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Survival outcomes, low awareness, and the challenge of neglected leptospirosis in dogs

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ABSTRACT

Background: Leptospirosis is a globally neglected zoonotic disease with significant morbidity and mortality in dogs, particularly in resource-limited settings.

Aim: This study aimed to characterize prognostic factors and survival outcomes in dogs with suspected leptospirosis, emphasizing the potential underestimation of disease burden.

Methods: This retrospective study was conducted using medical records of dogs diagnosed with urinary *Leptospira* polymerase chain reaction (PCR).

Results: Urinary *Leptospira* PCR was positive in 22 dogs and negative in 62. Azotemia was present in approximately two-thirds of both groups, with no predictive value identified between PCR-positive and PCR-negative dogs. However, PCR-positive dogs exhibited significantly shorter survival times for both all-cause mortality (median 60 days, range: 8–601 days) and leptospirosis-related death (median 27 days, range: 8–67 days) compared to PCR-negative dogs (median 402 days, range: 7–812 days) ($p < 0.01$). The neutrophil-to-lymphocyte ratio (NLR) in-dependently predicted leptospirosis-related death (HR = 1.073, 95%CI: 1.02–1.13, $p = 0.01$), while the BUN-to-creatinine ratio predicted all-cause mortality (HR = 1.02, 95% CI: 1.003–1.03, $p = 0.02$).

Conclusion: Our findings underscore the severity of leptospirosis in older dogs, particularly those with azotemia or positive PCR results. NLR and BUN to creatinine ratios could be valuable tools for risk assessment and guiding treatment strategies in this vulnerable population.

Keywords: Dog, *Leptospira*, Leptospirosis, PCR, Prognosis.

Introduction

Leptospirosis, a highly prevalent zoonotic bacterial infection and potentially fatal disease in both human and animal populations (Bharti *et al.*, 2003), has a specific susceptibility in dogs (Sykes *et al.*, 2011). It is caused by the motile spirochetal bacterium of the *Leptospira* genus, with approximately 250 different pathogenic serovars (Goldstein, 2010; Costa *et al.*, 2015). A considerable number of isolates across various species exhibit clinical manifestations, such as serovars Copenhageni and Icterohaemorrhagiae, while the others remain unidentified (Bharti *et al.*, 2003; Sykes *et al.*, 2011). It is difficult to determine the primary serovars responsible for illnesses in dogs or humans due to the common reliance on serological data (which may not precisely indicate the specific serovar responsible for the infection) in many studies (Levett, 2003; Reagan and Sykes, 2019). According to current scientific understanding, it is believed that six serovars (Grippotyphosa, Bratislava, Canicola, Icterohaemorrhagiae, Autumnalis, and Pomona) are frequently observed as potentially pathogenic in dogs (Adin and Cowgill, 2000; Goldstein, 2010). These serovars exhibit notable similarities to the pathogenic *Leptospira* serovars that have been recently documented in Thailand, which are known to be prevalent in the local population (Tangkanakul *et al.*, 2005; Suwancharoen *et al.*, 2016; Altheimer *et al.*, 2020).

In dogs, leptospires can penetrate intact mucosal surfaces or skin abrasions, leading to bacteremia that can last for up to 10 days (Greenlee *et al.*, 2005). The

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bacteria in the blood can then attack the kidneys, liver, and other organs, while also being excreted through urine (Sykes *et al.*, 2011). In the first week of infection, antileptospiral IgM becomes detectable, and their levels increase significantly as the illness progresses. Subsequently, anti-leptospiral IgG can be observed approximately 2 weeks after infection (Hartman *et al.*, 1984). Understanding the timing of bacteremia, bacteriuria, and IgM/IgG antibody production, particularly their relationship to the illness timeline, can assist veterinarians in choosing the appropriate diagnostic test and timing of sample collection (Reagan and Sykes, 2019). While acute kidney failure is indeed a common finding (80%–100%) (Stoddard *et al.*, 2009; Sykes *et al.*, 2023), the zoonotic nature of leptospirosis can indirectly influence the diagnostic process. Veterinarians might prioritize ruling out other non-zoonotic threats before considering leptospirosis, leading to delayed diagnosis. Additionally, the disease can manifest in various ways beyond kidney failure, with non-specific clinical signs. Therefore, considering risk factors, consistent clinical presentations, and appropriate diagnostic tests which fall into two categories: direct bacteria detection [culture, dark field microscopy, or detection of bacterial DNA using polymerase chain reaction (PCR)] and antibody identification using the microscopic agglutination test (MAT) (Reagan and Sykes, 2019). In clinical practice, PCR is typically conducted on blood or urine samples. It is recommended to submit whole-blood samples within the initial 10 days of the illness and urine samples after the first week, which correspond to the bacteremic and bacteriuric phases of the disease, respectively. If the infection timeline is uncertain, submitting both samples can enhance sensitivity. Positive PCR results, in the presence of consistent clinical signs and clinicopathologic changes, indicate leptospirosis (Reagan and Sykes, 2019).

The challenges of leptospirosis diagnosis and management are further compounded in developing countries. Limited resources, lack of diagnostic infrastructure, and lower awareness of the disease among both pet owners and veterinary professionals can contribute to underdiagnosis and delayed treatment (Al-Orry *et al.*, 2016; Goarant, 2016). Additionally, in resource-limited settings, pet owners may perceive the cost of veterinary care as a barrier. This could lead to neglecting to seek treatment for their dogs, even when potentially serious symptoms are present. These factors collectively increase the risk of severe disease progression and the potential for zoonotic transmission to vulnerable populations, especially in tropical and subtropical areas where the disease is most common, particularly within developing countries (Al-Orry *et al.*, 2016; Goarant, 2016; Flores Somarriba *et al.*, 2017; Bradley and Lockaby, 2023).

