Biofilm-Mediated Survival of *Leptospira* spp.: A Comprehensive Review on Molecular Basis and Control Strategies

Robby Wijayanto[1]*, Chalida Nahendra Zilfiarani[1], Moch. Ilham Riza Fahlefi[1]
[1]Division of Veterinary Public Health and Disease Sciences, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

Corresponding author: robbywijayanto006@gmail.com

ABSTRACT

Leptospirosis is a significant tropical zoonosis, with a considerable burden on morbidity and mortality in humans as well as animals. One of the possible explanations is biofilm formation, which is the accumulation of EPS, eDNA, and c-di-GMP signals that respond to various environmental, antibiotic, or host-immune challenges. This review focuses on the molecular mechanisms underlying biofilm formation, ecological significance, connection to antimicrobial resistance, and the public health implications. Elucidation of the c-di-GMP regulatory networks, adhesin gene and protein expression, and other metabolic shifts account for the survival of *Leptospira* biofilms in autochthonous populations of aquatic habitats and kidney colonization of reservoir hosts. In addition, biofilms have been associated with long-term bacterial colonization, chronic urine stream associated with persistent bacterial shedding, and failed antibiotics. This scenario, from an epidemiological perspective, facilitates the emergence of anthropogenic infections, and perpetuates the endemic nature of the disease. From a disease-control perspective, biofilms increase the burden associated with persistent infections. This review emphasizes that, as a survival strategy, biofilms represent several potential avenues for the implementation of novel control strategies, including the use of antibiofilm agents, quorum-sensing inhibitors, and multi-epitope vaccines. The functional and integrated dissection of biofilms positioned *Leptospira* spp. to novel One Health-based control strategies for the disease.

Keywords: *Leptospira,* biofilm, c-di-GMP, antibiotic resistance

Received: May 11, 2025 **Revised:** August 5, 2025 **Accepted:** November 29, 2025

INTRODUCTION

Leptospirosis is widespread and deadly purely zoonotic disease, with a greater prevalence among the human population, particularly in South and Southeast Asia. According to the World Health Organization, it is distinctly classified as a major new and re-emerging disease (Karpaham and Ganesh, 2020). The estimated global annual mean number of leptospirosis cases is approximately 1.03 million (Costa *et al.*, 2015). The disease is transmissible via infected animals, and the potential for transmission is greater as a result of urbanization and climate change (Cilia *et al.*, 2020).

The members of the genus *Leptospira* are able to persist within the environment by forming biofilms. Biofilms and their exopolymeric substances (EPS), extracellular DNA (eDNA), and cyclic di-GMP (c-di-GMP) represent of multiple levels of colonization, adherence, and persistence in the aquatic environment as well as the renal tubules which assists in the transmission and host reservoir infectious disease dynamics (Diaz and Pinna, 2025). The biofilms also contribute to the global spread of leptospirosis by allowing the pathogen to persist in soil and water. *Leptospira* are

able to survive outside a host for extended periods in soil and water by leveraging biofilms to combat UV light, temperature changes, and antimicrobials (Kumar *et al.*, 2015).

Difficulties associated with overcoming biofilms, and their contribution to *Leptospira* persistence, arise from the toughness and persistence of biofilms. The variation within and among bacterial species and their adaptive mechanisms results in biofilms being even more difficult to manage, thus greatly complicating efforts to mitigate contamination and infection (Muhammad *et al.*, 2020). The biofilm structure enables bacteria to resist antibiotics and evade the immune system, leading to persistence of clinically subverted infections. In the case of *Leptospira*, the chronic colonization in reservoir hosts facilitates relentless environmental shedding, thus posing a risk to potential infection of humans and other animals (de Carvalho *et al.*, 2023).

In the field of biofilm research, this review offers a comprehensive description of the works related to the genus *Leptospira* and its biofilms in the environment, although it concentrates mainly on the ecological context and in vitro approaches. Work outlining the mechanisms of biofilm formation and integration between biofilms, antibiotic resistance, chronic infection, and the control of leptospirosis under the One Health paradigm remains insufficiently developed and not well articulated.

