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Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

Molecular Study of Infectious Canine Hepatitis in Stray Dogs

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Abstract | Infectious Canine Hepatitis (ICH) is a viral disease that is considered a highly contagious and is life-threatening disease in dogs. Canine adenovirus type 1 (CAV-1) is a cause of ICH, which is caused infectious canine hepatitis and uveitis in canine. The study was conducted on 200 stray dogs from three provinces in Iraq (Baghdad, Najaf, Karbala, and Diwaniya). Clinical examinations and signs were documented. Blood samples were obtained from the cephalic vein of dogs with EDTA tubes for molecular assay, and without anticoagulant tubes for serological tests. Polymerase Chain Reaction (PCR) and Enzyme-linked immunosorbent assay (ELISA) tests were used to identify CAV-1. Specific primers targeting the E3 gene were employed for the diagnosis of canine adenovirus type 1 (CAV-1). The PCR results indicated the presence of canine adenovirus type 1 (CAV-1) in 9 out of the 200 animals tested, with the remaining 191 animals testing negative for the virus (4.5%) while the infection rate was five out of 200 stray dogs (2.5%) fixed by ELISA in the survey study. The partial sequences of the E3 gene of three isolates of CAV-1. These sequences of CAV-1 were submitted to the gene bank under accession numbers (PP900637.1, PP900638.1, and PP900639.1), the phylogenetic analysis of CAV-1 Iraqi isolates close to other isolates of isolates as India, Australia, U.K., Canada, Portugal, Italy. The results of the clinical study showed that the Mean of temperature, respiratory rates and pulse rates were significantly increased in the infected dogs with CAV-1. The clinical signs in the infected dogs with CAV-1 included loss of appetite, emaciation, respiratory signs, vomiting, pallor of mucous membranes, depression, and eye cloudiness. This study confirmed some of the clinical manifestations of CAV-1 infection and is considered the first molecular evidence of CAV-1 in Iraq.

Keywords | Molecular study, Adenovirus, Infectious canine hepatitis, Dogs, PCR**Received** | June 19, 2024; **Accepted** | November 05, 2024; **Published** | December 04, 2024***Correspondence** | Aiman Mohammed Baqir Al-Dhalimy, Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; **Email:** 1ayman.mohammed1101f@covm.uobaghdad.edu.iq**Citation** | Al-Dhalimy AMB, Mahmood AK (2024). Molecular study of infectious canine hepatitis in stray dogs. J. Anim. Health Prod. 12(s1): 202-208.**DOI** | <https://dx.doi.org/10.17582/journal.jahp/2024/12.s1.202.208>**ISSN (Online)** | 2308-2801**Copyright:** 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

INTRODUCTION

Infectious Canine Hepatitis (ICH) is a viral disease that is considered a highly contagious and threatening life disease to canine (Sykes, 2014). Canine adenovirus type 1 (CAV-1) is a cause of ICH, which is a dsDNA non-enveloped virus and is classified under the genus Mastadenovirus (Chander *et al.*, 2021). According to genetic and antigenic properties, CAV is classified into CAV-1 and CAV-2, CAV-1 is caused

infectious canine hepatitis and uveitis in canines and also affects the digestive system, while CAV-2 is caused by respiratory disease disorders (Hu *et al.*, 2001; Sykes, 2014). The Infectious Canine Hepatitis is characterized by acute enterohaemorrhagic hepatitis. However, it exhibits a broader spectrum of clinical manifestations, including corneal edema (blue eye), uveitis, and interstitial nephritis. The latter may occur following the acute phase of the disease due to the deposition of circulating immune

