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Analysis of Cerebrospinal Fluid Samples in Canine Tick Paralysis: Insights and Implications

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ABSTRACT

Tick paralysis, caused by the salivary neurotoxin of several species of ticks, is a non-infectious neurological syndrome. Its clinical findings are often confused with other acute flaccid paralyses (AFPs). Demographic data and cerebrospinal fluid (CSF) examination, along with routine analyses, can be used to increase the index of suspicion for tick paralysis. Thus, this study aims to elucidate neurological manifestations and mechanisms, offering diagnostic insights to enhance understanding of tick paralysis pathophysiology and inform treatment development and prevention. 15 dogs with tick paralysis and AFP findings were included. Anamnestic data were collected for all the dogs, followed by physical examination, complete blood count (CBC), and CSF examinations. It was observed that physical examination, CBC, and CSF analysis results except total protein level were within reference values. Unexpectedly, despite the non-infectious nature of tick paralysis, Staphylococcus lentus and Aeromonas sobria were detected in three CSF samples. Although these bacteria are not typically associated with tick paralysis, their presence in shepherd dogs was interpreted as contamination from livestock. In conclusion, although physical and laboratory examination findings often fall within reference values in tick paralysis cases, a comprehensive evaluation of clinical findings alongside demographic data may help increase the index of suspicion for tick paralysis, especially in atypical cases. While CSF analysis is crucial in diagnosing neurological disorders, it is also valuable for identifying potential complications in non-infectious cases such as tick paralysis. Furthermore, the risk of contamination during CSF collection, particularly in shepherd dogs, should be carefully considered.

Keywords: Acute flaccid paralysis, Aeromonas sobria, shepherd dog, Staphylococcus lentus, tick paralysis

Köpek Kene Felcinde Beyin Omurilik Sıvısı Örneklerinin Analizi: Görüşler ve Çıkarımlar

ÖZ

Çeşitli kene türlerinin tükürük nörotoksininin neden olduğu kene felci, bulaşıcı olmayan bir nörolojik sendromdur. Klinik bulguları sıklıkla diğer akut flassid paralizilerle (AFP) karıştırılır. Rutin analizlerin yanı sıra demografik veriler ve beyin omurilik sıvısı (BOS) incelemesi, kene felci şüphesini artırmak için kullanılabilir. Bu nedenle, bu çalışma nörolojik bulguları ve mekanizmaları aydınlatmayı, kene felci patofizyolojisi anlayışını geliştirmek ve tedavi geliştirme ve önleme konusunda bilgi sağlamak için tanısal bilgiler sunmayı amaçlamaktadır. Kene felci ve AFP bulguları olan 15 köpek çalışmaya dahil edildi. Tüm köpeklerden anamnez verileri alındı, ardından fiziksel muayene, tam kan sayımı (CBC) ve BOS incelemeleri yapıldı. Fiziksel muayene, CBC ve total protein düzeyi hariç BOS analiz sonuçlarının referans değerleri dahilinde olduğu görüldü. Kene felcinin bulaşıcı olmayan doğasına rağmen beklenmedik bir şekilde üç BOS örneğinde Staphylococcus lentus ve Aeromonas sobria tespit edildi. Her ne kadar bu bakteriler tipik olarak kene felci ile ilişkili olmasa da, çoban köpeklerinde bulunmaları çiftlik hayvanlarından kaynaklanan bir kontaminasyon olarak yorumlandı. Sonuç olarak, fiziksel ve laboratuvar muayene bulguları kene felci vakalarında sıklıkla referans değerleri içinde kalsa da, klinik bulguların demografik verilerle birlikte kapsamlı bir şekilde değerlendirilmesi, özellikle atipik olgularda, kene felci şüphesi indeksini artırmaya yardımcı olabilir. BOS analizi nörolojik bozuklukların teşhisinde önemli olsa da, kene felci gibi enfeksiyöz olmayan vakalarda olası komplikasyonları belirlemek için de değerlidir. Ayrıca, özellikle çoban köpeklerinde BOS örneği alımı sırasında kontaminasyon riski dikkatlice değerlendirilmelidir.

