Epidemiology of Canine Distemper and assessment of Homeopathic adjunct therapy in neurological cases

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Journal of Livestock Science (ISSN online 2277-6214) 17: 1014-1024
Received on 10/5/26; Accepted on 4/11/26; Published on 9/11/26
doi. 10.33259/JLivestSci.2026.1014-1024

Abstract

The dogs that presented with the classical signs of canine distemper, either neurological form alone or in association with systemic illness during January-December 2022, were taken up in this study. The incidence of canine distemper was high in the 0-6 months age group (32.21%), non-descript (72.48%), males (75.16%) and during summer (41.6%), with a highly significant difference ($P<0.01$) among age groups, sex, breeds and seasons. The therapeutic usefulness of homeopathic medicinal preparations (HMPs) in paralytic cases of canine distemper (CD) was reported. For this purpose, the dogs presenting with paralytic signs were confirmed by either lateral flow assay (LFA) or reverse transcription polymerase chain reaction (RT–PCR). The test-positive cases were categorized into three treatment groups (n=6 in each), which were treated (oral/parenteral) as follows: Group I: *Withania somnifera*, *Tinospora cordifolia*, *Cicuta virosa* and methylcobalamin; Group II: *Cicuta virosa*, methylcobalamin and enrofloxacin; Group III: Diazepam, methylcobalamin and enrofloxacin; and Group IV: Healthy dogs. Group I, post treatment, showed improved gait in paralytic cases with the absence of secondary complications, an increase in the mean lymphocyte count (prognostic indicator), a decrease in the neutrophil count, and a reduction in serum alkaline phosphatase (SAP) and alanine aminotransferase (ALT) were observed. Group I showed best scoring in terms of clinical improvement than that of other treatment groups. The synergistic effect of *Withania*, *Tinospora* and *Cicuta* was found to be effective in treating paralytic cases stimulating lymphocyte count and preventing secondary bacterial complications in neurological cases of canine distemper.

Keywords: Alternative medicine, Canine distemper, Diagnosis, Epidemiology, Haemato-biochemical changes

Introduction

Canine distemper is a highly contagious and globally distributed disease with high mortality in susceptible canids (Rendon-Marin et al., 2019). Canine distemper virus (CDV) is reported to cause the second highest mortality rate in domestic dogs (Palomino *et al*., 2022). The virus has multiple cellular tropisms affecting the digestive, respiratory, nervous system[3]and immune system that result in long-lasting immune suppression (Karki et al., 2022). Secondary infections associated with leukopenia commonly result in complications during the clinical course (Rendon-Marin et al., 2019).

It causes persistent infection within the central nervous system, resulting in progressive and multifocal demyelination and encephalitis (Sykes, 2014; Yilmaz et al., 2022). The neurologic form of canine distemper may occur at any age in dogs that have no history of systemic signs and can cause progressive neurologic dysfunction in older dogs. The systemic signs include anorexia, pyrexia, serous oculo-nasal discharge, impetigo on the ventral abdomen, occasional rashes, respiratory distress, vomiting and diarrhoea, anaemia, and thrombocytopenia, and these can occur in various combinations (Sykes, 2014).Neurological signs such as myoclonus (hyperkinesia), chewing gum fits, chorea, grand-mal seizures, hyperesthesia, cervical spasm, cerebellar signs, vestibular signs and paraplegia or tetraplegia are highly suggestive of canine distemper (Degene and Zebene, 2019).Neurological signs can be observed simultaneously with multisystemic illness or at 1-3 weeks after recovery from systemic illness.

Homeopathic medicines may have their own mechanisms of action and could offer an alternative approach to promoting healing in aquaculture without harmful chemical residues **(**Narayanan & Vidhya 2025; Narayanan & Iffath, 2026). The homeopathic medicinal preparations (HMP) of the herbal medicine *Withania somnifera* (Aswagantha) have an immunomodulatory effect, as they increase leukocyte count (WBC), haemoglobin (Hb), red blood cells (RBC) and platelets. It also has anticonvulscent, antibacterial, antioxidant, anti-inflammatory, hepatoprotective and psychotropic properties (Narinderpal et al., 2013; Meher et al., 2016). The herbal medicine *Tinospora cordifolia* (Guduchi) has immunomodulatory, antiviral, antioxidant, and increased phagocytic activity by macrophages and production of reactive oxygen species (Mittal et al., 2014; Afroz Patan et al, 2024). *Cicuta virosa* (CV, the water hemlock) is a GABA receptor antagonist, and the HMP of CV controls epileptic convulsions (Saeed et al., 2015; Mishra et al., 2021). Methylcobalamin (Vitamin B_{12}) has been used as a promising drug candidate in novel neuroprotective therapies (Li et al., 2021), nerve conduction, peripheral nerve repair and neuroprotective function due to its anti-necrotic and anti-apoptotic effects on neurons (Suresh et al., 2021). Further, methylcobalamin is the only form of B_{12} that can cross the blood brain barrier without biotransformation (Gupta and Sana, 2015).

