The Haryana Veterinarian (March, 2024) **63(SI),** 46-48 ISSN 0033-4359 (P) **Research Article**

MOLECULAR IDENTIFICATION OF CANINE ADENOVIRUS 1 INFECTION IN ADULT DOGS WITH HAEMATOLOGICAL PARAMETERS OF LEUKOPENIA AND THROMBOCYTOPENIA

G. SATHISH, M. PARTHIBAN, M. CHANDRASEKAR[1], P. RAJA and P.S.L. SESH[2]
Department of Animal Biotechnology, [1]Department of Veterinary Medicine,
[2]Department of Veterinary Biochemistry, Madras Veterinary College, Chennai-600007, Tamil Nadu, India

Received: 20.06.2023; Accepted: 09.08.2023

ABSTRACT

Nowadays canine adenovirus is uncommonly reported in India because of regular vaccination and is less notifiable comparing with other common viral diseases like canine parvovirus and canine distemper virus. Sometimes, longstanding clinical adult dog cases presented with leukopenia and elevated liver enzymes parameters even after treatment with other infectious causes make suspicious of other viral cause of disease. Here in this study, 50 suspected dogs presented to small animal clinics of Madras Veterinary College were screened for canine adeno virus (CAdV) with specific primers. Four cases exhibited positive amplification for CAdV-1 and further positive PCR products were analysed for sequencing. Sequencing results confirmed homology with specific canine adenovirus 1 sequences by BLAST analysis. Although the incidence rate of CAdV-1 in suspected cases is lower (8%), we cannot ignore the canine adenoviral infection in regularly vaccinated dog population. Further research is reviewed for causative factors and pathogenesis of canine adenovirus 1 infection.

Keywords: Canine adenovirus 1-PCR, Haemato biochemical parameters, Molecular identification

How to cite: Sathish, G., Parthiban, M., Chandrasekar, M., Raja, P. and Sesh, P.S.L. (2024). Molecular identification of canine adenovirus 1 infection in adult dogs with haematological parameters of leukopenia and thrombocytopenia. *The Haryana Veterinarian* **63(SI)**: 46-48.

Infectious canine hepatitis (ICH), a systemic deadly viral disease that was once regularly reported in dogs, is caused by canine adenovirus type 1 (CAdV-1). The veterinary researcher Carl Swen Rubarth initially identified ICH in Swedish dogs in the 1940s (Rubarth, 1947), and it was then widely reported in the years that followed as "Rubarth's disease" (Parry, 1950). The Adenoviridae family of viruses includes the non-enveloped icosahedral double-stranded DNA virus known as CAdV-1 (Appel, 1987). According to the International Committee on Taxonomy of Viruses (ICTV) (Adams *et al.*, 2014), the canine adenovirus species has just undergone a taxonomically correct name change to Canine mastadenovirus A. The canine adenovirus type 2 (CAdV-2) is closely linked to CAdV-1 both genetically and antigenically. Since after 1980, the canine adenovirus type 2 (CAdV-2) has been used as a vaccine strain to prevent CAdV type 1-induced vaccine-associated adverse events in dogs (Zhu *et al.*, 2022). CAdV-2 is the cause of the canine upper respiratory disease formerly known as "canine infectious tracheobronchitis" (Bergmann *et al.*, 2020). The Canidae (dogs, foxes, and wolves), Ursidae, and Mustelidae families, as well as the Mustelidae, have all been reported to have CAdV-1 infection to date (Decaro *et al.*, 2008, Olega *et al.*, 2022). Although vaccination has decreased the prevalence of CAdVs in the domestic dog population, isolated outbreaks of CAdV-1 infection have occurred occasionally but consistently been reported. This has led to ongoing concerns about the pathogen's current prevalence, circulation, and role at the domestic/wild animal interface, as well as potential risks for endangered wild species (Balboni *et al.*, 2014, Dowgier *et al.*, 2018). Occasionally identified alone or in co-infection with other microbiological pathogens (Mira *et al.*, 2022), CAdV-1 infection in domestic dogs has been linked to inadequate vaccination coverage in kennelled dogs or to unrestricted dog imports (Decaro *et al.*, 2008). The aim of this study was to evaluate the CAdV-1 infection in adult dogs with haematological signs parameters of leukopenia and thrombocytopenia.

