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Diagnostic Efficacy of Certain Physical Examination and Serum Biochemistry Parameters in Dogs with Tick Paralysis

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Abstract: Recognizing tick paralysis is crucial as it can be fatal, but no specific diagnostic test exists. Therefore, this study aimed to evaluate the diagnostic effectiveness of routine clinical examinations and selected laboratory analytes and to understand the impact of tick burden on the severity of symptoms in tick-paralyzed dogs. Fourteen dogs, whose diagnosis of tick paralysis was confirmed by the *ex juvantibus* method, were divided into Low Tick (1-4 ticks, n=7) and High Tick (5-60 ticks, n=7) subgroups based on the number of ticks. The healthy group consisted of dogs with no disease history (n=7). Physical examinations and certain serum biochemistry profile analyses of all the dogs were evaluated comparatively. The diagnostic efficiency of all the parameters was investigated by ROC-based diagnostic performance analysis. The respiratory rate of the dogs with tick paralysis was higher than that of the healthy dogs ($p<0.0001$). The body temperature and heart rate of the High Tick group dogs were higher than those in the other groups ($p<0.0001$). Capillary refill time (CRT) was shorter in the High Tick group than in the other groups ($p=0.018$). Total protein, blood urea nitrogen (BUN), and total bilirubin levels were higher in the High Tick group than in the other groups ($p=0.035$). It was concluded that as the number of ticks increases, the diagnostic performance of the investigated analytes improves potently due to the rate and volume of toxin secretion by the ticks. However, they should be evaluated together with other parameters due to their low specificity.

Keywords: *Acute flaccid paralysis, Diagnosis, Dog, Serum biochemistry, Tick paralysis.*

Kene Felci Olan Köpeklerde Bazı Fiziksel Muayene ve Serum Biyokimyası Parametrelerinin Tanısal Etkinliği

Özet: Kene felcini tanımak, ölümcül olabileceğinden çok önemlidir, ancak spesifik bir tanı testi bulunmamaktadır. Bu nedenle, bu çalışmada rutin klinik muayenelerin ve seçilmiş laboratuvar analizlerinin tanısal etkinliğinin değerlendirilmesi ve kene felci olan köpeklerde kene yükünün semptomların şiddeti üzerindeki etkisinin anlaşılması amaçlanmıştır. Kene felci tanısı *ex juvantibus* yöntemi ile doğrulanan 14 köpek, kene sayısına göre Düşük Kene (1-4 kene, n=7) ve Yüksek Kene (5-60 kene, n=7) alt gruplarına ayrıldı. Sağlıklı grup, hastalık geçmişi olmayan köpeklerden oluşturuldu (n=7). Tüm köpeklerin fiziksel muayeneleri ve bazı seçilmiş serum biyokimya profili analizleri karşılaştırmalı olarak değerlendirildi. Tüm parametrelerin tanısal etkinliği, ROC-tabanlı tanı performans analizi ile araştırıldı. Kene felci olan köpeklerin solunum hızı, sağlıklı köpeklere göre daha yüksekti ($p<0.0001$). Yüksek Kene grubundaki köpeklerin vücut sıcaklığı ve nabzı diğer gruplardaki köpeklere göre daha yüksekti ($p<0.0001$). Kapillar tekrar dolum zamanı (CRT) Yüksek Kene grubunda diğer gruplara göre daha kısaydı ($p=0.018$). Total protein, kan üre azotu (BUN) ve total bilirubin düzeyleri Yüksek Kene grubunda diğer gruplara göre daha yüksekti ($p=0.035$). Kene sayısı arttıkça, kenelerin toksin salgılama hızı ve hacmi nedeniyle araştırılan analitlerin tanı performansının güçlü bir şekilde arttığı kanısına varıldı. Fakat, özgüllüklerinin düşük olması nedeniyle bu parametrelerin diğer bulgularla birlikte değerlendirilmesi gerekmektedir.

Anahtar Kelimeler: Akut yumuşak paraliz, Kene paralizi, Köpek, Serum biyokimyası, Tanı.

Introduction

The clinical manifestation of acute flaccid paralysis (AFP) is a sudden onset of weakness, sometimes affecting swallowing and respiratory muscles. The lack of spasticity or other symptoms of abnormal motor tracts of the central nervous system, such as clonus, hyperreflexia, or extensor plantar reflexes, is referred to as flaccid (Bowley and Chad, 2019; Marx et al., 2000). Etiologies contributing to AFP encompass spinal cord injuries (infectious, inflammatory, compressive), neuromuscular junction disorders (myasthenia gravis, botulism, Lambert-Eaton Myasthenic Syndrome), muscle-related issues (muscular dystrophies, necrotizing myopathies, hypokalemia or severe hyperkalemia), and root or peripheral nerve disorders (Guillain-Barré Syndrome, polyneuropathy, tick paralysis) (Fadia et al., 2019). Tick paralysis, among the causes of AFP, is a rare neurological syndrome that is non-infectious and characterized by acute ataxia. Its clinical presentation often resembles Guillain-Barré syndrome, botulism, and acute idiopathic polyneuropathies, thereby complicating the diagnostic process (Malik and Farrow, 1991; Simon et al., 2023).

