B-4143
[1-5]

RESEARCH ARTICLE Indian Journal of Animal Research

Estimation of Myelin Basic Protein Levels in Natural Cases of Canine Distemper Encephalomyelitis

M. Ranjithkumar[1], S. Dey[2], M. Saini[3], R. Singh[4], M. Saravanan[1], M.U. Sivakumar[5]

10.18805/IJAR.B-4143

ABSTRACT

Background: Canine distemper virus (CDV) affects both white and grey matter in the central nervous system (CNS). It causes demyelination of white matter tracts, which releases myelin basic protein (MBP) into the cerebrospinal fluid (CSF). MBP levels in CSF could therefore indirectly indicate the extent of white matter damage. The objective of this study was to determine MBP levels in the CSF of dogs infected with natural cases of neurologic distemper.

Methods: In this study samples from healthy dogs, clinical cases of CDV and stray dogs from in and around the institute were collected for investigation. The infection of the CNS was established by estimating the albumin quotient and, for 16 cases, by histopathology. CSF biochemical parameters were analysed for 35 sero-positive clinical cases of neurologic distemper. MBP levels in samples were estimated by quantitative ELISA. Stray dogs (6 cases) were also screened for this purpose.

Result: MBP levels in CSF were significantly ($p \leq 0.05$) increased in natural cases of distemper encephalomyelitis and stray dogs relative to controls. Three of the neurologic distemper cases clinically recovered despite high MBP levels, and two stray dogs also had higher MBP concentrations. High CSF MBP may therefore not be a useful marker for predicting the outcome of neurologic distemper.

Key words: Albumin quotient, CSF, Encephalomyelitis, Myelin basic protein.

INTRODUCTION

Canine distemper (CD), caused by the canine distemper virus (CDV), is highly infectious and contagious disease (Degene and Zebene, 2019) and widely prevalent in Indian domestic and wild animals (Pawar *et al.,* 2011; Ashmi *et al.,* 2017; Mourya *et al.,* 2019). In a survey of free-ranging dog populations from the Great Indian Bustard Sanctuary, Maharashtra, India, 90.7% of dogs had been exposed to CDV (Vanak *et al.,* 2007). A high incidence was also recorded by Latha *et al.* (2007) in suspected CD clinical samples, where 70% of cases tested positive by Dot-ELISA.

Canine distemper virus causes a multifocal demyelinating disease in dogs (Lempp *et al.,* 2014), referred to as neurologic distemper when it progresses to the CNS. The virus has tropism for myelinated tissues of the brain and spinal cord (Carvalho *et al*., 2012). Common areas for demyelination include white matter areas of the cerebellum, periventricular areas (especially around the fourth ventricle) and the optic pathways and spinal cord (Beineke *et al.,* 2009). Demyelination can occur at multiple foci wherever the virus has seeded but is consistently found in fiber tracts near cerebrospinal fluid (CSF) pathways (Summers and Appel, 1994).

Myelin basic protein (MBP) is one of the specific proteins of the central nervous system. The levels of MBP or MBP-like material in CSF could be a marker for measuring the extent of myelin damage in the CNS (Kalistova *et al.,* 2003). In CDV infection, white matter injury is reflected by elevated levels of MBP in CSF (Summers *et al.,* 1987). As a supplemental CSF test, the MBP ELISA, therefore, has the potential for diagnosing and prognostication of demyelinating disorders in dogs (Oji *et al.,* 2007; Olby *et al.,* 2020).

[1]Department of Veterinary Clinical Medicine, Madras Veterinary College, Chennai-600 001, Tamil Nadu, India.
[2]Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243 122, Uttar Pradesh, India.
[3]Wild Life Section, Indian Veterinary Research Institute, Izatnagar-243 122, Uttar Pradesh, India.
[4]Division of Pathology, Indian Veterinary Research Institute, Izatnagar-243 122, Uttar Pradesh, India.
[5]Veterinary Officer, Hosamalangi, Kollegal-571440, Karnataka, India.

