

Pathological Investigations of Experimental Leptospirosis in Hamsters

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Abstract: Forty-eight Syrian hamsters (*Cricetus auratus*), aged 1.5-2 months, were used in this study. Forty of the animals were infected with *Leptospira interrogans* serovar *grippotyphosa* by intraperitoneal route. Eight hamsters were given physiological saline solution by same route. A systematic necropsy was performed on 9 animals that died during the study period and on 31 animals that were euthanized on days 7, 14, 21 and 28 of the trial. Remaining 8 animals was necropsied on day 28 as control group. Macroscopically, the hamsters included in Group 1. presented with mild icterus and marked anaemia throughout the study period. The lungs were swollen and dark red coloured, and some of the cases displayed haemorrhagic lesions. In Group 2., the liver was swollen and dark red-coloured and the kidneys were swollen and pale. Groups 3. and 4. presented with multiple greyish white foci that varied in number, in the kidneys. Microscopically, Hematoxylin-Eosine (HE) staining in Group 1. demonstrated the presence of haemorrhages in multiple organs, including primarily the lungs, as well as degenerative alterations, necrosis and mild neutrophil leukocyte and mononuclear cell infiltrations in the liver, and degenerative changes in the kidneys. In Group 2., degenerative changes and mononuclear cell infiltrations in the liver were observed to be of greater intensity. In Groups 3. and 4., lesions were generally limited to the kidneys and findings related to interstitial nephritis were observed. The presence of the infectious agent was detected in all trial groups, by the silvering methods and Avidin-Biotin Complex Peroxidase (ABC-P) staining.

Keywords: Experimental, hamster, *Leptospira grippotyphosa*, pathology

Hamsterlerde Deneysel Leptosirosiste Patolojik İncelemeler

Özet: Çalışmada; 1,5-2 aylık, 48 adet Suriye hamsteri (*Cricetus auratus*) kullanıldı. Hayvanlardan 40'ına intraperitoneal yolla *L. interrogans* serovar *grippotyphosa* verildi. Ayrıca 8 hamstere sadece steril fizyolojik tuzlu su verilerek kontrol amaçlı kullanıldı. Deney süresince ölen 9 hayvan ve 7., 14., 21. ve 28. günlerde ötenazi uygulanan 31 hayvanın sistemik nekropsileri yapıldı. Kalan 8 hayvan ise nekropsileri yapıldıktan sonra kontrol grubu olarak kullanıldı. Makroskobik olarak 1. gruptaki hayvanlarda deney süresince hafif sarılık ve belirgin anemi gözlemlendi. Akciğerler şişkin ve koyu kırmızı renkli olup, bazı olgularda kanamalı lezyonlara rastlandı. İkinci grupta; karaciğerler şişkin ve koyu kırmızı renkli, böbrekler şişkin ve solgundu. Üçüncü ve dördüncü gruplarda; böbreklerde değişen sayıda küçük boz-beyaz odaklara rastlandı. Kontrol grubu hayvanlarda ise herhangi bir makroskobik bulgu gözlenmedi. Mikroskobik olarak; grup 1'de HE boyamalarda, akciğerler başta olmak üzere birçok organda kanamaların yanı sıra, karaciğerde dejeneratif değişiklikler ve nekrozlar ile hafif nötrofil lökosit ve mononükleer hücre infiltrasyonlarına, böbreklerde ise dejeneratif değişikliklere rastlandı. Grup 2'de ise karaciğerde dejeneratif değişiklikler ve mononükleer hücre infiltrasyonlarının yoğunluğunun arttığı gözlemlendi. Grup 3 ve 4'te lezyonların çoğunlukla böbreklerle sınırlı kaldığı ve intersitsiyel nefritise ilişkin bulguların şekillendiği dikkati çekti. Etkenler tüm deneme gruplarında gümüşleme metotları ve Avidin-Biotin Kompleks peroksidaz boyamalarla dokularda tespit edildi.

Anahtar Kelimeler: Deneysel, hamster, *Leptospira grippotyphosa*, patoloji

Introduction

Leptospirosis is a spirochetal and zoonotic disease of domestic and wild animals caused by *Leptospira interrogans* serovars (Ellis et al., 1994; Arda et al., 1997; Hazıroğlu and Milli, 2001; Jones et al., 1997). Leptospirosis is a major cause of mortality from acute disease, characterized by septicaemia, hepatitis, nephritis and meningitis, as well as of abortions and stillbirth, in farm animals (Badiola et al., 1983; Ellis et al., 1983; Hazıroğlu and Milli, 2001; Krivoshein, 1989).

Leptospirosis is one of the most common diseases in the world, which is considered important in terms of both human and animal health and economic losses (Jones et al., 1997).

Many studies have been conducted on the pathological findings, diagnosis and treatment of the disease in hamsters by the use of several serovars (Barnett et al., 1999; Haake, 2000; Matsuo et al., 2000; Sitprijia et al., 1980; Van Den Ingh and Hartmann, 1986; Weber et al., 1956). There have been more studies about light and electron microscopic investigation of pulmonary haemorrhages caused by the serovar *grippotyphosa* (Berkin, 1982) and the investigation of liver and kidney lesions caused by experimental infection with several serovars, including *L. grippotyphosa* (Haake et al., 2000; Miller and Wilson, 1966; Scanziani et al., 1989; Schrickler and Hanson, 1961;). Recently there have been many studies about immunohistochemical investigation

of leptospirosis (Haake, 1999; Haake et al., 2000; Yener and Keles, 2001).