Tick paralysis develops due to neurotoxins secreted by the salivary glands of certain tick species. Rhipicephalus sanguineus, one of the most common tick species in dogs, is recognized as a principal vector associated with this syndrome (Padula, 2016; Ceylan et al., 2019; Gülersoy and Günal, 2022). In Turkey, due to geographic and climatic diversity, various tick species are encountered throughout the year, and tick infestations are widespread (Aydın and Bakırcı, 2007; İça and Çetin, 2016). Although tick paralysis is most commonly attributed to a single engorged tick, cases associated with multiple ticks have also been reported (Padula et al., 2020). The severity of the disease may correlate with the number of attached ticks and the total amount of neurotoxin released. Currently, there is no specific diagnostic test for tick paralysis. Therefore, diagnosis primarily relies on the identification of engorged ticks through physical examination and the exclusion of other similar clinical presentations, followed by an ex juvantibus approach. However, in cases where ticks are few or not visible, this diagnostic process becomes more challenging. Hence, investigating the diagnostic utility of routine clinical examination findings and selected serum biochemical parameters is essential for early detection and timely intervention (Atwell et al., 2001; Gülersoy et al., 2023). This study aimed to evaluate the relationship between tick burden and the severity of clinical symptoms in dogs diagnosed with tick paralysis-induced AFP and to assess the diagnostic performance of physical examination findings and selected serum biochemistry analytes in this context.

Materials and Methods

This study was approved by the Harran University Animal Experiments Local Ethics Committee on 09.05.2022 with session 2022/003 and decision number 01-06.

Animals: This study included 21 dogs, which will later be divided into Healthy, Low Tick and, High Tick groups, admitted to the Animal Hospital of the Faculty of Veterinary Medicine, Harran University. The affected dogs in the study presented neurological findings strongly indicative of AFP caused by tick paralysis. These findings included the sudden onset of weakness, difficulty in movement, hind limb incoordination, quadriplegia, and attached and engorged ticks. The control (Healthy) group comprised clinically healthy dogs with no known history of disease and were admitted to the animal hospital for vaccination and/or check-up purposes.

Physical Examinations

Within the scope of the physical examination, rectal body temperature, heart and respiratory rate, and gingival capillary refill time (CRT) were evaluated. In addition, a visual assessment of the neurological manifestations of the affected dogs was performed. Moreover, the body weight and body surface area (BSA) of each dog were calculated. By thumb counting anatomical body locations such as the head, neck, ears, thorax, abdomen, interdigital areas, fore and hind limbs, tail, axillary and, inguinal area, tick-paralyzed dogs were assessed for the presence of ticks. To minimize potential confounding effects on serum biochemical parameters, all dogs included in the study underwent a complete blood count (CBC) and microscopic examination of blood smears. Both buffy coat and peripheral blood smears were evaluated for the presence of *Anaplasma platys*, *Ehrlichia canis*, *Babesia* spp., and *Hepatozoon canis* inclusions. Smears were examined using a light microscope under 100× oil immersion magnification. As a result of clinical examinations, dogs with any blood parasites and findings such as thrombocytopenia and pancytopenia, which have been commonly reported in dogs previously infected with R. *sanguineus* (Otranto et al., 2012), were not included in the study.

Inclusion/Exclusion Criteria

Inclusion criteria for the study required that dogs had no prior history of illness, had not received any antiparasitic treatment within the preceding month, presented with an engorged tick at the time of examination, and exhibited clinical signs consistent with AFP. The present study classifies the following clinical findings as suggestive of AFP: an inability to contract due to motor pathway impairment extending from the cerebral cortex to muscle fibers; the absence of spasticity or other signs of disordered central nervous system motor tracts, such as hyperreflexia, clonus, or extensor plantar reflexes; and the sudden development and worsening of weakness within a few days, particularly affecting respiratory muscles and swallowing ability (Marx et al., 2000). The observed clinical presentation in the affected dogs prompts consideration of various potential causes of diffuse lower motor neuron diseases, including snake envenomation, botulism and, acute idiopathic polyneuropathy, among others (Malik and Farrow, 1991). In brief, botulism in dogs typically arises concurrently with the ingestion of spoiled food or carrion, a scenario not applicable to the dogs under consideration, as they were exclusively fed commercial food. Clinically, botulism manifests as challenges

in food prehension and swallowing, accompanied by hypersalivation and regurgitation (Shelton, 2002). It has been observed that dogs with a history of systemic disease or those exposed to raccoon saliva may develop acute idiopathic polyneuropathy, which is characterized by neurogenic muscle atrophy and hyperesthesia lasting more than five to seven days (Malik and Farrow, 1991). The breathing pattern in the aforementioned neuromuscular paralysis diseases is usually shallow and fast. However, in the present cases, it was marked by an expiratory effort, resembling the pattern observed in tick paralysis (Holland, 2008). As a result, the neurological symptoms listed here are more similar to those of tick paralysis than those linked to other prevalent possible causes of lower motor neuron disorders.

