Formosa Journal of Science and Technology (FJST)
Vol. 4, No. 3, 2025: 913-924

Virulence Factor of Leptospira sp. Causes of Leptospirosis and Its Management

Conny Riana Tjampakasari[1*], Wisiva Tofriska Paramaiswari[2]
[1]Department of Microbiology, Faculty of Medicine, Universitas Indonesia
[2]Magister Programme, Biomedical Science, Faculty of Medicine, Universitas Indonesia
Corresponding Author: Conny Riana Tjampakasari, connyrianat@yahoo.com

A R T I C L E I N F O

Keywords: Leptospirosis, Virulence Factor, Control

Received : 18, February
Revised : 4, March
Accepted: 18, March

A B S T R A C T

Leptospirosis is often found in tropical and subtropical countries with high rainfall. Leptospirosis outbreaks have been reported throughout the world and in Indonesia is still a problem with the outbreak of several Extraordinary Events in various regions. This bacterium has several virulence factors, including Leptopsiral Microbial Surface Component Recognizing Adhesive Matrix Molecules (MSCRAMM), Lipopolisakarida (LPS), Loa22, LipL32 or hemolysis-associated protein I (Hap I), Protein Len/Protein Lsa24, Protein LigA (Leptospiral Immunoglobulin-Like Proteins) and HemO. The diagnosis of leptospirosis is made based on laboratory examination, but not all countries, especially developing countries, are able to carry out examinations for Leptospira sp., so many cases go unreported. By studying the role of virulence factors, it is hoped that the self-defense mechanisms of bacteria in the host can be identified so that they can increase the potential to prevent their pathogenicity from causing disease. Control of Leptospirosis consists of primary prevention, namely protection of healthy people from VOID Leptospirosis, promotive in nature, and specific protection by means of vaccination; secondary prevention targets people who are already with Leptospira, by preventing complications that can cause death. Efforts to control Leptospirosis must be implemented thoroughly so that outbreaks do not continue.

DOI: https://doi.org/10.55927/fjst.v4i3.36
ISSN-E: 2964-6804
https://traformosapublisher.org/index.php/fjst

INTRODUCTION

Bacteria Leptospira sp. is an etiological agent that causes leptospirosis, one of the zoonotic infections that is widely found in various countries in the world. Leptospira is classified into two groups, namely, pathogenic and non-pathogenic. Rats (rodents) are the main reservoir of this bacterium, it is naturally maintained in the renal tubules and excreted through the urine. Severe leptospirposis (known as Weil syndrome) with a clinical picture of jaundice, impaired kidney function, and bleeding manifestations was identified by Adolf Weil in 1886.

Leptospira sp. It is viable in non-flowing water such as contaminated drinking water, water for bathing, water in swimming pools to water or puddles in floods and increases the risk of transmission of leptospirosis infection to humans, so leptspirosis is widely found in tropical or subtropical countries with high rainfall. Leptospirosis cases in the world are not known precisely, but it is estimated to reach 10-100 cases per 100,000 people living in tropical areas with high humidity. Rodent animal control is necessary to reduce the spread of Leptospira sp bacteria in leptospirosis endemic areas.

Leptospirosis outbreaks have been reported around the world, including Indonesia's Indians, Malaysia, Sri Lanka, Thailand, Europe, Africa, North and South America. This event is classified as a re-emerging disease.3 In 2019, 920 cases of leptospirosis were reported in Indonesia with 122 deaths. These cases were reported from nine provinces (Banten, DKI Jakarta, West Java, Central Java, DI Yogyakarta, East Java, Maluku, South Sulawesi, and North Kalimantan), the annual morbidity of leptospirosis in the recent population of Indonesia was estimated at 39.2 per 100,000 people.

Leptospirosis infection is asymptomatic in reservoir hosts because Leptospira sp colonizes the renal tubules in these animals so that they can be in large amounts of urine. The clinical manifestations of leptospirosis are non-specific, the symptoms found in patients resemble flu symptoms but can be fatal and the symptoms are similar to other infectious diseases such as dengue hemorrhagic fever or hemorrhagic disease due to viral infections. Jaundice due to liver infection is also one of the clinical manifestations that are widely found in leptospirosis patients so that it resembles symptoms of hepatitis infection. The diagnosis of leptospirosis must be assisted by laboratory tests, but not all countries, especially developing countries, are able to conduct examinations for Leptospira sp. The less specific clinical manifestations of leptospirosis and laboratory limitations in testing suspected infected samples have led to a small number of leptospirosis cases being reported in the word.

