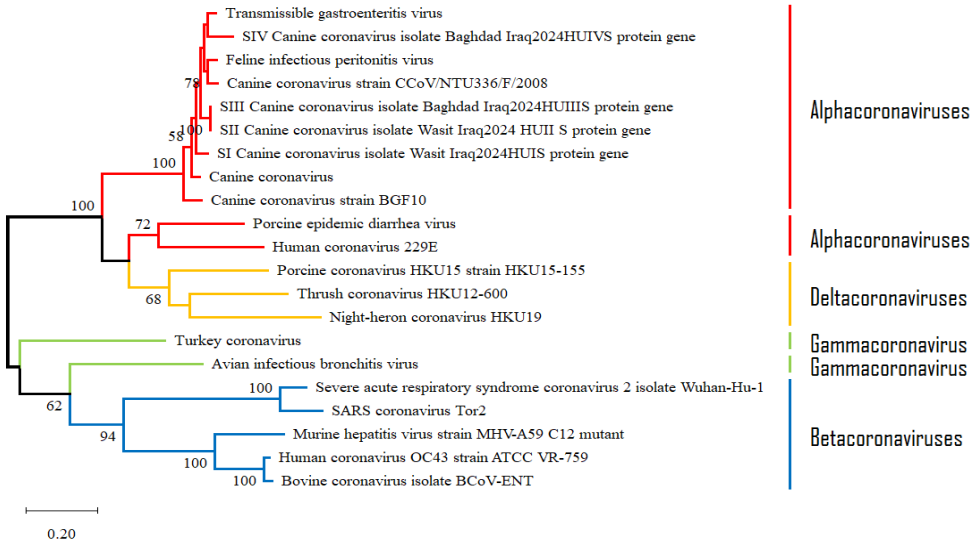

Figure 5. Phylogenetic Tree and Taxonomic Diversity of Coronaviruses: Representative Strains and Genera of the S gene

The S and M gene results from conventional PCR and Sanger sequencing were examined to identify genetic diversity, mutations, and conserved areas in coronaviruses. To clarify evolutionary links and host-specific adaptations of the viruses, phylogenetic analysis and bioinformatics are explored.

Discussion

Many researchers in Iraq have studied a variety of animal and human coronavirus species, such as those in humans (Shattab et al., 2022), dogs (Hussein & Al-Graibawi, 2024), birds (Altaee et al., 2023; Madhlom et al., 2025), and camels and cows (Altaee et al., 2023; Madhlom et al., 2025).

Over the past decade, several investigations have highlighted the role of CCoV as a significant etiological agent of enteritis in young dogs (Decaro & Buonavoglia, 2011; Le Poder, 2011; Madhloom et al., 2025; Ntafis et al., 2013). The central objective of the present study was to characterize and classify the prevalent CECoV strains circulating in Iraq through partial amplification and sequencing of the M and S genes.

Recurrent outbreaks have brought a great deal of interest to coronavirus studies, emphasizing how mutations can change organ-tissue tropism, as found in the cat-related infectious peritonitis virus, or cause serious infections in novel hosts, such as SARS-CoV-2.

CECoV infection rates in dog populations across the globe have fluctuated between 1.1–65.1% (Ntafis et al., 2013; Castanheira et al., 2014), with even higher rates up to 84.62% in a kennel population being noted (Timurkan et al, 2021). The authors suggest that the differing rates of infection could result from the dogs' varying opportunities for exposure to the virus and their interaction with other dogs (Naylor et al., 2001).

The total current positive results (13.5%) are believed to be higher than those reported by (Hussein & Al-Graibawi, 2024). It may be due to the dissimilarities of the dog populations in the two provinces, the longer time taken to research and find viruses, or some of the dog owners and dog sellers mismanaged their dogs.

The noted prevalence of CECoV in this study was portrayed differently in other reports. (Navarro et al., 2017) mentioned a 4.8% probability of CECoV infection in Saint Kitts and Nevis dogs suffering from

diarrhea. Similar research in Japan by (Takano et al., 2016) reported the presence of CECoV-II in 7.4% of the cases. Additionally, (Castanheira et al., 2014) presented two prevalence rates of 1.9% in 2010 and 1.1% in 2011, respectively. The differences in detection rates can be explained by the low viral load and sample collection time, fecal pH changes, or presence of ions and colloids that may render viral particles inactive, as pointed out by (Tennant et al., 1994). Recovered animals can act as asymptomatic carriers, intermittently shedding the virus and thereby sustaining environmental persistence (Stavisky et al., 2012).

