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Haemato-biochemical alterations of clinical leptospirosis in dogs#

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Abstract

Leptospirosis is a widespread zoonotic disease of global importance caused by pathogenic species of Leptospira, affecting a wide range of animals including dogs. In dogs, leptospirosis remains a significant infectious disease characterised by multisystemic involvement posing both veterinary and public health challenges. Blood and serum samples were collected from a total of 65 dogs showing clinical signs suggestive of leptospirosis—including myalgia, haematuria, icterus, and pyrexia. Out of 65 dogs, 21 dogs confirmed as positive for leptospirosis on real-time PCR. Among the positive animals, haematological findings showed leucocytosis, neutrophilia, monocytosis, lymphocytosis, thrombocytopenia, along with decreased haemoglobin levels, haematocrit and total RBC count. Serum biochemical evaluation indicated increased levels of alkaline phosphatase, serum albumin, and both direct and indirect bilirubin, as well as elevated serum creatinine and blood urea nitrogen. These findings highlight that in association with clinical presentation and epidemiological information, haemato-biochemical alterations can serve as tool for tentative diagnosis as well as prognosis indicator.

Keywords: *Leptospirosis, haemato-biochemical, bilirubin, creatinine, real time PCR*

Leptospirosis is a widespread, fatal and febrile illness caused by pathogenic *Leptospira* species, capable of infecting diverse domestic and wild animals (Sykes et al., 2023). Infection often results in multisystemic involvement, primarily targeting the liver and kidneys. Owing to its nonspecific clinical signs, laboratory evaluation remains a critical component for confirming diagnosis and assessing the extent of organ dysfunction (Howard, 2024).

Although the microscopic agglutination test is useful in confirming leptospiral infection, they lack the ability to discriminate between prior exposure and active disease. By using the dark field microscopy detects organisms directly in clinical samples, but it has low specificity and sensitivity. Conversely, antigen detection techniques such as conventional PCR and real-time PCR enable precise confirmation of infection even before the onset of detectable antibody production. Nevertheless, these advanced molecular diagnostic approaches necessitate sophisticated laboratory infrastructure and technically proficient personnel for accurate execution and interpretation (De Brito et al., 2018). Therefore, haematological

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and serum biochemical parameters provide valuable insight into the host response and the pathophysiological changes associated with leptospiral infection. These findings can offer a tentative diagnosis, enabling clinicians to initiate timely and appropriate treatment interventions (Knopfler et al., 2017; Wickramasinghe et al., 2025).

Furthermore, this present study strengthens existing knowledge by highlighting the diagnostic relevance of routine haematological and biochemical evaluations in leptospirosis, serving as practical complements to advanced molecular methods. The findings offer translational value for field veterinarians and diagnostic laboratories, facilitating early case recognition and improved therapeutic management of canine leptospirosis.

Materials and methods

Sixty-five dogs presented to teaching veterinary clinical complex (TVCC), Mannuthy, Thrissur, Kerala with clinical signs *viz*; pyrexia, jaundice, haematuria, episcleral congestion and myalgia formed the material for study. This study was conducted for a period of one year from June 2024 to July 2025.

The blood was collected from the cephalic vein of suspected dogs and transferred to EDTA and clot activator vials, respectively. The blood was subjected to the haematological evaluation using an automated haematology analyser, ORPHEE Mythic 5 Vet PRO, based on the principles of multi-angle laser scattering flow cytometry, electrical impedance, and colourimetry. The five-part differential count of white blood cells (WBCs) was determined using multi-angle laser scattering flow cytometry. Red blood cells (RBCs) and platelets (PLTs) were quantified by impedance technology, while haemoglobin (HGB) concentration was estimated by the colourimetric method. Furthermore, DNA extracted from the blood was subjected to real-time PCR for identification of leptospires by targeting the gene *lipl32* (Joseph et al. 2018). Serum was collected from the clot activator vials and subjected to serum biochemical analysis using RMS Analytica 705i semi-automated biochemistry analyser, which works on