THEORETICAL REVIEW

Morphology, Characteristics and Classification

Leptospira sp is a spiral-shaped Gram-negative bacterium with a curved tip, lansing, flexible, slow-growing in aerobic conditions, growing optimally at a temperature of 28°C-30°C, with a length of 5-25 µm, a diameter of 0.1-0.3 µm, and a wavelength of 0.5 µm. Leptospira bacteria have a distinctive internal flagella, so they can enter the tissues. Lipopolysaccharides (LPS) are the main antigens involved in serological classification.

These bacteria cannot be seen with a regular light microscope, but must use a dark field microscope. Similarly, this bacterium cannot be stained with Giemsa staining like other Gram-negative bacteria, but must use silver staining (Figure 1).

Figure 1. Microscopic observation of Leptospira sp bacteria using silver staining

This bacterium is divided into 2 species, namely L. interrogans which is a pathogenic strain that causes zoonotic infections and and L. biflexa which is a saprophytic strain from the environment. To distinguish between the two types of bacteria, several types of tests can be used, such as biochemical tests. The diversity of strains between Leptospira speies is differentiated based on serovar reactions in the form of agglutination that is cross-absorbed with homologous antigens using the principle of antigen-antibody reaction. Leptospira serovar is said to be different when >10% of homologous titers are at least 1 of 2 antiseras in repeated tests. The following is the classification of Leptospira sp:

- Filum : Spirochaetes
- Ordo : Spirochaetales
- Family : Leptospiraceae
- Genus : *Leptospira*

The molecular classification of Leptospira sp was carried out by sequencing the 16SrRNA gene, where the sequence is already available in the GenBank. Phylogenetic analysis of the 16SrRNA sequence showed the presence of 3 clades in bacteria of the genus Leptospira consisting of pathogen groups, saprophytes and groups whose pathogenicity is not yet clear (Figure 2).

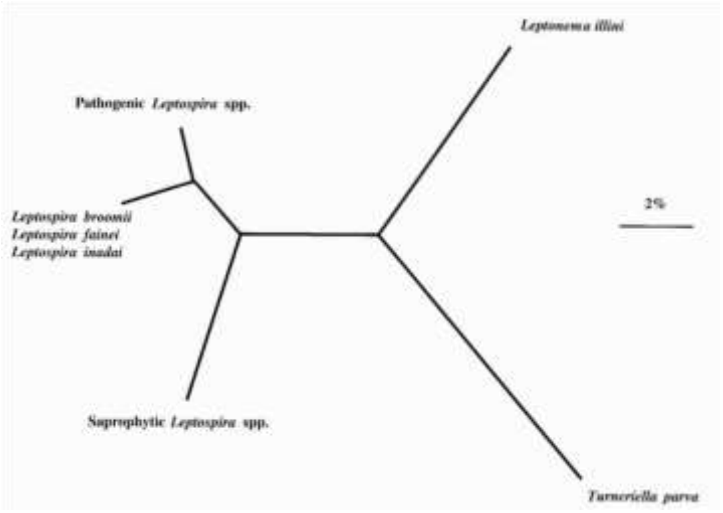

Figure 2. Phylogenetic relationships of bacteria of the genus Leptospira based on 16SrRNA10 gene sequencing

Pathogenesis and Virulence

Leptospira sp. infecting 2 types of hosts, namely reservoir hosts and incidental hosts (Figure 3). Chronic leptospirosis infection in endemic areas is mostly found in reservoir hosts. The reservoir host is a permanent host for the development of Leptospira sp. Each species and serovar of Leptospira sp. relate to specific reservoir hosts and it can be used as an epidemiological study. Specific host reservoirs of Leptospira sp. generally in the form of rodents and small mammals, while incidental host reservoirs such as humans and livestock are reservoirs that experience contact with materials exposed by Leptospira sp. such as urine or feces from a reservoir host and having leptospirosis infection.

Leptospira sp. It is a very thin and motile bacterium, so it can penetrate the mucous membrane through small incisions on the skin of its host and spread through the blood in the tissues and central nervous system. The bacteria that have entered then multiply rapidly and spread through the endothelium of the capillary blood vessels and can cause clinical manifestations such as meningitis, kidney dysfunction and bleeding. Leptospira sp. can be isolated from blood specimens and CSF in the early stages of infection, but when it enters the late stages of infection the bacteria can be isolated from urine specimens.

