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Molecular Epidemiological Investigation of Canine Distemper Infection in Dogs and its Effect on Haemato-biochemical, Oxidative and CNS Biomarkers

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ABSTRACT

The present study was conducted (July, 2022–June, 2025) in and around the Aizawl district of Mizoram to know the epidemiological status of canine distemper infection in dogs in the Aizawl district of Mizoram and its effect on haemato-biochemical, oxidative and CNS biomarkers. Canine distemper virus (CDV) infection was responsible for high morbidity and mortality in dogs worldwide. A limited number of studies have been carried out on canine distemper in Mizoram. Out of 1000, 95 cases (9.5%) were confirmed positive by a rapid diagnostic kit and molecular technique with a high case fatality rate (86.32%). CDV remains a serious threat to canine health, particularly in non-vaccinated, purebred, and young (1–2 years) dogs, with winter showing peak incidence in Mizoram. PCR amplification of positive cDNA yielded a 286 bp fragment of the partial nucleocapsid (N) gene of CDV which was identical to each other and showed 96.2–100% identity within the Asia-1 genotype of CDV, indicating low polymorphism. It may be responsible for hematobiochemical alterations, oxidative stress imbalance, changes in CNS-specific biomarkers, and cerebrospinal fluid abnormalities. The progressive elevation of myelin basic protein (MBP) and Neuron-specific enolase (NSE) in non-survivors indicates severe demyelination and neuronal damage in advanced CDV infection. Overall, integration of hemato-biochemical, oxidative, CNS biomarkers and CSF, data provides a comprehensive approach for assessing disease severity, predicting outcomes, and differentiating survivors from non-survivors.

KEYWORDS: Canine distemper, molecular technique, oxidant-antioxidant, neuron-specific enolase, myelin basic protein

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Canine Distemper (CD) is a highly contagious and often fatal viral disease of dogs, caused by the Canine Distemper Virus (CDV). CDV is responsible for significant mortality, particularly in young dogs, and is recognized as one of the most infectious and lethal viruses in canines, second only to the rabies virus in severity. Epidemiological studies of canine distemper play a vital role in controlling and treating outbreaks across different regions. Epidemiological studies have demonstrated that the prevalence of canine distemper Virus (CDV) varies significantly across different geographic regions. Reported prevalence rates include in Brazil: 23.60% (Barbosa et al., 2025), in Turkey: 9.30% (Gencay et al., 2004), in Iraq: 8.86% (Mohammad et al., 2022) and in Nigeria: 7.50% (Namroudi et al., 2013). These variations are likely influenced by local factors such as climate, vaccination coverage, public health infrastructure, and stray dog populations (Avizeh et al., 2007). The neurological form of CD is associated with high mortality (Greene, 2012). One of its hallmarks is multifocal demyelination of the white and grey matter, which is attributed to the excessive production of free radicals during viremia (Summer and Appel, 1994; Vandevelde and Zurbriggen, 2005; Karadeniz et al., 2008). Treatment for CDV-induced myelitis is generally unsuccessful (Ranjithkumar and Dey, 2021). Canine distemper virus (CDV) infection is associated with anaemia, primarily resulting from the virus's deleterious effects on the hematopoietic system (Pascutti et al., 2016). A consistent haematological finding in CDV infection is leukopenia, particularly lymphocytopenia, which is often profound (Schobesberger et al., 2005). Dogs infected with canine distemper virus (CDV) exhibit significant elevations in liver enzyme levels, particularly alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) (Willi et al., 2015). Biochemical analysis also reveals significant increases in blood urea nitrogen (BUN), creatinine and total bilirubin (Von, 2014). A significant decrease in total protein (TP) levels has been reported in CDV-infected dogs compared to healthy controls, suggesting hepatic dysfunction, protein-losing enteropathy, or increased catabolism (Saeed and Al-Obaidi, 2021). Free radicals and their derivatives, play a significant role in the pathogenesis of many diseases, including canine distemper (CD) (Vandevelde and Zurbriggen, 2005). This is supported by findings of elevated plasma levels of oxidative damage marker such as malondialdehyde (MDA), nitrates, nitrites, and, to a lesser extent, ceruloplasmin in CDV-infected dogs. Neuron-specific enolase (NSE) is considered a major biomarker of neuronal injury in human medicine (Yokobori et al., 2013). Its role as a marker is increasingly being explored in veterinary neurology, including in cases of canine distemper virus (CDV) infection, where neurological manifestations are prominent. CDV-induced demyelination causes the release of Myelin Basic Protein (MBP) into the CSF, where its concentration correlates with the severity of neurological involvement (Kalistova et al., 2003). Additional diagnostic methods for canine distemper include cerebrospinal fluid (CSF) analysis, virus isolation, immunohistochemistry (IHC), and PCR-based detection of viral nucleic acids (Greene 2012). CDV RNA has been successfully detected in peripheral blood mononuclear cells from suspected infected dogs (Shin et al., 1995). Based on these premises; the study has been carried out to know the epidemiological status of canine distemper infection in dogs in Aizawl district of Mizoram and its effect on haemato-biochemical, oxidative and CNS biomarkers.

2. MATERIALS AND METHODS

2.1. Selection of animals

A total of 1000 dogs that were brought for treatment to the Veterinary Clinical Complex, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Mizoram (VCC), Veterinary polyclinic, Khatla and private veterinary clinics of Aizawl, Mizoram state were screened based on symptoms like diarrhoea, ocular and nasal discharge, coughing, vomition, digital hyperkeratosis, biphasic fever and central nervous system signs in either single or mixed form was considered as suspected clinical cases of canine distemper and subjected to screening by rapid diagnostic kit test.

2.2. Epidemiological studies

For the epidemiological study, a total of 1000 dogs were examined, and age, sex, breed and history were recorded throughout 3 years from June, 2022 to May, 2025.

2.3. Collection of samples

2.3.1. Blood samples

Blood samples (around 4.0 ml) were collected from each dog by venipuncture of cephalic or lateral saphenous vein into vials with and without dipotassium ethylenediaminetetraacetic acid (K2-EDTA). The blood samples without anticoagulant were allowed to clot and then centrifuged at 2000 rpm for 10 min at 4°C to separate the serum, which was subsequently collected and stored at -20°C till analysis.