Traditional antiepileptic drugs (AEDs) pose a significant challenge in treatment due to their inability to control seizures in approximately 20% to 30% of epileptic dogs. Additionally, they are associated with adverse effects such as lethargy and ataxia in both dogs and humans, leading to a reduced quality of life (Wesssmann et al., 2016). Moreover, AEDs do not treat demyelinating encephalitis, lower motor neuron (LMN) dysfunction, or tetraplegia in canine distemper cases. In some instances, they may even exacerbate paralysis. Therefore, there is a pressing need for an alternative therapy for epilepsy management (Cameron et al., 2023) and alternatives for antimicrobials to prevent the CDV associated systemic signs, sepsis caused by secondary bacterial infections and risk of antimicrobial resistance (AMR) associated with long term usage of antibiotics in chronic cases. The objective of this paper is to carry out an epidemiological investigation on the incidence of canine distemper, assess the therapeutic usefulness of homeopathic medicines in the treatment of paralytic canine distemper cases and their immunomodulatory effect to prevent secondary complications or sepsis and increase the lifespan of paralytic cases.

Materials and methods

The dogs presented in Clinics, Veterinary College and Research Institute, Namakkal with the classical signs of canine distemper had a history of either no vaccination or incomplete vaccination against canine distemper. The systemic signs observed were anorexia, pyrexia, oculo-nasal discharge, congested mucosae, impetigo on the ventral abdomen, hyperkeratosis of the foot pad/nasal planum, genital discharge from the penile orifice/vagina, epididymitis, scrotal dermatitis, ocular signs and enamel hypoplasia and pulmonary infiltration by radiography (Figure 3A and 3B). The neurological form included signs such as focal or generalized myoclonus, chorea, status epilepticus, chewing gum fits, ataxia, cervical rigidity with head tilting (UMN dysfunction) and paraplegia (LMN dysfunction) (Figure 3A).

The immunochromatography-based lateral flow assay kits procured from Ubio Biotechnology systems Pvt. Ltd. (QuickVET®, Ubio Biotechnology Pvt. Ltd., Kochi) were used to confirm the paralytic cases to be selected for the treatment groups in this study. The nasal swabs were aseptically collected from the paralytic cases of CD into the buffer vials provided. Approximately 15 microliters of the test sample followed by two drops of the assay buffer using a sterile pipette were added into the sample hole of the test kit. The results were interpreted within 10 minutes. The

positive result is indicated by the red lines in both the control (C) and test (T) marks (Figure 3C), whereas the negative result is indicated by the red line only in the (C) mark.

The paralytic cases that were negative for CDV by lateral flow assay were subjected to reverse transcriptase polymerase chain reaction (RT–PCR), and the positives were selected for the treatment groups. Blood samples in EDTA vials/nasal swabs in buffered saline from the paralytic cases of CD were collected, and RNA was extracted by using a QIAGEN QIAamp® Viral RNA Mini kit (Qiagen Sciences, MD, USA) as per the protocol recommended. The primers for RT–PCR (Menezes et al., 2023) were custom synthesized (Eurofins and Sigma, India) with the following nucleotide sequences (5'-3') targeting the nucleoprotein gene of CDV: Forward– ACA GGA TTG CTG AGG ACC TAT and reverse- CAA GAT AAC CAT GTA CGG TGC with an amplicon size of 287 bp (Figure3D). The DNA extract was then amplified using the above primers and reaction mixture with the following cycling conditions: initial denaturation at 94°C/4 min, denaturation at 94°C/60 sec, annealing at 59.5°C/120 sec and extension at 72°C/60 sec with 40 cycles, and final extension at 72°C/5 min. The gel was visualized under a UV trans-illuminator.

The clinical cases presented with the neurological signs and confirmed by lateral flow assay test or RT–PCR were selected (n=18) and categorized into three groups: Group I (n=6): Treated with *Withania somnifera* cum *Tinospora cordifolia* (200C potency, 5 drops each, PO, q12hr: SBL, New Delhi), *Cicuta virosa* (200C potency, 5 drops, PO, q12hr; SBL, New Delhi) and Inj. Methylcobalamin (500 mcg, IV, q24hr); Group II (n=6): Treated with *Cicuta virosa* (200C potency, 5 drops, PO, q12hr; SBL, New Delhi), Inj. Methylcobalamin (500 mcg, 24hr) and Inj. Enrofloxacin (5 mg kg^{-1},IV, 24hr); Group III (n=6): Treated with Inj. Diazepam (5 mg kg^{-1}, IV, 24hr), Inj. Methylcobalamin (500 mcg, IV, q24hr) and Inj. Enrofloxacin (5 mg kg^{-1}, IV, 24hr); and Group IV (n=6): Healthy dogs negative by the tests were selected and no treatment was given. Blood samples were collected from all the groups on day 1 and day 7 post-treatment and subjected to haemato-biochemical analysis. All procedures were performed in compliance with relevant laws and institutional guidelines and that the appropriate institutional committee have approved them.

Statistical analysis

The data pertaining to the Epidemiological factors associated with the occurrence of canine distemper were expressed by frequencies and percentages and further statistically analysed by Chi-square test.