The present study was aimed at the detailed investigation of the clinical and pathological findings and pathogenesis of leptospirosis in a hamster experimental infection model established by administering the animals with *L. interrogans* serovar *grippotyphosa* by intraperitoneal route.

Materials and Methods

Animals, infectious agent and hyperimmune sera:

The infectious agent, experimental animals and hyperimmune serum used in the study were obtained from the Etlik Central Veterinary Control and Research Institute. Forty-eight Syrian hamsters (*Cricetus auratus*), aged 1.5-2 months and weighing 120-150 g, were used.

To increase the number and pathogenicity of the infectious agent, the serovar was passaged in the laboratories of the Institute using modified Johnson's synthetic medium (25 °C, pH 7.5) [*Leptospira* medium base EMJH (Bacto)] and was incubated at 30 °C over a period of 4-14 days. The viable *Leptospira* bacteria obtained by passaging were counted using a Thoma haemocytometer slide (Hawksley, London), and dilution was

performed such that the final concentration was $1-2 \times 10^8$ /ml. Forty of the hamsters were injected with 0.5 ml of the medium containing the *L. interrogans* serovar *grippotyphosa* strain at a concentration of 1×10^8 , while 8 of the animals were administered with an equal volume of sterile physiological saline by intraperitoneal route as following the decision of the Ethics Committee (Ankara University Faculty of Veterinary Medicine Commission Headship of the Ethics Committee 24.07.2002 - 2002/39).

The anti-*Leptospira interrogans* serovar *grippotyphosa* serum, used as the hyperimmune serum for immunoperoxidase stainings, was obtained by the administration of *Leptospira interrogans* serovar *grippotyphosa* antigens (Strain RM52) to rabbits in the laboratories of the same institute. The experimentally infected animals were allocated to four groups, which were examined on days 7, 14, 21 and 28 of the trial, respectively. Immediate post-mortem examination was performed on animals that died within the trial period, and animals that survived were euthanized for necropsy. Necropsy findings were recorded. The numbers of animals that died within the study period and that were euthanized for necropsy are presented in Table 1.

Table 1. Numbers of animals that died and were euthanized for necropsy.

| Groups | Mortality | Euthanasia | Control | Trial | |
|-----------------------|-----------|------------|---------|-----------|----------|
| | | | | Trial | Control |
| Group 1. (Days 1-7) | 8 | 2 | 2 | 10 | 2 |
| Group 2. (Days 8-14) | - | 10 | 2 | 10 | 2 |
| Group 3. (Days 15-21) | 1 | 9 | 3 | 10 | 2 |
| Group 4. (Days 22-28) | - | 10 | 3 | 10 | 2 |
| Total | | | | 40 | 8 |

Pathology: Macroscopic findings observed in the hamsters on which systematic necropsy was performed were recorded. Tissue samples taken from the organs were fixed in 10% buffered formalin solution. Following fixation, tissue samples were subjected to routine processing and embedded in paraffin. Sections of 5-7 µm thickness were cut from the paraffin blocks and were stained with Hematoxyline and Eosine H&E (Presnell and Schreibman, 1997).

Furthermore, to ensure improved observation of the infectious agent, all tissues were also stained with the Warthin-Starry and Levaditi methods (Presnell and Schreibman, 1997).

Immunohistochemistry: An anti-*Leptospira interrogans* serovar *grippotyphosa* hyperimmune

serum was used for the detection of leptospiral antigens in the sampled tissues. The Avidin-Biotin Complex Peroxidase (ABC-P) method was applied by the use of an anti-rabbit universal kit (DAKO, Cytomation LSAB2, System HRP, Code: K0672). For this purpose, as was for histopathological examination, sections were prepared from the paraffin tissue blocks pertaining to each animal. Subsequently, the anti-*Leptospira interrogans* serovar *grippotyphosa* hyperimmune serum, which was obtained from rabbits and used as the hyperimmune serum (microscopic agglutination test, 1/1200), was diluted at a rate of 1/64 and applied to the sections. At the final stage, the sections were kept in an AEC chromogen solution (DAKO Corporation, USA) for 7 minutes and

counterstained with Mayer's haematoxylin for 1 minute, and rinsed under running tap water. Finally, the sections were applied a water-based adhesive and covered with a coverslip. Tissue sections belonging to the control group used as negative controls were subjected to the same procedure.

Results

Clinical Findings

As from day 2 of the trial, the animals presented with various clinical symptoms, including inertia, ruffled it's feathers, spinal curvature (hollow-back), listlessness, inappetence, slowness of movements, delayed reaction to physical stimuli, reduced interest in feed and water consumption, huddling near the sides of the cages and the tendency to acquire dorsal recumbency. At the end of day 2, two of the animals were observed to have died. Some of the animals that died on days 4 and 5 displayed tonic-clonic convulsions in unconscious state and dorsal recumbency shortly before death. As from days 6 and 7, the animals were observed to show increased interest in water and feed consumption. The clinical symptoms were less severe in Group 2., whilst Groups 3. and 4. did not show any clinical symptom except from one dying in Group 3.

