Serogroup Distribution and High Seroprevalence of Bovine Leptospirosis in Southeastern Portalegre, Portugal

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Summary

Bovine leptospirosis is a globally distributed zoonotic disease with significant implications for animal health, public health, and livestock productivity. This study aimed to characterize the epidemiological status of leptospirosis in cattle from the Alto Alentejo region (Portugal), with particular emphasis on serogroup distribution and infection dynamics. Between February and June 2025, a total of 83 cattle from 11 farms were sampled across four municipalities. Serological analysis was performed using the microscopic agglutination test (MAT), while molecular detection of pathogenic Leptospira DNA was conducted using polymerase chain reaction (PCR) in selected calf samples. Overall, 81% of animals tested positive, suggesting widespread exposure and endemic circulation. The most prevalent serogroups were *L. Pomona* (27%), *L. Mozdok* (23%), and *L. Pyrogenes* (13%), accounting for over 78% of positive cases. Multiple serogroup reactivity was frequently observed, reflecting either repeated exposure or cross-reactivity. Infections were geographically widespread, with higher frequencies in extensive production systems. PCR confirmed active infection in calves, supporting the role of leptospirosis in acute disease and mortality events. Climatic conditions, particularly increased precipitation, likely contributed to environmental persistence and transmission. Overall, the findings suggest a complex epidemiological scenario involving multiple serogroups, environmental reservoirs, and host interactions in cattle from Alto Alentejo. However, the conclusions should be interpreted cautiously due to the relatively small sample size, limited geographic coverage, cross-sectional study design, and the restricted use of PCR analysis in selected samples. . These findings emphasize the need for improved surveillance, adapted vaccination strategies, and integrated control measures to mitigate the impact of bovine leptospirosis in southern Portugal.

Keywords: bovine leptospirosis; seroprevalence; MAT; PCR; epidemiology; Portugal; zoonosis

Resumo

A leptospirose bovina é uma doença zoonótica de distribuição mundial, com implicações significativas para a saúde animal, a saúde pública e a produtividade pecuária. O presente estudo teve como objetivo caracterizar a situação epidemiológica da leptospirose em bovinos da região do Alto Alentejo (Portugal), com especial enfoque na distribuição dos serogrupos e na dinâmica da infeção. Entre fevereiro e junho de 2025, foram amostrados 83 bovinos provenientes de 11 explorações pecuárias distribuídas por quatro concelhos. A análise serológica foi realizada através do teste de microaglutinação (MAT), enquanto a deteção molecular de ADN de *Leptospira* patogénica foi efetuada por reação em cadeia da polimerase (PCR) em amostras selecionadas de vitelos. No total, 81% dos animais apresentaram resultados seropositivos, sugerindo uma exposição generalizada e uma circulação endémica do agente. Os serogrupos mais prevalentes foram *L. Pomona* (27%), *L. Mozdok* (23%) e *L. Pyrogenes* (13%), representando, em conjunto, mais de 78% dos casos positivos. Foi frequentemente observada reatividade a múltiplos serogrupos, refletindo uma exposição repetida ou reações cruzadas. As infeções apresentaram uma distribuição geográfica alargada, com maior frequência em sistemas de produção extensivos. A PCR confirmou infeção ativa em vitelos, reforçando o papel da leptospirose em casos de doença aguda e episódios de mortalidade. As condições climáticas, em particular o aumento da precipitação, terão provavelmente contribuído para a persistência ambiental e a transmissão do agente. Globalmente, os resultados sugerem um cenário epidemiológico complexo, envolvendo múltiplos serogrupos, reservatórios ambientais e interações entre hospedeiros nos bovinos do Alto Alentejo. Contudo, as conclusões devem ser interpretadas com prudência, devido à dimensão relativamente reduzida da amostra, à cobertura geográfica limitada, ao desenho transversal do estudo e à utilização restrita da PCR apenas em amostras

selecionadas. Estes resultados reforçam a necessidade de melhorar os programas de vigilância, adaptar as estratégias de vacinação e implementar medidas integradas de controlo para mitigar o impacto da leptospirose bovina no sul de Portugal.

