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Clinical and Molecular Study of Canine Enteric Coronavirus in Iraq

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Abstract

anine enteric coronavirus (CECoV) is one of the most common causes of gastrointestinal disorders in dogs, especially unvaccinated young dogs. This study was designed to determine the infection rate of CECoV in the dogs by reverse transcriptase-polymerase chain reaction (RT-PCR) with phylogenetic analysis. Rectal swabs were collected from 211 dogs presenting at Baghdad Veterinary Hospital and private veterinary clinics with clinical signs of gastrointestinal disturbances during the period from February 2022 to June 2023; their ages ranged from 3 months to 7 years. Clinical and demographic data was recorded for each sample, including the dog's number in the household, signalment, with detailed clinical examinations, and diarrhoea's recent history. The molecular results revealed that 13 positive samples (6.2%) were detected; interestingly, seven of the infected dogs from three different households were aged less than 6 months. All the positive samples were classified as CECoV type II based on M gene analysis. The phylogenetic analysis results showed that the isolates of this study were closely related to CECoV universal isolates that were isolated from dogs with 98.37–97.02% identity. No previous study was conducted about the detection of CECoV in Iraq.

Keywords: dogs, RT-PCR, phylogenetic analysis, CECoV, Iraq.

Introduction

Coronaviruses are single-stranded (ss) RNA and are considered the largest family of viruses that usually cause acute to severe gastrointestinal, respiratory, and neurological diseases in different hosts, including companion animals and humans [1]. CECoV is classified into two biotypes: classical and pantropic. The classical biotype has two serotypes (I and II), and serotype II can be organised into four subtypes (a, b, c, and transmissible gastroenteritis like virus). The classical canine enteric coronavirus biotype (CECoV-IIa) causes enteritis restricted to the small intestine, while the pantropic biotype can infect other body systems, which leads to leukopenia, transmissible gastroenteritis like virus, and CECoV- IIb causes highly contagious mild enteritis [2]. Canine coronavirus infections cause a high morbidity and low mortality, except for younglings. The infection is mainly restricted to the intestinal tract; symptoms include loss of appetite, diarrhoea, vomiting, and eventually death. The death of animals is often a consequence of co-infection with canine parvovirus, canine distemper virus, or other pathogens [3]. The causes of diarrhoea in dogs were divided into infectious causes and non-infectious causes. The infectious causes include viral infections such as CECoV, Parvo virus, canine distemper, and Rota virus; bacterial infections such as *Salmonella spp., Escherichia coli*, and *Clostridium perfringens*; fungal infections such as Histoplasmosis and

*Corresponding author: Mohammed A. Hussein, E-mail: Mohammedalani@uofallujah.edu.iq . Tel.:009647709196681 (Received 23/12/2023, accepted 21/01/2024) DOI: 10.21608/EJVS.2024.257545.1745 ©2024 National Information and Documentation Center (NIDOC) Pythiosis; parasitic causes such as Helminths and Protozoa; and non-infectious causes (diet intolerance, allergy, and rapid dietary change). Nelson et al.[4] and Pratelli. [5] reported that shedding of CECoV occurs for as long as six months, which is an important factor that helps virus persistence in the dog population. Prevalence rates for canine enteric coronavirus vary depending on population studied, region, and method of diagnosis used. A study used polymerase chain reaction to investigate canine enteric coronavirus in dogs housed in four different UK dog kennels; prevalence ranged from 5.3% to 33.3% [6]. Other studies reported prevalence ranging from 0.1% [7] to 12% [8] to as high as 26.7% in Japan [9]. Numerous studies were conducted about coronaviruses in Iraq by using the PCR technique in humans [10-13] and in animals [14-20]. To the best of our knowledge, no previous studies were conducted about CECoV in Iraq.