Elimination of infection by Leptospira is caused by the host's humoral and cellular immune responses. The symptoms of the first phase are caused by tissue damage due to Leptospira, while the symptoms of the second phase arise due to the immune response of the host. Some of the organs that experience disorders due to Leptopsira toxin are the kidneys, eyes, liver, skeletal muscles, blood vessels and heart. When Leptopsira enters the brain membrane through the CSF, the clinical manifestation is meningitis. Meningitis is the most common neurological complication found in leptospirosis.

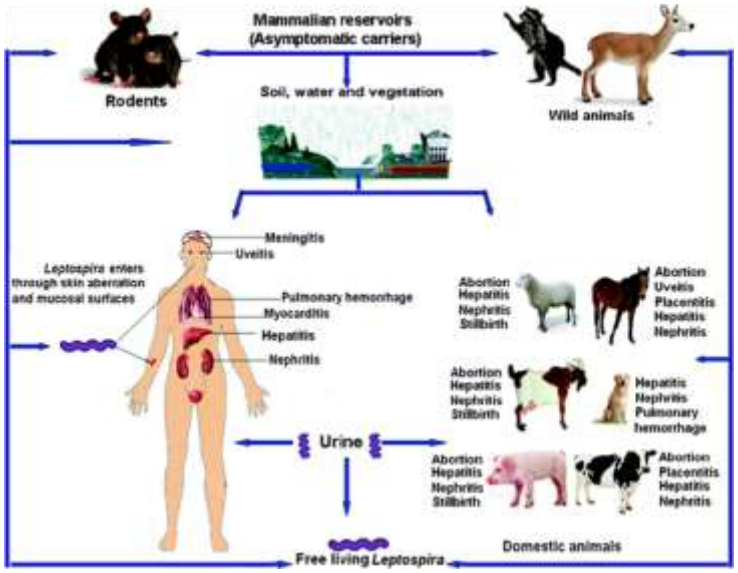

Figure 3. Life cycle of clinical manifestations of leptospirosis in reservoir hosts and incidental hosts.

Infection by Leptospira is facilitated by several virulence factors (Figure 4), namely:

1. The surface protein in leptospira or Leptopsiral Microbial Surface Component Recognizing Adhesive Matrix Molecules (MSCRAMM) which functions to bind various extracellular matrix (ECM) components and mediates the interaction between the host and Leptospira. Surface proteins help in adhesion, invasion and initiation of infection by the bacterium Leptospira sp. to the host.

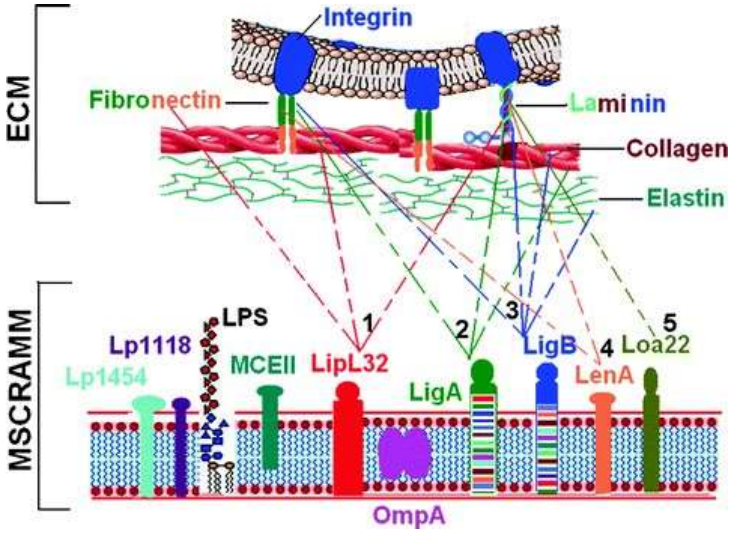

Figure 4. Interactions between Leptopsiral Microbial Surface Component Recognizing
Adhesive Matrix Molecules (MSCRAMM) with Extracellular Matrix membrane
Molecules (ECM) in the host

