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Journal Articles

VASCULAR PATHOLOGY ASSOCIATED WITH *LEPTOSPIRA ICTEROHAEMORRHAGIAE* SEROVAR LAI STRAIN LANGKAWI INFECTION IN GUINEA PIGS *(CAVIA PORCELLUS)*

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SUMMARY

Fifteen (15) guinea pigs were experimentally infected with *Leptospira icterohemorrhagiae* serovar Lai strain Langkawi, a new strain that was isolated from a human leptospirosis patient. Hematoxylin and Eosin ((H&E) staining showed haemorrhages, congestion and oedema in all internal organs examined (lungs, liver, spleen and kidneys) with inflammatory cell infiltration characterized by neutrophils, lymphocytes and macrophages. Hydropic degeneration and cell necrosis were also common in our findings. Leptospires were detected starting Day 2 p.i by silver staining and Transmission Electron Microscopy (TEM). Rise in antibody titres started on Day 5 p.i and leptospiral DNA was detected beginning Day 3 in the kidneys and Day 5 in the liver by Polymerase Chain Reaction (PCR) assay. The findings illustrated the pathogenesis of leptospirosis in guinea pigs which disclosed them as a suitable animal model for demonstration of clinical symptoms of leptospirosis and pathological changes after being infected with *Leptospira icterohaemorrhagiae* serovar Lai strain Langkawi, particularly pulmonary haemorrhages, a leading cause of mortality in human leptospirosis.

Keywords: Vascular, leptospira, infection, guinea pig

INTRODUCTION

Leptospirosis is a zoonotic disease of worldwide distribution (Trevejo *et al.,* 1998), caused by pathogenic *spirochetes* from the genus *Leptospira* which has a large number of species and more than 200 serovars of *L. interrogans* have been identified. Interaction between humans and the mammalian reservoirs is the main factor in the transmission of the disease, where humans can be infected through direct contact with infected animals or through exposure to waters or soils that have been contaminated by infected animal urine (Orpilla-Bautista and Panaligan, 2002).

It has been known that the basic pathomorphological changes in leptospirosis are endothelial damages which led to generalized vasculitis (Tappero *et al.,* 2000). However, studies on the vascular pathology caused by pathogenic leptospires are limited and this prompt us to study this critical stage of the infection which if not treated early, might lead to mortality. In this study, guinea pigs were infected with *L. icterohaemorrhagiae* serovar Lai strain Langkawi in order to study the clinical symptoms and pathological changes and to provide a better understanding of the pathogenesis of leptospirosis and vascular changes caused by pathogenic leptospires.

MATERIALS AND METHODS

Bacteria

The *Leptospira interrogans* serovar *Lai* strain Langkawi was isolated from a blood sample of human leptospirosis patient in one of the hospitals in the Netherlands in 2005, cultured in Ellinghausen and McCulough Johnson (EMJH) medium and provided to us by courtesy of the Royal Tropical Institute, Amsterdam, The Netherlands.

Animals

Animal experiments were with the approval of the Animal Care and Use Committee (ACUC), UPM (Reference No. 08R55/9 dated January 2009) to use seventeen guinea pigs (*Cavia porcellus*) age 3 weeks old, weighing from 250 to 300 grams. There were five treated groups (Groups 1-5) where each group consisted of 3 guinea pigs, injected with 10^6 of low-passage *L. icterohemorrhagiae* serovar Lai strain Langkawi in a final volume of 500 µl EMJH medium. Two guinea pigs were placed in the negative control group and were injected intraperitoneally with 500 µl of EMJH liquid alone. The animals were observed and recorded daily for clinical signs, appetite, the presence of discharge, feed intake, body weight, mobility, behaviour, respiratory distress, body temperature and clinical signs such as icterus. The animals were sacrificed serially beginning from Day 1 until Day 7 p.i.. On Day 0 and Day 7 p.i, one guinea pig from the negative control group was sacrificed for baseline data. Blood samples were taken for microscopic agglutination test (MAT) pre- and post-infections with the *Leptospira*.

Anaesthesia and Sample Collection

Serial killing was done on Day 1, 2, 3, 5 and 7 using ketamine (30 mg/kg) and xylazine 5 mg/kg for anaesthesia by intramuscular (IM) injection (ACUC,

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2011) then bled the animals by cardiac puncture. Blood sample was taken for MAT, hematological and biochemistry tests. Dissection was performed to remove the lungs, liver, kidneys and spleen for histopathology (H&E stain and silver stain), transmission electron microscopy (TEM) and PCR. Blood samples of infected and control animals were sent to the Veterinary Laboratory Services Unit, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary UPM for haematology and clinical biochemistry tests.