Dogs exhibiting clinical signs of AFP but without detectable tick infestations, those in which an alternative etiology for AFP was identified based on anamnesis, clinical evaluation, and laboratory findings, as well as dogs diagnosed with other causes of neurological disorders such as spinal cord compression, epidural abscesses, or exposure to plant or snake toxins were excluded from the study. Confirmation of AFP due to tick paralysis was achieved through the *ex juvantibus* method, as all dogs showed prompt and complete recovery (median: 24 hours, min: 16 h, max: 34 h) following acaricidal treatment and tick removal. Additionally, eight dogs were excluded from the study because they did not exhibit clinical improvement despite acaricidal treatment and tick removal. Based on the anamnesis, these cases were suspected to be related to clostridial neurotoxin (botulism). The collected ticks were stored individually in vials and later identified at the species level using morphological keys. Morphological identification was conducted using a light stereomicroscope, examining the following characteristics: idiosoma, dorsal scutum, basis capituli, angles of basis capituli, hypostomal dentition, female genital opening, dorsal tail of spiracular plates, lateral and postmedian grooves, internal and external cervical grooves, marginal lines, accessory plates, presence of a male caudal process and body color. All ticks were identified as R. sanguineus (Ceylan et al., 2019; Gülersoy and Günal, 2022; Soulsby, 2005). As a result, 14 dogs infested with R. *sanguineus*, with clinical findings compatible with tick paralysis, and with a confirmed tick paralysis diagnosis *ex juvantibus*, constituted the tick paralyzed group of the study. To understand the impact of tick burden on the severity of symptoms and guide treatment strategies accordingly, dogs with tick paralysis were divided into two subgroups based on tick count: dogs with 1–4 ticks comprised the Low Tick group, while those with 5–60 ticks comprised the High Tick group. Seven dogs with similar BSAs (p=0.714), which were admitted either for vaccination and/or check-up purposes, constituted the healthy Control Group.

In summary, tick paralysis was diagnosed by ruling out other conditions with similar presentations and confirmed using the *ex juvantibus* method, in addition to the presence of engorged ticks and clinical signs such as quadriplegia, facial paralysis, megaesophagus, vomiting, and regurgitation. As previously mentioned, ticks were identified morphologically as *R. sanguineus* (Soulsby, 2005).

Taking Venous Blood Samples and Serum Biochemistry Profiling

Venous blood samples were taken from all the dogs via vena cephalica (5–10 mL) venepuncture with minimal restraint in order not to cause stress. Tubes without anticoagulant were used for serum extraction (1500 x rpm for 15 minutes at 4 °C). Serum biochemistry profiling was performed on the serum samples obtained using an autoanalyzer (Arkray Spotchem EZ SP-4430 automatic dry biochemistry, Japan) in the central laboratory of the animal hospital.

Statistical Analysis

The statistical program SPSS 25.00 (SPSS for Windows®) was used to analyze all the data. The Kolmogorov-Smirnov test was performed on the sample data to ascertain whether the data were parametric. Non-parametric data were analyzed using the Mann-Whitney U and Kruskal-Wallis tests and reported as the median (min-max). Statistical significance between group means was evaluated via one-way ANOVA with post-hoc Tukey analysis. Utilizing Receiver Operating Characteristic (ROC) curve analysis, the diagnostic and/or prognostic effectiveness of the previously described biomarkers was examined. The diagnostic sensitivity and specificity (>70%), standard deviation (Std. error), area under the curve (AUC >0.600), and diagnostic sensitivity were among the metrics used to assess the diagnostic performance of biomarkers. It was accepted that an AUC of 0.5 indicates no discrimination (i.e., the test's capacity to distinguish between patients with and without the illness or condition), 0.6 to 0.8 was regarded as acceptable, 0.8 to 0.9 as excellent, and >0.9 as exceptional (Hosmer and Lemeshow, 2000). Additionally, Spearman correlation analyses of all the aforementioned parameters were also performed. Within this scope, it was accepted that 0.40 to 0.69 refers to a moderate correlation, 0.70 to 0.89 a strong correlation, 0.90 to 1.00 a very strong correlation (Schober et al., 2018). Statistical significance was accepted as $p<0.05$, CI=95% for all data.

Results

Animal Characteristics

All dogs of the present study were domestic dogs, unvaccinated, fed on commercial dry dog food, and taken outside for walks 2-3 times a day. Among the dogs, mostly of mixed breed, the ones with tick paralysis were 4 (2-7) months old and the healthy ones were 4.5 (3-6) months old (p=0.843). Anamnestic data revealed that the dogs had no previous history of disease. The symptom duration of tick-paralyzed dogs was 4 (2-7) days and it was learned that the owners attempted to remove a few ticks themselves before presenting them to the hospital. It was observed that the head of the tick remained in some dogs (2 out of 14 paralyzed dogs). During the clinical examination, further ticks were removed from the dogs, and to validate the *ex juvantibus* diagnostic approach, all of the dogs were treated with a spot-on formulation of Fipronil 10% / (S)-Methoprene

9% (Frontline Combo, Merial S.A.S., France). Serum biochemistry profiling was conducted on dogs suspected of tick paralysis and deemed appropriate for inclusion in the study. This profiling occurred during the initial assessment. Results that did not support tick paralysis based on the *ex juvantibus* method were excluded from the study. Consequently, in addition to the 14 dogs included, 8 dogs were excluded because they did not show clinical improvement despite acaricidal treatment and tick removal, as previously mentioned.

