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NECROPSY AND HISTOPATHOLOGICAL RESEARCH IN SWINE SALMONELLOSIS

NECROPSIE ŞI CERCETĂRI HISTOPATOLOGICE ÎN SALMONELOZA PORCINĂ

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ABSTRACT | REZUMAT

The study was conducted between October 2021 and February 2023 through the necropsy at the Discipline of Forensic Medicine and Necropsy Diagnosis of F.M.V. Timisoara of 87 pig cadavers aged between one week and 12 weeks, sampled from the intensive breeding system, in order to elucidate. the cause of death, of which 61 presented gastrointestinal lesions of an inflammatory nature. After evisceration, a thorough macroscopic examination of the organs under study was carried out. We took samples from the gastrointestinal mass for histopathological examination. This examination involved the recording of modified structural features (shape, size, colour, appearance, lobulation, consistency, and section examination) and the collection of samples for microscopic examination. The obtained histopathological preparations were examined under an Olympus Cx41 microscope with increasing magnifications (x6, x10, x20, and x40), the most significant lesions were microphotographed. Of the 87 necropsied pig carcasses aged between one week and 12 weeks, 61 (70.11%) presented gastrointestinal lesions of an inflammatory nature, and 48 (55.17% of the carcasses and 78.68% of corpses with gastroenteropathies) took on the appearance of haemorrhagic and fibrino-haemorrhagic inflammation. For an even more accurate diagnosis of salmonellosis, as well as to confirm the diagnosis, we performed a randomised bacterial identification exam - API (Analytical Profile Index) 20E within the Discipline of Infectious Diseases within the Faculty of Veterinary Medicine in Timișoara.

Keywords: pig, *Salmonella*, pathogenesis, gastrointestinal lesions, API Test

Cercetările s-au efectuat în perioada octombrie 2021 - februarie 2023, prin necropsierea la Disciplina de Medicină Legală și Diagnostic necropsic a F.M.V. Timișoara a 87 cadavre suine cu vârste cuprinse între o săptămână și 12 săptămâni, provenite din sistemul intensiv de creștere, în vederea elucidării cauzei morții, din care 61 au prezentat leziuni gastro-intestinale de natură inflamatorie. După eviscerare, s-a efectuat examinarea macroscopică amănunțită a organelor luate în studiu. Din masa gastro-intestinală am prelevat probe pentru examen histopatologic. Acest examen a vizat consemnarea particularităților structurale modificate (formă, mărime, culoare, aspect, lobulație, consistență și examen pe secțiune) și recoltarea probelor pentru examene microscopice. Preparatele histopatologice obținute au fost examinate la un microscop Olympus Cx41, cu obiective crescânde (x6, x10, x20, x40), leziunile relevante au fost microfotografiate. Din cele 87 cadavre suine cu vârste cuprinse între o săptămână și 12 săptămâni care au fost necropsiate, 61 (70,11%) au prezentat leziuni gastro-intestinale de natură inflamatorie, 48 (55,17% dintre total cadavre și 78,68% din cadavrele cu gastroenteropatii) îmbrăcând aspectul inflamației hemoragice și fibrino-hemoragice. Pentru o stabilire mai exactă a diagnosticului de salmoneloză suină, cât și pentru confirmarea diagnosticului, am efectuat examen randomizat de identificare a bacteriilor - API (Analytical Profile Index)20E în cadrul Disciplinei de Boli infecțioase din cadrul Facultății de Medicină Veterinară din Timișoara.

Cuvinte cheie: suine, *Salmonella*, patogeneză, leziuni gastrointestinale, Test API

The generic name of salmonellosis includes toxic-infectious diseases, produced by bacteria of the genus *Salmonella* with universal distribution that can affect the entire animal kingdom, including humans. In animals of economic interest, the disease evolves either in an acute septicaemic form or in a subacute or chronic form, with digestive or genital localization. Pulmonary, joint, and, less often, nervous localizations are also recorded (8, 10, 12, 22). In parallel with the fundamental entero-genito-tropism, salmonellae also show a remarkable reticulotropism, associated with a prolonged portage of germs.

The pig, among all domestic mammals, is the most frequently affected species and the host of the most numerous salmonella serotypes, most of which are identified during the examination of healthy animals

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slaughtered in the slaughterhouse, the clinical forms of the disease being the prerogative of a limited number of serotypes (5, 11, 17).