METHODS

This review intends to gather and systematically assess the scholarly literature regarding the biofilms of Leptospira in terms of molecular mechanisms and ecological roles, antimicrobial resistance, and biofilm management and control strategies. Publications from 2015 to 2025 were retrieved from the primary databases, PubMed, Scopus, ScienceDirect, and Google Scholar. The search was conducted using an array of search terms as follows: '*Leptospira* biofilm,' 'c-di-GMP,' 'antimicrobial resistance,' 'biofilm regulation,' 'biofilm formation,' '*One Health*,' and 'control strategies'.

For the reference selection, scientific relevance and validity were given priority. The review was limited to English peer-reviewed literature that discussed the phenomena of biofilm formation, molecular control mechanisms, ecological flexibility, antimicrobial resistance and adjunct vaccine/control strategies of Leptospira spp. In addition, grey literature, that is, non-scientific documents, conference abstracts, and papers with weak methodologies were omitted.

All relevant references underwent systematic review, and major findings were extracted and synthesised qualitatively. The analysis involved distinguishing common molecular pathways, regulatory frameworks, and socio-environmental factors associated with the persistence and virulence of Leptospira biofilms. Comparative analysis across studies was conducted to identify and emphasise emerging patterns, gaps in the literature, and prospective pathways within the *One Health* and disease control paradigm.

RESULT AND DISCUSSION

Molecular basis of biofilm formation in *Leptospira* spp.

Leptospira spp. regulation of biofilm formation on a molecular level entails the intricate coordination of gene expression and behavior for the adhesion, biofilm matrix synthesis, and biofilm matrix maintenance at the biofilm matrix. Within the biofilm matrix, the transition between the planktonic and biofilm forms of *Leptospira* spp. is mediated by the

second messenger, c-di-GMP. Formation of the biofilm matrix also requires additional activation of cellular transcription programs, as suggested by transcriptomic studies. In the case of biofilm matrix formation with polysaccharide biofilm matrix components, the transcription reprogramming encompasses adhesin proteins, exopolysaccharide synthesis, and various other control proteins. Lastly, structural and surface adhesive proteins that mediate adhesion to biotic and abiotic substrates comprise attachment factors, a crucial element in the initiation step of biofilm matrix formation (Davignon *et al.*, 2023).

For pathogenic *Leptospira*, biofilm formation is a major addition to our understanding of its virulence in infections in the environment. The biofilm formation process involves the cyclic-di-GMP (c-di-GMP) signaling molecule, which is produced by *diguanilate cyclases* (DGCs) and broken down by *phosphodiesterases* (PDEs). High concentrations of c-di-GMP in the cell activate the genes responsible for the motility and biofilm matrix formation, thereby inducing biofilm formation. The biofilm matrix is primarily composed of *extracellular* DNA (exDNA), which aids in the adhesion, cohesion and overall structural integrity of the biofilm. Thibeaux *et al.* (2020) reported that modulation of the c-di-GMP signaling pathway generates biofilm mutants that actively form biofilms with decreased stress tolerance, evidencing their competence for biofilm establishment and persistence under adverse conditions.

SAP and systems similar to SAP have been noted in both saprophytic and pathogenic *Leptospira*, albeit with varying degrees of entanglement. In relation to L. biflexa, there have been and continue to be more than 40 identified c-di-GMP-responsive proteins one of which includes the enzyme that degrades c-di-GMP to the physiologically inactive pGpG, and the other effector proteins, a member of which is the PilZ protein, suggesting more than one regulatory complex for which there are interconnections with other systems, notably the cAMP signaling system, the CsrA system, and flagella regulons. In the other instance, L. interrogans has 8 c-di-GMP related proteins shared with the other strain, which more so points to having a more stable basal function and thus less diversity. The more complex adaptation with relation to the environment may correspond to the saprophytic way of life and the pathogenic species may be employing more direct or narrow routes that are specific to their hosts (Vasconcelos *et al.*, 2023).

