

Pathological Effects of *Leptospira yasudae* Strain BJ3 Infection in Experimental Golden Syrian Hamster Isolated from Perak, Malaysia

(Kesan Patologi Jangkitan Strain BJ3 *Leptospira yasudae* pada Hamster Syria Emas Uji Kaji yang Dipencilkan dari Perak, Malaysia)

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Received: 4 March 2025/Accepted: 5 August 2026

ABSTRACT

Leptospirosis is a zoonotic disease caused by helical bacteria of the genus *Leptospira* that affects animals and humans. Recently, a newly identified isolate called *Leptospira yasudae* strain BJ3 has been discovered in soil samples obtained from a wildlife conservation center located in Malaysia. Currently, data on the virulence of *L. yasudae* in susceptible hosts is scarce. Hence, this study was conducted to identify the virulence of *L. yasudae* strain BJ3 based on the histopathological effects due to leptospirosis infection towards susceptible hosts. The strain was inoculated intraperitoneally in hamsters at different doses (1×10^8 cells, 2×10^8 cells, and 3×10^8 cells). Histopathological effects of hamsters infected with *L. yasudae* strain BJ3 were compared with negative control (uninfected) and positive control (infected with *L. interrogans* serovar Lai strain Langkawi) hamsters. The results demonstrated that infection with 1×10^8 *L. yasudae* strain BJ3 led to noticeable weight loss. Microscopically, pulmonary edema in the lung, sparse focal inflammatory cells infiltration but with normal hepatocytes architecture in the liver and mild tubular degeneration in the kidney were observed. More vital pathological changes occurred at higher dose (3×10^8 cells). The gross examination showed crucial weight and fur loss. Microscopic examination showed the presence of interstitial pneumonia and lung lesions, hepatocyte expansion, hypertrophy and hyperemia with severe lesions in kidney cells. In conclusion, infection caused by *L. yasudae* strain BJ3 may represent a noteworthy emerging local agent of leptospirosis due to its ability to induce virulence and cause histopathological alterations in the kidneys of susceptible hosts. To the best of our knowledge, this is the first report demonstrating that a novel *Leptospira* species isolated from soil can infect hamsters and induce histopathological changes in organs.

Keywords: Histopathology; hosts; *Leptospira yasudae*; Leptospirosis; virulence

ABSTRAK

Leptospirosis merupakan penyakit zoonotik yang disebabkan oleh bakteria heliks daripada genus *Leptospira* yang menjangkiti haiwan dan manusia. Terdapat satu pencilan baharu yang dikenali sebagai *Leptospira yasudae* strain BJ3 telah ditemui dalam sampel tanah yang diperolehi dari sebuah pusat pemuliharaan hidupan liar di Malaysia. Namun, tahap kevirulenan strain ini pada perumah yang rentan masih kurang difahami. Oleh itu, kajian ini dijalankan bagi mengenal pasti kevirulenan *L. yasudae* strain BJ3 berdasarkan kesan histopatologi akibat jangkitannya terhadap hos yang rentan. Strain ini diinokulasikan secara intraperitoneal kepada hamster pada dos berbeza (1×10^8 sel, 2×10^8 sel dan 3×10^8 sel). Kesan histopatologi pada hamster yang dijangkiti *L. yasudae* strain BJ3 dibandingkan dengan kumpulan kawalan negatif (tidak dijangkiti) dan kawalan positif (dijangkiti dengan *L. interrogans* serovar Lai strain Langkawi). Hasil kajian menunjukkan bahawa jangkitan dengan 1×10^8 *L. yasudae* strain BJ3 menyebabkan penurunan berat badan. Secara

mikroskopik, edema pulmonari pada paru-paru, penyusupan sel keradangan fokal yang jarang tetapi dengan struktur hepatosit yang masih normal pada hati, serta degenerasi tubul yang ringan pada buah pinggang telah diperhatikan. Perubahan patologi yang lebih ketara berlaku pada dos yang lebih tinggi (3×10^8 sel). Pemeriksaan makroskopik menunjukkan kehilangan berat badan dan bulu yang ketara. Pemeriksaan mikroskopik pula mendedahkan kehadiran pneumonia interstitial dan lesion pada paru-paru, pembesaran hepatosit, hipertrofi dan hiperemia serta lesion teruk pada sel buah pinggang. Kesimpulannya, jangkitan yang disebabkan oleh *L. yasudae* strain BJ3 berpotensi menjadi ancaman penting sebagai agen leptospirosis tempatan yang baharu kerana keupayaannya untuk menyebabkan penyakit pada hos yang rentan. Tambahan pula, kajian ini merupakan laporan pertama yang menunjukkan bahawa spesies *Leptospira* baharu yang dipencilkan daripada tanah mampu menjangkiti hamster dan menyebabkan perubahan histopatologi pada organ haiwan tersebut.

Kata kunci: Histopatologi; kevirulenan; *Leptospira yasudae*; Leptospirosis; perumah

INTRODUCTION

Leptospira is a pathogenic bacterium that causes the zoonotic disease leptospirosis and is prevalent in numerous countries worldwide, especially in tropical regions (Rajapakse 2022). Leptospirosis can be transmitted either through direct contact with the skin and mucous membranes or indirectly through exposure to contaminated water or soil (Mazni et al. 2023). Rats are the primary reservoir for *Leptospira* and can excrete around 5.7×10^6 bacteria/mL of urine and the urine volume is 3.3 mL/100 g body weight/day (Shilpa et al. 2024). Domestic and wild animals can also serve as reservoirs or incidental hosts depending on the specific serovar (antigen) of *Leptospira* that infects them (Cilia, Bertelloni & Fratini 2020; Hagedoorn et al. 2024). This is because different animals demonstrate varying responses to the same serovar (Putz & Nally 2020). Various host-serovar associations are commonly observed, such as rats (particularly *Rattus norvegicus* and *R. rattus*) being associated with serovars Icterohaemorrhagiae and Copenhageni, and mice (*Mus musculus*) being associated with the serogroup Ballum (Bharti et al. 2003). Among these, Icterohaemorrhagiae is the serogroup most frequently implicated in human infections (Picardeau 2013). The transmission cycle of this disease typically culminates with humans as incidental hosts (López-Robles et al. 2021).

