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Original article

Genomic and immunoinformatic characterization of canine adenovirus type 1 in parvovirus-infected dogs: A comprehensive study from northeast India

S.K. Behera[1], L. Maibam[1], M. Lalhmangaihzuali[1], B.D. Sahu[2], J. Sahu[2], A.N. Marak[1], J.B. Rajesh[1], P. Roychoudhury[3], G. Das[4], P. Chakraborty[5], W.K. Maibam[6], H. Prasad[1], P.K. Subudhi[3]

[1] Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry,
Central Agricultural University, Selesih-796 015, Aizawl, Mizoram, India
[2] Office of Deputy Director of Veterinary Services Ambikapur, Surguja, Chhattisgarh, India
[3] Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry,
Central Agricultural University, Aizawl, Mizoram, India
[4] Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry,
Central Agricultural University, Jalukie, Nagaland, India
[5] Department of Veterinary Medicine,
West Bengal University of Animal & Fishery Sciences, Kolkata – 700 037, West Bengal, India
[6] Department of Veterinary and Animal Husbandry Services, Imphal, Manipur, India

Correspondence to: S.K. Behera, e-mail: suv4505ivri@gmail.com, tel.: +917978035663

Abstract

Canine adenovirus type 1 (CAdV-1), the causative agent of infectious canine hepatitis (ICH), a fatal disease affecting domestic and wild canids, yet its prevalence and molecular characteristics remain underexplored in India's north eastern region (NER). This study presents the first comprehensive genomic and immunoinformatic analysis of CAdV-1 in dogs in the region. Out of 208 canine parvovirus type-2 (CPV-2) positive fecal samples, 36 (17.30%) tested positive for CAdV-1 by PCR. Of the 36 positive samples, 25 samples were sequenced. Deduced amino acid analysis revealed notable amino acid mutations, including Asn127Asp, His129Arg, Trp148Ser, Leu201Pro, Thr206Met and Gly215Glu. Sequence analysis of the 25 field samples revealed distinct regional clustering consistent with regional viral evolution. In terms of relatedness to global strains, the NER isolates showed highest similarity to Asian and European canid and wildlife-origin isolates with 96% to 100% amino acid homology. Selection pressure analysis revealed predominantly purifying selection. aBSREL and Contrast-FEL identified a few codons potentially experiencing weak or episodic positive selection, likely reflecting host immune adaptation. GARD analysis ruled out evidence of recombination. Immunoinformatic prediction identified B-cell epitope, "NKTTTEATIITY ISMTFLLVSLTLFLNLVTLTL," in most CAdV-1 sequences making it a suitable candidate for future vaccine development. The MHC-I binding peptide "LTFPNVLITLNNKY" (positions 83-96) demonstrated a strong affinity for the canine allele, suggesting its potential for triggering cytotoxic T-cell responses. These findings shed new light on the molecular epidemiology of CAdV-1 in the NER and highlight the critical need for multi-pathogen screening, molecular surveillance at wildlife – livestock interfaces, and future whole-genome studies to explicate viral evolution, host interactions, immune evasion, and regional strain diversity.

Keywords: CAdV-1, CPV-2 co-infection, epitope mapping, mutations, North Eastern India, phylogenomics

Introduction

Infectious canine hepatitis (ICH) is a highly contagious and often fatal disease that impacts dogs and wild canids globally, including India. It is caused by Canine adenovirus-1 (CAdV-1), a non-enveloped icosahedral double-stranded DNA virus belonging to the species *Mastadenovirus canida,* genus *Mastadenovirus* in the family *Adenoviridae* (International Committee on Taxonomy of Viruses, ICTV, https://ictv.global/taxonomy/taxondetails?taxnode_id=202402418&taxon_name=-Mastadenovirus%20canidae, accessed on October 2025). The disease was first identified in dogs by a Swedish veterinarian named Carl Swen Rubarth back in the 1940s, and has since been referred to as "Rubarth's disease" (Rubarth 1947). It spreads through direct contact with infected animals or indirectly through contaminated urine, faeces, saliva or respiratory secretions (Sykes et al. 2023).

Common signs are fever, depression, anorexia, mucosal congestion, vomiting, lymphadenopathy, jaundice, bloody diarrhoea, abdominal pain, epistaxis, ocular lesions, neurological signs, and may result in sudden death, particularly in unvaccinated or young dogs (Sykes et al. 2023). Mortality can vary from 10%-30% in unvaccinated populations and may rise with co-infections (Hornsey et al. 2019).