2. Lipopolysaccharides (LPS) are a virulence factor common in Gramnegative bacteria. LPS is a major component of the outer membrane of bacteria thus contributing to the structural integrity of bacteria. LPS in Leptospira has a similar composition to other Gram-negative bacteria but has lower endotoxic activity. LPS is a determinant of the antigenic diversity of the pathogen Leptopspira sp. due to the variation of O antigens among different serovars, thus serving as a key molecule for diagnostic tests. The presence of atypical lipid A (methylated phosphate) in LPS is not detected by TLR4 in humans causing human susceptibility to infection by Leptospira.
3. Loa22 is the first genetically determined Leptospira virulence factor. Loa22 is highly conserved among pathogenic leptospira and its mutants which are then virulent, both in leptospirosis models of guinea pigs and hamsters. Loa22 is a surface protein recognized by serum from human leptospirosis patients. Loa22 is able to bind laminin found in human extracellular membranes even though the interaction is weak.
4. LipL32 or hemolysis-associated protein I (Hap I) is a lipoprotein that makes up 75% of the outer membrane protein of Leptospira, so this protein is used as one of the specific antigens of Leptospira to establish diagnosis using a serology-based test with the principle of antigen-antibody reaction. The C-terminus portion of LipL32 binds to various ECM components such as laminin, fibronectin, and collagen. LipL32 also induces tubulointerstitial nephritis through a TLR2-dependent pathway followed by activation of the transcription factor Nf-kB, a mitogen-activated protein kinase inducing chemokine and cytokine differentiation as an immune response to infection of the tubuloinsterstitial found in the infected host kidney. LipL32 also induces the activation of the fibrosis pathway and causes the accumulation of fibronectin.
5. Len protein or Lsa24 protein is a 24-kDa protein that binds to laminin as well as complement factor H, fibrinogen and fibronectin. LenA is a member of the leptospiral protein family such as leptospiral endostatins consisting of LenB, LenC, LenD, LenE, and LenF proteins. Len protein functions to bind fibronektin to the extracellular membrane of the host.
6. 6. LigA (Leptospiral Immunoglobulin-Like Proteins) proteins that function as adhesins and determinants of invasion in other pathogenic bacteria such as intimin in Escherichia coli and invasin in Yersinia pseudotuberculosis. LigA proteins are members of the bacterial family of surface proteins that contain 12-13 tandem immunoglobulin-like repeat domains.
7. HemO or heme oxygenase which functions to degrade the tetrapyrol ring from the Heme molecule so that it releases iron in erythrocytes and makes it easier for Leptospira sp. to absorb the iron needed for growth during the infection process. HemO is encoded by the hemO

gene. Disruption of the hemO gene greatly attenuates the virulence of L. interrogans in the leptospirosis hamster model. This suggests that heme is the main source of iron that Leptospira needs when infecting its host.

METHODOLOGY

The method in this article is in the form of a literature review, which is an analysis and synthesis of various literature sources relevant to the research topic or study being conducted that aims to identify and understand existing concepts, theories, and discoveries related to the research topic; analyze and synthesize information from various sources to obtain a more complete and accurate picture of the research topic; identify gaps or shortcomings in previous research that can be addressed in the ongoing research and provide a theoretical and conceptual basis for the ongoing research.

RESULT AND DISCUSSION

Diagnosis

Leptospira sp is a difficult and time-consuming bacterium to cultivate, so molecular-based examinations such as real-time PCR 16S rRNA followed by a sequencing process or PFGE (pulsed-field gel electrophoresis) based examination using PulseNet are widely used to establish leptospirosis diagnosis. However, the bacteria Leptospira sp. can be cultured through specialized media such as Fletcher media, Ellinghausen-McCullough-Johnson-Harris [EMJH] media and Tween 80-albumin media. Leptospira grows for a relatively long time when cultured, with an incubation time of 28-30oC and can show positive results at an incubation time of 2 weeks. Growths can be seen under the surface of the medium in the form of small bands (Dinger's rings).

Leptospira sp. which is viable can survive in clinical specimens such as blood or CSF (cerebrospinal fluid) in the first 10 days of leptospirosis infection, while in urine it can survive within 1 week. However, the bacterial load of Leptospira sp. relatively low in all three clinical specimens so in order to isolate Leptospira from patients with suspected leptospirosis from clinical specimens such as blood or CSF, specimen collection should be performed as soon as possible.