Salmonella infection can be clinically inapparent or manifest clinically as septicaemia, enterocolitis, or pulmonary localizations, less often as meningoencephalitis or abortion (2, 7, 15, 21).

Etiologically, the serotype with the most important role in pig pathology is *S. choleraesuis,* with two varieties: monophasic or Kunzendorf, and biphasic, or American, from which *S. typhisuis* and *S. paratyphi* C are derived through selective mutations (3, 6, 21).

Salmonella choleraesuis causes most cases of salmonellosis in pigs, which, together with *Salmonella typhimurium*, account for over a third of swine salmonella outbreaks (5, 9).

Salmonella choleraesuis, although a pig-adapted species, has been described as causing disease in many other species of mammals, birds, and humans. The infection in humans is manifested by jaundice, pleuropneumonia, periostitis, and meningitis, with the highest morbidity and mortality recorded in children (7, 9, 12, 16).

The epidemiology of pig salmonellosis must be considered from several distinct perspectives, among which are: looking at the disease with clinical manifestations in live animals; salmonella contamination of pig carcasses during slaughter (13) or of derived products during their production technology, with a negative impact on public health. To all this can be added the ability of *Salmonella* to form bacterial biofilms that launch challenges and concerns for processors in the food industry (1, 20). Not separating these epidemiological aspects creates confusion, which is sometimes serious. Regarding the epidemiology of the slaughterhouse, regarding live and slaughtered pigs, a chain of risk factors must be noted. Thus, transport stress activates latent carriers, which will contaminate the environment in the slaughterhouse; the duration of the pre-slaughter period, which can increase by 50% for every 24 hours; and the prevalence of infection; the mechanical transfer of contamination between casings and machines. Sick animals, slaughtered out of necessity, may be sent for industrial processing. Sacrifice and utilisation for public consumption of animals that have passed through the disease, and been treated will be possible after 60 days, with adequate bacteriological control (18).

MATERIALS AND METHODS

The research was carried out between October 2021 and February 2023, through the necropsy at the Discipline of Forensic Medicine and Necropsy Diagnosis of F.M.V. Timișoara of 87 pig cadavers aged between one week and 12 weeks from the intensive breeding system in order to elucidate the cause of death, of which 61 presented gastrointestinal lesions of an inflammatory nature.

The necropsy was performed using specific swine techniques (2, 14). After evisceration, a macroscopic examination of the organs was performed. The histopathological examination was made after taking samples from the gastro-intestinal mass.

The samples were fixed in a 10% formaldehyde solution for 24 hours, then modelled and reintroduced into a new formaldehyde bath for final fixation for two days. The fixed samples were passed into the dehydration battery, consisting of alcohol in increasing concentrations, from 70 degrees to absolute alcohol. In each of the 5 baths, the samples were kept for 2 hours, after which the alcohol was removed by introduction into benzene to clarify the section, and then the paraffin bath was placed in a thermostat at 56 ° C. By paraffining, blocks were obtained containing samples (fragments) of injured organs, which were sectioned at the microtome at six micrometres. The obtained sections were fixed on well-degreased slides with Mayer albumin. The sections glued to the slides were stained by the Masson trichromic method (H.E.A.) modified by V. Ciurea with methyl blue for overall examination and the Giemsa method for cellular details (4, 19). Confirmation of the certainty of the diagnosis of salmonellosis was made with the help of the API (Analytical Profile Index) 20E test within the Discipline of Infectious Diseases within the Faculty of Veterinary Medicine in Timisoara (23) (Fig. 2).

RESULTS AND DISCUSSIONS

Out of the 87 necropsied pig carcasses aged between one week and 12 weeks, 61 (70.11% of the examined carcasses) presented gastrointestinal lesions of an inflammatory nature, and 48 (55.17% of the carcasses and 78.68% of corpses with gastroenteropathies) had the appearance of haemorrhagic and fibrino-haemorrhagic inflammation (Fig. 1).