complexes (De Jonge *et al.*, 2020; Essa *et al.*, 2020). The genetic and antigenic matching between CAV-1 and CAV-2 reached 75% nucleotide identity (Chander *et al.*, 2021). However, the genetic and antigenic relationship between the two types can be distinguished by DNA hybridization and restriction endonuclease analysis (Decaro *et al.*, 2008; Thanoon *et al.*, 2019). The target sites for CAV-1 replication are the endothelial cells of the vessels and parenchymal cells of the liver and kidneys. In contrast, the CAV-2 replication site is in the respiratory system and intestinal epithelia. The cause of ICH, mast-adenovirus A, formerly known as CAV-1, is an acute liver infection affecting dogs (Decaro *et al.*, 2008) but also wild Canidae (e.g., foxes, wolves, and coyotes), Ursidae (bears), and Mustelidae (e.g., martens, weasels, sloats) can be infected (De Jonge *et al.*, 2020). The virus spreads through nasal discharge, blood, urine, saliva, and faces. When it enters through the nose and mouth, it localizes and replicates in the tonsils before affecting the liver and kidneys (Greene, 2006; Sykes, 2014; Balboni *et al.*, 2022; Decaro *et al.*, 2008). The virus, an icosahedral double-stranded DNA virus, shares antigenic similarities with CAV-2, while interestingly, CAV-1 is not limited to canine hosts; it can also cause diseases in other animals such as wolves, coyotes, skunks, and bears (Majeed and Mahmood, 2024; Sykes, 2014). The latter may occur following the acute phase of the disease due to the deposition of circulating immune complexes (De Jonge *et al.*, 2020). The CAV-1 has a predilection for replicating in vascular endothelial cells and hepatocytes since this replication process leads to the development of acute necro hemorrhagic hepatitis, with a more pronounced clinical impact observed in young dogs compared to their adult counterparts (Pereira *et al.*, 2021). Adenovirus infections in dogs, those caused by CAV-1, exhibit a diverse array of clinical manifestations, underscoring the systemic nature of these infections. Furthermore, according to serologic surveys, CAV-1 infections can be caused by clinical acute or chronic disease (Evermann and Kennedy, 2011). The disease can impact multiple organs, including the liver, kidneys, eyes, respiratory tract, and neurologic system (Mohmoud, 2018; Polovitzer *et al.*, 2022).

The CAV-1 primarily targets the liver, inducing hepatic necrosis characterized by the death of liver cells (Greene, 2006). The clinical signs were recorded that related to hepatic injury, vomiting, fever, diarrhea, hepatomegaly, and abdominal pain (Decaro *et al.*, 2008; Pereira *et al.*, 2021). An effective early immune response can limit hepatic injury, but severe cases may progress to disseminated intravascular coagulation, causing a hemorrhagic diathesis (Goggs *et al.*, 2018). Chronic hepatopathy may develop in cases of partial immune responses, marked by persistent hepatic inflammation (Bergmann *et al.*, 2020; Mansour and Hasso, 2021).

MATERIALS AND METHODS

ANIMALS OF STUDY

The study was conducted on 200 stray dogs from three provinces in Iraq (Baghdad, Najaf, Karbala, and Diwaniya). These dogs were selected randomly through the period from April 2022 to December 2023. Clinical examinations and signs were documented. Blood samples were obtained from the cephalic vein of dogs with EDTA tubes for molecular assay, and without anticoagulant tubes for serological test. Polymerase Chain Reaction (PCR) and Enzyme-linked immunosorbent assay (ELISA) tests were used to identify CAV-1. The study aimed to detect the prevalence of ICH in stray dogs in some provinces of Iraq (Abd-Elmoneim and Mahmood, 2019). Samples were collected according to Valenciano *et al.* (2013). Eight ml of blood were divided into four milliliters with EDTA tube for PCR analysis and four milliliters without anticoagulant and centrifuged at 3000x g for 5 minutes to obtain serum for serological tests (ELISA) (Al-Dujaily and Mahmood, 2021).

THE CLINICAL EXAMINATION

Clinical manifestations were evaluated in all investigated animals. The clinical examination included: A general assessment: Measurement of heart rate, pulse rate, and body temperature, documentation of clinical symptoms, and recording any additional clinical symptoms observed during the examination (Al-Hadithy and Badawi, 2015).

DNA EXTRACTION METHOD

Genomic DNA was isolated from buffy coats using the ReliaPrep™ Blood gDNA Miniprep System (Promega, USA) according to the manufacturer's instructions. The purity and concentration of DNA were assessed using the Nanodrop device following Thermo Fisher Scientific instructions. Gel electrophoresis was performed according to Voytas (2000) with 1.2% of the gel and visualized the template DNA and bands positive under UV light.

PRIMERS OF THE STUDY

Specific primers targeting the *E3* gene were employed for the diagnosis of canine adenovirus type 1 (CAV-1). These primers were selected for their high specificity and sensitivity in detecting the presence of CAV-1 in the collected blood samples, ensuring accurate and reliable molecular analysis. The primers of the study were F: CGC GCT GAA CAT TAC TAC CTT GTC, and R: CCT AGA GCA CTT CGT GTC CGC TT; these primers were produced product size 508bp (Yoon *et al.*, 2010).