Light Microscopy

Samples of the lungs, liver, spleen and kidneys were collected and fixed in 10% formalin for 16 hours and then transferred into 70% alcohol. Samples were subsequently processed in an automatic tissue processor. Processed samples were then embedded in paraffin and 4µ thick sections on glass slides were stained with Hematoxylin and Eosin stain (De Brito *et al.,* 1966) and silver stain, and then covered with a drop of DPX and cover slip. The sections were then examined under a light microscope at 100x, 200x, 400x and 1000x magnifications.

Sample Preparation for Transmission Electron Microscope (TEM)

Samples of the lungs, liver, spleen and kidneys were diced into 1 mm^3 cubes and fixed in 2.5 % gluteraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 for 16 hours. Samples were then washed with the same buffer solution as above and post-fixed in 1% aqueous osmium tetroxide for 2 hours. Following washing with similar buffer solution the samples were dehydrated in ascending concentrations of acetone (35%, 50%, 75%, 95%, and 100%). Samples were then infiltrated with equal mixtures of acetone and resin overnight and embedded in 100% resin in beam capsules and polymerized at 60^o C in an oven for 16 hours. Semi-thin section were stained with methylene blue while ultrathin sections on copper grids were stained with uranyl acetate and lead citrate (De Brito *et al.,* 1966) and examined under a transmission electron microscope (LEO 912AB EFTEM) opening at 80 kV.

Serological Test (the Microscopic Agglutination Test)

The MAT was performed according to OIE (2005) as a confirmation test for the experimental diagnosis. Serum samples (control and infected) from 0.5 ml blood following intracardial puncture were tested with *L. icterohemorrhagiae* serovar Lai strain Langkawi as antigen. Positive results were confirmed by presence of microagglutination, while absence of microagglutination was considered as negative results.

Molecular Diagnosis (Polymerase Chain Reaction Assay)

Extraction: Two month formalin-prefixed tissue samples (kidneys and liver, 25 mg per sample) were extracted according to Qiagen Manual and kept at -20^o C until being used for PCR assay. Polymerase Chain Reaction (PCR) Assay: The PCR assay components consist of 25 µl master mix solution and 12 µl of it were mixed with 5 µL of extracted crude DNA as described by Shukla *et al,* (2003). All PCR processes were subjected to 35 cycles consisting of denaturation of the PCR mixture where temperature and time were set at 94^o C for 1 minute, annealing at 54^o C for 1 minute and extension in 72^o C for 2 minutes. The last extension was set at 72^o C for 10 minutes for 1 cycle respectively.

Primer Design: Available gene sequence of 16S rRNA genes of pathogenic serovars was retrieved from the gene bank. Shared sequences between 2 serovars at the region from 40 to 83 bp and 1164 to 1231 bp were chosen to design the primers using the DNasis Program. The set of designed primers specific for pathogenic *Leptospira* were synthesized by M/S Genetix, New Delhi.

Primer sequences of pathogenic *Leptospira* specific for the 16S rRNA gene were:

Forward primer 5' CGCTGGCGGCGCGTCTTAAA 3'
Reverse primer 3' AAGGTCCACATCGCCACTT 5'

They were located between the position at the region from 23 to 31 bp and 477 to 497 bp with expected product size of 631 bp. The DNA amplification was performed using Thermal Cycler (MyCycler, BioRad, USA). Not only the test samples were amplified, but the positive and the non-template control were also applied in every amplification assembly. The amplified products were then visualised in 15 % agarose gel electrophoresis. The visible DNA band in the gel imaging system (Gel Doc) (Biorad, USA) which has a size of 631 bp was considered positive for *L. icterohemorrhagiae* serovar Lai strain Langkawi.

RESULTS

The guinea pigs were seen huddling most of the time and were also anorexic. Mucous membrane of the eyes turned yellow (jaundiced). The animals showed droopy eyes from Days 5 to 7 p.i. Two guinea pigs died on Day 5 and Day 7 p.i respectively. Other guinea pigs were sacrificed on Days 1, 2, 3, 5 and 7 p.i.

The MAT done on serum samples before the guinea pigs were experimentally infected with *Leptospira icterohemorrhagiae* serovar Lai strain Langkawi showed no leptospiral antibody in all the guinea pigs indicating there was no history to leptospirosis. However, significant MAT titres were seen in the guinea pigs sacrificed on Day 5 p.i (2/3) and Day 7 p.i (2/3).