The data on the haematological and serum bio-chemical values were analysed by descriptive statistics in the form of Mean and Standard Error and further analysed by One-way ANOVA and the significant differences between groups were assessed by post-hoc test in the form of Duncan's multiple range test. The statistical analysis for this study were performed by statistical software IBM® SPSS® Ver.26.0 for Windows®.

Results and Discussion

The epidemiological determinants associated with the clinical cases (n= 298) presented with the systemic signs and classical neurological signs of canine distemper during January–December 2022 were analysed by retrospective study (Table 1, Figure 1, Figure 2). High positivity was observed in 0-6 month and 1-3 year age group. This finding was in accordance with that of Yilmaz et al. (2022) and Devi et al. (2022) who also recorded a high positivity in the below 6 months age group, whereas Menezes et al. (2023) reported a high incidence in adults (1-6 years). The higher incidence in young dogs may be related to incomplete vaccination and the decline of maternally derived antibodies (Vila Nova et al., 2018). Delayed vaccination of non-descript dogs by most pet owners around 4 to 6 months of age could be the reason for the decreased incidence at the age group of 6-12 months. Because many pet owners prefer to vaccinate only after experiencing the incidence of the acute and fatal canine parvo viral (CPV) enteritis. However, due to the neglectof revaccinationby most pet owners and the resultant waning of vaccinal immunity could be attributed to the increased incidence of CD again at the age of 1-3 years.

Most of the cases were male dogs (75.16%), as males were the most preferred by pet owners, and this finding was in agreement with that of Devi et al.(2022). Non-descript dogs (72.48%) were the most commonly presented among the positive cases, followed by other breeds, as non-descript dogs were the most preferred dogs compared to any other breed. This finding was in accordance with that of Menezes et al. (2023) and Devi et al. (2022). The reason could be the increased population of non-descript dogs and less attention and ignorance of vaccination paid to these dogs than breeds. Most cases were recorded in the summer season (41.61%), followed by other seasons. Previously, Devi et al. (2022) also recorded a high number of CD cases during Summer in 2021 and 2022 in Chennai of Tamil Nadu, whereas the incidence was reported high during winter in 2017 and 2018. This could presumably due to the climatic change associated with unexpected rainfall throughout the year in 2022 and the increased rate of virus spread by inhalation of aerosol in dry months. Statistically, significant difference ($P<0.01$) was observed among age groups, sexes, different breeds and different seasons (Table 1).

The clinical signs of systemic illness and demyelinating encephalitis observed in cases of canine distemper in this st udy were in accordance with that of previous authors (Sykes, 2014). Seizures and myoclonus have been

observed as the most frequent neurological findings, although they were observed in association with systemic signs in some cases (Sykes, 2014). Paraplegia and tetraparesis observed in this study could be attributed to multifocal encephalomyelitis, which lead to a chronic course in dogs, starting with incoordination and ataxia (Rendon-Marin et al., 2019). Demyelination could be associated with the immunosuppression caused by canine distemper virus (CDV) replication in the white matter (Sykes, 2014).

Immunochromatography-based lateral flow assay, as a quick test, was found to be useful in detecting the CDV antigen from conjunctival swabs/nasal swabs, with a sensitivity of 66.6% and specificity of 100.0% when compared with RT–PCR (Joshi et al., 2022) however, a negative result does not rule out canine distemper (Sykes, 2014). Hence, such point-of-care-based diagnostic aids are reported to be useful for early diagnosis, and molecular-based techniques such as RT–PCR can also be used for confirmatory diagnosis, although they are time consuming and laborious (Desai et al., 2021).

A custom clinical scoring system (Table 2) was developed for the response in all three groups before treatment (BT) and after treatment (AT), based on published literature describing the spectrum of neurological and systemic signs in canine distemper (Degirmencay, 2023). The clinical score reduced from 51 (BT) to 8 (AT) in group I, from 56 (BT) to 25 (AT) and in group III, 41 (BT) to 39 (AT). Hence, a best scoring was observed in Group I in terms of clinical improvement, when compared to other treatment groups. Group I: The clinical improvement from neurological signs shown by cases in group I was shown in Figure 4). Previously, Jeyalakshmi et al. (2021) recorded neurogenic effect of *Withania* in goats with idiopathic paresis and Saravanan et al. (2019) recorded the antispasmodic effect of *Cicuta* in a dog affected with tetanus. *Withania* acts as a GABA mimetic, promotes dendritic axonal growth and synaptogenesis, and prevents neuronal degeneration and dyskinesia (Singh et al., 2011). All these HMPs could have an additive effect in reducing the generalized myoclonus or seizures. Their antimicrobial, anti-inflammatory and anti-oxidant properties (Mittal et al., 2014 and Meher et al., 2016) may possibly have contributed n reducing the severity and course of multisystemic illness caused by CDV and secondary bacterial infections. Further, these HMPs could be the alternatives to conventional AEDS like phenobarbitones or gabapentins which on long term oral administration at high therapeutic doses can cause hepatotoxicity (Podell et al., 2016), sedation or incoordination (Vail et al., 2020). The AEDs could also result in ataxia and sedation, leading to lower perceived quality of life scores by the owners (Barry et al., 2021). Group II: Systemic illness/secondary infections, tremors and recurrent episodes of seizures (status epilepticus) could not be observed post treatment due to the continuous administration of broad spectrum antibiotic. Pre-renal azotemia could not be noticed post treatment and it may be due to the supportive therapy with fluids. Group III: Systemic illness and tremors could not be observed post treatment. However, the death observed could be due to either severe encephalitis as evidenced by status epilepticus or respiratory paralysis as evidenced by tetraplegia/ paraplegia in this group.