2.3.2. CSF samples

CSF was collected from the cerebello-medullary cistern (CMC), as described by Terlizzi and Plat (2006) in post-sedation cases. Dogs were anesthetised with ketamine (10 mg kg^{-1}) and diazepam (1 mg kg^{-1}) intravenously, and CSF specimens (approximately 1 ml) were collected into 1.5 ml tubes without anticoagulant from cerebello-medullary cistern after disinfection with povidone-iodine and 70% ethanol and stored at -20°C until further analysis.

2.4. Screening by rapid CDV antigen test kit

All the suspected cases were screened by a commercially available Canine CDV rapid antigen detection kit (Bionote, cat. No. RG1103DD, Gyeonggi-do, Korea, as per the manufacturer's instructions.

2.5. Reverse transcription polymerase chain reaction for detection of the N Gene of CDV

2.5.1. RNA isolation using commercial kits

Nasal swabs and blood collected from infected dogs were subjected to viral RNA extraction using commercially available RNA extraction kits (Nucleospin RNA mini kit, Macherey-Nagel) for reverse transcription polymerase chain reaction (RT-PCR) confirmation.

2.5.2. Reverse transcription-polymerase chain reaction for detection of Canine Distemper Virus (CDV) nucleic acid

2.5.2.1. Complementary DNA preparation

The template RNA solution was thawed on ice. The primer solution, RT buffer, dNTP mixture, and RNase-free water were thawed at room temperature and then stored on ice immediately. Each solution was mixed by vortexing. The PCR components were combined to create a reaction volume of 10.0 µl (Table 1).

Table 1: Prepared the following mixture in a 0.2 µl microtube

Reagent	Volume
Random 6 mers (50 um)	1 µl
DNTP mix (10 mM)	1 µl
Template RNA	200 ng
RNase-free water	Adjusted to 10 µl
Total	10 µl

1. Incubated 5 min at 65°C and immediately cooled in ice.

2. Prepared the reaction mixture in a total volume of 20 µl. (Table 2).

3. Mixed gently.

4. Incubate the reaction mixture using the following conditions. 30°C-10 min (required when using Random 6 mers), 42°C (50°C)-30–60 min.

5. Inactivated the enzyme by incubating at 95°C for 5 min, then cooled on ice.

Table 2: Preparation of reaction mixture

Template RNA primer	10 µl
5×Primscript buffer	4 µl
RNase Inhibitor	0.5 µl
Primescript RTase enzyme	1.0 µl
RNase-free water	4.5 µl
Total	20 µl

6. The c-DNA thus formed was properly labelled and stored at-20 °C till further use.

2.5.2.2. Amplification of partial nucleocapsid protein gene (N Gene) of CDV

The N gene (Partial) region in the cDNA samples was amplified by RT-PCR using specific primers (Frisk et al., 1999) to amplify a partial 286 nt region of canine distemper virus (Table 3). The PCR components were combined to create a reaction volume of 10.0 µl (Table 4). Different steps and conditions of the thermal cycler for amplification of the N gene were shown below (Table 5).

Table 3: Sequences of primer sets used for amplification of the N gene of CDV

Gene	Primer sequence	Product	Reference
"N" GENE	(F) 5' ACAGGATTGCTG AGGACCTAT 3' (R) 5' CAAGATAACCATG TACGGTGC 3'	286bp	Frisk et al., 1999

Table 4: Prepared reaction mixture for 10 µl in a 0.2 ml PCR tube for amplification of the N gene from cDNA

amaR one master mixture	5 µl
Reverse primer (10 pmole)	0.5 µl
Forward primer (10 pmole)	0.5 µl
DNA template	2 µl
Nuclease-free water	2 µl
Total	10 µl

2.5.3. Confirmation of PCR amplicon

To confirm the targeted PCR amplicon that was obtained, the PCR products were subjected to electrophoresis in a 1.7% agarose gel containing ethidium bromide (10 mg ml^{-1}) and visualized under UV light.

2.5.4. Sequence confirmation and phylogenetic analysis

Purified PCR products were sequenced by outsourcing, and the raw sequence data were annotated. Sequence analysis

Table 5: Different steps and conditions of the thermal cycler for amplification of the N gene

Step-1	95°C	3 min	Initial denaturation
Step-2	95°C	1 min	Denaturation
Step-3	59.5°C	1 min	Annealing
Step-4	72°C	1 min	Extension
Step-2-4		Repeated 35 times	
Step-5	72°C	7 min	Final extension
Step-6	4°C	Hold	Soak cycle

was carried out using the multiple alignment program of MEGA11 software. The phylogenetic tree was generated by using the Neighbour-Joining method, keeping a bootstrap consensus from 1000 replicates. Sequence comparison was carried out by retrieving the gene sequence from the gene bank. Retrieved sequences from the genebank in the present study were selected from different outbreaks in different geographical areas.

2.5.5. Virus isolation

2.5.5.2. Harvesting of virus

Nasal swab collected in virus transport medium was vortexed briefly and centrifuged at 12000 rpm for 10 min. Supernatant was collected and passed through a syringe filter (0.22 µm). Filtered harvest was treated with a 2× concentration of antibiotic-antimycotic cocktail mixture and incubated for 1 h before infection of Madin-Darby Derby Canine Kidney (MDCK) cell monolayer.

2.5.5.3. Virus isolation in madin-darby canine kidney (MDCK) cells

MDCK cells were purchased from NCCS Pune, Govt. of India and propagated in the laboratory using DMEM cell culture medium with 10% FBS. A monolayer of 80% confluency was used for virus inoculation. Harvested virus materials were inoculated (500 µl) in a T-25 cell culture flask and adsorbed for 1 h. After adsorption, briefly monolayer was washed with HBSS medium and replenished with cell culture maintenance medium. Cells were further incubated in a CO_2 incubator for 4 days. All the isolates were passed three times in a monolayer to observe the cytopathic effect.

2.6. Haemato-biochemical estimation

The haematological parameters viz. total erythrocyte count (TEC), haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), monocyte count, lymphocyte count and total platelet count of all the positive cases were measured by using an automated haematology cell counter (IDEEX, USA) as per manufacturer's instructions.

Serum samples of the positive cases were evaluated for the concentrations of total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine and serum enzymatic activities of alanine aminotransferase (ALT) by using commercially available test kits in an automated biochemical analyzer (Fuji Dri-Chem 4000i Biochemistry Analyzer, Japan) following the manufacturer's instructions. The globulin concentration in serum was derived from total protein and albumin concentrations by using Friedwald's equation.

