MOLECULAR DETECTION OF *LEPTOSPIRA* SPP. IN PIGS IN KOTA BHARU, KELANTAN

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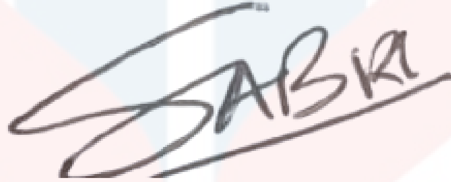
CERTIFICATION

This is to certify that we have read this research paper entitled **'Molecular Detection of *Leptospira* spp. in Pig in Kelantan'** by Corneylius Colodius, and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course DVT 55204 – Research Project.



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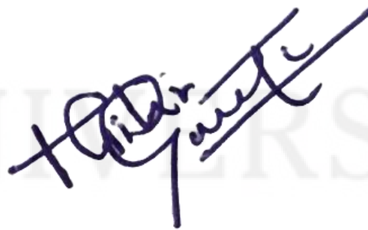

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Thank You

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DEDICATIONS

I would really like to dedicate this dissertation to my family, who have always been a source of support for me. A special thanks to my beloved parents Colodius and Juvina for always believing in me, as well as my siblings Adryna, Adry, Bonnydius, and Bibiannie.

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ABSTRACT

An abstract of the research paper was presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 55204 – Research Project.

Leptospirosis is a serious disease that affects livestock animals including pigs. It is a zoonotic illness with public health implications. This study was carried out to detect the presence of *Leptospira* spp. in pigs in Kelantan via molecular detection. Thus, 30 organ samples were collected, including 20 kidneys and 10 livers of pigs from wet market in Kelantan. Polymerase Chain Reaction (PCR) targeting 16S rRNA gene was used for molecular detection, which was followed by gel electrophoresis. From the results, there was no *Leptospira* spp. detected in collected organ samples from pig in Kelantan. In conclusion, *Leptospira* spp. were not found in samples of pig organs taken from Kelantan wet markets.

Keywords: Leptospirosis, PCR, Pig, 16S rRNA

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ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Leptospirosis adalah penyakit serius yang menyerang haiwan ternakan termasuk babi. Ia adalah penyakit zoonosis yang turut memberi implikasi kepada kesihatan awam. Kajian ini dijalankan untuk mengesan kehadiran *Leptospira* spp. dalam babi di Kelantan melalui pengesanan molekul. Justeru, 30 sampel organ telah dikumpul termasuk 20 buah pinggang dan 10 hati babi dari pasar basah di Kelantan. Polimerase Chain Reaction (PCR) yang menyasarkan gen 16S rRNA telah digunakan untuk pengesanan molekul, yang diikuti oleh elektroforesis gel. Daripada keputusan, tiada *Leptospira* spp. dikesan dalam sampel organ terkumpul daripada babi di Kelantan. Kesimpulannya, *Leptospira* spp. tidak ditemui dalam sampel organ babi yang diambil dari pasar basah Kelantan.

Kata kunci: Babi, Leptospirosis, PCR, 16S rRNA

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1.0 Introduction

Leptospirosis is a zoonotic disease caused by *Leptospira* spp. with any of potentially pathogenic serovars. The disease affects almost all mammals and has a wide range of clinical manifestations, ranging from mild, asymptomatic infection to multiple-organ failure and death (Lunn, 2022). *Leptospira* spp. are obligate aerobic, thin, tightly coiled spirochetes that move in a distinctly flexuous manner. The genus *Leptospira* spp. is divided into three broad categories; pathogenic, intermediate and non-pathogenic (Johnson, 2014). Infection with *Leptospira* spp. can result in leptospirosis, a bacterial disease that affects a wide range of domestic and wild animals, as well as humans worldwide, and has been reported in over 150 mammalian species (Hartmann, 2021). Leptospirosis can also affect farm animals such as cattle, sheep, goats, pigs, South American camelids, and horses (Services, 2021). According to a recent study by Diaz *et al.* (2022), leptospirosis causes major economic losses and an occupational threat in the pig industry. Mild leptospirosis in pigs often undetected on the farm because the associated clinical findings are mild and non-specific, such as light fever, ataxia, and depressed behavior. Chronic leptospirosis, on the other hand, causes abortions, stillbirths, small litter sizes, and weak piglets (Zhitnitskiy, 2015).