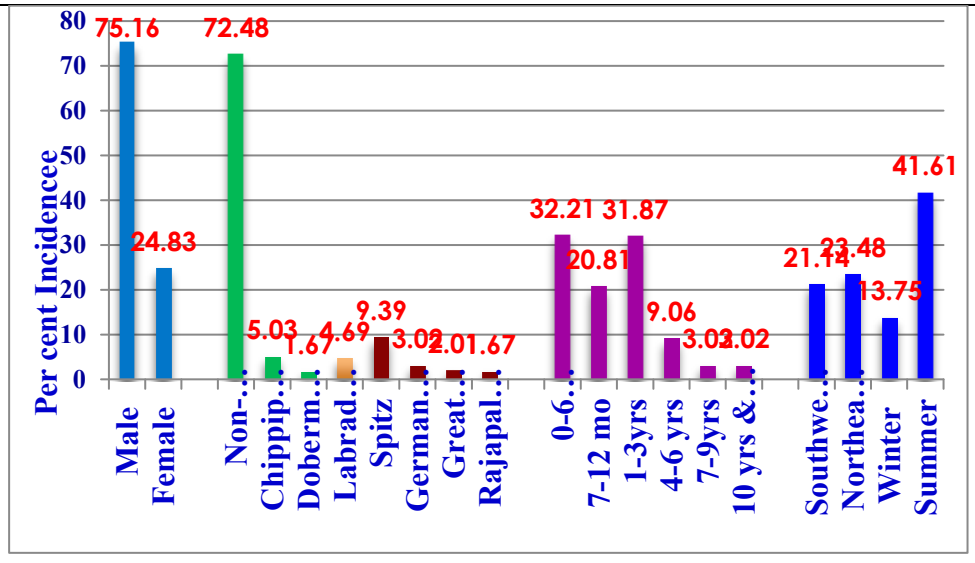


Figure 1. Epidemiological determinants associated with the incidence of canine distemper in dogs in Namakkal region

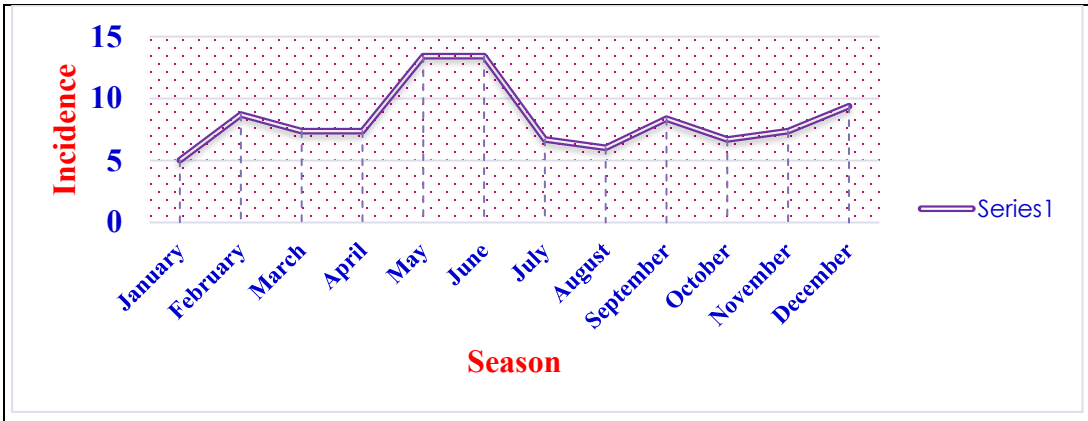


Figure 2. Temporal pattern of the incidence of canine distemper in dogs in Namakkal region