2.7. Evaluation of oxidative and antioxidant status of CDV-infected dogs

The serum levels of oxidative stress indices including lipid hydroperoxide (LPO) (Cayman® Lipid Peroxide (LPO) Colorimetric Assay Kit, Catalog No.: 705003), Superoxide dismutase (SOD) Colorimetric Assay Kit, (Cayman® Catalog No.: 706002) and total antioxidant capacity T-AOC Colorimetric Assay Kit, (Cayman®, Catalog No.: 709001) were estimated using spectrophotometer (Thermo ScientificTM, EvolutionTM 201 UV-Visible Spectrophotometer, USA).

2.8. Estimation of neuron-specific enolase (NSE) from serum of CDV-infected dogs

NSE in serum and CSF were estimated by using the canine NSE ELISA kit (Enolase, neuron specific code-ITLK09192, G-Biosciences, USA) as per the recommendation of the manufacturer. The plate was read at 450 nm using an automatic ELISA plate reader. For comparison, six healthy dogs were also selected.

2.9. Measurement of myelin basic protein (MBP) in CSF

Myelin basic protein (MBP) was estimated by using commercial Canine MBP (Myelin Basic Protein) ELISA Kit. The sandwich ELISA kit was purchased from G-biosciences, USA, Cat no: TLK09191. The test principle applied in this kit was a Sandwich enzyme immunoassay.

2.10. CSF analysis

All CSF samples were examined carefully for turbidity. Dipstick analysis of CSF samples (including leukocyte (WBC), total protein, and erythrocyte (RBC) parameters) was performed using URIT-31 Vet® (Accurex Biomedical Pvt. Ltd., India) strip analyser within 5–10 min after the sampling. After that, the remaining CSF samples were prepared for microscopic examination by cytocentrifuge (1500 rpm for 10 min at room temperature). After the Giemsa staining, the slides were first examined at ×100 magnification, then with immersion oil under a compound microscope at ×1000 magnification. WBC and RBC counts were roughly quantified as none (0/slide), rare (<10/slide), few (<1/oil immersion field [OIF]), moderate (1 to 10/OIF), or many (>10/OIF).

2.11. Statistical analysis

Statistical analysis was carried out as per Snedecor and Cochran (1994). The data analysis was done by one-way analysis of variance. The values are expressed as mean ± SEM. Superscripts * between the groups differ significantly (p<0.05). Post hoc analysis using Duncan's multiple range tests was conducted following one-way ANOVA to identify statistically significant differences in haematological, biochemical, and oxidative stress parameters among the three study groups. SPSS Software, version 20, was employed for calculations.

3. RESULTS AND DISCUSSION

3.1. Prevalence of canine distemper viral infection in dogs in aizawl district of Mizoram in relation to various risk factors

Out of 1000, 200 cases were suspected of CD, and 95 cases were confirmed positive by the rapid diagnostic kit and molecular technique. The prevalence of canine distemper virus infection in dogs in the Aizawl district of Mizoram was 9.5%.

Season wise prevalence of canine distemper virus infection in dogs in Aizawl district of Mizoram revealed that the prevalence of CDV infection in dogs of Aizawl district of Mizoram was significantly (p<0.05) (Chi-square test, X^2=60.2472, df=2, p=<0.00001) the highest in winter season (22.75% 53/233) followed by monsoon (10.67%, 27/235) and summer season (2.82%, 15/532) (Table 6) CDV infection occurred almost in all age groups starting from pup to above 3 years of old dogs. But 1–2 years age group showed significantly (p<0.05) the highest prevalence of CDV infection as compared to other three groups (Chi-square test, X^2=15.3206, df=3, p=0.0001562). The sex-wise prevalence of CDV infection in dogs of the Aizawl district of Mizoram showed no significant variance (Chi-square test, X^2=0.0007, df=1, p=0.978625). Breed wise prevalence of CDV infection in dogs of Aizawl district of Mizoram revealed that Purebred dogs showed significantly (p<0.05) the highest prevalence (21.56%, 58/269) followed by crossbred (5.50%, 35/636) and Mongrel (2.11%, 2/95) (Chi-square test, X^2=49.3787, df=2, p=<0.00001). The prevalence of canine distemper viral infection in dogs of Aizawl district, Mizoram, based on vaccination status from July 2022 to June 2025, revealed that non-vaccinated dogs were significantly (p<0.05) more susceptible to CDV infection than vaccinated dogs (Chi-square test, X^2=6.7966, df=2, p=0.03343) (Table 6).

Canine Distemper (CD) infection in dogs was a major problem with dog owners in India. The present study was conducted on a total of 1000 dogs of different ages, breeds and sexes in and around the Aizawl district of Mizoram. Out of 1000, 200 cases were suspected of CD, and 95 cases were confirmed positive by a rapid diagnostic kit and molecular technique. The prevalence of canine distemper virus infection in dogs in the Aizawl district of Mizoram was 9.5% which was higher than the previous study (Mousafarkhani et al., 2023). They reported only 4.04% of prevalence of CDV infection in Iran. This large variation in CDV prevalence may be related to the degree of specificity of the evaluation method utilized, the phase of distemper present, the immunological state of these dogs when evaluations were taken, and the peculiarities of each location. Hospital-based surveys might not reveal the true prevalence of canine distemper in urban dog populations when compared with field surveillance studies (Headley and Graça, 2000). The reduced CDV prevalence in animals attending Veterinary Hospitals, as was observed in this study, in comparison with other field survey reports, indicated that the true prevalence of canine distemper in normal animal populations was higher than has been revealed by hospital surveys. Thus, hospital-based surveys might not reveal the true prevalence of canine distemper in urban dog

Table 6 : Prevalence of canine distemper viral infection in dogs in Aizawl district of Mizoram in relation to various risk factors

	Variables	Total no. of animals examined	No. of positive cases	Prevalence rate (%)	X^2	p-value
Season	Winter (November–March)	233	53	22.75		
	Summer (April–June)	532	15	2.82	60.2472	<0.00001
	Monsoon (July–October)	235	27	10.67		
Age	0–1 year	421	37	8.79		
	1–2 years	125	25	20.00		
	2–3 years	234	20	8.55	15.3206	0.001562
	>3 years	220	13	5.91		
Sex	Male	654	62	9.48		
	Female	346	33	9.53	0.0007	0.978625
Breed	Purebred	269	58	21.56		
	Crossbred	636	35	5.50	49.3787	< 0.00001
	Mongrel	95	2	2.11		
Vaccination	Vaccinated	220	10	4.54	6.8833	0.0087
	Non-vaccinated	780	85	10.89		

populations when compared with field surveillance studies.

