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Acute Leptospirosis Outbreak in Cattle: A Case Report

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ABSTRACT

Leptospirosis is a globally distributed re-emerging zoonotic disease caused by *Leptospira* species. In cattle, the clinical course varies from a subclinical-chronic infection, commonly found in adult animals, to a severe acute syndrome mostly found in calves. The present study reports an outbreak of acute leptospirosis, following favourable climacteric conditions. Seven affected farms were monitored. Clinical observations from infected animals (n = 30) revealed two distinct patterns: lactating calves experienced hyperacute courses, often culminating in death, and older animals, mainly steers, displayed signs of apathy, anorexia, icterus and haemoglobinuria. Necropsies confirmed jaundice and haemoglobinuria, aligning with a presumptive diagnosis of leptospirosis. Haematology and serology results further supported this diagnosis. Pomona and Mozdok serovars were the most prevalent (62.5%) and had the highest mean agglutination titres, 1:1160 and 1:700, respectively. Antimicrobial treatment of sick animals consisted of oxytetracycline. Chemical metaphylaxis and prophylactic measures were established to control the outbreak in cohabiting animals. Environmental factors like climate change are expected to contribute to more frequent leptospirosis outbreaks. Comprehensive serological surveys are recommended to develop region-specific control measures, emphasising the importance of vaccination as a practical and effective prophylactic measure.

1 | Introduction

Leptospirosis is a re-emerging zoonotic disease caused by members of the genus *Leptospira* (Osorio-Rodríguez et al. 2024). It is globally distributed (Adler and de la Peña Moctezuma 2010), although most outbreaks occur in tropical climates or are linked with seasonal fluctuation in temperate regions where warm temperatures and high rainfall rates favour the survival of *Leptospira* in the environment (Samrot et al. 2021). The taxonomic classification is complex: genetic approaches have differentiated 68 *Leptospira* species that can be classified as pathogenic, intermediate or saprophytic (Vincent et al. 2019). According to the classical serological classification, there are 300 serovars which are then organised into 30 serogroups (Picardeau 2017). Cattle are natural hosts for the serovar Hardjo, but can also harbour serovars Pomona, Bratislava, Icterohaemorrhagiae, Grippotyphosa and Canicola (Ellis 2015; Delooz et al. 2018; Pinto et al. 2017; Sohm et al. 2023). Theoretically, any serovar can infect every animal species, but, in practice, it depends on the presence of risk factors that predispose to the onset of infection (Cilia et al. 2021).

Transmission occurs primarily through direct or indirect (i.e. via contaminated soil, water or food) contact with the urine of infected animals (Zamir et al. 2022). The same animal or species can function as a maintenance host for some serovars and an incidental host for others (Ibrahim et al. 2022). Small mammals such as rats, mice, voles, muskrats and nutrias are the most important maintenance hosts as they carry the bacteria in the renal tubules, where it multiplies and is excreted in the urine over long periods of time (Žele-Vengušt et al. 2021; Munoz-Zanzi et al. 2020). Other animals, such as pigs, cattle, dogs and horses, also excrete leptospires through their urine when infected. Among cattle, direct transmission is considered the main mechanism for the spread of *Leptospira interrogans* serovar Hardjo (Loureiro and Lilenbaum 2020). Indirect transmission is typically associated with incidental bovine leptospirosis caused by the serovars maintained by other domestic and free-living animals (Loureiro and Lilenbaum 2020).