Süreç

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Introduction

Certain tick species, including Rhipicephalus sanguineus, commonly known as the brown dog tick, can secrete neurotoxins through their salivary glands while blood feeding. These neurotoxins can cause tick paralysis, characterized by a quick, ascending acute flaccid paralysis (AFP). (Otranto et al, 2021). Tick paralysis is a potentially life-threatening condition (Greene, 2006). The clinical signs of tick paralysis typically begin with weakness and a lack of coordination, which progress to paralysis over time. Affected dogs may exhibit difficulty breathing, gagging, coughing, and in severe cases, respiratory failure and death. The severity of symptoms depends on factors such as the size of the tick, the duration of attachment, and the individual dog's sensitivity to the toxin (Harkin & Walshaw, 1999). In dogs, diagnosis of tick paralysis is based on clinical signs and a history of recent tick exposure. Identification of ticks on the dog's body or in the environment can support the diagnosis. Additionally, laboratory tests such as complete blood count (CBC), serum biochemistry, and cerebrospinal fluid (CSF) analysis may be performed to assess neurological function and rule out other conditions (Tipold, 1995).

Routine CSF tests include assessing protein and glucose levels, cell counts with differential analysis, microscopic examination, and culture. Considering the fact that tick paralysis primarily affects the nervous system, leading to weakness, paralysis, and other neurological symptoms, CSF analysis provides beneficial information about the extent and nature of neurological dysfunction in affected dogs (Hogan & Schatzberg, 2019). Also, CSF analysis can help differentiate tick paralysis from other neurological conditions with similar clinical signs. By evaluating CSF parameters, veterinarians can rule out alternative diagnoses and ensure appropriate management strategies are implemented (Greene, 2006; Otranto et al, 2021). Moreover, the results of CSF analysis may influence treatment decisions in dogs with tick paralysis (Mackenzie, 2011).

Increasing misdiagnoses of tick paralysis and other acute motor polyneuropathies may further delay or complicate management of this condition and lead to unnecessary treatments. Therefore, the purpose of this study is to obtain insights into the neurological manifestations and underlying mechanisms of this condition and to provide diagnostic information that enhances the understanding of the pathophysiology of tick paralysis and informs the development of effective treatment strategies or preventive measures.

Material and Methods

This study was approved by the Local Ethics Committee for Animal Experiments at Harran University (Ethics Committee Decision date and number: 09.05.2022, 2022/003-01/06).

Animals

This study involved a total of 15 dogs, all were admitted to Harran University Veterinary Faculty Animal Hospital between March and July 2023 with neurological findings that enough to suspect the presence of AFP, such as sudden onset of weakness, hind limb incoordination, difficulty in movement, quadriplegia, dogs on which a tick was detected during clinical examination, and admitted for diagnostic and treatment purposes.

Inclusion/Exclusion Criteria

Inclusion criteria for the present study were: no history of disease of the dog, had not received antiparasitic medication recently (<1 month), had an engorged tick present, and exhibited symptoms of AFP. The following are accepted as clinical findings indicating the presence of AFP; (i) weakness that develops suddenly but increases in severity within a few days, which is characterized by weakness of respiratory muscles and swallowing ability, (ii) lack of hyperflexia, (iii) lack of clonus, (iv) absence of central nervous system motor pathways such as extensor plantar reflexes, (v) lack of contraction due to impairment of motor pathways extending from the cortex to muscle fibers (Marx et al, 2000).

Dogs were excluded from the study if they exhibited clinical signs of diffuse lower motor neuron diseases unrelated to tick paralysis. This included cases of acute idiopathic polyneuropathy with a history of systemic disease or exposure to raccoon saliva (Malik & Farrow, 1992), as well as botulism characterized by difficulty in grasping and swallowing, hypersalivation, and regurgitation (Shelton, 2002). Other neurological disorders, such as spinal cord compression, epidural abscesses, or exposure to snake and plant toxins, also resulted in exclusion (Holland, 2008). Additionally, dogs with detectable blood parasites (including Babesia spp., Anaplasma platys, Ehrlichia canis, and Hepatozoon canis) or hematological findings like pancytopenia and thrombocytopenia were not included (Otranto et al., 2021). Dogs presenting signs of AFP without detectable ticks or identifiable alternative causes through clinical and laboratory assessments were also excluded. Only dogs that demonstrated rapid and full recovery after acaricidal treatment and tick removal, confirming a diagnosis of tick paralysis ex juvantibus (Soulsby, 2005), were included in the study. Ultimately, the study population consisted of dogs infested with Rhipicephalus sanguineus that exhibited clinical symptoms consistent with tick paralysis.