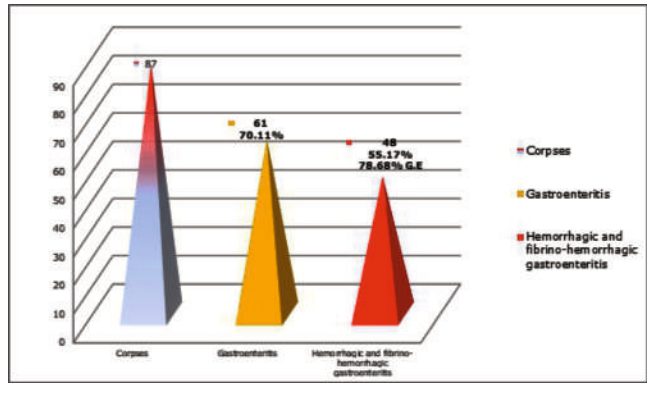

Fig. 1. Graphical representation of cases of gastroenteropathies in the cases studied

Gastrointestinal inflammatory lesions, correlated with lesions present at the level of other organs, but mainly in the liver, lung, and heart, led me to widen the scope of morphopathological investigations in order to record the structural changes in dynamics, both at the place of colonisation and internalisation of *Salmonella*, as well as some organs of vital importance, in order to establish the morphopathological diagnosis of porcine salmonellosis. Also, for an even more accurate diagnosis of swine salmonellosis, we performed a randomised bacterial identification exam - API (Analytical Profile Index) 20E, where we obtained confirmation of Salmonella (*Salmonella* Typhimurium, one case being *Salmonella* Choleraesuis, Spp. Arizonae) (Fig. 2).

Fig. 2. Results of the API test (Analytical Profile Index) 20E

Fig. 3. Pig carcasses: mediocre to bad maintenance condition, enolphthalmia, pallor of apparent mucous membranes, dehydration, and reddish areas (varioloid exanthema) on the skin surface

During the external examination of the corpses, we observed a mediocre to bad state of maintenance, enolphthalmia, pallor of the apparent mucous membranes, dehydration, reduced skin elasticity, and the presence of reddish coloured areas (varioloid exanthema) on the external face of the hocks, the temporomandibular region, ears, and the external face of the tarso-metatarsal joints (Fig. 3).

The hindquarters and anal region were soiled with brown faeces, with a repulsive smell. The internal examination revealed the presence of a yellowish, semitransparent liquid in the chest cavity (serous exudate). In the pericardial cavity, there was a greyish-yellowish, friable, matte, rugous mass adherent to the epicardium. In the abdominal cavity, the existence of a yellowish, citrine, slightly opalescent liquid was reported in the amount of approximately 100 ml (in 9 cases) - ascites. The macroscopic examination revealed changes in the physical-structural features in the stomach, colon, lungs, heart, liver, and kidneys. After examining the stomach on the surface, I opened it along the great curvature, along its entire length, from the cardia orifice to the pylorus. The content, mucosa, and wall per section were examined, assessing the colour, consistency, appearance and thickness.

Macroscopically, the wall of the stomach was thickened, swollen, and oedematous. It presented on the section a brown-ossific colour with a slight appearance of "coffee grounds". The content consisted of fodder mixed with a bloody mass,which after removal allowed the observation of the grey mucosa with extended areas of reddish colour, which remained even after washing under a jet of water - diffuse haemorrhagic gastritis (Fig. 4).

Fig. 4. Pig stomach: diffuse haemorrhagic gastritis; extensive areas of reddish colour, which remained even after washing under running water

Microscopically, the presence of haemorrhagic exudate in the mucosa, submucosa, and muscle is observed. In the lamina propria, there were atrophies, dystrophies, and necrosis of the structural components (glandular tubes, connective tissue, lymphoid follicles, etc.) (Fig. 5).

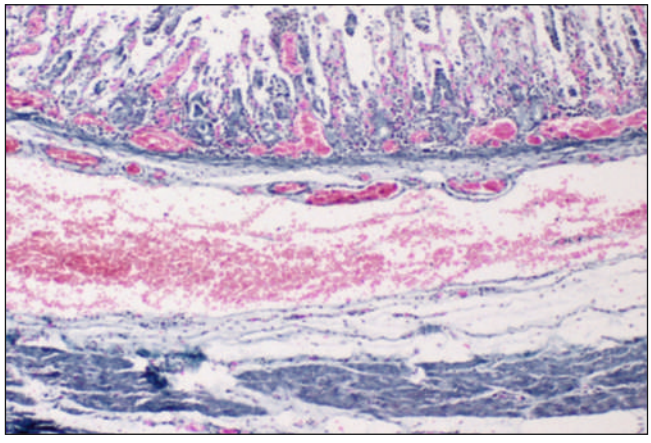

Fig. 5. Haemorrhagic gastritis: the presence of haemorrhagic exudate in the mucosa, submucosa, and muscle; atrophies, dystrophies, and necrosis of structural components (Haematoxylin-eosin and alcian blue staining, x10)