For molecular detection and typing, previous research has demonstrated a single primer pair targeting E3 gene region producing clearly distinguishable products, approximately 508 bp for CAV-1 and 1030 bp for CAV-2, allowing clear differentiation of the two serotypes (Hu et al. 2001). Additionally the E3 region encodes several immunomodulatory proteins that regulate host immune evasion, viral virulence and adaptation to different hosts, playing a crucial role in virus – host interactions (Wang et al. 2022). Earlier reports have also identified key amino acid substitutions in E3 region linked to CAV-1's virulence, behavior, and immune evasion mechanism (Balboni et al. 2022, Mira et al. 2022). While such mutations have been reported globally, their functional relevance and distribution among circulating Indian strains remain unknown.

To date, CAdV-1 infection has been described globally both in domestic and wild members of the families *Canidae*, *Mustelidae*, and *Ursidae* (Sykes et al. 2023). In India, it has been sporadically documented (Chethan et al. 2021, Syamily et al. 2023, Chakraborty et al. 2024a), with very scarce data from the North Eastern Region (NER). The NER's unique ecological setting, characterized by porous international borders, complex wildlife – livestock interfaces, inadequate vaccination coverage in owned pets, and unregulated animal movement may facilitate viral persistence and spillover (Barthakur et al. 2018, Balboni et al. 2022).

CAdV-1 often co-occurs with pathogens such as canine parvovirus-2 (CPV-2), canine distemper virus, canine adenovirus-2, canine circovirus, fungal infections, and *Pasteurella pneumotropica,* which complicate disease severity, diagnosis, and clinical outcomes (Headley et al. 2019, Balboni et al. 2022, Silva et al. 2023, Chakraborty et al. 2024b). Notably, only two cases from Mizoram have been reported by our team (Chakraborty et al. 2024a) recently, underscoring the critical need for better surveillance and molecular characterization to elucidate the virus's epidemiology and evolution.

Beyond epidemiological gaps, there is also a paucity of data on the immunogenic regions (epitopes) of CAdV-1 proteins that are essential for host – virus interactions and vaccine development. Immunoinformatics approaches including, B-cell and T-cell epitope prediction provide valuable insights for understanding viral evolution, protective immune targets, and promising vaccine candidates (Wang et al. 2022). Yet, such analyses remain largely unexplored to CAdV-1 strains circulating in India, leaving a significant knowledge gap.

To address these gaps, the objective of the study was to retrospectively examine CPV-2-positive fecal samples from dogs with gastroenteritis in Northeast India to detect CAdV-1. Additionally, the study focused to understand the molecular epidemiology of CAdV-1 by analyzing the evolutionary relationships of detected strains using phylogenomics and selection-pressure analysis, along with conducting immunoinformatic analyses, including epitope mapping to identify key antigenic regions.

Materials and methods

Ethical approval

All animal experiments were conducted following Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines and were approved by the Institutional Animal Ethics Committee (1476/GO/Re/SL/2011/CPCSEA dated 12th June 2023).

Study area, population and sample collection

This study retrospectively screened CPV-2 positive fecal samples from eight States in North Eastern Region (NER) of India including Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim (Fig. 1) for the presence of CAdV-1. The samples were collected over a period from 2015 through 2023. Total 208 CPV-2 positive fecal samples were screened for CAdV-1 by PCR.

