Microscopic changes in the kidneys of cows infected with *Leptospira* sp.

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Abstract

The article presents data on histopathological studies of the kidneys of cows, which either recovered or died from leptospirosis. Fragments of seven kidneys from slaughtered cows, positive for *Leptospira* antibodies in the microscopic agglutination test (MAT) (titres of 50 and higher) were used in the study. The MAT was conducted with eight serological groups of *Leptospira*: Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Sejroe, Tarassovi, and Australis. Microscopic changes in all morphological structures of the kidneys were presented. Micromorphological criteria, which can be used for post-mortem diagnosis of leptospirosis were established. They included: serous glomerulonephritis with granular dystrophy of podocytes, necrosis and collapse of the inner layer of Bowman's capsule, partial destruction of capsule and vascular glomeruli, granular and vacuolar degeneration and destruction of tubular epithelial cells, foci of interstitial oedema, and infiltrations predominantly with monocytes and isolated neutrophils. Microscopic changes in the kidneys suggest that the cows died from leptospirosis as a result of toxic shock syndrome.

Keywords: cattle, leptospirosis, kidneys, microscopic changes, toxic shock syndrome.

Introduction

Leptospirosis, caused by pathogenic *Leptospira* species, is one of the most widespread and best-known zoonoses. Although there is considerable genetic diversity among pathogenic *Leptospira*, the clinical manifestation of the disease is constant for all species and ranges from a mild, largely asymptomatic chronic infection, to an acute, potentially lethal one (8). At least 0.5 million human cases occur each year, with mortality rates ranging from 5% to 15% (14). They infect a wide spectrum of hosts, including mammals, reptiles, birds, and amphibians. A variety of clinical symptoms may be seen in cows infected for the first time: abortion, mastitis, loss of milk, stillbirth, or giving birth to weak or infected but clinically healthy calves. Infertility associated with persistent infection is the most important economic consequence. Infection is usually transmitted by direct contact with contaminated urine, run-off water, or abortion fluids from infected animals.

Leptospires usually survive in the renal tubules of many domestic and wild animals (1, 9). In acute infections, all organs may become infected. Bacterial passage from infected kidneys to the bladder and release of the bacteria during urination can expose new hosts to leptospires (8).

The disease is characterised by the development of vasculitis, endothelial damage, and inflammatory infiltrates composed of monocytic cells, plasma cells, histiocytes, and neutrophils. During gross examination, petechial or even extensive haemorrhages are common (2), and organs are often discoloured due to icterus (19). Histopathological lesions are the most visible in the liver, kidneys, heart, and lungs (6, 12, 16), but other organs may also be affected according to the severity of the individual infection. The overall structure of the liver is not significantly disrupted, but there may be
intrahepatic cholestasis. Hypertrophy and hyperplasia of Kupffer cells are evident, and erythropagocytosis has been reported. In the kidneys, interstitial nephritis is the major finding, accompanied by an intense cellular infiltration composed of neutrophils and monocytes (20).

Pathological findings in the heart include interstitial myocarditis with infiltration of predominantly lymphocytes and plasma cells, petechial haemorrhages (particularly in the epicardium), mononuclear infiltrations in the epicardium, pericardial effusions, and coronary arteritis (5, 7, 13). In the lungs, pulmonary congestion and haemorrhage are common (21, 23), and infiltrations of alveolar spaces by monocytes and neutrophils occur (14). In the brain, perivascular cuffings are observed (24).

The aim of the study was to investigate the microscopic lesions in the kidneys of cows infected with leptospirosis.

Material and Methods

Antigens. Reference strains of serological groups of *Leptospira interrogans*: Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Pomona, Sejroe, Tarassovi, and Australis were cultured to prepare the microscopic agglutination test (MAT). The reference strains are kept in the Laboratory of Leptospirosis at the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences in Kiev. Leptospires were cultivated in Kortgof liquid medium at 28-30ºC under aerobic conditions. The strains were subcultured every 7-10 d.

Microscopic agglutination test. The test was carried out according to the OIE Manual of Standards for Diagnostic Tests and Vaccines (4). The serum samples diluted 1:25 were mixed with an equal volume of each of the *Leptospira* serovars. Including this added antigen, the serum dilution used during the preliminary examination was 1:50. For samples reacting in the preliminary examination with one or more serovars, a series of twofold dilutions were prepared to titre end point: 50% agglutination. The samples with titres of 50 were recognised as positive.

Histological examination. Fragments of the kidneys of seven cows displaying specific antibody titres of 50 and higher in the MAT were fixed in 10% buffered formalin, dehydrated in rising concentrations of ethanol, and embedded in paraplast through chloroform. Sections were stained with haematoxylin and eosin (H.E.).