Physical Examination Findings

The abnormalities detected as a result of visual assessment of neurological manifestations were hind limb incoordination (6 dogs, 43%), quadriplegia (3 dogs, 21%), ataxia (2 dogs, 14%), paresis (2 dogs, 14%), and absence of reflexes (1 dog, 7%). The respiratory rate of the tick-paralyzed dogs was higher than the healthy dogs ($p<0.0001$) but no statistical difference was determined between the Low Tick and High Tick groups. The heart rate of the High Tick group dogs was higher and statistically different from those in the other groups ($p<0.0001$). CRT was shorter in the High Tick group than in the other groups ($p=0.018$). While rectal body temperature was not statistically different between the Low Tick and the healthy groups, it was higher in the High Tick group than the others ($p<0.0001$). Physical examination findings are presented in Table 1.

Table 1. Physical examination findings.

Parameters	Healthy Group n:7 median (min-max)	Low Tick Group n:7 median (min-max)	High Tick Group n:7 median (min-max)	p value
Respiratory rate (breaths/min)	**39 (24-46)^{b}**	**84 (72-88)^{a}**	**76 (68-87)^{a}**	**0.0001**
Heart rate (beats/min)	**79 (73-96)^{b}**	**96 (90-108)^{b}**	**120 (104-164)a**	**0.0001**
Capillary refill time (sec)	**3 (2-3)^{a}**	**2.5 (2-3)^{ab}**	**2 (1-3)^{b}**	**0.018**
Body temperature (°C)	**38.1 (37.7-38.5)^{b}**	**38.8 (38.1-39.5)^{b}**	**40.1 (39.2-40.5)^{a}**	**0.0001**
Body weight (kg)	6 (5-7)	6.5 (5-8)	6 (5-8)	0.706
Body surface area (m^2)	0.33 (0.30-0.37)	0.35 (0.30-0.40)	0.33 (0.30-0.40)	0.714

The formula used to estimate BSA from body weight in dogs is $BSA=10.\ W^{2/3}$, where W=body weight in grams then converted into kilograms. $^{a, b}$: Indicates statistical differences.

Table 2. Serum biochemistry profiling results.

Parameters	Healthy Group n:7 median (min-max)	Low Tick Group n:7 median (min-max)	High Tick Group n:7 median (min-max)	p value
Total protein (g/dL)	**6.1 (5.3-7.5)^{ab}**	**5.1 (4.2-5.5)^{b}**	**7.4 (6.1-10)^{a}**	**0.002**
Albumin (g/dL)	3.1 (2.8-3.6)	3 (2.1-3.8)	3.3 (2.1-5)	0.728
Total bilirubin (mg/dL)	**0.3 (0.2-0.7)^{b}**	**0.35 (0.2-0.8)^{b}**	**1 (0.2-1.6)^{a}**	**0.002**
AST (U/L)	25 (15-45)	94 (10-112)	41 (17-467)	0.274
ALT (U/L)	66 (43-74)	36 (10-88)	31 (10-280)	0.781
LDH (U/L)	389 (236-401)	192.5 (100-665)	500 (102-2059)	0.281
BUN (mg/dL)	**7.4 (5.7-11)^{b}**	**18.4 (8.3-26.4)^{ab}**	**22.8 (6.7-43.1)^{a}**	**0.035**
Creatinine (mg/dL)	1.3 (0.7-1.6)	0.8 (0.3-1.1)	1.5 (1-26.2)	0.335

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, $^{a, b}$: Indicates statistical differences.

Table 3. Diagnostic efficacies of clinical parameters of the tick paralyzed dogs in the Low Tick group.

Parameter	AUC	Std. Error	Asymp. Sig.	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Respiratory rate	0.863	0.084	0.012	0.698	1.000	70	100%	57.1%
Heart rate	0.482	0.129	0.902	0.230	0.734	89	100%	42.9%
Capillary refill time	0.589	0.132	0.536	0.330	0.848	1.5	100%	21.4%
Body temperature	0.435	0.129	0.650	0.182	0.687	38.55	66.7%	50%

AUC: Area under curve, Std: Standard, Asymp. Sig.: Asymptotic Significance.

Table 4. Diagnostic efficacies of serum biochemistry parameters of the tick paralyzed dogs in the Low Tick group.

Parameters	AUC	Std. Error	Asymp. Sig.	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Total protein	0.036	0.037	0.001	0.000	0.109	5.45	16.7%	7.1%
Albumin	0.440	0.166	0.680	0.116	0.765	3.35	50%	64.3%
Total bilirubin	0.369	0.125	0.364	0.124	0.614	0.35	50%	42.9%
AST	0.714	0.153	0.138	0.415	1.000	68	83.3%	85.7%
ALT	0.399	0.146	0.483	0.113	0.685	32.5	66.7%	28.6%
LDH	0.286	0.136	0.138	0.019	0.553	192.5	50%	21.7%
BUN	0.732	0.112	0.108	0.513	0.951	7.95	100%	50%
Creatinine	0.083	0.062	0.004	0.000	0.205	0.8	50%	7.1%

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, AUC: Area under curve, Std: Standard, Asymp. Sig.: Asymptotic Significance

Table 5. Diagnostic efficacies of clinical parameters of the tick paralyzed dogs in the High Tick group.

Parameter	AUC	Std. Error	Asymp. Sig	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Respiratory rate	0.665	0.123	0.235	0.425	0.905	57	100%	53.8%
Heart rate	0.984	0.023	0.000	0.939	1.000	101	100%	99.3%
Capillary refill time	0.181	0.107	0.022	0.000	0.391	2.5	14.3%	38.5%
Body temperature	0.973	0.033	0.001	0.909	1.000	39.1	100%	99.3%

AUC: Area under curve, Std: Standard, Asymp. Sig.: Asymptotic Significance

Table 6. Diagnostic efficacies of serum biochemistry parameters of the tick paralyzed dogs in the High Tick group.