Timurkan et al., 2021 reported CECoV-II prevalence in Brazil and Turkey to be 30.4% and 84.62%, respectively, as per (Alves et al., 2018).These findings are thought to be consistent with the continuous high infection rate recorded by Ntafis et al., 2013, who testified that the occurrence of CECoV was 65.1% in dogs with diarrhea in Greece and that the positive cases were more recurrently perceived in canines less than three months old.

The low infection rate in Iraq could be due to the occasional low virus circulation, the environmental instability of the virus, and very few virus particles in feces, as Castanheira et al. (2014) pointed out. The low number of virus particles during the fecal collection period, the fluctuations in pH, or the ions and colloids present in the feces, causing virus inactivation, may be some of the factors that lead to the CECoV detection limits as described by (Tennant et al., 1994). Recovered dogs can serve as asymptomatic reservoirs and intermittently shed the virus, allowing CECoV to endure in the environment for extended periods (Stavisky et al., 2012).

Upon sequencing all 23 CECoV-positive samples, the bioinformatics findings were documented in the NCBI GenBank. The nucleotide sequences obtained in this study were deposited in the GenBank database under accession numbers PV798545, PV798546, PV798547, PV799987, PV799988, and PV799989 (Table 2). When comparing the positive sample sequences with isolates from around the world to find out the level of relatedness, a phylogenetic tree was generated to investigate the M and S genes in Iraq. The evolutionary tree was drawn using MEGA 11 software, and it was displayed that sequenced isolates had the highest similarity with 94.59-96.23 percent identity to sequenced global Iraqi isolates. Sequencing of the S gene revealed the highest resemblance to sequenced Brazilian isolates worldwide, with 90.91–93.65 % identity.

Conclusion

To the best of our knowledge, this research represents the first detailed molecular characterization of Canine Enteric Coronavirus (CECoV) strains currently circulating in the Wasit and Baghdad regions of Iraq. CECoV was detected in 13.5% of the dogs analyzed with RT-PCR targeting the M and S genes, with a greater prevalence in puppies under six months of age. All positive samples were classified as CECoV type II, indicating the predominance of this genotype in the study regions. The phylogenetic and sequence identity analyses indicated that the Iraqi CECoV isolates are genetically very similar (94.59 – 96.23%) to the global strains based on the M gene and only slightly less (90.91–93.65%) in comparison to the Brazilian isolates based on the S gene. It can be inferred from these results that the CECoV strains in Iraq are not very different from the global lineages reported in the past, but they may represent an area where new variations can arise; thus, there is a need for constant monitoring. The paper points out the fact that CECoV continues to circulate in, and remains genetically diverse in, domestic dogs; thus, there is a need for conducting molecular monitoring and genomic sequencing to track the virus's evolution and potential recombination events. Regular surveillance and immunization programs are recommended to mitigate the risk of outbreaks, particularly among young and unvaccinated dogs. Moreover, the study adds to the understanding of CECoV's spread in the Middle East, and

the usage of both molecular and phylogenetic approaches in future veterinary virology studies is emphasized as a necessity.

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Author contributions

The individual work of each writer. Research Conception and design: Hussein A.M.B. Acquiring statistics: Hussein A.M.B. and Zainab D.K. Analyzing and/or interpreting data: Hussein A.M.B. Critical reviewing/revising: Zainab D.K. Writing the manuscript: Hussein A.M.B.

Conflict of interest

The authors reported no financial, personal, or other conflicts of interest that could compromise the study's integrity.

Ethical Approval

The faculty of the Veterinary Medicine of Wasit University Scientific Committee has given its permission to the present work (Ref. No. 139-2592021). Every animal usage procedure was executed strictly following institutional and national guidelines for animal welfare. Data and samples were collected only after obtaining informed consent from the dog owners.

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