RESEARCH ARTICLE Indian Journal of Animal Research, Volume 58 Issue 4: 676-680 (April 2024)

Prognostic Factorial Index for Dogs with Canine Distemper

T. Devi[1], M. Asokkumar[2], M. Vijaya Bharathi[1], A. Ramesh[1], K.G. Tirumurugaan[1], R. Venkataramanan[1], N. Pazhanivel[1]

10.18805/IJAR.B-5017

ABSTRACT

Background: Canine distemper (CD) is a highly fatal disease in dogs. Hence, it is important to document the factors which are significantly correlated with the poor prognosis amongst CD infected dogs to develop a prognostic index.

Methods: Forty dogs positive for CD virus by nested reverse transcriptase polymerase chain reaction (nRT-PCR) were included in our study at infectious disease unit (IDU), Madras Veterinary College Teaching Hospital, Chennai between March 2021 and August 2022. Two different herbal drugs were tried in two groups of dogs where each group comprised of 20 dogs and clinical responses were observed. Epidemiological and clinical data were collected and analysed between survival and non-survival groups using chi-square test. The statistically significant factors were further analysed by logistic regression to identify their strong association with the event of death. Kaplan-Meier curves for survival were constructed to explore differences in the survival time for different dogs.

Result: The overall case fatality rate was found to be 55% (22/40). The event of death due to CD was observed in young dogs having ocular discharge with strong correlation but the chances of survival were found to increase when age advanced.

Key words: Canine distemper, Case fatality rate, Herbal drugs, Prognostic index, Survival.

INTRODUCTION

Canine distemper virus (CDV) represents an important conservation threat to many canine species due to its high case fatality rate and has also contributed to the population decline of several wild animals. It is caused by a fragile RNA virus, Morbilli virus. Dogs are generally exposed to CDV through contact with infected oronasal secretions which may be shed by subclinically or clinically affected dogs (Sykes, 2013). Infection of dogs can lead to a severe, multisystemic disease that primarily affects lymphoid tissues followed by gastrointestinal, respiratory, neurologic and cutaneous systems. Moreover, duration and severity of the disease in domestic dogs depends mainly on the age, immune status of the animal and virulence of the virus strain involved. During the second phase of viremia which occurs generally 15 days post infection, animal shows various clinical manifestations such as conjunctivitis, nasal discharge, anorexia, respiratory signs, gastrointestinal signs and neurological deficits. Respiratory signs are a sequels of virus-induced rhinitis and interstitial pneumonia while vomiting, diarrhoea and dehydration are caused by gastrointestinal tract infection (Decaro *et al*., 2004). Enteric and respiratory signs are more often worsened by secondary bacterial infections. Since there is no specific therapy for animals with clinical form of CD (Deem *et al*., 2000) except supportive treatment with antibiotics which is quite helpful to prevent the secondary bacterial infections.

Early treatment especially before the onset of severe nervous signs may save the animal. In India, many herbal drugs have been recently tried along with the symptomatic treatment to reduce the severity of the clinical disease as well as to prolong the life time of the infected animal. In our study, we used two commercially available herbal drugs in treatment trials in 40 dogs and clinical responses of the dogs were observed. With the treatment trials, we also compared the clinical signs, laboratory RT-PCR test results and epidemiological data between survivor and non-survivor dogs. This comparative study leads to identify the factors which are strongly related to the poor prognosis of the dogs and also to develop a prognostic index which is a clinical tool to aid in predicting the prognosis for CD infected dogs.

[1]Department of Veterinary Preventive Medicine, Madras Veterinary College, Tamil Nadu Veterinary Animal Sciences University, Chennai-600 007, Tamil Nadu, India.

[2]Department of Public Health and Epidemiology, Madras Veterinary College, Tamil Nadu Veterinary Animal Sciences University, Chennai-600 007, Tamil Nadu, India.

