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Detection and characterization of canine corona virus infection: an enteric approach

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ABSTRACT

Canine corona virus (CCoV) infection is a new emerging disease affecting dogs. It results in low mortality but considerable morbidity in dogs with intermediate to moderate enteritis. In India, serological research on dogs has revealed the existence of antibodies, demonstrating the prevalence of the condition among canines. The aim of the present study was to investigate the prevalence of CCoV infection in dogs suffering from gastroenteritis, and genetic analysis of circulating virus strains. The current study was designed to test fecal samples of dogs with gastroenteritis suspected for the presence of CCoV. Using primers based on the M-gene, PCR was used to amplify the RNA (c-DNA) templates retrieved from the fecal samples. RT-PCR based detection of CCoV showed positivity of 30% (15/50). The phylogentic analysis revealed that two samples had nt/aa identity levels of 100%/100% to the CCoV isolate from China and 99.19/100% of nt/aa identity with that from Korea. One of the samples showed divergence and revealed 95.63%/94.37% nt/aa identity with the China isolate and 95.15%/97.06% nt/aa identity with Indian isolates. The recombination analysis indicated a mutation rate in one of the samples. In brief, the present study illustrates the circulation of canine corona virus in dogs suffering from gastroenteritis. The present findings emphasize the need for vaccination of dogs for prevention, as a high prevalence of viral gastroenteritis was found in these dogs.

Key words: RT-PCR; canine corona virus; diarrhea; phylogenetic analysis; genetic characterization

Introduction

Dogs can contract mild to severe gastroenteritis from the canine corona virus (CCoV), a single-stranded enveloped RNA virus belonging to the family Coronaviridae and order Nidovirales (APPEL, 1978). Canine Coronavirus (CCoV) type I and type II, which descended from Feline Coronavirus (FCoV) type I and type II, are two distinct coronaviruses that have been found in dogs. Furthermore, CCoV type I is genetically closer to FCoV type I than CCoV type II, and FCoV type II originated from a heterologous recombination between CCoV type II and FCoV

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type I (PRATELLI et al., 2003). Numerous infections with various pathogens, including Canine Parvovirus (CPV-2), canine adenovirus type 1, Canine Distemper virus (CDV), and CCoV, are linked to a higher fatality rate in animals despite the infection's high morbidity rate (DECARO and BUONAVOGLIA, 2012). Dogs affected with acute gastroenteritis are thought to be most commonly affected by CCoV (McELLIGOTT et al., 2011). However, a very lethal, multi-systemic, and virulent strain of CCoV was discovered in puppies a few years ago (BUONAVOGLIA et al., 2006; DA COSTA et al., 2019). CCoV-2a has evolved into two separate pathogenic subtypes (enteric/classical and pantropic), based on virulence. In 2005, a pantropic CCoV strain of the highly contagious CCoV-2a virus was discovered in deceased puppies from an Italian pet store. Puppies that have this strain can be seen postmortem with severe clinical symptoms and organ damage. The severity of the illness was observed to differ depending on the age and immunological status of the animals in experimental infections in dogs (DECARO et al., 2010). More specifically, it has been claimed to make the condition worse and, over time, cause lymphopenia (MARINARO et al., 2010)

Four distinct structural proteins, M (membrane protein), S (spike protein), N (nucleocapsid protein) and E (envelope protein) are encoded by the coronavirus genome. Additionally, a membrane-anchored HE (hemagglutinin-esterase) protein is present in several betacoronaviruses (TIMURKAN et al., 2021). The membrane (M) glycoprotein, which crosses the membrane bilayer three times and leaves a long COOH terminus inside the virion and a short NH2-terminal portion outside the virus, is the most common structural protein (DECARO and BUONAVOGLIA, 2008). The M protein causes complement-mediated virus neutralization that is antibody-dependent. M appears to be essential for its budding and assembly, although its exact function is not fully understood. Strong immunological reactions can be triggered by the M glycoprotein of CCoV. The M gene alterations may provide some form of growth advantage or prevent host immunity (PRATELLI et al., 2003). The M protein can be targeted for molecular identification of recombinant events in the virus population.

RT-PCR assay would allow for more rapid diagnosis of Canine Corona Enteritis than virus isolation in cell cultures, and it would eliminate the necessity for electron microscopy, which could yield inaccurate results. Additionally, the RT-PCR technique enables the identification/detection of denatured CCoV in fecal samples (PRATELLI et al., 1999). Many RT-PCR assays based on the M gene and S gene are available for detection of CCoV from dogs (TIMURKAN et al., 2021). Many clinical cases of viral enteritis are reported regularly in dogs in spite of timely vaccinations. It is possible that the viral strains incorporated in the vaccines are different from the field strains, or some new genotype or variant is circulating, against which the vaccines need to be updated (NANDI et al., 2008). Additionally, quick diagnosis is necessary to isolate affected dogs and provide supportive care in order to lower morbidity and mortality (CHO et al., 2006). Therefore, studies on the characterization and molecular epidemiology of CCoV of dogs are very limited in the state of Haryana.