Palavras-chave: leptospirose bovina; seroprevalência; teste de microaglutinação; reação em cadeia da polimerase; epidemiologia; Portugal; zoonose.

Introduction

Leptospirosis is a neglected zoonotic disease caused by pathogenic spirochetes of the genus Leptospira, affecting a wide range of domestic and wild animals, as well as humans. In cattle, the disease has significant economic impact, being associated with reproductive disorders such as abortion, infertility, stillbirths, and reduced milk production (Bolin, 2003; Ellis, 2015). Chronic infection may also result in persistent renal colonisation, contributing to environmental contamination and sustained transmission cycles (Ko et al., 2009).

Despite its importance, leptospirosis remains underdiagnosed due to its frequently subclinical presentation and the limitations of laboratory diagnostics, particularly cross-reactivity in the microscopic agglutination test (MAT) (Levett, 2001; Picardeau, 2017). In Portugal, epidemiological data remain limited, especially in extensive production systems such as those predominant in the Alentejo region (Lilenbaum & Martins, 2014). Nevertheless, recent outbreaks and serological surveys in Portugal highlight the circulation of multiple pathogenic serogroups in cattle populations, particularly under extensive production systems and favorable climatic conditions (Paixão et al., 2025).Therefore, bovine leptospirosis is considered an endemic zoonotic disease in Portugal, with studies demonstrating the circulation of multiple pathogenic Leptospira serogroups among domestic and wild animal hosts, highlighting the epidemiological complexity and persistence of infection in Portuguese cattle production systems (Ferreira et al., 2020), which the present study aims to address.

Recent observations of increased calf mortality in the study area raised concerns regarding the possible involvement of leptospirosis, particularly given the increased susceptibility of young animals to acute infection (Haake & Levett, 2015). Understanding circulating serogroups is essential for effective disease control, particularly in regions where multiple transmission pathways coexist.

Therefore, this study aimed to characterise the epidemiology of bovine leptospirosis in the Alto Alentejo region, with a focus on seroprevalence, serogroup distribution, and infection dynamic.

Material and Methods

The study was conducted in the southeastern region of the Portalegre district, Portugal, encompassing the municipalities of Elvas, Campo Maior, Monforte, and Arronches. A total of 83 cattle from 11 farms were included (Figure 1). Written informed consent for sample collection, diagnostic procedures, and publication of anonymized data was obtained from all animal owners prior to the study. The farms included in the study were predominantly extensive production systems, with 10 farms dedicated to beef production involving different purebred and crossbred beef cattle breeds, and one semi-intensive dairy farm composed of Holstein–Friesian cattle. The studied population included animals from different age groups and was divided into two categories:

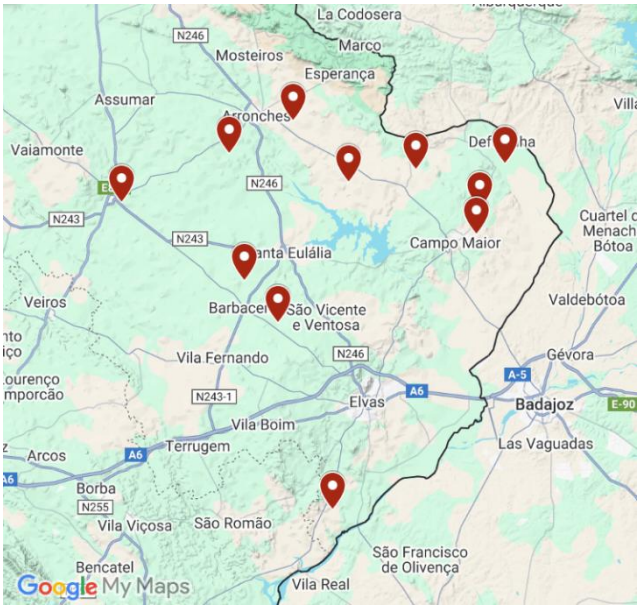

Figure 4. Representative map of the locations of the 11 farms in the municipalities of Elvas, Campo Maior, Monforte, and Arronches. Source: Google My Maps.