As an anthropozoonosis, leptospirosis constitutes an important threat to humans (Zarantonelli et al. 2018) and equally affects a broad range of domestic and wild mammals (Adler 2015). In cattle, it leads to notable economic losses since it can affect reproductive and productive efficiency (Loureiro and Lilenbaum 2020; Di Azevedo et al. 2023). During infection by *Leptospira interrogans*, two stages can be distinguished (Rajapakse 2022). After an incubation period of 3–20 days, the septicaemia/bacteraemia phase begins, characterised by the distribution and multiplication of leptospires in various organs (Samrot et al. 2021), especially the kidneys (Adler and de la Peña Moctezuma 2010; Adler 2015) or genital tracts, in ruminants (Pires et al. 2018; Silva et al. 2019; Lilenbaum et al. 2008). At the beginning of this phase, a considerable amount of haemolysin is produced, resulting in generalised intravascular haemolysis (Adler and de la Peña Moctezuma 2010), especially in some serogroups such as Pomona and Icterohaemorrhagiae (Adler 2015). The septicaemia phase ends with the appearance of anti-leptospiral antibodies in the circulation, which help the phagocytosis and removal of the leptospires from the bloodstream–immune phase (Ellis 2015). In cattle, the clinical course of the disease varies with respect to the animal's age and the infecting serovar. It can be perceived as a reproductive subclinical-chronic infection, namely bovine genital leptospirosis, commonly found in adult cattle, or a severe multi-systemic acute syndrome found mostly in calves (Sohm et al. 2023).

2 | Case Presentation

2.1 | History

Several occurrences of sudden death in lactating calves and acute illness in fattening steers were reported to Vet+ veterinary clinic, located in Montemor-o-Novo, southern Portugal (Latitude: 38° 38′ 42 N, Longitude: 8° 12′ 56″ W).

The clinical team monitored seven affected farms. One was a dairy farm, while the rest were beef, two of which were dedicated to rearing and fattening, and four were suckling herds. Most kept their livestock in extensive rearing. The size of the affected herds ranged from 109 to 649 animals. None of the properties was adjacent to another. Affected cattle were mainly beef crossbreeds, except for two farms, where they used Holstein–Frisian crosses.

2.2 | Clinical Signs and Post-Mortem Examination

The clinical signs are exhibited in Table 1. The most common finding among affected animals was death (67%). The animals seen alive were mostly aged between 6 and 7 months old (90%).

TABLE 1 | Demographic and clinical characteristics of affected cattle (n = 30).

Variables	Affected animals (n = 30)
Age (months), mean (SD)	3.5 (2.2)
Sex, *n* (%) male	17 (57)
Clinical manifestation, *n* (%) (%)manifestation, *n* (%)	
Apathy	8 (27)
Anorexia	7 (23)
Icterus	3 (10) + 5 (17)[a]
Haemoglobinuria	4 (13) + 5 (17)[a]
Tachycardia	6 (20)
Found dead	20 (67)

[a]Pathological alterations found during necropsy.

FIGURE 1 | Sick steer with haemoglobinuria.

Exceptionally, a 2-month-old calf with the clinical signs described above was seen alive and treated. All of the sick animals presented at least three of the following symptoms: apathy, anorexia, tachycardia, icterus and haemoglobinuria (Figure 1). Of these, apathy and anorexia were the most common. Haemoglobinuria was observed in 40% of treated animals.

Nine necropsies were performed. All revealed a similar pattern: jaundice (Figure 2), dark red-coloured urine (Figure 3) and haemorrhages from natural orifices.

2.3 | Laboratory Testing

Blood samples were taken from animals presenting clinical symptoms. In animals that were found dead, mainly lactating calves, blood samples were collected, preferably from the mother of the dead calf or, if not possible, from a cohabitant. Samples were sent, refrigerated, to the National Institute of Agricultural and Veterinary Research (INIAV), to perform a microscopic agglutination test (MAT). The MAT was performed as described

FIGURE 2 | Necropsy of dead calf. Jaundice is seen in intra-abdominal fat and subcutaneous tissues.