Statistical analysis using the chi-square test revealed a significant difference in prevalence across seasons, indicating that seasons influenced the occurrence of canine distemper. Similar to the present study, a higher prevalence of canine distemper in winter season was also reported by Mousafarkhani et al. (2023). As the canine distemper virus was an enveloped virus, it was sensitive to high temperatures and has a longer shelf life at low temperatures. Therefore, high rates of suspected and confirmed referrals cases were expected to be in autumn and/or winter (Naveenkumar et al., 2025) and additionally, cold weather might trigger stress-induced immunosuppression in young animals, increasing their susceptibility to infection (Menezes et al., 2023).

The study revealed that CDV infection occurred in almost all age groups, but 1–2 years age group showed significantly (p<0.05) the highest prevalence. The susceptibility of 1–2 years or above age group to infection could be because of poor development of vaccine antibody which might be because of interference of maternal antibodies during the primary vaccination or could be because of poor handling of the vaccine (Latha et al., 2007).

In the present study, a comparison between gender and CDV revealed that no significant variance between males and females for the prevalence of distemper. In other studies, contradictory results were reported between males and females, so that some studies similar to this study have reported that the prevalence rate in females was higher than in males (Ogbu et al., 2023) and some concluded that there was no difference between male and female dogs (Zengin et al., 2025). These findings supported the theory that the sex of the host does not have any effect on CDV prevalence.

Purebred dogs showed significantly (p<0.05) the highest prevalence followed by crossbred and mongrel. Although the exact reason for purebred predominance in CDV infection was unknown, people of this region mostly prefer purebred animals as pets, and this might be the possible reason. However, the present observations were contrary to the findings of others who found that there was no breed predilection (Behera et al., 2014)

The results revealed that non-vaccinated dogs were significantly (p<0.05) more susceptible to CDV infection than vaccinated dogs. This result indicated the importance of vaccination for preventing the disease. Other reports have also highlighted the importance of vaccination (Martella et al. 2008; Latha et al., 2007), who reported that vaccinated dogs were also infected with CDV.

3.2. Evaluation of the fatality and recovery rate of the distemper disease

Out of 95 dogs with a final diagnosis of distemper, 82 dogs (86.32%), died, and 13 (13.68%) survived (Table 7). From the table, it was observed that the fatality rate of CDV-infected dogs was 86.32% and the recovery rate was 13.86% There was a significant difference (p<0.05) in fatality and recovery rate in CDV-infected dogs.

Table 7: Recovery and fatality rates in dogs with distemper

Dis-temper	Outcome	No. of dogs	Reco-very rate (%)	Fatality rate (%)	p-value
Positive	Survive	13	13.68	-	
	Non-survive	82	-	86.32	<.00001
	Total	95	-	-	

There was a significant difference (p<0.05) in fatality and recovery rate in CDV-infected dogs. This indicated that a significant number of dogs died despite treatment. The recovery rate could vary, based on factors like age, vaccination status, and the severity of the disease. Very few reports revealed the accurate fatality and mortality rates of distemper in dogs. In a study, the mortality rate of distemper in vaccinated dogs was estimated at 30% (Headley and Graça, 2000). In another article, the observed distemper mortality rate was 50% (Beineke et al., 2009).

3.3. Diagnosis of canine distemper virus infection in dogs

The diagnosis was carried out by both a rapid diagnostic kit and a molecular technique. Out of 200 samples collected, 35 (17.5%) samples tested positive for CDV infection by rapid diagnostic Ag kit, whereas 95 (47.5%) samples (including rapid test positive) tested positive for CDV infection by PCR (Table 8).

Table 8: Percentage of positive samples for canine distemper infection in dogs diagnosed by rapid antigen kit and PCR

Total no. of samples screened	No. of positive cases (%)	
	Rapid antigen detection kit	PCR
200	35 (17.5%)	95 (47.5%)

3.3.1. Molecular detection of CDV

Out of 200 samples, ninety-five (47.5%) were found positive for CDV. Positive cDNA could be amplified by PCR to obtain a 286 bp fragment of the partial N gene of CDV (Figure 1).

PCR purified product (10 samples) upon sequencing confirmed nucleocapsid protein gene (N gene) of CDV. The 10 Nucleocapsid protein gene (N gene) of CDV was submitted to the NCBI GenBank, and an accession number was obtained. The accession numbers were given as PX112323, PX112324, PX112325, PX112326,

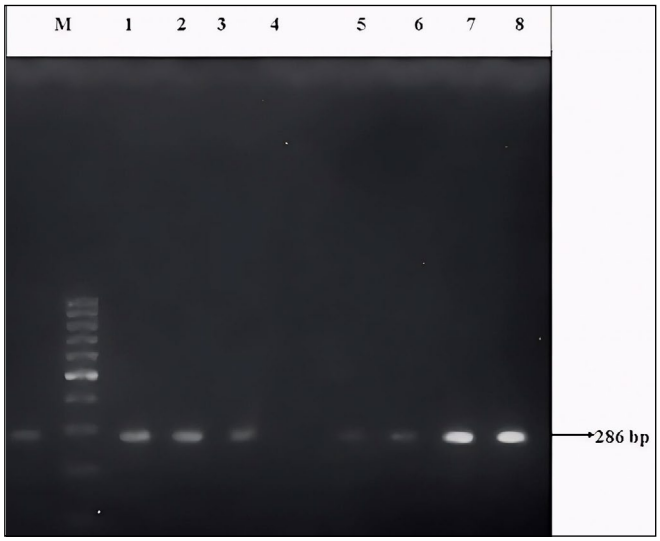

Figure 1: 1.5% Agarose-based gel electrophoresis of the PCR amplicon of partial N gene of CDV

PX112327, PX112328, PX112329, PX112330, PX112331 and PX112332; were identical to each other. The N gene of CDV showed low polymorphism with 96.2 to 100% identity within the species of different isolates across the globe (Figure 2).

Sequence data of 237 bp were used for the generation of a phylogenetic tree and to identify the genotype of CDV detected in Mizoram. The phylogenetic tree revealed all the isolates belonged to Asia1 genotype of CDV (Figure 3).

3.3.2. Virus isolation

Out of 95 samples collected, 10 samples were processed for virus isolation, and virus could be isolated from the entire all ten positive samples. After three passages, virus isolates were started showing typical cytopatheic effect of syncytia (Figure 4) formation and subsequence total disruption of monolayer. All the isolates were further confirmed by PCR. The cell culture passaged virus also gave positive amplification in RT-PCR for the CDV N gene in both outer and nested PCRs.