For primer preparation, deionized distilled water was added to lyophilized primers (IDT Company, USA) to form a concentration of 100 µM for each primer and ten picomoles for each primer. This was achieved by adding ten µL of primer

stock to 90 μ L of deionized distilled water. The primers were then ready for use in the PCR master mix reaction.

PCR thermocycler conditions include denaturation (15 minutes at 95°C, annealing (1 minute at 58 °C), then extension (1 minute at 72°C) for 35 cycles. The final extension was five minutes at 72°C. Gel electrophoresis was run on 1.2% agarose gels at 90 Vol. for 40 minutes to visualize PCR products (Yoon *et al.*, 2010). The master mix protocol was done according to the protocol of green master mix kits (Promega, USA) including Master mix reagent 12.5 μ L, Primer (10 picomol) Forward 1 μ L and Reverse 1 μ L, Template DNA 4 μ L, Nuclease-free water 6.5 μ L, and final total reaction 25 μ L.

THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

The enzyme-linked immunosorbent assay (ELISA) method was employed to detect and quantify the presence of canine adenovirus type 1 (CAV-1) in serum samples. Sample preparation involved allowing the collected whole blood to clot and then centrifuging it to obtain serum. Test conducted according to instructions of (SunLong Biotech Co., LTD, China).

Statistical analyses

Statistical analyses were conducted using SPSS version 20 software. One-way ANOVA was used to compare the means between the two groups. The Odds ratio was chosen to detect the risk factor of the infection between studied tests. The results were considered statistically significant if the p-value was less than 0.05.

RESULTS AND DISCUSSION

MOLECULAR TEST

DNA extraction was done for all blood samples of dogs. The results of extraction were very good, and an assessment of the quality of the DNA was performed by using agarose gel electrophoresis as displayed in Figure 1. The bands of DNA were appeared as clear and regular edges. The purity and concentrations of the template DNA were measured by Nano drop, the purity ranges between 1.6 to 2 and concentrations ranges of DNA between 63.1 to 89.4 ng/mg. The PCR results indicated the presence of canine adenovirus type 1 (CAV-1) in 9 out of the 200 animals tested, with the remaining 191 animals testing negative for the virus. This finding suggests a prevalence rate of approximately 4.5% for CAV-1 infection among the sampled stray dog population in Iraq (Figure 2).

The partial sequences of E3 gene of three isolates of CAV-1. These sequences of CAV-1 submitted to gene bank under accession number (PP900637.1, PP900638.1, and PP900639.1), the phylogenetic analysis of CAV-1 Iraqi

isolates close to other isolates of isolates as India, Australia, U.K., Canada, Portugal, Italy (Figure 3).

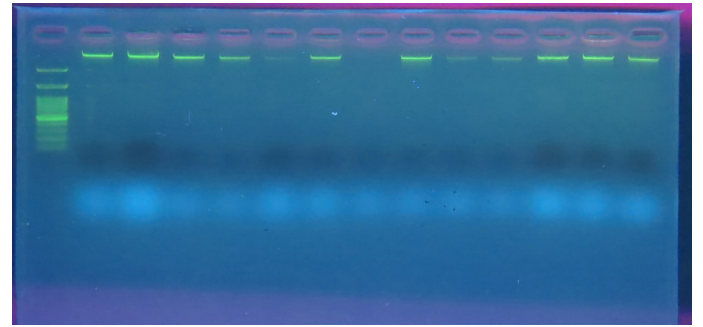


Figure 1: Template DNA loading on agarose gel 1.2% loading with red safe stain.

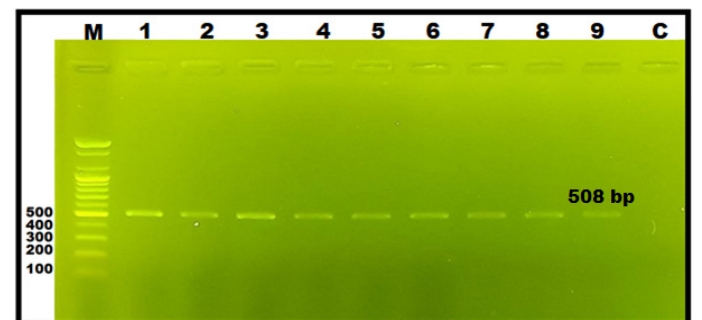


Figure 2: Results of gel electrophoresis 1.2% with red safe stain at 80 A and 120 Vol. of fragment 508bp E3 gene of CAV-1 in dogs of survey. A: Lane M: Molecular weight marker (Sizer 100bp). Lane 1-9: positive cases. Lane C: Negative control.