Leptospira is a motile organism capable of penetrating damaged skin and mucous membranes. Once it successfully enters the host, *Leptospira* spreads through the bloodstream, leading to systemic infection (Wunder et al. 2016a). This can result in mild clinical symptoms like fever or more severe conditions such as Weil's disease and pulmonary hemorrhage (Chacko et al. 2021; Dong & Chen 2021). While some patients experience progressive and potentially fatal infections, there are also cases of chronic infection where the bacteria persist asymptotically (Picardeau 2017). Furthermore, susceptible hosts may show manifestations of acute leptospirosis infection. On the other hand, reservoir hosts can experience chronic

infection, with the bacteria residing in the proximal renal tubules before being shed into the environment through urine. However, these animals do not experience kidney damage such as interstitial nephritis during the infection. This indicates that renal colonization and the pathogenicity of *Leptospira* are host dependent (Dimroth et al. 1999; Fraser et al. 1998).

Leptospira is a highly complex genus that comprises 64 species. Based on the latest taxonomic classification, this genus is divided into four main groups: (i) pathogenic group 1 (P1), which includes strains that cause disease; (ii) pathogenic group 2 (P2) (formerly known as intermediates due to unclear pathogenicity); (iii) saprophytic group 1 (S1) and (iv) saprophytic group 2 (S2) consisting of strains that do not cause disease in infected hosts (Ko, Goarant & Picardeau 2009; Vincent et al. 2019). Previously, a novel *L. yasudae* strain BJ3 was isolated from the soil of an *ex-situ* wild animal conservation area in Perak, Malaysia. Genome sequence analysis indicated that the strain is within the pathogenic (P1) clade of the genus *Leptospira*. However, limited information is currently available regarding the virulence of *L. yasudae* in susceptible hosts. Therefore, the objective of this study was to shed light on the virulence of *L. yasudae* strain BJ3 through experimental infection in golden Syrian hamsters.

MATERIALS & METHODS

BACTERIAL STRAINS

Leptospira yasudae strain BJ3 was isolated from soil collected from a wildlife rehabilitation center in Malaysia (Shamsusah et al. 2021). The strain was maintained in Ellinghausen–McCullough–Johnson–Harris (EMJH) media (BD, USA) at 30 °C under aerobic conditions (Nair & Gomes-Solecki 2020). In this study, *L. interrogans* serovar Lai strain Langkawi, obtained from the Institute for Medical Research (IMR), Malaysia was used as a positive control and were cultured in EMJH as well. To sustain virulence, the bacteria were passaged in hamsters fewer than 10 times (Segawa et al. 2014; Villanueva et al. 2014).

ANIMAL HOSTS

Six- to eight-week-old Golden Syrian hamsters (*Mesocricetus auratus*) (n = 27; 13 males and 14 females) were obtained from the IMR and maintained at its animal research facility under a 12-h light/12-h dark cycle with *ad libitum* access to food and water (Villanueva et al. 2014). Following a one-week acclimatization period, hamsters were anesthetized with 30% (v/v) isoflurane in propylene glycol (Sigma-Aldrich, USA). A total of nine groups were included, with three hamsters per group (n = 3). Sample size was determined based on preliminary experiments and ethical considerations to minimize animal usage and no formal power calculation was performed. Hamsters were intraperitoneally injected with 1 mL of *L. yasudae* strain BJ3 or *L. interrogans* serovar Lai suspensions containing 1×10^8 , 2×10^8 , or 3×10^8 leptospiral cells, respectively. Cell density was determined by dark-field microscopy and adjusted based on preliminary dose-optimization experiments to reproduce acute or chronic infection. Negative control hamsters received 1 mL EMJH medium only. The infected and negative control hamsters were observed three times a day for a period of up to 28 days.

A small amount of blood was collected from the hamsters' cranial vena cava as described by Picazo, Benito and García-Olmo (2009), within four days post-inoculation, for direct culture in the EMJH medium (Shetty, Kundu & Gomes-Solecki 2021). Their body weight was monitored daily, and they were examined for any signs of clinical illness. Endpoint criteria included reduced food and water intake, ruffled fur, external bleeding, weight loss of $\geq 10\%$ relative to the maximum recorded body weight and a moribund state (Coutinho et al. 2014; Guedes et al. 2021; Villanueva et al. 2014). Body weight was recorded every 7 days throughout the study and percentage body weight change was calculated as:

$$\% \text{ body weight change} = \frac{\text{Body weight at day } x - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

Hamsters that developed endpoint criteria were euthanized by isoflurane inhalation followed by cardiac puncture. Hamsters that survived beyond day 28 of infection were also euthanized using similar method. Blood samples were collected from each hamster via a 23 G needle (Terumo, Japan). Internal organs, including the lungs, liver, kidney and spleen were collected and fixed in 10% neutral buffered formalin (NBF) (Sigma-Aldrich, USA) at room temperature for histological analysis. This study received approval from the UKM Animal Ethics Committee (UKMAEC) with code: FST/2020/HANI KARTINI/16-JAN./1081-JAN.-2020-NOV.-2022.

STRAIN ISOLATION FROM BLOOD OF INFECTED HAMSTERS

A drop of blood was inoculated into EMJH medium (BD, USA) and then incubated at a temperature of 28-30 °C. The culture was examined using dark field microscopy (Nikon Eclipse, Japan). In the case of any contamination, it was eliminated by sub-culturing into fresh EMJH medium through a syringe filter (Pall, USA) with a diameter of either 0.45 µm or 0.22 µm, depending on the level of contamination. The cultures were subsequently observed on a weekly basis for a period of three months.

STAINING AND HISTOPATHOLOGY ANALYSIS

Fixed tissues were embedded in paraffin (Sigma-Aldrich, USA) and sectioned at a thickness of 7 µm. To identify any potential pathological alterations, the tissue sections were subjected to hematoxylin and eosin (H&E) staining (Merck, Germany). Fontana silver staining, as described by Gunasekara et al. (2017), was employed to detect the presence of *L. yasudae* strain BJ3 and *L. interrogans* Lai in the tissues. The stained specimens were sent to the histopathology department at Makmal Bioserasi UKM for further analysis.

STATISTICAL ANALYSIS

The statistical analysis of weight changes in hamsters was conducted using GraphPad Prism 9.0.0 software with a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values are considered significant when $p \leq 0.05$ (Coutinho et al. 2014).