One of the serology-based tests used in reference laboratories for the diagnosis of leptospirosis is the microscopic agglutination test (MAT). The MAT examination uses an agglutination reaction due to an antigen-antibody reaction by the patient's serum against the Viabelle Leptospira bacteria. The agglutination reaction is carried out specifically for certain serotypes so that the agglutination examination uses a pool system of leptopsira antigens and is observed using a microscope. The patient is said to be infected when agglutinin is detected at the patient's serum dilution up to 1:800 (4x antibody titers).

The latest method that is often used is the DNA-based method, namely Polymerase Chain Reaction (PCR). Molecular examination is also used to accelerate the acquisition of microbiological laboratory examination results, which are very important in the management of infectious diseases. These tests are generally very accurate, but they must be performed by experienced

technicians under the supervision of an infectious disease molecular biologist in a qualified facility to obtain accurate results. Specimens that can be examined for molecular microbiology examination of Leptospira are urine and blood. Leptospira circulates in the blood for 10 days after the onset of the disease. The bacteria appear on other body fluids such as urine and cerebrospinal fluid a few days after the onset of the disease. This examination procedure detects genetic material, namely deoxyribonucleic acid (DNA) from disease-causing bacteria through amplification techniques (multiply).

Governance

Treatment with appropriate antibiotics is carried out since the suspect case is clinically established. Therapy for mild cases of Leptospirosis is an antibiotic of choice in the form of Doxycycline 2x100mg for 7 (seven) days except in children, pregnant women, or when there are contraindications. If doxycycline cannot be given, the alternative antibiotic is Amoxicillin 3x500mg/day in adults or 10-20mg/kgBB per 8 hours in children for 7 (seven) days and if there is a history of Amoxicillin allergy, Macrolide is the antibiotic of choice.

In the case of severe Leptospira, antibiotics that can be given include, Ceftriaxone 1-2 grams intravenously for 7 (seven) days; Penicillin Procaine 1.5 million units im per 6 hours for 7 (seven) days; Ampicillin 4 x 1 gram intravenously per day for 7 (seven) days. Supportive therapy is needed when there are complications such as kidney failure, organ bleeding (lung, gastrointestinal, urinary tract, cerebral), shock and neurological disorders.

Risk Factor Control

Leptospirosis control consists of 2 ways, namely, primary prevention and secondary prevention. Primary prevention is the protection of healthy people to avoid Leptospirosis, so that the activities are promotive, and specific protection by means of vaccination. Meanwhile, secondary prevention, which targets people who have been infected with Leptospira, is prevented so that the person avoids complications that will later cause death.

Leptospirosis risk factor control activities are carried out on: sources of infection; transmission channels between the source of infection and humans; and health promotion.

Sources of Infection (Various types of rodents, farm animals, pets)

Rat control is an act of controlling the source of infection. The use of chemicals (rodenticides) should be done wisely by choosing products that are safe for human health and the environment. Rodentides are not automatically used directly, but it is necessary to pay attention to human social environmental factors. In controlling rats, it is recommended to use personal protective equipment in the form of protective clothing, waterproof gloves, masks and hats. Prevention of Leptospirosis transmission will obtain optimal results with integrated rat control, namely combining the various control techniques mentioned above.

Leptospirosis control for certain workers, such as slaughterhouse workers, dairy farmers, veterinarians, garbage/sewer workers, plumbers, and

miners can be done by vaccination. In Indonesia, the use of vaccines on domestic animals (dogs and cats) is commonly carried out as a prevention of Leptospirosis in domestic animals. The Leptospira vaccine for animals is an inactivated vaccine in liquid form (bacterial) which also acts as a solvent because generally the Leptospira vaccine is combined with other vaccines, such as distemper and hepatitis. The Leptospira vaccine in dogs circulating in Indonesia consists of two types of serovars, namely L. canicola and L. ichterohemorrhagiae. The Leptospira vaccine in dogs is given when the dog is 12 weeks old and repeated when the dog is 14-16 weeks old. The immune system after vaccination lasts for 6 months, so dogs need to be vaccinated again every six months. No vaccine provides protection.

Breaking the Flow of Transmission Between Sources of Infection and Humans

This method is carried out by disinfecting water reservoirs and bodies natural water, using chlorine in water reservoirs and water bodies, or using sodium hydroxide.