Necropsy Findings

Most pronounced necropsy finding was petechial haemorrhage on the serous membranes of the internal organs in the 8 animals that died naturally and the 2 animals that were sacrificed end of the 7 th days of the trial. In all of the cases, the lungs were dark red in colour and swollen. In 3 of the animals, the cranial and caudal lobes have displayed multifocal petechial and ecchymotic haemorrhage (Figure 1a). The 3 animals that died on day 5 presented with a aspect of mild icterus in body fat, the peritoneum and subcutaneous tissues. The blood was light red coloured and showed weak clotting activity. In this trial group, the liver was enlarged, dark red-coloured, swollen and rounded edge, and it was observed that blood leaked from the cross sections. The kidneys were also swollen and dark red-coloured with marked congestion of the subcapsular vessels, and oedema and congestion of the cross sections. In Groups 2. and 3. the liver and kidneys were swollen and pale. In Group 3., one animal died and presented with multiple pinhead-sized yellowish white, confined

foci that did not exceed a few millimetres in diameter and were scattered across the hepatic lobes (Figure 1b). It was noted that the kidneys were swollen and friable and had a mottled appearance (Figure 1c). Sand-like greyish white foci were scattered across the cortex. The prescapular and popliteal lymph nodes were enlarged and their cross sections had a moist appearance. Animals included in Group 4. presented with swelling and paleness of the liver and a mottled appearance of the kidneys. In some of these animals, indistinguishable greyish white foci, some of which reached the size of sand particles, were present below the capsule (Figure 1d). In the kidneys, the proportion of the cortex to the medulla was observed to have increased in favour of the cortex. No macroscopic finding was observed in the control animals that were necropsied in pairs on days 7, 14, 21 and 28 of the trial.

Microscopic Findings

As the microscopic findings varied with the different phases of the infection and the severity of the lesions, the organs with the most severe lesions were examined in the first place. In this respect, the infection was investigated under three phases, namely, the acute destructive, subacute and chronic phases.

1. Acute destructive phase: In this phase, the organs that were affected at the highest level by the infectious agent after septicaemia, and the damage associated with infection, were examined. Group 1. was examined in this phase of the infection. The animals in Group 1. demonstrated the septicaemic phase of the infection and presented with evident and characteristic pulmonary lesions; including, hyperaemia of the interalveolar capillaries and interlobular arteries and veins, diffuse haemorrhagic areas and mild acute catarrhal bronchopneumonia. The Levaditi, Warthin-Starry method and immunoperoxidase stainings demonstrated the presence of the infectious agent and it's antigenic structure in the lumen and wall of interalveolar capillaries, haemorrhagic areas and the alveolar wall. In the heart, which presented with no macroscopic finding, hyperaemia of the blood vessels was observed, and in almost each case the muscle bundles were swollen and their cytoplasm displayed a granular appearance. In three of the animals, in some areas free erythrocytes were observed between muscle bundles.