This study aims to identify predictors of both urinary *Leptospira* PCR positivity and leptospirosis-related mortality in dogs. We utilized a comprehensive dataset encompassing medical records and urine samples from dogs with suspected leptospirosis. Employing this data, we systematically investigated potential risk factors associated with a positive urinary *Leptospira* PCR test. Furthermore, we seek to uncover distinct clinical and laboratory markers within the PCR-positive group that predict leptospirosis-related death. Additionally, we aim to assess owner awareness of leptospirosis transmission risks, as it is a zoonotic disease.

Materials and Methods

Recruitment obtained from urine sample submission and medical records

In this retrospective study, we meticulously curated submitted urine specimens for the traditional PCR targeting *Leptospira*, sourced from The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals (MoZWE), Faculty of Veterinary Science, Mahidol University, from May 2012 to March 2014. A comprehensive review of medical records from Prasu Arthorn Veterinary Teaching Hospital (which is affiliated with the esteemed Faculty of Veterinary Science, Mahidol University) was conducted, wherein laboratory findings pertaining to conventional PCR-based *Leptospira* diagnosis were scrutinized. Laboratory parameter references are listed in Supplementary Table 1. Pertinent information pertaining to the health status of each of the selected dogs was collected from the medical records or through phone calls to their respective owners, all before July 31, 2014.

Leptospirosis diagnostic testing (2020-2023)

We retrospectively analyzed data from the computerized database at Prasu Arthorn Veterinary Teaching Hospital. Our analysis focused on determining the total number of dogs presented to the hospital and the number of dogs that underwent leptospirosis diagnostic testing between January 1st, 2020, and December 31st, 2023.

DNA extraction and the PCR assay

Urine samples were obtained from dogs with varying sample volumes ranging from 5 to 10 ml, through catheterization or cystocentesis. The entire volume of collected urine from each individual underwent DNA extraction, centrifuged at 5,000 rpm for 5 minutes to remove cellular debris, and then at 12,000 rpm for 20 minutes to retrieve the pellet. DNA was extracted from the urine pellets utilizing the QIAamp® Viral RNA Mini Kit (QIAGEN, Hilden, Germany).

The detection of the *Leptospira* DNA in canine urine samples was performed using PCR primers specific to 16s rRNA, *ligA*, and *lipL32* (Stoddard *et al.*, 2009). The nucleotide sequences of the amplified products were validated through DNA sequencing.

Outcome definitions and survival analysis

Survival times were calculated from the date of positive PCR sample collection to the date of death. For the survival analysis, dogs that were still alive at the end of the study (July 2014) were censored. The primary

outcome was leptospirosis-related death, defined as a death directly attributed to leptospirosis complications while undergoing active treatment and monitoring. Cases where a positive PCR test preceded death, but the death was attributed to a separate, unrelated illness (as determined during subsequent veterinary care), were categorized as non-leptospirosis deaths. Mortality in the urinary PCR-negative group was also categorized as non-leptospirosis-related death.

Statistical analysis

A statistical software program (SPSS 18.0 for Windows, Chicago, IL, USA) was used for all statistical analyses. The threshold for statistical significance was set at $p < 0.05$. Continuous variables are presented as median values with interquartile ranges, while categorical variables are presented as frequencies and percentages. Continuous and ordinal variables were compared between groups using the Mann–Whitney U test, whereas the chi-square test was used for categorical variables.

To identify potential risk factors for *Leptospira* PCR positivity, a univariable logistic regression approach was employed. Odds ratios and 95% confidence intervals (CIs) were calculated. Variables with *p*-values of < 0.05 in the univariable analysis were included in a backward multivariable logistic regression model to determine the most likely predictive risk factors for leptospirosis in the study. The model fit was assessed using the Hosmer-Lemeshow test.

Kaplan-Meier analysis was used to generate survival curves and estimate survival probabilities over time. Due to a high proportion of censoring among PCR-positive dogs with non-leptospirosis-related deaths, Kaplan-Meier curves were analyzed with censoring removed. This provided a more focused assessment of the direct impact of leptospirosis on survival. The log-rank test was used to compare survival distributions between groups.

For the survival analysis of urinary *Leptospira* PCR positivity, the potential risk factors were entered into univariable Cox proportional hazard models to determine whether they were associated with survival, considering leptospirosis mortality. Cox proportional hazards analysis was performed to address the censoring issue. The same approach was employed to investigate all-cause mortality of both urinary PCR-positive and PCR-negative groups, ensuring analytical consistency. Variables with $p < 0.05$ in the univariable analysis were included in a manual backward-selection stepwise multivariable Cox proportional hazard analysis. Hazard ratios and 95% CIs were calculated from Cox proportional hazard analyses.

Ethical approval

The Animal Care and Use Committee of the Faculty of Veterinary Science, Mahidol University, Thailand, asserted that no authorization was necessitated for the undertaking of this study. The requirement for informed consent was waived due to the retrospective nature of the study.