Corresponding Author: M. Asokkumar, Department of Public health and Epidemiology, Madras Veterinary College, Tamil Nadu Veterinary Animal Sciences University, Chennai-600 007, Tamil Nadu, India. Email: drashokpmd@gmail.com

How to cite this article: Devi, T., Asokkumar, M., Bharathi, M.V., Ramesh, A., Tirumurugaan, K.G., Venkataramanan, R. and Pazhanivel, N. (2024). Prognostic Factorial Index for Dogs with Canine Distemper. Indian Journal of Animal Research. 58(4): 676-680. doi: 10.18805/IJAR.B-5017.

Submitted: 12-09-2022 **Accepted:** 19-12-2022 **Online:** 23-12-2022

MATERIALS AND METHODS

Dogs brought to Infectious Disease Unit (IDU), Madras Veterinary College Teaching Hospital with the clinical symptoms suggestive of CD were tested by nRT-PCR against CDV for a period of 18 months from March 2021 to August 2022. From them, forty dogs which were detected to be positive by nRT-PCR with at least one of the five samples collected from each dog such as conjunctival, nasal, genital swab, buffy coat and urine made the study population.

Conjunctival, nasal and genital swabs were collected in a sterile tube with 1 ml of phosphate buffered saline (PBS, pH 7.4) and stored at -20°C until processing. RNA was extracted from epithelial cells from conjunctival, nasal and vaginal/prepucial swabs by Trizol method (Agnihotri *et al*., 2017). Peripheral blood mononuclear cells were separated by gradient centrifugation of 3 ml of blood collected in a EDTA vacutainers and were used for RNA extraction within 2 hours of collection (Zhu and Murthy, 2014). Urine samples were collected in a sterile container using a catheter and then the sample was centrifuged at 8000 × g for 8 minutes prior to RNA extraction (Tozato *et al*., 2016). QIAamp viral RNA mini kit (QIAGEN, Cat No: 52904) was used for RNA extraction from buffy coat and urine (Zanian *et al*., 2021).

Bio-Rad i-Script CDNA synthesis kit was used for cDNA conversion. Upon RNA extraction, the reaction recipe was prepared for the total volume of 20 µl. The cyclical condition followed for conversion of cDNA includes priming (5 minutes at 25°C); reverse transcription (20 minutes at 46°C) reverse transcription inactivation (1 minute at 95°C). The synthesized cDNA was stored at -20°C until use. Primers of nRT-PCR and cyclical conditions were designed as per Alcalde *et al.* (2013). Puppy DP (Nobivac) vaccine was used as a control.

Epidemiological data such as age, breed, sex, season and vaccination status and data on clinical manifestations of 40 dogs were also collected. Conium, a homeopathic drug prepared from the plant poison Hemlock (Schwabe India Pvt. Ltd.) and is available in granules was given to Group-1 consisting of 20 dogs @ 5 granules twice a day (Naveenkumar *et al*., 2019) for a month. Candist, a herbal syrup was given to Group-2 consisting of 20 dogs @ 5 ml twice a day for a month. Candist contain the extracts of *Andrographis paniculata*, *Tinospora cordifolia*, *Curcuma longa* and *Piper nigrum* (Phyto specialities Pvt. Ltd.). Both drugs were tried along with supportive therapy including antibiotics. Clinical response after 3 months of treatment was observed and dogs that were alive when the study ended *i.e.* after 3 months of treatment were categorized as the censored cases (survived cases).

Presence of clinical signs, nRT-PCR results and epidemiological data of survivor and non-survivor dogs were compared. Variables were compared by Chi-square test for continuous variables and Fisher's exact test for categorical variables. A two-sided P value<0.05 was considered significant. Then significant factors were further, analysed by logistic regression analysis to incorporate these factors with the event of death. All of the statistical analyses were performed with commercially available statistical software, IBM, SPSS software version 26. Kaplan-Meier curves for survival were constructed to explore differences in the survival time for different dog subgroups stratified according to time to death in days.