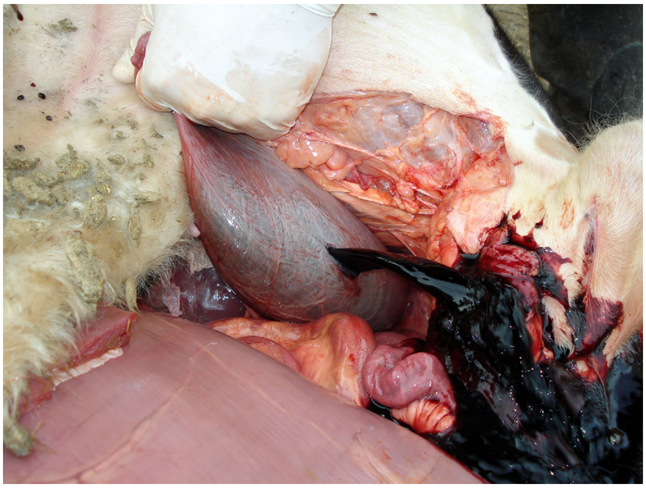

FIGURE 3 | Necropsy of dead steer. Dark red-coloured urine is seen at the time the bladder was pierced.

by the WOAH guidelines (WOAH 2018), with an initial serum dilution of 1:10, using the following antigens from 17 live serovars: Autumnalis, Ballum, Bataviae, Bratislava, Canicola, Celledoni, Copenhageni, Cynopteri, Grippotyphosa, Hardjo, Hebdomanis, Icterohaemorrhagiae Mozdok, Pomona, Pyrogenes, Tarassovi and Shermani.

Exceptionally, blood from three affected steers was taken duplicate to an Ethylenediaminetetraacetic acid (EDTA) collection tube for in-clinic haematology (Fujifilm haematology dri-chem 4000i).

2.4 | Differential Diagnosis

The main differential diagnoses considered were bacillary haemoglobinuria and babesiosis. Despite sharing symptoms, the course of the latter is considerably longer, and no ixodids were found. Bacillary haemoglobinuria was theoretically ruled out as all herds performed routine vaccination with multi-clostridial vaccines.

The clinical signs, the acute course with high mortality and the alterations observed during necropsies allowed to establish a presumptive diagnosis of leptospirosis.

2.5 | Treatment

All the animals showing clinical symptoms were treated with a single dose of long-acting (LA) oxytetracycline 20 mg/kg liveweight (LW) via intramuscular. In three (43%) of the monitored farms, an identical antimicrobial therapy was performed in the remaining animals from the same group as a metaphylactic treatment.

Vaccination was performed on all cohabiting animals susceptible to developing an acute form of leptospirosis, and on three farms, the entire herd. Two different vaccines were used: Triangle 9 vaccine, which contains bacterins from five serovars of *Leptospira*: Pomona, Hardjo, Grippotyphosa, Canicola and Icterohaemorragiae and Leptavoid vaccine, which solely consists of *L. interrogans* serovar Hardjo bacterins.

In all cases, it was suggested to change the herd, or group of animals affected, to a new pasture or paddock, as far away as possible from where they remained when the first cases happened. It was advised to isolate all non-lactating animals showing clinical signs from the rest of the group.

2.6 | Laboratory Results

The most relevant haematology results were as follows: two (66%) of the sampled animals had normocytic normochromic anaemia (HCT<16%), and one (33%) presented marked leucocytosis (3.9×10^9 cells). The MAT results are summarised in Table 2. Peak titres were recorded for Pomona (≥1:3200). Three out of seven sampled sick animals showed agglutination titres higher than 1:200. Eight samples (50%) did not show any agglutination reactions (data not shown).

2.7 | Outcome Follow-Up

All animals showing clinical symptoms, treated with antimicrobial therapy, recovered remarkably. In some cases, a week after treatment, animals that were previously apathetic and anorectic, with haemoglobinuria and jaundice, did not show any of the referred clinical signs. Following metaphylaxis and vaccination, no further records of deaths or illness could be related to this outbreak.

3 | Case Discussion

From the beginning of the outbreak, there was a perception of the possibility of a serious zoonosis. All necessary biosecurity precautions were followed to safeguard the health and safety of the clinical team and the farm workers.