Corresponding Author: M. Ranjithkumar, Department of Veterinary Clinical Medicine, Madras Veterinary College, Chennai-600 001, Tamil Nadu, India. Email: clmranjith@gmail.com

How to cite this article: Ranjithkumar, M., Dey, S., Saini, M., Singh, R., Saravanan,M., Sivakumar, M.U. (2022). Estimation of Myelin Basic Protein Levels in Natural Cases of Canine Distemper Encephalomyelitis. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4143.

Submitted: 13-04-2020 **Accepted:** 29-12-2021 **Online:** 15-03-2022

MBP levels in dogs with natural clinical cases of distemper encephalomyelitis have not previously been established. Because MBP levels could potentially serve as a measure of white matter damage, the objective of this study was to determine the level of MBP in the CSF of dogs infected with natural cases of neurologic distemper.

MATERIALS AND METHODS

This study was conducted on a sample of apparently healthy dogs, clinical cases of CDV and stray dogs (free-ranging or community-owned dogs) from in and around the Referral Veterinary Polyclinic, Indian Veterinary Research Institute, UP, India during the period 2008-2011. Ten healthy dogs

maintained at the Division of Nutrition were randomly selected as controls. Their health status was analysed based on written records. Stray dogs were randomly chosen with the help of a dog catcher. Ethical approval for healthy and stray dogs was obtained via No.F.1-53/2004-JD (Res), dated 09.04.2010 and No.F.1-53/2004-JD (Res), dated 16.1.2012. Clinical cases were brought to the Institute for treatment and therefore did not require any administrative ethical approval, but the owner's written consent was obtained for these cases. The quality of a dog's life is highly associated with its owner's socioeconomic status and all the cases considered in this study (59 cases) were not vaccinated against CDV. Cases were identified by their institutional case number.

Diagnosis

Sick dogs presented to the outpatient unit with clinical signs suggestive of neurologic distemper were subjected to serological diagnosis. In brief, the neurological signs include epilepsy, head pressing, decerebellate rigidity, circling, champing of jaw, temporal twitching, myoclonus of limbs, posterior paresis and signs of meningitis were the observed in these dogs. The serological diagnosis consisted of the recombinant nucleocapsid distemper virus-specific immune-enzymatic assay for IgM and an indirect immuno-enzymatic assay for IgG. Both test kits were procured from Inmunologia y Genetica Aplicada (Ingenasa, Madrid, Spain) and tests were performed following the manufacturer's guidelines. Cases were considered sero-positive for distemper when they were positive either on the IgM assay alone or on both the IgM and IgG assays. Only sero-positive animals were considered for further studies. The infection of the nervous system was established by estimating the albumin quotient (Thomas, 1998; Furr, 2002). Blood samples were collected saphenous or cephalic veins and separated serum samples were stored in aliquots at -20°C for further analysis. Serum biochemical parameters were analysed by commercial kits in 47 neurologic distemper cases and 7 stray dogs. For 16 cases that either died naturally or were euthanized, CNS infection was further confirmed by histopathological examination. Brain samples were sectioned, stained with haematoxylin and eosin and observed under a microscope.

CSF collection and analysis

CSF was collected (n=33) mostly from the cerebellomedullary cistern (CMC) or the caudal lumbar subarachnoid space, as per earlier reports (Di Terlizzi and Platt, 2009). All samples were aliquoted and stored at -20°C until further analysis. Total protein, albumin, potassium, sodium and calcium levels in the CSF samples were estimated by spectrophotometry using commercial kits. The albumin quotient was calculated using a previously described method (Thomas, 1998):

$$\text{Albumin quotient (AQ)} = \frac{\text{CSF albumin (mg/dl)}}{\text{Serum albumin (g/dl)} \times 10}$$

MBP levels in the CSF samples were quantified using a two-step sandwich ELISA. The ELISA kit was purchased from Diagnostic Systems Laboratories, TX, USA and the standard curve was calculated using human MBP. The ELISA results were obtained following established protocols (Oji *et al.,* 2007).