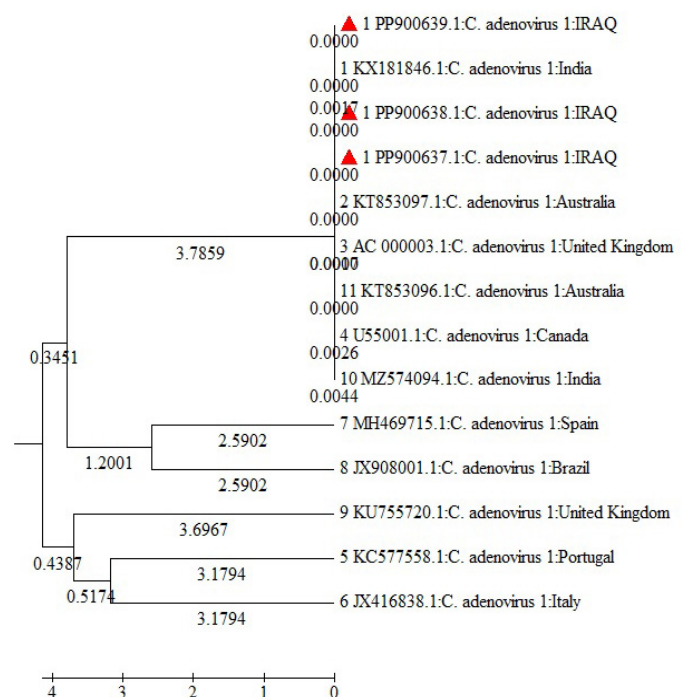


Figure 3: Phylogenetic tree depends on the partial sequence of E3 gene of 3 isolates of CAV-1 created by using the Maximum Composite Likelihood method. Evolutionary analyses were generated by using MEGA6 software.

CLINICAL SIGNS

The results of the clinical study showed that the Mean of temperature, respiratory rates, and pulse rates were significant increase in the infected dogs with CAV-1 compared to the non-infected dogs (Table 1). The clinical signs in the infected dogs with CAV-1 included loss of appetite, emaciation, respiratory signs, vomiting, pallor of mucous membranes, depression, and eye cloudiness (Figure 4).

Table 1: Physical examination values of dogs in CAV-1 infection.

Groups/ Test	Non-infected (191)	Infected dog (9)
Temperature °C	38.73± 0.03 B	39.10± 0.18A
Pulse rate/minute	96.85± 2.08 B	103.37± 8.65A
Respiratory rate/minute	53.02± 2.01 B	74.87± 14.45A

The differences in capital letters horizontally statistically significant at $P \geq 0.05$.

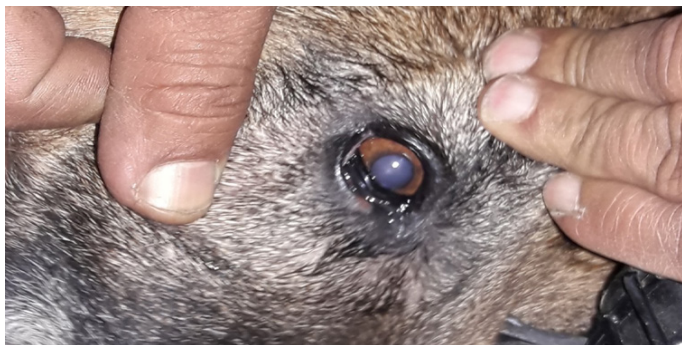


Figure 4: Male dogs aged 1.5 years suffering from eye clouding, this infected by CAV-1.

SEROLOGICAL DIAGNOSIS

The serological study detected five positive dogs, all positive results showing optical density higher than the Cut-off value of optical density of the standard positive. The infection rate was five out of 200 stray dogs (2.5%) fixed by ELISA in the survey study, which is lower non-significantly when compared to the PCR diagnosis. Therefore, the molecular diagnosis produced higher results than the ELISA technique (Table 2).

Table 2: The infection rate of CAV-1 according to ELISA and PCR.