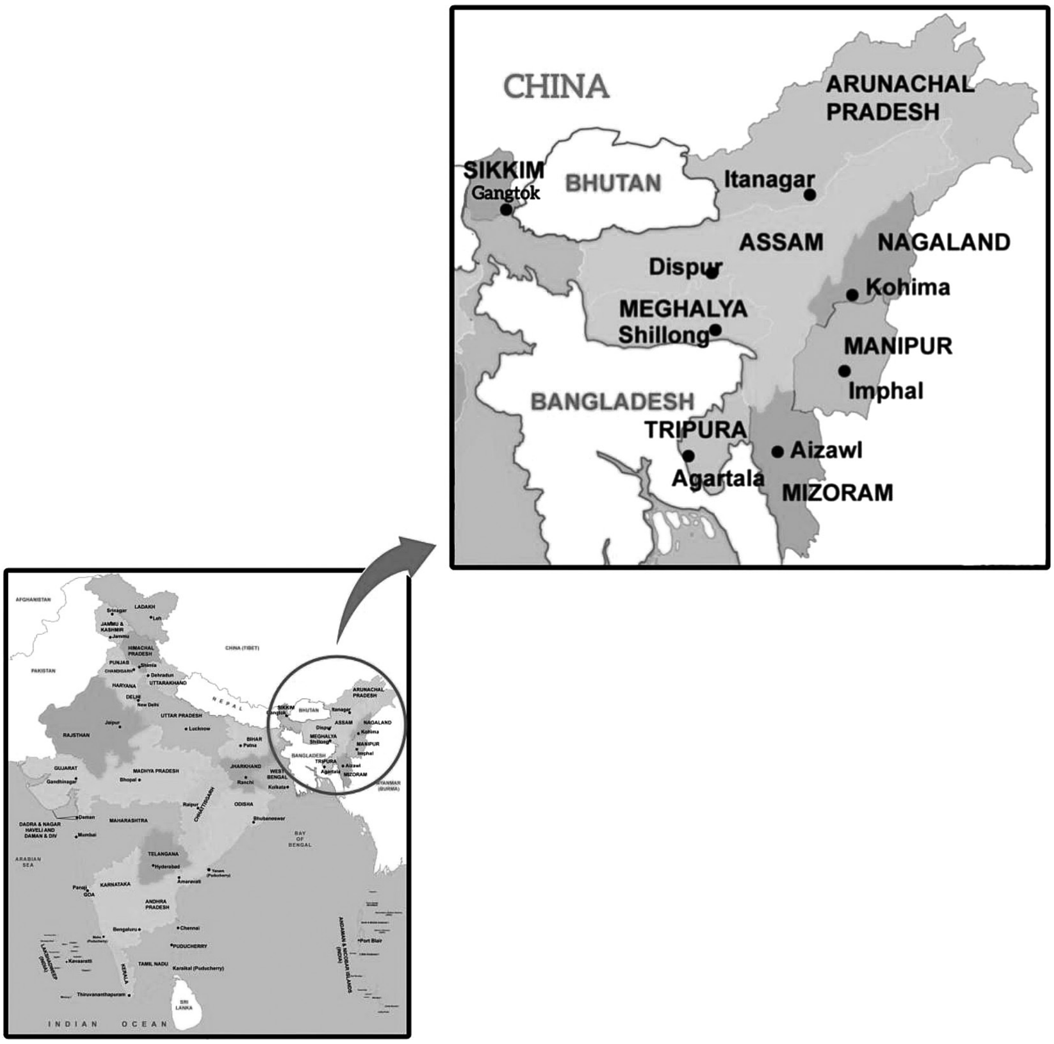

Fig. 1. The map illustrates the Northeastern Hill (NEH) states of India that were included for sample collection in the present study.

PCR screening and amplification of E3 gene fragment

Viral DNA was extracted from stool and vomit samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen), and stored at -20°C until further use. Extracted DNA was screened for CAdV-1 using published E3 gene-specific primers (FP-HA1: CGCGCTGAA CATTACTACCTTGTC, RP-HA2: CCTAGAGCACTT CGTGTCCGCTT) amplifying a 509 bp fragment (Hu et al. 2001). PCR was carried out in a 25 µL reaction mixture containing 50-100 ng template DNA, 12.5 µL of 2x PCR master mix (Thermo Fisher Scientific), 1 µL each of forward and reverse primer (10 pmol/µL), and nuclease-free water. The cycling conditions were: 95°C for 5 min, 35 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 90 s, and final extension at 72°C for 10 min. Amplicons were resolved on 1.5% agarose gel with a 100 bp marker and visualized under UV using a gel documentation system (Vilber Bioprint, France).

Cloning of PCR products

Among the 36 CPV-2 and CAdV-1 co-infected samples, 25 were selected for sequencing based on sufficient DNA quantity and purity, clearly visible PCR amplicons on the gel and absence of non-specific amplification. Samples that resulted weak amplification, or degraded DNA were excluded from sequencing. The 25 positive PCR products were purified from agarose gel using the GeneJET Gel Extraction Kit (Thermo Scientific, K0702) and cloned with the CloneJET PCR Cloning Kit (Thermo Scientific, K1232) following the manufacturer's protocol. Recombinant plasmids confirmed by PCR, were outsourced for sequencing.

Sequence analysis

Nucleotide sequences were edited and checked for vector contamination using NCBI VecScreen, then analyzedin BioEdit v 7.2.5. Sequence identity was verified using BLAST against the GenBank and multiple alignments of nucleotide and deduced amino acid sequences