Table 1. Statistical analysed data of haematological parameters of confirmed leptospirosis group and clinically healthy group

Haematological parameters	Confirmed leptospirosis group (n=21) (Mean value ± SE)	Clinically healthy group (n=12) (Mean value ± SE)	t - value	p - value
Total leucocyte count (10^3/µL)	37.43 ± 4.50	11.02 ± 0.58	5.811	<0.001**
Neutrophils	31.48 ± 4.83	8.04 ± 0.46	4.830	<0.001**
Monocytes	2.06 ± 0.48	0.72 ± 0.07	2.770	0.012*
Lymphocytes	4.52 ± 0.89	1.52 ± 0.14	3.334	0.003*
Total Red blood cells count (10^6/µLl)	4.70 ± 0.36	6.05 ± 0.13	3.446	0.012*
Haemoglobin (gm/dL)	10.28 ± 0.76	14.59 ± 0.42	4.903	<0.001**
Haematocrit(%)	30.46 ± 2.11	41.52 ± 0.94	4.765	<0.001**
Platelets (10^3/µL)	186 ± 33.12	347.83 ± 29.8	3.125	0.004*

**- Highly significant (p<0.01), *- Significant (p<0.05)

Table:2 Statistical analysed data of serum biochemical parameters of confirmed leptospirosis group and clinically healthy group

Serum biochemical parameters	Confirmed leptospirosis group (n=21) (Mean value ± SE)	Clinically healthy group (n=12) (Mean value ± SE)	t - value	p - value
Alanine aminotransferase (IU/L)	55.32 ± 17.56	38.61 ± 4.97	0.665	0.510^{ns}
Alkaline phosphatase (IU/L)	192.78 ± 32.74	42.32 ± 9.95	4.396	<0.001**
Serum creatinine (mg/dL)	4.79 ± 1.15	0.93 ± 0.07	3.345	0.003*
Blood urea nitrogen (mg/dL)	35.54 ± 8.29	14.14 ± 3.14	2.414	0.022*
Serum albumin (g/dL)	2.15 ± 0.11	2.83 ± 0.08	3.883	<0.001**
Total protein (g/dL)	5.24 ± 0.24	5.40 ± 0.30	0.502	0.619^{ns}
Direct bilirubin (mg/dL)	1.41 ± 0.29	0.11 ± 0.02	4.915	<0.001**
Indirect bilirubin (mg/dL)	2.99 ± 1.27	0.07 ± 0.01	2.297	0.031*

Superscript 'ns' indicate mean value does not differ significantly at 0.05 level (p>0.05).

** - Highly significant (p<0.01), * - Significant (p<0.05)..

the principle of absorbance photometry. The following serum biochemical parameters were analysed, namely, alkaline phosphatase (IU/L), alanine aminotransferase (IU/L), albumin (g/dL), total protein (g/dL), total bilirubin (mg/dL), direct and indirect bilirubin (mg/dL), creatinine (mg/dL), blood urea nitrogen (mg/dL).

Based on the results of the real-time PCR assay, the study population was stratified into two groups: dogs with confirmed leptospirosis and those negative for leptospirosis. A comparative evaluation of haematological and serum biochemical parameters was subsequently conducted between the confirmed leptospirosis group and the clinically healthy group. Statistical analyses were performed using SPSS software (version 24), employing the independent *t*-test to determine the significance of differences observed.

Results and discussion

Among the 65 dogs examined, 21 were confirmed positive for leptospirosis using real-time polymerase chain reaction (PCR) with Ct values ranging from 29.86 to 33.56. A Ct value of 32.0 or lower was considered indicative of a positive reaction for leptospirosis (Rahman et al., 2023). Based on the standard curve established from the reference serovar *Leptospira interrogans* serovar Autumnalis, a Ct threshold of 33.88 or below was also classified as a positive result. Melt curve analysis of selected samples, which exhibited distinct single peaks, is illustrated in Fig. 1. The comparative analysis of haematological and serum biochemical parameters between the confirmed leptospirosis cases (n = 21) and clinically healthy controls (n = 12) is presented in Tables 1 and 2, respectively.