Detection of *L. icterohemorrhagiae* serovar Lai strain Langkawi gene was determined by PCR assay. From 30 tissue samples made up of 15 livers and 15 kidneys from infected guinea pigs, positive results were presented. The *Leptospira* gene was found in the kidneys of guinea pigs that were sacrificed on Day 7 p.i and in the liver of guinea pigs that were sacrificed on Day 3 p.i.

In lungs, the tunica media of the blood vessel wall were thickened which partially or completely obliterated the blood vessel lumina on Day 3 p.i. There were also margination of neutrophils and lymphocytes in the lumen of the blood vessels. Thrombi, marked by homogenous reddened areas attached to the wall of the vessel appeared for the first time (Figure 1). The presence of leptospires in

the lungs could only be shown by TEM. Leptospires were detected for the first time in the lumen of the alveolar capillary on Day 3 p.i and neutrophils just adjacent to the leptospires were also be seen. Severe haemorrhages in the alveolar lumen, emphysema and occasional bronchiolar necrosis were seen in the 3 guinea pigs on Day 7 p.i. (Figure 2). The tunica media and adventitia of blood vessel walls were thickened. Perivascular fibrin deposition and hyperplasia of bronchiole epithelial cells were observed (Figure 3).

In the liver, thickening of vascular wall with necrosis of vascular layers and perivascular fibrosis were noted on Day 1 p.i (Figure 4), resulting in diminished lumina of the blood vessels. Intimal proliferation of necrotic vascular wall of arteries marked by hyperchromatic elongated nuclei which projects into the vascular lumen were also noted in the affected blood vessel (Figure 5). Congestion of the central vein was an obvious finding in the liver since Day 1 p.i, grossly showed microscopic evidence of many red blood cells in the sinusoids and central veins (Figure 6). Many blood vessels with some showing disappearance of the tunica intima and tunica media cells (karyolysis) were observed (Figure 7). By silver staining, leptospires were found adjacent to the central veins and in the bile canaliculi in between hepatocytes (Figure 8). Many leptospires were also found in the bile canaliculi near the central vein, at the sites of centrilobular necrosis (Figure 9).

On Day 1 p.i, red blood cells were observed at the interstitial area of the kidneys and near glomeruli (Figure 10). Some blood vessels showed karyolysis and pyknosis of the vascular walls. The cells at the tunica adventitia of the blood vessels showed vacuoles suggesting degeneration (Figure 11). Leptospires could be detected with silver staining, at the interstitium of the collecting duct on Day 2 p.i (Figure 12). TEM showed leptospires adjacent to degenerate tubular cells at the interstitial junction. Degenerated tubular cells showed the mitochondrial cristae were less prominent and loss their characteristic feature of arrangement (Figure 13).

The spleens of 3 guinea pigs showed venous sinus at the red pulp were packed with red blood cells (splenic congestion) at Day 1 p.i (Figure 14) and Day 2 (Figure 15). Leptospires were detected in red and white pulps, attached into sinusoid of the red pulps and lymphoid cells and vascular wall both in red and white pulps. TEM showed leptospires in the endothelial cells leading membrane cell rupture, swollen rough endoplasmic reticulum and the mitochondria lost their characteristic cristae.

Figure 1. Photomicrograph of a lung section of a guinea pig on Day 3 p.i. Note the blood vessel wall at the tunica media is thickened (double arrow). Neutrophils (N) are seen marginating the wall of this blood vessel. Early thrombus (T) formation is seen as homogenously reddened areas which are attached to the wall of the vessel. H and E. 1,000x magnification.

Figure 2. Photomicrograph of a lung section of a guinea pig sacrificed on Day 7 p.i. Severe focal haemorrhage into the alveolar lumina (H) and emphysema are evident (double arrow). H and E. 100x magnification.