Results

Urine sample submission and medical records

Eighty-four urine samples were included in the study, all of which were submitted to the MOZWE laboratory for conventional PCR analysis to detect *Leptospira* DNA. Among these samples, 22 (26.2%) were PCR-positive and 62 (73.8%) were PCR-negative. The detailed demographic information, physical examination findings, and laboratory results for hematology and serum biochemistry were collected on the same date of urine sampling for the *Leptospira* PCR analysis. The data collected, along with the health status and mortality outcomes of the *Leptospira* PCR-positive group and the *Leptospira* PCR-negative group were summarized and compared in Table 1. Overall, baseline variables were comparable between the two groups. However, the was a significant difference in leptospirosis death ($p < 0.01$). Additionally, plasma protein levels were higher in the PCR-negative group ($p = 0.048$). Clinical characteristics, concurrent diagnoses, and type of antibiotic prescribed in dogs categorized by their *Leptospira* PCR test results were summarized in Table 2. Urine samples were collected from dogs on antibiotics, encompassing those with ongoing or pre-existing treatment. Clinical findings observed in each group during physical examination are summarized in Supplementary Table 2. Due to the difficulty in assessing severity, formal comparisons between groups were not conducted.

Owner awareness of leptospirosis transmission

We assessed owner awareness of leptospirosis transmission through a telephone questionnaire of 84 dogs' owners. Thirty seven percent (36.9%) of dog owners correctly identified that leptospirosis can infect dogs. About half of the owners (51.2%) recognized the potential for animal-to-human transmission of leptospirosis. Interestingly, we compared awareness levels between owners of dogs with positive and negative PCR results for leptospirosis. Owners of dogs testing negative for leptospirosis exhibited a significantly lower awareness (56.4%) of the zoonotic risk compared to owners of dogs with positive PCR results (27.3%). This difference was statistically significant ($p = 0.02$).

Leptospirosis diagnostic testing (2020–2023)

During the period 2020–2023, a total of 33,304, 34,809, 34,982, and 38,464 dogs were presented at Prasu Arthorn Veterinary Teaching Hospital in each respective year. Of the dogs that underwent leptospirosis diagnostic testing using urinary PCR (43, 85, 62, and 64 in each respective year), 5, 6, 2, and 3 samples were positive. This corresponds to positivity percentages of 11.63%, 7.06%, 3.23%, and 4.69% across this period. No blood PCR or MAT tests were submitted.

Table 1. Baseline characteristics of dogs tested for urinary *Leptospira* polymerase chain reaction (PCR).

		***Leptospira* PCR**		
Factor	**Variable**	**Positive *n* = 22**	**Negative *n* = 62**	***p*-value**
Dog characteristics	Age (years)	9.5 (5.3–12.3)	8 (5.1–10)	0.10
	Sex (M/F) (%)	11/11 (50/50)	38/24 (61.29/38.71)	0.36
	Breed (pure/mixed) (%)	11/11 (50/50)	33/29 (62.9/37.1)	0.80
	Desexed (Yes/No) (%)	7/15 (31.82/68.18)	14/48 (22.58/77.42)	0.39
Physical examination variable	General appearance (alert & responsive/depressed) (%)	7/15 (31.82/68.18)	15/47 (24.19/75.81)	0.22
	Body weight (kg)	15.3 (9.2–27.2)	16.8 (8.8–22.3)	0.54
	Heart rate (beats per minute)	120 (108–132)	120 (110–137)	0.51
	Mucous membrane (pink/yellow) (%)	17/5 (77.27/22.73)	41/21 (66.13/33.87)	0.33
Hematology & biochemistry	WBC (10^3/µl)	16.15 (8.28–21.28)	17.85 (9.57–31.15)	0.25
	Monocytes (10^3/µl)	0.30 (0.00–0.77)	0.49 (0.11–1.64)	0.14
	Neutrophils (10^3/µl)	12.18 (7.25–17.30)	15.24 (8.06–25.57)	0.25
	Lymphocytes (10^3/µl)	1.22 (0.72–3.02)	1.52 (0.87–3.09)	0.56
Hematology & biochemistry	Basosinophils (10^3/µl)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.40
	Band neutrophils (10^3/µl)	0.00 (0.00–0.03)	0.00 (0.00–0.03)	0.90
	NLR	6.3 (4.4–18.1)	7.7 (5.5–15.0)	0.52
	Erythrocytes (10^6/µl)	4.4 (3.1–5.5)	4.7 (3–5.7)	0.79
	Hemoglobin (g/dl)	8.8 (7–12.3)	10.6 (7.5–13.4)	0.46
	PCV (%)	27 (20–35)	31 (23–39)	0.29
	MCV (fl)	66.4 (61.1–69.6)	67.6 (65.7–71.2)	0.09
	MCH (pg)	22.2 (21.3–23.7)	23 (21.5–24.4)	0.19
	MCHC (g/dl)	33.9 (31.8–34.9)	33.5 (31.8–34.9)	0.58
	Platelets (10^3/µl)	171 (105–209)	114 (63–222)	0.22
	RDW (%)	18.0 (16.6–19.7)	16.5 (15.2–19.6)	0.11
	Plasma protein (g/dl)	8.9 (8.3–10.0)	9.6 (8.8–11)	0.045
	ALP (U/l)	204 (82–372)	268 (120–579)	0.20
	ALT (U/l)	80 (59–110)	100 (49–268)	0.40
	BUN (mg/dl)	80 (28–130)	92 (39–134)	0.45
	Creatinine (mg/dl)	2.5 (1.1–6.3)	3.4 (1.2–7.7)	0.52
	BUN to creatinine ratio	24.3 (18.1–31.5)	23.1 (15.6–32.6)	0.78
	Platelet smear (decreased/adequate/ increased) (%)	9/8/2 (47.37/42.1/10.53)	37/21/2 (61.67/35/3.33)	0.34
Treatment	Antibiotic (Yes/No) (%)	20/2 (90.9/9.1)	47/15 (75.8/24.2)	0.13
Vaccination	Currently vaccinated for leptospirosis (Yes/No) (%)	7/15 (31.8/68.2)	23/39 (37.1/62.9)	0.80
Survival	Leptospirosis death (Yes/No) (%)	8/14 (36.4/63.6)	0/62 (0/100)	<0.001
	Non-Leptospirosis death (Yes/No) (%)	5/9 (35.7/64.3)	32/30 (51.6/48.4)	0.28
	All cause mortality (Yes/No) (%)	13/9 (59.1/40.9)	32/30 (51.6/48.4)	0.62

WBC: total white blood cell count; NLR: neutrophil to lymphocyte ratio; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; ALP: alkaline phosphatase; ALT: alanine aminotransferase; BUN: blood urea nitrogen.