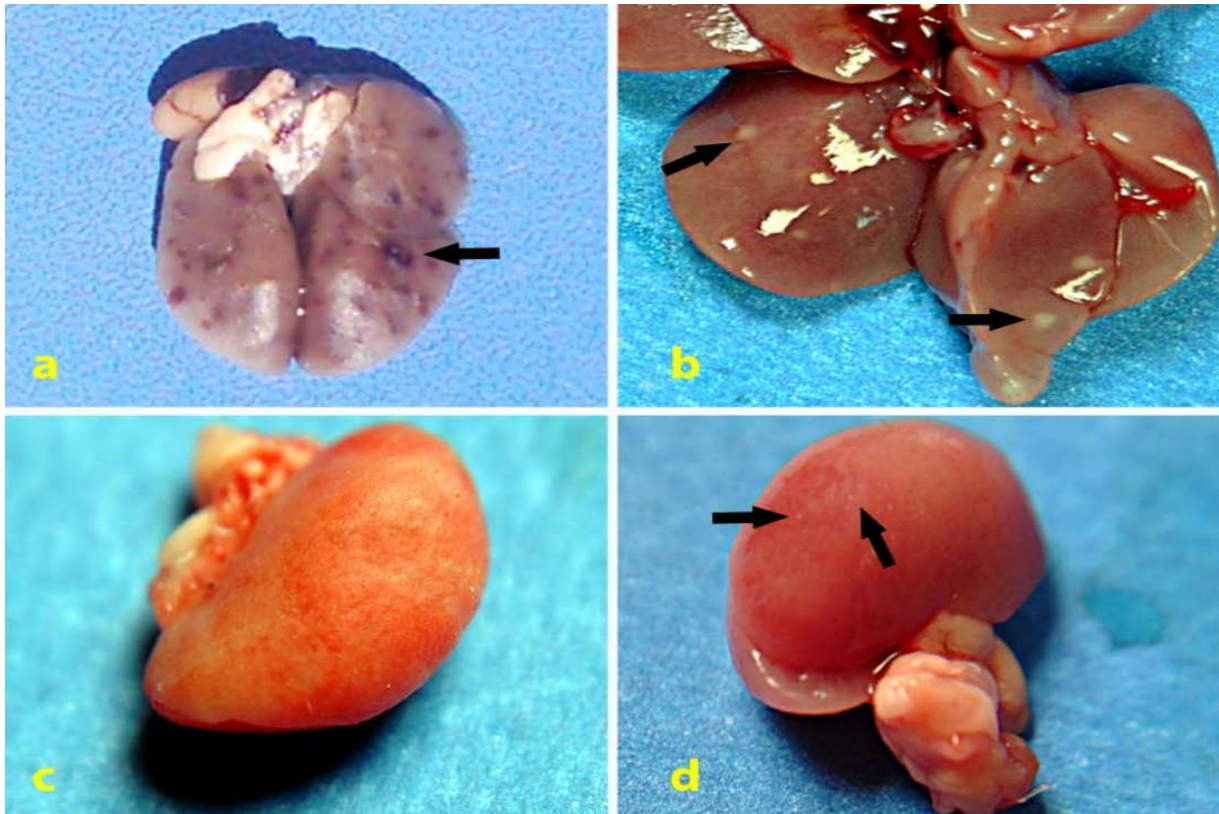


Figure 1. Macroscopical aspects: a) Lungs, Group I, Diffuse petechial and ecchymotic hemorrhages (Arrow), b) Liver, Group III, multifocal gray-white areas dispersed in lobes (Arrows), c) Kidney, Group III, Pale and multicolored appearance, d) Kidney, Group IV, multicolored appearance and gray-white foci in shape of grits (Arrows).

In Group 1., the vena centralis, vena interlobularis and sinusoids were observed to have been greatly enlarged, and in some areas thrombosis had occurred. The Remark cords were dissociated, the sinus endothelium was swollen, and the Kupffer cells were hyperplastic. In some of the animals, apart from the listed findings, granular degeneration and solitary cell necroses were observed in hepatic epithelial cells. Surrounding these lesions, cell infiltrations, composed generally of macrophages and lymphocytes, and to a less extent, neutrophils, were observed. Similar inflammatory cells were also present in small numbers in the sinusoids. Immunoperoxidase staining demonstrated the presence of the antigenic structure in this regions.

Renal findings were more pronounced in Group 1. In almost each case, marked hyperaemia of the renal blood vessels and evident glomerular alterations were observed. Enlargement of the glomerular capillaries and Bowman's space, proliferation of the mesangial cells, the presence of proteinaceous materials in Bowman's space associated with free erythrocytes in some regions were also determined. The proximal convoluted tubule epithelium contained fine granules and light coloured vacuolisation in certain regions. In some of the tubules, the lumen contained aggregates of

proteinaceous material and epithelial debris. In three cases, aggregates of free erythrocytes were observed in the interstitium. The Levaditi method and Warthin-Starry stainings demonstrated at pulmonary haemorrhages and the presence of brown-black coloured aggregates of the infectious agent that resembled a spiral in shape, in the bronchiolar epithelium, interalveolar capillaries, hepatic sinusoids, Disse spaces, renal glomeruli and proximal tubuli. Furthermore, many immunopositive areas were detected by immunoperoxidase staining in the glomeruli, lumen of the intertubular arteries and proximal tubules, as well as in the hepatic sinusoids, Disse spaces and cytoplasm of the Kupffer cells. No immunopositivity was detected in any of these regions in the control group.

Brain lesions were not specific, but in one animal, aggregates of free erythrocytes were observed in the submeningeal tissue, which extended towards the neuropile.

Findings observed in the spleen included hyperplasia of the lymph follicles, oedema in the trabeculae, infiltrations mainly composed of macrophages and also a few neutrophil leukocytes, and marked increase in the red pulp.

In the prescapular, popliteal and mesenteric lymph nodes, the cortical and medullar sinuses

were observed to have enlarged and their lumen contained macrophages and a few neutrophil leukocytes. In some animals free erythrocytes were present in the parenchyma.

2. Subacute phase: This phase was characterized by the continuation of destructive processes at a progressively decreasing level and the strengthening of the immune response. Group 2. was examined in this phase of the infection.

In the animals included in Group 2., the organs most affected by the infection were the liver and kidneys. Degeneration and necrosis were again observed in the liver, and mononuclear cell foci of greater number and concentration were present in the parenchyma. In almost each case, apart from vacuolar degeneration, mononuclear cells were observed, which were mainly composed of macrophages and also contained a few neutrophil leukocytes, and were ascertained to have formed 2 or 3 foci in a single microscopic field (X10 objective). The foci contained necrotic hepatocytes in the centre and were located particularly in the periportal and periacinar regions (Figure 2a). The vena centralis, portal veins and sinus endothelium were swollen and contained macrophages in their lumen. The immunoperoxidase technique produced immunopositive stainings (Figure 2b). Control stainings produced no immunopositive area.

Renal lesions were characterized mostly by glomerular alterations, proximal tubular degeneration and necrosis. It was important point that the tubular degenerative alterations had developed particularly in a few tubules surrounding the glomeruli. In these areas, tubular epithelial cells were swollen and their cytoplasm was light coloured. Some of these cells contained vacuoles with indefinite periphery and displayed patches of pink coloured granules (Figure 2c). In some areas, the lumen of the tubules contained epithelial debris and proteinaceous materials.

The Levaditi method and Warthin Starry staining demonstrated the presence of the infectious agent in the form of masses in hepatic sinusoids and Disse spaces, and also as adhered to the tubular epithelium in the proximal and distal tubules of the kidneys. Immunoperoxidase stainings produced immunopositive areas on the sinusoidal surface of hepatocytes, within sinusoids, and in the cytoplasm of Kupffer cells and macrophages in the liver; and in the proximal tubules (Figure 2d), the lumen of intertubular blood vessels and areas visualized with pycnotic nuclei in the corticomedullar region in the kidneys.

The control stainings did not produce any immunopositive area.