RESULTS

VIRULENCE OF *L. yasudae* STRAIN BJ3 IN HAMSTERS

Infection of six- to eight-week-old Golden Syrian hamsters with *L. yasudae* strain BJ3 and *L. interrogans* serovar Lai strain Langkawi at doses ranging from 1×10^8 - 3×10^8 cells did not result in mortality in the tested animals. However, two out of three hamsters infected with 1×10^8 *L. yasudae* strain BJ3 experienced a significant weight loss by day 28 ($p = 0.03$) (Figure 1). Similar effects were observed in one hamster infected with 3×10^8 cells of *L. yasudae* strain BJ3 (day 14 post-infection; $p = 0.008$) (Figure 1) and two hamsters infected with 3×10^8 cells of *L. interrogans* serovar Lai strain Langkawi (day 28 post-infection; $p = 0.01$). On the other hand, weight gain can be seen at dose of 2×10^8 cells of both strains (Figure 1).

The symptoms of decreased appetite and water intake were notable exclusively among hamsters infected with 1×10^8 cells of *L. interrogans* serovar Lai strain Langkawi (n = 2) and *L. yasudae* strain BJ3 (n = 3), respectively. Furthermore, infection with 3×10^8 cells of *L. interrogans* serovar Lai strain Langkawi and

L. yasudae strain BJ3 resulted in significant fur loss in one hamster each, as illustrated in (Figure 2). The blood cultures indicated a positive presence of *L. interrogans* serovar Lai strain Langkawi in 8/9 samples, while *L. yasudae* strain BJ3 was found in 9/9 samples.

Gross observations of the infected organs mostly showed normal morphology. However, one of the hamsters infected with 2×10^8 *L. yasudae* strain BJ3 showed hemorrhagic spots and yellowish tissue discoloration in the lungs (Figure 3).

Microscopic observations showed the presence of spiral-like structures (*L. interrogans* serovar Lai strain Langkawi and *L. yasudae* strain BJ3) in the liver and kidney at dose 1×10^8 - 3×10^8 cells (Figure 4). Furthermore, Table 1 presents detailed histopathological findings of organ tissues from hamsters infected with these strains at specific doses.

At a dosage of 1×10^8 cells, the *L. interrogans* serovar Lai strain Langkawi infection resulted in edematous lesions in the lung, hepatocyte vacuolation, cell hyperplasia in the spleen and severe tubular necrosis in the kidney (Figure 5(e)-5(h)). On the other hand, infection of 1×10^8 *L. yasudae* strain BJ3 resulted in pulmonary edema in the lung, normal hepatocytes architecture with sparse focal inflammatory cells infiltration in the liver and mild tubular degeneration in the kidney (Figure 5(i)-5(l)). More serious effects of *L. yasudae* strain BJ3 infection can be observed at a dosage of 3×10^8 cells, including interstitial pneumonia and lung lesions, hepatocyte expansion, hypertrophy and hyperemia with severe lesions in kidney cells (Figure 6(i)-6(l)).

Glomerular shrinkage was exclusively observed in the kidneys of hamsters infected with *L. interrogans* serovar Lai strain Langkawi (1×10^8 - 3×10^8 cells). Conversely, this particular feature was not detected in the kidneys of hamsters infected with *L. yasudae* strain BJ3. Furthermore, the majority of *L. interrogans* serovar Lai strain Langkawi and *L. yasudae* strain BJ3 infections also resulted in inflammatory cell infiltration and vacuolation of kidney and liver cells.

DISCUSSIONS

Leptospira yasudae was initially reported in Brazil (Casanovas-Massana et al. 2020) and has subsequently been isolated in Malaysia from soil samples obtained from an *ex-situ* wild animal conservation site in Perak (Shamsusah et al. 2021). Classified within the P1 lineage, *L. yasudae* is considered to have potential pathogenicity in both humans and animals, although its pathogenic characteristics remain poorly understood. Perak continues to record notably high leptospirosis incidence and mortality rates. Reported incidence rates ranged from 11.41 to 13.60 cases per 100,000 population in 2016-2017 (Philip & Ahmed 2023), while case fatality

rates between 2011 and 2016 ranged from 3.14% to 14.3% (Fann et al. 2020). Furthermore, pathogenic *Leptospira* were detected in 57% of environmental samples collected from recreational forests in the state (Yap et al. 2021), suggesting that these environments may facilitate bacterial survival and contribute to ongoing disease transmission.

Golden Syrian hamsters were chosen for this study because they are highly susceptible to Leptospirosis infection and exhibit clinical characteristics similar to human infections (Haake 2006). Both *L. yasudae* strain BJ3 and *L. interrogans* serovar Lai strain Langkawi were successfully identified in the blood of infected hamsters through positive blood culture. According to Andrade et al. (2007), *Leptospira* infection leads to its dissemination in the bloodstream, resulting in weight loss two weeks after infection. The symptom of weight loss may also be attributed to dehydration caused by reduced fluid intake and impaired sodium reabsorption in the proximal renal tubule due to Leptospirosis (Andrade et al. 2007).

However, the absence of significant weight loss in hamsters infected with 2×10^8 cells of *L. yasudae* strain BJ3, despite effects observed at lower and higher doses, suggests a non-dose-dependent response influenced by biological variability in host susceptibility, immune activation and physiological adaptation. Differences in innate immune recognition and evasion, including interactions with TLR4, TLR5, and NOD1 pathways (Bonhomme & Werts 2022), may also contribute. In addition, distribution-related mechanisms may contribute to this paradoxical pattern. Previous research by Hullahalli and Waldor (2021) supports our observation that leptospiral pathogenicity is not strictly proportional to inoculum size but depends on successful persistent colonization. At 2×10^8 , rapid immune activation may enhance clearance and prevent stable colonization, resulting in no weight loss (Wunder et al. 2016b). At 1×10^8 , optimal establishment and clonal expansion from few founding cells may promote significant weight loss (Hullahalli & Waldor 2021), whereas at 3×10^8 , the bacterial burden may overwhelm early host defenses, allowing sustained infection.

