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Initial Clinical Manifestations of Dogs with Neurological Distemper

Erdem GÜLERSOY^{*,1,a}, Canberk BALIKÇI^{1,b}, İsmail GÜNAL^{1,c}, Adem ŞAHAN^{1,d}, Kerem YENER^{2,e}, Esma KISMET^{1,f}, Meral ÇIKMA^{1,g}

¹ Harran University, Faculty of Veterinary Medicine, Department of	Abstract: The canine distemper virus (CDV) causes demyelination
Internal Medicine, Sanliurfa, Türkiye.	within the central nervous system, gastrointestinal and/or
² Harran University, Faculty of Veterinary Medicine, Department of	determining the initial clinical manifestation of dogs with
Surgery, Sanliurfa, Türkiye.	neurological CDV of different ages can be used to increase the index of suspicion of CDV, especially in triage. 44 dogs, aged 2-8 months, with clinical findings suggesting the presence of neurological CDV were used, and 38 were enrolled. The dogs were divided into 2
	subgroups based on their age. Accordingly, dogs aged 2-4 months were included in Group 1 (n:16), and dogs aged 5-8 months were
^a ORCİD:0000-0001-8511-0150	included in Group 2 (n:22). The body temperature of Group 1 was higher than that of Group 2 (p <0.000). Within the scope of
^b ORCID:0000-0001-7473-5163	extraneural findings, fever, anorexia, depression, ocular discharge,
°ORCID:0000-0003-3679-4132	nasal discharge, diarrhea, uveitis, and pneumonia were detected. Neural findings included behavioral change, head tilt, quadriplegia,
^d ORCİD:0000-0002-4779-0893	convulsion, ataxia and myoclonus. Thoracic radiography revealed a
°ORCİD:0000-0002-6947-0356	cranioventral alveolar pulmonary pattern and diffuse interstitial pneumonia. The presence of the foreign body in the gastrointestinal
^f ORCID:0000-0002-2906-074X	tract was prominent in Group 2. On thoracic ultrasonography, B
^g ORCID:0000-0002-3859-433X	lines (>3 lines) were prominent in both groups. Abdominal ultrasonography revealed dilated intestine segments. It was concluded that the evaluation of the initial clinical manifestation in
	combination with non-invasive imaging methods might be used to increase the index of suspicion of neurologic CDV before proceeding to laboratory analyses, especially in triage.
Received: 07.06.2023	Keywords. Diagnosis, Dog, Radiography, Okrasouna
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Introduction

In the field of veterinary neurology, various etiologies such as metabolic, degenerative, nutritional, autoimmune, inflammatory and infectious can affect the brain and cause encephalopathy (Dewey, 2008). Canine distemper virus (CDV), which has an important place among infectious encephalopathies of dogs, is a re-emerging agent that can cause an epidemic even in vaccinated dogs worldwide (Lan et al., 2006). In addition, diagnosing CDV-associated encephalomyelitis is clinically difficult without traditional findings such as systemic manifestations (Amude et al., 2012). Systemic findings associated with CDV are loss of appetite, fever, nasal/ocular discharge, cough, dyspnea, vomiting and diarrhea. These findings may be in combination with each other and neurological findings may occur without systemic findings (Tipold et al., 1992). In previous studies, it was reported that conjunctivitis, fever, respiratory and gastrointestinal system-related extraneural findings were detected in two-thirds of the dogs admitted to the hospital, while no extraneural findings were detected in one-third (Amude et al., 2012; Tipold et al., 1992).

Myoclonus is a characteristic and typical sign in dogs with CDV-related encephalomyelitis. However, the nervous form of CDV infection may occur without myoclonus and systemic involvement. Although there are many studies on dogs with CDV-related neuropathy (Amude et al., 2012; Appel, 1994; Gülersoy et al., 2022), data evaluating the clinical syndromes of CDV infection are available only in classical textbooks or reviews (Amude et al., 2012; Braund, 1994). The number of studies evaluating the whole clinical manifestation is limited.

The present study aimed to determine the initial clinical manifestation of dogs of different ages with clinical findings that would suggest neurological CDV infection, and to determine which clinical findings could be used to increase the index of suspicion of CDV in triage.