Materials and methods

*Collection of samples***.** The dogs in the current study were brought to the canine section of the veterinary clinical complex at Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, with a history of anxious symptoms and diarrhea. The study was conducted from with a total of 50 dogs showing symptoms of gastroenteritis. Diarrheic fecal samples were collected using sterile swabs and were added to 1 ml (10% W/V) fecal suspension of sterile PBS (pH 7.4), vortexed and preserved at -80°C till further processing. The PBS containing suspensions were later centrifuged at 10,000 rpm for 10 min, and the supernatant was processed for extraction of viral RNA using the TRIzol reagent (Invitrogen, Waltham, Massachusetts, USA) for the detection of CCoV by conventional RT-PCR assay. The remaining samples were stored in 5 volumes for RNA ladder (Invitrogen, Waltham, Massachusetts, USA) for future use.

History, Physical and Clinical Observation. The study comprised 50 canines/dogs that were up to one year old or younger, and had a history of diarrhea, nervous symptoms and vomiting. Signalment of the affected dogs was recorded, including the dogs' breed, age, sex, and coat color, along with the name, address and contact number of the owner. The full history of affected cases was recorded, including the length of the illness, frequency of vomiting, appetite and diarrhea, the consistency and color and of the vomit and feces, immunization and deworming schedule, any prior treatments given, the vaccine name and type given, and other pertinent information.

Extraction of Viral RNA. For screening of CCoV in different samples, RNA was extracted by the TRIzol (Invitrogen, Waltham, Massachusetts, USA) method with some modifications. Briefly, 200µl of the fecal material supernatant in PBS was treated with an equal volume of TRIzol reagent(Invitrogen, Waltham, Massachusetts, USA). Further, 40µl of chloroform (SRL,Gurugram, Haryana, India) was added, followed by vigorous vortexing to avoid formation of insoluble aggregates. The mixture was centrifuged at 12,000 rpm for 15 minutes to separate the aqueous phase, which was collected in a 1.5 ml Eppendorf tube without disrupting the organic phase or interphase. The extracted RNA was precipitated by the addition of an equal volume of isopropanol (SRL, Gurugram, Haryana, India). The precipitated RNA was centrifuged and pelleted, followed by addition of 1 ml of 70% chilled ethanol (SRL, Gurugram, Haryana, India) for washings (to remove excess salts). The pellet was directly dissolved in nuclease free water (NFW) by heating at 50°C for 5-10 min in a water bath, and was used further for synthesis of the first strand complementary DNA (c-DNA).

c-DNA Synthesis for RT-PCR. c-DNA of the extracted fecal RNA samples was prepared using a c- DNA synthesis kit (K1622) (Thermo-scientific, Waltham, Massachusetts, USA). Briefly, a master mix was prepared consisting of 4µl RT Buffer (5X); 2µl dNTP's; 1µl Random Hexamers; 1µl RT Enzyme; 1µl Ribolock and 6µl NFW. The secondary structures of 5µl of RNA template were first melted by being heated at 65°C for 5 minutes, followed by immediate snap chilling on ice. The Final Reaction Volume (20µl), consisting of 15 µl master mix and 5µl snap chilled template, was initially preheated for primers annealing at 25°C for 10 min, followed by the reverse transcription step carried out at 42°C for 60 minutes in a programmable 96 well thermal cycler (Applied Biosystem, Waltham, Massachusetts, USA). The reverse transcriptase was heat inactivated at 70°C for 5 min. The c-DNA was kept at -20°C until use.

Screening of fecal samples for CCoV RNA. The fecal samples were examined for the presence of viral RNA by amplifying the prepared c-DNA using a conventional RT-PCR primer pair (AGNIHOTRI et al., 2018). The PCR was carried out in a 12.5 µl reaction using a thermal cycler (Applied Biosystem, Waltham, Massachusetts, USA).and included 3 µl of template DNA, 6.25 µl of Master mix 2X concentration GoTaq® Green Master Mix-(Promega, Madison, USA), 0.5 µl of forward and reverse primer (10 pmoles concentration), and 2.25 µl NFW. The CCoV cycling conditions included a single initial denaturation cycle lasting 5 minutes at 94°C, 30 seconds at 94°C, then 40 cycles of denaturation lasting 30 seconds, annealing for 1 minute at 55°C, extension for 1 minute, and final extension lasting 15 minutes at 72°C. The commercially synthesized genome specific CCoV gene construct from the available NCBI gene bank (Bio Link, New Delhi, India) used as a positive control for CCoV and Non template control, was used as the negative control (NCBI, 2023).

