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Other

Study on Seroprevalence and Leptospiral Antibody Distribution Among Livestock Breeders and Farmers in Golestan Province

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ABSTRACT

Background: The high prevalence of leptospirosis in humans is of great public health concern, particularly in tropical and subtropical regions.

Objective: This study aimed to determine the seroprevalence of leptospiral antibodies and the distribution of serovars in livestock breeders and farmers in Golestan province.

Methods: Seventy samples of serum collected from farmers and ranchers suspected of leptospirosis were examined using an ELISA method for surveying Immunoglobulin M (IgM) anti-leptospiria. Also from samples, DNA was extracted and PCR was performed using by primers for *16s rRNA*. Demographic properties of positive patients were analysed.

Results: Chi-square statistical test shows a statistically significant difference between the gender and prevalence leptospirosis (p-value = 0.004). Also, by examining the age, it was shown that 68.57% of patients are in the middleaged rang. According to the results obtained from the study and investigation of blood serum IgM-and Immunoglobulin G (IgG) in people suspected of leptospirosis, 3 cases of the patients had IgM higher than 11 and were known to be positive for leptospirosis. Also, by examining the IgG level of patients, 5 cases had intermediate results, and 2 cases were found to be positive for IgG. The PCR results showed that 41.42% of patients tested positive for the *16s rRNA*.

Conclusion: Leptospirosis is a common disease among farmers, and in Golestan province, considering traditional farming methods, it is considered an important infectious disease. Therefore, health and safety measures should be expanded to control and prevent this disease. Also, by employing mechanised agricultural methods, the prevalence of leptospirosis in this region can be significantly reduced.

1 | Introduction

Leptospirosis is a potentially fatal bacterial infection recognised as an emerging zoonotic spirochetal disease and has major impacts on both humans and animals (Harran et al. 2022; Haake and Levett 2015). The disease is also considered as a re-emerging global public health issue of worldwide importance, especially in tropical and subtropical countries (Md-Lasim et al. 2021). The exact number of human cases in the world is not precisely known because of the lack of surveillance data worldwide. This disease is caused by pathogenic spirochetes from the family Leptospiraceae and from the genus *Leptospira*. The death rate of this disease reaches more than 20%–25% in some areas (Cilia et al. 2021; Md-Lasim et al. 2021).

The genus *Leptospira* is divided into two species, *Leptospira introgans* (pathogenic) and *L. biflexa* (non-pathogenic), whose pathogenic species cause disease with various clinical symptoms in humans and animals (Becirovic et al. 2020). This disease is considered an occupational disease due to direct transmission from animals to humans, and people such as slaughterhouse workers, farmers, livestock farms, veterinarians and veterinary students who are exposed to direct contact with animals are more at risk than others. Occupational contact covers 30%–50% of human cases of leptospirosis (Becirovic et al. 2020; Cook et al. 2017). This disease is one of the most important infectious diseases shared between humans and animals, which has various clinical symptoms. Rapid diagnosis of this disease is very important because only early treatment is effective, and delay in treatment leads to numerous and serious complications, especially in the kidney, and can lead to the death of the patient (Ellis 2015).

Leptospira are excreted from the blood in the first days of the disease and from the urine after about 14 days. Isolation using a specific culture medium requires 6 to 14 days and in some cases up to 5 weeks. The serological response usually occurs 5 to 7 days and sometimes up to 10 days or more after the onset of the disease (Ridzuan, Aziah, and Zahiruddin 2016). Specific Immunoglobulin M (IgM) against surface antigens anti-*Leptospira* can often be detected in the blood from the sixth day, but Immunoglobulin G (IgG) appears 2 weeks after the beginning of the infection and lasts for months (Sahimin et al. 2019). Using of serological methods such as ELISA, which is a method with quantitative and qualitative tests used to detect antibodies in serum, and is a useful method for screening, which is useful and very sensitive in seroepidemiological studies of leptospirosis (Hartskeerl, Collares Pereira, and Ellis 2011; Sahimin et al. 2019; Vernel-Pauillac et al. 2021). Maintaining the purity and stability of the antigenic composition of *Leptospira* spp. reference strains are crucial for accurate leptospirosis diagnosis. Sequencing the *16s rRNA* gene is a reliable method for the molecular characterisation of bacterial species, including *Leptospira* spp. (Baimova et al. 2023).