Parameter	AUC	Std. Error	Asymp. Sig	Asymptotic 95% Confidence Interval		Cut-off value	Sensitivity	Specificity
				Lower Bound	Upper Bound			
Total protein	0.874	0.078	0.007	0.721	1.000	5.95	100%	69.2%
Albumin	0.533	0.147	0.812	0.245	0.821	3.25	57.1%	61.5%
Total bilirubin	0.879	0.109	0.006	0.665	1.000	0.75	85.7%	99.3%
AST	0.593	0.139	0.501	0.321	0.866	35.5	71.4%	53.8%
ALT	0.319	0.139	0.191	0.045	0.592	44	42.9%	38.5%
LDH	0.582	0.171	0.552	0.246	0.918	450.5	57.1%	99.3%
BUN	0.654	0.143	0.267	0.374	0.933	11.9	71.4%	69.2%
Creatinine	0.852	0.087	0.011	0.682	1.000	1.15	85.7%	69.2%

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen, AUC: Area under curve, Std: Standard, Asymp. Sig.: Asymptotic Significance

Serum Biochemistry Findings

Statistical differences were found in total protein, bilirubin, and BUN levels ($p<0.05$) among the parameters evaluated in the serum biochemistry profile. However, no differences were detected in the other parameters ($p>0.05$). Total protein level was higher in the High Tick group than in the Low Tick group ($p<0.002$). Total bilirubin level was statistically different and higher in the High Tick group compared to the other groups ($p<0.002$). Although the BUN level was numerically different between the dogs with tick paralysis, no statistical difference was determined. However, the BUN level was statistically different and higher in the High Tick group than in the Healthy group ($p<0.035$). Serum biochemistry profiling results are presented in Table 2.

ROC Analysis Results

As a result of the ROC analysis of the dogs with fewer than 5 ticks detected, it was determined that only respiratory rate, one of the clinical examination parameters, had diagnostic efficacy in making the clinical distinction of the disease (AUC=0.863; excellent diagnostic performance). ROC analysis and ROC curves of the clinical examination parameters investigated in the present study as a result of the grouping based on the number of ticks detected on the body (Low Tick Group, number of ticks < 5) are presented in Table 3 and Fig. 1.

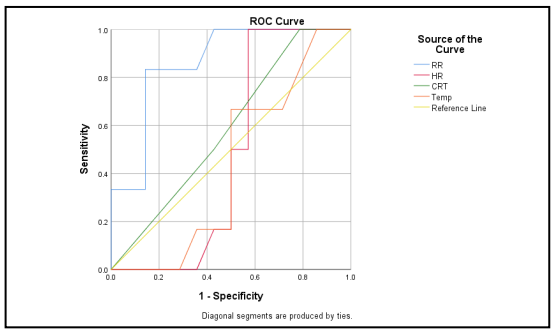

Figure 1. ROC curves of clinical examination parameters of the tick paralyzed dogs in the Low Tick group. RR: Respiratory rate, HR: Heart rate, CRT: Capillary refill time, Temp: Temperature

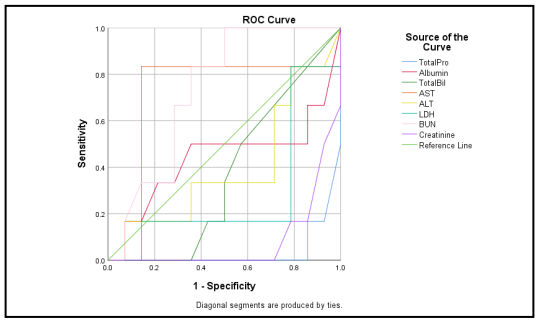

Figure 2. ROC curves of serum biochemistry parameters of the tick paralyzed dogs in the Low Tick group. TotalPro: Total protein, TotalBil: Total bilirubin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, BUN: Blood urea nitrogen

As a result of the ROC analysis of serum biochemistry parameters of the dogs with fewer than 5 ticks detected, it was determined that the diagnostic efficacies of aspartate aminotransferase (AST) and BUN levels for clinical differentiation of the disease were acceptable (AUC=0.714 and AUC=0.732; acceptable diagnostic performance). ROC analysis and ROC curves of the serum biochemistry parameters investigated in the present study as a result of grouping based on the number of ticks detected on the body (Low Tick Group, number of ticks<5) are presented in Table 4 and Figure 2. As a result of the ROC analysis of the dogs with more than 5 ticks detected, it was determined that respiratory rate had acceptable (AUC=0.665) and heart rate and body temperature had outstanding (AUC=0.984, AUC=0.973) diagnostic efficacies in clinically distinguishing the disease among the clinical examination parameters. ROC analysis and ROC curves of the clinical examination parameters investigated in the present study as a result of the grouping based on the number of ticks detected on the body (High Tick Group, number of ticks>5) are presented in Table 5 and Figure 3.