Material and Methods

This study protocol was approved by the ethics committee of the Faculty of Veterinary Medicine, Harran University (session and permit number: 2021-005/01-14). This article was produced from the study entitled Evaluation of the Efficacy of Blood Gases and Hemogram Parameters in the Diagnosis of non-Neurogenic Distemper and Parvoviral Enteritis in Dogs with Acute Gastroenteritis.

Animal Material: This study consisted of 44 dogs, aged 2-8 months, with clinical findings such as respiratory and gastrointestinal signs, fever and neurologic disorders that would suggest CDV infection. All animals were client-owned (30 males, 14 females; 38 mix breed, 3 Labrador, 2 Rotweiler, 1 Pointer) and admitted to Harran University Faculty of Veterinary Animal Hospital for diagnostic and/or treatment purposes. The owner's consent was obtained before the clinical evaluation and sample collection.

Anamnestic Data: None of the dogs were neither vaccinated nor dewormed. All animals were fed on commercial dry dog food. Of the dogs deemed suitable for

inclusion in the study, 24 were outdoor, and 20 were indoor dogs. The mean duration of clinical symptoms of all the dogs was 8 (3-22) days.

Clinical Examinations: Body temperature, gingival capillary refill time, evaluation of palpable lymph nodes, as well as heart and lung auscultation were measured for all dogs eligible for inclusion in the study. Also, fecal samples were obtained from all the dogs with sterile swabs to microscopically investigate the presence of parasites (light microscope, x40 magnification, light microscope, Olympus[®]). Findings detected as a result of physical examinations were classified as extraneural and neural. Thoracic/abdominal radiographic (Fujifilm VXR, Japan) and ultrasonographic (Mindray Z60, China) examinations were performed following the physical examination.

The imaging depth for ultrasonographic examination of the thorax was set at 3–6 cm depending on the animal. The focus position was set to the pleural line. All examinations were performed in sternal positions or standing without clipping, after the application of alcohol and coupling gel on the chest. Multiple scan fields were evaluated for a complete examination of the thorax (using 8 MHz microconvex probe). The patient was placed in dorsal recumbency for the abdominal ultrasonography and gently restrained. Cranioventral abdominal hair was clipped and wetting the skin with water, a tincture of 70% isopropyl alcohol, followed by ultrasound gel that permits obtaining an improved image (using 6-8 MHz microconvex probe).

Blood Sampling and Application of Rapid Diagnostic Test Kits: To eliminate any diseases causing similar symptoms CDV, Canine Adenovirus 2, Canine Influenza virus, Canine Coronavirus (Asan Easy Test CAV2/CIV/CCV Ag, ASAN Pharm. Co., Ltd. Gyeonggi-do Korea, relative sensitivity: 93.10%, relative specificity: 97.50%) and Canine Parvovirus antigen tests (Asan Easy Test CPV Ag, ASAN Pharm. Co., Ltd. Gyeonggi-do Korea, relative sensitivity: 97.96%, relative specificity: 97.50%) were performed according to the manufacturer's instructions. All results were negative. CDV Ag test (Asan Easy Test CDV Ag, ASAN Pharm. Co., Ltd. Gyeonggi-do Korea, relative sensitivity: 97.96%, relative specificity: 97.50%) was performed by using both ocular and nasopharyngeal secretions were obtained with sterile wet swabs according to the manufacturer's instructions to confirm suspicion of CDV infection.

Inclusion/Exclusion Criteria: Dogs with clinical findings that would suggest CDV infection at first admission, whose clinical findings were classified as extraneural and neural, with a positive CDV Ag and a negative CAV2/CIV/CCV test result, were included in the study. Dogs with any concurrent disease or with parasites/parasite eggs in feces were not included. As a result, 38 out of 44 dogs with CDV-related clinical findings were enrolled in the study.

Forming Subgroups: 38 dogs, whose CDV infection was confirmed by clinical and rapid diagnostic test kits were divided into 2 subgroups based on age. Accordingly, dogs aged 2-4 months were included in Group 1 (n:16), and dogs aged 5-8 months were included in Group 2 (n:22).