Material and Methods

A total of 211 rectal swabs were collected from 161 dogs with diarrhoea and from 50 apparently healthy dogs (as a control group) from Baghdad veterinary hospital and private veterinary clinics in Baghdad city, Iraq, from February 2022 to June 2023. Their ages ranged from less than 3 months to 7 years. A questionnaire for every dog owner, including the following: sex, age, breed, being housed in groups or individually, and history of vaccination, was recorded. The rectal swabs were collected in a cooled box using ice bags and transferred to the laboratory of the Razi Centre-Ministry of Industry and Minerals. Rectal swabs were centrifuged at 1500 RPM for 10 minutes at 4 °C after dilution in 1.5 ml of phosphatebuffered saline, and each specimen supernatant was transferred to a 1.5 ml tube. RNA extraction was performed by using a viral nucleic acid extraction kit according to the manufacturer's instructions (Geneaid, Taiwan) and performing complementary DNA (cDNA), which was kept frozen at -20 °C until the RT-PCR test was performed. Complementary DNA (cDNA) was synthesized with the commercial kit (Accupower RT PreMix; Bioneer, Korea) as instructed by the manufacturer, molecular detection of CECoV was conducted by using RT-PCR targeting an 409 bp fragment of the M gene of CECoV according to description by Pratelli et al., (1999), the oligonucleotide used in this study CCV1 (337–356 nucleotides) sense primer (5'-TCCAGATATGTAATGTTCGG-3') and CCV2 antisense primer (726–746 nucleotides) (5'-TCTGTTGAGTAATCACCAGCT-3') primers designed by [21], after purification of PCR products, they were directly subjected to Sanger sequencing, and each sample was sequenced 3 times, and PCR products were submitted to (Macrogen, Korea) for Sanger sequencing, Partial M protein gene sequences were directly submitted to the NCBI GenBank database.

Results and Discussions

The present study reports the first molecular detection of CECoV in dogs in Iraq. CECoV was detected by RT-PCR in 13 out of 211 (6.2%) dogs in Baghdad city. All the positive samples were classified as CECoV type II based on M gene analysis; the majority of the positive samples (7/13) were from puppies under 6 months of age (table 1) (Figure 1), The infection rate of CECoV in various populations of dogs in the world has ranged from 1.1–65.1% [22, 23] to as high as 84.62% recorded in the kennel population [24]. The variation in the infection rate may be attributed to differences in the opportunity for exposure of the dogs to the virus and social interaction [25].

Our results coincide with those of other studies, such as Navarro *et al.*[26], who found a prevalence of CECoV of 4.8% in diarrheal young and adult dogs on the Caribbean island of Saint Kitts and Nevis (KNA), and Takano *et al.*[9], who reported a prevalence rate of CECoV-II by RT-PCR of 7.4% in Japan. Castanheira *et al.*[22] reported that the prevalence in their study was 1.9% in 2010 and 1.1% in 2011. While Ntafis *et al.*[23] reported that the prevalence of CECoV was 65.1% in dogs with diarrhoea in Greece and the positive cases more commonly detected in dogs younger than 3 months old, Timurkan *et al.*[24] reported that the prevalence of CECoV-II in dogs in Turkey was 84.62% and the prevalence rates founded by Alves *et al.*[27] at 30.4% in Brazil.

The low infection rate may be attributed to a low virus circulation, the instability of the virus in the environment, and the low number of virus particles in faeces [22]. Tennant *et al.*[28] mentioned that the detection limits of CECoV are possibly due to the low number of virus particles present in the faeces sampling time, or may be due to pH changes or the presence of colloids and ions in the faeces, which lead to virus inactivation. The recovered dogs from infection with CECoV may act as reservoirs asymptomatically and periodically shed the virus, leading to continued and persistent environmental circulation of CECoV [6].

All 13 RT-PCR positive samples of CECoV were sequenced, and BLAST outcomes were documented in the NCBI Gene Bank (National Centre for Biotechnology Information). The nucleotide sequences generated in our study were deposited in the GenBank with the following accession numbers: OR147197, OR421198, OR421199, and OR421200 (Figure 2). When comparing our 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 gene in Iraq. The evolutionary tree was drawn using MEGA 11 software and displayed that 19 sequenced isolates had the highest similarity with 98.37-96.48 percent identity to sequenced universal isolates.

CECoV isolates showed a high identity of 98.37% with isolate ID (KF309007) from diarrheal puppies under two months of age in Brazil [8], 97.56% with The current results of the phylogenetic analysis of isolate ID (AY884048, AY864662, and AY884049) isolated from healthy dogs, foxes, and raccoon dogs in China [29], and 97.02% identity with isolate ID (EU856362) from dogs that had died with gastroenteritis in Italy [30]. Also, our isolates showed 97.02% identity with isolates from Swedish dogs ID (DQ431017) [31].