3. Chronic regenerative-proliferative phase: Groups 3. and 4. were examined in this phase of the infection. The hepatic lesions observed in the animals included in Group 3. differed from those observed in Groups 1. and 2. Findings related to hepatic degeneration continued but with progressive decrease, while the dissociation of the Remark cords and regenerative alterations in the hepatic epithelial cells had increased. It was determined that the number and concentration of the mononuclear cell foci found in the periportal and periacinar regions had decreased. In some areas the bile ducts were hyperplastic. The number of hepatocytes with double and large nuclei had increased.

Renal findings also differed from those observed in the first two groups. With respect to the distribution of lesions, glomerular alterations were of less importance and existing lesions were mostly at tubular-interstitial level. Intracytoplasmic vacuoles and desquamation were still observed in the lumen of the tubules, but interestingly, cell infiltrations had started to emerge in the interstitium. Cell infiltrations were observed generally around the subcapsular, periglomerular and convoluted tubules, as well as around the arteries in the corticomedullar region, and were observed to be less severe than those observed in Group 4. Immunoperoxidase stainings produced immunopositive areas in the sinusoids and the sinusoid-facing surface of epithelial cells in the liver; and in the tubular epithelial cells of the cortex and cytoplasm of the macrophages in the interstitium in the kidneys. Control stainings did not produce any immunopositive area.

In Group 4., nonsuppurative interstitial nephritis, a typical symptom associated with the disease, was observed at varying intensity in all animals, and was much severe than that observed in Group 3. The liver displayed both degenerative and regenerative findings, and periportal and periacinar mononuclear cell infiltrations were either very scarce or did not exist.

Although renal lesions did not display significant in-group differences, the distribution and severity of the lesions varied. The lesions were predominated by interstitial nephritis, which varied from subacute to chronic in course. In the periphery of the proximal and distal convoluted tubules and interlobular arteries found in the periglomerular region, both interstitial oedema and mononuclear cell infiltrations were observed.

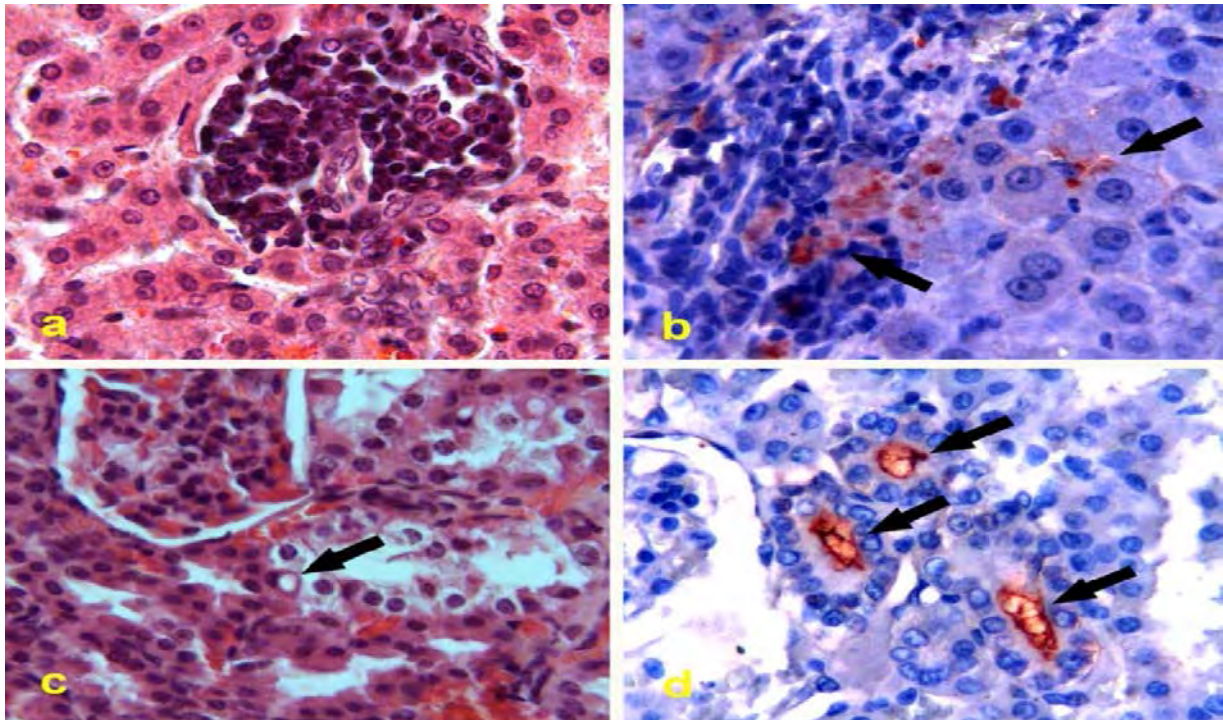


Figure 2. Histopathological view of sections for H&E and Immunohistochemical staining. a) Liver, Group II, focal mononuclear cell infiltrations in parenchyma, H&E, X400, b) Liver, Group II, immunopositive stainings in cytoplasm of mononuclear cells and sinusoids, (Arrows), ABC-P, X 400, c) Kidney, Group II, vacuolar and hydropic degenerations in proximal tubules in areas near glomerulus (Arrow), H&E, X400, d) Kidney, Group II, immunopositive stainings in tubule lumens around glomerulus (Arrows), ABC-P, X 400.