Statistical analysis

A one-way ANOVA was performed using SPSS software, version 21, to compare the mean biochemical levels among study groups (*SPSS* Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412). Post hoc multiple comparisons were performed with the Tukey test.

RESULTS AND DISCUSSION

Of 59 suspected cases of neurologic distemper, 47 (79.66%) were sero-positive either for IgM or both IgM and IgG. However, only 35 positive cases were considered for CSF parameters; CSF was not collected in 12 cases due to the owner's poor compliance, blood contamination, or failed sampling attempts. The 35 neurologic distemper cases were classified into three groups based on clinical signs and age, as per an earlier report (Thomas, 1998). Classifying animals proved to be difficult because many animals exhibited multiple clinical signs. In addition to these clinical cases, seven stray dogs and 10 healthy dogs were also screened for CDV-specific antibodies. All the stray dogs had low to median titer values of IgM (1/20-1/80) and high titer values of IgG (≥ 1/320). The healthy dogs were negative for IgM antibodies and had low titer values for IgG (1/20-1/40).

Biochemical changes

Serum biochemical levels for the various groups are shown in Table 1. Compared to healthy controls, there was a significant ($p \leq 0.05$) reduction in both albumin levels and the

Table 1: Serum biochemical parameters in various groups of dogs.

Parameters	Healthy control (n=10)	Acute Encephalomyelitis (n=25)	Chronic Encephalomyelitis (n=16)	Old dog Encephalitis (n=6)	Stray dogs (n=7)
Total protein (g/dl)	7.15 ± 0.40^{ab}	6.33±0.37^{a}	8.02±0.39 ^{ab}	8.72±1.04^{b}	7.43±0.30^{ab}
Albumin (g/dl)	3.67 ± 0.20^{b}	2.86±0.15^{ab}	2.95±0.14 ^{ab}	2.89±0.20^{ab}	2.31±0.18^{a}
Globulin (g/dl)	3.41 ± 0.39^{a}	3.47±0.33 ^{a}	5.07±0.35 ^{ab}	5.84±1.04 ^{b}	5.12±0.17^{ab}
A:G ratio	1.23 ± 0.20^{b}	0.88±0.09 ^{ab}	0.67±0.06 ^{a}	0.56±0.09^{b}	0.45±0.03^{a}

Means bearing different superscripts in the same row differ significantly ($P \leq 0.05$).

albumin-globulin (A: G) ratio in stray dogs and the A: G ratio in dogs with chronic encephalomyelitis. The reduction in albumin values in the chronic encephalomyelitis group was non-significant. Biochemical levels in CSF for the various groups are shown in Table 2. There was a significant ($p \leq 0.05$) increase in total protein and albumin in dogs with chronic encephalomyelitis. There was also a significant increase in mean albumin quotient (AQ) values in dogs with acute or chronic encephalomyelitis compared to controls, which confirmed infection of the nervous system. However, 15 cases (42.86%) in our study showed AQ values less than 2.35.

MBP levels in control animals were less than 1 ng/ml (0.33±0.11 ng/ml). There was a significant increase ($p \leq 0.05$) in MBP values in dogs with acute (26.82±9.27 ng/ml) and chronic (58.62±18.82 ng/ml) distemper encephalomyelitis and in stray dogs (2.39±1.65 ng/ml) when compared to the controls. MBP levels were especially high in the chronic encephalomyelitis group. One animal in the acute encephalomyelitis group had the lowest CSF MBP value (0.872 ng/ml), while another two animals had high values (165.1 and 166 ng/ml). MBP levels in the chronic encephalomyelitis group ranged from 13.88 to 342.425 ng/ml. Three cases in our study with high CSF MBP values have since completely recovered. These cases were followed up with after 1.5 years, 7 months and 2 years and their initial CSF MBP values were 16.75, 48.955 and 166 ng/ml, respectively. Two stray animals in our study also had high CSF MBP values (1.693 and 10.568 ng/ml).

