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Therapeutic efficacy of herbal drugs on Canine Distemper infected animals

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Abstract

Background: Canine Distemper (CD) is an important viral disease of dogs and seasonal prevalence of the disease can be predicted every year in Chennai. Since nervous signs of CD require long time of treatment, it is advisable to utilize herbal drugs. With this aim, the present study was conducted in CD infected dogs.

Methods: Dogs (40) positive for CD by nRT-PCR were selected and they were divided into two groups consist of 20 dogs each. Group - 1 and Group -2 were treated with Conium and Candist respectively for a month. Clinical response was observed and Kaplan–Meier curves were constructed to explore the effectiveness of treatment trials.

Results: Out of 40 dogs, totally 18 had survived (12 in Group-2 and 6 in Group -1). Candist was found to give the better result compared to conium.

Conclusion: Candist saved 60 per cent of the dogs in the trial.

Keywords: Canine distemper, herbal drugs, candist, conium

1. Introduction

Canine Distemper (CD) is an endemic disease in India and the disease occurrence is a quite common particularly in Chennai during the transition period between winter and summer. Generally, there is no specific therapy for animals with clinical form (Deem *et al.*, 2000) [3] except supportive treatment with antibiotics, rehydration with fluids. Moreover, prolonged treatment with anti-convulsant is required to minimize CNS signs like seizures and chorea. Further, residual symptoms like temporal twitching and champing of jaws require a year-long treatment. Hence, it is wise to use indigenously available herbal drugs in the prolonged duration of treatment instead of allopathic drugs to reduce the possible renal damage induced by the latter. With this context the present study utilized the dogs (40) which were positive for Nested RT-PCR (nRT-PCR), an effective test amongst molecular diagnostics for CD and the efficacy of the drugs were compared.

2. Materials and Methods

2.1 Collection of samples

A sterile two mL tube with one mL of sterile Phosphate Buffer Saline, PBS (1x, pH 7.2) was used to collect ocular, nasal and prepuccial/vaginal swabs and stored at -20 °C until further processing. About three mL of blood was collected from the saphenous/brachiocephalic vein of the dogs and transferred to EDTA coated tubes for Peripheral Mononuclear Cells (PMC)/Buffy coat separation. *Intra-vitam* urine was collected by catheterization using sterile catheter. All swabs, buffy coat and urine were stored at -20 °C until further processing.

2.2 RNA extraction and Complementary DNA (cDNA) synthesis

The CDV RNA was extracted from epithelial cell swabs (conjunctival, nasal, vaginal and prepuccial swabs) using Trizol method. RNA extraction from buffy coat and urine were done using QI Amp viral RNA mini kit (QIAGEN, catalogue # 52906). The cDNA synthesis was performed from the extracted RNA using iScript Reaction Mix (Bio-Rad).

2.3 Polymerase Chain Reaction (PCR)

2.3.1 Primers

Primers used for nRT-PCR targeting N gene obtained from Sigma-Aldrich, Bengaluru, India. Details of the primers were

listed in Table 1 and reaction volume and concentration of PCR components were followed as per (Alcalde *et al.*, 2013) [1].

Table 1: Primers used in Nested RT-PCR

Gene Target	Name of the Primers	Sequences of primers used	Amplicon size in bp
CDV N gene	Outer Forward	TAA GCT GGG TCA AAG TAA GAT CG	336
	Outer Reverse	GAA TTG CTG AAA TGA TTT GTG AT	
	Inner Forward	TTG GCA TTG AAA CTA TGT ATC C	234
	Inner Reverse	CGA AAC CCA ACC CTC CCA TG	

2.4 Therapeutic study

The dogs (40) positive for CDV by nRT-PCR were selected for the treatment trials and they were divided into two groups consist of 20 dogs each. Group - 1 treated with Conium, homeopathic drug @ 5 granules twice a day for a month whereas Group - 2 was treated with Candist, phytomedicine @5 ml twice a day for a month. The dogs (20) which were treated with only antibiotics were considered as control. The details of the drugs are as follows:

2.4.1 Candist: (Phyto specialities Pvt. Ltd.): It contains the extracts of *Andrographis paniculata*, *Tinospora cordifolia*, *Curcuma longa* and *Piper nigrum*.