Purification of PCR products. PCR products were purified using a QIA quick® gel extraction kit (Qiagen, Germantown MD, USA) to remove unused primers and other PCR ingredients, before sequencing. Fifty µl of PCR product was loaded on the 1.5% gel and the gel was run at 5V/cm for 60 minutes to separate the product. The area of gel containing the product was excised with a clean, sharp scalpel and taken in a 1.5 ml microcentrifuge tube and 3 volumes of buffer QG was added to 1 volume of gel. The tube was incubated for 10 minutes at 50°C to dissolve agarose and vortexed every 2-3 min during incubation. After incubation, 1 gel volume of isopropanol was added to the sample and mixed. The sample was applied to the

column and centrifuged at 12000 rpm for 1 minute and flow through was discarded. Five hundred µl of buffer QG was added to the column and centrifuged at 12000 rpm for 1 minute to remove all traces of agarose and the flow through was discarded. After that 750µl of buffer PE was added to the column and was allowed to stand for 5 minutes, then centrifuged at 12000 rpm for 1 minute and the flow through was discarded. The column was centrifuged at 12000 rpm for an additional 2 minutes to remove residual buffer. The column was placed into a clean 1.5 ml micro centrifuge tube and 30 µl of elution buffer was added to the column. The column was allowed to stand for 15 minutes and then centrifuged for 3 minutes at 12000 rpm to elute the PCR product. The purified PCR product was analyzed on the gel by adding 1 volume of loading dye to 5 volume of purified PCR product.

Automated nucleotide sequencing. The gene-specific primers designed for each positive sample were used for sequencing using Big Dye® Terminator v 3.1Cycle Sequencing Kit (ABI) in an automated DNA sequencer (Applied Biosystem 3130XL Genetic Analyzer, Waltham, Massachusetts, USA) available at the Department of Animal Biotechnology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana.

Sequence and phylogeny analysis. Contigs were created using the raw data from the sequencer. The contigs were examined using the NCBI BLAST online software tool accessible on the internet (http://www.ncbi.nlm.nih.gov/BLAST/). The M gene of the CCoV sequence was aligned with DNA sequences obtained from the NCBI database using the Clustal W (THOMPSON et al., 1994) and Bio-Edit programs (HALL et al., 1999). The proposed phylogeny and reference sequences (besides similar sequences obtained by BLAST) were taken from GAN et al. (2021). The CLUSTAL W software was operated to evaluate the aligned sequences and 1,000 replicates were used to calculate bootstrap probabilities. The software program Mega version X was used to create the phylogenetic tree using the neighbor-joining approach and a distance matrix with two parameters (KUMAR et al., 2018). A Recombination Detection Program (RDP) 5.0 was used to examine the M gene sequence's probable recombination events (MARTIN et al., 2015). The RDP software employed seven techniques: RDP, Chimaera, SiScan, MaxChi, and 3Seq. The breakpoints and similarity values were also defined by the RDP.

Results

Clinical profile and history of dogs positive for CCoV. The dogs suffering from gastroenteritis were chosen for detection of CCoV. The symptoms of the dogs found positive for CCoV are given in Table 1. The dogs that are found positive were about 6m-1 year of age. All the dogs are unvaccinated, and even their mothers had not received any vaccine. These puppies had never left the country, and had never had any experience with other dogs because they were kept apart from their mother. The comparison of the hematological data with the mean control values for the relevant parameters showed relative neutrophilia (85%) and lymphopenia (11%), along with leucocytosis ($40x10^3$/L). Additionally, it was noted that the levels of hemoglobin (8.00-10 g/dL), packed cell volume (25-30%), and total platelet count (150-180 x 10^3/L) were lower than the mean control reference values, which are 11.9-18.9 g/dL, 35-57%, and 211-621 x 10^3/L, respectively. All the dogs were unvaccinated and born to a non-vaccinated mother. In these dogs, there were decreased average values of total protein, globulin, albumin and serum electrolytes (sodium, potassium, and chloride), but increased average values of ALT, AST, BUN, and serum creatinine (Table 2).