The increased effectiveness of PCR's depends on the number of cycles, the quality of the starting material, the length of the target DNA and the variability of the annealing and elongation temperatures.

The tentative diagnosis of distemper was typically based on the animal's history, clinical signs, and laboratory findings. A definitive diagnosis, however, could be achieved using various techniques, including serological, histopathological, and molecular methods that detected antibodies, antigens, or nucleic acids (Greene, 2012). Serological assays that detected antibodies (such as virus-neutralizing antibodies) might produce false-positive results if the animal had previously been vaccinated or has recovered from the disease (Józwik and Frymus, 2002). Currently, reverse transcription polymerase chain reaction (RT-PCR) offered a rapid

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1		96.2	97.0	96.2	99.2	96.6	96.2	96.2	96.2	96.2	96.2	96.2	96.2	97.0	97.0	97.0	97.0	1	DQ003302.1 india
2	3.9		99.2	100.0	96.6	98.7	97.9	98.3	96.2	96.2	96.2	98.7	98.7	99.2	98.3	96.2	97.5	2	EU715244.1 China
3	3.0	0.8		99.2	97.5	97.9	97.9	97.5	97.0	97.0	97.0	97.9	97.9	98.3	98.3	96.2	98.3	3	JN896331.1 China
4	3.9	0.0	0.8		96.6	98.7	97.9	98.3	96.2	96.2	96.2	98.7	98.7	99.2	98.3	96.2	97.5	4	KJ848781.1 China
5	0.9	3.5	2.6	3.5		97.0	97.0	96.6	96.6	96.6	96.6	97.0	97.0	97.5	97.5	97.5	97.5	5	MH536200.1 China
6	3.5	1.3	2.1	1.3	3.0		98.3	98.7	96.6	96.6	96.6	100.0	100.0	99.6	99.6	96.6	96.2	6	Mizoram-CDP1
7	3.5	2.1	2.1	2.1	3.0	1.7		99.6	96.6	96.6	96.6	98.3	98.3	98.7	98.7	96.6	96.2	7	Mizoram-CDP2
8	3.9	1.7	2.6	1.7	3.5	1.3	0.4		96.2	96.2	96.2	98.7	98.7	99.2	98.3	96.2	95.8	8	Mizoram-F1
9	3.9	3.9	3.0	3.9	3.5	3.5	3.5	3.9		100.0	100.0	96.6	96.6	97.0	97.0	95.4	95.8	9	Mizoram-F2
10	3.9	3.9	3.0	3.9	3.5	3.5	3.5	3.9	0.0		100.0	96.6	96.6	97.0	97.0	95.4	95.8	10	Mizoram-F4
11	3.9	3.9	3.0	3.9	3.5	3.5	3.5	3.9	0.0	0.0		96.6	96.6	97.0	97.0	95.4	95.8	11	Mizoram-F7
12	3.5	1.3	2.1	1.3	3.0	0.0	1.7	1.3	3.5	3.5	3.5		100.0	99.6	99.6	96.6	96.2	12	Mizoram-M9A
13	3.5	1.3	2.1	1.3	3.0	0.0	1.7	1.3	3.5	3.5	3.5	0.0		99.6	99.6	96.6	96.2	13	Mizoram-M10B
14	3.0	0.9	1.7	0.9	2.6	0.4	1.3	0.8	3.0	3.0	3.0	0.4	0.4		99.2	97.0	96.6	14	Mizoram-M16A
15	3.0	1.7	1.7	1.7	2.6	0.4	1.3	1.7	3.0	3.0	3.0	0.4	0.4	0.8		97.0	96.6	15	Mizoram-M23
16	2.6	3.5	3.5	3.5	2.2	3.0	3.0	3.5	4.4	4.4	4.4	3.0	3.0	2.6	2.6		95.8	16	MN128876.1 India
17	3.0	2.6	1.7	2.6	2.6	3.9	3.9	4.4	4.4	4.4	4.4	3.9	3.9	3.5	3.5	4.0		17	MT905031 India
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		

Figure 2: Homology analysis of nucleotide sequences (A) among CDV strains

and accurate method for diagnosing infectious diseases from biological samples. The results of phylogenetic tree construction of the local Aizawl CDV virus isolates were found to be closely related to NCBI-Blast reference CDV isolates belonged to Asia1 genotype of CDV, including KJ994343.1, KJ848781.1. EU715244.1, China, and DQ522030.1, Taiwan. The current research demonstrated how these strains could spread through the Aizawl area by importing infected domestic dogs from different countries.

3.4. Haematological status in CDV-infected dogs compared with survival, non-survival and health control

According to the severity, infected dogs were divided into two groups, viz. survival and non-survival. The values were compared with healthy groups. The value of Hb, PCV, lymphocytes and monocytes of non-survival were significantly decreased (p<0.01) as compared to survival and healthy groups (Table 9). There was no significant difference in TLC value among the groups. The eosinophil values of non-survival groups (1.01±0.09) and survival group (2.82±0.44) were significantly (p<0.01) decreased as compared to the healthy (3.92±0.46) group. In case of neutrophil value, the non-survival group were significantly (p<0.01) increased as compared to the survival and healthy groups (Table 9). The platelet values of the non-survival group (157.96±3.97) were significantly (p<0.01) decreased,

followed by the survival group (335.15±32.78) as compared to the healthy group (445.53±44.81).

In this study, CDV-infected dogs were categorized into two groups based on disease severity: survival and non-survival, and their hematological values were compared to those of a healthy control group. Significant decreases

Table 9: Comparison of haematological parameters among survival, non-survival group of canine distemper infected dogs and healthy control (mean±SE)

Parameters	Survival	Non-survival	Healthy	*p*-value
Hb (g dl^{-1})	12.88±0.51[a]	8.020±0.16[b]	15.333±0.56[c]	<.001*
TLC (×10^3 μl^{-1})	8.65±0.80	8.69±0.59	10.37±0.55	0.722
PCV (%)	36.52±0.99[a]	25.04±0.54[b]	46.17±1.85[c]	<.001*
Lymphocytes (%)	16.46±0.96[a]	7.62±0.27[b]	16.67±1.20[ac]	<.001*
Monocytes (%)	5.15±0.56[a]	2.19±0.09[b]	5.33±0.88[ac]	<.001*
Basophils (%)	1.26±0.04[a]	1.27±0.02[ab]	1.58±0.14[c]	<.001*
Eosinophil (%)	2.82±0.44[a]	1.01±0.09[b]	3.92±0.46[c]	<.001*
Neutrphils (%)	76.55±0.31[a]	81.46±0.05[b]	75.03±0.26[ac]	<.001*
Platelets (×10^3 μl^{-1})	335.15±32.78[a]	157.96±3.97[b]	445.53±44.81[c]	<.001*