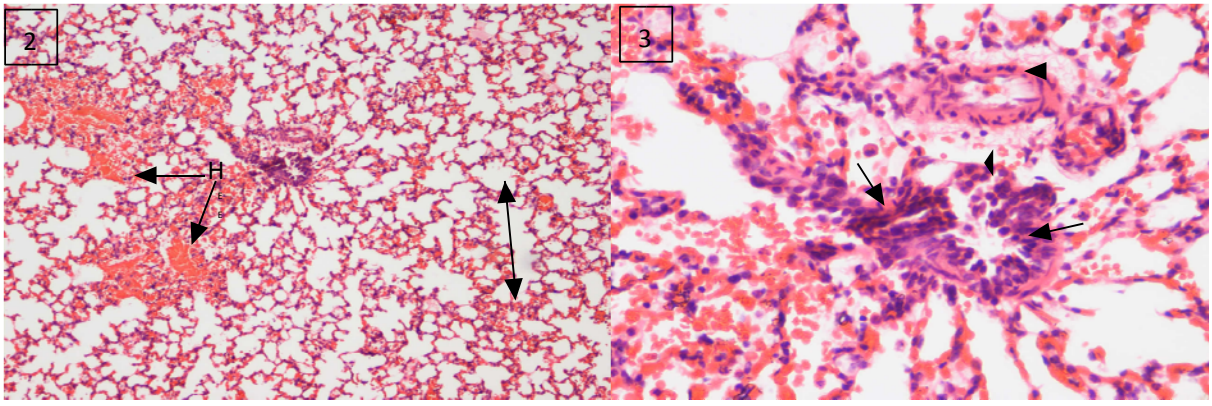

Figure 3. Photomicrograph of a lung section of a guinea pig sacrificed on Day 7 p.i showing a bronchiole epithelial hyperplasia (arrow). Note perivascular fibrin deposition (arrow head) of adjacent blood vessel. H and E. 400x magnification.

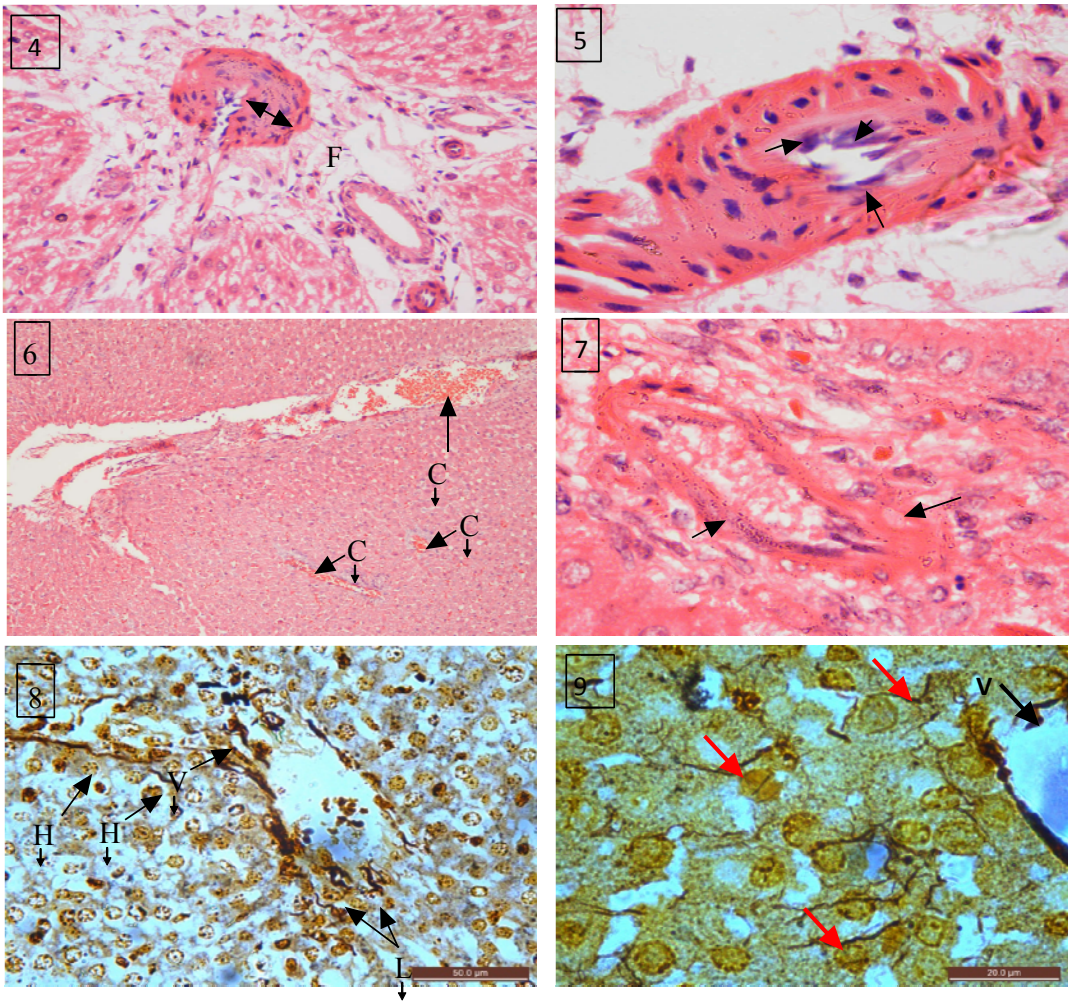

Figure 4. Photomicrograph of a liver section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 1 p.i. Note thickening of vascular wall. There is also necrosis of vascular layers and perivascular fibrosis (F). H and E. 400x magnification.