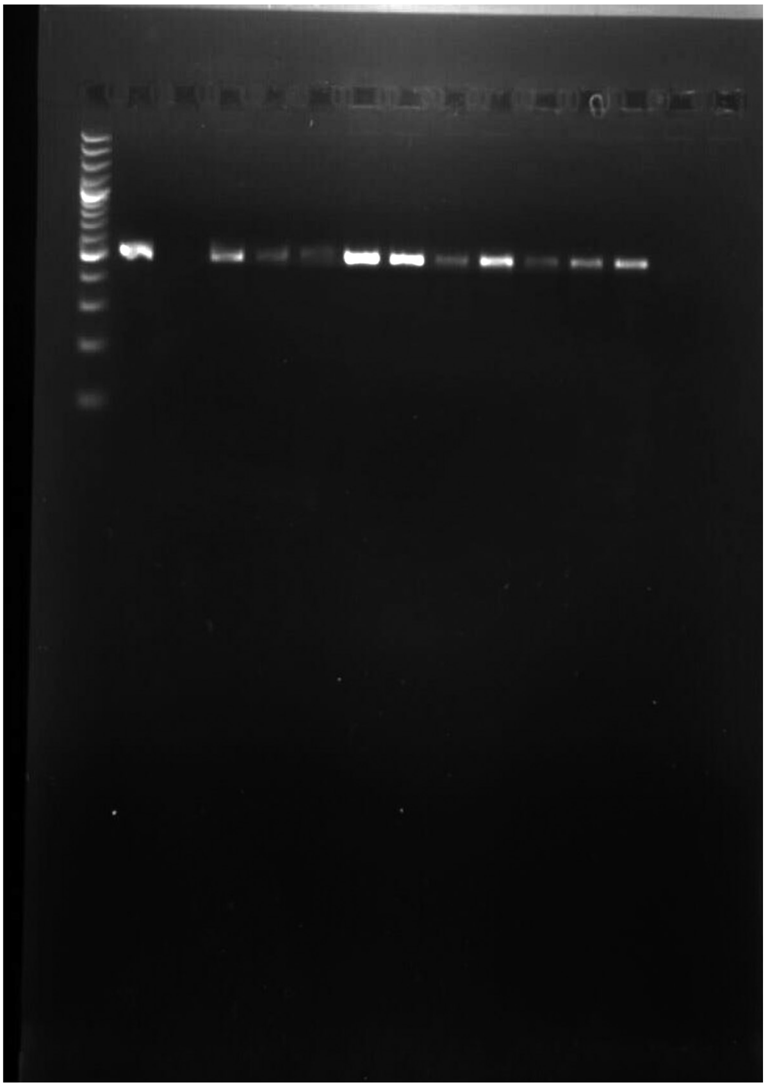

Fig. 2. PCR amplification of the E3 gene of CAdV-1 showing 509 bp product. Lane 1: 100bp plus Gene Ruler, Lane 2: positive control (vaccine strain of CAdV-1), Lane 3: Negative control Lane 4-13: samples showing positive amplification.

were carried out using ClustalW in MEGA XI. Thealigned partial E3gene sequences were submitted to Genbank for accession numbers.

Phylogenetic analysis

Phylogenetic analysis was inferred using the Neighbor-Joining method, applying the Tamura 3-parameter model along with 1000 bootstrap replicates in MEGA XI. E3 gene sequences from this study were analyzed alongside reference sequences from GenBank to assess similarity and divergence. The reference CAdV-1 sequences were selected based on availability of partial E3 region sequences of comparable length in GenBank, representation of diverse geographic regions, inclusion of both domestic dog and wild canid isolates, inclusion of E3 region of Indian CAdV-1 isolates to facilitate regional comparison and coverage of different years to capture temporal diversity.

Selection pressure analysis

Selection pressure on E3 gene was analyzed by examining the ratio of nonsynonymous (dN) to synonymous (dS) mutations (dN/dS) using Single-Likelihood Ancestor Counting (SLAC) via the Datamonkey web server (http://www.datamonkey.org). A dN-dS ratio of <1.0, 1.0, and >1.0 signifies negative, neutral, and positive selection, respectively. Additional analyses were conducted using Fixed Effects Likelihood (FEL), Fast Unconstrained Bayesian Approximation (FUBAR), Mixed Effects Model of Evolution (MEME), aBSREL (adaptive Branch-Site Random Effects Likelihood) analysis, Contrast-FEL and GARD (Genetic Algorithm for Recombination Detection) methods on the same platform (Weaver et al. 2018). Sites were considered significant at posterior probability >0.9 (FUBAR) or p<0.1 (FEL, SLAC, MEME).

Table 1. Descriptive statistics of the canine adenovirus type-1 (CAdV-1)affected dogs included in the study population (n=25) and canine parvovirus type-2 (CPV-2) variant co-infections.