*Corresponding author: sg20685@gmail.com

MATERIALS AND METHODS

Collection of suspected samples:

Collection of blood samples in MVC clinics from adult dogs (more than 1 year of age) with clinical signs of fever, in appetence and vomition and subjected to analysis of haematological parameters like thrombocytopenia ($<150\times10^3/\mu L$), leukopenia ($<4.5\times10^3/\mu L$) and elevated liver enzymes (ALT>60 U/L, ALP>150 U/L). Recently adeno viral vaccinated dogs, dogs suspected with parvoviral enteritis and canine distemper were excluded in this study.

Screening of canine adenovirus using differential PCR:

Genomic DNA was extracted from blood as per described protocol in Sathish *et al.*, 2021. DNA samples were screened for presence of canine adenovirus type I and II by differential PCR with already published primers (Hu *et al.*, 2001). The reaction mixtures were prepared in 20.0 µl volume as Red dye master mix (2X)-10.0 µl, Canine adenovirus Forward primer (10 pmol/µl) - 1.0 µl, Canine adenovirus Reverse primer (10 pmol/µl) - 1.0 µl, DNA

template - 2.0 µl, nuclease free water - 6.0 µl. Following were the cycling condition of the PCR reaction: Initial denaturation at 94° C for 5 min; 32 cycles of denaturation at 94° C for 45 sec, annealing at 58° C for 1 min, extension at 72° C for 1 min and final extension at 72° C for 7 min.

Organisms	Primer	CAdV Type	Disease	Product length
Canine Adenovirus	5'-CGCGCTGAACAT TACTACCTTGTC-3'	CAdV-1	Infectious Canine Hepatitis	508 bp
	5'-CCTAGAGCACTT CGTGTCCGCTT-3'	CAdV-2	Infectious Canine Laryngo-tracheitis	1030 bp

Sequencing of PCR products

Positive PCR products of canine adenovirus 1 identified in above study were further sequenced for confirming similarities by comparing it with reference strains available in the Genbank database.

RESULTS AND DISCUSSION

The suspected samples for screening of canine adenovirus 1 were identified initially based on haematological parameters. Later on liver function test parameters mainly Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) levels were included in this study. White Blood Cell (WBC) and thrombocytes count of collected blood were categorized and presented in Table 1. ALT and ALP serum parameters were also categorized and presented in Table 1. All the samples were screened for canine adenovirus 1 using previously reported differential PCR primers for differentiation of canine adenovirus 1 and 2. Four samples were showed positive amplification of 508 bp PCR product which confirms the presence of canine adenovirus-1 (Fig. 1). All the 4 samples were leukopenic with less than 5×10^3/µl WBC count. While thrombocytopenia was taken in account that 3 out of 4 PCR positive samples were shown less than 75×10^3/µl category and one was 75-150$\times10^3$/µl range of category. When ALP and ALT level were taken in account, ALP of all the 4 samples were more than 350 U/L, ALT of 3 samples were more than 250 U/L and one sample was 61-250 U/L. No samples were showed positive for CAdV-2 which causes localized respiratory tract infection in dogs called as kennel cough.

By using BLAST analysis, which revealed 99% similarity with other known sequences in the NCBI database, it was also established that the sequencing of purified PCR products contained canine adenovirus 1 (Fig. 2). A non-coding segment and the U-exon gene sequences make up the majority of the CAdV-1 sequences that are currently obtainable and stored in the GenBank database. Among the investigated genes, this target, which is