Physical Examinations

Rectal body temperature, heart rate, respiratory rate, and capillary refill time (CRT) were measured. Body weight and body surface area (BSA) were also calculated using the formula [BSA in square meters = K × (body weight in grams2/3) × 10-4, K = constant (10.1 for dogs)] for each dog. Dogs with tick paralysis were examined for ticks by inspecting anatomical body regions, including the head, ears, neck, fore and hind limbs, thorax, abdomen, interdigital areas, tail, axillary, and inguinal regions.

Blood Sampling and Complete Blood Count Analyses

Venous blood samples (2–5 mL) were collected from all dogs via cephalic vein puncture with minimal restraint

to avoid causing stress. Tubes with anticoagulant (K3EDTA) were used for CBC using an autoanalyzer (Sysmex® pocH 100i, Japan) in the central laboratory of the animal hospital.

Collection of Cerebrospinal Fluid Samples

Before cerebrospinal fluid tap, all tick paralyzed dogs were sedated by intramuscular injection with xylazine (at a dose of 1 mg / kg, Xylazin Bio® 2%, Bioveta) after blood sampling. CSF samples (1–2 mL) were taken between the occipital and atlas bones with the appropriate procedure (using a 1.5 inch stylet spinal needle, 22 gauge) (Gülersoy et al., 2022). To prevent airway obstruction, excessive flexion of the head was avoided. The procedure was completed without any complications.

Physical and Microscopic Examinations, Dipstick Analysis and Bacterial Culture of CSF Samples

All CSF samples underwent meticulous visual inspection for turbidity. Dipstick analysis was conducted on each CSF sample, assessing leukocyte (WBC), glucose, specific gravity (Sg), total protein, hydrogen ion concentration (pH) and erythrocyte (RBC) levels, utilizing a strip analyzer (URIT-31 Vet®, Accurex Biomedical, India). This analysis was completed within 5–10 mins of sampling. Subsequently, a portion of CSF samples were prepared for microscopic examination using a cytocentrifuge at 1500 rpm for 10 minutes at room temperature. Following preparation, slides were stained with May-Grünwald-Giemsa and examined initially at x100 magnification, followed by further examination at x1000 magnification using a light microscope (CX23LEDRF, binocular light microscope, Olympus®, Japan). WBC and RBC counts were categorized as none (0/slide), rare (<10/slide), few (<1/oil immersion field [OIF]), moderate (1 to 10/OIF), or many (>10/OIF). The remaining CSF sample was utilized for bacterial culture. Two series of cultivations were performed on 5% sheep blood agar (Oxoid) and MacConkey agar (Oxoid). One series was incubated at 37°C for 48 hours, one under aerobic conditions and the other under microaerophilic conditions (5% CO2). After incubation, colonies observed on sheep blood agar (comprising two different colony forms: transparent and small, S-type creamy whitish colonies) and on MacConkey agar (lactose positive) under microaerophilic conditions were identified. Gram-positive cocci and Gram-negative rods were observed in microscopy (using a light microscope under x100 magnification with immersion oil, Olympus, Japan) using the Gram staining method (Quinn et al., 1994). Genera identified through classical methods were further analyzed at the species level using the VITEK 2 identification system (bioMérieux, USA).

Statistical Analyses

Data of the tick paralyzed dogs of the present study were processed using SPSS version 25.00 (SPSS for Windows®) statistical software. The distribution of the present data was assessed with the one-sample Kolmogorov-Smirnov test to determine whether it followed a parametric or non-parametric distribution. Descriptive statistics were performed to summarize the central tendency and variability within the dataset. Specifically, the median, minimum, and maximum values were calculated for each variable using the One-Sample Wilcoxon Signed Rank Test. Certain demographic data were expressed as percentages (% n/total).