Management of soils contaminated with pathogenic Leptospira bacteria. Wet soils that have the potential to be exposed to Leptospira bacteria can be a source of transmission for irrigation workers, sugarcane farmers, laboratory workers, veterinarians, slaughtering workers, forest survey workers, and mine workers. To avoid transmission, these workers are recommended to wear special clothing that can protect contact with contaminated soil/materials, such as boots, masks and gloves. It is recommended after work, especially laboratory workers and slaughtering animals to wash work tools with diluted sodium hypochlorite 1:4000 or with detergent.

Health Promotion

Promotive efforts, for the control of Leptospirosis, are carried out by means of education, where between one region and another have different serovars and Leptospirosis epidemics. As Leptospirosis is a classic zoonotic disease in animals that is the main source of infection, therefore any educational program must involve the health/medical professions, veterinarians and social groups of the community involved.

CONCLUSION

Leptospirosis in Indonesia is still a problem with the outbreak of several Extraordinary Events (KLB) in various regions. Many deaths of Leptospirosis cases are caused by delays in early detection and delays in referral to hospitals. Leptospirosis outbreaks that continue to occur in various regions that result in death in humans will cause anxiety in people in Indonesia. By studying the role of virulence factor, it is hoped that the bacterial self-defense mechanism in the host can be known so that it can increase the potential to prevent its pathogenicity in causing disease. In Indonesia, environmental factors have been suspected as risk factors for being infected with Leptospirosis such as flood-prone areas, poor environmental sanitation, areas with a high rat population, so it is feared that KLB will continue to occur if Leptospirosis control efforts are not implemented completely.

RECOMMENDATION

From the results of the literature, this study recommends that leptospira eradication is related to determining the source of infection and then cutting off transmission and health promotion.

FURTHER STUDY

In the future, by knowing the virulence factors of Leptospira, its risk factors and its transmission, eradication can be improved so that the existence of leptospira can be minimized as much as possible.

REFERENCES

Antony VS, Tan CS , Karanam SB, Chamarthy SS, SaiPriya C, Raji P, Emilin RR, Suresh KS and Pooi LM. Leptospiral Infection, Pathogenesis and Its Diagnosis—A Review. Pathogens. 2021:10, 145. https://doi.org/10.3390/pathogens10020145

Asih R, Yos AP, Kurnia D. Correlation of Serology Test Result Against Leptospira Sp. to The Representation of Histopathological Lesions on The Cattle Kidney. Journal of Medical Laboratory Science Technology. Juli 2021:4(1);30-34

Barkah W, Agus I, Agustina H, Kasiyanto, Marleni, Purwaning R, Tri Wahyuningsih. Edukasi Untuk Meningkatkan Pengetahuan dan Kesadaran Masyarakat Dalam Pencegahan Leptospirosis. Jurnal Salingka Abdimas. Juni 2023: 3(1); 177-182

Davignon G, Cagliero J, Guentas L, Bierque E, Genthon P, Gunkel-Grillon P, Juillot F, Kainiu M, Laporte-Magoni C, Picardeau M, Selmaoui-Folcher N, Soupé-Gilbert M-E, Tramier C, Vilanova J, Wijesuriya K, Thibeaux R and Goarant C. Leptospirosis: toward a better understanding of the environmental lifestyle of Leptospira. Frontiers in Water. Juli 2023:5; 1195094.

Fragma AS, Munfika M, Karlina SS. Severe Leptospirosis. Cermin Dunia Kedokteran 298. 2021:48(11):353-356

Guntari TM, Sri H, Hastari W, Ida T, Yuriadi, Irkham W, Yanuartono, Hary P, S Indarjulianto, Slamet R, Alfariza N, Angeline G, Yeo SJ. Identification of Serovar that Caused Canine Leptospirosis in Yogyakarta. Jurnal Sain Veteriner. Desember 2019:37(2);227-231

Isnaini AZ, Dyah MS. Analisis Spasial Kasus Leptospirosis Berdasar Faktor Epidemiologi dan Faktor Risiko Lingkungan. Higeia Journal of Public Health Research and Development. 2020:4(4);587-598

Isnaini AZ, Dyah MS. Analisis Spasial Kasus Leptospirosis Berdasar Faktor Epidemiologi dan Faktor Risiko Lingkungan. Higeia Journal of Public Health Research and Development. 2020:4(4);587-598