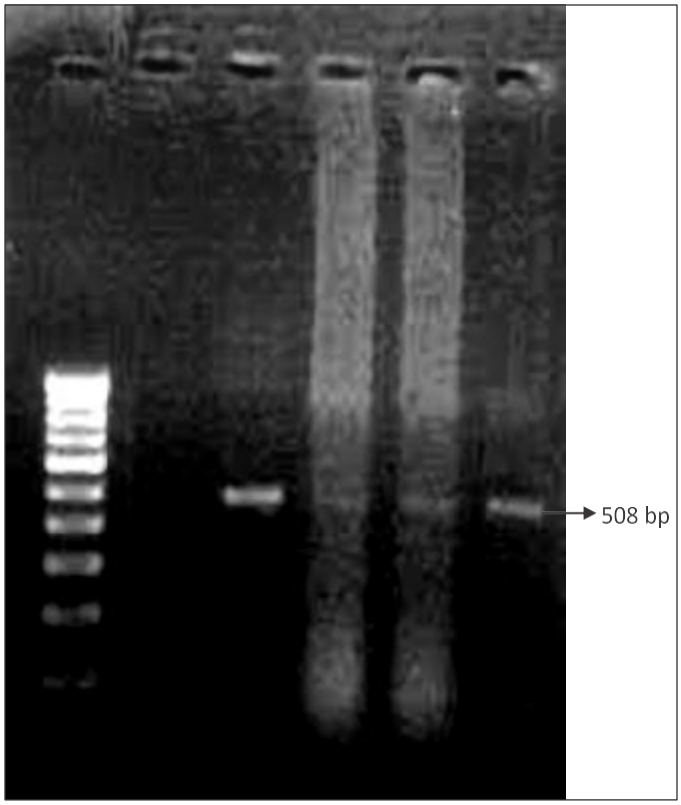

Fig. 1. Screening of suspected blood DNA samples for CAdV-1 by PCR
Lane 1: 100 bp DNA molecular weight marker
Lane 2: Nuclease free water
Lane 3, 4, 5, 6: CAdV-1positive blood DNA

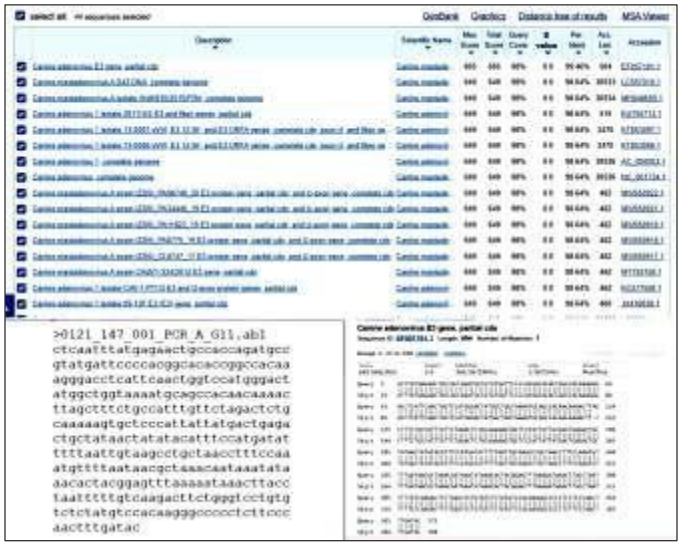

Fig. 2. BLAST analysis of Canine Adenovirus sequence obtained from sequencing of purified positive PCR product

frequently discussed in the present literature for diagnostic assays able to distinguish between CAdV-1 and CAdV-2 (Balboni *et al.*, 2015), displayed the lowest nucleotide and amino acid divergences. A wider diagnostic panel should be taken into consideration to prevent the underestimating of any condition due to the similarities of clinical signs and lesions caused by several enteric viruses as well as the

Table 1. Categorization of hematological analysis and PCR screening of CAdV-1 infection for suspected samples

Parameters	Category	WBC COUNT (×10^3/µl) (leukopenia)	
		BELOW 5	5-7
Platelet count	Below 75 (x 10^3/µl)	10	13
	76-150 (× 10^3/µl)	12	8
	Above 150 (× 10^3/µl)	3	4
Alanine transaminase (ALT)	61-250 U/L	11	13
	Above 250 U/L	14	12
Alkaline phosphatase (ALP)	151-350 U/L	12	10
	Above 350 U/L	13	15
CAdV-1 PCR positive		4	-

1 2 3 4 5 6

potential superimposition of findings in co-infections (Mira *et al.*, 2022).

Additionally, due to immunosuppressive effects and the high probability of failure in the study's test dogs, as previously noted (Alves *et al.*, 2018), co-infections may have contributed to the pathogenicity of the illness. Out of 4 positive cases only one case was recovered after treatment. Other 3 clinical cases were diagnosed at later stage of treatment and were not recovered. In conclusion, it is important to recognize CAdV-1 as a potential cause of domestic dog infection given its ongoing circulation and the genetic characteristics presented in this investigation. These findings give us comprehensive understanding of the epidemiology and development of the many genetic variations of CAdV-1 for future comparative investigations.

CONCLUSION

The samples suspected for viral etiology based on haemato-biochemical parameters showed 8% positive for canine adenoviral infection by molecular diagnosis. Although the incidence rate of CAdV-1 in suspected cases is lower, the ignorance of the canine adenoviral infection in well equipped regularly vaccinated dog population should be reviewed for causative factors and pathogenesis of canine adenovirus 1 infection.