A molecular diagnosis and serology are the most often used diagnostic methods for leptospirosis. Molecular tests, such as polymerase chain reaction (PCR), can offer a prompt and accurate diagnosis of leptospirosis, particularly early phase in the illness. PCR can be run on a variety of samples, including blood, urine, cerebrospinal fluid, and tissue samples. Serologic tests, on the other hand, are used to assess the presence of specific immunoglobulin IgM and IgG antibodies against *Leptospira* spp. in serum (Day, 2022).

Leptospirosis is often spread indirectly from infected animals to other susceptible hosts such as animals and humans via contact with water or soil contaminated by infected animal's urine. The bacteria can also be transmitted directly by direct contact with contaminated animal urine, blood, or tissue. Bacteria can enter the body through skin or mucous membrane on the interior of the mouth, nose, or eyes (Vohra, 2020). In the recent years, studies on porcine leptospirosis in Malaysia is very limited including in the north-eastern region. Hence, molecular detection of leptospirosis in pigs, is investigated further in the current study.

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2.0 Research problem

Leptospirosis in pigs has the potential to have a large impact on public health and the economy of pig farms. Infected pigs may develop symptoms or clinical evidence of leptospirosis, which can lead to major health concerns. If the diseased pig spread the bacteria to other pigs on the farm as well as farm workers, a zoonotic risk would emerge. As a result, there is a risk of infection if workers do not take efforts to control leptospirosis in pigs with the help of a veterinarian. Several studies have been carried out to detect *Leptospira* spp. in pigs, but none in Kelantan, Malaysia. As a result, the purpose of this study is to determine the presence of *Leptospira* spp. in pigs in Kelantan using molecular detection.

3.0 Research question

What is the detection rate of *Leptospira* spp. in pigs in Kelantan?

4.0 Research hypothesis

Leptospira spp. in pigs in Kelantan can be detected using a molecular method

PCR.

5.0 Research objective

To detect the presence of *Leptospira* spp. in pigs in Kelantan using the molecular method.

6.0 Literature review

6.1 Etiology of leptospirosis

An infection with spirochete bacteria belonging to the genus *Leptospira* spp. results in leptospirosis. *Leptospira* spp. are tightly coiled spirochetes that range in size from 0.1µm x 6 to 0.1 x 20µm, while certain cultures may contain much longer cells. The helical amplitude is about 0.1 to 0.15µm, while the wavelength is about 0.5µm. The organism have pointed tips that are typically bent into a characteristic hook. The periplasmic space has two axial filaments (periplasmic flagella) with polar insertions. The flagellar proteins have a complex structures (Levett, 2001).

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6.2 Pathogenesis of leptospirosis

Leptospira spp. enter the host through mucous membranes or cuts on the skin or exposure to contaminated water (Adler, 2014). *Leptospira* spp. will quickly enter the bloodstream after infecting the host, causing bacteremia, and spread to variety of organs, including the liver, spleen, kidneys, eyes, central nervous system, and urogenital tract (Sessions & Greene, 2004). The spread of bacteria to multiple organs causes the infected organ to dysfunction, resulting in multiple organ failure and, eventually, death of the infected host (Samrot *et al.,* 2021).

6.3 Predisposing factors in maintenance and transmission of leptospirosis

Reservoir and carrier hosts, such as rodents, can produce a high number of *Leptospira* spp. in their urine, contaminating large and small bodies of water as well as soil. Leptospirosis outbreaks have been related to a variety of circumstances, with contaminated water sources responsible for a high proportion of cases. Open water bodies such as ponds, rivers, lakes, surface water, and moist soil provide a suitable environment for *Leptospira* spp. to live (Wynwood *et al.,* 2014). It can also be found in stagnant or slow moving water, and heavy rainfall or flooding might allow it to survive longer in the environment (Rudolph, 2013). Flooding can also be a risk factor for contamination of water bodies with infected animal urine. Then, without suitable protection, animal-human interaction can pose a risk of infection from occupational or recreational activities (Sulaiman & Lokman, 2011). *Leptospira* spp. is spread through direct contact with infected animal urine or through indirect contact with urine-contaminated soil or water. Farm animals such as cattle, pigs, and goats are common carriers of leptospirosis, but it can also be transmitted by wild animals such as raccoons and porcupines, as well as domesticated dogs (Wang *et al.,* 2020).