On colon, macroscopically, on the surface, on the grey background, you could see pinkish-reddish areas with slightly increased elastic consistency. After the opening, the intestinal contents were brownish-green in colour and foul-smelling. After removing the contents, the mucosa was observed, which presented grey-yellow deposits on the surface, stratified, diffusely arranged, reliable, and similar to "bran" - diffuse diphtheroid colitis.

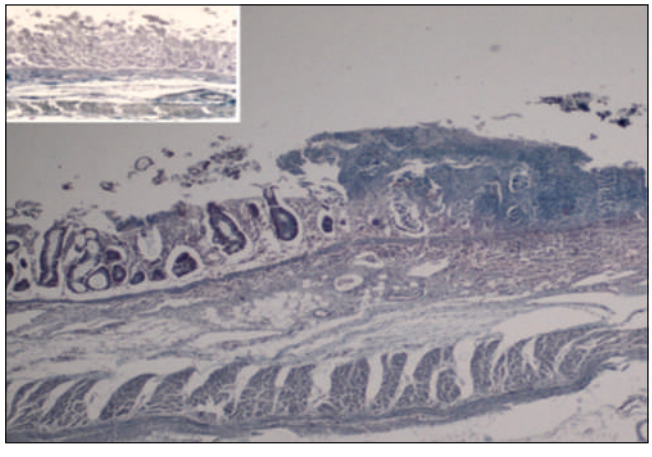

Fig. 6. Diffuse diphtheroid colitis: fibrinous exudate on the surface of the mucosa, necrosis, and desquamation of the epithelium (Haematoxylin-eosin and alcian blue staining, x10)

Microscopically, it is revealed: the presence of a fibrino-necrotic exudate on the surface of the mucosa and in the lumen; necrosis of the covering and glandular epithelium; necrosis of lymphoid follicles and surrounding tissue; massive leukocyte infiltrate at the base of the mucosa; a fibrin network strongly anchored in the lamina propria and submucosa; and hypertrophy of the submucosa, following vascular changes (congestion, oedema, fibrinous exudate, leukocyte infiltrate) (Fig. 6).

Macroscopically, in 12 cases, both lungs increased in volume and weight, and the pleura was tense. Some pulmonary lobules had a red-cherry colour on the surface, and on the section, the colour was identical to that on the surface, with pasty consistency, and the docimasia "between two waters" - passive pulmonary congestion and inflammatory oedema. Other pulmonary lobules had a red-brown colour and a slightly mosaic appearance, with whitish adherent deposits and a "brackish" appearance on the surface of the pleura, alternating with white-yellowish areas (interstitial enema). The section surface was dry, with a matte appearance, finely granular (rough), and with colour differences between the densified tissue and the bronchial apparatus, which had a lighter shade compared to the red-brown background of the lungs, giving it a marbled appearance. Docimasia in brown areas with a mosaic appearance is positive - fibrinous pleurobronchopneumonia (Fig. 7).