Although the hamsters infected with 2×10^8 cell *L. yasudae* strain BJ3 remained asymptomatic, one of the lungs showed hemorrhagic spots and yellowish tissue discoloration. These findings are consistent with previous studies that reported lung haemorrhage in cases of *L. interrogans* and *L. mayottensis* infections (Cordonin et al. 2019; Villanueva et al. 2014). Haemorrhagic complications are often associated with abnormal blood clotting caused by Leptospirosis (Gomes-Solecki, Santecchia & Werts 2017). *Leptospira* disrupt the blood clotting system by acquiring host plasminogen and utilizing its active form (plasmin) to degrade fibrin clots in the infected animal's organs, resulting in continuous

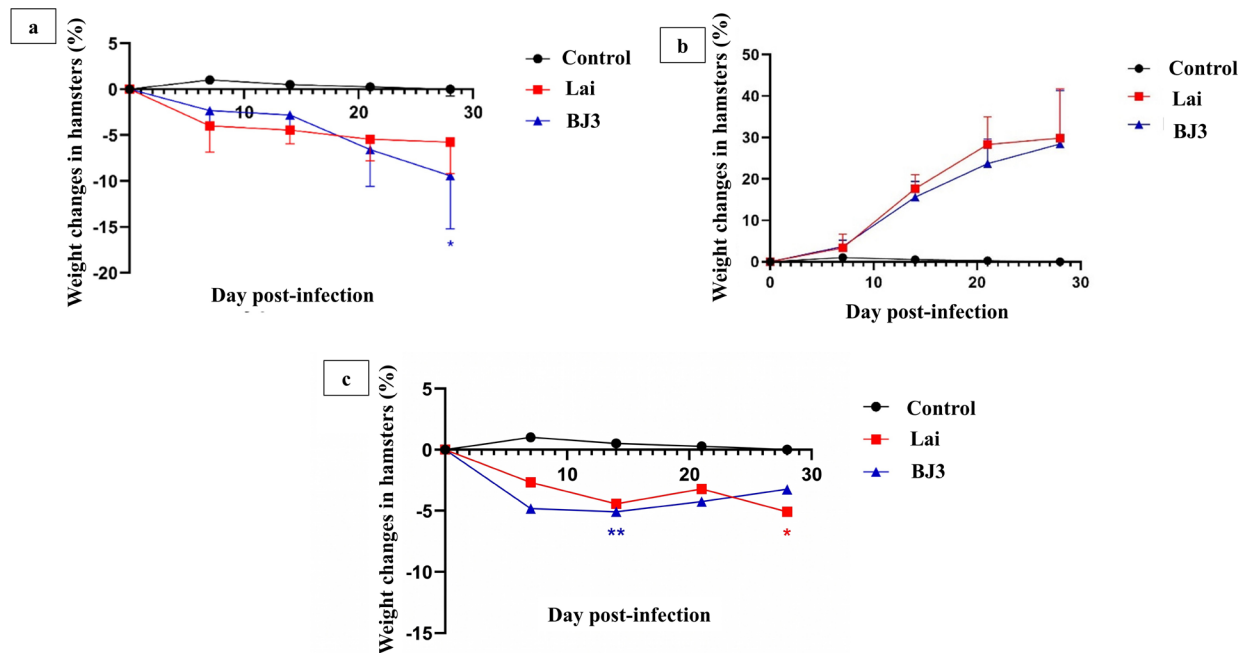


FIGURE 1. Changes in hamster body weight following infection with *Leptospira*. Each group consisted of three hamsters ($n = 3$), and body weight is presented as the percentage change from baseline over a 28-day observation period. Dose-dependent responses were evaluated for *L. yasudae* strain BJJ3 and *L. interrogans* serovar Lai at inoculation doses of 1×10^8 , 2×10^8 , and 3×10^8 cells, with corresponding control groups ($n = 3$). Significant weight loss was observed in 2/3 hamsters at (a) 1×10^8 cells of *L. yasudae* BJJ3 and in 1/3 hamsters at (c) 3×10^8 cells for both *L. yasudae* BJJ3 and *L. interrogans* Lai. In contrast, no significant weight changes were observed in any hamsters (3/3) at (b) 2×10^8 cells for either strain. Statistical significance is indicated as $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

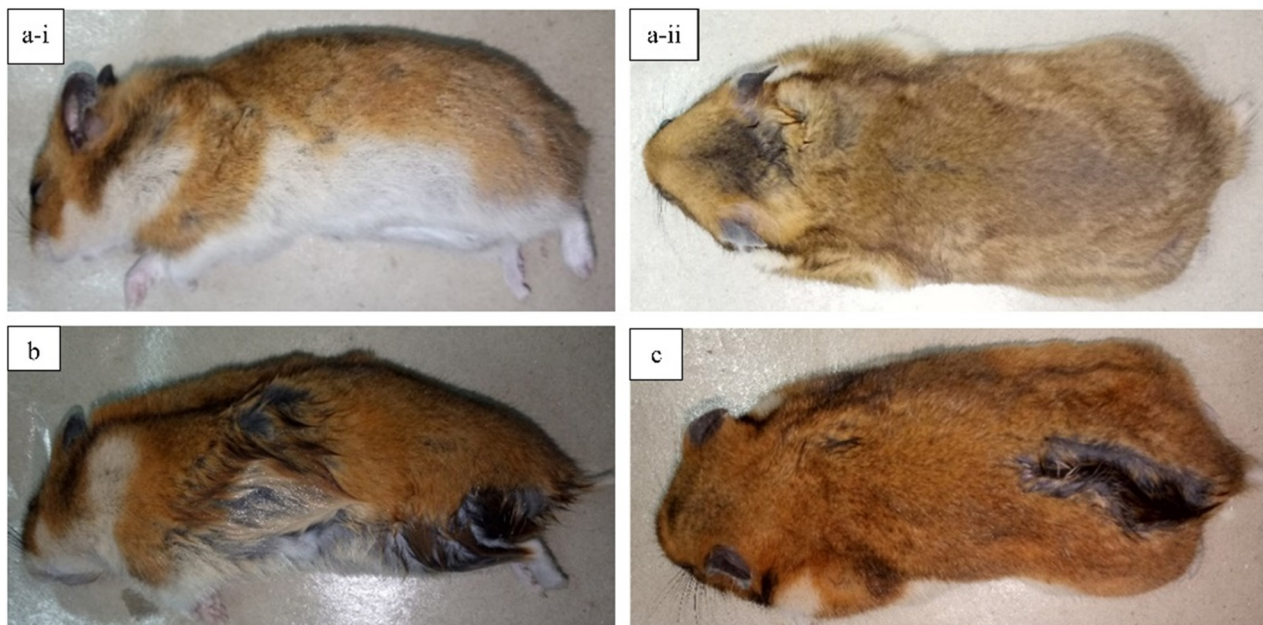


FIGURE 2. Comparison between (a-i, a-ii) normal hamsters and (b-c) infected hamsters. The animals experiencing fur loss after being infected with 3×10^8 *L. interrogans* serovar Lai strain Langkawi (b; $n = 1/3$, day 22 post-infection) and 3×10^8 *L. yasudae* strain BJJ3 (c; $n = 1/3$, day 27 post-infection)