Since the rapid diagnosis of this disease can help in the prevention of the disease and has a significant role in the health of society, therefore the purpose of this research is to determine the frequency of anti-*Leptospira* antibodies in the serum samples of farmers and ranchers working in Golestan province.

2 | Materials and Methods

2.1 | Study Design and Population

A cross-sectional study was conducted in 2019 in the Golestan province in the northeast region of Iran. The study was granted ethical approval by the Research and Ethics Committee at Golestan University of Medical Sciences (IR.GOUMS.REC.1399.018). All the farmers involved voluntarily signed the informed consent form after they were given a detailed explanation about the procedure and adequate time to decide. There are not any included and excluded criteria for this study. Also, a questionnaire form was designed that included some demographic and job information that was provided to the people.

2.2 | Blood Samples and Serologic Tests

Venous blood samples were tested for the presence of antileptospiral antibodies using the ELISA method following standard methods (Ridzuan, Aziah, and Zahiruddin 2016; World Health Organization 2007).

The EDTA whole blood was centrifuged at 3000 rpm for 5 min to obtain plasma. 10 μL of the plasma was used for IgM ELISA to detect anti-leptospiral antibody, while about 200 μL of plasma was used for DNA extraction and subsequent detection. ELISA was carried out using the automated set of analyser Washer 470 and Reader 270 (BioMérieux) and the *Leptospira* IgM ELISA kit, EIA-4715 (DRG International Inc). None of the plasma samples were excluded from ELISA analysis because of anticipated interference due to sample properties (cloudy lipemic samples) or too little volume to proceed with DNA extraction. The procedure was performed and interpreted according to the manufacturer's instructions. For IgM ELISA, all the samples were run in duplicates and checked whether the replicates gave the same cutoff result. Besides the reading OD by machine, the wells were checked visually with reference to positive and negative control against a white background and the intensity of color formed graded according to the kit insert.

2.3 | Extraction DNA and PCR

DNA was extracted from the plasma using the QIAamp DNA Mini, 250 (Qiagen), following the manufacturer's protocol. Internal positive and negative control samples were included in each batch of DNA extraction procedure and PCR. In this study, a pair of primers specific to *16s rRNA* gene of the *Leptospira* pathogenic was used, amplifying fragments of 650 nucleotides in length (Hartskeel et al. 2001; Honarmand, Khoramizadeh, and Eshraghi 2009) (Tabe 1).

2.4 | Statistical Analysis

Data were entered and analysed using IBM SPSS version 22. All continuous variables were described using means and standard

TABLE 1 | Primer sequences and PCR conditions for amplification of *16s rRNA* gene.

Target	Primer (5'- 3')	Program PCR	Ref
16s rRNA gene	F: GAATCTCTCTTTTGATCTTCG- R: GAGTTAGAGCTCAAATCTAAG	Initial denaturation 7 min Denaturation phase 60 s Annealing phase 60 s. 35 cycle Extension phase 90 s Final extension 10 min	Hartskeel et al. (2001), Honarmand, Khoramizadeh, and Eshraghi (2009)

deviations. Frequencies and percentages were presented for categorical variables. Seroprevalence of leptospirosis was described with a 95% CI. The difference between categories was considered statistically significant when the *p*-value was less than 0.05 (Table 1).

3 | Results

The frequency of participants in the study is different in terms of gender, and male gender constitutes 67.14% of the subjects under study, and the chi-square statistical test shows a statistically significant difference between the participants' gender ($p = 0.004$).

Also, by examining the age of the subjects, it was shown that most of them are in the middle-aged age range (68.57%) and the chi-square test showed two statistically significant differences between the ages of the people suspected of leptospirosis ($p < 0.001$).

