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Determination of fibrin age in pathologically changed tissues in porcine reproductive and respiratory syndrome

V V Stafford and Ya B Strel'tsova

Federal State Budget Institution "Federal Scientific Center VIEV", 24-1, Ryazanskiy avenue, Moscow, 109428, Russia

E-mail: stafford.v.v@gmail.com

Abstract. The paper presents data supplementing the pathogenesis of porcine viral reproductive and respiratory syndrome. It was shown that the pathological changes taking place are characteristic both for animals infected under natural conditions and for experimental infection of piglets. Pathological data reflecting the development of pathology in the skin and in the internal organs of piglets indicate the development of systemic pathology affecting the hepato-linear, immune, respiratory and vascular system. The use of specific staining for fibrin showed its effect on the development of pathology not only in the respiratory system, but also in immunocompetent tissues. Specific staining for fibrin in histological sections of lung and lymph node tissues made it possible to determine that "young" fibrin coagulates in the tissues for 6 hours and becomes yellow-orange; "Mature" fibrin stays longer in the body for up to 24 hours and is painted depending on the period in orange-red (6-12 hours), bright red (12-18 hours) and red-violet (18-24 hours), then turning into the "old" fibrin, which is painted in purple (more than 24 hours) and grey-blue (more than 48 hours).

1. Introduction

Pig production is one of the most rapidly developing agricultural sectors in the Russian Federation. Due to the intensification of technological processes of fattening and raising animals, pig farms provide uninterrupted supply of food products [1]. However, the prosperous development of this industry, like any other branch of animal husbandry, is hampered by the occurrence of infectious diseases that cause major economic damage [2, 3]. Among infectious diseases of pigs, one of the leading positions is occupied by diseases caused by viral agents [4, 5]. These infections include porcine reproductive and respiratory syndrome (PRRS) and circovirus infiltration of the second type of pigs (PCV-2) [2, 6, 3]. Each of these diseases causes severe pathological changes in the organs, leading to death. In Russia and abroad, great attention is paid to the diagnosis of PRRS, however, despite the rather large-scale research in the field of virology and molecular biology, questions remain about the nature of the pathological effect of the PRRS virus on the animal's organism [1, 6, 7]. The main problem of studying the effect of PRRS virus on the pig organism and the pathway for the development of the pathogenesis of the disease is the high degree of variability of the virus within one type [1, 7, 5].

2. Materials and methods

The work was performed with piglets having respiratory pathology from pig farms of the Russian Federation, where PRRS is regularly registered. The aim of the study was to determine the presence of



fibrin in the test samples, determine the time of fibrin coagulation in the tissues and find out the effect of the disease on the pathogenesis.

The autopsy of the animals was performed according to the rules of the autopsy, along the midline. A visual inspection of the organocomplex was performed, the correctness of the anatomical topography of the organs of the thoracic and abdominal cavities were inspected. For histological examination, pathological material was collected from lungs and bronchial lymph nodes. From the obtained pathological material, samples of organs were cut, fixed in 10% buffered formalin. Paraffin filling of samples was performed in a carousel-type automatic installation. Paraffin blocks were cut to a thickness of 5 microns and stained routinely (hematoxylin-eosin). Routine staining of histosections was performed in a linear automatic installation of Thermo Scientific company; specific staining for fibrin was performed by an instrument set of BioVitrum company, by manual method.

3. Results

The most striking pathoanatomical changes characteristic of PRRS were noted in the case of a lethal outcome. External examination of the corpses showed the following changes of the epithelium:

On the skin, in the area of the facial part of the skull, dark-purple color of soft tissues, patch, blue skin of the upper and lower jaw were revealed (figure 1). Similar skin color changes spread along the ventral surface of the neck, affecting the lateral parts of the mandible angle (figure 2).



Figure 1. Dermal pathology in the facial area of the skull.



Figure 2. Dermal pathology of the ventral neck, lateral and medial surfaces of the thoracic limbs.