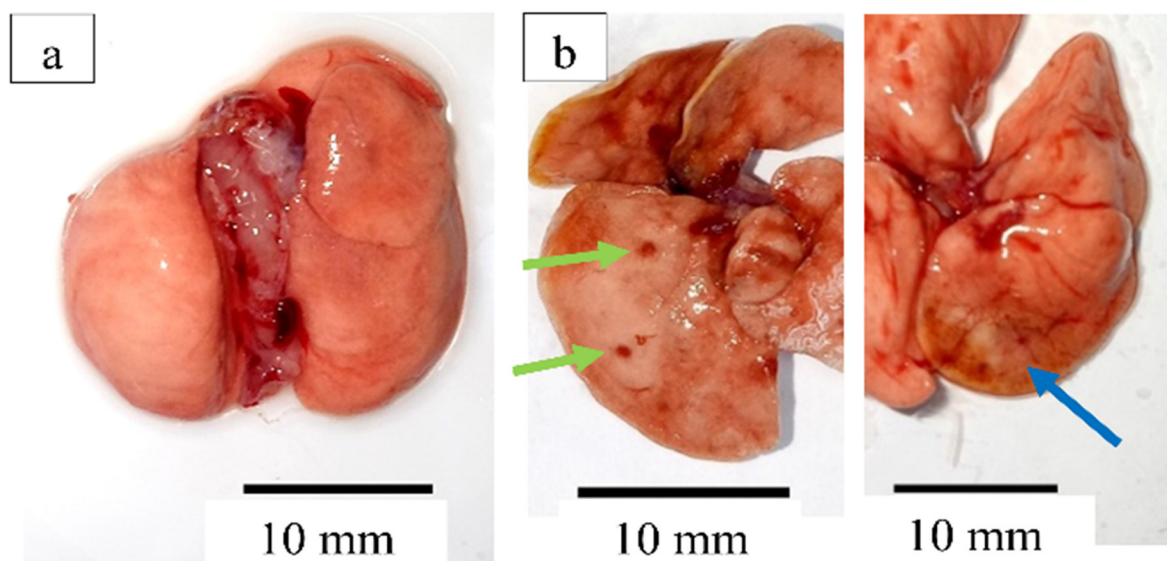


FIGURE 3. Gross examination of (a) normal lungs and (b) infected lungs. The lungs of a hamster infected with 2×10^8 *L. yasudae* strain BJ3 (n = 1/3) which showed hemorrhagic spots (green arrow) and yellowish tissue discoloration (blue arrow)

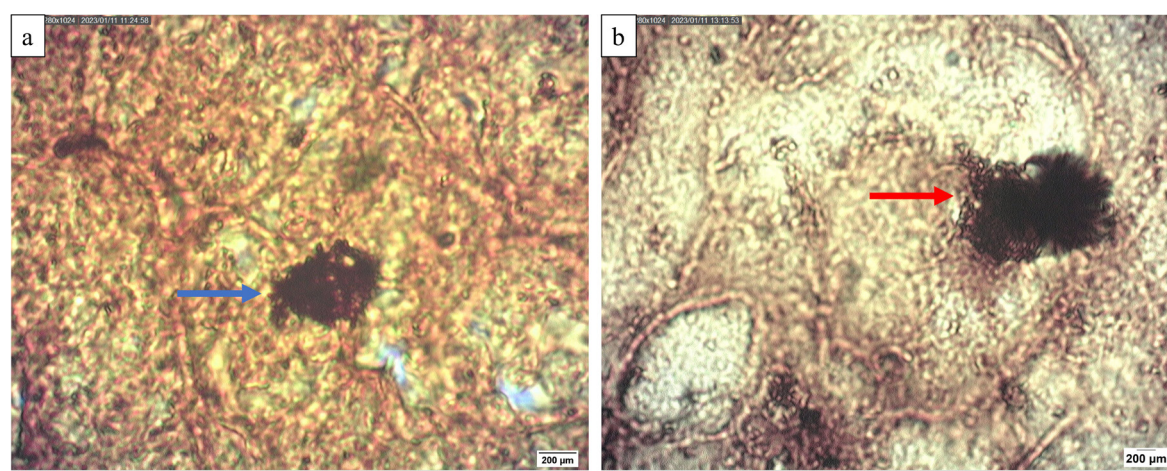


FIGURE 4. The presence of *Leptospira* in infected organs. (a) *L. interrogans* Lai aggregates in infected liver (blue arrow) and (b) *L. yasudae* strain BJ3 aggregates in infected kidney (red arrow) (400×)

bleeding (Castiblanco-Valencia et al. 2012; Vieira et al. 2017). Yellowish discoloration of hamsters' tissues, indicating jaundice, was also observed in the study by Philip et al. (2022).

In this study, *L. yasudae* strain BJ3 and *L. interrogans* serovar Lai strain Langkawi were successfully detected in the kidneys, liver and lungs, as well as the occurrence of histopathological tissue changes, indicate bacterial invasion during the infection. This is because pathogenic *Leptospira* organisms can move rapidly through the bloodstream (septicemia) (Sun, Liu & Yan 2020) upon invasion and subsequently enter the internal organs for replication, thereby leading to a more

aggressive infection (Casanovas-Massana et al. 2020; Lunn 2022). However, contrary to this study, previous researches have indicated that novel *Leptospira* species isolated from soil, such as *L. yasudae* strain F1T (Thibeaux et al. 2018), *L. barantonii*, *L. adleri*, and *L. ellisii* (Day & Edward 2010), were not detected in the kidneys of infected hamsters, suggesting that these strains are not virulent thus making this the first report of such occurrences.