Table 2. Clinical characteristics, concurrent diagnoses, and type of antibiotic prescribed in dogs tested for urinary *Leptospira* polymerase chain reaction (PCR).

Clinical characteristics/ concurrent diagnoses/ antibiotics	Positive (*n* (%))	Negative (*n* (%))
Azotemia	14 (63.6%)	34 (62.9%)
Anemia	13 (59.1%)	33 (53.2%)
Thrombocytopenia	11 (50.0%)	40 (64.5%)
Leukocytosis	9 (40.9%)	32 (51.6%)
Ehrlichia canis antibody positive	0	25 (40.3%)
Babesia canis volgeli blood smear positive	0	4 (6.4%)
Neoplasia	0	1 (1.6%)
Pancreatitis	0	4 (6.4%)
Heartworm antigen positive	0	2 (1.6%)
Cystitis	0	2 (3.2%)
Prostate gland involvement	0	2 (3.2%)
Constipation	0	1 (1.6%)
Pyometra	0	1 (1.6%)
Shock	0	1 (1.6%)
Liver involvement	0	1 (1.6%)
Dermatitis	0	1 (1.6%)
Beta lactams	3 (13.6%)	5 (8.1%)
Tetracyclines	7 (31.8%)	19 (30.6%)
Quinolones	10 (45.4%)	23 (37.1%)

Logistic regression to identify risk factors for leptospirosis

Univariable analysis did not identify any significant risk factors for *Leptospira* PCR positivity. Therefore, multivariable analysis was not performed to avoid potential overfitting and spurious associations. The results of the univariable logistic regression analyses are presented in Supplementary Table 3.

Survival analysis

Out of the 84 dogs included in this study, 45 (53.6%) died Table 3 summarizes the outcome for all 84 dogs included into the retrospective study. Mortality was higher in the PCR-positive group (13/22 dogs, 59.1%) compared to the PCR-negative group (32/62 dogs, 51.6%) (Fig. 1). Of the urinary PCR-positive group, leptospirosis-related-death has met the criteria in 8 cases (36.4% of the urinary PCR-positive dogs). Among the 37 dogs with non-leptospirosis-related deaths, the majority were PCR-negative (32 dogs), with the remainder being being PCR-positive (5 dogs). Table 4 presents a detailed summary of mortality outcomes categorized by cause of death and PCR status.

The median study period was 309 days (range: 8–812 days). Dogs in the urinary PCR-positive group had a shorter median survival time of all-cause mortality (60 days, range: 8–601 days) compared to the urinary PCR-negative group (402 days, range: 7–812 days) ($p < 0.01$, Fig. 1). Similarly, the median survival time to leptospirosis-related death (27 days, range: 8–67 days) was shorter than both the urinary PCR-positive dogs with non-leptospirosis-related death (238 days, range: 166–601 days) ($p < 0.01$, Fig. 2) and the urinary PCR-negative dogs with non-leptospirosis-related death (402 days, range: 7–812 days) ($p < 0.01$, Fig. 2). There was no significant difference in time to non-leptospirosis-related death between the PCR-positive and PCR-negative groups ($p = 0.09$, Fig. 2).

Cox proportional hazards analysis of leptospirosis-related death

The results of the univariable analysis of the predictive value of continuous and categorical variables for leptospirosis-related death are presented in Table 5. Only three variables, ALT, BUN to creatinine ratio, and neutrophil-to-lymphocyte ratio (NLR), met the criteria from the univariable analysis as potential predictors of leptospirosis-related death. However, multivariable analysis revealed NLR as the only independent predictor, with a unit increase in the NLR ratio translating to a 7.3% rise in the hazard of death (HR = 1.073, 95% CI: 1.02–1.13, $p = 0{\cdot}01$). Baseline characteristics of dogs with positive urinary *Leptospira* PCR, grouped by leptospirosis-related death and non-leptospirosis-related death or alive are compared in Supplementary Table 4.

Cox proportional hazards analysis of all-cause mortality

The univariable analysis identified the BUN to creatinine ratio as the sole predictor for all-cause mortality (Supplementary Table 5). Each unit increase in the ratio was associated with a 2% increased hazard of death (HR = 1.02, 95% CI: 1.003–1.03, $p = 0.02$). Table 5 presents a detailed summary of mortality outcomes categorized by cause of death and PCR status.

Discussion

Leptospirosis, a significant zoonotic bacterial disease affecting both humans and animals (Bharti *et al.*, 2003), poses a particular threat to dogs due to various pathogenic serovars (Sykes *et al.*, 2011; Sykes *et al.*, 2023). While numerous serovars are associated with canine leptospirosis, definitively identifying the culprit remains challenging, often relying on serological data with limitations (Adin and Cowgill, 2000; Levett, 2003; Goldstein, 2010; Reagan and Sykes, 2019; Altheimer *et al.*, 2020). In Thailand, the National Institute of Animal Health (NIAH) and university laboratories routinely employ PCR testing on blood, urine, or tissue samples

Table 3. Outcome for all 84 dogs included into the retrospective study.