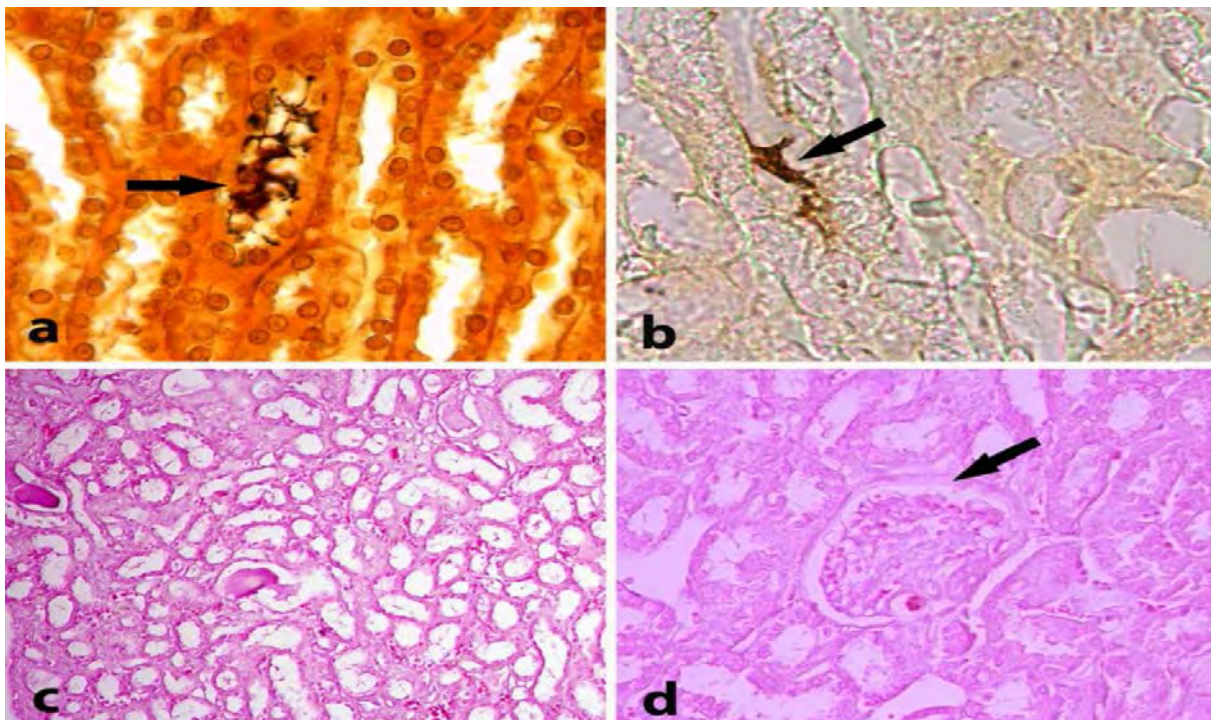


Figure 3. Histopathological view of sections for H&E, Levaditi and Warthin Starry staining. a) Kidney, Group IV, spiral shaped leptospiras in piles hanging on tubule lumens (Arrow), Levaditi X400, b) Liver, Group IV, agents hanging tubule epithels (Arrow), Warthin- Starry, X1000, c) Kidney, Group IV, connective tissue proliferations in interstitium and proteinaceous deposits in tubule lumens, H&E, X100, d) Kidney, Group IV, thickening in parietal leaf of Bowman capsule (Arrow), H&E, X400.

The Levaditi method and Warthin-Starry stainings demonstrated the presence of the spiral-shaped infectious agent, mostly in the form of clusters, in the epithelial cells and lumen of the tubules as well as in the interstitium (Figure 3a, 3b). In some of the euthanized animals, thickening and partial adhesion of the glomerular basal membrane were observed (Figure 3d), whilst some of the glomeruli were atrophic. Furthermore, mild proliferation of the connective tissue in the interstitium and the presence of pink homogenous proteinaceous materials in part of the cortical tubular epithelium were observed (Figure 3c). In two of the animals, syncytial cell formations, which were composed of sloughed epithelial cell groups, were observed in the cell infiltrations located in the interstitium.

Immunoperoxidase stainings produced a few weakly stained areas in the liver of some of the animals. No immunopositive stainings were determined in the other animals. In the kidneys, immunopositive areas were determined within macrophages and in the lumen of tubules. The control stainings did not yield any immunopositive area in the liver or kidneys.

Discussion

As a spirochetal infection that threatens both human and animal health, leptospirosis has been known for more than a century. Various researches have been conducted on the pathogenesis, diagnosis and treatment of the disease (Abdu and Sleight 1965; Hubbert and Miller, 1967; Barnett et al., 1999; Haake et al., 2000) and multiple methods have been developed for protection against the infection (Avila et al., 1985; Brenot et al., 2001; Matsuo et al., 2000). The pathogenesis of the lesions caused by leptospiral agents has drawn the attention of researchers for many years and the focus of previous research has been the investigation of whether the lesions associated with the disease are caused by the infectious agent itself. As regards this issue, some researchers have claimed that the lesions result from the damage caused by the spiral movements of the leptospiral agents to the blood vessel wall and tissues (Barnett et al., 1999; Haake et al., 2000; Pereira et al., 1997; Sitprijja et al., 1980;), while some other researchers (Miller et al., 1974; Van Den Ingh and Hartmann, 1986) have suggested that immunoglobulins produced against leptospiral exotoxins activate the complement system, and thereby lead to the degranulation of neutrophil leukocytes, which brings about the breakdown of the wall of blood vessels and the formation of thrombosis, and have thus, claimed that the degeneration and

haemorrhages associated with leptospirosis are caused by ischemia.