The maturation of biofilms involves downregulation of both motility and metabolism genes, while genes linked to stress response and homeostasis of the redox state escalate expression. Such configurational stress responses allow *Leptospira* to withstand unfavorable conditions. After dispersal of the biofilm, bacterial cells regain their rapid metabollic state and retain the virulent phenotype (Irola *et al.*, 2016; Davignon *et al.*, 2024). In addition, multiple other interactions within the cAMP signaling pathway and the flagellar assembly also play a significant role in biofilm regulation (Phoka *et al.*, 2021; Vasconcelos *et al.*, 2023).

Ecological and environmental roles of biofilm

Protective and adaptive roles of *Leptospira* biofilms. The biofilm matrix gives structural support and houses the bacteria. The matrix being composed of polysaccharides, lipids, nucleic acids, and inorganic matter constitutes the extracellular biofilm framework. This matrix creates a biofilm shelter which

enplies the bacteria assist them to avoid the bacterial's host's immune system (Skariyachan *et al.*, 2020). The presence of biofilms almost certainly contributes to the bacteria's resistance to the biofilm-embedded bacteria. Antimicrobial resistance is generated when bacteria use their defense mechanisms to avoid therapy. Their mechanisms of resistance serve to inhibit horizontal transfer of resistance genes. bacteria exocellular biofilms can up-regulate their efflux systems, synthesizing resistance enzymes such as β-lactamase, thereby degrading the bioactive antimicrobial agents trapped within the biofilm 255 (de Carvalho *et al.*, 2023).

In the initial phases of maturation, the production of exopolymers develops and the formation of a water channel biofilm is accomplished, enabling the bidirectional flow of nutrients and oxygen and the expulsion of metabolites. After bacteria join a biofilm, they have the capacity for regulated and more battery-efficient expression of stress response genes linked to decreased motility and energy expenditure (Diaz and Pinna, 2025). The combined impact of the above factors results in a greater, nonhomogeneous, and non-minimal increase in the biofilm bacteria's resistance when compared to its planktonic counterpart. This disparity results in a higher chance of treatment failure and an increase in the duration of treatment, resulting in an increase in treatment-associated multidrug resistance (Diaz and Pinna, 2025).

The programmed adjustments within the *Leptospira* biofilms are arguably reflections of the integrated configurations of biofilm protection and transformation at the structural level. Changes in *Leptospira* biofilm metabolism are correlated with reprogramming of the energy metabolism because of limited and challenging environmental conditions. When assessed against planktonic forms, *Leptospira* biofilms exhibit unique protein expression patterns, including the synthesis of stress response proteins such as GroEL. The primary function of GroEL is to refold and mend proteins damaged by environmental stress, thereby aiding biofilm survival and stability. Changes in metabolism are also characterized by the increased expression of proteins involved in the degradation of carbohydrates, lipids, and the biosynthesis of EPS. Collectively, these adaptation mechanisms involve the regulation of energy, extracellular polysaccharide (EPS), and protective adhesive, anchoring, and maturation proteins that facilitate biofilm maturation (Kumar *et al.*, 2017).

Interactions among members of *Leptospira* biofilms are incorporated as biofilm structural components and affect the survival of constituent biofilm members *Leptospira*. For example, Azospirillum brasilense, which associates with *Leptospira* biofilms, helps in building biofilms of increased structural strength and in the survival of biofilms during harsher environmental conditions. Increased survival of *Leptospira* as biofilms during co-attachment/symbiotic biofilm establishment is also recognized in Sphingomonas, which has also been reported in river waters. In contrast to the previously mentioned biofilm constituent members, Deliftia and Flavobacterium are speculated to have a negative influence on *Leptospira* survival. In the literature, the soil microbe Azospirillum is associated with *Leptospira* spp when described as producing a biofilm that likely offers increased environmental protective biofilm features (Meganathan *et al.*, 2022) like resilience to UV light,

temperature variation, or antibiotic exposure.