Day	n	Leptospirosis-related death (PCR positive)	Non-leptospirosis-related death (PCR positive)	Non-leptospirosis-related death (PCR negative)	Still alive	Total censored
0	84	0	0	0	84	0
1–60	84	7	0	2	75	0
61–365	75	1	4	12	39	19
366–730	39	0	1	15	5	13
> 730	5	0	0	3	2	2

for diagnosis. However, the MAT considered the gold standard for serovar identification, is primarily used at the NIAH for research purposes or by a special request and is not readily available in general veterinary practices. This limited accessibility, even within veterinary teaching hospitals, could contribute to lower submission rates for MAT testing or even PCR.

This study holds significance by being the first to report on leptospirosis in dogs from Thailand and to explore pet owner perceptions regarding its zoonotic nature. Our findings reveal a concerning knowledge gap, with a substantial proportion of owners unaware that leptospirosis can infect both dogs and humans. Notably, owners of dogs testing negative by PCR displayed a significantly lower awareness of the zoonotic risk. This suggests that a positive diagnosis might prompt increased communication and education from veterinarians about zoonotic transmission routes (Hartskeerl *et al.*, 2011). However, it also underscores the critical need for proactive educational campaigns targeting all pet owners, regardless of their dog's leptospirosis status. By raising awareness about the zoonotic potential of leptospirosis and its transmission routes, we can empower pet owners to take preventive measures such as vaccination, ultimately reducing the risk of zoonotic transmission and protecting public health (Hartskeerl *et al.*, 2011; Raghavan *et al.*, 2012). Vaccination is a challenging preventative measure due to its partial immunity, cost, and limited availability in some countries. In humans, it offers only partial protection against the Icterohaemorrhagiae serovar. Dog vaccination, while protective against various serovars, is most valuable in reducing renal colonization and urinary shedding of targeted serogroups. This reduction in shedding has been demonstrated in dogs and is crucial for mitigating the risk of human infection (Broughton and Scarnell, 1985).

Unexpectedly, we found higher plasma protein levels in dogs that were urinary *Leptospira* PCR-negative compared to the PCR-positive group. Although this difference was not strongly significant, it deserves attention. The clinicopathological abnormalities observed in both groups (hyperproteinemia, neutrophilia, lymphopenia, leukocytosis, anemia, thrombocytopenia, kidney dysfunction, and elevated ALP) are consistent with either canine leptospirosis (Rentko *et al.*, 1992; Harkin and Gartrell, 1996; Birnbaum *et al.*, 1998; Geisen *et al.*, 2007; Mastrorilli *et al.*, 2007; Adin and Cowgill, 2000; Kohn *et al.*, 2010; Tangeman and Littman, 2013; Knöpfler *et al.*, 2017; Raj *et al.*, 2021; Griebsch *et al.*, 2022) or another underlying inflammatory or infectious process. In PCR-negative dogs, other infections, immune-mediated diseases, or even cancer should be carefully investigated (Schuller *et al.*, 2015; Sykes *et al.*, 2011; Reagan and Sykes, 2019; Sykes *et al.*, 2022; Sykes *et al.*, 2023). These findings highlight the advantages of a careful clinical assessment, particularly when leptospirosis is suspected, especially in dogs that are exposed to rats, have outdoor access, and have consumed contaminated water (Ricardo *et al.*, 2020; Abdul Rahman *et al.*, 2021; Smith *et al.*, 2022). Measuring these parameters, especially in conjunction with elevated levels of BUN, creatinine, ALT, or ALP, can provide useful diagnostic information (Sykes *et al.*, 2023). It is possible that some PCR-negative dogs were in an earlier or resolving phase of *Leptospira* infection. Bacterial shedding might be intermittent or below the detection threshold of PCR, while ongoing physiological changes (like inflammation) could still elevate plasma proteins (Goldstein, 2010; Schuller *et al.*, 2015; Reagan and Sykes, 2019; Sykes *et al.*, 2023). If the plasma protein levels were measured at different stages in the disease course for PCR-positive and PCR-negative dogs, this could possibly explain the discrepancy. The dynamics of protein change during illness should be considered. Further studies on increased plasma protein and these clinicopathological abnormalities, including MAT to assess recent or past *Leptospira* exposure and potential chronic infections; testing for other infectious agents such as tick-borne diseases; and collecting follow-up samples from both PCR-positive and PCR-negative groups. Monitor changes in plasma protein levels, PCR status, and other hematological/biochemical markers over time to reveal potential dynamic patterns related to disease progression or resolution (Tangeman and Littman, 2013; Schuller *et al.*, 2015; Barthélemy *et al.*, 2017; Knöpfler *et al.*, 2017; Reagan and Sykes, 2019). The univariable logistic regression did not reveal any significant risk factors for *Leptospira* PCR positivity.