Some researchers have reported that the haemorrhages observed particularly in the lungs and the serous membranes (Miller et al., 1974), and the renal lesions (Van Den Ingh and Hartman, 1986) associated with leptospirosis, develop as a result of the damage to blood vessel walls and thrombosis caused by leptospiral toxins (Arean et al., 1964). However, in the present study, Warthin-Starry and immunoperoxidase stainings having demonstrated haemorrhagic areas in the lungs and the presence of the infectious agent and its antigenic structure in the interalveolar capillaries and alveolar epithelium, the renal glomerular and tubule epithelium in the kidneys, as well as in other organs in the animals included in Group 1; together with the observation of interstitial cell infiltrations and immunopositive areas in these cells in Groups 3. and 4., supported the opinion that lesions are caused by the infectious agent itself (Barnett et al., 1999; Pereira et al., 1997; Sitprijja et al., 1980).

Another aspect that has attracted the attention of researchers in the past years is the route the leptospiral agents use to reach the lumen of renal tubules. While some researchers have suggested that the infectious agent passes from the blood vessels to the interstitium and from the interstitium to the lumen of the tubules (Alves et al., 1991; Sitprijja et al., 1980), some other have claimed that the infectious agent passes into the interstitium from the lumen of the tubules and triggers the interstitial reaction (Barnett et al., 1999). Researchers have not been able to fully explain how the causative agent passes the epithelial barrier, but have put forward several theories. Barnett et al., (1999) have suggested 3 theories. According to the first, by causing the degeneration of the tubular epithelium, the infectious agent increases the permeability of the basal membrane, and thereby, passes into the interstitium. On the other hand, the second theory suggests that the agent has access to the cytoplasm of epithelial cells by means of its spiral movements, and from here is passed into the interstitium via intracytoplasmic vacuoles by means of intracellular transport. According to the third theory, the agent passes from the tubular epithelial cells into the interstitium by active transport.

In the present study, immunoperoxidase stainings in Group 2. having produced immunopositive areas that appeared as granules in the cytoplasm of renal tubular epithelial cells, and the inexistence of positive staining in the vacuoles found within these epithelial cells, do not conform

with the claim of Barnett et al., (1999) that “the infectious agent passes into the interstitium within intracytoplasmic vacuoles”; but rather suggest that these vacuoles could be signs of acute cellular swelling resulting from the effect of leptospiral agents on the tubular epithelium. Furthermore, as leptospiral agents have the capability of rotational movement (Arda et al., 1997) and given that their residues are toxic (Miller et al., 1974; Sitprijia et al., 1980; Masuzawa et al., 1990), the theory that these agents pass from the tubular epithelial cells into the interstitium by active transport and without causing any damage to cells is not considered likely.

Researchers, who have detected the presence of leptospiral antigens in the periphery of renal interstitial blood vessels within the first 3 hours of infection, the lumen of tubules by the end of the 9th hour of infection (Sitprijia et al., 1980), in free form in the lumen of blood vessels and the interstitium in the corticomedullar region on the 4th day of infection, within macrophages in the interstitium, in the periphery of tubules, in the tubular basal membranes and tubular epithelial cells on the 5th day of infection, and in the lumen of tubules on the 6th day of infection (Alves et al., 1991) have reported the distribution of the infectious agent in the renal tissue to occur from

the blood vessels in the interstitium towards the tubules, and have also indicated the inflammatory reaction to result from the movement of the infectious agent in the interstitium. It was noteworthy that in Groups 1. and 2., the first region that was affected by the disease was the tubular epithelium in the periphery of the glomeruli. In this phase, mononuclear cell infiltrations were not encountered in the interstitium. Leptospiral antigens were detected in the glomeruli, the walls surrounding the lumen of adjacent tubules, and the wall, lumen and periphery of interstitial blood vessels. These findings suggest that the infectious agent may access the interstitium by both blood vessels and tubules, and that apart from the agent and its residues, the degeneration of the tubules and blood vessels may also have influence on the development of the interstitial reaction.

The findings other than haemorrhage, which were observed in the other organs, were not considered as typical symptoms of the disease, and were assessed as nonspecific findings.