RESULTS AND DISCUSSION

Out of Forty dogs, 18 had survived and 22 were dead. But all forty dogs were found to be positive for CDV by Nested RT-PCR with either one of the five samples from each dog (Table 1 and Fig 1). Out of five samples, conjunctival swab gave positive results in 17 non-survivors out of 22 dogs and it is statistically significant (p<0.05) compared with the results of non-survivor dogs.

In non-survivor group, nineteen out of twenty-two (19/22) were aged below one year and had ocular discharge. But in survival group, ocular discharge found in only seven dogs which were aged above 5.5 years. Significant difference was also obtained between survival and non-survival groups with the appearance of nasal discharge (p<0.05). Clinical signs other than ocular and nasal discharge were not statistically significant and could not correlate with the event of death (Table 3).

Dogs of non-survival group are found younger than dogs that have survived. Age and ocular discharge were identified as strong prognostic factors by logistic regression (Table 4). Although the present study shown that summer season had highest record of death, season, breed, sex and vaccination status were not statistically significant as prognostic factors. Since total infected animals were mostly male dogs (29/ 40), there was no significant difference in sex while comparing survival and non-survival groups. Similarly, majority of animals were non-descriptive (25/40) and unvaccinated (30/40), significant difference was not obtained in breed and vaccination status of the two groups (Table 2).

Eighteen dogs had survived out of 40 dogs, *i.e.* six from Group-1 and twelve from Group-2 and total case fatality rate was found to be 55%. Kaplan Meier survival curve showed that both treatment trials were not significantly effective though 60 and 30 per cent of animals had survived in Group 2 and Group 1 respectively. Survival curve also revealed the differences in survival time of different survivors in days (Fig 2).

Table 1: CDV positive samples detected in the survival and non-survival groups of dogs.

Total number of experimental animals	Number of survivors	Number of dead animals	
40	18	22	
nRT-PCR results of variables	Positive per cent in survival group	Positive per cent in non-survival group	p values (2-Sided)
Conjunctival swab	38.88% (7/18)	77.27% (17/22)	0.023*
Nasal swab	33.33% (6/18)	31.18% (7/22)	1.000^{NS}
Genital swab	33.33% (6/18)	54.54% (12/22)	0.216^{NS}
Buffy coat	38.88% (7/18)	36.36% (8/22)	1.000^{NS}
Urine	38.88% (7/18)	50% (11/22)	0.755^{NS}

*significant at p<0.05; NS: Non-significant.

Table 2: Epidemiological characteristics in the survival and non-survival groups of dogs with Canine distemper.

Variables	Survival group	Non-survival group	p value (2-sided)
Age (Median in yrs)	2.5 (1 m-12 yrs)	1 (4 m-5 yrs)	0.004*
Sex			0.300^{NS}
Male	66.66% (12/18)	81.81% (18/22)	
Female	33.33% (6/18)	18.18% (4/22)	
Breed			1.000^{NS}
Non-descriptive	61.11% (11/18)	63.63% (14/22)	
Crossbred	38.88% (7/18)	36.36% (8/22)	
Vaccination status			0.253^{NS}
Unvaccinated	66.66% (12/18)	81.81% (18/22)	
Season			0.639^{NS}
June-Sept	11.11% (2/18)	4.5% (1/22)	
Oct-Nov	5.5% (1/18)	4.5% (1/22)	
Dec-Feb (Winter)	61.11% (11/18)	50% (11/22)	
March-May (Summer)	22.22% (4/18)	40.90% (9/22)	

*Significant at p<0.05; NS: Non significant.

Table 3: Clinical manifestations in the survival and non-survival groups of dogs with canine distemper.