In relation to the education level, 88.57% of the participants were illiterate, and their literacy level was below diploma, which indicated that people suspected of leptospirosis had a lower literacy level. By examining the frequency of underlying diseases in the patients, it was found that 18.57% of the participants had underlying diseases. And in this sense, the underlying disease cannot play a role in the occurrence of symptoms suspected of leptospirosis. It was also shown that suffering from suspected leptospirosis is related to the number of years of working in agriculture, and its prevalence in people with less than 5 years of experience is 24.29%, and in people with 5 to 10 years of experience, is 34.29%. And it was equal to 34.29% in people with 10 to 15 years of experience. However, there was no statistically significant difference between the study participants in terms of residence and type of agricultural land ($p < 0.05$) as mentioned in (Tables 2 and 3).

According to the results obtained from the study and investigation of blood serum immunoglobulin M and G in people suspected of leptospirosis, 3 cases (4.29%) of the patients had IgM higher than 11 and were known to be positive for leptospirosis. Also, by examining the IgG level of patients, 5 cases (7.14%) had intermediate results, and 2 cases (2.86%) were found to be positive for IgG (Figure 1).

Among 70 samples of patients, 29 samples had a specific and positive band, and the remaining samples did not have any bands (Figure 2).

TABLE 2 | Demographic characteristics of suspicious patients to leptospirosis.

Variable	Subgroups	Abundance (%)	*p*-value
Gender	*Male*	67.14	0.004
	Female	32.86	
Age	*Teenager*	0	>0.001
	Young	20	
	Middle-aged	68.57	
	Oldster	11.43	
Educational degree	*Illiterate*	7.14	>0.001
	Under diploma	81.43	
	Diploma	7.14	
	Associate degree	4.29	
Habitat	*City*	44.29	0.339
	Village	55.71	
Underlying disease	*Yes*	18.57	>0.001
	No	81.43	
Number of occupation year	*Less than 5*	24.29	0.003
	5–10	34.29	
	10–15	*34.29*	
	More than 15	*7.14*	
Type of agriculture land	*Rice field*	*54.29*	*0.473*
	Non-rice field	*45.71*	

4 | Discussion

Leptospirosis is a potentially fatal bacterial infection. This disease is considered an occupational disease due to direct transmission from animals to humans. The use of serological methods such as ELISA, which is a method with quantitative and qualitative tests used to detect antibodies in serum, is a useful method for screening, which is useful and very sensitive in seroepidemiological studies of leptospirosis. The current study is a type of descriptive and analytical study, and while investigating the prevalence of leptospirosis in rice farmers in the province and subordinate cities, we investigated the underlying factors and risk factors of leptospirosis.

In studies conducted in Iran and the world, the prevalence of *Leptospira* has been investigated. The prevalence of *Leptospira*

TABLE 3 | Frequency of risk factors in subjects under study.

Abundance (%)	Abundance	Subgroups	Variable
35.71	25	Never	Have you ever entered a paddy field with bare feet?
44.29	31	Sometimes (less than once a week)	Have you ever drank paddy field water?
20	14	Always (more than once a week)	Have you ever washed your hands and face with paddy field water?
68.57	48	Never	Have you ever washed your dishes with paddy field water?
27.14	19	Sometimes (less than once a week)	Have you ever washed your clothes with paddy field water?
4.29	3	Always (more than once a week)	
44.29	31	Never	Have you ever entered a paddy field with bare feet?
44.29	31	Sometimes (less than once a week)	Have you ever drank paddy field water?
11.43	8	Always (more than once a week)	Have you ever washed your hands and face with paddy field water?
55.71	39	Never	Have you ever washed your dishes with paddy field water?
40	28	Sometimes (less than once a week)	Have you ever washed your clothes with paddy field water?
4.29	3	Always (more than once a week)	
58.57	41	never	Have you ever entered a paddy field with bare feet?
35.71	25	Sometimes (less than once a week)	Have you ever drank paddy field water?
5.71	4	Always (more than once a week)	Have you ever washed your hands and face with paddy field water?
57.14	40	Never	Have you ever washed your dishes with paddy field water?