Sample screening. Out of the 50 samples screened with conventional RT-PCR, CCoV was detected in 15 samples, and three of them were sequenced for characterization. These samples were chosen as they showed typical signs of gastroenteritis caused by suspected CCoV infections, and they represented different age groups. Agarose gel (1.5 percent) exposed a single, uniform band for CCoV with amplicon 321 bp size (Fig. 1). The total percentage prevalence of CCoV was found to be 30%. In mixed breed dogs, CCoV was shown to be most prevalent.

Table 1: History of samples positive for canine coronavirus

Sr no	Breed	Age	Gender	Clinical symptoms	Vaccination status
1.	Pakistan bully	3.5 month	Male	Twitching of muscles, anorexia, gastroenteritis	Nil
2.	Labrador	6 month	Male	Nervous signs, anorexia, gastroenteritis	Nil
3.	Pomeranian	3 month	Male	Anorexia, gastroenteritis, diarrhea	Nil
4.	Labrador	9 month	Male	Anorexia, vomiting, diarrhea	Nil
5.	Rottweiler	6 month	Male	Anorexia, hemorrhagic gastroenteritis, diarrhea	Nil
6.	Mixed breed	1.5 years	Male	Anorexia, nervous signs	Nil
7.	Labrador	3 month	Male	Twitching of muscles, anorexia, gastroenteritis	Nil
8.	Mixed breed	3.5 month	Male	Anorexia, gastroenteritis, diarrhea	Nil
9.	Labrador	3 year	Female	Anorexia, vomiting	Nil
10.	Pit bull	10 month	Female	Twitching of muscles, anorexia, gastroenteritis	Nil
11.	Mixed breed	5 month	Female	Nervous signs, anorexia	Nil
12.	Mixed breed	11 month	Male	Gastroenteritis, diarrhea	Nil
13.	Mixed breed	9 month	Female	Anorexia, gastroenteritis, diarrhea	Nil
14.	Mixed breed	10 month	Female	Anorexia, nervous signs	Nil
15.	Golden retriever	6 Year	Male	Anorexia, vomiting, fever	Nil

Table 2: Biochemical profile of a dog diagnosed with CCoV gastroenteritis

Parameters	Dog affected with CCoV	Normal Reference range (Source: The Merck's Veterinary Manual,11th edition)
AST (IU/L)	44.9	13-15
ALT (IU/L)	55.30	10-109
TP (g/dl)	3.9	5.4-7.5
Albumin (g/dl)	1.16	2.3-3.1
Globulin (g/dl)	1.89	2.7-4.4
BUN (mg/dl)	53.40	8-28
Creatinine (mg/dl)	1.68	0.5-1.7
Sodium (mEq/L)	135	142-152
Potassium (mEq/L)	2.65	3.9-5.1
Chloride (mEq/L)	106.10	110-124

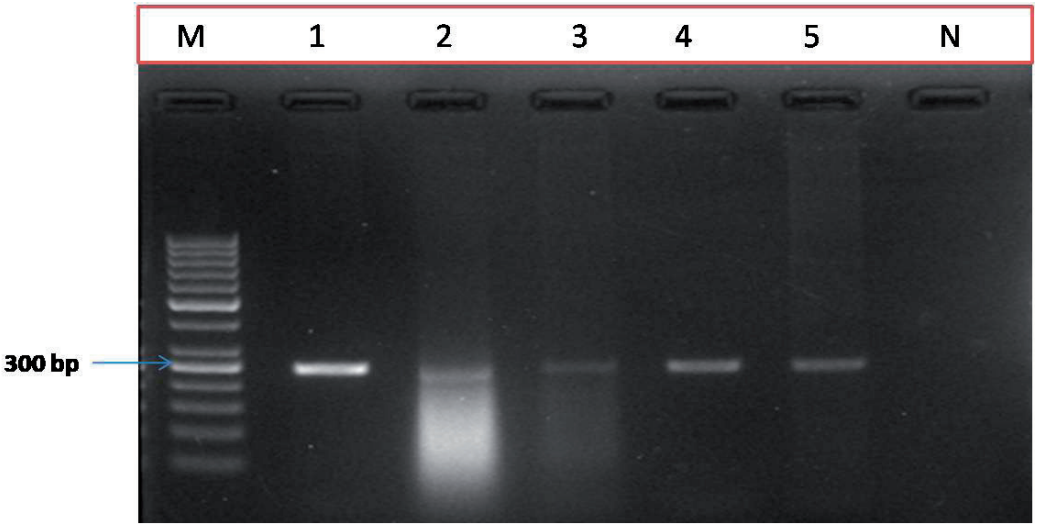

Fig. 1. Agarose gel electrophoresis showing purified PCR product from CCoV positive samples (amplicon size- 321bp)
M: Ladder 50 bp, N: Negative PCR control, Lane 1: Positive control
Lanes: 2- 5, Field samples (positive)