The values have been expressed as Mean±SE; Superscripts a, b, and c among the groups differ significantly (p<0.05)

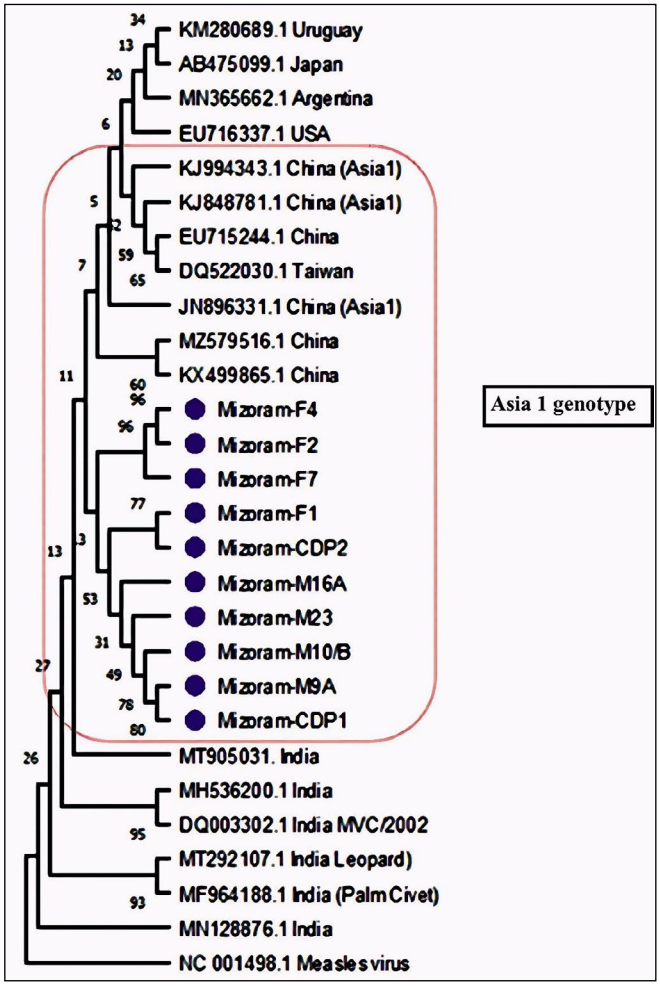

Figure 3: Phylogenetic analysis of CDV nucleocapsid protein gene (partial, 237 bp) of 10 field viruses

(p<0.05) in haemoglobin (Hb), packed cell volume (PCV), lymphocytes, monocytes, basophils, eosinophils, and platelet counts were observed in the non-survival group compared to controls. In contrast, neutrophil counts were significantly increased (p<0.05) in the non-survival group, consistent with findings by Headley and Sukura (2009). Interestingly, in the survival group, levels of Hb, lymphocytes, monocytes, and neutrophils showed no significant difference from the control group. These haematological changes in non-surviving dogs viz, anemia, leukopenia, neutrophilia, and thrombocytopenia, suggest bone marrow suppression, likely due to viral persistence and erythroid hypoplasia (Pascutti et al., 2016). CDV has been shown to replicate in the bone marrow, leading to its pathology and contributing to anaemia (Saeed and Al-Obaidi, 2021; Willi et al., 2015). Leukopenia in early-stage CDV infections is believed to result from lymphocytic destruction in lymphoid organs and excessive leukocyte recruitment. As the disease progresses, lymphocytosis and leukocytosis may develop, reflecting a shift in immune response (Saeed and Al-Obaidi, 2021). Thus, both leukopenia and leukocytosis can be features of CDV, depending on the stage of infection. Thrombocytopenia, also observed in this study, is likely due to bone marrow suppression caused by chronic CDV infection (Saeed and Al-Obaidi, 2021). Therefore, while CBC analysis provides valuable supportive data, it is not a definitive method for CDV diagnosis, and should be interpreted alongside clinical signs and confirmatory tests.

3.5. Biochemical status in CDV-infected dogs compared with survival, non-survival and health control

The study revealed total protein (TP) albumin and globulin

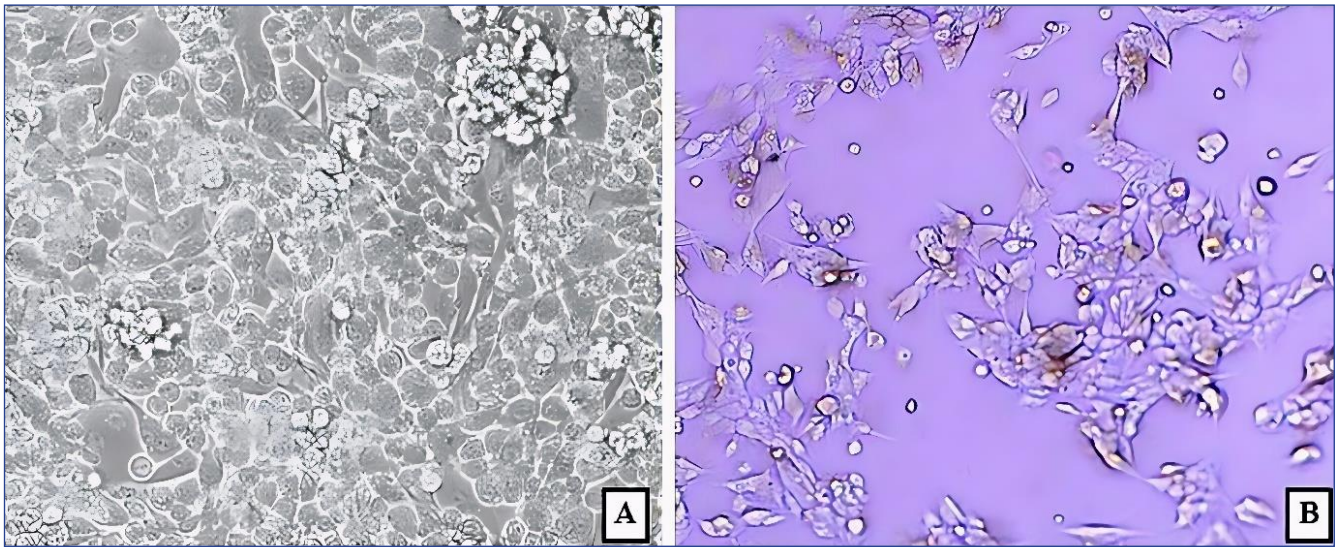

Figure 4: A: Normal (uninfected) MDCK cell monolayer in culture (400×); B: Infected cells after 72 h of infection showing cyncytia and total disruption of monolayer cells (400x)

level of CDV infected non-survival group was significantly (p<0.05) decreased as compared to survival dogs and healthy dog (Table 10). In terms of Kidney function parameters, viz, blood urea nitrogen (BUN) and creatinine were markedly elevated (p<0.01) in non-survival dogs as compared to survival dogs and Healthy dogs (Table 10).