Figure 5. Photomicrograph of a liver section of a guinea pig sacrificed on Day 1 p.i. The arteriolar wall is thickened and necrotic and there is proliferation of intimal cells with hyperchromatic elongated nuclei (arrow) which projects into the diminished diameter of the vascular lumen. H and E. 1,000x magnification.

Figure 6. Photomicrograph of a liver section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 3 p.i, showing hepatic congestion. Many red blood cells are seen in the central vein (C). H and E. 100x magnification.

Figure 7. Photomicrograph of liver a section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 5 p.i. Note a thickened artery at the portal triad area with necrotic (N) vascular wall and intimal proliferation that slightly obliterated the vascular lumen. H and E. 1,000x magnification.

Figure 8. Photomicrograph of a liver section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 3 p.i, showing leptospires (arrow) between hepatocytes in the bile canaliculi (H), adjacent to a central vein (V). Some leptospires are seen close to the lumen of the central vein (L). Silver stain. 400x magnification.

Figure 9. Photomicrograph of a liver section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 7 p.i, showing many *Leptospira* (red arrows) in the bile canaliculi (red arrows) near the central vein (V). One end of a Leptospira is in the lumen of the central vein (black arrow). Silver stain. 1,000x magnification.

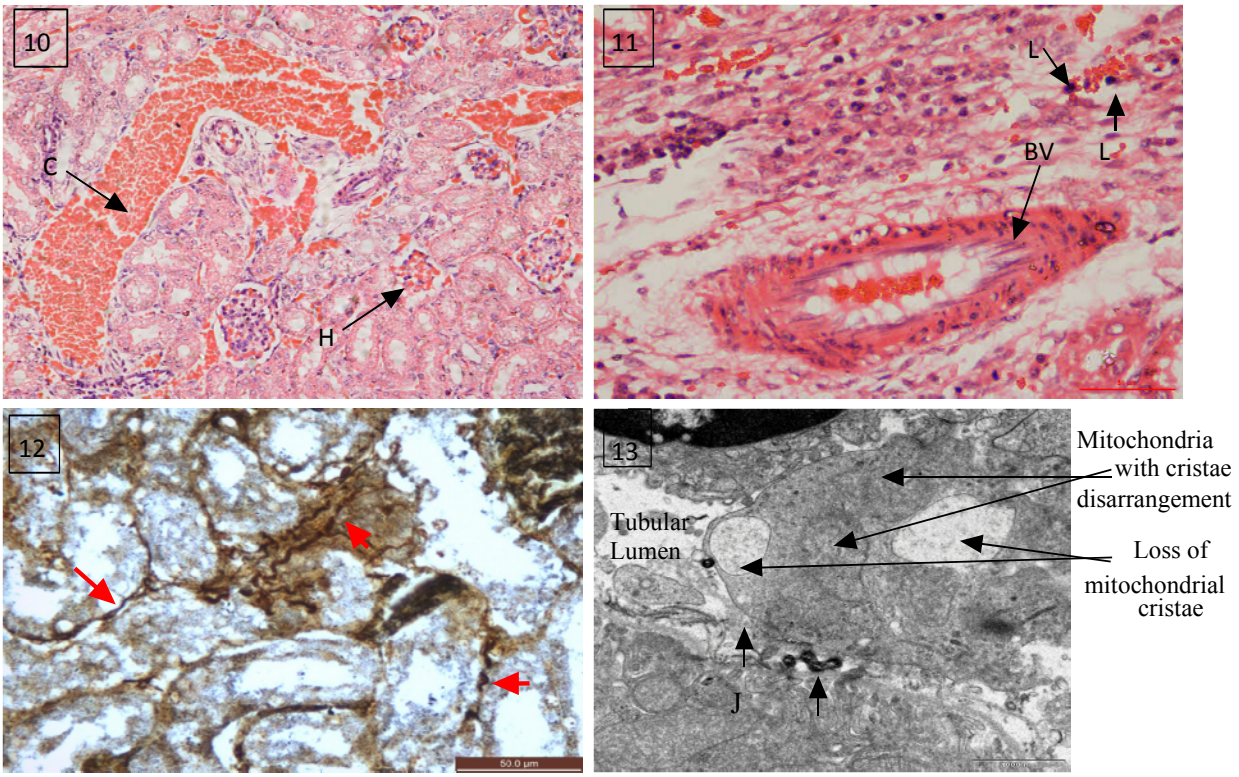

Figure 10. Photomicrograph of a kidney section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 1 p.i, showing a very congested kidney (C) and hemorrhage (H) at the interstitial area near convoluted tubules and renal corpuscle. H and E. 200x magnification.

Figure 11. Photomicrograph of a kidney section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 7 p.i, showing higher magnification of the focal inflammation with lymphocyte infiltration (L) and perivascular fibrosis (PF). The blood vessel wall (BV) is thickened, necrotic (pyknosis and karyolysis) and showed presence of spaces (degeneration). H and E. 200x magnification.

Figure 12. Photomicrograph of kidney tissue infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 2 p.i, showing several *Leptospira* (red arrows) at the interstitium of the collecting tubule. Silver stain. 400x magnification.