Phylogenetic analysis. On the basis of the partial sequence of the CCoV, phylogenetic analysis was carried out by comparing the partial sequences of the M gene (321 bp) with other sequences available in the databank. The sequences of three samples were submitted to the gene bank with Accession Numbers ON873743, ON873744 and ON873745. The CCoV/IND/HSR-2/2022 revealed nucleotide (nt) identity levels of 100% with the CCoV isolate from China (Accession Number MT250819), 99.19% with the CCoV isolate from Korea (Accession Number HM450122.1) and nt identity of 98.39% with that of the Italy isolate (Accession no. OQ565699). The Indian CCoV/IND/HSR-3/2023 revealed maximum nt changes in 13 places resulting in only 94.66% nt identity with the CCoV isolate from Italy (Accession no. OQ565699), 95.63% nt identity with the CCoV isolate from Taiwan (Accession Number MK986734), 94.66% nt identity with the China isolate (Accession Number OP422903) and 94.61% with the CCoV isolate from Korea (Accession Number HM450123.1). The CCoV/IND/HSR-4/2023 revealed nucleotide identity levels of 97.24% with the CCoV isolate from China (Accession Number MT250819), 97.60% with the CCoV isolate from Korea (Accession Number HM450122.1), 97.95% nt identity with the CCoV isolate from Taiwan (Accession Number MK986734) and 99.38 % nt identity with the CCoV isolate from Italy (Accession no. OQ565702). The phylogenetic analysis indicated these samples were very close to the isolates from China and Taiwan (Fig. 2).

Genetic analysis of Canine coronavirus. The partial sequence obtained for three CCoV isolates encoded a polyprotein of 80 amino acids (aa). The Indian CCoV/IND/HSR-2/2022 and CCoV/IND/HSR-4/2023 revealed aa identity levels of 100% with the CCoV isolate from China (Accession Number AAU29528) and aa identity of 92% with that from Korea (Accession Number AHA51662). The CCoV/IND/HSR-3/2023 isolate showed major changes in amino acid substitution at three places (Y-L, K-L and A-R) revealing only 97.06% aa identity with the CCoV isolate from Brazil (Accession Number ADB82633), 94.37% aa identity with the CCoV isolate from China (Accession Number QSL97386), and 90.14% aa identity with one of the Indian isolates (Accession Number UZT75485). The phylogenetic analysis based on amino acid indicated these samples were close to the China and Brazil isolates (Fig. 3).