While our isolate revealed 96.75% identity with isolates ID (MW718811, MW718810, MW722877, MW722872, MW722866) in China [32], and isolate ID (MZ320954) in China [33], and isolate ID (GU146061) in Italy [34], and isolate ID (KP981644) in Italy [35], and isolate ID (DQ112226) in Italy [36],

and isolate ID (JF682842) in Greece [37], another studies showed 96.74% and 96.48% identity to CECoV isolates from dogs in UK and China [38] isolate ID (FJ009114)[39] isolates ID (MT114542, MT114541) respectively.

Conclusions

This study concluded that CECoV was more prevalent in dogs younger than 6 months; in addition, all the positive samples were classified as type II, there is not sufficient published data about the molecular study of CECoV in neighboring countries of Iraq; our isolates, compared with universal isolates only, are worthwhile. The current isolates were recovered from dogs imported from various.



Age group	Dogs with diarrhea	Healthy dogs	Positive in pcr
Less than 6 months	52	15	7
6-12 months	35	8	4
1-2 years	23	12	2
2-5 years	39	10	0
More than 5 years	12	5	0
Total	161	50	13

*p $\leq 0.05 \chi^2 = 2.92$ non-significant



Fig. 1. PCR products (409 bp) of different coronavirus samples using CCV1-CCV2 primers. Lane 1, marker (Gene RulerTM100 bp DNA Ladder); lane 2 to lane 7 fecal sample CECoV positive.



Fig. 2. Phylogenetic tree based on canine coronavirus M gene sequences from Iraq and reference sequences from GenBank. Sequence from Iraq of enteric CCoV with accession number OR147197, OR421198, OR421199 and OR421200 and reference sequences from around the world are indicated by their accession numbers.

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The authors declare that the present study has no financial issues to disclose.

Conflict of interest: None

Authors contributions:

Mohammed Ali Hussein: Practical work

Mawlood Abbas Ali Al-Graibawi: study design and editing.

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دراسة سريرية وجزيئية لفيروس كورونا المعوي الكلابي في العراق

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يعد فيروس كورونا المعوي الكلبي (CECoV) أحد الأسباب الأكثر شيوعًا لاضطرابات الجهاز الهضمي لدى الكلاب، وخاصة الكلاب الصغيرة غير الملقحة. تم اعداد هذه الدراسة للكشف عن نسبة انتشار الإصابة بفيروس كورونا المعوي الكلبي في الكلاب في مدينة بغداد عن طريق استخدام اختبار تفاعل البوليميراز المتسلسل للنسخ العكسي (RT-PCR) مع التحليل الجيني الجزيئي, حيث تم جمع مسحات براز من مستقيم 211 كلباً من مستشفى بغداد البيطري وكذلك من العيادات البيطرية الخاصة بالحيوانات الإليفة وكانت هذه الكلاب تظهر علامات سريرية تشمل اضطرابات الجهاز المهمي وخاصة الاسهال خلال الفترة من شباط 2022 إلى حزيران 2023؛ وتراوحت أعمار الحيوانات بين 3 أشهر إلى 7 سنوات وتم تسجيل البيانات السريرية ومنطقة تواجد الكلب واعداد الكلاب لكل منزل مع القيام بالفحص السريري الدقيق لكل حالة وقد أظهرت نتائج التحليل الجزيئي ان نسبة انتشار الفيروس هي 6.2 % (لا همام أن سبعة من الكلاب المصابة من ثلاث منازل مختلفة كانت أعمار ما فقل من العادم من العيادات

تم تصنيف جميع العينات الموجبة على أنها فيروس كورونا الكلبي المعوي من النوع الثاني II CECoV)) بناءً على تحليل الجزيئي لجين M كما أظهرت النتائج أن عزلات هذه الدراسة كانت مرتبطة ارتباطًا وثيقًا بالعزلات العالمية لـهذا الفيروس والتي تم عزلها من الكلاب وبنسبة تطابق تتراوح من 98.37 الى 97.02٪. علما انه لم يتم إجراء أي دراسة سابقة حول الكشف عن فيروس كورونا الكلبي المعوي في العراق.

ا**لكلمات الدالة:** الكلاب، تفاعل البوليمراز المتسلسل للنسخ العكسي ، التحليل الوراثي، فيروس كورونا المعوي الكلابي ، العراق.