6.4 Diagnosis of leptospirosis

For diagnosing of leptospirosis, microscopic agglutination test (MAT) is the gold standard (Hsu & Yang, 2022). In order to perform MAT, patient serum is incubated with various *Leptospira* spp. serovars. Different serum dilutions are tested with the positive serovar to determine MAT titers. The infectious serovar is thought to be the one that responds in higher titer with patient serum (Chirathaworn *et al.,* 2014). A molecular test, Polymerase Chain Reaction (PCR) can also be used to identify leptospirosis. DNA extraction was carried out on the sample that was taken from the

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animal that was suspected to have leptospirosis. The *Leptospira* spp. were amplified using PCR utilising a thermocycler after the DNA was extracted. Then, gel electrophoresis analysis was performed on the PCR products (Ahmed *et al.,* 2012). Ellinghausen-McCullough-Johnson-Harris (EMJH) agar is a widely used standard to culture these organisms (Chideroli *et al.,* 2017). Cerebrospinal fluid, blood, or urine cultures can be used to diagnose leptospirosis; however, this approach has low sensitivity. Isolation of the organism is successful in 5 to 50 percent of cases and may take several weeks (Day, 2022). Dark-field microscopy is used to inspect cultures once a week for up to 13 weeks as they are incubated at 28°C to 30°C before being discarded (Levett, 2001). Histopathology is another method for identifying leptospirosis. Organ samples from the kidney, liver, and lungs were taken and sent for histopathology (Prakoso *et al.,* 2020). *Leptospira* spp. in clinical specimens have been found using a number of histological stains. Silver stains and Warthin-Starry stains were the most commonly used staining methods for histopathological examination (Budihal, 2014).

6.5 Leptospirosis infection in humans in Malaysia

The first human case of severe leptospirosis was identified in Malaysia in 1927. Since its classification as a notifiable disease in 2010, at least 33,000 cases have been reported, with 450 deaths (Neela *et al.,* 2020). Malaysia is endemic for leptospirosis, and recent human fatalities have raised concerns among Malaysian medical professionals. According to Sabah-based studies, people who live close to a national park have a high seroprevalence (25.75%), most likely as a result of exposure to or interaction with wild mammals. In 2011, 186 human cases of leptospirosis, including 13 fatal cases, were reported in Sarawak, increased from 49 cases a year before (Thayaparan e*t al.,* 2013).

6.6 Leptospirosis in pigs

There are very few published data on leptospirosis in pig industry in Malaysia. Previous study reported 25.5% of domestic animals in West Malaysia that underwent a cross-sectional serological screening tested positive for agglutinating antibodies to one or more *Leptospira interrogans* antigens. The prevalence of infection in pigs in West Malaysia was reported to be 16%. Throughout the study period, it was shown that the prevalence of infection in pigs decreased, and it is believed that pigs in West Malaysia are the maintenance host for serovar Pomona (Bahaman *et al*., 1987). In

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addition, according to the study of leptospirosis in pigs in Malaysia by Benacer et al. (2017), only 5 (6%) of 81 urine samples analysed from female pigs were positive for leptospirosis using the PCR method, and one sample was definitively identified as *L. interrogans* serovar Pomona using MAT. Using PCR and DNA sequencing, four positive samples were identified as *L. interrogans* serovar Pomona and one as *L. interrogans* serovar Bratislava with a similarity of up to 99%. Another study from Thailand was conducted in which 400 sera were collected from sows in central Thailand between August 2004 and January 2005 and examined to 20 reference *Leptospira* serovars using a microscopic agglutination test (MAT). The results showed that 10% of the samples had a positive reaction (Niwetpathomwat *et al*., 2006). Acute and chronic infections in pig are primarily described in terms of the reproductive impairment, abortion, stillbirth, and perinatal mortality that cause economic loss. However, descriptions of the general condition which includes haemorrhage, haematuria, renal damage, and death have also been presented. Pigs also appear to be asymptomatically carrying the bacteria, which enables undetected spread and maintenance of the pathogens on farms (Naudet *et al.,* 2022). The important *Leptospira* spp. in pigs comprise two species and numerous serovars namely *L. interrogans* serovars Pomona, Icterohaemorrhagiae, Canicola, and Bratislava, and *L. borgpetersenii* serovars Sejroe and Tarassovi (Davies, 2022).

6.7 Zoonotic impact

Worldwide, leptospirosis is a zoonotic and waterborne illness with regard to morbidity and death in both humans and animals. It is a neglected, re-emerging illness of global public health relevance. Leptospirosis is becoming a major contributor to acute febrile disease in many developing nations because of neglect, rapid, unplanned urbanization, and poor sanitation. As domestic and wild animals can spread *Leptospira* spp., everyone is at risk of contracting the disease. The at-risk population includes medical professionals, animal caregivers, farmers and agricultural workers, fishermen, rodent catchers, water sports enthusiasts, National Disaster Response Force (NDRF) personnel, volunteers for rescue efforts in flood-affected areas, sanitary and sewage workers (Karpagam & Ganesh, 2020).