TABLE 2 | Agglutination titres from positive serum samples by microscopic agglutination test (MAT), titres <1:10 not presented (n = 16).

ID	Sample	Agglutination titre								
		Har	Pom	Moz	Gri	Bal	Ict	Can	Bra	Aut
1446	Dead			1:20						
6066	Sick		1:400	1:200						
2024	Sick					1:20				
3201	Sick		1:200	1:100						
8006	Sick		1:3200	1:1600	1:20		1:20	1:20	1:10	1:20
4422	Mother	1:400								
9040	Mother		1:400	1:800	1:100					
0980	Cohabitant		1:1600	1:800		1:40				

The course, symptoms and thus the outcome of this disease depend mainly on the age of the animal (Sohm et al. 2023) and its adaptation to the infecting leptospiral serovar (Ellis 2015). The initial reports and signs pointed towards an incidental leptospirosis infection. In fact, a total of 20 deaths from seven herds were linked to this outbreak, which corresponded to an overall mortality rate of 67%. Most of these reported deaths (85%) corresponded to calves younger than 5 months old. Although it could have been useful for scientific purposes, the duration of the course of the disease in these animals was not investigated as, in most farms, cattle were reared extensively. Contrarily, almost all the reported sick animals had between 6- and 7-month-old. No illnesses or deaths were reported in adult cattle.

Several antimicrobials are referred to as effective in treating Leptospirosis in cattle: dihydrostreptomycin-penicillin G (Sykes et al. 2011; Bautista et al. 2022), cefiofur (Liegeon, Delory and Picardeau 2018), tilmicosin or a single injection of oxytetracycline (Alt, Zuerner and Bolin 2001). The latest combines the practicality of a unique, LA injection with its reasonable cost. In companion animals, β-lactam and tetracyclines are consensually accepted to treat acute leptospirosis (Schuller et al. 2015). The treatment used in this outbreak, Oxytetracycline LA at a dose of 20 mg/kg LW, proved to be curative, confirming Alt (Alt, Zuerner and Bolin 2001) study, although urinary shedding of leptospires after treatment was not investigated. In fact, all of the animals treated in the initial phase responded positively to the treatment, including a 2-month-old calf. The same antimicrobial was used as a metaphylactic treatment. This technique was performed in cohabiting calves and steers with a higher risk of getting the disease, trying to ensure the elimination of infection in possible kidney carriers or animals during the incubation period. Metaphylactic antimicrobial therapy for acute bovine leptospirosis is undescribed in the literature. Nevertheless, the technique is well-accepted to control human outbreaks (Guzmán Pérez et al. 2021; Goarant 2016).

For medical prophylaxis, adult animals with a lower risk of acquiring clinical disease were vaccinated. Knowing the most prevalent serovars beforehand could have been useful for choosing the commercial vaccine. Nonetheless, facing an acute leptospirosis outbreak, we should prioritise the most common non-adapted serovars bacterins that cause incidental infections in cattle (Pomona, Bratislava, Icterohaemorrhagiae, Grippotyphosa and Canicola). Vaccination provides humoral immunity to animals so they are protected against the clinical manifestations of leptospirosis, preventing the disease from being transmitted between animals and humans.

Eight sampled animals had detectable antibodies (≥1:10). From these, two had low titres (<1:100), and six had titres higher than 1:100. Circulating antibody titres greater than 1:100 are considered positive according to WOAH criteria (WOAH 2018). Five of these positive samples (83%) had a positive agglutination titre (≥1:100) for serovars Mozdok and Pomona. Both serovars Pomona and Mozdok belong to the Pomona serogroup. This corroborates Rocha (Rocha 1998), where a high seroprevalence of the Pomona serogroup was observed in the same region of the outbreak, in southern Portugal. In another study on horses, Rocha (Rocha et al. 2004) found that the highest percentages of titres were observed in the Australis and Pomona serogroups. In contrast, in a meta-review considering the whole Europe (Sohm et al. 2023), serogroup Pomona was only the fifth most reported. Only two animals displayed positive reactions to the serovars Hardjo and Grippotyphosa, which are the most reported in the literature for cattle in Europe (Sohm et al. 2023). To our knowledge, no further bovine leptospirosis serological surveys were performed in Portugal.