Biofilm and host interaction

The biofilm has been noted to aid in the preservation of *Leptospira* in the rat kidney, where it could go into brief and highly stressful situations, providing conditions so that leptospires are under these low stress levels reflected below since an undetermined period, up- regulation of other chronic processes. The biofilm complex is situated in the renal tubules, keeping *Leptospira* hidden from the immune system and even antimicrobials. This leads to chronic infection where the colonization biofilm is elaborate so that it creates a sealed environment that is incarcerating the bacteria within soft host tissue. *Leptospira* excretion in urine was significantly lower during biofilm formation compared to planktonic bacteria, indicating a reservoir function of biofilm-committed *Leptospira* for colonization, and probably, continued transmission (Santos *et al.*, 2021).

Renal tubules can manifest fusiform masses of L. interrogans, in which the bacteria adopt arrangements akin to in vivo biofilm formations. Various renal environmental factors induced by low and restricted iron availability trigger the formation of such masses. Studies using TEM demonstrate the role of membrane vesicles in cell-to-cell interaction and community formation, enhancing the persistence and survival of the bacterial pathogens during an infection. Such arrangements allow *Leptospira* to reside and multiply, establishing persistent colonization within the renal interstitium. Therefore, biofilm and aggregate formations are mechanisms of survival, which may extend functionality to virulence, or serve as a protective shield against host immunity (Yamaguchi *et al.*, 2018).

Biofilm and antimicrobial resistance

The formation of biofilms by *Leptospira* spps. contributes to persistent infections despite antibiotic treatments. In biofilms, bacteria become encased in an extracellular polymeric matrix, which effectively protects them from antibiotic treatments and immune system attacks. As a result, bacteria may survive even when successfully killing infected cells or alleviating aberrant immune responses. Moreover, biofilm bacteria exhibit altered phenotypic and reduced metabolic activity, enhancing their toleration to xenobiotic pressure. *Leptospira* in horses with RAO, according to Ackermann *et al.* (2021), primarily resides in immune-evading biofilm structures. This immune evasion facilitates persistence although there are high intraocular antibiotic levels and antibody titers.

Extracellular Polymeric Substances (EPS) considerably bolster the penetration of antibiotics and other antimicrobial agents. ExDNA and polysaccharides, which are constituents of the biofilm matrix, contribute to the protective biofilm's density and thus the material becomes more challenging to penetrate for antibiotics. For example, the efficacy of exDNA clearing compounds and DNase I enzyme treatment spa removal increases susceptibility of both saprophytic and pathogenic biofilm architecture in vitro and neutrophil differentiation, the latter potentially targeted for antimicrobial therapy marked by biofilm, confirming EPS' active role in mediating the antimicrobial resistance biofilm (Thibeaux *et al.*, 2020).

Theoretically, the elimination of drug compounds via the efflux pump system should inhibit the effect antibiotics have on intracellular influx. Active biofilm development has been associated with higher expression of

efflux pumps, although these pumps also function in the protective response to external environmental stressors and antibacterials. The c-di-GMP system has been described in the literature as a regulator of biofilm formation and antibiotic resistance in a number of bacterial species. This regulator has a range of effects on gene expression pertaining to bacterial adhesion, biofilm matrix synthesis, and motility. Generally, high concentrations of c-di-GMP enhance the solidity of the biofilm matrix, reducng the efficacy of antibacterials and increasing bacterial resistance to the treatment (Iraola *et al*, 2016).

De Carvalho *et al*. (2023) indicated that the resistance of antibiotics increased in oligotrophic *Leptospira* interrogans biofilms. Resistance was especially observed among biofilm producing strains in comparison to non biofilm forming bacteria. This was indicated by the high MIC_{90} (minimum inhibitory concentration of 90% of the isolates) values that stood out especially in the disuctive ranges of ≥1600 µg/mL for amoxicillin, ampicillin, doxycycline and ciprofloxacin. This again confirms that biofilms provide a greater resistance and the killing of bacteria in biofilms is more difficult to destroy than in a planktonic state. The over all findings pointed out that biofilm production directly adds to the high resistance observed in *Leptospira*.