Microscopic observations of the kidney showed that at a dosage of 1×10^8 cells, *L. interrogans* serovar Lai strain Langkawi infection resulted in severe tubular necrosis whereas *L. yasudae* strain BJ3 only caused

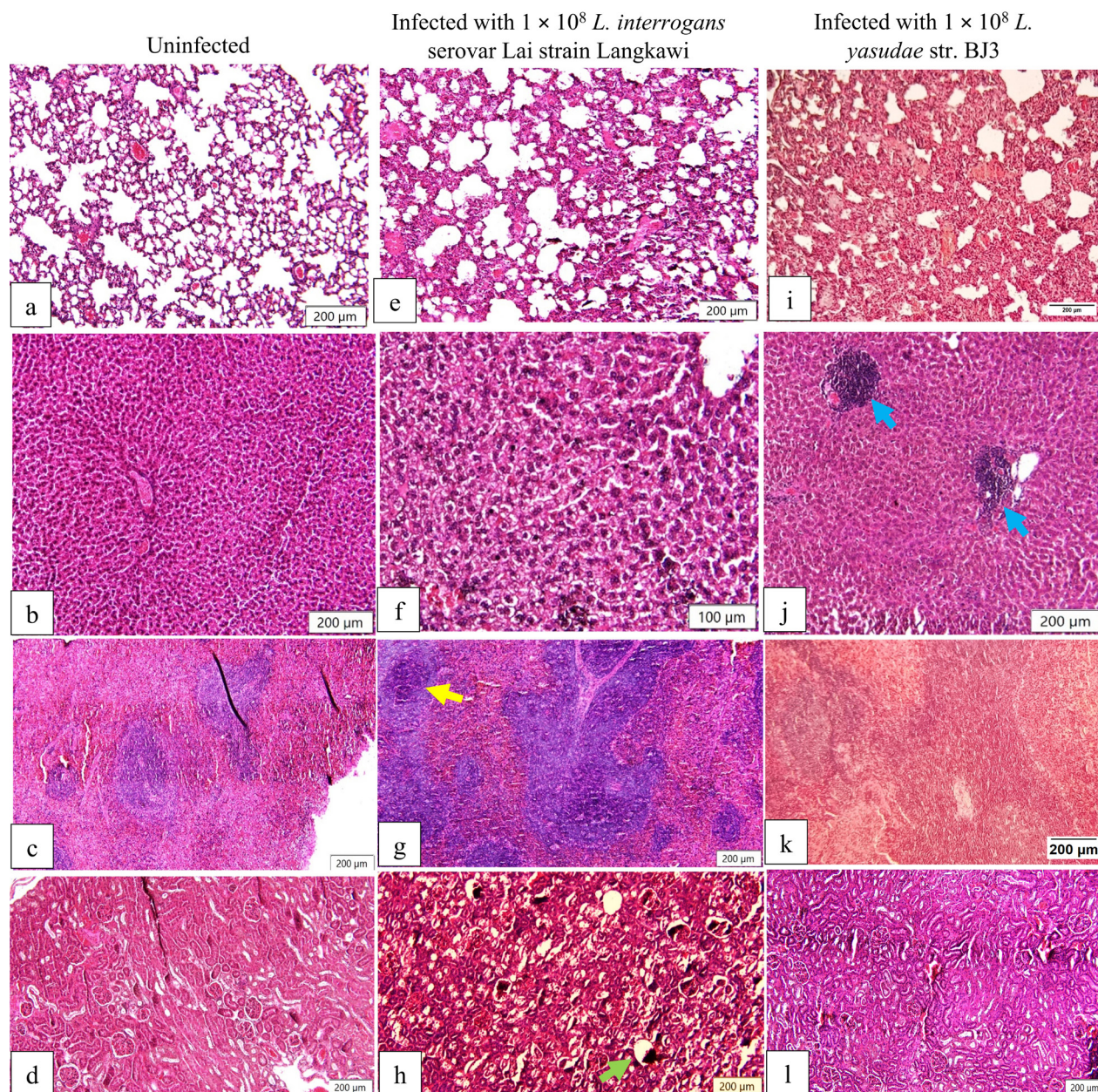


FIGURE 5. Microscopic examinations of organs of uninfected and infected hamsters. Uninfected hamsters: (a) normal lung cells (100 $\times$), (b) normal hepatocytes architecture (100 $\times$), (c) normal spleen cells (100 $\times$) and (d) normal tubules and glomeruli in kidney (100 $\times$). Hamsters infected with 1×10^8 *L. interrogans* Lai: (e) edematous lesions and inflammatory cell infiltration of alveolar septa in infected lung (100 $\times$), (f) hepatocyte vacuolation in infected liver (200 $\times$), (g) cell hyperplasia (yellow arrow) in infected spleen (100 $\times$) and (h) severe tubular necrosis and mild glomerular shrinkage (green arrow) in infected kidney (100 $\times$). Hamsters infected with 1×10^8 *L. yasudae* strain BJ3: (i) pulmonary edema in infected lung, (j) a few blood vessels were cuffed by the mononuclear inflammatory cells (blue arrows) with normal hepatocytes in infected liver (100 $\times$), (k) normal spleen cells structure (100 $\times$) and (l) mild tubular degeneration in infected kidney (200 $\times$)