Biochemical profiling of CDV-infected dogs, compared to healthy controls, revealed significant alterations, particularly in the non-survival group. These included hypoproteinemia, hypoalbuminemia, hypoglobulinemia, and elevated levels of blood urea nitrogen (BUN) and creatinine, when compared to both surviving and healthy dogs. However, some studies have reported that total bilirubin (TB), creatinine, and total protein (TP) levels remained within normal ranges, with only BUN showing a significant increase in CDV-infected dogs (Willi et al., 2015). As BUN was a major nitrogenous waste product of protein metabolism, its levels could rise due to liver or kidney dysfunction. However, such changes were often considered non-specific and might vary among individual dogs. In the present study, no significant changes in alanine aminotransferase (ALT) levels were observed in CDV-positive dogs. These findings suggested that blood biochemical parameters have a limited and non-specific correlation with CDV infection and were likely influenced by factors such as age, general health status, and the presence of concurrent internal diseases.

3.6. Comparison of oxidant-antioxidant status among survival, non-survival group of canine distemper-infected dogs and healthy controls

The Mean±SE value of LPO was significantly (p<0.05) increased in CDV-infected non-survival dogs and survival dogs as compared to healthy dogs (Table 10). But SOD and TOAC value was significantly (p<0.05) decreased in CDV-infected non-survival dogs and survival dogs as compared to healthy dogs (Table 11).

In the present study, CDV-infected dogs exhibited marked increases in oxidative stress markers, such as lipid peroxidation (LPO), and significant reductions in antioxidant parameters, including superoxide dismutase (SOD) and total antioxidant capacity (TOAC), in both survival and non-survival groups when compared to healthy controls. Similar findings were previously reported by Viscone et al. (2022). The

Table 10: Comparison of biochemical parameters among survival, non-survival groups of canine distemper-infected dogs and healthy controls (mean±SE)

Parameters	Survival	Non-survival	Healthy	p-value
TP (g dl^{-1})	5.01±0.31^{a}	4.13±0.15^{b}	5.55±0.28^{ac}	0.005*
Albumin (g dl^{-1})	2.69±0.15^{a}	1.99±0.05^{b}	2.63±0.15^{ac}	<.001*
Globulin(g dl^{-1})	2.29±0.15^{a}	2.08±0.07^{ab}	3.45±0.22^{c}	<.001*
ALT (u l^{-1})	67.33±3.16	75.46±2.14	66.47±6.81	0.207
BUN (mg dl^{-1})	31.65±0.47^{a}	134.54±0.88^{b}	32.41±0.08^{ac}	<.001*
Creatinine (mg dl^{-1})	0.66±3.86^{a}	1.15±10.41^{b}	0.42±1.05^{ac}	<.001*

The values have been expressed as Mean±SE; Superscripts a, b, and c among the groups differ significantly (p<0.05)

Table 11: Comparison of oxidant-antioxidant status among survival, non-survival group of canine distemper-infected dogs and healthy controls

Parameters	Survival (n=13)	Non-survival (n=82)	Healthy (6)	*p*-value
TOAC (mMTE)	1.97±0.04[a]	1.61±0.01[b]	4.77±0.20[c]	$p<0.001^{*}$
LPO (nmol)	4.98±0.17[a]	5.641±0.07[b]	0.28±0.06[c]	$p<0.001^{*}$
SOD (U ml^{-1})	0.12±0.01[a]	0.08±0.01[b]	2.30±0.10[c]	$p<0.001^{*}$

The values have been expressed as Mean±SE; Superscripts a, b, and c among the groups differ significantly ($p<0.05$)

elevated levels of malondialdehyde (MDA) observed in this study provide additional evidence of oxidative stress and excessive ROS accumulation in CDV-infected dogs. According to Griot et al. (1990), oligodendrocytes were particularly vulnerable to ROS, and myelin was especially sensitive to ROS-induced lipid peroxidation, a key factor in demyelination. The decrease in plasma SOD and overall antioxidant levels observed in this study suggested excessive ROS production, resulting in the consumption and depletion of antioxidant defenses. This imbalance, characterized by elevated oxidative damage markers (LPO) and reduced antioxidant capacity (SOD), provides clear evidence of oxidative stress-induced impairment of the antioxidant system during CDV infection.

3.7. CSF analysis of survival and non-survival CDV infected dogs

Grossly, all CSF samples obtained through cerebellomedullary cistern were colourless and clear in this study. In CSF analysis, the value of leucocyte cells in survival CDV-infected dogs were significantly ($p<0.05$) increased (Table 12). Few red blood cells (RBCs) in CSF were present in both survival and non-survival CDV-infected dogs. Glucose concentration of CSF in case of the non-survival group was

Table 12: CSF analysis findings of the CDV-infected survival and non-survival groups of dogs

Parameters	Survival (n=13)	Non-survival (n=82)	*p*-value
Leucocyte cells mm^{-3} (<1/OIF)	1.54±0.22	1.95±0.08	0.45
Glucose (mg dl^{-1})	61.65±2.36	69.70±1.03	0.003
Total protein (mg dl^{-1}) (<30 g dl^{-1})	33.33±0.42	45.90±0.61	<0.001
Specific gravity	1.024±0.00	1.025±0.00	0.633
pH	7.08±0.09	7.16±0.04	0.382
RBC cells mm^{-3} (<1/OIF)	1.15±0.25	1.43±0.10	0.378

significantly ($p<0.05$) increased (Table 12). The protein concentration in non-survival CDV infected dogs was significantly ($p<0.01$) increased than survival group.