The most notable changes (significant at $p<0.01$) were leucocytosis, neutrophilia, reduced haemoglobin and haematocrit levels, elevated alkaline phosphatase, direct bilirubin and marked hypoalbuminemia. Other significant findings ($p<0.05$) were monocytosis, lymphocytosis, a decrease in total RBC count, thrombocytopenia, elevated indirect bilirubin, blood urea nitrogen, and serum creatinine. Importantly, alanine aminotransferase and total protein were statistically non-significant.

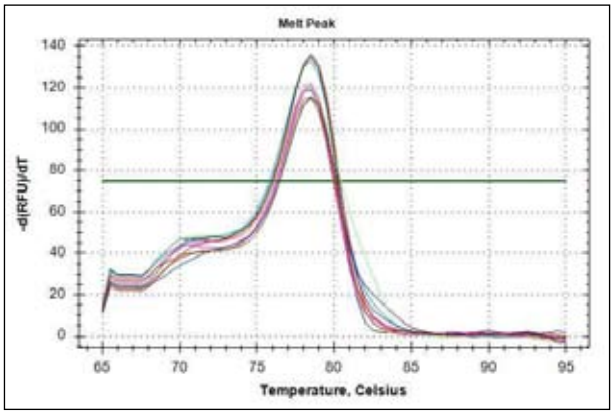

Fig.1. Melt curve analysis of representative positive samples indicating single peaks

A marked leucocytosis was observed which was in consistent with the observations of Tansakul et al. (2024), who reported leucocytosis in 26.2% of affected dogs and attributed it to the upregulation of inflammatory mediators and activation of immune cells during the acute phase of bacterial infection. Marked neutrophilia was observed in the present study, which aligns with the findings of Papa and Kotrotsiou (2015) and Alikhani et al. (2022). These authors explained that *Leptospira* species possess microbe-associated molecular patterns (MAMPs), including lipopolysaccharides and outer membrane proteins, that are recognised by host pattern recognition receptors (PRRs). This recognition triggers a robust intracellular signalling cascade, leading to the initiation of an acute inflammatory response. During this phase, neutrophils act as the primary effector cells, playing a pivotal role in the rapid elimination of pathogens from the circulation. The occurrence of monocytosis parallels the reports of Novak et al. (2022) and Sykes et al. (2023), suggesting an intensified mononuclear response as part of the host's defence mechanism.

A significant elevation in lymphocyte counts was observed in the present study, which contrasts with the findings of Duran-Galea et al. (2024), who reported lymphocytopenia as a consistent haematological feature in dogs affected by leptospirosis rather than lymphocytosis. The lymphocytosis detected in the infected animals of this study may indicate a recovery or convalescent phase, or reflect individual variations in immune responsiveness, as suggested by Sykes et al. (2023). Furthermore, Barry et al. (2006) proposed that the proliferation of atypical lymphocytes, particularly gamma-delta lymphocytes, could contribute to the observed lymphocytosis in affected dogs.

A noticeable decrease in total erythrocyte count, haemoglobin concentration, and haematocrit reflecting the haemolytic nature of leptospiral anaemia. Leptospiral toxins, particularly sphingomyelinases, possess potent pore-forming activity that compromises the integrity of erythrocyte membranes, ultimately leading to their lysis and a decline in circulating red cells (Narayanavari et al., 2012). Following the onset of the humoral immune response, the majority of leptospires are cleared from the bloodstream; however, a subset of organisms evades immune elimination and localises within the renal tubules. The persistence of these organisms induces injury to peritubular interstitial fibroblasts, impairing the synthesis and secretion of erythropoietin. The resultant deficiency in erythropoietin-mediated signalling diminishes bone marrow stimulation and suppresses erythroid progenitor activity, further exacerbating anaemia (Bovens et al., 2014; Schuller et al., 2015).