REFERENCES

Adams, M.J., Lefkowitz, E.J., King, A.M.Q. and Carstens, E.B. (2014). Ratification Vote on Taxonomic Proposals to the International Committee on Taxonomy of Viruses. *Arch. Virol.* **159**: 2831-2841.

Alves, C.D.B.T., Granados, O.F.O., da F. Budaszewski, R., Streck, A.F., Weber, M.N., Cibulski, S.P., Pinto, L.D., Ikuta, N. and Canal, C.W. (2018). Identification of Enteric Viruses Circulating in a Dog Population with Low Vaccine Coverage. *Braz. J. Microbiol.* **49**: 790-794.

Appel, M.J. (1987). Canine adenovirus type 1 (infectious canine hepatitis virus). In Virus Infections of Carnivores; Elsevier Science: Amsterdam, The Netherlands. pp. 29-43.

Balboni, A., Dondi, F., Prosperi, S. and Battilani, M. (2015). Development of a SYBR Green Real-Time PCR Assay with Melting Curve Analysis for Simultaneous Detection and Differentiation of Canine Adenovirus Type 1 and Type 2. *J. Virol. Methods.* **222**: 34-40.

Balboni, A., Mollace, C., Giunti, M., Dondi, F., Prosperi, S. and Battilani, M. (2014). Investigation of the Presence of Canine Adenovirus (CAdV) in Owned Dogs in Northern Italy. *Res. Vet. Sci.* **97**: 631-636.

Bergmann, M., Freisl, M., Zablotski, Y., Speck, S., Truyen, U. and Hartmann, K. (2020). Antibody Response to Canine Adenovirus -2 Virus Vaccination in Healthy Adult Dogs. *Viruses.* **12**: 1198.

Decaro, N., Martella, V. and Buonavoglia, C. (2008). Canine Adenoviruses and Herpesvirus. *Vet. Clin. North. Am. Small Anim. Pract.* **38**: 799-814.

Dowgier, G., Lahoreau, J., Lanave, G., Losurdo, M., Varello, K., Lucente, M.S., Ventriglia, G., Bozzetta, E., Martella, V., Buonavoglia, C. and Decaro, N., (2018). Sequential Circulation of Canine Adenoviruses 1 and 2 in Captive Wild Carnivores, France. *Vet. Microbiol.* **221**: 67-73.

Hu, R.L., Huang, G., Qiu, W., Zhong, Z.H., Xia, X.Z. and Yin, Z. (2001). Detection and differentiation of CAV-1 and CAV-2 by polymerase chain reaction. *Vet. Res. Commun.* **25**: 777-84.

Mira, F., Puleio, R., Schirò, G., Condorelli, L., Di Bella, S., Chiaramonte, G., Purpari, G., Cannella, V., Balboni, A., Randazzo, V., Antoci, F., Vicari, D. and Guercio, A. (2022). Study on the Canine Adenovirus Type 1 (CAdV-1) Infection in Domestic Dogs in Southern Italy. *MDPI Pathogens.* **11**: 1254.

Oleaga, A., Balseiro, A., Espí, A. and Royo, L.J. (2022). Wolf (Canis lupus) as Canine Adenovirus Type 1 (CAdV-1) Sentinel for the Endangered Cantabrian Brown Bear (Ursus arctos arctos). *Transbound. Emerg. Dis.* **69**: 516-523.

Parry, H.B. (1950). Viral Hepatitis of Dogs (Rubarth's Disease). I. Clinical and Pathological Observations on a Spontaneous Epidemic. *Vet. Rec.* **62**: 359-365.

Rubarth, S. (1947). An Acute Virus-Disease with Liver Lesion in Dogs (Hepatitis Contagiosa Canis), a Pathologico-Anatomical and Etiological Investigation. *Acta Pathol. Microbiol. Scand.* https://www.ncbi.nlm.nih.gov/nlmcatalog?cmd=PureSearch&term=0012136R%5Bnlmid%5D.

Zhu, Y., Xu, J., Lian, S., Zhang, R., Hou, J., Wang, M. and Yan, X. (2022). Difference Analysis Between Canine Adenovirus Types 1 And 2. *Front. Cell. Infect. Microbiol.* **12**: 854-876.