Figure 13. Electronmicrograph of kidney tissue of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 5 p.i. Note *Leptospira* (arrow) at the intercellular junction (J) between renal tubular cells. Adjacent tubular cell shows mitochondria appeared to loose their normal cristae arrangement. The tubular lumen contains cellular debris. Lead citrate and uranyl acetate. TEM 4,000x magnification.

DISCUSSION

This study clearly showed that the guinea pigs were susceptible to the new strain, namely *Leptospira icterohaemorrhagiae* serovar Lai strain Langkawi. From the 15 infected guinea pigs, 2 guinea pigs died on Day 5 p.i (1 guinea pig) and the other guinea pig died on Day 7 p.i. Thirteen guinea pigs which survived were sacrificed on Day 1, 2, 3, 5 and 7 p.i. These findings were supported by PCR results which showed the presence of the pathogen in the liver on Day 3 p.i and in the kidneys on Day 7 p.i. Silver staining revealed early presence of the leptospires in the kidneys on Day 2 p.i and in the liver and spleen on Day 3 p.i and 5 p.i. Antibody detection with MAT showed antibody production on Day 5 p.i until Day 7 p.i. No antibody was detected on Day 1, 2, and 3 p.i. It is believed that the leptospires were transmitted to various organs via blood and lymphatic circulation following the intraperitoneal injection (Carvalho and Bethlem, 2002). This is believed to be the invasive stage where aggressive spread occurs without any inhibition by antibody (Stuart, 1952), made possible by the ability of the leptospires to multiply intravascularly (Marshal, 1974).

The PCR findings revealed *Leptospira* genes were detected in the liver on Day 3 p.i and in the kidney on Day 7 p.i. It is believed that during leptospiremia, the pathogen multiplied intravascularly and were then disseminated to all organs (Nally *et al.*, 2005). It has also been reported that leptospiremia occurs on the first week of illness (Levett, 2001). The detection of leptospires in the liver by PCR suggested that the liver is a conducive environment for leptospires to localize and multiply

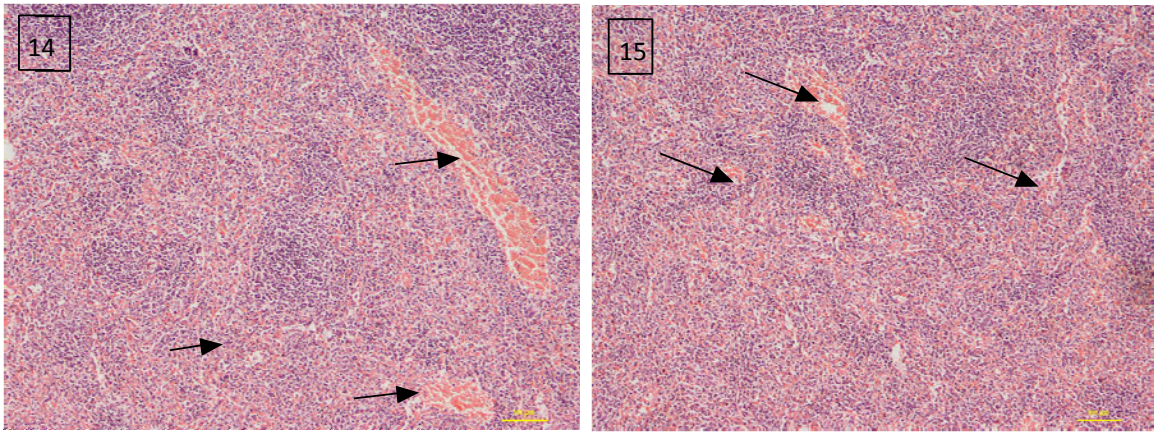

Figure 14. Photomicrograph of a spleen section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 1 p.i, showing blood vessels and the venous sinuses are packed with red blood cells (congested areas) (arrows). H and E. 100x magnification.