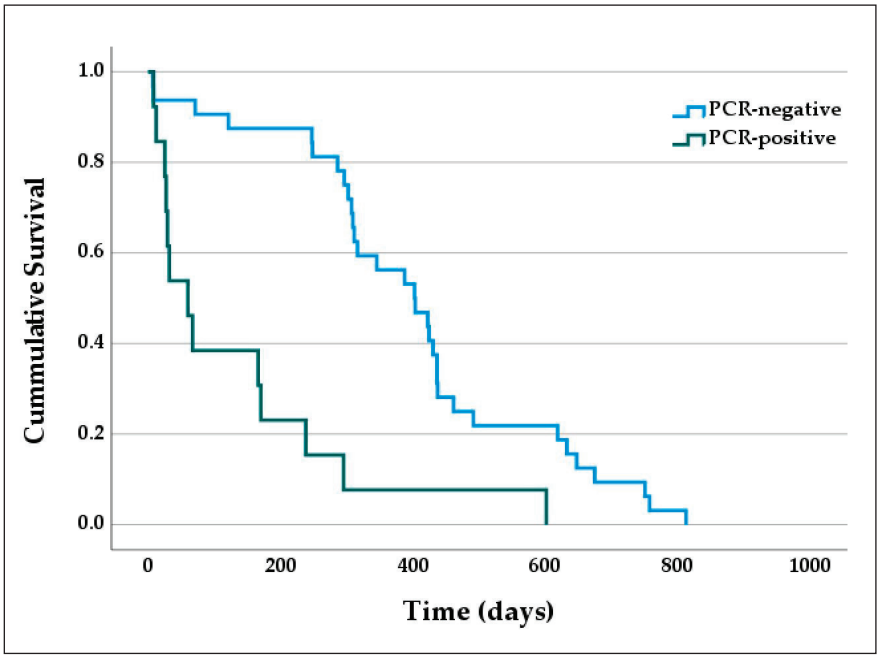

Fig. 1. Kaplan-Meier survival curves comparing dogs with positive and negative urinary PCR results for all-cause mortality. Dogs in the urinary PCR-positive group had a shorter median survival time of all-cause mortality (60 days, range: 8–601 days) compared to the urinary PCR-negative group (402 days, range: 7–812 days) ($p < 0.01$).

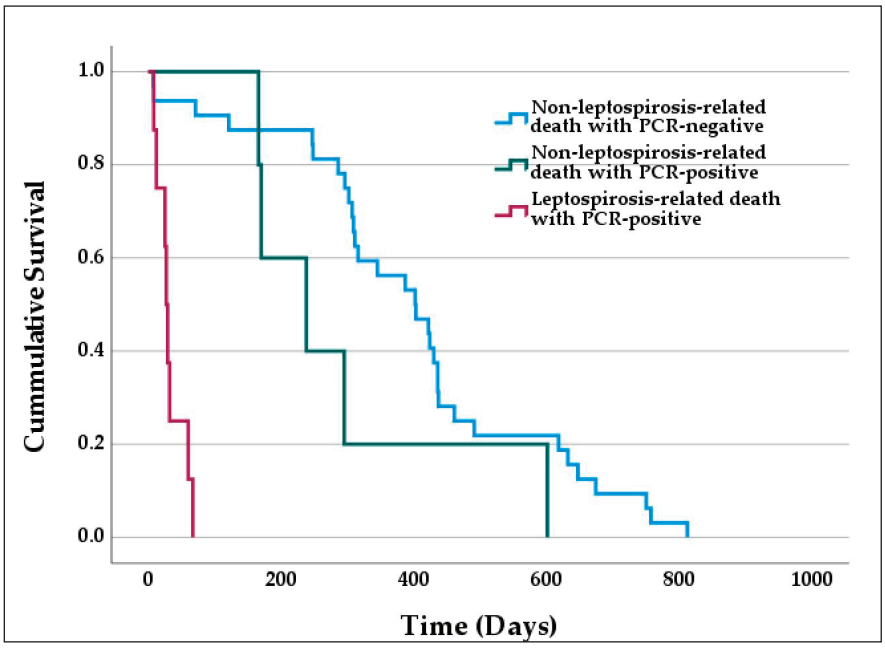

Fig. 2. Kaplan-Meier survival curves comparing dogs with positive and negative urinary PCR results for leptospirosis related death with PCR-positive, non-leptospirosis-related death with PCR-positive, and non-leptospirosis-related death with PCR-negative. Dogs with leptospirosis-related death with PCR positive had median survival time 27 days (range: 8–67 days) was shorter than non-leptospirosis-related death with PCR positive had median survival time 238 days (range: 166–601 days) ($p < 0.01$), and non-leptospirosis-related death with PCR negative had median survival time 402 days (range: 7–812 days) ($p < 0.01$).

Table 4. Summary of causes of death obtained from 45 dogs.

Causes	Leptospirosis-related death (PCR positive)	Non-leptospirosis-related death (PCR positive)	Non-leptospirosis-related death (PCR negative)
Leptospirosis	8	0	0
Natural	0	0	14
Neoplasia	0	1	0
Kidney involvement	0	2	11
Heart & kidney involvements	0	1	0
Anemia & leukocytosis	0	0	1
Pyometra	0	1	0
Kidney involvement & blood parasites	0	0	2
Chronic cystitis	0	0	1
Hepatic involement	0	0	1
Kidney & liver involvements	0	0	1
Hit by car	0	0	1

Among 8 dogs with leptospirosis-related death, complex abnormalities were detected, including anemia (n = 5), azotemia (n = 5), leukocytosis (n = 5), thrombocytopenia (n = 4), and jaundice (n = 3). All dogs exhibited at least one abnormality.

This finding, in the context of an older dog population (median age above 8 years in both groups, with only 25% of them younger than 5 years) (Willems *et al.*, 2017; Harvey, 2021), warrants further investigation. The lack of identifiable risk factors for urinary *Leptospira* PCR positivity within our predominantly older dog population is unexpected. The finding contradicts findings from a meta-analysis that indicated a higher risk in dogs over 4 years old, albeit with weak statistical significance (Ricardo *et al.*, 2020). However, a study by Smith et al (2022) highlights the increased risk in dogs under 5 years (Smith *et al.*, 2022). This discrepancy suggests a complex relationship between age and *Leptospira* susceptibility.