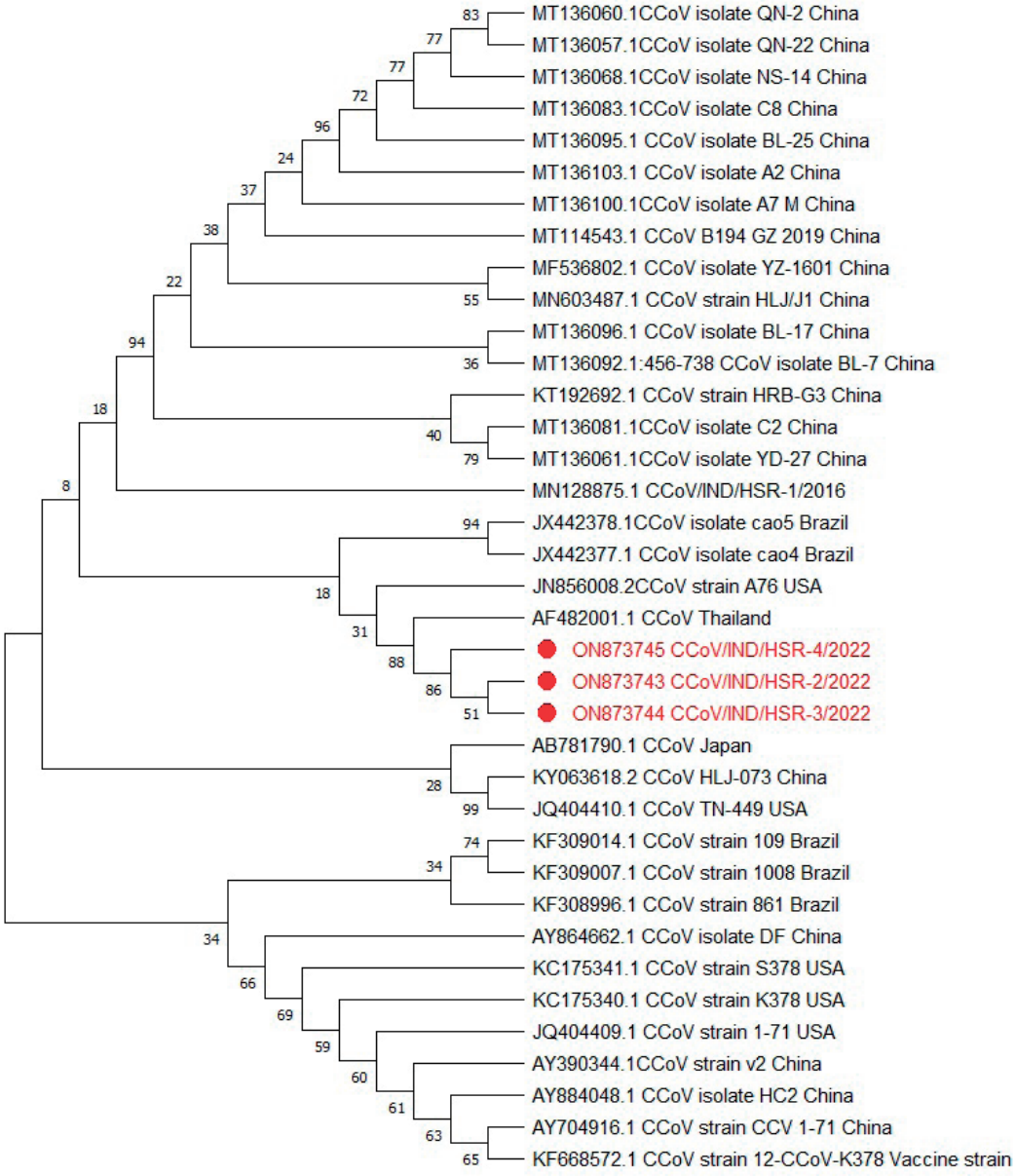

Fig. 2. Phylogenetic analysis of canine corona virus strains using the nucleotide sequences of the partial M gene (241bp) of CCoV

The 2022 CCoV sequence is shown in red and dots indicate important nodes that received 100% bootstrap. All the sequences were analyzed in the MEGA X program using the Neighborhood joining method, with 1000 bootstrap replicates. The name and accession number of each strain are shown. The proposed phylogeny and reference sequences were obtained from GAN et al., 2021.