Results and Discussion

Animals

The dogs included in the study were aged 6 (3-11) months, with 7 males and 8 females. Anamnestic data revealed that the duration of symptoms in the dogs was 5 (3-11) days. It was found that 13 of the dogs were shepherd dogs and were housed with sheep and goats, while the remaining 2 dogs were kept in the garden and taken for walks for toilet purposes at least twice a day. Consequently, the study sample consisted of dogs of both genders, with an average age of 6 months, predominantly mixed breeds (10 mixed breed, 3 Gureghs, 2 Anatolian Shepherds), and fed with commercial dry dog food. Anamnesis and demographic data results are presented in Table 1.

Table 1. Anamnesis, physical examination findings and demographic data

Parameters	Results median (min-max)
Rectal body temperature (°C)	38.8 (37.7-39.6)
Heart rate (beats/min)	105 (94-164)
Respiratory rate (breaths/min)	91 (79-99)
Capillary refill time (sec)	2 (1-3)
Body weight (kg)	9.5 (7-14)
Body surface area (m^2)	0.437 (0.370-0.590)
Age (mos)	6 (3-11)
Symptom duration (days)	5 (3-11)
Purpose of housing	13 shepherd, 2 companion

Physical Examination Results

Voice change (%40) was observed in 6 of the tick-paralyzed dogs included in the study, altered breathing patterns were noted in 4 (%26.6), gagging occurred in 5 (%33.3), and vomiting was observed in 2 (%13.3). Regarding neurological findings, all dogs exhibited incoordination (%100). Furthermore, quadriplegia was evident in 5 dogs (%33.3), while hind limb weakness was observed in 6 dogs (%40). The results of the parameters investigated within the scope of physical examination, along with demographic data, are presented in Table 1.

Complete Blood Count Res

All investigated CBC parameters were found to be within the reference values (Klaassen, 1999). The results of the parameters investigated within the scope of CBC analysis and reference values are presented in Table 2.

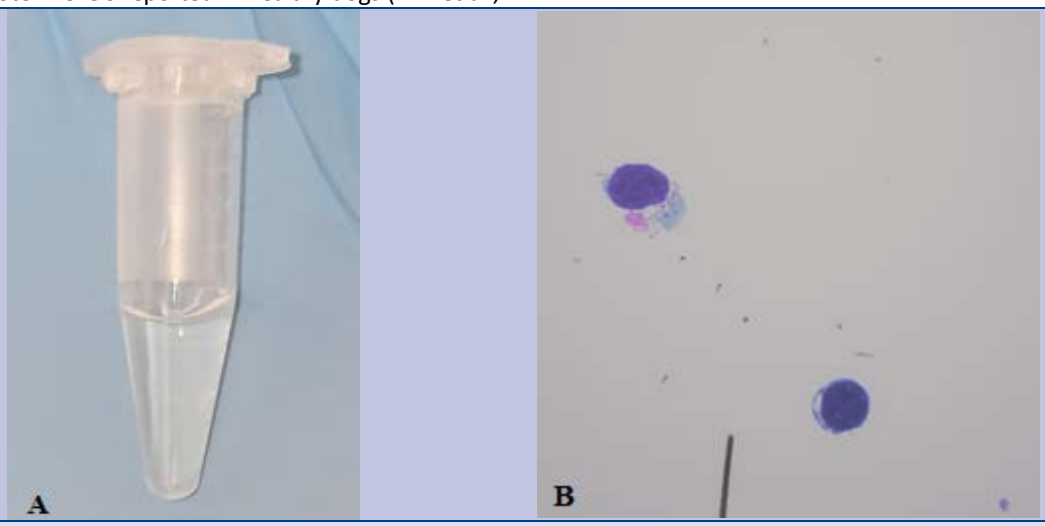
Table 2. CBC analysis results

Parameters	Results median (min-max)	Reference values*
WBC (K/µL)	7.42 (2.84-18.50)	3-14.8
Lymphocyte (K/µL)	3.75 (1.01-5.82)	1.2-8
Monocyte (K/µL)	0.39 (0.05-1.70)	0-0.6
Neutrophil (K/µL)	3.4 (0.59-13.4)	2.5-8.5
RBC (M/µL)	6.90 (5.27-8.62)	5.92-14.8
MCV (fL)	63.85 (48.70-71.42)	37-61
Hct (%)	45.78 (34.3-55.2)	29-48
Hb (g/dL)	15 (11-19)	9.3-15.9
Plt (K/µL)	591 (389-1016)	200-600