6.8 Treatment for leptospirosis

Leptospirosis has been extensively treated with antibiotics in cattle, humans, swine,

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and dogs. Initial studies reveal that penicillin is both inhibitory and bacteriostatic in action. Other antibiotics that inhibit *Leptospira* include oxytetracycline, streptomycin, chlortetracycline, chloramphenicol, erythromycin, and tetracycline hydrochloride (Hanson, 1960). The first line of treatment for leptospirosis in human is regarded to be antibiotics, particularly those belonging to the penicillin group. A few clinical trials have also examined the effects of other antibiotics like cephalosporin, chloramphenicol, doxycycline, and azithromycin. The majority of pigs are treated by a single dose of streptomycin at 25 mg/kg of body weight. For new breeders, even those who appear healthy, it is advised in addition to vaccination (Fisheries, 2018).

7.0 Materials and method

7.1 Sample collection

A total of 30 organ samples (20 kidneys and 10 livers) were collected from a wet market in Kota Bharu, Kelantan, Malaysia in October 2022. The samples were individually stored in a sterile seal bag and dispatched to the laboratory in an ice box. Samples were stored and froze at -20°C until further analysis.

7.2 DNA extraction

DNA extraction was performed using a commercial gSYNC^{TM} DNA extraction kit following manufacturer's instruction. Tissue dissociation was performed on each organ, which involved cutting up 30mg of organ tissue and transferring it into a 1.5ml microcentrifuge tube. The organ was ground to a pulp with a micro pestle. The sample tissue was homogenized by grinding after 200µl of GT Buffer was added to the tube. The sample mixture was administered with 20µl of Proteinase K, and the tube was vigorously shaken. The tissue was incubated for one night at 60°C. Lysis was performed following an overnight incubation. The tube was filled with 200µl of GBT Buffer and vigorously shaken for 5 seconds. The lysate was incubated at 60°C for 20 minutes to ensure it was clear. At the same time, an Elution buffer was heated to 60°C for use in the last phase of DNA extraction. Following the incubation of the tissue sample, 200µl of absolute ethanol was added to the tube and vigorously shaken for 10 seconds. A GS column was placed in a 2ml collection tube. The mixture then transferred to the GS column and centrifuged at 14000x g for 2 minutes. The 2ml collection tube was discarded, and the GS column was moved to a new 2ml

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collection tube. 400µl of W1 Buffer was added to the GS column and centrifuged at 14000x g for 30 seconds. The flow through was discarded, and the GS column was reinserted into the 2ml collecting tube. The GS column was loaded with 600µl of wash buffer and centrifuged for 30 seconds at 14000x g. The flow through was then discarded, and the GS column was reinserted into the 2ml collecting tube. The column matrix was then centrifuged again for 3 minutes at 14000x g to dry. The dried GS column was put into a clean 1.5ml microcentrifuge tube for DNA elution. In the centre of the column matrix, 100µl of pre-heated elution buffer was added. To verify that the elution buffer was thoroughly absorbed, the GS column was kept standing for 5 minutes. The GS column was centrifuged again for 30 seconds at 14000x g to elute the pure DNA and extracted DNA was stored at -20 °C.

7.3 Polymerase Chain Reaction (PCR)

The molecular detection of *Leptospira* spp. was performed by PCR targeting 16S rRNA gene. The 16S rRNA gene sequence of the forward primer was 5'-GAA CTG AGA CAC GGT CCA T-3' and sequence for the reverse primer was 5'-GCC TCA GCG TCA GTT TTA GG-3'. The 16S rRNA gene primer is a universal primer (Lu *et al.*, 2015). This primer is designed to target the 16S ribosomal RNA subunit (Gokmen *et al.*, 2016). Each sample, including positive and negative controls, was prepared in a PCR tube. 5µl of DNA sample was placed into the PCR tube, followed by 12.5µl of master mix solution, 1µl forward primer, 1µl reverse primer, and 5.5µl of nuclease free water. Instead of the sample, 5µl of nuclease-free water was added to the negative control and *Leptospira* spp. DNA was added to the positive control. After preparing the positive control, negative control, and all DNA samples, PCR was carried out using a thermal cycler. PCR amplification was initiated at 94˚C for 5 minutes, followed by 30 cycles at 94˚C for 1 minute, 58˚C for 45 seconds, 72˚C for 30 seconds and final elongation at 72˚C for 6 minutes.