Also, it was determined those in terms of RBC count in the CSF samples of the survival CD group (Table 13), 13 dogs were categorized as none (30.77%), 3 dogs were categorized as rare (23.08%) and 2 dogs were categorized as moderate (15.38%), according to the cell count per slide or OIF. Similarly in terms of RBC count in the CSF samples of the non-survival CD group, 20 dogs were categorized as none (24%), 26 dogs were categorized as rare (32%) and 10 dogs were categorized as moderate (12%), in terms of WBC count of the CSF samples of the survival CD group (Table 13), 6 dogs were categorized as none (46.15%), 4 dogs were categorised as rare (30.77%), 2dogs were categorised as few (15.38%) and 1 dog was categorized as moderate (7.69%). On the other hand, 26 dogs were categorised as none (32%), 30 dogs were rare (37%), 13 dogs were under few (16%) and 11 dogs were under moderate (13%) in case of non-survival groups.

In the present study, the total protein level in the CSF of the CDV-infected group was notably higher than reported levels in healthy dogs (Kim et al., 2008). Similarly, Gama et al. (2007) found significant increases in CSF total protein and albumin quotient, demonstrating disruption of the blood-brain barrier (BBB) in nervous distemper, which aligned with our findings. Dogs that were lethally infected exhibited higher CSF protein concentrations than those that survived. Morphological evidence of albumin localization around parenchymal vessels in the brain further supported the occurrence of BBB leakage. In healthy dogs, several red blood cells (RBCs) might be present in CSF samples due to dural puncture during collection (Thomson et al., 1990). Normal CSF findings in healthy dogs include total protein concentrations below 30 mg dl^{-1} (Kim et al., 2008). Several parameters in the current study exceeded previously reported normal ranges in healthy dogs (Kim et al., 2008). The observed leukocytosis and elevated total protein concentrations in CSF were interpreted as signs of BBB damage caused by viral invasion (Gulersoy et al., 2020).

Table 13: RBC and WBC count in the CSF samples of survival and non-survival CDV-infected dogs

	Survival (n=13)	Non-survival (n=82)
RBC		
None (0/slide)	30.77% (4/13)	24% (20/82)
Rare (<10/slide)	23.08% (3/13)	32% (26/82)
Few (<1/oil immersion field [OIF]	30.77% (4/13)	32% (26/82)
Moderate (1 to 10/OIF)	15.38% (2/13)	12% (10/82)
Many (>10/OIF)	-	-
WBC		
None (0/slide)	46.15% (6/13)	32% (26/82)
Rare (<10/slide)	30.77% (4/13)	37% (30/82)
Few (<1/oil immersion field [OIF]	15.38% (2/13)	16% (13/82)
Moderate (1 to 10/OIF)	7.69% (1/13)	13% (11/82)
Many (>10/OIF)	-	-

These alterations correlated strongly with neurological involvement and poor prognosis.

3.8. Comparison of CNS biomarkers among survival, non-survival groups of canine distemper-infected dogs and healthy controls

The MBP level was significantly (p<0.01) increased in non-survival groups, followed by survival groups as compared to healthy dogs (Table 14). Similarly, NSE level was also significantly (p<0.01) increased in non-survival groups, followed by survival groups as compared to healthy dogs (Table 14).

In canine distemper virus (CDV) infection, particularly in its neurological form, elevated NSE levels in cerebrospinal fluid (CSF) and plasma were indicative of neuronal damage. In the present study, neuron-specific enolase (NSE) concentrations in both cerebrospinal fluid (CSF) and plasma were found to be significantly elevated in dogs infected with canine distemper virus (CDV) compared to healthy controls. These findings suggested that NSE could serve as a useful biomarker for assessing neuronal degeneration in canine distemper. The increase in NSE was presumed to result from its leakage from injured neurons into the extracellular space, from where it entered the CSF and subsequently the bloodstream (Elias et al., 2019). However, some conflicting results have been reported. Ranjithkumar et al. (2022) noted that several CDV-infected serum samples showed zero or undetectable NSE levels, with no significant increase observed when compared to control or stray dogs. This raised concerns about the diagnostic reliability of serum NSE alone, suggesting that cerebrospinal fluid (CSF) measurements might offer a more accurate and dependable assessment of neuronal damage. Nevertheless, in our study, NSE values were significantly (p<0.05) elevated in most dogs in both the survival and non-survival groups compared to healthy dogs, supporting its potential value as a biomarker in CDV-related CNS damage. Similarly, myelin basic protein (MBP), a structural protein of the myelin sheath, was also found to be elevated in the CSF of dogs with neurologic distemper. Increased MBP levels were consistent with demyelination, a hallmark of CDV-associated leukoencephalopathy. The present study showed that MBP concentrations in CSF were significantly higher in CDV-infected dogs than in healthy controls, suggesting severe myelin damage in natural cases of neurological distemper. Previous studies have shown that dogs with subacute to chronic CDV infections develop demyelinating leukoencephalopathy, which correlated with elevated CSF MBP levels (Lempp et al., 2014). Overall, the findings of this study suggested that both NSE and MBP levels in CSF might serve as valuable diagnostic and prognostic markers in dogs with neurological manifestations of CDV infection. While serum NSE levels might be less reliable due to variability in detection, CSF-based measurements of these markers provided a more direct and accurate reflection of CNS pathology. The progressive elevation of MBP and NSE in non-survivors indicated severe demyelination and neuronal damage in advanced CDV infection. These biomarkers, therefore, provided valuable prognostic indicators, with higher levels correlating with poor outcomes and increased neurological involvement.

Table 14: Comparison of CNS biomarkers among survival, non-survival group of canine distemper-infected dogs and healthy controls

Parameters	Survival (n=13)	Non-survival (n=82)	Healthy (6)	p-value
MBP (ng ml^{-1})	16.80±1.34^{a}	26.64±0.82^{b}	0.35±0.04^{c}	p<0.001*
NSE (ng ml^{-1})	6.57±0.97^{a}	8.97±0.70^{b}	1.51±0.08^{c}	p=0.009

The values have been expressed as Mean±SE; Superscripts a, b, and c among the groups differ significantly (p<0.05)

4. CONCLUSION

An overall prevalence of 9.5% and a high case fatality rate (86.32%), CDV was a serious threat to canine health, particularly in non-vaccinated, purebred, and young (1–2 years) dogs, with winter showing peak incidence in

Mizoram. The concurrent elevation of CNS biomarkers such as myelin basic protein (MBP) and neuron-specific enolase (NSE), along with oxidative stress markers, strengthens the understanding of underlying neurodegeneration.

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