WBC: Leukocyte, RBC: Erythrocyte, MCV: Mean corpuscular volume, Hct: Hematocrit, Hb: Hemoglobin, Plt: Platalet. *Reference values (Klaassen J.K., 1999).

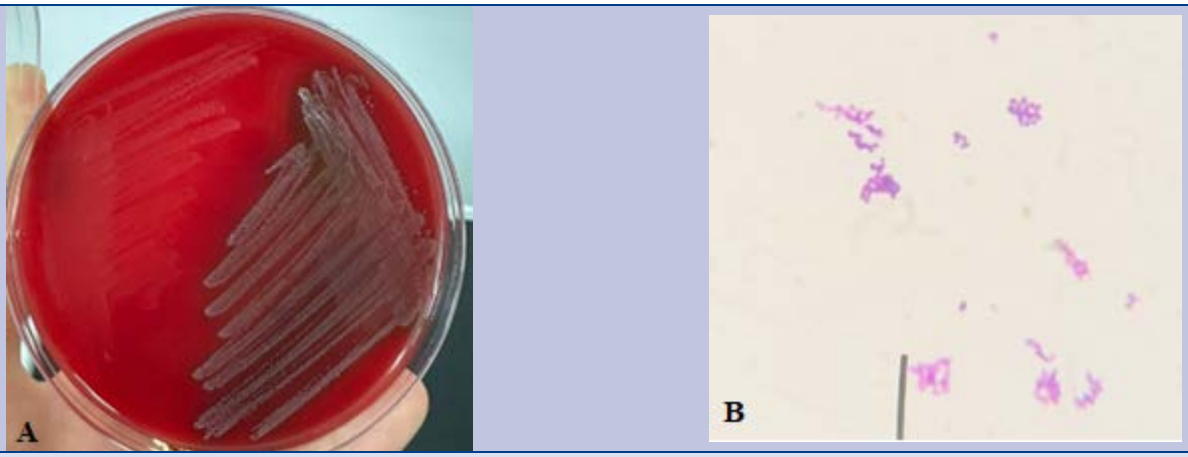
Physical and Microscopic Examination, Dipstick Analysis and Bacterial Culture results of CSF Samples

Overall, in this study, all CSF samples obtained via the cerebellomedullary cistern were found to be colorless and clear (Figure 1A). Additionally, light microscopic examination revealed the presence of gram-positive coccobacilli (Figure 1B). As a result of CSF dipstick analysis, in comparison to the CSF total protein levels reported in healthy dogs (Kim et al., 2008; Di Terlizzi & Platt, 2009), the total protein level observed in the tick-paralyzed dogs in this study was slightly elevated. The results of the CSF dipstick analysis are presented in Table 3. Following bacterial cultivation, isolation was observed in three samples. Using the VITEK 2 identification system (bioMérieux, USA), Staphylococcus lentus and Aeromonas sobria were detected in two samples, while only Staphylococcus lentus was found in one sample. (Figure 2).