Variables	Survival group	Non-Survival group	p values (2-Sided)
Ocular discharge	38.88% (7/18)	86.36% (19/22)	0.003**
Nasal discharge	27.27% (5/18)	68.18% (15/22)	0.025*
Fever	11.11% (2/18)	22.72% (5/22)	0.427^{NS}
Temporal twitching	33.33% (6/18)	31.81% (7/22)	1.000^{NS}
Champing of jaws	33.33% (6/18)	18.18% (4/22)	0.300^{NS}
Chorea	0	9% (2/22)	0.492^{NS}
Myoclonus	27.27% (5/18)	13.63% (3/22)	0.430^{NS}
Abdominal pustule	5.5% (1/18)	9% (2/22)	1.000^{NS}
Hard pad	0	9% (2/22)	0.492^{NS}
Diarrhoea	16.16% (3/18)	18.18% (4/22)	1.000^{NS}
Vomiting	11.11% (2/18)	9% (2/22)	1.000^{NS}
Seizures	16.16% (3/18)	13.63% (3/22)	1.000^{NS}
Osteosclerosis of long bones	27.77% (5/18)	22.72% (5/22)	1.000^{NS}
Respiratory distress	5.5% (1/18)	9% (2/22)	1.000^{NS}

**Significant at p<0.01 *Significant at p<0.05; NS: Non significant.

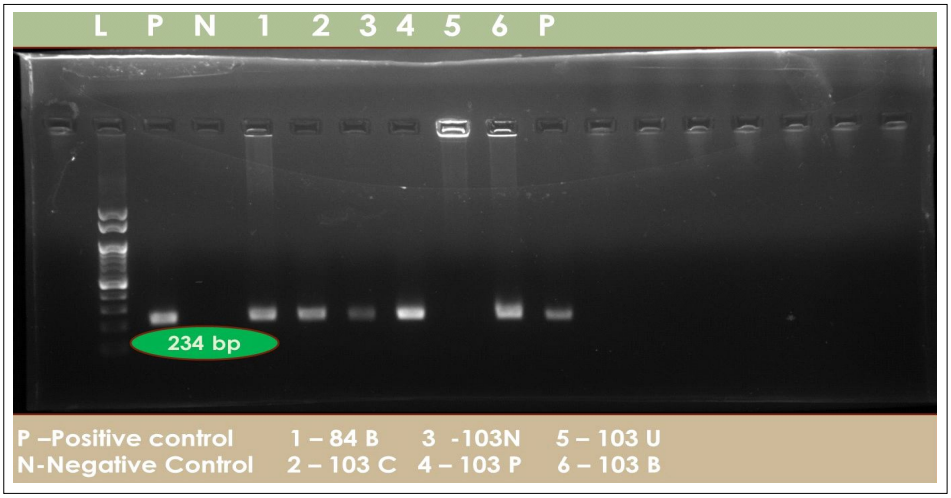


Fig 1: nRT-PCR results of dogs (sample No: 84 and 103).

In the present study, we selected the dogs based on positive nRT-PCR results because nested PCR with the products of a RT-PCR had the potential of increasing the sensitivity of diagnostic limit against CD (Schulze and Baumgartner, 1998). Nested RT-PCR was also used as a gold standard test for the comparison of results of immunochromatography based assay for the earlier diagnosis of Canine Distemper (An *et al*., 2008). Negrao *et al*. (2007) opined that more than one type of clinical sample should be evaluated for the diagnosis of CDV by RT- PCR considering the different clinical manifestations of the disease. Hence, we have tested five samples from each dog in our study and we obtained more percentage of nRT-PCR positivity in conjunctival swabs (Table 1). Kim *et al.* (2006) also mentioned that conjunctival swabs had higher detection rates than the other specimens ($p<0.05$). Moreover, CDV persists in the conjunctival epithelium longer than in other tissues and it makes the conjunctival swab as a sample of choice for diagnosing CDV.