As demonstrated by Kumar *et al*. (2016), the biofilms formed by *Leptospira* are significantly contributing factors in the resistance to multiple antibiotic compounds that are clinically relevant such as penicillin G, ampicillin, tetracycline, and doxycycline. This demonstrates that the resistance of biofilm bacteria exceeds that of the planktonic cells by five to six times. The biofilms are considerably more resistant than the planktonic bacteria which are about 800-1600 mg/mL or µg/mL, while the planktonic bacteria are in the 25 to 100 mg/mL or µg/mL range.

Epidemiological and public health implications

Leptospira biofilms enhance the viability and transmission potential of leptospirosis. Biofilms promote the survival of bacteria in the presence of antibiotics and aid in developing resistance. This assists the kidney-associated and environmental biofilm-forming *Leptospira* remaining infectious and extending the window for long-range dissemination. This long-range dissemination is increasingly difficult for epidemiological control. Kidney biofilms allow *Leptospira* to persist in chronically infected natural reservoirs domestic and wild hosts enhancing transmission of the pathogen to humans via water and soil. Biofilms grant *Leptospira* buoyancy and additional defense mechanisms against control and therapeutic interventions, perpetuating this epidemiological condition and fostering an environment resistant to surveillance, treatment, and disease spread (Ratet *et al.*, 2014)

The role of carrier animals in the chronic transmission of *Leptospira* via urine is critical to the epidemiology and transmission to other hosts of leptospirosis. In this situation, *Leptospira* is repetitively passed in urine reservoir animals and hosts without sufficient clinical manifestations. Given this situation, the risk of transmission between animals is more considerable, and direct transmission to humans may occur through urine aerosol, soil contact, and broken skin or mucous membranes. From an epidemiological standpoint, chronic excretion of the urine extends the period of infectivity within a population, increasing the chances of *Leptospira* survival and

epidemiological spread. *Leptospira* survival is further exacerbated by its presence in moist, impure urine, complicating control efforts, and extending the period of the disease-endemic. Differences in the excretion of hosts (asymptomatic and long-lived) and incidental hosts (temporary and less severe symptoms) influence the overall disease transmission dynamics differently (Monahan *et al.*, 2009).

The factors of the environment determining biofilm generation in extreme settings include the diverse components of the environment that influence the gene expression of microbes and promote biofilm generation. Biofilm formation offers RP against ultraviolet radiation inducing the removal of biofilm matrix. Increased and impeded temperatures promote and impede the formation of biofilm micropollutant degraders through altering the composition of the biofilm. At low and high extremes of the pH scale, biofilms promote the active cells of bacteria to restructure the extracellular matrix of slime to adapt and overcome adverse environmental conditions. Elevated salinity promotes the granulation of biofilms in bacteria which makes the cells more difficult to destroy. Increased pressure makes more biofilm polysaccharides which permits the cells to be more persistent. Quorum sensing, and second messenger nucleotides of intracellular polysaccharides, control gene expression. These environmental characteristics result in microbial adaptation to less desirable conditions (Yin *et al.*, 2019).

Control strategies targeting biofilm

The development of biofilm control strategies involves the application of tailored structural disruptions to enhance therapy. Antimicrobial biofilm-penetrating agents, including certain nanoparticles and natural antibiofilm agents, are a major focus in this area. Other strategies focus on targeting specific biofilm proteins, including the chaperone GroEL, or interactions with extracellular matrix molecules, which inhibit biofilm formation and increase antibiotic susceptibility. The development of quorum-sensing inhibitors that attenuate biofilm formation in *Leptospira* is also a novel strategy. The use of natural, environmentally friendly, and non-toxic (plant-based) additives in the chemical production and use of antibiofilm agents is an underexplored approach. Biofilm formation and proliferation lead to increased antibiotic resistance and reduced effectiveness of *Leptospira* therapy. Therefore, approaches that directly target biofilms are an effective way to improve therapeutic efficacy (Dias and Pinna, 2025).