A.Clear, colorless and watery appearance of the CSF sample B.Low cellular content (a few lymphocytes) of CSF sample, 20 µm

Figure 1A-B. Physical appearance and microscopic examination images of CSF sample



A. Two different colony forms: transparent and small, S-type creamy whitish colonies
B. Presence of gram-positive coccobacilli in CSF sample colonies

Figure 2A-B. CSF bacterial culture results and microscopic image

Table 3. Dipstick analysis results of CSF samples

Parameters	Results median (min-max)	Reference values*
WBC (cells/mm^3)	1 (0-2)	≤5/OIF**
Glucose (mg/dL)	55.65 (50.54-79.5)	50-80
Total Protein (mg/dL)	36 (18-36)	≤30 mg/dl
Specific gravity	1.030 (1.020-1.030)	1.030-1.060**
pH	7 (6.5-8.5)	6.9-7.45**
RBC (cells/mm^3)	1 (0-3)	≤1/OIF*

WBC: Leukocyte, OIF: Oil immersion field, , pH: Power of hydrogen, RBC: Erythrocyte. * Reference values (Gülersoy E. et al, 2022), **Reference values (Di Terlizzi & Platt, 2009)

In the present study, demographic data, physical examination findings, CBC, and microscopic, dipstick and bacterial culture results of CSF samples were evaluated from dogs with tick paralysis confirmed by the ex juvantibus method. Findings within the reference ranges for physical examination, CBC, and CSF analysis—except for the total protein level, which may indicate albuminocytological dissociation—suggest an absence of an inflammatory response to tick paralysis. Unexpectedly, despite the non-infectious nature of tick paralysis, Staphylococcus lentus and Aeromonas sobria were detected in 3 CSF samples. Although these bacteria are unrelated to tick paralysis pathogenesis, their presence may indicate contamination of shepherd dogs with livestock. Therefore, in cases of tick paralysis, including atypical cases where ticks are not detectable, a comprehensive evaluation of demographic data, along with physical and laboratory findings, may help raise the index of suspicion and identify potential complications. Additionally, while CSF analysis is diagnostically important for neurological disorders, careful consideration of contamination risk is warranted.

Tick paralysis is an AFP characterized by sudden weakness, with severity typically peaking within a few days. It involves weakness of respiratory muscles and can impair the ability to swallow. If left untreated, AFP is not only permanent but can cause death due to respiratory failure. Thus, accurate diagnosis is important for both prognosis and treatment (Gülersoy et al., 2022; Pienaar et al., 2018). In addition to the non-specific clinical findings observed in cases of tick paralysis, other findings that increase the mortality rate up to 100% in untreated cases are due to cardiovascular, gastrointestinal and respiratory system problems (Padula, 2016). In a clinical study conducted on Beagle dogs, it was reported that clinical findings began to appear within 5.5-7 days following 3-4 tick bites (Ilkiw & Turner, 1987). It has been reported that ascending flaccid paralysis develops in clinically ill dogs, and these dogs typically die within 18-32 hours. Clinical findings reported in the initial period include changes in vocalization and hind leg ataxia. During this phase, appetite may remain normal, vomiting may be absent, and the dog may appear otherwise healthy. As the paralysis progresses, standing becomes increasingly difficult, and the dog remains in a lateral lying position. During this, vocalization abnormalities intensify, moaning sounds may be heard, and swallowing reflexes are affected. In the final stages of paralysis, death typically occurs within 2 hours, marked by changes in mucous membrane color, pupillary dilation, and loss of all body reflexes, including respiratory reflexes (Ilkiw & Turner, 1987; Padula, 2016). The clinical findings, including voice change, gagging, and hind limb weakness, detected at a higher incidence in the tick-paralyzed dogs, were consistent with those of previous studies, taking into account the symptom duration of the dogs included in the present study (Marx et al., 2000; Webster et al., 2013). The absence of systemic findings such as tachycardia, collapse, and respiratory failure (Padula, 2016) may also be related to the time to hospital admission.