Statistical Analysis: The data were evaluated using statistical software (SPSS 25.00 for Windows). One sample Kolmogorov-Smirnov test was applied to determine whether all data were non-parametric or parametric. Non-parametric data were presented as median (min, max) with Mann Whitney U, Kruskal-Wallis test. Statistical significance was considered as p<0.05 for all data.

Table 1. Physical examination findings.

Results

Physical Examination Findings: As a result of physical examinations, the body temperature of Group 1 was higher than that of Group 2 (p<0.000). Physical examination findings are presented in Table 1. In Group 1, within the

Clinical Parameters	Group 1	Group 2	
	(n:16) median (min-max)	(n:22) median (min-max)	p value
Symptom duration (days)	6.5 (3-19)	9 (3-22)	0.140
Body weight (kg)	7.85 (5.3-11.2)	10.75 (7-16)	0.000
Body temperature (°C)	39.25 (38.8-39.8)	38.45 (36.6-39.6)	0.000
Capillary refill time (sec)	2 (1-3)	2 (1-4)	0.071
Respiration rate (breath/min)	69 (44-93)	89 (46-99)	0.189
Heart rate (beat/min)	101.5 (68-124)	86 (65-144)	0.101

scope of extraneural findings, fever (8 out of 16, 50%), anorexia (14 out of 16, 87.5%), depression (15 out of 16, 93.75%), ocular discharge (13 out of 16, 81.25%), nasal discharge (13 out of 16, 81.25%), diarrhea (5 out of 16, 31.25%), uveitis (5 out of 16, 31.25%) and pneumonia (10 out of 16, 62.5%) were detected. Neural findings included behavioral change (14 out of 16, 87.5%), head tilt (10 out of 16, 62.5%), quadriplegia (5 out of 16, 31.25%), convulsion (8 out of 16, 36.36%), ataxia (5 out of 16, 31.25%) and myoclonus (15 out of 16, 93.75%). The distribution of clinical symptoms of Group 1 is presented in Figure 1. Extraneural



Figure 1. Distribution of extraneural and neural clinical symptoms of Group 1.

findings of Group 2 were determined to be fever (8 out of 22, 36.36%), anorexia (16 out of 22, 72.72%), depression (18 out of 22, 81.81%), ocular discharge (16 out of 22, 72.72%), nasal discharge (15 out of 22, 68.18%), diarrhea (14 out of 22, 63.63%), uveitis (13 out of 22, 59.09%), hardpad (1 out of 22, 4.54%), and pneumonia (11 out of 22, 50%). Within scope of neural findings of Group 2, behavioral change (14 out of 22, 63.63%), head tilt (10 out of 22, 45.45%), quadriplegia (5 out of 22, 22.72%), convulsion (8 out of 22, 36.36%), ataxia (5 out of 22, 22.72%) and myoclonus (15 out of 22, 68.18%) were detected. The distribution of clinical symptoms of Group 2 is presented in Figure 2.



Figure 2. Distribution of extraneural and neural clinical symptoms of Group 2.

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Radiographic Findings: Thoracic radiography revealed a cranioventral alveolar pulmonary pattern (4 out of 16, 25%), diffuse interstitial pneumonia (5 out of 16, 31.25%), and enlarged mediastinal lymph node (3 out of 16, 18.75%) in Group 1. In Group 2, caudodorsal alveolar pattern (9 out of 22, 40.99%) and diffuse interstitial pneumonia (10 out of 20, 45.45%) were evident. Abnormal thoracic radiographic

findings are presented in Figure 3. According to the abdominal radiographs, intestinal distension (11 out of 16, 68.75%) was evident in Group 1. In contrast, gas and dilated stomach (8 out of 22, 36.36%) and foreign body in the gastrointestinal tract (18 out of 22, 81.81%) were prominent in Group 2. Abnormal abdominal radiograph findings are presented in Figure 4.



A. Alveolar pattern, which is characterized by the existence of more or less broad portions of the lung more opaque than normal due to partial or complete alveolar filling. B. Interstitial pneumonia, which is characterized by lung volume loss and an apicobasal gradient of peripheral septal thickening and bronchiectasis.





A. Gas-filled stomach, and intestinal distention which is characterized by multiple, small, randomly distributed, gaseous foci scattered throughout the abdomen. B. Intestinal distension, and foreign body causing radio-opaque appearance in the intestines.