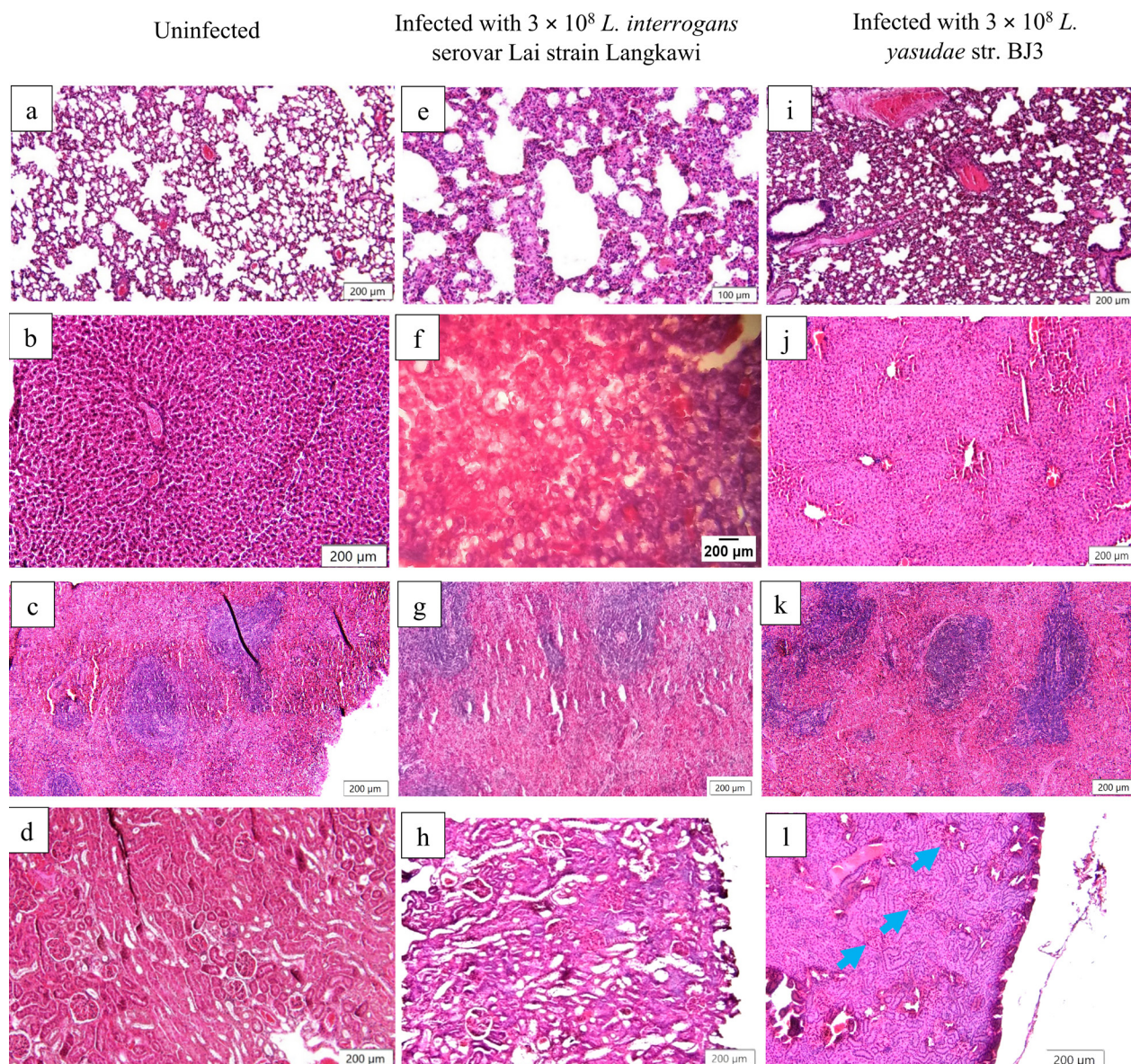


FIGURE 6. Microscopic examinations of organs of uninfected and infected hamsters. Uninfected hamsters: (a) normal lung cells (100×), (b) normal hepatocytes architecture (100×), (c) normal spleen cells (100×) and (d) normal tubules and glomeruli in kidney (100×). Hamsters infected with 3×10^8 *L. interrogans* serovar Lai strain Langkawi: (e) pneumonia interstitial, thickening of alveolar septa and inflammatory cell infiltration in infected lung (200×), (f) hepatocyte vacuolation in infected liver (400×), (g) normal spleen cells structure (100×) and (h) severe tubular necrosis and moderate glomerular shrinkage (100×). Hamsters infected with 3×10^8 *L. yasudae* strain BJ3: (i) mild interstitial pneumonia with spiral-like lesions in the lung parenchymal (100×), (j) generalize ballooning of hepatocytes which reduce the sinusoid spaces (100×), (k) normal spleen cells structure (100×) and (l) hypertrophy of the glomeruli (blue arrows) with mononuclear cells infiltration (100×)

TABLE 1. Histopathological details of organs in hamsters infected by *L. interrogans* serovar Lai Langkawi and *L. yasudae* strain BJ3 at specific doses

Organs	Details	<i>L. interrogans</i> serovar Lai			<i>L. yasudae</i> strain BJ3		
		1×10^8	2×10^8	3×10^8	1×10^8	2×10^8	3×10^8
Lung	The presence of <i>Leptospira</i> in cells	0	0	0	0	0	1
	Edema lesions	1	0	0	0	0	0
	Inflammatory cell infiltration	1	0	1	0	0	1
	Interstitial pneumonia	0	0	1	0	0	0
	Increase alveolar septa thickness	0	0	1	0	0	0
	Presence of giant cells (Langhans)	0	0	0	0	0	1
Liver	The presence of <i>Leptospira</i> in cells	2	0	1	2	1	2
	Hepatocyte vacuolation	1	1	0	1	2	0
	Haemorrhage	0	0	0	0	0	0
	Inflammatory cell infiltration	0	0	0	1	0	0
	Hepatocyte enlargement	0	0	0	0	0	1
Kidney	The presence of <i>Leptospira</i> in cells	0	0	0	1	0	1
	Renal tubule vacuolation	1	2	1	0	1	2
	Renal tubule necrosis	1	0	1	0	1	0
	Renal tubule degeneration	0	2	1	1	1	0
	Glomerulus shrinkage	1	1	1	0	0	0
	The presence of fibrin in the glomerulus	0	0	0	0	1	0
	Hypertrophy of the glomerulus	0	0	0	0	0	1
	Hyperemia in the interstitial area	0	0	0	0	0	1
Spleen	The presence of <i>Leptospira</i> in cells	0	0	0	0	0	0
	White pulp hyperplasia	1	0	0	0	0	0

The number of replicates per dose = 3 individuals. The numbers 0, 1, and 2 indicate the number of individuals that showed histopathological effects after *Leptospira* spp. infection

mild tubular degeneration. Tubular necrosis is a form of damage that occurs in the renal tubular cells due to the movement of *Leptospira* within the organ (Thibeaux et al. 2018). This finding is in line with the histopathological observations of tubular necrosis in humans (Abdulkader & Silva 2008), sheep (Almeida et al. 2019) and dogs (Dash et al. 2018) because of Leptospirosis infection.

Moreover, only hamsters infected with *L. interrogans* serovar Lai strain Langkawi (1×10^8 - 3×10^8 cells) exhibited glomerular shrinkage in their kidneys, while this specific characteristic was not observed in hamsters infected with *L. yasudae* strain BJ3. Generally, Leptospirosis infection rarely affects the renal glomeruli (Chancharoenthana et al. 2022). However, glomerular shrinkage occurs in severe cases of Leptospirosis infection, resulting in increased collagen, deposition of mesangial matrix and loss of glomerular capillaries, ultimately leading to the expansion of Bowman's space (Benavente 2010; Tan 1983). Previous studies on rodent infections have also indicated histological evidence of glomerular shrinkage resulting from infections caused

by *L. interrogans* Pomona (Krangvichian et al. 2022) and Copenhageni (Nair & Gomes-Solecki 2020).