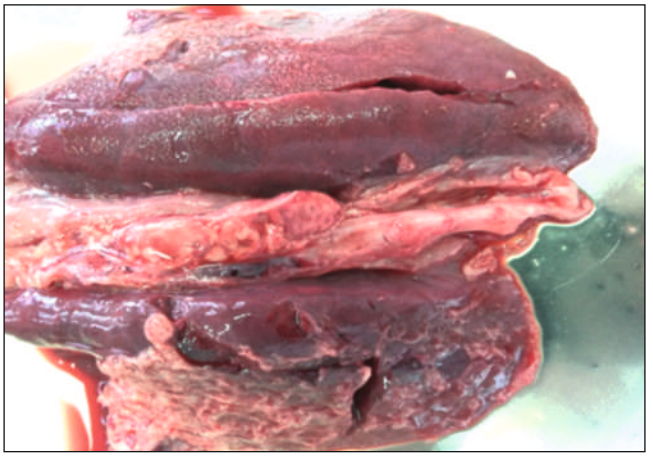

Fig. 7. Pig lung: fibrinous pleurobronchopneumonia

Microscopically: hyperaemia of alveolar capillaries; fibrino-haemorrhagic exudate in the bronchial and parabronchial lumen; fibrino-haemorrhagic exudate in the pulmonary parenchyma (intraalveolar and intralobular); the alveolar exudate is made up of a network of fibrin in the mesh of which there are numerous red blood cells, rare leukocytes, and desquamated alveolar cells, which completely eliminate the air from the alveoli. These aspects circumscribe croupal bronchopneumonia - the red hepatization phase (Fig. 8).

In six cases, the affected lung areas presented aspects almost identical to those of the previous stage, but the colour was grey, the surface of the section was wet and shiny, the marbling was more pronounced, and a yellowish creamy liquid was expressed on compression due to the accumulation of neutrophils in the alveoli. Microscopically: capillary hyperaemia was less

or absent (alveolar capillaries were flattened due to intraalveolar exudate); a large number of neutrophils were present in the lumen of the alveoli, and fibrin was very little or absent; the predominance of neutrophils in the lumen of the bronchi and alveoli explains the absence of fibrin, which was lysed by proteolytic enzymes; the alveolar endothelium is completely gone – croupal bronchopneumonia, grey hepatization phase (Fig. 9).

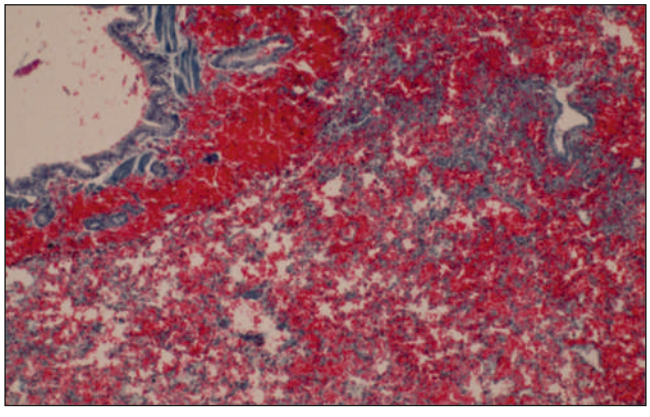

Fig. 8. Fibrinous bronchopneumonia: fibrinous exudate-peribronchial haemorrhage, in the lumen of the bronchus and interlobular (Haematoxylin-eosin and alcian blue staining, x20)

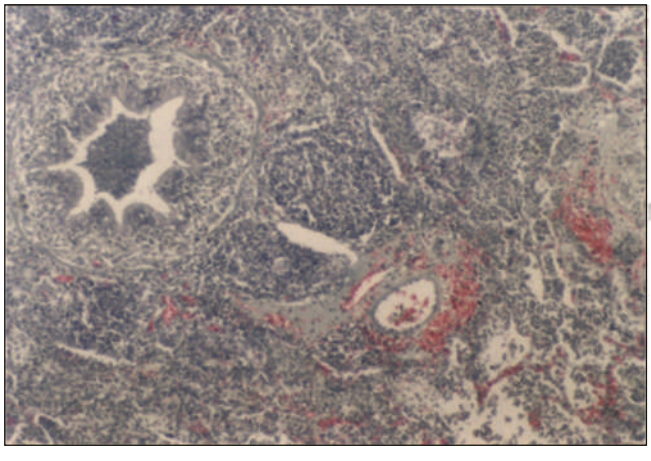

Fig. 9. Fibrinous bronchopneumonia, gray hepatization phase – predominantly intrabrochial, intraalveolar, and interlobular nephrofilc exudate (Haematoxylin-eosin and alcian blue staining, x10)

Macroscopically, the pericardial sac was distended and slightly adherent to the epicardium due to the yellowish-grey fibrinous exudate. After opening, the presence of a fibrinous exudate was observed in the pericardial cavity, in the structure of which small amounts of serous exudate, haemorrhage, or purulent were present. The surface of the pericardial serosa was opaque, thickened, rough, and lacked luster due to fibrin deposits - fibrinous pericarditis (Fig. 10).