Figure 4. Abdominal radiographic findings detected.

Ultrasonographic Findings: On thoracic ultrasonography, B lines (>3 lines) were prominent in both groups (Group 1, 12 out of 16, 75%; Group 2, 14 out of 22, 63.63%). In Group 2, the loss of the A lines (5 out of 22, 22.72%) was also evident. Lung sliding was detected in both groups. Abnormal thoracic ultrasonography findings are presented in Figure 5. On the other hand, in abdominal ultrasonography, dilated intestine segments with gas (10 out of 16, 62.5%) were detected in Group 1, while acoustic shadowing, which is characterized by a clear shadow that suspects the presence of a foreign body in the stomach and intestines was evident in Group 2 (17 out of 22, 77.27%). Abnormal abdominal ultrasonography findings are presented in Figure 6.



A. Multiple B lines origination from pleura. B. Loss of A lines along with the presence of B lines which is also known as comet-tail artifact indicating subpleural interstitial edema.





A. Clear acoustic shadow indicating the presence of a foreign body in the stomach. B. Dirty shadow associated with the presence of gas in the intestines.

Figure 6. Abnormal abdominal ultrasonography findings.

Discussion

In this study, the initial clinical manifestations and imaging results of dogs with neurological CDV were evaluated in triage, and important findings were achieved. While the incidence of extraneural findings such as diarrhea, uveitis and neural findings such as quadriplegia were lower in 2-4 month-old dogs, the same findings had a higher incidence in 5-8 month-old dogs. Non-specific findings such as depression and anorexia were the most critical extraneural findings in both groups whereas myoclonus and behavioral change were the most prominent neural findings. In accordance with these results, it was concluded that the evaluation of initial clinical manifestation with non-invasive imaging methods such as radiography and ultrasonography could be used to increase the index of suspicion of neurological CDV before proceeding to laboratory analyses such as complete blood count, serum biochemistry and rapid diagnostic test applications, especially in triage.

CDV causes a progressive, multifocal demyelinating disease within the central nervous system. CDV infection may also lead to gastrointestinal and/or respiratory signs (Amude et al., 2006). After the entrance of the virus into the body via nasal or oral route, it rapidly replicates in the lymphoid tissue and causes immunosuppression. Transient fever peaks 3-6 days after infection and during this period, loss of appetite, depression, ocular and nasal discharges and tonsillitis can be detected. 6-9 days after infection, the virus spreads to the epithelium of most organs with cellassociated viremia (Appel et al., 1982; Winters et al., 1984). At this stage, the severity of the symptoms depends on the virulence of the virus strain, the immune status and the age of the dog. If the dog has a weak immune response, the virus attains epithelial cells and the central nervous system (CNS). The initial clinical findings disappear; the virus persists for long periods in the neurons, uvea, urothelium and soles of the feet (hard pad). If the dog shows no immune response, the virus continues to replicate and spreads throughout the body, and usually becomes more severe with secondary bacterial infections (Green and Appel, 1990). Neurological symptoms such as head tilt, circling, nystagmus, complete or partial paralysis, convulsions, behavioral changes and dementia begin 20 days after the onset of infection. Involuntary muscle contractions, twitching and gum chewing are considered typical symptoms for CDV infection. In some dogs, neurologic signs may take up to 40-50 days (Green and Appel, 1990; Vandevalde and Zurbriggen, 2005). Considering the duration of symptoms of naturally CDV-infected dogs of the present study (8 (3-22) days), the wide range of neural and extraneural findings, including gastrointestinal and respiratory symptoms, can be attributed to the properties of CDV's antigenic recognition by hemagglutinin protein (H), SLAM and nectin-4-mediated lymphoid tissue interaction and multiple cell tropism (Rendon-Marin et al., 2019). In addition, the higher body temperature detected in Group 1 compared to Group 2 was associated with the duration of onset of infection (Table 1) and incomplete immune maturation of the puppies (Handl et al., 2009).