Significant thrombocytopaenia was observed, which was echoing the observations of Schuller et al. (2015), and they explained that Leptospira-induced vascular endothelial damage plays a central role in this condition. The damaged endothelial lining expresses tissue factor (Factor III), initiating the extrinsic coagulation cascade, while leptospiral toxins simultaneously trigger endothelial activation and the release of platelet-activating factors. These events collectively accelerate platelet utilization and clearance from circulation. Moreover, Vieira and Nascimento (2020) proposed that immune-mediated platelet destruction and direct cytotoxic effects of *Leptospira* further intensify the thrombocytopaenic response.

While alanine aminotransferase (ALT) is an intracellular enzyme released into circulation as a consequence of hepatocellular destruction (Umesha et al., 2014), the observed difference in the present study was not statistically significant. The absence of a marked increase in ALT suggests that the hepatic involvement in these cases was predominantly inflammatory or cholestatic, rather than indicative of extensive hepatocellular necrosis. These findings were in accordance with the findings of Sulistiawati et al. (2022), who reported elevated alkaline phosphatase activity accompanied by normal ALT levels in serum samples from ten dogs affected by leptospirosis.

A significant elevation of alkaline phosphatase (ALP) was suggesting the presence of cholestatic hepatitis or hepatic stress, likely associated with injury or inflammation caused by leptospiral endotoxins. Inflammatory processes can lead to narrowing of the bile ducts, resulting in bile reflux into the hepatic parenchyma, which in turn provokes hepatocellular injury and stimulates the release of ALP into the bloodstream (Klosowski & Bohn, 2023).

Hypoalbuminaemia was prominently observed in leptospirosis-positive animals, which may be attributed to hepatocellular dysfunction leading to decreased albumin synthesis, or to increased renal loss resulting from nephritic damage and subsequent leakage of albumin through compromised glomeruli (Lees & van Dongen, 2013). The absence of a significant difference in total protein levels is likely due to a compensatory rise in gamma globulins associated with the robust humoral immune response characteristic of leptospiral infection. As Hoskins (2004) noted, the elevation of serum antibodies can offset the decline in albumin concentration, thereby maintaining overall total protein levels within the normal range

Hyperbilirubinemia was corroborating with previous research of Abdullatheif et al. (2018), where they found hyperbilirubinemia in eleven confirmed cases and explained that predominance of the direct fraction was characteristic of cholestatic hepatitis induced by leptospirosis. Andrade et al. (2020), described that leptospiral-induced cholangitis which impedes normal bile excretion, it was reflected by elevated serum bilirubin levels.

Involvement of high levels of blood urea nitrogen in association with creatinine rise was suggestive of azotaemia related renal injury in leptospirosis affected dogs, consistent with the observations of Abdullatheif et al. (2018) and Tansakul et al. (2024). According to the Chou et al. (2023), leptospiral outer membrane protein, LipL32 directly binds to the host toll-like receptor-2 (TLR-2) expressed on renal tubular epithelial cells, it triggers inflammatory signalling pathways that lead to renal tubular injury, reflected clinically by elevated creatinine levels. Sykes et al. (2023) reported that reduced glomerular filtration rate (GFR) was attributed to the azotaemia, which might be due to the intrinsic renal injury and/or extrinsic factors like decreased water intake due to pyrexia and fluid loss from the body through vomiting.

Conclusion

Haematological and biochemical evaluations of canine leptospirosis revealed abnormalities consistent with its systemic nature and multi-organ involvement. Thrombocytopaenia, anaemia, and leucocytosis with neutrophilia indicated vascular injury and inflammatory responses. The thrombocytopaenia likely resulted from endothelial damage and increased platelet consumption, while the anaemia was attributed to haemolysis induced by leptospiral toxins and reduced erythropoietin production secondary to renal impairment. Biochemical alterations, including marked hyperbilirubinaemia, elevated alkaline phosphatase, and hypoalbuminaemia, reflected hepatic dysfunction, whereas increased creatinine and blood urea nitrogen concentrations confirmed renal involvement. Collectively, these haematological and biochemical parameters serve as valuable diagnostic indicators and markers of disease severity, facilitating early therapeutic intervention and improving the prognosis in affected dogs.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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