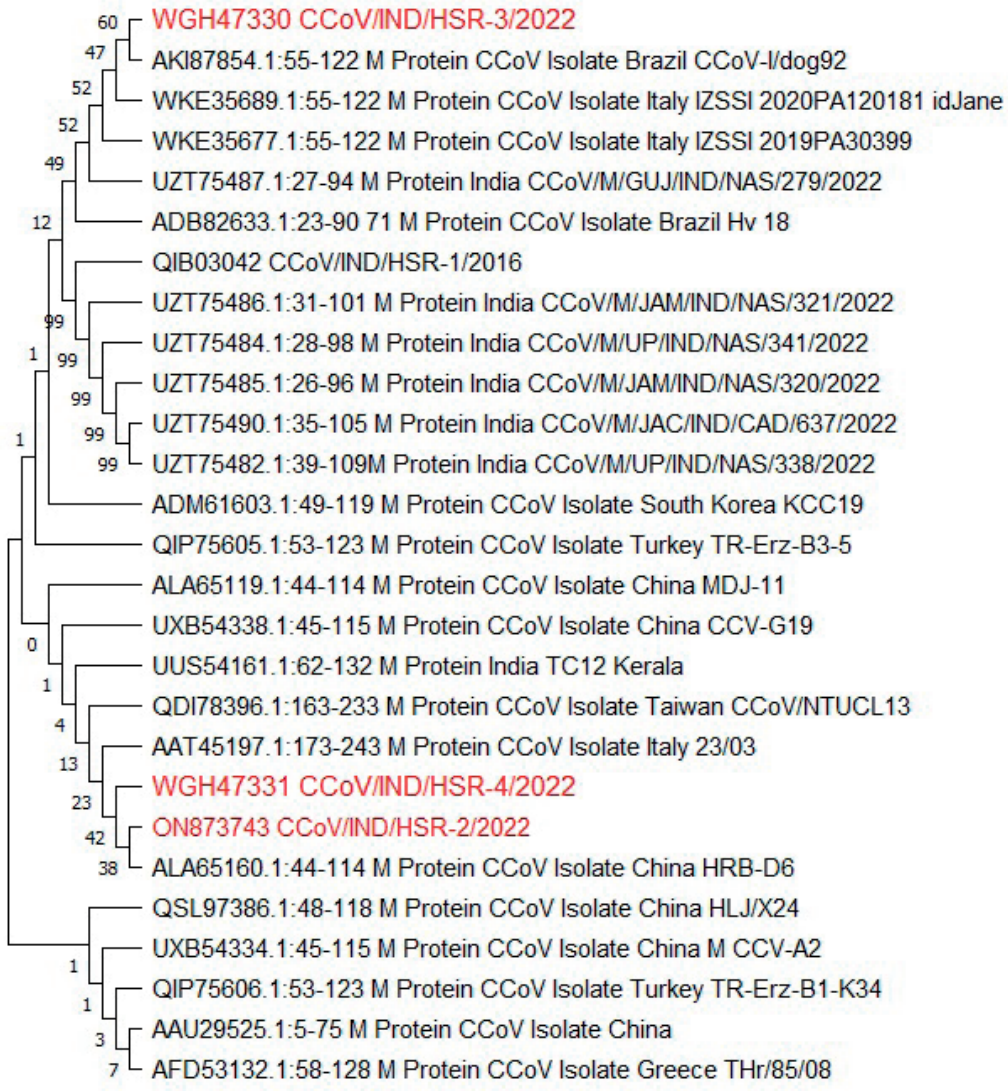

Fig. 3. Phylogenetic analysis of canine corona virus strains using the amino acid sequences of the partial M gene (241 bp) of CCoV

The 2022 CCoV sequence is shown in red which codes for 80 amino acids. All the sequences were analyzed in the MEGA X program using the Neighborhood joining method, with 1000 bootstrap replicates. The name and accession number of each strain are shown

*Recombination analysis***.** To demonstrate the recombination between CCoV isolates from different regions of the world, similarity plots and bootscanning studies were carried out. The three CCoV isolates were used as a query sequence, and compared with other reported CCoVs worldwide. The likelihood of recombination in the M gene was also confirmed by the consistency of the results of the bootstrapping and phylogenetic studies (Fig. 3). The probability of recombination using MaxChi and Chimaera was 1.57 x 10^{-02}, RDP, 2.768 x 10^{-08} based on SiScan, and 2.484 x 10^{-04} based on 3Seq. This indicates the recombination event was frequent in this virus genotype.

Discussion

Currently, severe acute respiratory syndrome/ Middle East respiratory syndrome and Coronavirus have become emerging infectious diseases. The genetic characterization of these emerging pathogens is necessary due to the diverse properties of infectious viruses, including changing phenotypes, shifting pathogenicity, and their escape mechanisms from host immune responses. Enteric coronavirus is responsible for causing diarrhea in cats due to its affinity for enterocytes in the intestine. It can also lead to lethal multi-systemic diseases, such as peritonitis, granulomatosis and organ damage (OĞUZOĞLU et al., 2013). The majority of studies on canine CCoV infections are based on serological studies, as there have been relatively few molecular studies on the subject (YEŞILBAĞ et al., 2004). Numerous studies have looked into how different viral agents contribute to the development of diarrhea in puppies and adult dogs. For the first time in Europe, DECARO et al. (2010) reported a highly virulent pantropic strain of CCoV-2a that was deadly to puppies. CCoV type I and type II genotypes were found in canine/dog feces samples by PRATELLI et al. (2004) in their sequencing research with the M and S gene of CCoV.

For the current investigation, we carried out molecular characterization and phylogenetic analysis of enteric CCoV strains circulating in the canine population. The majority of investigations identify enteric agents as being present as coinfections. However, virus infections themselves can result in serious disease in cases where the virus's virulence is high. A recent study has also shown that CCoV-positive puppies exhibit hemorrhagic enteritis, as seen with CPV-2 infections (PINTO et al., 2014). In the present study, CCoV infection was detected in 15 of the 50 dogs that had severe diarrheal symptoms. Therefore, while making a differential diagnosis for an animal exhibiting gastrointestinal symptoms, enteric viruses such as CCoV and CPV-2 should always be taken into account.