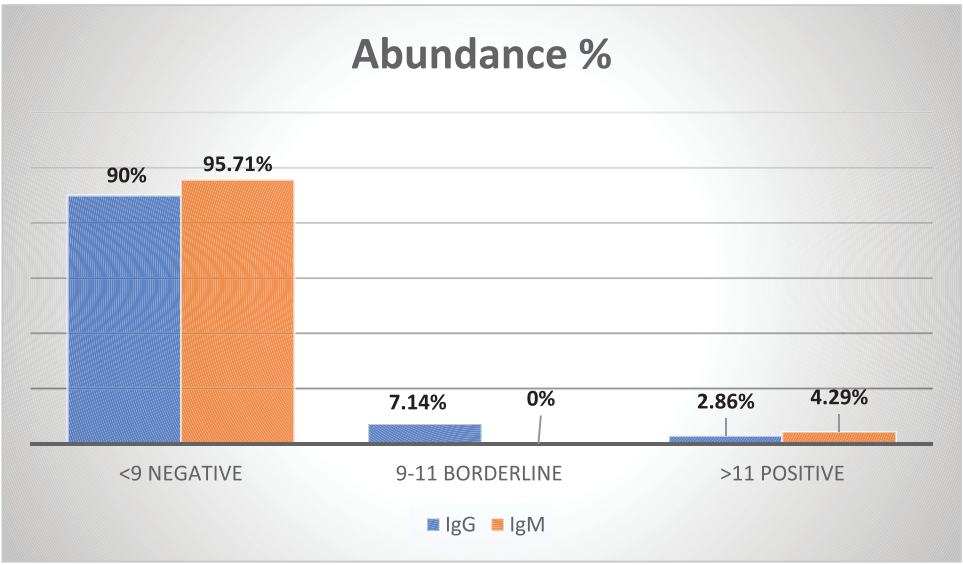

FIGURE 1 | The result of leptospirosis IgG and IgM test in farmers.

serovar Harjo and Gripotyphoza by Ebrahimi et al. is the highest and the lowest prevalence, respectively. In addition, the level of pollution in women is reported to be higher than in men. The high prevalence of leptospirosis in nomadic women is probably due to their hard work such as farming and animal husbandry, while our study shows a high level of pollution in males (Ebrahimi, Nasr, and Kojouri 2004). Leptospirosis has been documented among slaughterhouse workers globally. Risk factors include smoking and drinking on the job, as well as performing tasks like cleaning offal. Cook et al. found that the seroprevalence of antibodies to *Leptospira* spp. was 13.4%. Identified risk factors included having wounds, smoking, eating at work, cleaning offal, and using a borehole for personal water. At the slaughterhouse level, risk factors included having a roof and drawing water from a well

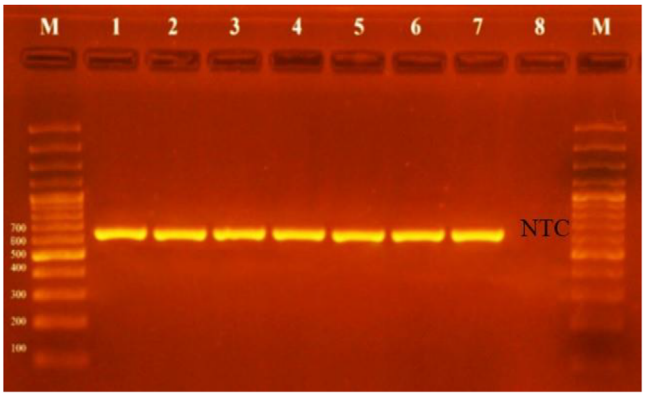

FIGURE 2 | Amplification of *16s rRNA* gene in leptospirosis isolates. Lane M: 100 bp plus DNA ladder; Lanes 1–7: *16s rRNA* amplicons of leptospirosis isolates; Lane 8: negative control.