7.4 Gel electrophoresis

Following the PCR, gel electrophoresis was performed. The gel was prepared using agarose powder. A weighing boat was used to weigh 0.9g of 1.5% agarose powder. The 1.5% agarose powder was dissolved in a Schott bottle with 60ml of 1x TBE Buffer. The mixture was microwaved for 3 minutes, or until the 1.5% agarose powder was completely dissolved. The agarose solution was mixed with 1µl of

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Midori Green dye. The solution was poured into a gel tray while a well comb was in place. The gel was left to solidify for 30 minutes at room temperature. The well comb was removed once it solidified, and the agarose gel was placed in an electrophoresis tank. After that, the tank was filled with 1x TBE Buffer until the gel was completely covered. The 5µl of 100bp DNA ladder was loaded into the first lane of the gel. The PCR products were loaded to the remaining available wells of the gel, which the last two wells were loaded with positive control (*L. interrogans* serovar Pomona) and negative control (sterile distilled water). The gel was run for 40 minutes at 100V. After 40 minutes, the gel was taken from the tank and the DNA fragment was observed using the GelDoc^{TM} EZ Imager, which referred to the DNA fragment as a band according to its presence on the gel which is at the level of 350 base pair (bp).

8.0 Results

Amplification of the 16S rRNA gene of *Leptospira* spp. showed that all the samples were negative for PCR. **Figure 1** shows all samples were negative for leptopsiral 16S rRNA gene. If *Leptospira* spp. is present, a band should visible on the DNA sample on the same level as the positive control. Only the positive control exhibits a band, indicating the presence of a *Leptospira* spp. DNA at the level of 350bp.

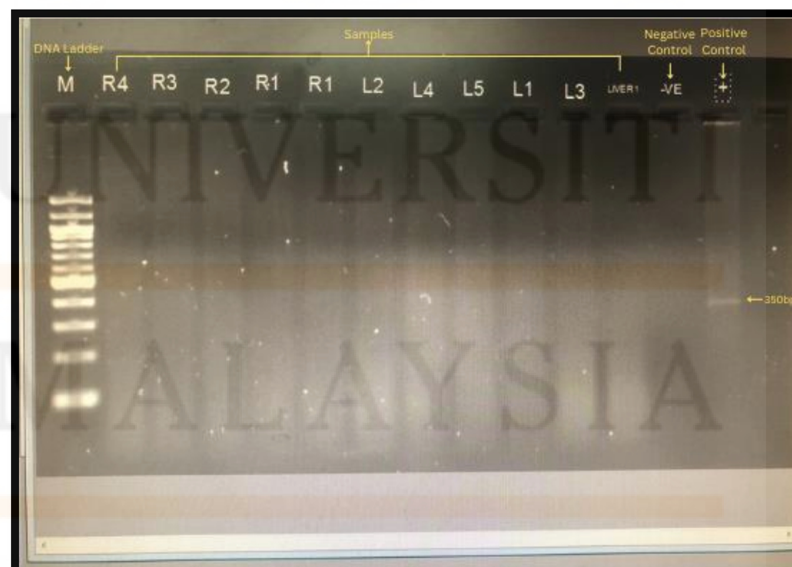

Figure 1: Representative Agarose gel electrophoresis of 16S rRNA gene amplification from the organ samples of pigs. All samples were negative for *Leptospira* spp. 16S rRNA gene. (M= DNA Ladder), (R4, R3, R2, R1, L2, L4, L5, L1, L3, LIVER 1= PCR product of the organ samples), (-ve= Negative control), (+ = Positive control).

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9.0 Discussion

Pig leptospirosis can be caused by different species and serovars of *Leptospira* spp. and is a widespread zoonotic disease. According to a study by Ngugi *et al.* (2019), there is a significant prevalence of leptospirosis positive pigs at slaughterhouses. The possibility for cross-species transmission of pathogenic serovars is highlighted, as is the potential for occupational exposure to slaughterhouse staff. In order to figure out the potential zoonotic risk, current study was carried out to detect the *Leptospira* spp. in pig kidney and liver samples.