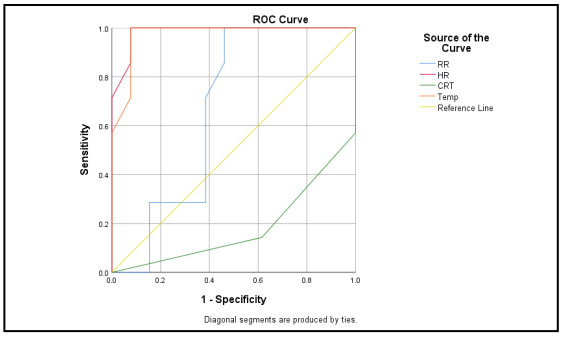

Figure 3. ROC curves of clinical examination parameters of the tick paralyzed dogs in the High Tick group.

As a result of the ROC analysis of serum biochemistry parameters of the dogs with more than 5 ticks detected, it was determined that the diagnostic efficacies of total protein, total bilirubin and creatinine levels in clinical differentiation of the disease were excellent (AUC=0.874, AUC=0.879 and AUC=0.852). The diagnostic efficacy of BUN level in distinguishing the disease was acceptable (AUC=0.654). ROC analysis and ROC curves of the serum biochemistry parameters investigated in the present study as a result of grouping based on the number of ticks detected on the body (High Tick Group, number of ticks>5) are presented in Table 6 and Figure 4.

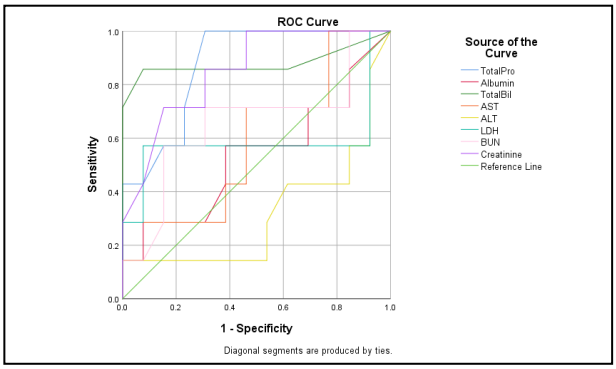

Figure 4. ROC curves of serum biochemistry parameters of the tick paralyzed dogs in the High Tick group

As a result of the Spearman correlation analysis of the parameters investigated within the scope of the present study, a strong negative correlation (Lim, 2020) was determined between albumin level and body weight (r=-0.713) and albumin and BSA (r=-0.713). A very strong positive correlation (Lim, 2020) was determined between body weight and BSA (r=1.000). The result of the Spearman correlation analysis is presented in Supplementary file.

Discussion

In the present study, routine examination parameters were comparatively evaluated and the diagnostic efficacies of these analytes were investigated for tick paralysis. An increase in tick burden was associated with corresponding elevations in pulse rate and body temperature, as evidenced by physical examination findings. Additionally, elevated levels of BUN and total bilirubin were detected in serum biochemistry parameters, potentially attributable to the rate and volume of toxin secretion by the ticks. As a result of the ROC-based diagnostic performance analysis of the investigated parameters, it was observed that when the number of ticks was less than 5, the respiratory rate showed excellent, while BUN and AST evaluation showed acceptable diagnostic performance. As the number of ticks increased, the diagnostic performance of respiratory rate, heart rate and body temperature increased. Among the serum biochemistry parameters, it was determined that total protein, total bilirubin and creatinine had excellent, and BUN had acceptable diagnostic performance. As a result, it was determined that the diagnostic performances of the investigated routine examination parameters increased as the number of ticks increased, contrary to previous reports (Padula, 2016). However, since these parameters have low specificity in atypical cases where ticks cannot be detected (Gülersoy and Günal, 2022), it was concluded that their diagnostic use is controversial and specific diagnostic tests should be developed by combining them with other parameters.

The clinical signs of tick paralysis, such as ascending flaccid paralysis, typically emerge 5-9 days post-tick attachment. The progression starts with hind limb weakness and advances to quadriplegia within the subsequent 24-72 hours. Failure to remove ticks may lead to respiratory paralysis and subsequent death within 1-5 days. However, removing all ticks generally leads to improvement in clinical manifestations within 24 hours and complete recovery within 72 hours (Atwell et al., 2001; Soulsby, 2005). According to previous reports, tick removal and supportive care alone can often lead to a full recovery in cases of tick paralysis when diagnosed early and treated promptly. On the other hand, if neglected, it can worsen and lead to respiratory failure and even death. These characteristic timeframes were also observed in the present study, where the diagnosis was confirmed using the *ex juvantibus* approach—clinical recovery following tick removal and acaricidal treatment. This underscores the critical importance of prompt tick identification and removal. While supportive care alone may lead to recovery, the reliance on *ex juvantibus* diagnosis in clinical settings where no specific test exists further highlights the need for objective diagnostic criteria (McGee, 2012).