Common histopathological changes in the brain tissue of cases with neurologic distemper included sponginess or status spongiosa, along with astrogliosis (Fig 2 and 3). Gemistocytic astrocytes or gemistocytes and gitter cells were occasionally present. Brain sections also showed an apparent proliferation of capillaries, as well as distension and congestion of blood vessels and loss of the surrounding parenchyma. Degenerative changes in the neurons, such as pyknosis, chromatolysis, gliosis and neuronophagia (Fig 4, 5 and 6), were also common. Neuronal necrosis in the cerebral and cerebellar cortexmarked perivascular cuffing and meningeal infiltration were also observed. Ten animals in our study had intranuclear inclusion bodies in astrocytic neurons (Fig 1). Another animal contained cysts of protozoal bradyzoites.

Canine distemper continues to be a major threat to domestic and wild animal populations in developing countries, including India. The neurological signs (Wyllie *et al.,* 2016; Degene and Zebene, 2019) and histopathological lesions observed in our study were similar to earlier reports of dogs with CDV (Jones *et al.,* 1997, Koutinas, 2002). CDV affects both white and grey matter in the CNS (Carvalho *et al.,* 2012; Lempp *et al.,* 2014). This study explored the possibility of establishing biochemical

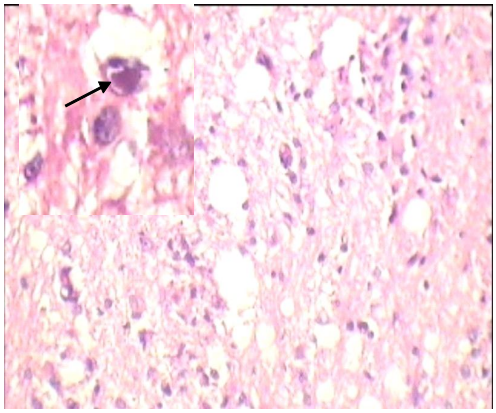

Fig 1: Intranuclear inclusion body in cerebellum 100x H&E.

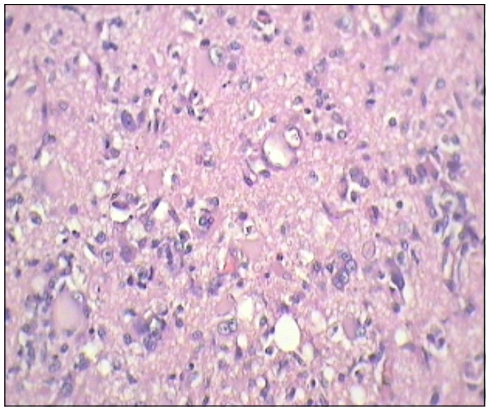

Fig 2: Astrogliosis in cereberum 40x H&E.

Table 2: Cerebrospinal fluid biochemical parameters in various groups of dogs.

Parameters	Healthy control (n=6)	Acute Encephalomyelitis (n=23)	Chronic Encephalomyelitis (n=10)	Stray dogs (n=6)
Total protein (g/dl)	0.03±0.01^{a}	0.51±0.06^{bc}	0.70±0.12^{c}	0.35±0.09^{b}
Albumin (mg/dl)	10.50± 1.06^{a}	77.22±7.55^{b}	108.90±17.19^{b}	14.22±3.55^{a}
Chloride (mEq/L)	124.22± 8.95	119.09±6.34	128.18±4.19	112.01±15.57
Sodium (mEq/L)	146.37±3.14	143.42±16.12	108.35±10.82	151.67±7.03
Potassium (mEq/L)	2.13±0.43	3.02±0.24	2.93±0.49	3.13±0.50
MBP (ng/ml)	0.30±0.11^{a}	26.82±9.27 ^{c}	58.62±18.82^{c}	2.39±1.65^{b}
AQ	0.24±0.07 ^{a}	2.76±0.30 ^{b}	3.67±0.68^{b}	0.58±0.24