Figure 15. Photomicrograph of a spleen section of a guinea pig infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi on Day 2 p.i, showing splenic congestion in the red pulp (arrows). H and E. 100x magnification

(De Brito *et al.*, 1979) and the detection of the pathogens by this technique indicateed that the leptospires are circulating before antibodies could be detected in the blood (Ooteman *et al.,* 2006). When antibody has not been produced as yet, the leptospires were able to multiply and spread in various organs and expressed their virulence inside the tissues results in lesions and related clinical symptoms. These findings were supported by the MAT results, which exhibited no antibody production against the *Leptospira icterohaemorrhagiae* serovar Lai strain Langkawi on Day 1 up to Day 3 p.i.

Leptospires were successfully observed inside the alveolar capillary by TEM on Day 3 p.i contrary to a study by Nicodemo *et al.* (1997), who showed higher number of leptospires in the lungs. It is assumed, that the small sampling size 1 mm x 1 mm in TEM, may influence the frequency and number of leptospires seen in the lung samples. Infrequent presence of the organisms in the lungs may be contributed by the oxygen-rich alveolar environment which is not a suitable environment for a micro-aerophilic bacterium, like *Leptospira* (Levett, 2001).

Capillary congestion shown by blood accumulation in the capillaries. Haemorrhages were the common findings in most organs examined such as the lungs, liver, spleen and kidneys, leading to cell necrosis. These findings have been reported in previous findings by Arean (1962) and Marinho *et al.* (2009).

In this study the severity of infection increased continuously from Day 1 up to Day 7 p.i.. However, because of limited distribution of leptospires in the lungs, the toxin produced by the leptospires was considered as the main damaging factor as reported by Arean (1964), resulting in lung hemorrhages due to toxin-mediated capillary vasculitis (Luks *et al.,* 2003). The toxin was believed to affect the vascular wall integrity by altering the permeability of the capillaries and caused the escape of red blood cells resulting in haemorrhages. This vascular wall injury is believed to promote the interaction of blood cells, antigens and antibodies (Young *et al.,* 2006). Haemorrhages and congestion were also the frequent findings in human leptospirosis (Marinho *et al.,* 2009). In this study, haemorrhages were not the only finding, but there was also oedematous fluid with fibrin observed in the alveolar lumina. These features were the result of both endothelial damage caused by leptospires or an inflammatory response (vasculitis) provoked by leptospiral toxin. Damage to the endothelial cell membranes of small blood vessels is believed to be due to the leptospiral toxin that cause loss of intercellular junctions between cells, followed by the leakage of proteinaceus fluid and leptospires into extravascular spaces. Erythrocytes would be extravasated during a severe and extended damage (De Brito *et al.*, 1979). The mechanism of haemorrhages had been proven to be associated with genes responsible for the production of collagenase by leptospires that cause vascular injuries. Other virulence factors involved were von Willebrand factor (vWF), platelet activating Factor (PAF) acetylhydrolase and paraxonase that are known to activate loss of homeostasis (Ren *et al.,* 2003) with two mechanisms of bleeding disorder: (1) direct activation of haemostatic pathways, leading to a consumption coagulopathy, and (2) insufficiency of autoimmune response stimulation against host haemostatic factors by vWF of leptospires (Bharti *et al.,* 2003). Even though the leptospires were not sufficiently detected in the lungs in this study, it has been known that leptospires were able to cause pulmonary vascular damage in guinea pigs (de Brito *et al.,* 1979) leading to haemorrhages possibly due to enzymatic digestion by collagenase to tissues including blood vessels leading to death in humans (Segura *et al.,* 2005).