Although physical examination findings provide limited information compared to specific tests, they can be useful in increasing the clinical suspicion of diseases (Ilkiw and Turner, 1987). In dogs with tick paralysis, in addition to neurological findings, clinical examinations reveal signs such as fever, altered mental status, and increased respiratory and heart rates (Atwell et al., 2001). In severe cases of tick paralysis, the main clinical anomaly and most likely the primary cause of mortality is respiratory failure (Shaffran, 2008). Adverse effects on the cardiovascular system include elevated blood pressure and heart rate, arrhythmias, and coagulopathies (Eyer and Zilker, 2007). The respiratory rate of the tick-paralyzed dogs in the present study was higher than that of the healthy dogs ($p<0.0001$), but no statistical difference was detected between the Low Tick and High Tick groups. The heart rate of the dogs in the High Tick group was higher and statistically different from the dogs in the other groups ($p<0.000$). Capillary refill time was shorter in the High Tick group than in the other groups ($p<0.018$). While rectal body temperature was not statistically different between the Low Tick group and healthy group, it was higher in the High Tick group than the others ($p<0.000$). In addition, in the ROC-based diagnostic performance analysis of the clinical examination parameters of dogs with fewer than 5 ticks detected, it was determined that only respiratory rate had a diagnostic efficacy in the diagnosis of the disease and this was excellent (AUC=0.863). When the number of ticks detected was more than 5, heart rate and body temperature were among the parameters with diagnostic efficacy (AUC=0.984, AUC=0.973; outstanding diagnostic performance) and the efficacy of respiratory rate decreased (acceptable diagnostic performance; AUC=0.665). In the present study, abnormal cardiovascular, respiratory, catabolic and metabolic rates, which are exacerbated by the increasing tick numbers, may be related to tick neurotoxin, which increases in conjunction with the increase in tick numbers (Padula, 2016). In addition, body temperature, whose diagnostic performance improves with increasing tick numbers, may be associated with intense skeletal muscle hypermetabolic reaction, which correlates with higher tick neurotoxin levels (Barrett and Topol, 2016).

The perception that physical examination holds limited value has become prevalent, despite ample literature data showcasing the diagnostic utility of many of its components. Physical examination remains essential to diagnosis, with its omission risking clinical errors. Despite criticism over its variability and calls for more sensitive tests like serum biochemistry, it remains fundamental to patient care and the therapeutic relationship (Nathwani et al., 2005). For this reason, not only the respiratory rate, previously highlighted as a prominent physical examination finding during the initial triage assessment of dogs with tick paralysis (Fadia et al., 2019; Shaffran, 2008), but also heart rate and body temperature can offer valuable clinical insights, enhancing the diagnostic suspicion index for the disease. Neuroimaging in tick paralysis typically reveals no abnormalities unless an

undetected tick is visualized. Cerebrospinal fluid and leukocyte (WBC) counts are usually normal. In cases with respiratory compromise, blood gas and pulmonary function tests assist in evaluating the need for intubation. Electromyography often shows reduced compound muscle action potential amplitudes, while repetitive nerve stimulation results are generally normal (McGee, 2012). Previously, hematochemical analysis findings in dogs with tick paralysis have revealed increases in hemoglobin concentration, red blood cell (RBC) and WBC counts, elevated BUN and creatinine concentrations attributed to dehydration, and elevated glucose and cholesterol levels due to sympathetic stimulation of the adrenal medulla (Shaffran, 2008). These previous data may highlight that the efficacies of routine tests including CBC and serum biochemistry should be investigated comparatively (Padula et al., 2020). In the serum biochemistry profiling of the present study, the total protein level exhibited a significant increase in the High Tick group compared to the Low Tick group ($p=0.002$). Moreover, the total bilirubin level was found to be statistically higher in the High Tick group than in the other groups ($p<0.002$). Additionally, BUN levels showed a significant elevation in the High Tick group compared to the Healthy group ($p<0.035$). Furthermore, upon conducting ROC based diagnostic performance analysis of serum biochemistry parameters in the dogs with fewer than 5 ticks detected, it was determined that the diagnostic efficacy of AST and BUN levels in clinically distinguishing the disease was deemed acceptable (AUC=0.714 and AUC=0.732, respectively; acceptable diagnostic performance). As a result of the ROC based diagnostic performance analysis of serum biochemistry parameters of the dogs with more than 5 ticks detected, it was determined that the diagnostic efficacies of total protein, total bilirubin and creatinine levels in clinical differentiation of the disease were excellent (AUC=0.874, AUC=0.879 and AUC=0.852, respectively). The diagnostic efficacy of BUN level in distinguishing the disease was acceptable (AUC=0.654). It was previously thought that in situations of tick paralysis, there would be minimal changes to biochemical parameters. However, given that these indices have not been previously measured, it was deemed necessary to conduct a detailed investigation to ascertain any potential changes. While many of these changes may be difficult to interpret in isolation, taken as a whole, they might represent the adrenal medulla's biochemical reaction to sympathetic stimulation. This stimulation can lead to the release of adrenaline and nor-adrenaline or the release of adrenocorticotrophic hormone, subsequently stimulating the adrenal cortex to secrete corticosteroids (Shaffran, 2008). None of these alterations, it was noted, is specific to tick paralysis and don't signify the severity or prognosis (Atwell et al., 2001; Padula et al., 2020). In the present study, findings such as elevated BUN and creatinine levels observed due to dehydration were consistent with previous reports (Atwell et al., 2001; Shaffran, 2008). Additionally, increased AST levels could be associated with recumbency-related rhabdomyolysis. It was reported that increased ALT levels could also be associated with rhabdomyolysis since it was reported (Guiloff et al., 1980) that along with AST, ALT levels also increase in case of acute muscular damage. Dehydration might be anticipated in animals exhibiting signs of reduced water and food intake, accompanied by vomiting. However, it is crucial to emphasize that if dehydration occurs in tick paralysis cases, it tends to be mild (Shaffran, 2008). Thus, the higher total protein level of serum samples of the dogs with more than 5 ticks on their bodies in the present study may be associated with dehydration and prolonged recumbency period (Lim, 2020). Although there was no statistical difference, the AST level, which has acceptable diagnostic performance in the diagnosis of the disease, was interpreted as a result of muscle damage (Guo et al., 2021). Furthermore, creatinine has been identified as an independent predictive biomarker for muscle damage; as a sign of malnutrition, it rises in the early stages of the damage and falls in the later stages (Boffey and Paterson, 1973). Although the creatinine level was not statistically different in the intergroup comparison similar to the AST level, its efficacy in the diagnosis of the disease was excellent and this finding was associated with muscle damage and dehydration (Atwell et al., 2001; Padula et al., 2020; Shaffran, 2008). Findings related to muscle damage were associated with tick toxin caused muscle damage by interference with cellular energy metabolic pathways (Terrault et al., 2018). In the present study, ALT concentrations of tick paralyzed dogs, which were statistically insignificant in the intergroup comparison but numerically lower than the healthy dogs, may be associated with higher mortality prediction (Bradbury, 2017). Nevertheless, studies on survival probability in tick paralyzed dogs may further elucidate the efficacies of these parameters.