Considering variables such as the type of neurological signs, the presence of concomitant systemic disease, age, vaccination and immunity status of the dog, and the frequency of neurological signs, five neurological syndromes associated with CDV were identified. These are: canine distemper encephalomyelitis in immature dogs (CDEID), multifocal distemper encephalomyelitis in mature dogs (MDEMD), chronic relapsing distemper encephalomyelitis (CRDE), post vaccinal distemper encephalomyelitis (PVDE) and old dog encephalomyelitis (ODE) (Headley et al., 2009). In acute cases, primary demyelination is not associated with inflammation due to the absence of prominent perivascular reduced myelin synthesis in CDV-infected cuffs, oligodendrocytes and metabolic dysfunction and activation of microglia cells (Vandevalde and Zurbriggen, 2005). In cases, demyelination is associated chronic with inflammation triggered by CDV-specific immune response (Griot et al., 1989). In ODE-like encephalitis, which is a rare form, progressive cortical derangement with multifocal parenchymal lymphoplasmacytic perivascular and encephalitis is evident in the cerebral hemispheres (Plattet et al., 2005). Diagnosing CDV-associated encephalomyelitis is difficult in the absence of CDV-specific neurologic findings such as myoclonus with respiratory/gastrointestinal signs. It was reported that chewing-gum movement in CDV cases is due to contraction of facial and masticar muscles and generalized partial seizures are due to tonic-clonic movements of all skeletal muscles. In addition, behavioral changes are reported to be associated with cerebral dysfunction in dogs with CDEID. Additional findings reported in this context include loss of consciousness, blindness and spastic tetraparesis (Braund, 1994). Loss of consciousness and behavioral changes have been associated with ascending reticular activating system (ARAS) dysfunction in the brain stem (Dewey, 2008). Nevertheless, the neurological signs are related to the affected area of the brain (Braund, 1994; Vite, 2005). The involvement of cortical and subcortical regions of the brain can explain the behavioral changes of the present study. In contrast, the loss of consciousness finding can be explained by the involvement of the brain stem (Vite, 2005). The lower incidence of findings, such as quadriplegia and ataxia, may be related to the rarer spinal cortex damage in dogs with CDEID (Braund, 1994). Although previous studies reported that gastrointestinal signs such as vomiting, diarrhea and respiratory signs such as cough are frequently observed in dogs with CDVID (Vite, 2005), diarrhea was found to be a less common finding in the present study, especially in 2–4-month-old dogs. Diarrhea, which has a relatively higher incidence in 5–8-month-old dogs, and anorexia, when evaluated with other compatible findings, can be used to increase the CDV suspicion index in older dogs.

CDV affects both gray and white matter in the CNS. For this reason, neurological findings vary and seizures, behavioral changes, cerebellar (cranial and truncal ataxia, hypermetria, tremor), vestibular (falling, head tilt, rotation and nystagmus) and visual disturbances, paresis, paralysis, myoclonus and limb weakness may be observed (Amude et al., 2007). Seizures and chewing gum fits are associated with the region where the cerebellum is affected (Greene and Appel, 1998). Myoclonus is the rhythmic twitching of a single or group of muscles. It is a characteristic finding of neurological CDV in young dogs (Braund, 1994). However, it has also been reported to occur episodically in other inflammatory diseases in which the CNS is affected (Tipold et al., 1992). The lower motor neurons of the spinal cord's and cranial nerve nuclei's degenerative alterations are thought to cause the myoclonus. These rhythmic muscle contractions are caused by this lesion, which develops an independent pacemaker. However, pathological changes are minimal in these gray matter regions suggesting afunctional problems may be implicated (DeLahunta and Glass, 2009). In a clinicopathological analysis, no lesions in the neural gray column of the related spinal cord segments were observed in 5 out of 13 spontaneous distemper patients with myoclonus (Koutinas et al., 2002). In the present study, myoclonus was the neural sign with the highest incidence in both groups. This sign may be related to pathologic changes of the cranial nerve nucleus in younger dogs and of the spinal cord in older dogs, considering the high incidence of quadriplegia.

Ataxia is the most common finding in dogs with MDEMD, with paresis, especially in the hind limbs (Shell, 1990). MDEMD is defined as multifocal encephalomyelitis with a chronic course and lower incidence. In this form of CDV, the cerebellum, brainstem and spinal cord are the affected areas (Braund, 1994). Spastic paraplegia, truncal or head tilt, dysmetria and hypermetria have been reported as common findings when the CNS is affected with multifocal lesions (Amude et al., 2007). The head tilt in the 2–4-monthold dogs of the present study may be associated with multifocal pathologic changes of the cerebellum, although the cases were not identified as MDEMD.