Means bearing different superscripts in the same row differ significantly ($P \leq 0.05$).

values, especially MBP levels, as indicators of white matter damage. Results of haematology and serum chemistry profiles are normal or non-specific in many animals with CNS inflammatory diseases (Mahajan *et al.,* 2018). In our study, the significant decreases in albumin levels and the A: G ratio in the acute encephalomyelitis group, as well as the non-significant increase in globulin values, indicate a non-specific immune reaction to CDV infection. Dogs mounting a partial immune response may recover from acute illness but fail to eliminate the virus, leading to a spectrum of more chronic diseases (Sykes, 2010). The significant reduction in the A: G ratio and albumin levels in stray dogs might be due to a lack of positive energy balance or to a shared response to various infections.

The CSF biochemical changes observed in this study matched previous reports of neurologic distemper (Thomas *et al.,* 1993; Thomas, 1998; Gama *et al.,* 2007; Mahajan *et al.,* 2018). The higher mean albumin quotient values in acute and chronic encephalomyelitis cases prove that there was damage to the blood-brain or blood-CSF barriers, confirming CNS infection in these animals (Furr, 2002). The significant increase in CSF total protein in stray dogs might be due to past CDV infection, which correlates with serum IgG levels. There were no CSF electrolyte changes in animals with distemper.

The levels of CSF MBP in a previous specific-pathogen-free experimental dog model ranged from 0 to 15.55 ng/ml. In an experimental distemper infection, dogs with severe demyelination had elevated values of immunoreactive MBP, while dogs with only mild inflammation had little or none (Summers *et al.,* 1987). The levels of CSF MBP in natural distemper cases in our study were significantly higher compared to this experimental model and other degenerative myelopathy studies. These findings indicate that there is severe demyelination in natural cases of neurologic distemper. Dogs with subacute to chronic distemper virus infection of the CNS have demyelinating leukoencephalopathy (Lempp *et al.,* 2014) and thus the elevated MBP in CSF.

Despite initially high levels of CSF MBP, three animals in our study clinically recovered using conventional therapy. Their recovery suggests that the level of CSF MBP may not predict the clinical outcome of neurologic distemper. The presence of high CSF MBP values in two stray dogs further suggests that MPB levels are not predictive of clinical outcomes. These five animals might have recovered due to an enhancement of the endogenous remyelination process performed either by residual oligodendrocytes or their progenitors (Duncan *et al.,* 1997). Extensive remyelination followed by functional recovery has already been reported in a cat model (Duncan *et al.,* 2009).

From our study, we conclude that the level of MBP in CSF was significantly ($p \leq 0.05$) higher in natural cases of canine distemper encephalomyelitis. Despite high MBP

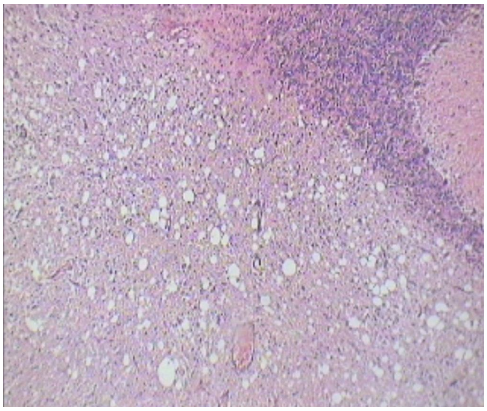

Fig 3: White matter spongiosis with diffuse gliosis 10x H&E.

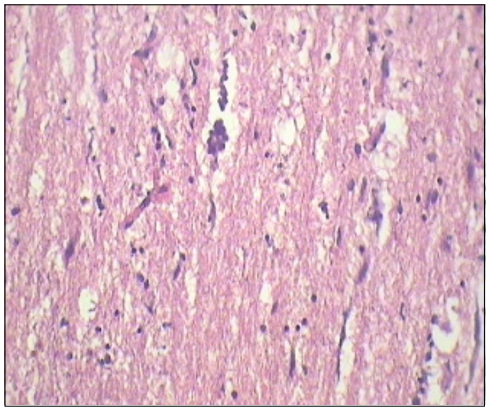

Fig 5: Demyelination of nerve tract in Spinal cord 40x H&E.