Hyperbilirubinemia refers to the excessive accumulation of bilirubin in the bloodstream, typically stemming from impaired bilirubin metabolism. Bilirubin, a byproduct of hemoglobin breakdown, results from the degradation of red blood cells by the mononuclear phagocytic system. Initially, water-insoluble, unconjugated bilirubin binds to albumin for transport to the liver, where it undergoes conjugation via glucuronidation within hepatocytes. Subsequently, bilirubin glucuronides are actively transported into bile canaliculi, stored in the gall bladder, and eventually excreted primarily in feces. Hyperbilirubinemia manifests as the accumulation of bilirubin pigment in the blood and tissues, leading to jaundice (icterus), and can be categorized as pre-hepatic, hepatic, or post-hepatic (Bhutani and Johnson, 2009). During the mid-twentieth century, the impact of bilirubin on the central nervous system (CNS) remained largely unexplored. Physicians noted notable motor deficits in neonates with severe hyperbilirubinemia, prompting investigations into how bilirubin affected motor systems in the developing brain. The CNS was implicated, suggesting that bilirubin might cross the blood-brain barrier (BBB) and damage the neurons linked to movement (Terrault et al., 2018). The globus pallidus and subthalamic nuclei are two brain locations where bilirubin has been shown to have a neurotoxic effect. This effect causes motor-related sequelae that can range from severe movement abnormalities to loss of coordination (Lim, 2020; Shapiro, 2012). The higher total

bilirubin level of the High Tick group may be related to reduced hepatocyte function, intrahepatic cholestasis and accumulation of conjugated and unconjugated bilirubin (Bhutani and Johnson, 2009). At physiological levels, bilirubin plays a crucial role in brain function as a potent antioxidant, protecting neural tissues from oxidative damage by neutralizing reactive oxygen species (ROS). Furthermore, it contributes to immune regulation by modulating microglial activation, cytokine release, complement system activity, Fc receptor function, and MHC II expression, thereby reducing the risk of inflammatory and autoimmune reactions in the CNS (Kaur et al., 2025). Given these roles, monitoring total bilirubin levels—which exhibit excellent diagnostic performance based on the present ROC analysis—may help predict CNS damage in dogs with tick paralysis, clarify motor pathway dysfunction, and aid in prognosis assessment.

The fact that the CBC results evaluated in the present study were used only as inclusion/exclusion criteria and selected parameters were evaluated within the context of serum biochemistry profiling can be considered a limitation. A key limitation of the present study is the relatively small number of animals included, which may restrict the generalizability of the findings. Therefore, future studies should include a larger sample size and incorporate a broader range of clinical and laboratory parameters for a more comprehensive evaluation.

Conclusions

In this study assessing the diagnostic efficacy of routine physical and selected serum biochemistry parameters in tick paralysis, notable considerations emerged regarding heart rate, body temperature, and respiratory rate in the assessment of clinical manifestations. Furthermore, total protein, total bilirubin, and creatinine levels demonstrated excellent diagnostic performance, while BUN levels exhibited acceptable performance. Consequently, the findings suggest that heightened exposure to tick neurotoxins, associated with an increased tick burden, may exacerbate the disease advanced stages, thereby amplifying the diagnostic efficacy of the parameters above through tissue and organ damage.

Conflict of Interest

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

Similarity Rate

We declare that the similarity rate of the article is 17% as stated in the report uploaded to the system.

Ethical Approval

This study was approved by the Harran University Animal Experiments Local Ethics Committee (09.05.2022, 2021/003 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

Author Contributions

Motivation / Concept: EG, CB
Design: EG, CB
Control/Supervision: EG, CB
Data Collection and / or Processing: EG, CB, AŞ, İG, EK
Analysis and / or Interpretation: EG, CB, AŞ, İG, EK
Literature Review: EG, CB, AŞ, İG, EK
Writing the Article: EG, CB, AŞ, İG, EK
Critical Review: EG, CB, AŞ, İG , EK

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