Data on behavioral changes in neurological CDV cases is limited. In a previous study, findings such as aggression, head leaning against objects, circling, altered state of consciousness, unconscious gait, and interest in foreign objects were reported within the scope of behavioral changes. These findings were defined as ODE-like syndrome and reported to occur due to progressive panencephalitis with a rare subacute-chronic course (Shell, 1990; Vite, 2005). In addition, a low incidence of spinal cord-related findings, such as ataxia and quadriplegia, were reported in dogs with ODE-like syndrome (Braund, 1994). In all groups of the present study, the highest incidence of neural findings was behavioral change, and Group 1, which consists of younger dogs, also had a lower incidence of findings associated with spinal cord injury. Within the scope of the behavioral changes determined in the present study, barking at night, interest in foreign objects, head leaning, and aggression were learned from the anamnestic data. In addition, radiographic and ultrasonographic examinations of all dogs revealed the presence of bones and stones in the stomach and intestines (appearance characterized by a clear shadow on ultrasound, which is suspicious for the presence of a foreign body.). The ingestion of materials such as stones, plastic, rope, wood, etc., termed pica by the animal, does not provide any physical benefit and may be associated with some medical, nutritional and behavioral disorders. The higher presence of gastrointestinal foreign bodies in puppies (aged 2-4 months) included in Group 1 of the present study, combined with etiologic factors such as teething, anxiety and lack of enrichment (Masson et al., 2021), may be used to consider the presence of panencephalitis in combination with other CDV compatible findings and to increase the index of suspicion for neurologic CDV.

Following epithelial localization, the virus is known to cause respiratory symptoms such as pneumonia and gastrointestinal symptoms such as diarrhea (Green and Appel, 1990). Previous studies reported a general, moderate, slightly heterogeneous increase in pulmonary opacity, multiple thickened bronchial ring shadows, and decreased delineation of peripheral pulmonary vessels within the thoracic radiographic findings of dogs with CDV (Willi et al., 2015). Sonographic evaluation of the thoracic and abdominal cavities provides diagnostic information. Thoracic radiographic findings, such as alveolar pulmonary pattern interstitial pneumonia, and thoracic and diffuse ultrasonographic findings, such as the presence of B lines (>3 lines) along with loss of A-lines in the present study were compatible with the presence of pneumonia (Mazzola et al., 2021). Abdominal radiographic findings such as intestinal distention and gas-filled stomach, together with abdominal ultrasonographic findings such as acoustic shadowing characterized by a clean shadow in the intestine may be related to the aerophagia as a result of dyspnea due to pneumonia, and foreign body affinity due to abnormal mental status in dogs with CDV (Harjes et al., 2018).

The fact that the neurological findings determined in the present study were not histopathologically demonstrated and that other etiologies of the presence of gastrointestinal foreign body associated with behavioral changes, which is among the important clinical findings, were not investigated, can be considered as limitations. Therefore, investigating hematologic, biochemical and trace element concentrations in dogs with neurologic CDV with the aforementioned clinical findings may reveal predisposing factors as well as disease pathogenesis.

Conclusion

The clinical diagnosis of neurologic CDV infection applies to traditional cases. Cases without traditional

symptoms require further diagnostic methods. Therefore, evaluation of the initial clinical manifestation allows early diagnosis and thus, treatment of CDV cases. In the present study, extraneural findings such as diarrhea and uveitis, and neural findings such as quadriplegia had a higher incidence in 5-8 month-old dogs; depression, anorexia, myoclonus, and behavioral changes along with the presence of gastrointestinal foreign body had a high incidence in all the dogs. It was concluded that the evaluation of the initial clinical manifestation in combination with non-invasive imaging methods such as radiography and ultrasonography could be used to increase the index of suspicion of neurologic CDV before proceeding to laboratory analyses, especially in triage.

Conflict of Interest

The authors stated that they did not have any real, potential, or perceived conflict of interest.

Ethical Approval

Permission was received for this study under HADYEK number 2021-005/01-14. Additionally, the authors declared that Research and Publication Ethics were complied with.

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