Leptospira biofilm destruction can be regulated through vaccination against antigens that affect biofilm formation and maintenance while managing the host immune response. The most prominent adhesion and biofilm formation factors, LipL32, LipL21, and LipL41, and the vaccination targets, can be strategically neutralized by biofilm-protective factors, thereby preventing *Leptospira* adhesion and infection establishment in host tissues or the surrounding environment. Contemporary vaccination approaches, namely multiepitope or chimeric vaccines, designed from multiple antigens and previously developed molecular adhesin vaccines, including OmpL1, OmpL37, and OmpA-like proteins that stop *Leptospira* at the early stages of colonization, also show promise. Other examples include TLR adjuvants, such as NLRs, DNA, mRNA, and chemical adjuvants, which significantly improve vaccine yield. Overall, this may be the first to combat biofilm formation, reduce chronic

infection and environmental containment, antibiotic resistance, and biofilm carriage (Barazzone *et al.*, 2022).

CONCLUSION

Biofilm formation is the primary means by which *Leptospira* spp. survive in the environment and within the host. Molecular regulation of biofilm formation, including c-di-GMP, EPS, and adhesion proteins, assists cells in developing biofilm structures that confer resistance to antibiotics and the immune system. The interaction between the host and the biofilm allows chronic colonization, and the biotic components of the biofilm facilitate the persistence of pathogenic *Leptospira*, broadening the epidemiology of the disease. Overall, the current review highlights the importance of an integrative approach to understanding the molecular determinants, ecological functions, and pathogenic impacts of biofilms in the context of developing control measures. Previous research descriptions have largely been in-depth syntheses of biofilm mechanisms and their potential implications for epidemiology.

Acknowledgment

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Author Contribution

All authors participated to all aspects of this work, including preparation, research, data collecting and analysis, manuscript drafting, and publication approval.

Competing Interest

None.

Ethical Approval

Not applicable.

REFERENCES

Ackermann, K., Kenngott, R., Settles, M., Gerhards, H., Maierl, J., and Wollanke, B. 2021. In vivo biofilm formation of pathogenic *Leptospira* spp. in the vitreous humor of horses with recurrent uveitis. Microorganisms, *9*(9), 1915.

Barazzone, G. C., Teixeira, A. F., Azevedo, B. O., Damiano, D. K., Oliveira, M. P., Nascimento, A. L., andamp; Lopes, A. P. 2022. Revisiting the development of vaccines against pathogenic *Leptospira*: innovative approaches, present challenges, and future perspectives. Frontiers in Immunology, *12*, 760291.

Cilia, G., Bertelloni, F., and Fratini, F. 2020. *Leptospira infections in Domestic and Wild Animals* (p. 128). MDPI-Multidisciplinary Digital Publishing Institute.

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., and Ko, A. I. (2015). Global morbidity and mortality of leptospirosis: a systematic review. PLoS neglected tropical diseases, *9*(9), e0003898.

Davignon, G., Cagliero, J., Guentas, L., Bierque, E., Genthon, P., Gunkel-Grillon, P., and Goarant, C. (2023). Leptospirosis: toward a better understanding of the environmental lifestyle of *Leptospira*. Frontiers in Water, *5*, 1195094.

Davignon, G., Pietrosemoli, N., Benaroudj, N., Soupé-Gilbert, M. E., Cagliero, J., Turc, É., ... and Thibeaux, R. 2024. *Leptospira* interrogans biofilm transcriptome highlights adaptation to starvation and general stress while maintaining virulence. *npj Biofilms and Microbiomes, 10(*1), 95.

de Carvalho, R. R. M., Dias, C. S., Paz, L. N., de Lima Fires, T. M., Figueira, C. P., Damasceno, K. A., and Pinna, M. H. 2023. Biofilm formation in vitro by *Leptospira* interrogans strains isolated from naturally infected dogs and their role in antimicrobial resistance. Heliyon, 9*(*3).

Dias, C. S., and Pinna, M. H. 2025. *Leptospira* biofilms: implications for survival, transmission, and disease management. Applied and Environmental Microbiology, *91*(2), e01914-24.

Iraola, G., Spangenberg, L., Lopes Bastos, B., Graña, M., Vasconcelos, L., Almeida, Á.,and Naya, H. 2016. Transcriptome sequencing reveals wide expression reprogramming of basal and unknown genes in *Leptospira* biflexa biofilms. Msphere, 1*(*2), 10-1128.