Pratelli (2011) mentioned that Canine distemper presented a variable progression which made the animal to develop either a restricted or a full set of clinical signs based on the virus strain. After 9 days post infection, the outcome of the infection and the severity of the signs vary markedly on the basis of strain virulence, the age of the animal and the immune status (Martella *et al*., 2008). We had also observed various combination of clinical signs in CD infected animals, but ocular discharge was observed 19 out of 22 non-survivors. This is in agreement with findings of Kim *et al*. (2006) who pointed out that CDV in conjunctiva was not subjected to rapid elimination by immune system but replicated in the conjunctival sac or orbital cavity. Appel and Gillespie (1972) stated that the conjunctiva and eye probably become infected at the time of generalized viremia from the early course of the disease. Summers *et al.* (1978) had also demonstrated a more widespread ocular involvement with CDV-infected cells in the conjunctival epithelium, corneal endothelium and iris in dogs with subacute fulminating canine distemper encephalomyelitis. This could be the reason for the presence of ocular discharge in most of the CD infected dogs in our study. Presence of nasal discharge in more numbers of non-survival cases revealed that pneumonia might contribute to the event of death but nasal discharge was not strongly correlated with the event of death.

CDV infection in younger age was strongly correlated with the event of death. We had 55% of case fatality in our study and majority of survivors are adults. This is in agreement with findings of Peserico *et al*. (2019) who also recorded high morbidity and mortality rates mainly in young dogs affected with Canine distemper. Younger pups with a decline in maternal derived immunity are more susceptible to infection and thus, more easily succumbed to death.

Our result is in concurrence with finding of Mahajan *et al*. (2018) who stated that male dogs were 2.29 times more prone to CD than bitches. Our study (Table 2) showed higher mortality rates in summer (40.90 per cent death between March and May) and this might also be due to heat stress CDV causes high morbidity and mortality worldwide, particularly in unvaccinated dogs or dogs with incomplete immunization (Shabbir *et al.,* 2010). Although, the treatment trials were ineffective in treating CD, the herbal drugs have to be tried without antibiotics and supplements to evaluate their effectiveness *per se* and further study is required to assess the antiviral properties of the herbal drugs through *in vitro* assay.

Table 4: Results of logistic regression analysis of significant factors.

Variables	p value	Odds ratio
Age	0.01*	0.220
Conjunctival swab RT-PCR result	0.14	3.718
Ocular discharge	0.03*	24.498
Nasal discharge	0.11	3.544

*Significant at $p<0.05$; NS: Non significant.

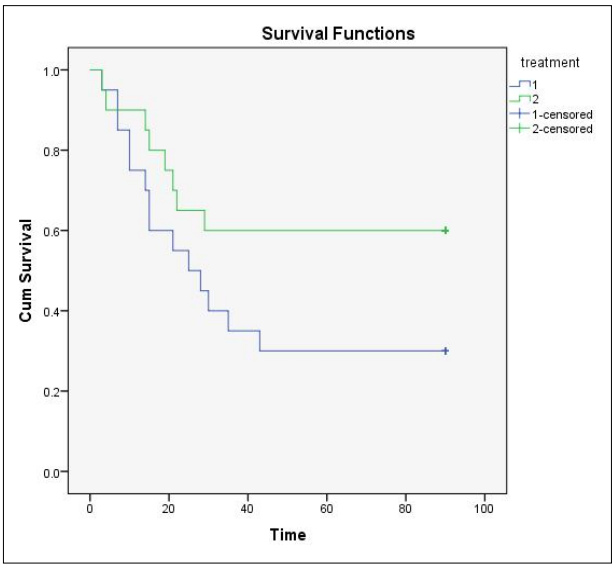


Fig 2: Survival curve.

CONCLUSION

In the present study, ocular discharge and younger age (below one year) were identified as prognostic markers for the poor prognosis in CD infected dogs. The odds ratio indicates that presence of ocular discharge has 24 times more probability of having death when compared with other possible overt clinical signs whereas the probability of survival is also increased when age advances. Hence, utmost care is needed on observation of ocular discharge particularly in animals aged below one year.

Conflict of interest

All authors declared that there is no conflict of interest.

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