Veterinarians should exercise particular caution when assessing older dogs with azotemia, as our study found approximately 30% of these dogs to be PCR-positive for *Leptospira*. This urinary PCR positivity could signal active leptospirosis or past exposure, even without traditional risk factors. This finding underscores the significant risk of zoonotic disease transmission that veterinarians face. Additionally, studies suggest that veterinarians could be exposed to antibiotic-resistant bacteria like *E.coli* with ESBL-associated genes, further compounding the occupational hazard within the veterinary field (Buranasinsup *et al.*, 2023; Marco-Fuertes *et al.*, 2023).

Our results significantly contribute to the comprehension of the survival and mortality rates of dogs with leptospirosis, a zoonotic bacterial infection that can cause severe illness and death. The study's median duration was 309 days (range: 8–812 days). This extended follow-up period enabled us to observe the long-term consequences of canine leptospirosis. We observed a significantly shorter median survival time for both all-cause mortality of 60 days (range: 8–601 days) in urinary PCR-positive dogs compared to the PCR-negative group (median 402 days, range: 7–812 days), and specifically leptospirosis-related death of 27 days (range: 8–67 days), compared to the urinary PCR-positive dogs with non-leptospirosis-related death (median 238 days, range: 166–601 days). These findings underscore the severity of active leptospiral infections, suggesting that even urinary shedding of *Leptospira* DNA may be associated with a poorer prognosis and an increased risk of mortality from various causes. Factors contributing to this shorter survival could include the virulence of the infecting *Leptospira* strain, the extent of organ damage at the time of diagnosis, delays in treatment initiation, and individual patient factors like age and immune status (Azócar-Aedo and Monti, 2016; Knöpfler *et al.*, 2017; Ricardo *et al.*, 2020; Griebsch *et al.*, 2022; Ioannou, *et al.*, 2024).

The leptospirosis-related mortality rate of 36.4% observed in our study is in line with previous findings from diverse geographical regions over the past two decades (Reagan and Sykes, 2019; Harvey, 2021; Griebsch *et al.*, 2022), highlighting the importance of early diagnosis and treatment, particularly in dogs exhibiting suspicious clinical manifestations (Dourmashkin *et al.*, 2023; Marco-Fuertes *et al.*, 2023). These findings reinforce the effectiveness of detecting pathogenic Leptospires in urine using nucleic acid amplification tests alone, as our study yielded comparable survival outcomes to those of studies utilizing a combination of *Leptospira* MAT titers and PCR for leptospiral DNA detection (Reagan and Sykes, 2019; Harvey, 2021; Griebsch *et al.*, 2022). This study highlights the gravity of leptospirosis and the

Table 5. Univariable Cox proportional hazards analysis of factors predictive of leptospirosis mortality.

Variable	Hazard Ratio (HR)	95.0% confidence interval of the hazard ratio	*p*-value
Age (years)	1.07	0.92–1.25	0.40
Sex (M/F)	0.57	0.14–2.40	0.44
Breed (pure/mixed)	2.24	0.53–9.42	0.27
Desexed (Yes/No)	0.42	0.10–1.68	0.22
General appearance (alert & responsive/depressed)	3.20	0.39–26.06	0.28
Body weight (kg)	0.98	0.91–1.05	0.51
Heart rate (beats per minute)	1.00	0.99–1.02	0.69
Mucous membrane (pink/yellow)	2.52	0.60–10.59	0.21
WBC (10^3/µl)	1.00	1.00–1.00	0.40
Monocytes (10^3/µl)	1.00	1.00–1.00	0.85
Neutrophils (10^3/µl)	1.00	1.00–1.00	0.12
Lymphocytes (10^3/µl)	1.00	1.00–1.00	0.69
Eosinophils (10^3/µl)	1.00	1.00–1.00	0.91
Basosinophils (10^3/µl)	NA	NA	NA
Band neutrophils (10^3/µl)	1.00	1.00–1.00	0.72
NLR	1.07	1.02–1.13	0.01
Erythrocytes (10^6/µl)	0.66	0.38–1.15	0.14
Hemoglobin (g/dl)	0.81	0.62–1.08	0.15
PCV (%)	0.96	0.90–1.03	0.22
MCV (fl)	1.02	0.94–1.11	0.68
MCH (pg)	1.07	0.78–1.47	0.67
MCHC (g/dl)	1.00	0.72–1.39	1.00
Platelets (10^3/µl)	1.00	1.00–1.01	0.81
RDW (%)	1.03	0.93–1.15	0.53
Plasma protein (g/dl)	0.67	0.39–1.14	0.14
ALP (U/l)	1.00	1.00–1.00	0.45
ALT (U/l)/100	2.17	1.00–4.67	0.048
BUN (mg/dl)	1.01	1.00–1.03	0.15
Creatinine (mg/dl)	1.03	0.85–1.25	0.73
BUN to creatinine ratio	1.05	1.01–1.09	0.01
Antibiotic (Yes/No)	0.04	0.00–691.03	0.52
Currently vaccinated for leptospirosis (Yes/No)	1.49	0.35–6.24	0.59

sex (M/F): female compared to male as the reference; breed (pure/mixed): mixed compared to pure as the reference; (Yes/No): no compared to yes as the reference; general appearance (alert & responsive/depressed): depressed compared to alert & responsive as the reference; mucous membrane (pink/yellow): yellow compared to pink as the reference; (normal/abnormal): abnormal compared to normal as the reference; WBC: total white blood cell count; NLR: neutrophil to lymphocyte ratio; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; ALP: alkaline phosphatase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; /10 implies that the hazard ratio is the increase in hazard per ten unit increase in the value of the measured variable; /100 implies that the hazard ratio is the increase in hazard per one hundred unit increase in the value of the measured variable.

urgency of early intervention. Prompt diagnosis and treatment can significantly improve survival outcomes and prevent fatality. Veterinarians should prioritize early detection and aggressive treatment strategies, particularly in dogs exhibiting signs indicative of leptospirosis. Additionally, the effectiveness of nucleic acid amplification tests in the detection of pathogenic Leptospires in urine is further validated, providing a valuable tool for the diagnosis of this potentially life-threatening infection.