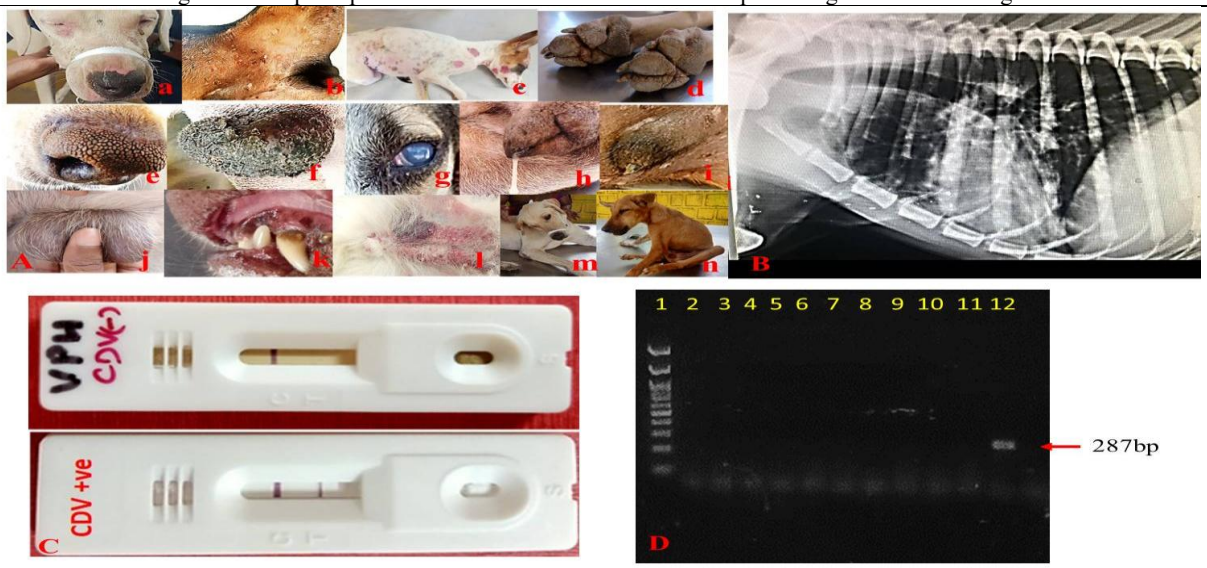


Figure 3 (A):Clinical signs in dogs with canine distemper showing (a) mucoid oculo-nasal nasal discharge, (b) impetigo on the ventral abdomen, (c) generalized pyoderma, (d) and (e) hyperkeratosis of foot pad and nasal planum, (f) Scrotal dermatitis, (g) Uveitis, (h) and (i) purulent discharge from male and female genitalia, (j) testicular swelling, (k) enamel hypoplasia, (l) gangrenous mastitis in a Spitz, (m) torticollis, (n) paraplegia; Figure 3(B):Pulmonary infiltration, Figure 3(C):Lateral flow assay test showing positive and negative results, Figure 3(D):RT-PCR: Lane 1: DNA ladder-100 bp;Lane 2-13 : CDV testsamples; Lane 12: Positive for CDV showing amplicon at 287 bp