Two apparently healthy adult cows had considerably high agglutination titres, despite not showing any visible clinical signs. The number of positive cows could have been greater if a complementary diagnostic had been used. According to Jones (Jones, Johnson and Heuer 2023) and Aymmée (Aymée and Lilenbaum 2024), MAT has some sensitivity limitations in endemic areas or in high prevalence herds. Despite not having performed a comprehensive survey due to the lack of reproductive records in most of the herds, there is a strong chance of chronically infected breeders on these farms. It is essential to consider this disease, particularly when reproductive performance fails. It is necessary to implement control measures to minimise production losses in cattle herds and simultaneously protect public health by reducing the risk of human infection.

Environmental risk factors such as extreme weather conditions (wet and warm), season (spring), animal movement and sharing of pastures and watercourses with wildlife are thought to increase the risk of the onset of infection in susceptible animals (Zamir et al. 2022). Biosecurity measures (Fávero et al. 2017), animal characteristics like age, sex, breed or type of production are also considered risk factors (Sohm et al. 2023) In this outbreak, many of these risk factors were present: the outbreak started in mid-spring, initially over an extended period of heavy rainfall (monthly average of 95.5 mm) followed by warm days (average daily temperature 14.4°C); animals were raised outside, sometimes in high-density feedlots, sharing waters troughs, feeders and pasture; there was no wildlife or rodent control. In this regard, it was recommended that farmers isolate sick animals, destroy all contaminated products and change the pasture or paddock location for cohabiting cattle.

The climate changes experienced in recent years have caused an increase in the average temperature and an abnormal distribution of rainfall throughout the seasons. These alterations have developed favourable conditions for the survival of leptospires in the environment. Therefore, more frequent outbreaks of leptospirosis are expected. Given the impossibility of eradicating the agent from the environment, implementing vaccine prophylaxis is recognised as the most effective and practical method of controlling leptospirosis. Even though, it is essential that the applied vaccines include the regional pathogenic strains. Therefore, it becomes crucial to develop more comprehensive serological surveys, collecting data on the prevalence of certain serovars by region, making it possible to provide an up-to-date characterisation of the infecting population. In essence, these studies would allow for the implementation of control measures according to the situation encountered. Combining the latest studies with updated clinical reports becomes essential to tackle bovine leptospirosis during field outbreaks.

Author Contributions

Gustavo Paixão: Conceptualisation; writing–review and editing (lead); methodology (lead). **Sofia Botelho-Fontela**: writing–review and editing (equal). **Filipe Gandra**: formal analysis (lead). **Joana Reis**: writing–review and editing (equal).

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Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been followed.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

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References

Adler, B. 2015. "History of Leptospirosis and Leptospira." *Current Topics in Microbiology and Immunology* 387: 1–9.

Adler, B., and A. de la Peña Moctezuma. 2010. "Leptospira and Leptospirosis." *Veterinary Microbiology* 140, no. 3–4: 287–296.

Alt, D. P., R. L. Zuerner, and C. A. Bolin. 2001. "Evaluation of Antibiotics for Treatment of Cattle Infected With Leptospira borgpetersenii serovar hardjo." *Journal of the American Veterinary Medical Association* 219, no. 5: 636–639.

Aymée, L., and W. Lilenbaum. 2024. "Comments on the Sensitivity Variation of Serology to Diagnose Bovine Leptospirosis: Facing the Chronic Infection." *Preventive Veterinary Medicine* 224: 106136.