Fig. 10. Pig heart, fibrinous pericarditis: greyish-yellowish mass, friable, matte, rough adherent to the epicardium

Microscopically, it was observed: a fibrin network (in the meshes of which there were micro- and macrophages) attached to the swollen and oedematous epicardium, partially or totally desquamated mesothelium; subepicardial mesenchymal reaction; myocardium in cross section (Fig. 11).

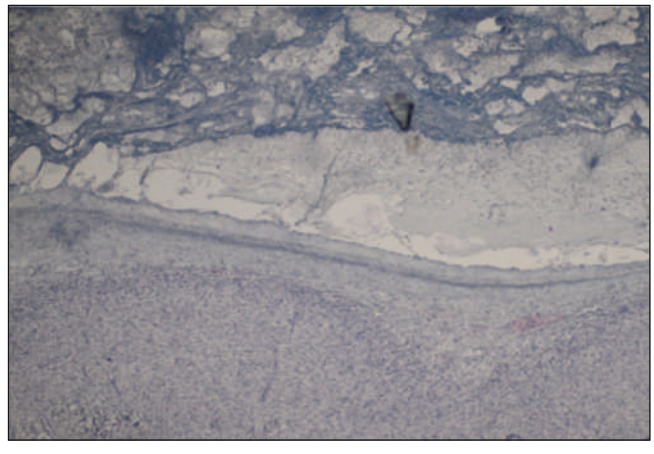

Fig. 11. Fibrinous pericarditis: fibrin network attached to the oedematous epicardium (Haematoxylin-eosin and alcian blue staining, x10)

Macroscopically, the liver was enlarged in volume and weight, with a tense and shiny capsule. On a uniform yellow background, both on the surface and on the section, there were numerous small, yellowish foci, the size of a "millet grain", friable - panlobular hepatic steatosis, and necrotic miliary hepatitis.

Microscopically, the hepatocytes in most of the lobules showed numerous optically empty vacuoles in the cytoplasm, of different shapes and sizes, which caused compressions on the nucleus (small, angular, or absent). These vacuoles represent the place where

the lipids are stored, which were solubilized in organic solvents (toluene, xylene, and benzene) by the paraffin technique, and thus the place remained empty. Some liver lobules occasionally show small, achromatic areas (hepatocyte necrosis). In the periphery and intralobular areas, hyperplasia of the connective fibres can be observed, which represents the initial phase of cirrhosis (Fig. 12).

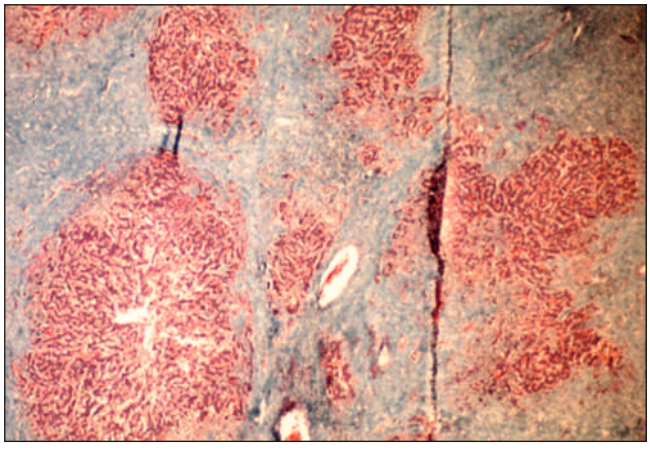

Fig. 12. Pig liver: atrophies, dystrophies, and necrosis, followed by fibroconjunctival hyperplasia (cirrhosis) ((Haematoxylin-eosin and alcian blue staining, x10)

The kidneys, macroscopically, were enlarged in volume with a globular, turgid, reddish-brown appearance, with small diffuse areas of yellowish colour and small spots the size of a whitish-yellowish needle and slightly larger white spots, increased consistency where the sectioning is more difficult (areas of fibrous interstitial nephritis), and on section the appearance was moist, homogeneous, the cortex brown with a reddish tinge, with small whitish foci, the medullary reddish – renal dystrophies and necrosis (Fig. 13).