(Cook et al. 2017). The prevalence rate of *Leptospira* serovars in this study is different from our results, although the results in this study and the results obtained in our survey indicate a high prevalence of *Leptospira* serovars.

Ellis et al. investigated the prevalence of leptospirosis in high-risk groups in India, and out of 25 samples of rural farmers, 8 cases (32%) were reported to be positive for leptospirosis (Ellis et al. 2015). Brockman et al. in Germany showed that people in water sports (especially swimming) are susceptible to leptospirosis and determined that the most common way of leptospirosis transmission of these athletes is through wounds (Brockmann et al. 2010). In another study, Ridzuan et al. revealed that a high prevalence of leptospiral antibodies was found among oil palm plantation workers, particularly among fruit collectors. The most common infecting serovar identified in these workers was serovar Sarawak. Also, the total seroprevalence of leptospiral antibodies was 28.6% (Ridzuan, Aziah, and Zahiruddin 2016). Based on the results above and the current study, it was determined that the prevalence of antibodies in this study (6.89%) is lower than in other studies, with most reports indicating negative results among the participants in the study.

Another study in France shows that the epidemic condition of leptospirosis changes under different weather conditions. Also, a severe form of hepatonephritis was reported in the north of France, which was caused by drinking water contaminated with leptospirosis (Sahimin et al. 2019).

Hope et al. conducted a study that revealed that the prevalence of anti-leptospiral IgM among neonates, as determined by ELISA, was 4.3%. All affected neonates presented with lethargy and poor feeding. Additionally, no pathogenic *Leptospira* species DNA was detected using qPCR (Hope et al. 2022). In the conducted study, results showed that 29 out of 70 patients tested positive for the *16s rRNA* gene. Additionally, the ELISA results indicated that 4.29% of the patients had IgM levels above 11 and were diagnosed as positive for leptospirosis. Additionally, 10% of the farmers tested positive for IgG, indicating past infection and the development of immunity in their bodies.

The study conducted by Samsudin et al. revealed that seroprevalence of leptospirosis among healthy workers was 46.3%. Detection of seropositivity was higher by MAT (46%) than ELISA (15%). We observed high seropositivity among local workers (49%), food handlers (49.5%), females (60.8%) and those aged 34 years and older (46.3%). The workplace places susceptible individuals at risk of leptospirosis (Samsudin et al. 2018). Chintana et al. (2014) reported that sera from some patients can react with more than one serovar. This finding could be due to cross-reaction among various serovars by different exposure times. The highest antibody titer we found was 1 > 1600 for serovar Patoc, in an 18-year-old food handler. The ELISA result also showed the highest optical density (OD) for both IgM and IgG antibodies, indicating that he may have had a true infection although there was no sign or symptoms of leptospirosis during the study period. The results of this study showed that suspected leptospirosis infection was associated with the number of years engaged in farming. The prevalence rate was 29.24% among those with less than 5 years of experience, 34.29% among those with 5 to 10 years of experience, and 34.29% among those with 10 to 15 years of experience. Also, males constituted the most of the individuals in the study. The chi-square statistical test indicated a significant statistical difference between the genders of the participants.

Leptospirosis is a widespread zoonotic disease that affects multiple organs in humans and can result in a range of clinical outcomes. Prasad Dahal et al. reported that 178 serum samples from patients suspected of leptospirosis were tested using the Panbio IgM ELISA at the National Public Health Laboratory in Kathmandu. Among these, 51 samples (28.65%) were positive for the anti-*Leptospira* IgM antibody. Leptospirosis was more prevalent among individuals in their 20s and 30s, who made up 56.86% of the total positive cases. The majority of those who tested positive were farmers, followed by students and housewives. Both animal and water contact appeared to significantly contribute to disease transmission. The symptoms were non-specific, with the most common being fever, headache, muscle pain, abdominal pain, vomiting, jaundice and diarrhoea (Prasad Dahal et al. 2016). In line with the results of the above study, the results of this study also showed that the frequency of IgM antibodies in patients is higher, compared to IgG, but the frequency is lower, compared to the previous study, as a larger population was examined.