It is believed that the pathogen and its toxin cause thickening of the walls (Marinho *et al.,* 2009) of blood vessels that reduce the lumina size and also intraluminal detachment of necrotic endothelial cells. In the lungs, this will affect the gas exchange, where the exhaled gas cannot

flow out from the lungs. Therefore, the gas will be trapped in the alveolus and cause emphysema as observed in this study starting on Day 3 p.i. The emphysema could be associated with weakening of necrotic and inflamed alveolar walls, which led to the emphysema due to alveolar wall rupture. The emphysema also decreased the function of the nearest small blood vessels in distributing oxygen in the lungs (Morris and Sheppard, 2006) producing the clinical symptoms of struggling for breath and resulting in the pale mucous membrane in the affected guinea pigs.

In the spleen, the leptospires initially enter the central artery and penetrated the capillary wall, and were distributed into the white pulps and red pulps through the sinusoids. The presence of the leptospires in the white pulps and red pulps resulted in mild to focal necrotic areas. The leptospires could rupture vascular walls causing haemorrhages of the white pulps in Syrian hamsters infected with *Leptospira interrogans* serotype *icterohaemorrhagiae* (van den Ingh and Hartman, 1986) and in this study in the guinea pigs infected with the *Lai* strain.

The inoculated leptospires caused severe pathological changes in the lungs and spleen of guinea pigs in this study. However, the kidneys and liver have been known as the major organs affected causing increase of liver and kidney specific enzymes (Greene *et al.*, 1998). When the infection was in the acute stage, haemorrhagic diathesis would be generated and decreased the liver and kidney functions (Higgins, 1981). Haemorrhages were also frequently observed in organs examined possibly by toxin-mediated vascular injuries (Luks *et al.*, 2003).

When the leptospires reached the liver via the blood stream, the leptospires were believed to penetrate the vascular wall and enter the interstitium and they were seen intercellularly between hepatocytes, and also in the bile canaliculi and sinusoids, leading to hepatocellular degeneration and necrosis as seen in this study possibly by the ability of the leptospires to produce toxin when in the extracellular location (Higgins and Cousineau, 1977). Haemorrhages and jaundice were obviously observed in this study, even though jaundice and haemorrhages are not always evident in leptospirosis (Kobayashi, 2001). However, hemolysin produced by the leptospires is able to lyse RBC and can cause hemolytic anemia but it is not always associated with jaundice (Hickey, 2010).

The perivascular fibrosis that was seen in the liver would activate the stellate cells to secrete TGF-$ß_1$ which is a potent activation of fibrotic response, resulting in connective tissue proliferation and ultimately causing blood flow obstruction (Ijzer *et al.*, 2008). The evidence of thrombi in the liver is related to DIC where intravascular blood clotting occurred following injuries to blood vessels since Day 2 p.i until Day 7 p.i. The endothelial cell injury would also reduce prostacyclin synthesis resulting in progression of trombocyte activation in clotting, thus reducing the number of circulatory platelets (consumptive coagulopathy) (Edwards *et al.*, 1986). It has been reported that the endotoxin of the leptospires was able to activate platelets, leucocytes, and complement that promote intravascular coagulation. Endotoxin is also responsible for the deposition of fibrin and other blood elements in the formation of a blood clot (thrombus) in the blood vessels (Davison *et al*., 2005).

In the kidneys, degeneration and necrosis were observed in the proximal and distal convoluted tubules, renal corpuscles and collecting tubules. At these lesion sites, as evidence by silver staining, many leptospires were predominantly seen at the interstitium between collecting duct cells and the lumina of proximal and distal convoluted tubules. How the bacterium enters the lumen of the tubules and ducts is yet to be explained, but it is believed to involve migration from the interstitium intercellularly into tubular cells and then extracellular exit or via intracellular junction into the tubular or ductal lumina.

The presence of microthrombi in the glomerular capillaries of the guinea pigs could be associated with the bile duct obstruction due to distortion and subsequent bile duct obstruction due to the contractile feature of fibrous tissues that was evident at the periportal area (periportal fibrosis).

CONCLUSION

From the results of this study, most of the findings were directed to reveal the vascular pathological changes caused by *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi indicating that the *Leptospira* and its toxins were able to cause vascular damages in both humans and guinea pigs. This suggests that guinea pig can be a good model to understand the pathogenesis of human leptospirosis, particularly the development of pulmonary haemorrhages leading to mortality. The findings on the clinical symptoms and pathological changes in the guinea pigs infected with *L. icterohaemorrhagiae* serovar *Lai* strain Langkawi has helped to illustrate the development of the infection and the urgency for rapid and intensive medical treatment.

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CONFLICT OF INTEREST

None of the authors have any potential conflicts of interest to declare.

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