Karpagam, K. B., and Ganesh, B. 2020. Leptospirosis: a neglected tropical zoonotic infection of public health importance—an updated review. European Journal of Clinical Microbiology and Infectious Diseases, *39*(5), 835–846.

Kumar, K. V., Lall, C., Raj, R. V., Vedhagiri, K., and Vijayachari, P. 2015. Coexistence and survival of pathogenic leptospires by formation of biofilm with Azospirillum. *FEMS* Microbiology Ecology, *91*(6), fiv051.

Kumar, K. V., Lall, C., Raj, R. V., Vedhagiri, K., Kartick, C., Surya, P., and Vijayachari, P. 2017. Overexpression of heat shock GroEL stress protein in *Leptospira* biofilm. Microbial Pathogenesis, *102*, 8-11.

Kumar, K. V., Lall, C., Raj, R. V., Vedhagiri, K., Sunish, I. P., and Vijayachari, P. 2016. In vitro antimicrobial susceptibility of pathogenic *Leptospira* biofilm. Microbial Drug Resistance, *22*(7), 511–514.

Meganathan, Y., Vishwakarma, A., and Ramya, M. 2022. Biofilm formation and social interaction of *Leptospira* in natural and artificial environments. Research in Microbiology, *173*(8), 103981.

Monahan, A. M., Callanan, J. J., and Nally, J. E. 2009. Host-Pathogen Interactions in the Kidney during Chronic Leptospirosis. Veterinary pathology, *46*(5), 792-799.

Muhammad, M. H., Idris, A. L., Fan, X., Guo, Y., Yu, Y., Jin, X., and Huang, T. 2020. Beyond risk: bacterial biofilms and their regulating approaches. Frontiers in microbiology, *11*, 928.

Phoka, T., Fule, L., Da Fonseca, J. P., Cokelaer, T., Picardeau, M., and Patarakul, K. 2021. Investigating the role of the carbon storage regulator A (CsrA) in *Leptospira* spp. Plos one, *16*(12), e0260981.

Ratet, G., Veyrier, F. J., Fanton d'Andon, M., Kammerscheit, X., Nicola, M. A., Picardeau, M., and Werts, C. (2014). Live imaging of bioluminescent *Leptospira* interrogans in mice reveals renal colonization as a stealth escape from the blood defenses and antibiotics: PLoS Neglected Tropical Diseases, 8*(*12), e3359.

Santos, A. A. N., Ribeiro, P. D. S., da França, G. V., Souza, F. N., Ramos, E. A. G., Figueira, C. P., and Ristow, P. 2021. *Leptospira* interrogans biofilm formation in Rattus norvegicus (*Norway rats*) natural reservoirs. PLoS Neglected Tropical Diseases, *15(*9), e0009736.

Thibeaux, R., Soupé-Gilbert, M. E., Kainiu, M., Girault, D., Bierque, E.,

Fernandes, J., and Goarant, C. 2020. The zoonotic pathogen *Leptospira* interrogans mitigates environmental stress through cyclic-di-GMP-controlled biofilm production. npj Biofilms and Microbiomes, 6*(*1), 24.

Vasconcelos, L., Aburjaile, F., Andrade, L., Cancio, A. F., Seyffert, N., Aguiar, E. R., and Ristow, P. 2023. Genomic insights into the c-di-GMP signaling and biofilm development in the saprophytic spirochete *Leptospira* biflexa. Archives of Microbiology, *205(*5), 180.

Yamaguchi, T., Higa, N., Okura, N., Matsumoto, A., Hermawan, I., Yamashiro, T., and Toma, C. 2018. Characterizing interactions of *Leptospira* interrogans with proximal renal tubule epithelial cells. BMC microbiology, *18*(1), 64.

Yin, W., Wang, Y., Liu, L., and He, J. 2019. Biofilms: the microbial “protective clothing” in extreme environments. International Journal of Molecular Sciences, *20*(14), 3423.

***