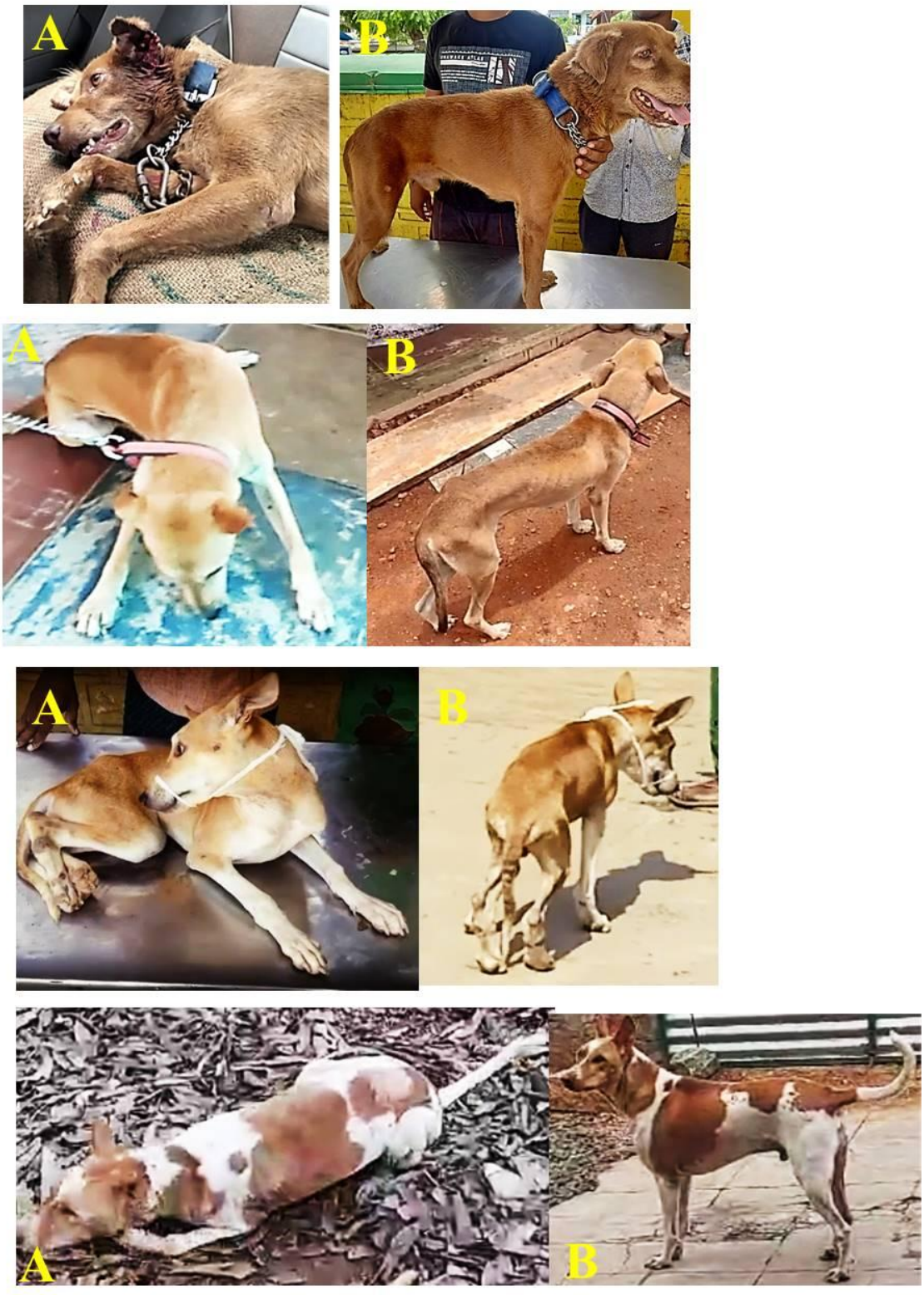


Figure 4: Clinical improvement in paralytic cases of canine distemper before (A) and after treatment (B) in group I

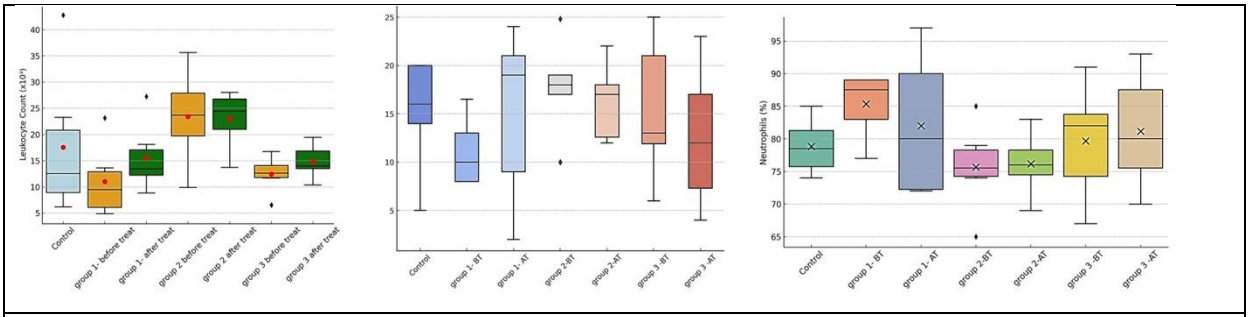


Figure 5: Box-whisker plot analysis of leukocyte, neutrophil and lymphocyte counts pre and post treatment in the treatment and healthy groups

Table 1. Epidemiological factors associated with the occurrence of canine distemper in Namakkal district

Epidemiological determinants		Total positives (n=298)	Per cent positives	Chi square Value
Age	0-6 months	96	32.21	197.52**
	7-12months	62	20.81	
	1-3years	95	31.87	
	4-6 years	27	9.06	
	7-9years	9	3.02	
	10 years and above	9	3.02	
Sex	Male	224	75.16	151.01**
	Female	74	24.83	
Breed	Non-descript	216	72.48	852.66**
	Chippiparai	15	5.03	
	Dobermann pinscher	5	1.67	
	Labrador retriever	14	4.69	
	Spitz	28	9.39	
	German Shepherd	9	3.02	
	Great Dane	6	2.01	
	Rajapalayam/Rottweiler/Pug	5	1.67	
Season	Southwest monsoon	63	21.14	66.67**
	Northeast monsoon	70	23.48	
	Winter	41	13.75	
	Summer	124	41.61	

**P<0.01, Statistically significant at 1% level

Table 2. Score card for the clinical outcome of neurological canine distemper cases before treatment (BT) and after treatment (AT)

Neurological Signs	Score	Treatment Group 1 (n=6)				Treatment Group II (n=6)				Treatment Group III (n=6)			
		BT		AT		BT		AT		BT		AT	
		Cases	Score	Cases	Score	Cases	Score	Cases	Score	Cases	Score	Cases	Score
Mild tremors	1	1	1	0	0	2	2	0	0	1	1	0	0
Myoclonus on Temporal muscles	2	4	8	2	4	3	6	2	4	3	6	2	4
Chewing-gum fits	3	4	12	1	3	3	9	0	0	2	6	1	3
Paraparesis/Tetraparesis	3	3	9	0	0	3	9	3	9	3	9	3	9
Generalized seizures	4	-	-	-	-	-	-	-	-	-	-	-	-
Status epilepticus	5	1	5	0	0	1	5	0	0	1	5	1	5
Paraplegia	4	1	4	0	0	1	4	1	4	1	4	1	4
Tetraplegia	5	1	5	0	0	1	4	1	4	1	4	1	4
Systemic illness													
Nasal discharge -Mucoid	2	2	4	0	0	3	6	0	0	3	6	0	0
Mild hepatocellular injury (Elevated ALT/AST levels)	1	3	3	1	1	5	5	4	4	0	0	0	0
Renal azotemia (Elevated BUN/ creatinine levels)	3	0	0	0	0	2	6	0	0	0	0	0	0
Death	10	0	0	0	0	0	0	0	0	0	0	2	10
Total score			**51**		**8**		**56**		**25**		**41**		**39**