2.4.2 Conium: (Schwabe India Pvt. Ltd.): A homeopathic drug was prepared from the plant poison Hemlock which is available in granules.

Both groups were concurrently treated with antibiotics as supplements. Clinical response post 3 months of treatment was observed and dogs that were alive when the study ended were categorized as the censored cases (survived cases). Kaplan–Meier curves were constructed to explore the effectiveness of treatment trials.

2.4.3 Statistical Analysis: All statistical analyses were performed with commercially available statistical software, SPSS, version 26 (IBM). Kaplan–Meier curves for survival

were constructed to explore differences in the survival time for different dog subgroups stratified according to time of death in days.

3 Results

3.1 Therapeutic experimental study of clinical epidemiology

The positive result of n-RTPCR is shown in Fig.1. Out of 40 dogs with therapeutic treatment trials, totally 18 had survived and it comprised of 12 (60 percent) and 6 (30 per cent) dogs from Group-2 and 1 respectively. In both the trials, 22 dogs were dead and the total case fatality rate was found to be 55 per cent (22/40). In control group only one dog was survived. In our study, candist was found to give the better result compared to conium. Although there was a difference noticed in survival per cent between two groups, the Kaplan Meier survival curve showed that both treatment trials were not significantly effective ($p < 0.01$). It also revealed the differences in survival time for different survivors in days.

The number of bends in curve 1 and 2 (Fig. 2) directly indicated the number of deaths. Whereas, censored cases were dogs that have survived till the end of the trial (90 days). The curve -1 which represented the Group-1 had more bends (14) compared to that (8) of curve -2 (Group -2). Moreover, it was noticed that all fatalities occurred within 42 and 30 days post treatment in Group-1 and Group -2 respectively and thereafter no death happened in both the groups.