Sl. No.	Isolate ID	Year	State	Breed	Age (months)	Sex	Vaccination	Accession no.	CPV-2 variant co-infection
1.	Arunachal Pradesh 1/ICH/India	2015	Arunachal Pradesh	Mixed	3	M	No	PV743807	2a
2.	Arunachal Pradesh 11/ICH/India	2021	Arunachal Pradesh	Mixed	3	M	No	PV743808	2c
3.	Arunachal Pradesh 24/ICH/India	2020	Arunachal Pradesh	Mixed	2	M	No	PV743809	2a
4.	Assam 3/ICH/India	2021	Assam	Golden Retriever	2.5	M	No	PV743806	2c
5.	Manipur 1/ICH/India	2016	Manipur	German Shepherd	2	F	No	PV743810	2c
6.	Manipur 35/ICH/India	2021	Manipur	Mixed	2.5	F	No	PV743811	2c
7.	Manipur 38/ICH/India	2021	Manipur	Mixed	7	M	No	PV743812	2c
8.	Manipur 40/ICH/India	2021	Manipur	Beagle	2	M	No	PV743813	2a
9.	Mizoram 68/ICH/India	2015	Mizoram	Mixed	6	M	No	PV743814	2b
10.	Nagaland 12/ICH/India	2021	Nagaland	Mixed	3	F	No	PV743815	2c
11.	Nagaland 17/ICH/India	2021	Nagaland	Non-descript	3	F	No	PV743816	2c
12.	Nagaland 19/ICH/India	2021	Nagaland	Non-descript	3	F	No	PV743817	2c
13.	Nagaland 23/ICH/India	2021	Nagaland	Non-descript	18	M	No	PV743818	2a
14.	Nagaland 28/ICH/India	2022	Nagaland	Non-descript	4	F	No	PV743819	2c
15.	Nagaland 29/ICH/India	2022	Nagaland	Non-descript	4	F	No	PV743820	2c
16.	Nagaland 44/ICH/India	2022	Nagaland	Non-descript	5	M	No	PV743821	2a
17.	Sikkim8/ICH/India	2021	Sikkim	Non-descript	3	M	No	PV743822	2c
18.	Sikkim9/ICH/India	2021	Sikkim	Non-descript	4	F	No	PV743823	2c
19.	Sikkim11/ICH/India	2021	Sikkim	Mixed	2.5	F	No	PV743824	2c
20.	Tripura 3/ICH/India	2020	Tripura	Non-descript	5	M	No	PV743825	2c
21.	Tripura 7/ICH/India	2020	Tripura	Non-descript	3.5	M	No	PV743826	2c
22.	Tripura 9/ICH/India	2016	Tripura	Labrador	2	M	No	PV743827	2a
23.	Tripura 22/ICH/India	2020	Tripura	Non-descript	3	M	No	PV743828	2c
24.	Tripura 31/ICH/India	2021	Tripura	Mixed	2	M	No	PV743829	2c
25.	Mizoram 2/ICH/India	2023	Mizoram	Golden Retriever	11	M	No	PQ634665	2c

B and T cell epitope prediction

Putative B-cell epitopes were predicted using BepiPred-2.0 (v2.24) (El-Manzalawy et al. 2008). MHC-I binding peptides were obtained using the IEDB Analysis Resource (Ndiana et al. 2022) for the canine allele DLA-8803401, using 14 amino acids (aa) peptides with the source species mentioned as dog. Results were ranked by binding scores and displayed in XHTML format.

Results

Molecular epidemiology data

Of 208 CPV-2 positive fecal samples, 36 (17.3%) were PCR-positive for CAdV-1 (Fig. 2) and 25 were cloned and sequenced. Table 1 provides a summary of the descriptive statistics for the dogs in the study population. The median age of the dogs was 3 months (range: 2-18) and majority of the population was unvaccinated (24/25, 96%). Prevalence was highest in non-descript (11/25, 44%) followed by mixed breed (8/25, 32%) and pure-breed (6/25, 24%) dogs. Seasonally, cases peaked in spring (44.4%), followed by winter (30.6%) and summer (25%).

Sequencing, nucleotide substitution and amino acid mutation

A 509 bp fragment of the E3 gene was sequenced, yielding a 101-aa sequence compared with 27 CAdV-1 references from NCBI. Notable non-synonymous aa mutations included Asn127Asp, His129Arg, Trp148Ser, Leu201Pro, Thr206Met and Gly215Glu (Table 2). Nucleotide substitutions detected were T→C (348, 477, 602, 642), A→G (379, 386, 579), G→C (443), G→A (644), and C→T (617) (Table 2).

Phylogenetic and homology analyses

Phylogenetic analysis based on deduced aa sequences (Fig. 3) revealed multiple distinct clusters among

Table 2. Nucleotide and amino acid substitutions in the CAdV-1 E3 gene of north eastern region (NER) isolates compared to reference strains.

Isolate ID	Accession No.	Nucleotide Substitutions (Position → Change)	Amino Acid Substitutions (Position → Change)
Nagaland 19	PV743817	348 T→C	-
Sikkim 9	PV743823	386 A→G, 644 G→A	129 H→R, 215 G→E
Nagaland 23	PV743818	379 A→G	127 N→D
Nagaland 28	PV743819	443 G→C, 617 C→T	148 W→S, 206 T→M
Sikkim 8	PV743822	617 C→T	206 T→M
Manipur 35	PV743811	477 T→C, 602 T→C, 642 T→C	201 215 L→P
Arunachal Pradesh 1	PV743807	443 G→C, 617 C→T	148 W→S, 206 T→M
Tripura 31	PV743829	579 A→G	-

Table 3. Selection pressure analysis on the sequenced E3 gene of CAdV-1.