Isnaini AZ, Dyah MS. Analisis Spasial Kasus Leptospirosis Berdasar Faktor Epidemiologi dan Faktor Risiko Lingkungan. Higeia Journal of Public Health Research and Development. 2020:4(4);587-598

Isnaini AZ, Dyah MS. Analisis Spasial Kasus Leptospirosis Berdasar Faktor Epidemiologi dan Faktor Risiko Lingkungan. Higeia Journal of Public Health Research and Development. 2020:4(4);587-598

Lester JP, Saraswathi L, Vanessa JD, Richard LF, Carol WM. A Validated Multiplex Real-Time PCR Assay for the Diagnosis of Infectious Leptospira spp. : A Bovel Assay for the Detection and Differentiation os Strains from Both Pathogenic Groups I and II. Forntiers in Microbiology. March 2020:11(457); 1-18

Muhammad FM , Mochammad H. Identifikasi DNA Leptospira sp Pada Sampel Air dan Tanah Di Kota Makassar. As-Syifaa. Juli 2018: 10 (01);7-43. ISSN : 2085-4714

Mujahidah, Maria EK, Desvita S , Subakir. Leptospirosis dengan Gangguan Ginjal : Laporan Kasus. Jambi Medical Journal. 2023: 11(1);117-122.

Muslimin B, Syifa MP, Putri MR, Ramdesima K, Dian W. Study Of Risk Factors And Leptospira Detection Of Sanitary Workers In Jakarta, Indonesia. Biomedika. Aguatus 2022:14(2); 118-126

Muslimin BS , Dian W, Karimulloh. Identification of Leptospira Bacteria in Human Urine According to Islamic View. International Islamic Medical Journal. December 2020:2(1);24-29

Muslimin BS , Dian W, Karimulloh. Identification of Leptospira Bacteria in Human Urine According to Islamic View. International Islamic Medical Journal. December 2020:2(1);24-29

Noraini Philip1, Jaeyres Jani2, Nurul Natasya Azhari1,, Zamberi Sekawi1 and Vasantha Kumari Neela. In vivo and in silico Virulence Analysis of Leptospira Species Isolated From Environments and Rodents in Leptospirosis Outbreak Areas in Malaysia. Forntiers in Microbiology. March 2021:2(753328); 1-15

Novie HR. Leptospirosis. Jurnal Biomedik. November 2016:8(3);143-150

Rima T, Sechan A, Mohammad AA. Penerapan Metode Naïve Bayes Dalam Mendiagnosa Penyakit Leptospirosis. Journal of Computer System and Informatics. August 2022:3(4);521-526

Rima T, Sechan A, Mohammad AA. Penerapan Metode Naïve Bayes Dalam Mendiagnosa Penyakit Leptospirosis. Journal of Computer System and Informatics. August 2022:3(4);521-526

Riyandini P, Susanti, Andi M. Efek Desinfektan Terhadap Leptospira Interrogans Serovar Hardjo. Prosiding Seminar Nasional Teknologi Dan Agribisnis Peternakan X: "Peningkatan Kapasitas Sumberdaya Peternakan Dan Kearifan Lokal Untuk Menghadapi Era Society 5.0" Fakultas Peternakan Universitas Jenderal Soedirman, 20 – 21 Juni 2023, Hal. 322-327

Senaka Rajapakse. Leptospirosis: clinical aspects. Clinical Medicine. 2022:22(1): 14–7

Syamsuar M, Hasnawati A, Sri IR. Identification of Leptospira Serovar in Leptospirosis Suspect Serum in Manggala Subdistrict, Makassar City. Open Access Maced Journal of Medical Sciences.May 2021:14 (9B):407-410.

Tri R, Diah W. Identifikasi Serovar Bakteri Leptospira Pada Tikus Kaitannya Dengan Kejadian Leptospirosis Di Beberapa Kabupaten Di Provinsi Jawa Tengah. Jurnal Pembangunan Manusia. Desember 2014: 8 (3):189-201

Widjayanti W. Epidemiologi, diagnosis, dan pencegahan Leptospirosis. JHECDs.2019: 5 (2) 2019, 62-68

Yolanda H, Evita M. Relationship between Knowledge and Attitude with Behavior of Preventing Leptospirosis in Healthcare Workers at Binjai City. Sumatera Medical Journal. 2022:5(1): 1 – 7