Based on the result, the detection rate is 0% for the pig organ samples collected from the wet market of Kelantan. However, this result could be a false negative due to a lack during the conduct of the study, such as a small sample size and a lack of sample types; collected samples that were not fresh and, not adequate diagnostic approaches for leptospirosis. According to a previous study of leptospirosis in pig in Malaysia by Benacer *et al.* (2017), only 5 (6%) of 81 urine samples analysed from female pig were leptospirosis positive based on the PCR approach, and 1 sample was definitively identified as *L. interrogans* serovar Pomona using MAT. With up to 99% similarity, 4 positive samples were identified as *L. interrogans* serovar Pomona and 1 as *L. interrogans* serovar Bratislava using PCR and DNA sequencing. In comparison to this study, the previous study showed a low detection rate of leptospirosis in pigs despite collecting more samples and doing more diagnostic workup. Thus, this study is expected to have a very low detection rate of 0%, which could be due to a small sample size and an insufficient variety of sample types.

Insufficient sample size and variety of samples can result in falsely negative leptospirosis detection results. In this study, just 30 pig's organ samples were obtained and analysed for leptospirosis from a wet market in Kelantan, which is insufficient to determine the presence or absence of leptospirosis. According to Villumsen *et al.* (2012), polymerase chain reaction testing of blood and urine samples allowed for a highly sensitive diagnosis of leptospirosis. As a result, it is preferable to obtain the sample from an abattoir or pig farm in order to gather a larger number of samples with a variety of types of samples such as blood, urine and organ samples.

Since the samples collected for this study were collected from a wet market in Kelantan, it is highly likely that the samples are not fresh because it is unknown for how long the seller kept the organs before the organs were collected. Furthermore,

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the organ sample that was collected was likely manipulated by the seller by washing it, which could affect the results of the study in detection of *Leptospira* spp.

Next, diagnosing leptospirosis by PCR alone is not sufficient due to the fact that PCR is not the gold standard for leptospirosis detection. Microscopic agglutination test (MAT) is the gold standard for diagnosing leptospirosis. In addition, there are a multiple of diagnostic approaches that are suggested in order to diagnose leptospirosis. Approaches like culture and histology could be carried out in order to increase the chance of detecting leptospirosis. According to a study, the sensitivity and specificity of PCR were 52% and 79%, respectively, when compared to the microscopic agglutination test (MAT), which is the gold standard for detecting leptospirosis (Mullan, 2016). Combination of other detection methods with current PCR might be a better solution to overcome false negatives.

10.0 Conclusion

In conclusion, there was no *Leptospira* spp. detected in organ samples of pigs collected from Kota Bharu's wet markets. Further extensive study enrolling samples from pig farms and slaughter houses in all district in Kelantan is recommended.

11. 0 Recommendations

Several obstacles were encountered while completing this research, including the inability to locate a pig abattoir in Kelantan. As a result, another option for acquiring the organ sample was to collect it from a wet market in Kelantan. In the future, it is recommended to collect samples from the abattoir or pig farm in order to collect fresh organ samples as well as other pig samples such as blood and urine. For the future study, the sample size recommended to be increase using relevant sample size determination to produce accurate results. Lastly, samples from the environment, such as stagnant water and moist soil around the wet market, pig farms and slaughterhouses as well as the samples from people who work with pigs, such as farmers, veterinarians and butchers can be collected to estimate the risk of occupational hazard and zoonotic potential of pig leptospirosis.

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References

Adler, B. (2014). Pathogenesis of leptospirosis: Cellular and molecular aspects. *Veterinary Microbiology*, *172*(3-4), 353–358. https://doi.org/10.1016/j.vetmic.2014.06.015

Ahmed, S. A., Sandai, D. A., Musa, S., Hoe, C. H., Riadzi, M., Lau, K. L., & Tang, T. H. (2012). Rapid diagnosis of leptospirosis by multiplex PCR. *The Malaysian Journal of Medical Sciences : MJMS*, *19*(3), 9–16. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3629659/

Bahaman, A. R., Ibrahim, A. L., & Adam, H. (1987). Serological prevalence of leptospiral infection in domestic animals in West Malaysia. *Epidemiology and Infection*, *99*(2), 379–392. https://doi.org/10.1017/s0950268800067868

Benacer, D., Thong, K., Ooi, P., Souris, M., Lewis, J., Ahmed, A., & Zain, M. (2017). Serological and molecular identification of Leptospira spp. in swine and stray dogs from Malaysia. *Tropical Biomedicine*, *34*(1), 1–9. https://eprints.um.edu.my/18323/1/Serological_and_molecular_identification_of_Leptospira_spp..pdf

Budihal, S. V., & Perwez, K. (2013, June 17). *Leptospirosis Diagnosis: Competancy of Various Laboratory Tests*. PubMed Central (PMC). https://doi.org/10.7860/JCDR/2014/6593.3950