Our study identified the NLR as a strong independent predictor of leptospirosis-related death in dogs. Univariable analysis suggested ALT and the BUN-to-creatinine ratio as potential predictors; however, only NLR retained its significance in the multivariable model. Elevated NLR was associated with a 7.3% increase in the hazard of death for each unit increase in the ratio. This finding highlights the prognostic value of NLR in assessing the severity of *Leptospira* infections and the potential for fatal outcomes. An elevated NLR reflects a heightened inflammatory state (neutrophilia) and relative lymphopenia, which can indicate immune dysregulation or stress (Hodgson *et al.*, 2018; Conway *et al.*, 2021; Dinler Ay, 2022; Dourmashkin *et al.*, 2023). In severe leptospirosis, the systemic inflammatory response can become overwhelming, leading to multi-organ dysfunction and an increased risk of mortality. Our results suggest that the magnitude of this inflammatory imbalance, as reflected in the NLR, could correlate with the severity of the infection (Buser *et al.*, 2019; Dinler Ay, 2022; Dourmashkin *et al.*, 2023). While initially identified as potential predictors, the ALT and BUN-to-creatinine ratio lost their independent predictive value in the multivariable analysis. This might suggest that while liver and kidney damage are frequent complications of leptospirosis (Major *et al.*, 2014; Schuller *et al.*, 2015; Azócar-Aedo and Monti, 2016; Knöpfler *et al.*, 2017; Ricardo *et al.*, 2020; Sykes *et al.*, 2023), their influence on mortality risk may be mediated through other factors, or that the NLR serves as a more robust overall indicator of systemic illness severity. The NLR, a simple and widely available hematological marker, could be integrated into the initial assessment of dogs with suspected leptospirosis. Dogs with higher NLR values may warrant closer monitoring, more aggressive treatment, and intensive supportive care. Serial monitoring of the NLR could provide insights into treatment response and the development of complications. Persistent elevation or a rising NLR might indicate a need for adjustments in the management strategy.

Our analysis revealed the BUN-to-creatinine ratio as a predictor of all-cause mortality. This finding suggests that kidney dysfunction, a common complication of leptospirosis, significantly increases mortality risk, regardless of the immediate cause of death (Zamagni *et al.*, 2020; Sykes *et al.*, 2023; Uribe-Restrepo *et al.*, 2023). This is especially relevant in our study population of older dogs, where pre-existing kidney disease may exacerbate the severity of leptospiral infection.

Nevertheless, the present study has several limitations that should be acknowledged. First, the retrospective nature of the study may have introduced biases due to the reliance on existing medical records and the potential for incomplete or inaccurate data. Second, certain parameters from the anamnesis (such as the vaccination status and lifestyle of the dogs) and serum biochemistry were not consistently available for all dogs, limiting our ability to fully assess the prognostic value of these variables. Third, data regarding the tests submitted to veterinary teaching hospitals compared to the time of the survival study differed in the timeframe, although the number of sample submissions was similar. Additionally, the study was conducted at a single center, potentially restricting the generalizability of the findings to other regions or populations with different risk factors for leptospirosis. Furthermore, the study relied solely on urinary PCR for the diagnosis of leptospirosis without incorporating other diagnostic modalities such as serology (MAT) or bacterial culture. While urinary PCR is a sensitive and specific technique for the detection of leptospiral DNA, it may miss cases of early infection or those with a low bacterial load (Hartman *et al.*, 1984; Reagan and Sykes, 2019).

Despite these limitations, our study identified several hematological and biochemical markers with potential prognostic value in dogs with suspected leptospirosis. Of particular interest were the NLR and the BUN to creatinine ratio, which emerged as independent predictors of leptospirosis-related death and all-cause mortality, respectively. These can assist veterinarians in assessing the severity of the infection, predicting the risk of death, and guiding decision-making. Further research is warranted to validate these findings in larger, multicenter studies and investigate the impact of these predictive and prognostic factors on diagnostic performance and treatment outcomes.

Conclusion

This study provides insights into the prognostic factors and survival outcomes in older dogs with suspected leptospirosis. We found that azotemia and urinary *Leptospira* PCR positivity were associated with shorter survival times. By highlighting the risk factors associated with leptospirosis in older dogs with azotemia and emphasizing the zoonotic potential, this study encourages increased vigilance among veterinarians and pet owners. Addressing the limitations of diagnostic tools and promoting owner education can contribute to earlier diagnosis, improved treatment outcomes, and reduced risk of zoonotic transmission.

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Authors' contributions

Conceptualization, research design, methodology, investigation, data curation, data analysis, interpretation and discussion the results, M.T., P.S., P.B., O.K., M.Th., K.C., and W.S.; validation and resources, M.T., M.Th., K.C., and W.S.; writing—original draft preparation, M.T., K.C., and W.S.; writing—review and editing, M.T., P.S., P.B., O.K., M.Th., K.C., and W.S.; supervision, project administration, and funding acquisition, W.S. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

Supplementary Tables (Tables S1–S5) are available and can be requested from the Corresponding Author.

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