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References

- Abdu MTF, Sleight SD, 1965: Pathology of experimental leptospira pomona infection in hamsters. *Cornell Vet*, 55, 74–86.
- Alves VA, Gayotto LC, Yasuda PH, Wakamatsu A, Kanamura CT, De Brito T, 1991: Leptospiral antigens (L. interrogans serogrup icterohaemorrhagiae) in the kidney of experimentally infected guinea pigs and their relation to te pathogenesis of the renal injury. *Exp Pathol*, 42, 81–93.
- Arda M, Aydın N, Ilgaz A, Minbay A, Kahraman M, İzgür M, Leloğlu N, Akay Ö, Diker KS, 1997: Spiroketler. In: *Veterinary Microbiology*. Ed.: Mustafa Arda, Medisan Press, Ankara, Turkey. pp. 257-274.
- Arean VM, Sarasin G, Green JH, 1964: The pathogenesis of leptospirosis: toxin production by *Leptospira icterohaemorrhagiae*. *Am J Vet Res*, 25, 836-842.
- Avila FA, Bechara GH, Santa Rosa CA, Avila SHP, Santana AE, 1985: Evaluation of the immune humoral response in swine experimentally vaccinated against *Leptospira interrogans* serotypes pomona and canicola. *Ars Vet*, 1, 51-55.
- Badiola J, Thiermann AB, Cheville NF, 1983: Pathologic features of leptospirosis in hamsters caused by *Leptospira interrogans* serovar hardjoand szwajizak. *Am J Vet Res*, 44, 91-99.
- Barnett JK, Barnett D, Bolin CA, Summers TA, Wagar EA, Cheville NF, Hartskeerl RA, 1999: Expression and distribution of leptospiral outer membrane components during renal infection of hamsters. *Infect Immun*, 67, 853-861.
- Berkin Ş, 1982: Hamsterlerde deneysel leptospiroziste (*L.grippytyphosa*) akciğer kanamalarının ışık ve elektron mikroskopik incelenmesi. *Ankara Üniv Vet Fak Derg*, 29,188-205.
- Brenot A, Trott D, Girons IS, Zuerner R, 2001: Penicillin-binding proteins in *Leptospira interrogans*. *Antimicrob Agents Chemoter*, 3, 870-877.
- Ellis WA, Bryson DG, Neill SD, Mcparland PJ, Malone FE, 1983: Possible involvement of leptospires in abortion, stillbirths and neonatal deaths in sheep. *Vet Rec*, 112, 291-293.
- Ellis GR, Partington DL, Hindmarsh M, Barton MD, 1994: Seroprevalence to *Leptospira interrogans* serovar hardjoin merino stud rams in south Australia. *Aust Vet J*, 71, 203- 206.
- Haake DA, 1999: Expression and distribution of leptospiral outer membrane components during renal infection of hamsters. *Infect Immun*, 67, 853-861.
- Haake DA, 2000: Spirochaetal lipoproteins and pathogenesis. *Microbiol*, 146, 1491–1504.

- Haake DA, Chaogzuerner RL, Barnett JK, Barnett B, Mazel M, Matsunaga J, Levett P, Bolin CA, 2000: The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. *Infect Immun*, 68, 2276–2285.
- Haziroglu R, Milli ÜH, 2001: Urinary system. In: *Veterinary Pathology* Eds., Haziroğlu R, Milli UH, Vol. I., Tamer Press, Ankara, Turkey.
- Hubbert WT, Miller JN, 1967: Effect of antibiotic therapy on experimental leptospirosis infection and development of acquired resistance in guinea pigs. *Am J Vet Res*, 28, 861–864.
- Jones TC, Hunt RD, King NW, 1997: *Veterinary Pathology*. Sixth Ed. Lippincott Wilkins, USA.
- Krivoshin YS, 1989: Spirochetes. *Handbook on microbiology laboratory diagnosis of infection disease*. Mir Publishers, United Kingdom.
- Masuzawa T, Nakamura R, Sehimizu T, Yanagihara Y, 1990: Biological activities and endotoxic activities of protective antigens (Pags) of leptospira interrogans. *Zbl Bakt*, 274, 109-117.
- Matsuo K, Isogai E, Araki Y, 2000: Control of immunologically crossreactive leptospiral infection by administration of lipopolysaccharides from a nonpathogenic strain of leptospira biflexa. *Microbiol Immunol*, 44, 887–890.
- Miller KG, Wilson RB, 1966: Electron microscopy of the liver of the hamster during acute and chronic leptospirosis. *Am J Vet Res*, 27, 1071–1081.
- Miller NG, Allen JE, Wilson RB, 1974: The pathogenesis of hemorrhage in the lung of the hamster during acute leptospirosis. *Med Microbiol Immunol*, 160, 269–278.
- Pereira MM, Andrade J, Lacerda MD, Batoreu NM, Marchevsky RS, Dos Santos RR, 1997: Demonstration of leptospiral antigens on tissues using monoclonal antibodies and avidin-biotin peroxidase staining. *Exp Toxic Pathol*, 49, 505–511.
- Presnell JK, Schreibman MP, 1997: *Staining microorganisms*. Humason's animal tissue techniques. Johns Hopkins Univ. Press. Ltd., USA.
- Scanziani E, Sironi G, Mandelli G, 1989: Immunoperoxidase studies on leptospiral nephritis of swine. *Vet Pathol*, 26, 442–444.
- Schricker RL, Hanson LE, 1961: Effect of cortisone on leptospira pomona infection in the guinea pig. *Am J Vet Res*, 22, 580–586.
- Sitprija V, Pipatanagul V, Mertowidjojo K, Boonpucknavig V, Boonpucknavig S, 1980: Pathogenesis of renal disease in leptospirosis: Clinical and experimental studies. *Kidney Int*, 17, 827–836.
- Van Den Ingh TS, Hartman EG, 1986: Pathology of acute leptospira interrogans serotype icterohaemorrhagiae infection in the syrian hamster. *Vet Microbiol*, 12, 367–376.
- Weber WJ, Creamer HR, Bohl EH, 1956: Chemotherapy in hamsters cronicly infected with Leptospira canicola. *J Am Vet Med Assoc*, 15, 271-273.
- Yener Z, Keles H, 2001: Immunoperoxidase and Histopathological Examinations of Leptospiral Nephritis in Cattle. *J Vet Med A*, 48, 441-447.
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