The nucleotide sequences of the CCoV strains achieved in this study were matched to reference strains present in GenBank. WANG et al. (2016) compared the sequence of the partial M gene of 57 CCoV strains from China, and found nucleotide homologies between them, ranging from 88.4 to 100% and 88.7 to 96.24% identity between the 57 CCoV strains and the Chinese reference strain HF3. CCoV/IND/HSR-2/2022 and CCoV/IND/HSR-4/2023 revealed nt/aa identity levels of 100%/100% to the CCoV isolate from China (Accession Number MT250819), nt/aa identity of 98.39%/ 97.56% with the isolate from Italy (Accession no. OQ565681) and nt/aa identity of 99.19/100% with that from Korea (Accession Number HM450122). The Indian CCoV/IND/HSR-3/2023 revealed 95.63%/ 95.59 nt/aa identity with the CCoV isolate from Italy (Accession Number OQ565703), 97.94%/94.37% nt/aa identity with the CCoV isolate from Taiwan (Accession Number MK986738), 95.63%/94.37% nt/aa identity with the isolate from China (Accession Number OP422903) and 95.15%/97.06% nt/aa identity with the Indian isolate (Accession Number OP858684). SAKULVERA et al. (2003) showed that their CCoV S gene sequence (AF482001) had 94.9% nucleotide identity to CCoV strains reported from the United Kingdom and Germany (Accession number D13096). Phylogenetic analysis of an Australian field sample showed the lowest identity to all other strains compared, with only 86.1% homology to the most closely related strains (NAYLOR et al., 2001). Phylogenetic analysis of Indian CCoV strains showed that these were similar to those found in other countries, such as Taiwan and China, remaining far away from USA strains.

Compared to DNA viruses, RNA viruses have higher rates of mutation. In addition to small insertions and deletions in the genome containing both structural and non-structural proteins, the high frequency of recombination, which encourages the spread of numerous virus strains with various biological and immunological functions, is the dominant force in the microevolution of positive RNA viruses (PRATELLI et al., 2003). The coronavirus genome is predicted to accumulate several nucleotide alterations per cycle of replication as a result of RNA-dependent RNA polymerase errors (JEOUNG et al., 2014). CCoVs have the capacity to adapt quickly to adverse conditions, such as those imposed by the immune system, because of their relatively high mutation frequency. M gene modifications showed amino acid changes that were exclusive to CCV strains from particular isolates, such as the Korean isolate. (JEOUNG et al., 2014). The CCoV/IND/HSR-3/2023 isolate showed major changes in amino acid substitution at three places, indicating a recombination event occurred in the virus. This finding highlights the fact that there may be frequent recombinations within the virus population circulating in dogs.

Conclusions

In conclusion, molecular techniques are very reliable and sensitive for diagnosing viral enteritis. Diseases reported in non-vaccinated dogs suggested that vaccination programs and an awareness campaign are required for pet owners in order to decrease the prevalence of this viral disease.

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Declaration of competing interest

No potential conflicting interest was reported by the authors

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BATRA, K., P. KUMAR, A. SEHRAWAT, D. AGNIHOTRI, S. MAAN: Detekcija i karakterizacija infekcije psećim koronavirusom: enteralni pristup. Vet. arhiv 94, 475-486, 2024.

SAŽETAK

Infekcija psećim koronavirusom (CCoV) nova je emergentna bolest koja se pojavljuje u pacijenata. U pasa s blagim do umjerenim enteritisom ona uzrokuje malu smrtnost i znatan morbiditet. Serološka su istraživanja na psima u Indiji otkrila postojanje protutijela i time ukazala na pitanje prevalencije u populaciji. Cilj je ovog rada bio istražiti prevalenciju infekcije virusom CCoV u pasa koji boluju od gastroenteritisa i provesti gensku analizu soja cirkulirajućeg virusa. Metodologija je uključivala uzorkovanje izmeta pasa s gastroenteritisom u kojih se sumnja na CCoV. Upotrebom početnica temeljenih na M-genu primijenjen je PCR za amplifikaciju predložaka RNA (c-DNA) dobivenih od uzoraka izmeta. RT-PCR za detekciju CCoV-a pokazao je pozitivan rezultat od 30% (15/50). Filogenetska analiza otkrila je da dva uzorka nt/aa imaju 100%/100%-tnu identičnost s izolatima CCoV iz Kine i 99,19/100%-tnu identičnost nt/aa s onima iz Koreje. Jedan je od uzoraka pokazao divergenciju i 95,63%/94,37%-tnu nt/aa identičnost s izolatima iz Kine i 95,15%/97,06%-tnu nt/aa identičnost s izolatima iz Indije. Rekombinacijska je analiza u jednom od uzoraka otkrila mutaciju. Ukratko, u radu je prikazano cirkuliranje psećeg koronavirusa u populaciji pasa s gastroenteritisom. Rezultati upućuju na potrebu za cijepljenjem pasa u svrhu prevencije s obzirom na otkrivenu visoku prevalenciju virusnog gastroenteritisa.

Ključne riječi: RT-PCR; pseći koronawirus; proljev; filogenetska analiza; genska karakterizacija