Chideroli, R. T., Gonçalves, D. D., Suphoronski, S. A., Alfieri, A. F., Alfieri, A. A., de Oliveira, A. G., de Freitas, J. C., & Pereira, U. de P. (2017). Culture Strategies for Isolation of Fastidious Leptospira Serovar Hardjo and Molecular Differentiation of Genotypes Hardjobovis and Hardjoprajitno. *Frontiers in Microbiology*, *8*, 2155. https://doi.org/10.3389/fmicb.2017.02155

Chirathaworn, C., Inwattana, R., Poovorawan, Y., & Suwancharoen, D. (2014). Interpretation of microscopic agglutination test for leptospirosis diagnosis and seroprevalence. *Asian Pacific Journal of Tropical Biomedicine*, *4*(Suppl 1), S162–S164. https://doi.org/10.12980/APJTB.4.2014C580

Davies, P. R. (2022, February). *Leptospirosis in Swine - Generalized Conditions*. Veterinary Manual. https://www.msdvetmanual.com/generalized-conditions/leptospirosis/leptospirosis-in-swine

Day, N. (2022, October 31). *UpToDate*. Www.uptodate.com. https://www.uptodate.com/contents/leptospirosis-epidemiology-microbiology-clinical-manifestations-and-diagnosis#H15

FYP FPV

FYP FPV

Diaz, E. A., Luna, L., Burgos-Mayorga, A., Donoso, G., Guzman, D. A., Baquero, M. I., Pearson, T., & Barragan, V. A. (2022). First detection of Leptospira santarosai in the reproductive track of a boar: A potential threat to swine production and public health. *PLOS ONE*, *17*(9), e0274362. https://doi.org/10.1371/journal.pone.0274362

Fisheries, A. (2018, November 29). Leptospirosis in pigs. Www.business.qld.gov.au. https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/biosecurity/animals/diseases/guide/leptospirosis-in-pigs#:~:text=A%20single%20dose%20of%20streptomycin

Gokmen, T. G., Soyal, A., Kalayci, Y., Onlen, C., & Koksal, F. (2016). Comparison of 16S rRNA-PCR-RFLP, LipL32-PCR and OmpL1-PCR Methods in the Diagnosis of Leptospirosis. *Revista Do Instituto de Medicina Tropical de São Paulo*, *58*(0). https://doi.org/10.1590/s1678-9946201658064

Hanson, L. E. (1960). Bovine Leptospirosis. A Review. *Journal of Dairy Science*, *43*(4), 453–462. https://doi.org/10.3168/jds.s0022-0302(60)90187-9

Hartmann, K. (2021). *Leptospira spp. infection* /. Abcdcatsvets.org. http://www.abcdcatsvets.org/leptospira-spp-infection/

Hsu, S.-H., & Yang, C.-W. (2022). Insight into the Structure, Functions, and Dynamics of the Leptospira Outer Membrane Proteins with the Pathogenicity. *Membranes*, *12*(3), 300. https://doi.org/10.3390/membranes12030300

Johnson, R. C. (2014). *Leptospira*. Nih.gov; University of Texas Medical Branch at Galveston. https://www.ncbi.nlm.nih.gov/books/NBK8451/

Karpagam, K. B., & Ganesh, B. (2020). Leptospirosis: a neglected tropical zoonotic infection of public health importance—an updated review. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*(5), 835–846. https://doi.org/10.1007/s10096-019-03797-4

Levett, P. N. (2001). Leptospirosis. *Clinical Microbiology Reviews*, *14*(2), 296–326. https://doi.org/10.1128/cmr.14.2.296-326.2001

Lu, Y.-Z., Ding, Z.-W., Ding, J., Fu, L., & Zeng, R. J. (2015). Design and evaluation of universal 16S rRNA gene primers for high-throughput sequencing to simultaneously detect DAMO microbes and anammox bacteria. *Water Research*, *87*, 385–394. https://doi.org/10.1016/j.watres.2015.09.042

Lunn, K. F. (2022, February). *Leptospirosis in Animals - Overview - Generalized Conditions*. MSD Veterinary Manual. https://www.msdvetmanual.com/generalized-

conditions/leptospirosis/leptospirosis-in-animals-overview#:~:text=Leptospira%20are%20maintained%20in%20nature

Mullan, S. (2016). Polymerase Chain Reaction: An Important Tool for Early Diagnosis of Leptospirosis Cases. *JOURNAL of CLINICAL and DIAGNOSTIC RESEARCH*. https://doi.org/10.7860/jcdr/2016/22462.9010