5 | Conclusion

The use of serological methods such as ELISA, which is a method with quantitative and qualitative tests used to detect antibodies in serum, is a useful method for screening, which is useful and very sensitive in seroepidemiological studies of leptospirosis, and it can also be used for initial screening. It is possible to prevent the disease by raising awareness and training people at risk and personnel in the health and treatment system of the region, preventing human contact with contaminating things and vaccinating livestock and animals, and in case of diagnosis, quick and timely treatment is possible.

Author Contributions

Conceptualised and designed the experiments; performed the data analysis; wrote the manuscript; approved the revision: Seyed Amir Soltani. Conceptualised and designed the experiments; performed the data analysis: Reza Hoseinpour. Performed the data analysis; wrote the manuscript; collected data; read and approved the article's final draft; approved the revision: Malihe Naderi. Wrote the manuscript; read and approved the article's final draft: Vahideh Hamidi Sofiani. Collected data; read and approved the article's final draft: Amir Mohammad Alborzi.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Statement

The study was approved by the Ethics Committee of the Golestan University of Medical Sciences (Ethics code: IR.GOUMS.REC.1399.018), and all methods were carried out in accordance with relevant guidelines and regulations.

Data Availability Statement

All data associated with the article are available in the manuscript.

Peer Review

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References

Baimova, R. R., Y. U. V. Ostankova, O. V. Blinova, N. A. Stoyanova, and N. K. Tokarevich. 2023. "Molecular and Genetic Characterization of *LEPTOSPIRA* spp. Collection Strains From the St. Petersburg Pasteur Institute Based on 16s rRNA Gene Sequencing Data." *Russian Journal of Infection and Immunity* 13, no. 6: 1040–1048. https://doi.org/10.15789/2220-7619-MAG-17028.

Becirovic, A., F. Numanovic, F. Dzafic, and D. Piljic. 2020. "Analysis of Clinical and Laboratory Characteristics of Patients With Leptospirosis in Five-year Period." *Materia Socio-Medica* 32, no. 1: 15–19. http://doi.org/10.5455/msm.2020.32.15-19.

Brockmann, S., I. Piechotowski, O. Bock-Hensley, et al. 2010. "Outbreak of leptospirosis Among triathlon participants in Germany, 2006." *BMC Infectious Diseases [Electronic Resource]* 10: 91. https://doi.org/10.1186/1471-2334-10-91.

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. https://doi.org/10.3390/ani11010191.

Chintana, C., I. Rajada, P. Yong, and S. Duangjai. 2014. "Interpretation of Microscopic Agglutination Test for Leptospirosis Diagnosis And Seroprevalence." *Asian Pacific Journal of Tropical Biomedicine* 4, no. Suppl. no. 1: 15–17.

Cook, E. A., W. A. de Glanville, L. F. Thomas, S. Kariuki, B. M. Bronsvoort, and E. M. Fèvre. 2017. "Risk Factors for Leptospirosis Seropositivity in Slaughterhouse Workers in Western Kenya." *Occupational and Environmental Medicine* 74, no. 5: 357–365. http://doi.org/10.1136/oemed-2016-103895.

Ebrahimi, A., Z. Nasr, and G. A. Kojouri. 2004. "Seroinvestigation of Bovine Leptospirosis in Shahrekord District, Central Iran." *Iranian Journal of Veterinary Research* 5: 110–113.

Ellis, W. A. 2015. "Animal leptospirosis." *Curr Top Microbiol Immunol* 387: 99–137. http://doi.org/10.1007/978-3-662-45059-8_6.

Haake, D. A., and P. N. Levett. 2015. "Leptospirosis in humans." *Current Topics in Microbiology and Immunology* 387: 65–97. http://doi.org/10.1007/978-3-662-45059-8_5.