Sites of positive selection				Sites of purifying/negative selection				dN/dS
FEL[1]	SLAC[1]	FUBAR[2]	MEME[1]	FEL[1]	MEME[1]	FUBAR[2]	SLAC	
None	None	None	None	Negative selection in eight sites (18, 29, 35, 63, 71, 76, 84, 85)	Negative selection in eight sites (18, 29, 35, 63, 71, 76, 84, 85)	None	None	0.503

[1] P-value threshold of 0.1, and [2] Posterior probability of ≥0.90

NER isolates. One cluster (Arunachal Pradesh 11, Manipur 40, Nagaland 17 and 19, Tripura 9, 7 and 31 and Manipur 38) was closely related to isolates from India (KY272053), China (KJ451612), and Italy (MW650917), suggesting Asian–European links. Another group, including Tripura 3 and 22, Sikkim 11, Nagaland 12 and 44, Manipur 1, and Assam 3 shared ancestry with Indian bear and dog sequences. Sikkim 9 and Mizoram 2 clustered with red fox isolates from Italy, while Nagaland 23 grouped with an Indian dog and an Iberian wolf from Spain. Nagaland 29, Mizoram 68, and Arunachal Pradesh 24 formed a cluster with an Iraqi isolate. Another distinct cluster comprised of Sikkim 8, Nagaland 28, and Arunachal Pradesh 1 clustered with an Indian strain (MZ574094). Overall, isolates shared 98.3-100% aa identity among themselves and 96-100% with reference strains.

Selection pressure analysis

Selection pressure analysis of the E3 gene showed most codons under purifying selection. Codons 18, 29, 35, 63, 71, 76, 84, and 85 were consistently negatively selected (Table 3). aBSREL detected weak/episodic diversifying selection at codons 18, 63, 76, and 85, while Contrast-FEL suggested a few codons may be shifting toward positive selection. GARD found no significant recombination, though seven potential breakpoints were inferred at ~50, 85, 105, 185, 210, 225, and one additional site.

B and T cell epitope prediction

Nearly all sequences contained the conserved C-terminal B-cell epitope motif (NKTTTEATIITY ISMTFLLVSLTLFLNLVTLTL) highlighting strong immunogenicity. Sequences PV743815–PV743821 and PV743825–PV743829 showed broad epitope coverage, identifying them as potential vaccine targets (Table 4). IEDB MHC-I prediction highlighted LTFPNVLITL NNKY (positions 83-96) as the top binder to canine allele DLA-8803401, with additional peptides at positions 14-27 suggesting an immunodominant region (Table 5).

Discussion

This study reports the first comprehensive genomic and immunoinformatic analysis of CAdV-1 infection in the NER of India, a region where the virus remains under-evaluated. Despite widespread canine vaccination reducing disease incidence, the disease still re-emerges in both domestic and wild canid populations across India, Iran, Italy, the United Kingdom, Switzerland, the United States and Turkey (Balboni et al. 2022) raising concerns about vaccine gaps, waning immunity, or viral evolution (Mira et al. 2022).

Of 208 CPV-2 positive fecal samples, 17.3% were positive for CAdV-1, confirming its ongoing circulation and pathogenic potential (Balboni et al. 2022). Co-infec-