Bautista, J. M., M. Aranda Estrada, L. Gutiérrez Olvera, R. Lopez Ordaz, and H. Sumano López. 2022. "Treatment of Bovine Leptospirosis With Enrofloxacin HCl 2H(2)O (Enro-C): A Clinical Trial." *Animals (Basel)* 12, no. 18: 2358.

Cilia, G., F. Bertelloni, S. Albini, and F. Fratini. 2021. "Insight Into the Epidemiology of Leptospirosis: A Review of Leptospira Isolations From "Unconventional" Hosts." *Animals (Basel)* 11, no. 1: 191.

Delooz, L., G. Czaplicki, F. Gregoire, et al. 2018. "Serogroups and Genotypes of Leptospira Spp. Strains From Bovine Aborted Foetuses." *Transboundary and Emerging Diseases* 65, no. 1: 158–165.

Di Azevedo, M. I. N., A. Borges, F. Kremer, J. de Melo, F. A. Carvalho-Costa, and W. Lilenbaum. 2023. "Genetic Analysis of LigA, LipL32, Loa22, and OmpL1 Antigens From Leptospira Spp. Sejroe Serogroup: How Close We Are to a Vaccine against Bovine Leptospirosis?" *Current Microbiology* 80, no. 9: 310.

Ellis, W. A. 2015. "Animal Leptospirosis." *Current Topics in Microbiology and Immunology* 387: 99–137.

Fávero, J. F., H. L. de Araújo, W. Lilenbaum, et al. 2017. "Bovine Leptospirosis: Prevalence, Associated Risk Factors for Infection and Their Cause-effect Relation." *Microbial Pathogenesis* 107: 149–154.

Goarant, C. 2016. "Leptospirosis: Risk Factors and Management Challenges in Developing Countries." *Research and Reports in Tropical Medicine* 7: 49–62.

Guzmán Pérez, M., J. J. Blanch Sancho, J. C. Segura Luque, F. Mateos Rodriguez, E. Martínez Alfaro, and J. Solís García Del Pozo. 2021. "Current Evidence on the Antimicrobial Treatment and Chemoprophylaxis of Human Leptospirosis: A Meta-Analysis." *Pathogens* 10, no. 9: 1125.

Ibrahim, N. A., B. M. Alrashdi, Y. F. Elnaker, et al. 2022. "Serological Investigation and Epidemiological Analysis of Bovine Leptospirosis in Egypt." *Tropical Medicine and Infectious Disease* 7, no. 9: 208.

Jones, G., W. O. Johnson, and C. Heuer. 2023. "Modelling Variation in Test Sensitivity for Monitoring Leptospirosis in Beef Cattle." *Preventive Veterinary Medicine* 221: 106074.

Liegeon, G., T. Delory, and M. Picardeau. 2018. "Antibiotic Susceptibilities of Livestock Isolates of leptospira." *International Journal of Antimicrobial Agents* 51, no. 5: 693–699.

Lilenbaum, W., R. Varges, F. Z. Brandão, et al. 2008. "Detection of Leptospira Spp. In Semen and Vaginal Fluids of Goats and Sheep by Polymerase Chain Reaction." *Theriogenology* 69, no. 7: 837–842.

Loureiro, A. P., and W. Lilenbaum. 2020. "Genital Bovine Leptospirosis: A New Look for an Old Disease." *Theriogenology* 141: 41–47.

Munoz-Zanzi, C., E. Groene, B. M. Morawski, et al. 2020. "A Systematic Literature Review of Leptospirosis Outbreaks Worldwide, 1970–2012." *Revista Panamericana De Salud Pública* 44: e78.

Osorio-Rodríguez, E., D. Rodelo-Barrios, C. Rebolledo-Maldonado, et al. 2024. "Acute Kidney Injury Associated With Severe Leptospirosis: Fatal Re-Emerging Disease in Latin America." *Kidney and Dialysis* 4, no. 2: 78–92.