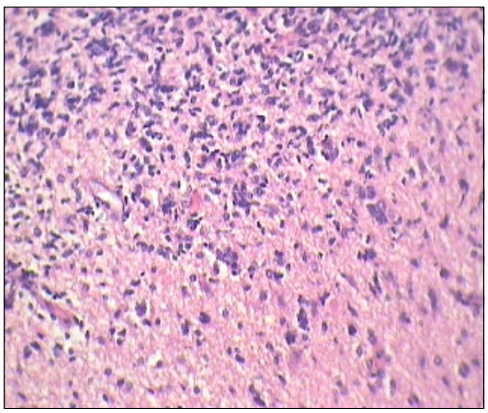

Fig 4: Extensive microgliosis 40x H&E.

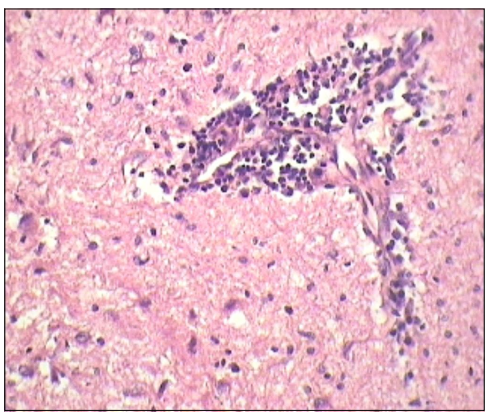

Fig 6: Perivascular cuffing in cerebrum 40x H&E.

values, three of the neurologic distemper cases clinically recovered. Two stray dogs also had higher MBP concentrations in their CSF. High CSF MBP may therefore not be a useful marker for determining the outcome of neurologic distemper.

Conflict of interest: None.

REFERENCES

Ashmi, J.M., Thangavelu, A., Senthilkumar, T.M.A., Manimaran, K. (2017). Molecular characterization of canine distemper virus from Tamil Nadu, India. Indian Journal of Animal Science. 87: 1062-1067.

Beineke, A., Puff, C., Seehusen, F., Baumgärtner, W. (2009). Pathogenesis and Immunopathology of Systemic and Nervous Canine Distemper. Veterinary Immunology and Immunopathology. 127: 1-18.

Carvalho, O.V., Botelho, C.V., Ferreira, C.G.T., Scherer, P.O., Soares-Martins, J.A.P., Almeida, M.R., Silva Junior. A.S. (2012). Immunopathogenic and Neurological Mechanisms of Canine Distemper Virus. Advances in Virology. 2012: 1-10.

Degene, B. and Zebene, M. (2019). Canine distemper: A review. International Journal of Advanced Research in Biological Sciences. 6(7): 12-19.

Di Terlizzi, R. and Platt, S.R. (2009). The function, composition and analysis of cerebrospinal fluid in companion animals: Part II - analysis. The Veterinary Journal. 80: 15-32.

Duncan, I. D., Greve, W. E. and Zhang, S. (1997). Repair of myelin disease: Strategies and progress in animal models. Molecular Medicine Today. 3(12): 554-561.

Duncan, I.D., Brower, A., Kondo, Y., Curlee, J.F. Jr. and Schuitz, D. (2009). Extensive remyelination of the CNS leads to functional recovery. PNAS 106(16): 6832-6836.

Furr, M. (2002). Antigen-specific antibodies in cerebrospinal fluid after intramuscular injection of ovalbumin in horses. Journal of Veterinary Internal Medicine. 16(5): 588-592.

Gama, F.G.V., Nishimori, C.T., Sobreira, M.R., Santana, A.E. (2007). Evaluation of electrophorertic profile and albumin quota in the cerebrospinal fluid of dogs with distemper showing or not nervous sings. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 59: 77-80.