Group A: adult females, used for serological testing, aleatory selected from each herd; and Group B: seven calves, whose tissues were used for post-mortem analysis. Calves submitted to necropsy were between 1 and 6 months of age and had a history of sudden death or clinical signs compatible with acute disease.

Blood samples were collected from adult females by coccygeal venepuncture into plain tubes for serum separation. Samples were refrigerated immediately after collection and transported under controlled conditions to the laboratory.

In necropsied calves, urine samples were obtained by cystocentesis, and tissue samples (liver and kidney) were collected aseptically. All samples were stored at approximately 4°C until laboratory analysis.

Serological detection of Leptospira spp. was performed using the microscopic agglutination test (MAT), the reference method for leptospirosis diagnosis. A titre ≥1:100 was considered positive. Analyses were carried out at the National Institute for Agrarian and Veterinary Research (INIAV), Évora.

Statistical analyses were performed using Jamovi software (version 2.7). Data are presented as mean ± standard deviation (SD).

Results

A total of 83 cattle from 11 farms located in the Alto Alentejo region were tested for Leptospira spp. infection (Supplementary material, Figure 5). Overall, 67 animals (81%) were positive, indicating a high level of exposure within the studied population.

Table 1. Descriptive statistics of frequency of serovars/serogroups detected by the reference diagnostic method MAT in samples obtained from cattle in the Alto Alentejo region, titers reflected as 100, 200, 400 and 800.

	L. H	L. Po	L. Ca	L. M	L. G	L. Py	L. Co	L. B
N	17	41	8	36	6	20	3	22
Mean	106	161	100	156	100	100	100	123
SD	24.3	188	0.00	168	0.00	0.00	0.00	68.5
Min.	100	100	100	100	100	100	100	100
Max.	200	800	100	800	100	100	100	400

L. H = Hardjo; L. Po = Pomona; L. Ca = Canicola; L. M = Mozdok; L. G = Grippotyphosa; L. Py = Pyrogenes; L. Co = Copenhageni; L. B = Bratislava.

Spatially, infections were observed across all sampled municipalities (Elvas, Campo Maior, Monforte, and Arronches) (Supplementary material, figure 4), with no clear restriction to a geographic cluster. In Elvas, Monforte and Campo Maior, the most prevalent serovars were L. Pomona and L. Mozdok, while in Arronches, serovars L. Hardjo and L. Bratislava presented similar prevalencies as the previous ones (Figure 3).

Table 2. Descriptive statistics of frequency of Serovars/serogroups detected by the reference diagnostic method MAT in samples obtained from cattle in the Alto Alentejo region, in the four counties.

	County	L. H	L. P	L. Ca	L. M	L. G	L. Py	L. Co	L. B
N	Elvas	2	11	2	10	2	7	1	4
	Monforte	0	5	1	4	0	3	2	1
	Arronches	12	15	5	12	2	7	0	12
	Campo Maior	3	10	0	10	2	3	0	5
Mean	Elvas	100	255	100	100	100	100	100	225
	Monforte		100	100	100		100	100	100
	Arronches	100	100	100	100	100	100		100
	Campo Maior	133	180		300	100	100		100

Multiple serogroup reactivity was frequently observed within individual animals, with several cases showing simultaneous detection of two to five serogroups. This pattern may reflect co-exposure in the field, environmental contamination, or serological cross-reactivity inherent to the MAT technique.