Harran, E., C. Hilan, Z. Djelouadji, and F. Ayral. 2022. "Epidemiology of Leptospirosis: The First Literature Review of the Neglected Disease in the Middle East." *Tropical Medicine and Infectious Disease* 7: 260. https://doi.org/10.3390/tropicalmed7100260.

Hartskeerl, R., M. Collares Pereira, and W. Ellis. 2011. "Emergence, Control and Re-Emerging Leptospirosis: Dynamics of Infection in the Changing World." *Clinical Microbiology and Infection* 17, no. 4: 494e501.

Hartskeel, R. A., H. Smits, H. Korver, M. Goris, and W. J. Terpstra. 2001. *Instruction Booklet of International Course on Laboratory Methods for the Diagnosis of Leptospirosis*. Amsterdam, The Netherlands: KIT Royal Tropical Institute Publication.

Honarmand, H., M. Khoramizadeh, and S. Eshraghi. 2009. "Evaluation of Patients Sera for Early Diagnosis of Leptospirosis by PCR." *Journal of Ardabil University of Medical Sciences* 9, no. 4: 353–359. http://jarums.arums.ac.ir/article-1-248-en.html.

Hope, D., S. Businge, S. Kyoyagala, et al. 2022. "Prevalence of Anti-Leptospiral IgM and Detection of Pathogenic *Leptospira* Species DNA in Neonates Presenting With Clinical Sepsis in Southwestern Uganda." *European Journal of Medical Research* 27: 268. https://doi.org/10.1186/s40001-022-00902-w.

Md-Lasim, A., F. S. Mohd-Taib, M. Abdul-Halim, A. M. Mohd-Ngesom, S. Nathan, and S. Md-Nor. 2021. "Leptospirosis and Coinfection: Should We Be Concerned?" *International Journal of Environmental Research and Public Health* 18, no. 17: 9411. https://doi.org/10.3390/ijerph18179411.

Ridzuan, J. M., B. D. Aziah, and W. M. Zahiruddin. 2016. "Study on Seroprevalence and Leptospiral Antibody Distribution Among High-risk Planters in Malaysia." *Osong Public Health and Research Perspectives* 7, no. 3: 168–171. https://doi.org/10.1016/j.phrp.2016.04.006.

Prasad Dahal, K., S. Sharma, J. Bahadur Sherchand, B. Prasad Upadhyay, and D. Raj Bhatta. 2016. "Detection of Anti-*Leptospira* IgM Antibody in Serum Samples of Suspected Patients Visiting National Public Health Laboratory, Teku, Kathmandu." *International Journal of Microbiology* 2016, no.2: 1–4. https://doi.org/10.1155/2016/7286918.

Sahimin, N., S. A. Sharif, I. R. Mohd Hanapi, et al. 2019. "Seroprevalence of Anti-*Leptospira* IgG and IgM Antibodies and Risk Assessment of Leptospirosis Among Urban Poor Communities in Kuala Lumpur, Malaysia." *American Journal of Tropical Medicine and Hygiene* 101, no. 6: 1265–1271. https://doi.org/10.4269/ajtmh.19-0003.

Samsudin, S., S. N. S. Sakinah, O. Malina, et al. 2018. "Seroprevalence of Leptospiral Antibodies Among Market Workers and Food Handlers in the Central State of Malaysia." *Tropical Medicine & International Health* 23, no. 3: 327–333. https://doi.org/10.1111/tmi.13033.

Vernel-Pauillac, F., G. L. Murray, B. Adler, I. G. Boneca, and C. Werts. 2021. "Anti-*Leptospira* Immunoglobulin Profiling in Mice Reveals Strain Specific IgG and Persistent IgM Responses Associated With Virulence and Renal Colonization." *PLOS Neglected Tropical Diseases* 15, no. 3: e0008970. https://doi.org/10.1371/journal.pntd.0008970.

World Health Organization. 2007. *Leptospirosis: Laboratory Manual*. New Delhi: WHO.