Jones, T.C., Hunt , R.D. and King, N.W. (1997). Canine Distemper. In: Veterinary Pathology, 6th edn. Lippincott Williams and Wilkins, Baltimore, Maryland. USA: 311-315.

Kalistova, M., Havrdova, E., Uhrova, J., Zeman, D., T'Yblova, M. and Mrazova, K. (2003). Myelin basic protein in multiple sclerosis and other neurological disorders. Journal of Neurology. 250: 874-875.

Koutinas, A.F., Polizopoulou, Z.S., Baumgartner, W., Lekkas, S. and Kontas, V. (2002). Relation of clinical signs of pathological changes in 19 cases of canine distemper encephalomyelitis. Journal of Comparative Pathology. 126: 47-56.

Latha, P., Srinivasan, S.R., Thirunavukkarasu, P.S., Gunaselan, L., Ramadas, P. and Narayanan, R.B. (2007). Assessment of canine distemper virus infection in vaccinated and unvaccinated dogs. Indian Journal of Biotechnology. 6: 35-40.

Lempp, C., Spitzbarth, I., Puff, C., Cana, A., Kegler, K., Techangamsuwan, S., Baumgärtner, W. and Seehusen, F. (2014). New Aspects of the Pathogenesis of Canine Distemper Leukoencephalitis. Viruses. 6: 2571-2601.

Mahajan, S., Dey, S., Kumar, A. and Panigrahi, P.N. (2018). Nitrosative stress indices in dogs with neurological form of canine 9(64): 229-232.

Mourya, D.T., Yadav, P.D., Mohandas, S., Kadiwar, R.F., Vala, M.K., Saxena, A.K., Shete-Aich, A., Gupta, N., Purushothama, P., Sahay, R.R., Gangakhedkar, R.R., Mishra, S.C.K. and Bhargava, B. (2019). Canine distemper virus in Asiatic Lions of Gujarat State, India. Emerging Infectious Diseases. 25(11): 2128-2130.

Oji, T., Kamishina, H., Cheeseman, A. and Clemmons, R.M. (2007). Measurement of myeline basic protein in the cerebrospinal fluid of dogs with degenerative myelopathy. Veterinary Clinical Pathology. 36: 281-284.

Olby, N.J., da Costa, R.C, Levine, J.M., Stein, V.M. and the Canine Spinal Cord Injury Consortium (CANSORT SCI) (2020). Prognostic Factors in Canine Acute Intervertebral Disc Disease. Frontiers in Veterinary Science. 7: 1-14

Pawar, R.M., Raj, G.D., Gopinath, V.P., Ashok, A. and Raja, A. (2011). Isolation and molecular characterization of canine distemper virus from India. Tropical Animal Health and Production. 43(8): 1617-1622.

Schobesberger, M., Zurbriggen, A., Summerfield, A., Vandevelde, M. and Griot, C. (1999). Oligodendroglical degeneration in distemper: apoptosis or neurosis? Acta Neuropathologica. 97: 279-87.

Summers, B.A. and Appel, M.J.G. (1994). Aspects of canine distemper virus and measles encephalomyelitis. Neuropathology and Applied Neurobiology. 20: 525-534.

Summers, B.A., Whitaker, N. and Appel, M.J.G. (1987). Demyelinating canine distemper encephalomyelitis: measurement of myelin basic protein in cerebrospinal fluid. Journal of Neuroimmunology. 14: 227-233.

Sykes, J.E. (2010). Immunodeficiencies caused by infectious diseases. Veterinary Clinics: Small Animal Practice. 40: 409-423.

Thomas, W.B. (1998). Inflammatory diseases of the central nervous system in dogs. Clinical Techniques in Small Animal Practice. 13(3): 167-178.

Thomas, W.B., Sorjonen, D.C. and Steiss, J.E. (1993). A retrospective evaluation of 38 cases of canine distemper encephalomyelitis. Journal of the American Animal Hospital Association. 29: 23-28.