Results

In histological studies of the kidneys of cows which died from leptospirosis, the presence of significant microscopic changes in all morphological structures was observed.

All blood vessels were dilated and filled with blood. Endothelial cells of arteries and veins were in a state of granular degeneration, some of them collapsed. In most cases, disruption of the walls of blood vessels of various types was registered, resulting in a haemorrhage which originated in the stroma (Fig. 1).

In all renal corpuscles extracapillary serous glomerulonephritis was observed. Its early stage was characterised only by accumulation of serous fluid in the Bowman’s capsule.

With the development of this process in the liver, the severity of microscopic changes increased markedly. Capillaries of vascular glomeruli were
clearly expanded, and they became swollen (Fig. 2). Granular degeneration and decomposition of podocytes were observed. All or almost all cells of the inner layer of the Bowman’s capsule were necrotic and collapsed. Part of the cells of the outer layer of Bowman’s capsule were in a state of granular degeneration and some of them were destroyed.

Later, accumulation of a large volume of serous fluid was observed in the cavity of the Bowman’s capsule. This led to an oedema in the glomerular vasculature (Fig. 3). A significant number of mesangiocytes were in a state of granular degeneration or destroyed. In some dystrophic cells of mesangium there was a margination of chromatin, which is, according to the modern view, the precursor of cell death. The accumulation of serous fluid also resulted in partial destruction of glomerular vessels and partial destruction of the Bowman’s capsule.

The nature of the microscopic changes in different nephron tubules was clearly dependent on the degree of microscopic changes in renal corpuscles - the more pronounced were the changes in the renal corpuscles, the more severe were the changes in the renal tubules.

In all parts of the nephron tubules in the renal corpuscles, where there was the initial stage of serous extracapillary glomerulonephritis, the majority of cells of the epithelium displayed visible signs of progressing granular degeneration (Fig. 1). In some areas of these tubules, separation of epithelial cells from the basement membrane and full decomposition of epithelial cells were identified. In all parts of the nephron tubules in the renal corpuscles in which significant microscopic changes were registered, cells of the epithelium were subjected to vacuolar degeneration and gradual destruction (Fig. 4). In one part of the tubules, epithelial cells were completely or partially destroyed and the destruction of the basal membrane of the epithelium was also recorded.

The renal interstitium was swollen in some places and a large number of monocytes and isolated neutrophils were found in these places (Fig. 5).

Discussion

Leptospirosis is a worldwide disease except Antarctica (1). This disease affects humans, wildlife, and domestic animals, including cattle. In the severe forms of the disease renal impairment is frequent. In some cases it leads to the incidence of acute renal insufficiency (11). It occurs when the kidneys become unable to filter waste products from the blood. When the kidneys lose their filtering ability, dangerous levels of metabolites may accumulate and the balance of the blood’s chemical composition may be destructed (15).

Examinations of the main necropsy material of fatal cases of leptospirosis under light and electron
microscopies in consort with determination of infectious and immune complexes demonstrated that leptospires are capable of causing toxic-infectious shock (TIS) with typical changes in the microcirculatory bed and organ parenchyma (3, 10).

The results of our histological studies indicate that the kidneys of cows with leptospirosis displayed significant changes in the microscopic structure. In part of the renal glomeruli, a destruction of the Bowman's capsule and necrosis of a part of their cells were recorded. In the epithelium of the renal tubules degenerative changes and destruction of epithelial cells were registered. We believe these changes reflect the effects of leptospira toxins in renal parenchymal patterns. It is confirmed by the case report where the presence of microscopic changes typical for necrosis, but not for cell apoptosis, was observed (22). This leads to condensation, nuclear fragmentation, and externalisation of membrane phospholipids, giving a signal for these cells to be engulfed by macrophages (18).

Additionally, the dystrophic cells lose their specific functions. Therefore, degenerative and necrotic changes in the cells of different parts of the nephron reflect the total or partial cessation of function of affected nephrons, resulting in acute renal failure. Defeat of the endothelial cells in blood vessels, in our opinion, was also due to the influence of leptospira toxins circulating in the blood. The presence of leptospira toxins and renal insufficiency causes the development of the general intoxication (17).

It is known that toxic shock syndrome (TSS) is the potentially fatal illness caused by different bacterial toxins. Different bacterial toxins may cause the syndrome, depending on the situation. Microscopic changes in the kidneys suggest that the death of cows from leptospirosis might occur as a direct result of TSS.

References