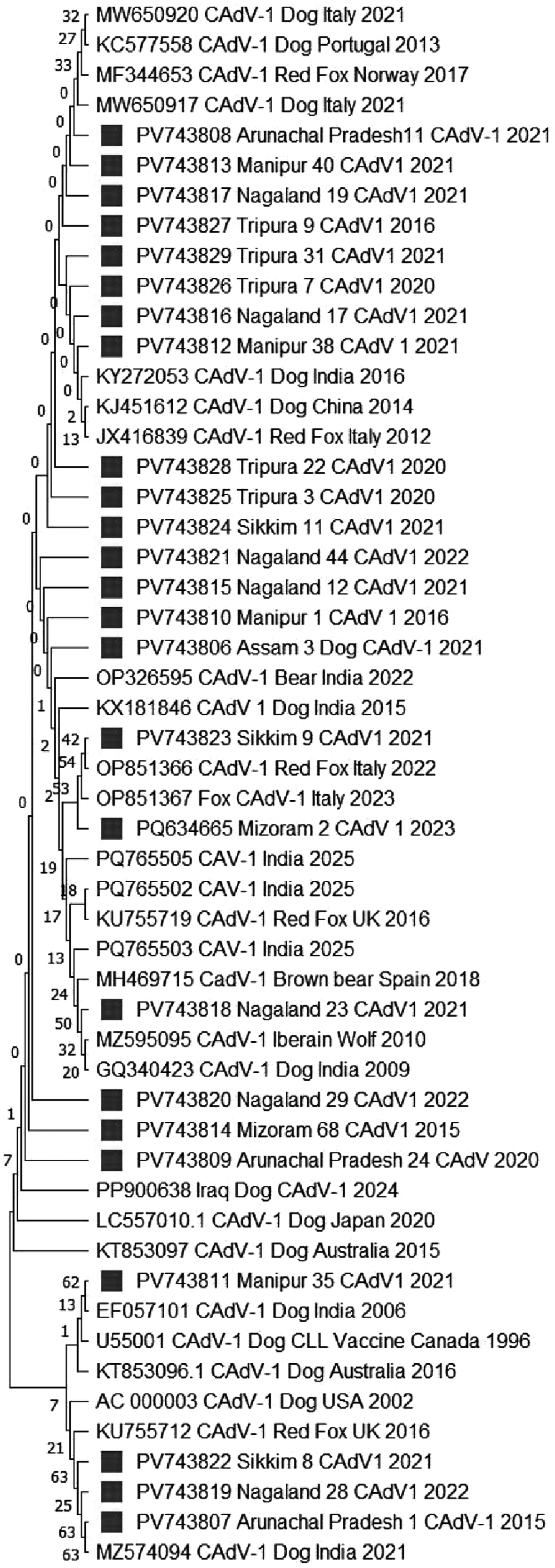

Fig. 3. Phylogenetic analysis of CAdV-1 partial E3 sequences of NEH isolates against reference sequences using the Neighbor-Joining method method with 1000 bootstrap replications (MEGA v11.0). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Study isolates are marked with blue squares. Bootstrap values ≥70 indicate strong support. Analysis involved 52 amino acid sequences with 243 positions in the final dataset (pairwise deletion applied).

Table 4. CAdV-1 B-cell liner epitope prediction with epitope threshold of 0.5 by BepiPred.

Accession number	Start-End Positions of Predicted Epitopes	Predicted Epitope Regions (Amino Acid Sequence)	Approximate Epitope length (amino acid residues)
PGB34665	10-95	NKTTTEATIITYISMTFLLVSLTLFLNLVTLT	33
PV743824	10-100	NKTTTEATIITYISMTFLLVSLTLFLNLVTLT LNKYKHYGV	42
PV743823	50-70	NKTTTEATIITYIP	14
PV743815 PV743816 PV743817 PV743818 PV743819 PV743820 PV743821	6-95	NKTTTEATIITYISMTFLLVSLTLFLNLVTLTLNKYKHYGV	41
PV743806 PV743811 PV743808 PV743813 PV743814	6-95	NKTTTEATIITYISMTFLLVSLTLFLNLVTLTL	33
PV743807 and PV743812	6-100	NKTTTEATIITYISMTFLLVSLTLFLNLVTLTLNKYKHYGV	38
PV743809 PV743810	6-95	NKTTTEATIITYISMTFLLVSLTLFLNLVTLTL	33
PV743825 PV743826 PV743827 PV743828 PV743829	6-100	NKTTTEATIITYISMTFLLVSLTLFLNLVTLTLNKYKHYGV	41

Table 5. Immune Epitope Database and Analysis Resource (IEDB) CAdV-1 MHC-I binding prediction analysis.

Peptide	Start-End	Binding Core	Score
LTFPNVLITLNNKY	83-96	LTFPNVLKY	0.007040
RQMPYDSPRHTGHK	14-27	RQMPHTGHK	0.006633
HQMPYDSPRHTGHK	14-27	HQMPHTGHK	0.001351

tions with immunosuppressive viruses like CPV-2, may worsen disease severity (Vita et al. 2018), highlighting the importance of considering co-infections in the diagnosis and management of canine enteritis and hepatitis.

Partial E3 gene analysis revealed key non-synonymous mutations (Asn127Asp, His129Arg, Trp148Ser, Leu201Pro, Thr206Met, Gly215Glu), some previously reported in Indian and European canid and wildlife isolates (Syamily et al. 2023, Chakraborty et al. 2024a), suggesting shared evolutionary pressures or cross-species transmission. The functional effects of these mutations are unclear, but their recurrence in both domestic and wild hosts indicates possible involvement in immune evasion or host interaction (Decaro et al. 2008). NER isolates showed high sequence similarity, consistent with the low mutation rate typical of double-stranded DNA viruses and the stability of conserved structural and functional proteins (Vita et al. 2019). Observed transitions (A↔G, T↔C) align with typical DNA virus mutational patterns, while recurrent substitutions (C→T at 617, G→C at 443) may reflect regional selection or shared ancestry (Sanjuán et al. 2016, Mira et al. 2022). Some substitutions, though silent at the nucleotide level, can result in amino acid changes that affect protein function or immune recognition (Balboni et al. 2022).