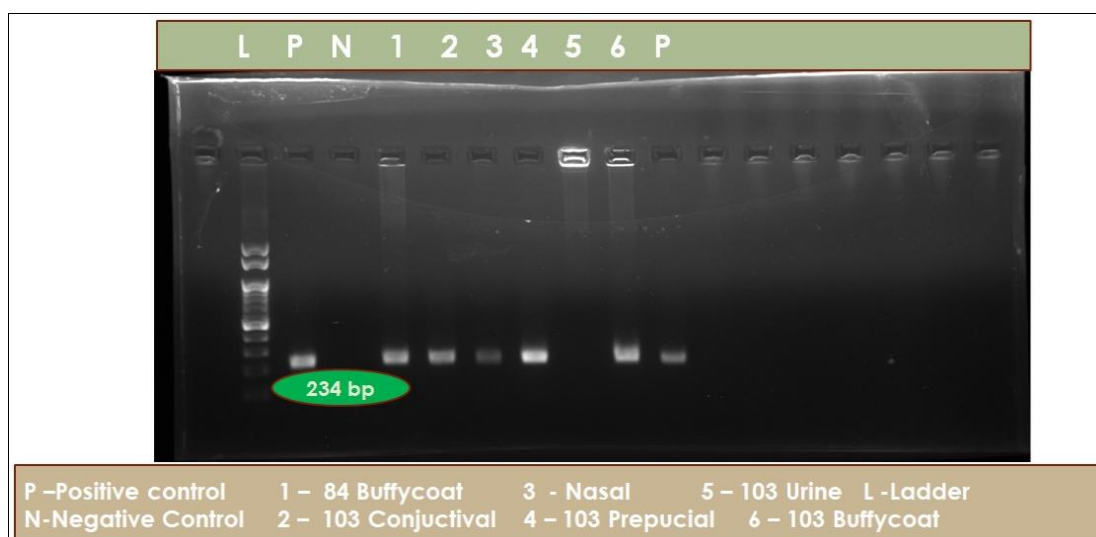


Fig 1: Results of nRT-PCR

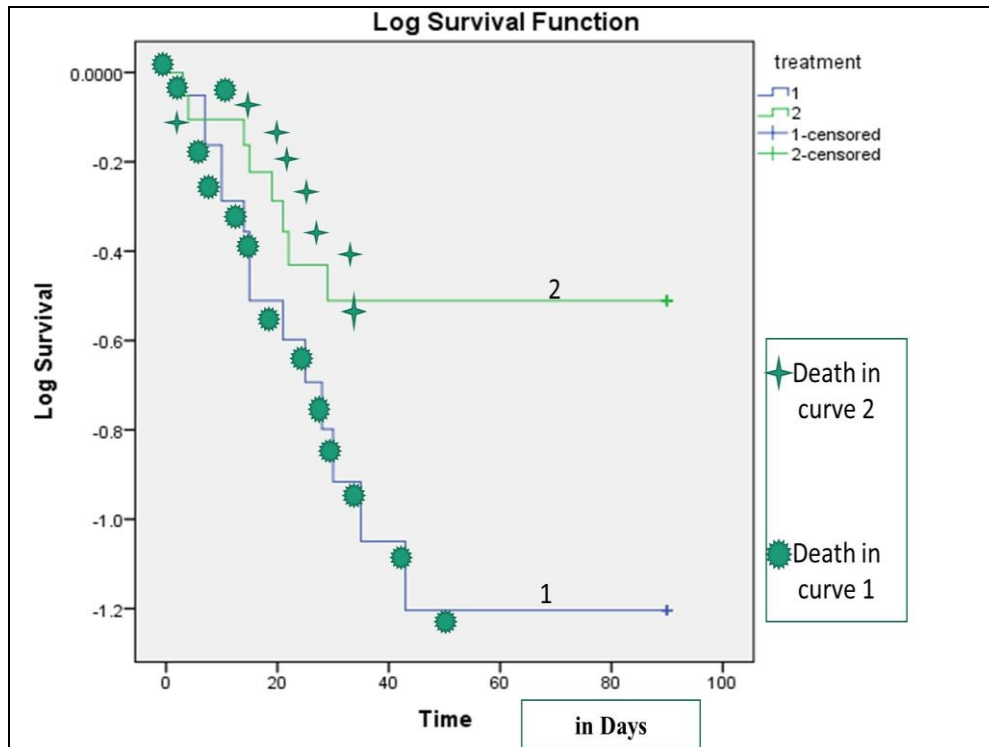


Fig 2: Kaplan Meier Survival Curve

4. Discussions

4.1 Therapeutic experimental study of clinical epidemiology

As there is no specific treatment for CD and the therapeutic management is based on symptomatic treatment aimed at limiting secondary bacterial invasion, supporting electrolytes balance and controlling neurologic manifestations (Degene and Zebene, 2019) [4]. The prognosis of nervous distemper is generally poor although some dogs could recover from the disease with residual burden and hence, the treatment is largely supportive and symptomatic (Tipold *et al.*, 1992) [8].

The present experimental trials studied with ethno-veterinary medicines, the conium and candist as adjunct therapy along with routine therapeutics showed that 18 out of 40 dogs survived in both groups comprising six in Group 1 (30%) and twelve in Group 2 (60%) out of survived dogs. The survival possibility was comparatively found to be higher in Group 2 treated with candist than Group 1 (Conium). These results are supported with Manjunath (1948) [5] and Chopra *et al.* (1956) [2] who had used *Conium maculatum* to treat spasmodic and inflammatory disorders and to relieve nervous excitation and significant analgesic and anti-inflammatory activity at a dose of 200 mg/kg (Reecha and Kumar, 2012) [7]. The empirical approach by Naveenkumar *et al.* (2019) [6] reported that acute clinical signs with systemic involvement due to CD was reduced post first week treatment but noticed residual deficits of neurological signs.

Although these drugs were not offering statistically significant encouraged results, candist saved 60 per cent of the dogs. All fatalities occurred within 42 and 30 days post treatment in Group-1 and Group-2 respectively and not after that. It implies that earlier start of treatment with herbal drugs along with standard symptomatic therapy may save CD infected animals. At the same time, it is necessary to carryout further study to assess their anti-viral property *in vitro* and also to evaluate therapeutic effectiveness without antibiotics and supplemental therapy per se.

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