Table 3: Hemato-biochemical changes (Mean±SE) in different groups of dogs affected with neurological canine distemper

Values	Control group (Healthy)	Group I		Group II		Group III		F value /Significance CI: 95%
		BT (Day 1)	AT (Day 7)	BT (Day 1)	AT (Day 7)	BT (Day 1)	AT (Day 7)	
Hb (g/dl)	16.95±0.41^{b}	10.83±0.89^{a}	10.05 ± 0.43^{a}	9.850±0.95^{b}	6.86±0.87^{a}	11.200±1.00^{a}	11.01±0.86^{a}	8.7/0.000**
HCT (%)	46.30 ±3.38^{b}	36.35±2.50^{a}	34.61±1.43^{a}	34.00±2.88^{b}	24.53±2.59^{a}	37.83±3.19^{a}	36.10±2.63^{a}	2.4/0.044*
RBC (×10^{6}/µl)	6.78 ±0.55^{b}	5.28±0.372^{a}	4.94±0.23^{a}	5.25±0.461^{a}	4.06±0.54^{a}	5.36±0.44^{a}	5.20±0.40^{a}	1.9/0.107^{NS}
WBC (×10^{3}/µl)	17.56 ±5.60^{ab}	11.00±2.77^{a}	16.52±2.33^{ab}	23.44±3.63^{bb}	20.87±3.58^{ab}	12.45±1.39^{a}	14.82±1.32^{ab}	2.4/0.043*
Neutrophils (%)	75.83 ±1.70^{a}	85.35±1.99^{b}	73.05±1.27^{a}	75.70±2.65^{a}	76.03±4.04^{a}	79.66±3.55^{ab}	81.30±3.64^{ab}	2.3/0.052^{NS}
Lymphocytes (%)	15.66 ±2.34^{ab}	9.91±1.78^{a}	20.40±1.21^{b}	17.30±1.98^{b}	16.53±3.23^{ab}	15.15±2.77^{ab}	15.14±2.90^{ab}	2.2/0.064^{NS}
Monocytes (%)	2.66 ±0.66^{a}	4.73±0.69^{ab}	5.88±1.043^{b}	7.00±0.73^{b}	6.33±1.33^{b}	6.98±2.26^{b}	5.15±0.75^{b}	2.8/0.022*
Platelets (x10^{3})	288.16 ±46.18^{a}	368.66±56.96^{ab}	751.66±275.80^{b}	325.83±40.25^{a}	436.00±74.48^{ab}	357.16±38.97^{ab}	305.83±77.10^{a}	1.7/0.132^{NS}
Total Protein (g/dl)	7.51±0.59^{b}	6.42±0.42^{ab}	5.90±0.70^{ab}	7.80±0.99^{b}	5.16±0.92^{a}	6.86±0.46^{ab}	7.28±0.35^{b}	1.8/0.129^{NS}
Albumin (g/dl)	2.93±0.19^{a}	3.07±0.36^{a}	3.16±0.36^{a}	4.18±1.18^{a}	2.63±0.48^{a}	3.30±0.22^{a}	3.56±0.19^{a}	0.8/0.527^{NS}
ALT (U/L)	75.66±10.27^{a}	70.15±34.09^{a}	68.23±25.34^{a}	59.16±24.93^{a}	88.66±66.44^{a}	38.23±6.54^{a}	70.66±25.89^{a}	0.3/0.923^{NS}
SAP (U/L)	62.66±7.33^{a}	150.16±61.55^{ab}	128.33±57.33^{ab}	359.00±143.21^{bc}	405.50±301.50^{c}	115.83±30.61^{a}	127.66±46.06^{ab}	2.3/0.055^{NS}
BUN (mg/dl)	30.17±2.79^{ab}	10.33±0.98^{a}	13.45±1.73^{a}	62.70±31.12^{b}	7.66±1.66^{a}	15.96±2.62^{a}	15.33±3.61^{a}	2.8/0.027*
Creatinine (mg/dl)	1.18±0.24^{a}	0.8667±0.15^{a}	0.87±0.18^{a}	4.93±2.52^{b}	0.700±0.11^{a}	0.89±0.11^{a}	0.95±0.07^{a}	2.7/0.029*
Calcium (mg/dl)	10.91±0.52^{a}	14.38±0.68^{a}	10.75±1.78^{a}	10.79±2.29^{a}	11.43±2.87^{a}	11.33±0.79^{a}	10.76±0.79^{a}	0.9/0.469^{NS}
Phosphorus (mg/dl)	4.13±0.47^{a}	4.99±0.72^{a}	5.80±0.61^{a}	12.06±3.74^{b}	6.38±.95^{a}	3.77±0.74^{a}	6.70±1.56^{a}	3.0/0.018*
Glucose (mg/dl)	125.16±13.08^{a}	153.20±51.32^{a}	95.0±10.15^{a}	94.80±12.83^{a}	91.33±3.17^{a}	111.16±19.73^{a}	109.83±15.16^{a}	0.7/0.598^{NS}
Sodium (mmol/L)	148.04±8.17^{b}	141.25±3.25^{b}	156.86±15.95^{ab}	121.00±9.082^{ab}	132.66±1.33^{b}	79.62±27.40^{a}	131.00±3.62^{b}	2.2/0.068^{NS}
Pottassium(mmol/L)	3.51 ±0.21^{a}	3.38±0.24^{a}	4.53±2.34^{b}	3.70±3.30^{a}	3.26±0.17^{a}	3.75±0.361^{a}	3.13±0.36^{a}	0.7/0.043*