Picardeau, M. 2017. "Virulence of the Zoonotic Agent of Leptospirosis: Still Terra Incognita?" *Nature Reviews Microbiology* 15, no. 5: 297–307.

Pinto, P. S., C. Pestana, M. A. Medeiros, and W. Lilenbaum. 2017. "Plurality of Leptospira Strains on Slaughtered Animals Suggest a Broader Concept of Adaptability of Leptospires to Cattle." *Acta Tropica* 172: 156–159.

Pires, B. C., J. Berzin Grapiglia, L. Moreira, L. H. Jaeger, F. A. Carvalho-Costa, and W. Lilenbaum. 2018. "Occurrence of Uterine Carriers for Leptospira Interrogans on Slaughtered Cows." *Microbial Pathogenesis* 114: 163–165.

Rajapakse, S. 2022. "Leptospirosis: Clinical Aspects." *Clinical Medicine (London)* 22, no. 1: 14–17.

Rocha, T. 1998. "A Review of Leptospirosis in Farm Animals in Portugal." *Revue Scientifique Et Technique* 17, no. 3: 699–712.

Rocha, T., W. A. Ellis, J. Montgomery, C. Gilmore, J. Regalla, and S Brem. 2004. "Microbiological and Serological Study of Leptospirosis in Horses at Slaughter: First Isolations." *Research in Veterinary Science* 76, no. 3: 199–202.

Samrot, A. V., T. C. Sean, K. S. Bhavya, et al. 2021. "Leptospiral Infection, Pathogenesis and Its Diagnosis—A Review." *Pathogens* 10, no. 2: 145.

Schuller, S., T. Francey, K. Hartmann, et al. 2015. "European Consensus Statement on Leptospirosis in Dogs and Cats." *Journal of Small Animal Practice* 56, no. 3: 159–179.

Silva, A., P. J. A. Farias, M. Silva, et al. 2019. "High Frequency of Genital Carriers of Leptospira Sp. in Sheep Slaughtered in the Semi-arid Region of Northeastern Brazil." *Tropical Animal Health and Production* 51: 43–47.

Sohm, C., J. Steiner, J. Jöbstl, et al. 2023. "A Systematic Review on Leptospirosis in Cattle: A European Perspective." *One Health* 17: 100608.

Sykes, J., K. Hartmann, K. F. Lunn, G. E. Moore, R. A. Stoddard, and R. E. Goldstein. 2011. "2010 ACVIM Small Animal Consensus Statement on Leptospirosis: Diagnosis, Epidemiology, Treatment, and Prevention." *Journal of Veterinary Internal Medicine* 25, no. 1: 1–13.

Vincent, A. T., O. Schiettekatte, C. Goarant, et al. 2019. "Revisiting the Taxonomy and Evolution of Pathogenicity of the Genus Leptospira Through the Prism of Genomics." *PLoS Neglected Tropical Diseases* 13, no. 5: e0007270.

WOAH. 2018. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 8th ed. Vol. 1–3. 1092–1106. Paris, France: Office international des epizooties.

Zamir, L., M. Baum, S. Bardenstein, et al. 2022. "The Association Between Natural Drinking Water Sources and the Emergence of Zoonotic Leptospirosis Among Grazing Beef Cattle Herds During a human Outbreak." *One Health* 14: 100372.

Zarantonelli, L., A. Suanes, P. Meny, et al. 2018. "Isolation of Pathogenic Leptospira Strains From Naturally Infected Cattle in Uruguay Reveals High Serovar Diversity, and Uncovers a Relevant Risk for human Leptospirosis." *PLOS Neglected Tropical Diseases* 12, no. 9: e0006694.

Žele-Vengušt, D., R. Lindtner-Knific, N. Mlakar-Hrženjak, K. Jerina, and G. Vengušt. 2021. "Exposure of Free-Ranging Wild Animals to Zoonotic Leptospira Interrogans Sensu Stricto in Slovenia." *Animals (Basel)* 11, no. 9: 2722.