Phylogenetic analysis revealed NER isolates forming distinct clusters, with some closely related to both Indian and European canid and wildlife-origin isolates, suggesting endemic persistence, transboundary movement and potential spillover.

Notably, one cluster showed a strong connection to sequences derived from an Indian bear (OP326595) and domestic dog (KX181846). In addition, isolates like Sikkim 9, Mizoram 2, and Nagaland 23 grouped with strains from both domestic and wild canids in India and Europe. These patterns indicate cross-species transmission driven by shared habitats and highlight the virus's broad host adaptability and geographic spread (Knies et al. 2008, Balboni et al. 2022). Certain isolates formed unique NER clusters, suggesting multiple introduction events or co-existing viral lineages (Wang et al. 2022).

Selection pressure analysis showed most E3 codons were under purifying selection, consistent with the gene's conserved function. This pattern is expected for essential viral genes involved in immune response and host interaction, where harmful changes are removed to keep the gene's function intact (Vita et al. 2019). The predominance of purifying selection also fits with the slow evolutionary rate of double-stranded DNA viruses which have more stable genomes compared to RNA viruses (Sanjuán et al. 2016). However, a few codons showed signs of weak or episodic positive selection, likely reflecting host immune adaptation. Similar patterns have been described in other adenoviruses where immune-exposed proteins accumulate adaptive mutations at low rates (Wang et al. 2022). In our data, these positively selected codons matched specific amino acid changes (e.g., Asn127Asp, His129Arg, Trp148Ser, Leu201Pro, Thr206Met, Gly215Glu). Some of these mutations have also been reported previously in Indian and European isolates (Balboni et al. 2022, Syamily et al. 2023, Chakraborty et al. 2024a), suggesting that similar evolutionary pressures operate in different regions and host species. The presence of shared amino-acid mutations in both domestic dogs and wildlife-origin isolates hints the possibility of cross-species transmission. This cross-species transmission often causes bursts of adaptation as the virus adjusts to new immune environments (Mira et al. 2022). To evaluate whether recombination contributed to the observed genetic patterns, GARD analysis was carried out, but found no evidence of it (Vita et al. 2019). This supports the idea that the E3 region evolves mostly through point mutations rather than recombination, a pattern consistent with studies on related viruses (Vita et al. 2019).

Overall, the selection-pressure findings show that while the E3 gene is highly conserved and functionally constrained, it also harbors specific sites potentially undergoing adaptive evolution. These changes likely reflect host immune pressures, co-infections, or interactions between wildlife and domestic animals in the NER.

Identifying conserved B-cell epitopes is crucial for designing effective vaccines. In this study, a highly conserved B-cell epitope motif, NKTTTEATIITYISMT FLLVSLTLFLNLVTLTL, was identified across nearly all sequences, suggesting functional constraints and strong potential for epitope-based vaccines (Ndiana et al. 2022). MHC-I binding prediction revealed LTFPNVLITLNNKY (positions 83-96) as the top binder for canine allele DLA-8803401, with additional strong-binding peptides at positions 14-27, suggesting an immunodominant region for early cytotoxic T-cell (CTL) recognition (Wang et al. 2022).

Together, these findings highlight that CAdV-1 harbors both conserved B-cell epitopes and high-affinity CTL peptides, supporting the feasibility of designing vaccines that can trigger both antibody and cell-based immune responses.

Conclusion

This study presents the first comprehensive genomic and immunoinformatic characterization of CAdV-1 in India's NER, confirming its circulation, often alongside CPV-2. E3 gene analysis revealed genetic relatedness with Asian and European canid and wildlife-origin isolates, suggesting transboundary transmission and shared ancestry. The study also highlights the immunogenic regions that may serve as vaccine candidates. The findings underscore the need for multi-pathogen screening in diagnosis and broader molecular surveillance at wildlife-livestock interfaces, and future whole-genome studies to clarify viral evolution, host interactions, immune evasion, and regional strain diversity.

Acknowledgments

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Author Declarations

Ethics approval

The study was conducted in accordance with the Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines and ethical approval was obtained from Institutional Animal Ethics Committee (1476/GO/Re/SL/2011/CPCSEA dated 12th June 2023).

Use of generative artificial intelligence

No generative artificial intelligence tools were used in the preparation, writing, analysis, or editing of this manuscript.

Conflict of interest

The authors declare that they have no known financial, personal or institutional relationships that could have appeared to influence the work reported in this manuscript.

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