Meteorological data from the study period showed marked fluctuations in precipitation, with periods of intense rainfall in January, March, and April 2025, exceeding the climatological average. These wet conditions, followed by warm temperatures and short dry periods, likely contributed to environmental persistence and dissemination of Leptospira spp., particularly in grazing areas and water-accumulating soils typical of extensive farming systems in the region.

From the seven cases of neonatal death in calves presenting lesions consistent with leptospirosis at necropsy, five presented lesions compatible with leptospirosis (Figure 2) supporting the involvement of pathogenic Leptospira in the acute infection and sudden mortality events observed.

Figure 25. Organs of calves after necropsy. Kidney (A) and liver (B) showing changes in color and consistency.

Discussion

The high seroprevalence observed in this study (81%) suggests that bovine leptospirosis is endemic in the Alto Alentejo region. This value exceeds those reported in several European studies, suggesting that local environmental and management conditions are particularly favourable for pathogen persistence and transmission (Costa et al., 2015). Extensive production systems, which dominate the region, likely contribute to this scenario by increasing exposure to contaminated water and soil.

The predominance of L. Pomona, L. Mozdok, and L. Hardjo and L. Bratislava represents a notable epidemiological pattern. While Pomona has been historically associated with livestock infections, the prominence of other serogroups may indicate evolving transmission dynamics involving wildlife reservoirs and multi-host interactions. This shift has also been reported in other endemic regions and reflects increasing ecological complexity (Vincent et al., 2019).

The detection of multiple serogroups within individual animals further supports the hypothesis of repeated exposure or co-circulation of strains. However, this pattern may also be influenced by cross-reactivity inherent to MAT, definitive association between Leptospira infection and the observed mortality events could not be established.

This study has several limitations that should be considered when interpreting the results. First, the relatively small sample size and the inclusion of farms from a restricted geographical area limit the generalisability of the findings to the wider cattle population in Portugal. Second, the cross-sectional design provides only a snapshot of infection status and does not allow assessment of temporal trends, transmission dynamics, or causal relationships. Third, serological diagnosis based on MAT may be influenced by cross-reactivity between serogroups and cannot distinguish between past exposure and active infection. Despite these limitations, the study provides valuable insights into the epidemiology of bovine leptospirosis in the Alto Alentejo region and supports the need for further longitudinal and large-scale investigations.

From a control perspective, the diversity of circulating serogroups and the apparent mismatch with commonly used vaccines represent a significant challenge. Current vaccination strategies may not provide adequate protection against locally prevalent strains, reinforcing the need for region-specific epidemiological data to inform control measures (Loureiro & Lilenbaum, 2020).

Conclusions

This study demonstrates that leptospirosis is apparently prevalent in cattle farms in southeastern Alentejo, with data compatible with endemic circulation and serogroup diversity. Overall, the findings suggest a complex epidemiological scenario involving environmental persistence, multiple reservoirs, and continuous exposure. Effective control of bovine leptospirosis in this context will require integrated approaches combining surveillance, vaccination, and improved management practices.

highlighting the need for complementary diagnostic approaches to improve epidemiological resolution (Levett, 2001).

Higher numbers of positive cases were recorded in extensive systems, where animals have greater environmental exposure and contact with potentially contaminated water sources.

Environmental conditions appear to play a central role in disease dynamics. The increased precipitation observed during the study period likely enhanced bacterial survival in soil and water, facilitating indirect transmission. This relationship between climatic factors and leptospirosis has been consistently demonstrated in both animal and human studies (Trueba et al., 2004).

Necropsy findings in calves were compatible with lesions that may occur in cases of acute leptospirosis, a disease to which young animals are particularly susceptible due to its potential for rapid systemic dissemination and fatal outcomes (Haake & Levett, 2015). Nevertheless, because no molecular testing was performed on calf tissues, a

The predominance of non-vaccine serogroups and the confirmation of active infections emphasise the need for improved surveillance and adapted control strategies. Future efforts should focus on integrating serological and molecular diagnostics, as well as developing vaccination programmes tailored to regional epidemiological profiles.

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