More serious effects of *L. yasudae* strain BJ3 infection can be observed at a dosage of 3×10^8 cells, including the presence of severe lesions, hypertrophy and hyperemia in renal cells. This finding aligns with the discovery by Palaniappan et al. (2006) that the glomeruli of hamsters infected with *L. interrogans* Pomona exhibit hypertrophy in the parietal epithelium lining Bowman's capsule. To add, both *L. yasudae* strain BJ3 and *L. interrogans* serovar Lai strain Langkawi also caused infiltration of inflammatory cells in organs during infection. This is a significant pathological finding in Leptospirosis as it is essential for the body's natural defense against pathogens (Burg & Pillinger 2001; Chen et al. 2017; Daher, de Abreu & da Silva Junior 2010). These cells infiltrate infected tissues and release antimicrobial substances, contributing to the elimination of the pathogens (Sheshachalam et al. 2014). The infiltration of inflammatory cells typically occurs during the early stages of infection (Visith & Kearkiat 2005).

In the liver, hepatocyte vacuolation was consistently observed in *L. interrogans* serovar Lai strain Langkawi and *L. yasudae* strain BJ3 infections at each dose. Similar vacuolation has also been reported in dogs infected with Leptospirosis (Ashna et al. 2019; McCallum et al. 2019). Within individual hepatocytes, numerous small vacuoles (microvesicular) can be seen surrounding the nucleus, while in other hepatocytes, larger vacuoles merge, pushing the nucleus to the periphery and forming a ring-like shape (macrovesicular) (Ashna et al. 2019). Additionally, hepatocyte enlargement was observed in the livers of hamsters infected with a high dose of *L. yasudae* strain BJ3 (3×10^8 cells). Similar findings have been reported in previous studies, where scattered multinuclear giant hepatocytes were observed in equine parenchyma (Poonacha et al. 1993), and severe hepatocyte enlargement led to the disruption of sinusoidal architecture in hamsters (Tyagita et al. 2019) as a result of Leptospirosis infection.

Furthermore, interstitial pneumonia also occurred in the lungs of hamsters infected with 3×10^8 *L. interrogans* serovar Lai strain Langkawi and *L. yasudae* strain BJ3, respectively. Interstitial pneumonia is characterized by clustered interstitial fibrosis alternating with normal lung areas, along with architectural changes in the lungs due to chronic scarring or the formation of 'honeycomb' patterns (American Thoracic Society 2002). Interstitial pneumonia caused by Leptospirosis can be observed in humans (Jayakrishnan et al. 2013) and dogs (Gendron et al. 2014; Kohn et al. 2010). Other identified histopathological changes include the presence of pulmonary edema resulting from *L. interrogans* Lai and *L. yasudae* strain BJ3 infections at 1×10^8 cells, respectively. According to Andrade et al. (2007), pulmonary edema occurs as a result of severe Leptospirosis infection, leading to excessive fluid accumulation in the alveolar walls and alveolar spaces of the lungs (Iqbal & Gupta 2022). Previous studies have reported the detection of pulmonary edema in dogs (Rissi & Brown 2014), rodents (Gonçalves-de-Albuquerque et al. 2014) and humans (Gancheva 2019) due to Leptospirosis infection. Additionally, high-dose infection with *L. yasudae* strain BJ3 (3×10^8 cells) has led to the formation of giant cells in the lungs. This finding is consistent with previous studies showing the presence of giant cells in the kidneys of chronically Leptospirosis-infected cattle (Burdin 1963; Yener & Keles 2001).

In hamster spleen, the infection of 1×10^8 *L. interrogans* serovar Lai strain Langkawi led to an increase in white pulp tissue, a condition known as white pulp hyperplasia. This reaction is indicative of host immune system activation and involves a combination of lymphocytes and macrophages (Suttie 2006). Similarly, previous research conducted

by Humphries et al. (2014) demonstrated macrophage hyperplasia in the white pulp of spleens from hamsters infected with *L. interrogans* Canicola.

The absence of mortality despite the presence of pathological lesions suggests that both *Leptospira* strains caused sublethal but clinically significant infections in hamsters. The observed weight loss was likely associated with organ dysfunction due to leptospiral colonization and tissue injury, particularly in the lungs, liver and kidneys (Cordonin et al. 2019; Liu, Chen & Chen 2024). Generally, lesions produced in hamsters infected with *L. yasudae* strain BJ3 and *L. interrogans* serovar Lai strain Langkawi likely impaired physiological functions, reduced appetite and water intake and contributed to weight loss. Kidney damage may have disrupted fluid and electrolyte balance, causing dehydration (Karnik & Patankar 2021), while liver lesions may have impaired metabolic function (Omar et al. 2025). Pulmonary lesions may also have increased physiological stress and reduced oxygen exchange (Chen et al. 2025), further weakening the infected hamsters.

However, this study has several limitations. Histopathological analysis was limited to the lung, liver, kidney, and spleen, excluding other potentially affected organs such as the brain and heart. Although mortality was monitored, formal survival analyses and long-term follow-up were not performed due to the absence of lethal outcomes, limiting understanding of disease progression. In addition, only a narrow inoculum range ($1 \times 10^8 - 3 \times 10^8$ cells) was tested; inclusion of lower and higher doses may better define the dose-response relationship and pathogenic threshold of *L. yasudae* strain BJ3 compared with *L. interrogans* serovar Lai strain Langkawi. Last but not least, the characterization of virulence-associated genes and molecular determinants of pathogenicity through whole genome sequencing should be considered when interpreting the findings to further elucidate pathogenic mechanisms.

CONCLUSION

In contrast to predominant strains like *L. interrogans*, significant pathological effects resulting from *L. yasudae* strain BJ3 infection are observed at a higher dose. Nonetheless, caution should be exercised as *L. yasudae* strain BJ3 may represent a noteworthy emerging local agent of leptospirosis due to its ability to induce virulence and cause histopathological alterations in the kidneys of susceptible hosts. Importantly, this study provides new insights into the pathogenic potential of *L. yasudae*, contributing to the limited knowledge regarding the virulence characteristics of emerging *Leptospira* species and highlighting the need for continued surveillance and further investigations into their clinical and public health significance.

ACKNOWLEDGEMENTS

We extend our appreciation to the Bacteriology Unit of IMR Malaysia and Makmal Bioserasi UKM for their invaluable technical guidance and support during the course of this project. We affirm that there are no conflicts of interest involved. All supplementary data is available upon requests from the corresponding author.

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