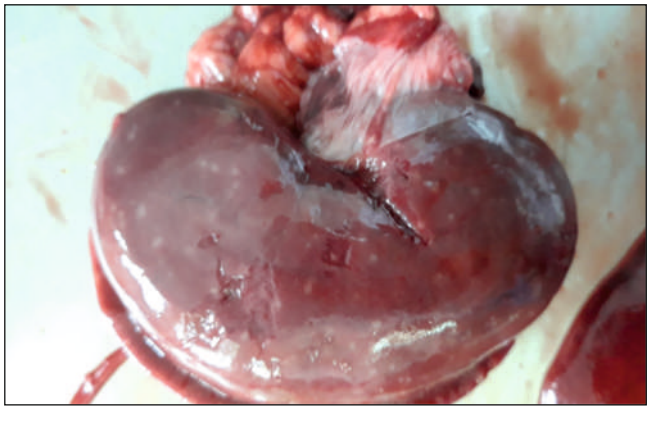

Fig. 13. Pig kidneys: renal dystrophies and necrosis

Microscopically, numerous extravascular hematomas were found in the interstitial space (peritubular and periglomerular), which cause atrophy, dystrophy, and necrosis of the glomeruli and urinary tubules - interstitial haemorrhages and tubular necrosis (Fig. 14.).

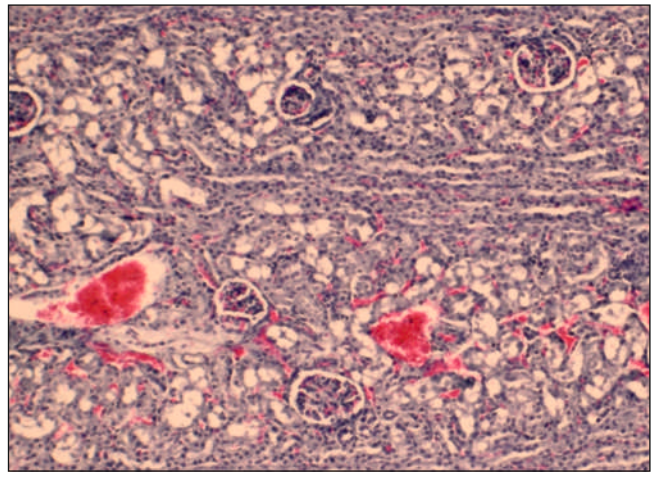

Fig. 14. Section through the kidneys: peritubular and periglomerular hemorrhages, and necrosis of the urinary tubules epithet. (Haematoxylin-eosin and alcian blue staining, x20)

The macroscopic and microscopic aspects highlighted by the images correspond to those mentioned in the specialized literature (7, 9, 16, 21), correlated with other non-specific lesions of equally great importance, with effects on the whole organism, which proves the significant economic impact.

CONCLUSIONS

From a total of 87 necropsied pig carcasses aged between one week and 12 weeks, 61 (70.11%) presented gastrointestinal lesions of an inflammatory nature, and 48 (55.17% of the carcasses and 78.68% of corpses with gastroenteropathies) had the appearance of haemorrhagic and fibrino-haemorrhagic inflammation. Morphopathologically, specific lesions were highlighted: haemorrhagic gastritis in acute and subacute evolutions; diffuse diphtheroid colitis; haemorrhagic and/or fibrino-haemorrhagic gastritis; fibrinous bronchopneumonia; fibrinous pericarditis; steatosis; and necrotic miliary hepatitis in subacute-chronic evolutions and non-specific lesions: hydrothorax, ascites, interstitial haemorrhages and renal tubular necrosis. The morphopathological diagnosis of porcine salmonellosis was established on the basis of specific macro- and microscopic lesions, correlated with anamnetic data and randomised laboratory examinations, through which the serotypes present were established: *Salmonella* Typhimurium and *Salmonella* Choleraesuis with an increased predominance of *Salmonella* Typhimurium.

Due to the fact that current methods do not allow the detection of carrier pigs, which makes it extremely difficult to prevent the introduction of salmonellosis into a herd, it is recommended: the initial selection of drugs used in treatment based on antimicrobial sensitivity testing; ensuring the microclimate and avoiding exposure of the body to stress factors, very important factors in the treatment and control of the disease; vaccination, corroborated with good management practices such as carrying out sanitary-veterinary actions to prevent and combat infectious diseases (according to the Strategic Programme of ANSVSA and Order no. 35 of March 30, 2016).

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