BT-Before treatment, AT- After treatment,Values with similar superscripts do not differ statistically between groups;
* - P<0.05, **P - <0.01, and NS - P>0.05

In the haemato-biochemical analysis (Table 3), the mean haemoglobin (Hb) and haematocrit (HCT) levels and RBC count pre-treatment were lower in groups I, II and III than in the control group. This could be attributed to the persistence of the CDV on bone marrow, interfering erythropoiesis (Sykes, 2014). A highly significant difference (P=0.000) for the mean Hb levels and a significant difference (P=0.04)) for mean HCT levels were observed in groups I, II and III when compared to the control group. However, there was no increase in mean Hb levels, HCT values or RBC count post-treatment in groups I, II and III.

The mean WBC count pre-treatment was significantly lower (P=0.043) in group I, followed by group III, than in the control group. Post-treatment, a significant increase in the mean WBC count returning to normal level was observed in group I, followed by group III. In contrast, post-treatment, a reduction in the mean WBC count was observed in group II. The mean lymphocyte (prognostic indicator) count pre-treatment was lower but within range in group I than in all other groups, since lymphopenia and neutrophilia are reported to be common in canine distemper cases (Sykes, 2014). Post treatment, a remarkable increase in the mean lymphocyte count was observed in group I, whereas no or mild change in the count could be observed in groups II and III. These changes in group I post-treatment could be associated with the immunomodulatory property of *Withania* and *Tinospora*. The mean neutrophil count pre-treatment in groups I and III was higher than (P<0.05) that in the control group and above the normal level. Post-treatment, a significant decrease (P<0.05) in the count returning to normal level could be observed in group I, indicating the recovery from secondary bacterial complications. In contrast, an increase in the mean count was observed in groups II and III, indicating the persistence of secondary bacterial infections, despite antibiotic therapy. The mean platelet count, pre-treatment in groups I was within the normal range and post-treatment, a significant increase in count (thrombocytosis) could be observed. This might be due to the effect of *T. cordifolia* which can significantly increase the platelet count within a shorter duration (Singh and Yadav, 2024), whereas a slight increase in the count was noticed in group II which did not include *Tinospora*. The decrease in platelet count noticed in group III could be associated with CDV which can lead to thrombocytopenia (Jesus et al., 2021). The mean monocyte count pre and post-treatment in groups I, II and III showed no deviation from normal levels. Change in the count of leukocytes, neutrophils (N) and lymphocytes (L) pre and post treatment in the treatment and healthy groups was interpreted by box-whisker plot analysis (Figure 5).

The mean levels of sodium and potassium pre-treatment in all treatment groups were below normal (hyponatremia and hypokalaemia) due to anorexia/hyporexia associated with pre-renal azotemia. However, post-treatment in all treatment groups, the mean sodium levels increased due to continuous parenteral electrolyte therapy in all groups, whereas the mean potassium level increased in only group I and decreased further in groups II and III, as Sykes (2014) and Vahlenkamp (2017) also reported electrolyte imbalance due to kidney dysfunction.A reduction in the enzyme levelsof ALT and SAP though within range, in group I post-treatment (P<0.05)could be due to the antiviral and hepatoprotective effect of *Tinospora* on CDV (Mittal et al, 2014), as CDV is known to cause hepatic necrosis

(Bastos et al., 2020). In contrast, the increase in these enzyme levels in group II and III post-treatment might be due to the perpetuating CDV infection. Elevated blood urea nitrogen (BUN) (p=0.027), creatinine (p=0.029) and hyperphosphatemia in group II pre-treatment could be the signs of pre-renal azotemia associated with the effect of CDV on urogenital epithelia (Palomino et al, 2022) or IgM mediated immune complex deposition (Silva et al., 2022), nevertheless, a reduction in these levels was noticed post-treatment. Hyperglycaemia was observed in group I pre-treatment and the reduction post-treatment, though it is not a feature of CDV infection. This could be associated with *Withania* and *Tinospora*, which are reported to be effective in enhancing insulin sensitivity and useful in diabetic management (Makhlouf et al., 2024 and Afroz Patan et al., 2024).

Conclusion

The HMPs, *Withania* and *Tinospora* had synergistic effect in treating the paralytic stage, contributing antimicrobial effects to combat CDV/secondary bacterial infections, and thus it could prevent the risk of septicaemia. The *Withania* together with *Cicuta* could be synergistic in reducing the frequency of convulsive episodes and could be the alternative to traditional AEDs, as the latter fail to prevent the relapse of seizures and can lead to hepatotoxicity upon long-term administration. Thus, HMPs were found to be useful in extending the lifespan of neurological cases of canine distemper.

Acknowledgement: The authors would like to thank the Dean, Veterinary College and Research Institute, Namakkal, for providing diagnostic facilities to carry out this study.

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