Tests	ELISA	PCR
Positive cases	5 (2.5%)	9 (4.5%)*
Negative cases	195	191
Total	200	200

*Non-significant, O.R. 1.83. confidence interval (CI 95%) = 0.60-5.58

The findings of this study are crucial for several reasons; firstly, they provide a clear indication of the presence and prevalence of CAV-1 in stray dogs in Iraq, contributing

to the broader understanding of the epidemiology of this virus in the region. Secondly, the significant differences in clinical signs between infected and control groups highlight the importance of thorough clinical examinations in the early detection and management of CAV-1. The increase in body temperature in the infected dogs is one of the findings of canine adenovirus infections which Decaro (2021) reported similar result in infected dogs, also (Al-Hyani and Al-Hasan, 2019), the increase in the temperature refer to inflammatory processes in the viral infection. High body temperature is a physiological response of the body, the biphasic fever in CAV-1 infections considered importance diagnostic indicator (Doronin *et al.*, 2024; Romon-Ochoa *et al.*, 2023). Previous study by Pereira *et al.* (2021) detected changes in pulse rate in CAV-1 infection in dogs, but the pulse rate not be considered diagnostic marker. However, it may still be needed to study other symptoms of disease for clinical diagnosis (Badawi and Yousif, 2020; Decaro, 2021). The significant increase in respiratory rate in the infected dogs consistent with findings by Brown *et al.* (2018), the increase in respiratory rate because of increased oxygen intake during CAV infection. The importance of respiratory rate in the monitoring of animal state and clinical assessment (Al-Obaidi *et al.*, 2021; Matsuu *et al.*, 2020; Priestnall and Sykes, 2021).

The PCR results detecting 9 out of 200 dogs positive for CAV-1 (4.5%). The numbers of studies in Italy documented prevalence of CAV-1 2.9% (4/138 dogs) in stray dead dogs (Cardillo *et al.*, 2020), while other research had 7.8% percentage (4/51 dogs) in alive dogs (Balboni *et al.*, 2014). In Iraq/ Nineveh province when used same ELISA kit of Canine adeno virus constituted 9% seropositive of 78 different dogs (Al-Jumaa *et al.*, 2020a). Although these studies had high infection rate when compared to our studies because of our study conducted on crossbreed without any history to vaccination, but in Italy study on stray dogs not highly variation with current study in Iraq. On the other hand, some reports recognized the seroprevalence of CADV-1 in red foxes about 19% -64.4% in the UK (Thompson *et al.*, 2010; Walker *et al.*, 2016), 3.5% in Germany (Truyen *et al.*, 1998), 23.2% in Australia (Robinson *et al.*, 2005), and 59.6% in Scandinavia (Åkerstedt *et al.*, 2010). The investigation of CAV-1 in these studies was varied in the percentage of infection, and this study suggest the percentage of infection was depended on the type of test, samples, animals, and stage of the disease. The role of CAV-1 in threat life of in dog populations need that for increasing control and vaccination efforts (Abdous *et al.*, 2021; Day *et al.*, 2020; Isvand *et al.*, 2020). The ELISA results, indicating a 2.5% infection rate of CAV-1. This lower rate in ELISA may be due to increase in sensitivity of the PCR. Studies had established the useful the PCR, indicating its use as a importance diagnostic method for diseases in dogs (Al-Jumaa *et al.*, 2020; Badawi, 2022).

On phylogenetic analysis, CAV-1 in Iraq were clustered in same groups, India isolates, Australia isolates, and sequences of UK and Canada isolates formed in the same-group of phylogenetic tree. Spain, Brazil, UK (Accession no. KU755720.1), Italy and Portugal strains formed a different subgroup separated from the Iraq group. The study of sequences of Iraq group revealed an evolutionary divergence 0.0017 with the Indian strain (Accession no. KX181846.1) and 0.00002 the Austerlia strain (Accession no. KT853097.1). However, this study is deepened on E3 region but the partial sequence therefore, further studies with long sequence data are improved to become the sequences analysis a clear picture in Iraq.

CONCLUSION

In conclusion, this study confirmed some the clinical manifestations of CAV-1 infection and considered first molecular evidence of CAV-1 in Iraq.

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NOVELTY STATEMENT

This study is the first to provide molecular evidence of Canine Adenovirus Type 1 (CAV-1) in stray dogs in Iraq, shedding light on the epidemiology and clinical impacts of Infectious Canine Hepatitis (ICH) in this region. Our findings contribute new insights into the prevalence and phylogenetic positioning of CAV-1 in Iraq, establishing a foundation for future research and preventative strategies.

AUTHOR'S CONTRIBUTION

AMBA-D conducted the experimental research, field data collection, and initial data analysis. AKM, as the supervisor, guided the research design, oversaw the laboratory analysis, contributed to data interpretation, and provided substantial input in manuscript preparation and revisions. Both authors were actively involved in the study's final analysis and manuscript completion.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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