There are no specific tests for the diagnosis of tick paralysis. Generally, the diagnosis of tick paralysis is made by finding an engorged tick on a patient. However, tick paralysis can develop even after a tick has dropped off. For this reason, while detecting the presence of a tick in typical cases facilitates diagnosis, atypical cases in which the tick cannot be detected pose difficulties due to the lack of a specific test for tick paralysis. Laboratory analyses such as CBC, serum biochemistry, and urine analysis can be utilized to assess the health profile and detect systemic abnormalities (Burke et al., 2005). Previous reports have indicated that these analyses yield normal results, attributed to the non-infectious nature of tick paralysis (Simon et al., 2023). It was determined that the CBC analysis results of the tick paralyzed dogs of the present study were within reference values. The normal hematological findings observed in cases of tick paralysis can be attributed to the pathogenesis of acute flaccid paralysis, which is characterized by the disruption of acetylcholine release and neuromuscular blockade at the motor end plate caused by the neurotoxin present in the tick's saliva (Diaz, 2015). The diagnosis of tick paralysis based on CBC results is often missed or delayed, resulting in unnecessary treatments, interventions, and improper management. Moreover, it's not uncommon for patients to undergo unnecessary procedures and investigations, further delaying accurate diagnosis and treatment and exposing them to potentially catastrophic consequences (Salman et al., 2023). For this reason, CSF analysis can serve as a crucial diagnostic tool in cases presenting neurological findings such as Guillain-Barré syndrome and tick paralysis. It can support the diagnosis and aid in the differential diagnosis process (Solomon et al., 2019). In the current study, CSF samples from tick-paralyzed dogs were comprehensively analyzed. It was determined that they exhibited a physically clean appearance with dipstick analysis results within the reference range, except CSF total protein level, and low cellularity upon microscopic examination. The slightly elevated CSF total protein level detected in the present study was associated with albuminocytological dissociation, defined as an increase in total protein without a corresponding increase in total nucleated cell count in the CSF. Although idiopathic polyradiculoneuritis, extradural compressive lesions, or spinal cord compression may be

involved in the etiology, further research is recommended, as these findings are anecdotal (Suñol et al., 2021). Interestingly, gram-positive coccobacilli were observed in the microscopic examination of the CSF samples from three tick-paralyzed dogs included in the study. Bacterial culture resulted in the growth of Staphylococcus lentus and Aeromonas sobria.

Staphylococcus lentus is widely distributed in nature and is a commensal bacterium colonizing the skin of several animal species. It has commonly been isolated from food-producing animals, including poultry and dairy animals and from their food products (Huber et al., 2011). In a study of the 20 individuals involving solely Staphylococcus lentus from several bodily fluids including CSF, had clinical signs of infection including leukocytosis, fever and pain (Mazal & Sieger, 2010). Aeromonas sobria is also commonly found in natural environments like water, soil, and feces, and serves as a conditional pathogen for humans, aquatic animals, livestock, and poultry. Infection in humans or animals occurs through ingestion of contaminated food or direct contact, leading to symptoms such as bacteremia, tissue damage, pneumonia, meningitis, gastroenteritis, or septicemia, particularly in individuals with weakened immune systems (Song et al., 2019). The fact that the aforementioned bacteria grew in only 3 CSF samples (3 out of 15, 20%) in the present study can be considered as contamination, considering that there are no signs of systemic infection in the dogs. The growth of contaminant organisms in culture samples from sterile sites may create a significant clinical dilemma. It was reported that false-positive CSF culture rates range from 2.7% to 8.3% in institution-based reports (Dunbar et al., 1998). For this reason, it is important to remember that bacteria found in CSF samples may be contaminated because shepherd dogs often interact with livestock and are more exposed to ticks (Sahu et al., 2013).

The low number of animal material, the fact that the paralysis-causing tick species is only Rhipicephalus sanguineus, and the lack of further analysis such as biochemical and biomarker research of CSF samples can be considered as limitations in this study.

Conclusion

This study evaluated demographic data, physical examination findings, CBC, and CSF analysis results from dogs with tick paralysis. Voice changes and hind limb weakness were the most prominent findings. While the CBC results were normal and the CSF total protein was increased due to albuminocytological dissociation, Staphylococcus lentus and Aeromonas sobria were detected in three CSF samples, possibly due to contamination in shepherd dogs exposed to livestock. This situation suggests that shepherd dogs have a higher probability of encountering ticks, and tick paralysis should be considered if AFP findings are present. It was concluded that, since examination results often remain within reference values, a comprehensive evaluation of clinical and demographic data may help raise the index of suspicion for tick paralysis. Additionally, CSF analysis is important for diagnosing and differentiating neurological disorders and can identify potential complications in non-infectious cases like tick paralysis.

Conflict of Interest

The authors declare no conflict of interest.

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