Naudet, J., Crespin, L., Cappelle, J., Kodjo, A., & Ayral, F. (2022). Circulating serogroups of Leptospira in swine from a 7-year study in France (2011–2017). *Porcine Health Management*, *8*(1). https://doi.org/10.1186/s40813-022-00257-y

Neela, V. K., Philip, N., & Sekawi, Z. (2020). Leptospirosis: Malaysia Leptospirosis Research network experience. *International Journal of Infectious Diseases*, *101*, 544–545. https://doi.org/10.1016/j.ijid.2020.09.1413

Ngugi, J. N., Fèvre, E. M., Mgode, G. F., Obonyo, M., Mhamphi, G. G., Otieno, C. A., & Cook, E. A. J. (2019). Seroprevalence and associated risk factors of leptospirosis in slaughter pigs; a neglected public health risk, western Kenya. *BMC Veterinary Research*, *15*(1), 403. https://doi.org/10.1186/s12917-019-2159-3

Niwetpathomwat, A., Luengyosluechakul, S., & Geawduanglek, S. (2006, August). *A serological investigation of leptospirosis in sows from central Thailand*. ResearchGate.https://www.researchgate.net/publication/6677348_A_serological_investigation_of_leptospirosis_in_sows_from_central_Thailand

Prakoso, Y. A., Widyarini, S., & Kurniasih, K. (2020). Histopathology of the Organs from Cattle with Leptospirosis. *Advances in Animal and Veterinary Sciences*, *8*(11). https://doi.org/10.17582/journal.aavs/2020/8.11.1220.1224

Rudolph, L. W. (2013, July). *VetFolio*. Www.vetfolio.com. https://www.vetfolio.com/learn/article/leptospirosis-in-small-animals

Samrot, A., Sean, T. C., Bhavya, K., Sahithya, C., Chan-Drasekaran, S., Palanisamy, R., Robinson, E. R., Subbiah, S., & Mok, P. (2021). Leptospiral Infection, Pathogenesis and Its Diagnosis—A Review. *Pathogens*. https://doi.org/10.3390/pathogens10020145

Services, S. C. V. (2021, August 14). *Leptospirosis—A Shared Disease Among Pets, Farm Animals, and Humans*. Sale Creek. https://salecreek.vet/leptospirosis-a-shared-disease-among-pets-farm-animals-and-humans/

Sessions, J. K., & Greene, C. E. (2004, August). *VetFolio*. Www.vetfolio.com. https://www.vetfolio.com/learn/article/canine-leptospirosis-epidemiology-pathogenesis-and-diagnosis

FYP FPV
UNIVERSITI MALAYSIA KELANTAN

Sulaiman, B., & Lokman, H. (2011). *Guidelines For The Diagnosis, Management, Prevention And, Control of Leptospirosis in Malaysia.* Retrieved February 18, 2023, from https://www.moh.gov.my/moh/resources/auto%20download%20images/589d71cb177d8.pdf

Thayaparan, S., Robertson, I., Fairuz, A., Suut, L., & Abdullah, M. (2013). Leptospirosis, an emerging zoonotic disease in Malaysia. Malaysian Journal of Pathology, 35(2), 123–132.

Villumsen, S., Pedersen, R., Borre, M. B., Ahrens, P., Jensen, J. S., & Krogfelt, K. A. (2012). Novel TaqMan® PCR for detection of Leptospira species in urine and blood: Pit-falls of in silico validation. *Journal of Microbiological Methods*, *91*(1), 184–190. https://doi.org/10.1016/j.mimet.2012.06.009

Vohra, S. (2020). *Leptospirosis*. State.il.us. http://www.idph.state.il.us/public/hb/hblepto.htm

Wang, S., Stobart Gallagher, M. A., & Dunn, N. (2020). *Leptospirosis*. PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK441858/#:~:text=Leptospirosis%20is%20caused%20by%20an

Wynwood, S. J., Graham, G. C., Weier, S. L., Collet, T. A., McKay, D. B., & Craig, S. B. (2014). Leptospirosis from water sources. *Pathogens and Global Health*, *108*(7), 334–338. https://doi.org/10.1179/2047773214y.0000000156

Zhitnitskiy, D. V. M. P. (2015). Leptospirosis. *Open.lib.umn.edu*. https://open.lib.umn.edu/swinedisease/chapter/leptospirosis/

FYP FPV