Further, the reddening extended to the lateral and medial surfaces of the free limbs (figures 3 and 4) and had a diffuse character, from the proximal to the distal sections.



Figure 3. Dermal pathology in the inguinal region and on the lateral and medial surfaces of the pelvic limbs.



Figure 4. Extensive and targeted hemorrhages on the skin.

We found that, despite the diffuse nature, the areas with altered skin colour had clear boundaries, located in the form of dotted reddenings of various sizes, merging in some places into one large spot. Similar changes were observed on the skin from the ventral surface in the region of the sternum (figure 4), starting from the region of the handle of the sternum, and ending in the pubic region.

Macroscopically, the parenchyma of the organs looked slightly edematous, and in the lung tissue (figure 5), in addition to all, areas of hemorrhages were found impregnating tissue deep into the organ, and abundant dark red discharge was observed at the section. Bronchial lymph nodes had a loose consistency, with hemorrhages under the organ capsule (figure 6).



Figure 5. Lung with diffuse areas of haemorrhage.



Figure 6. Bronchial lymph node.

After evaluating the macroscopic changes in the parenchymal organs, pathological material was selected for further study of pathological changes in the internal organs.

4. Pathomorphological study of organ and tissue samples using specific staining for fibrin

Our findings on pathological and pathological changes were similar to experimental data and mainly reflected the chronic course of the disease. As a result, we used specific histochemical staining methods.

When staining for fibrin detection, the following results were obtained: - in bronchial lymph nodes, hemosiderin was observed in the parenchyma of the organ and perivascular and intravascular fibrin deposition, which filled almost the entire lumen of the vessel (figure 7).

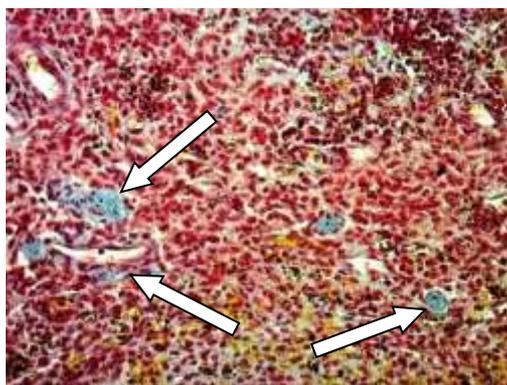


Figure 7. Bronchial lymph node. Fibrin deposition of over 48 hours. Colouring on fibrin. H. 100.

In the lumen of the large bronchi extensive areas of necrosis of the mucous and submucous layer with a large amount of fibrin of different ages and deep alteration of the bronchial mucous layer were revealed (figure 8). Alveolar atelectasis was noted, lymphocytic infiltration was detected in interalveolar septa with a small amount of fibrin on the alveoli mucosa (figure 9).

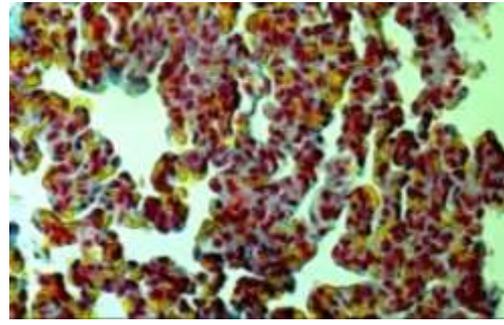
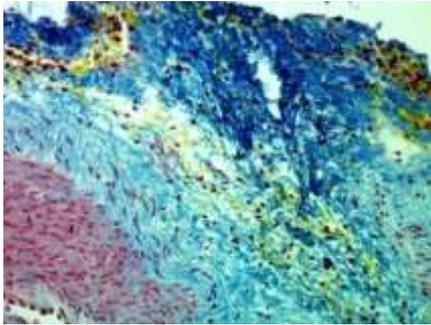


Figure 8. The lumen of the large bronchus. **Figure 9.** Alveoli of the lung. Fibrin deposition of 24-48 hours. Colouring on fibrin. H. 100. Fibrin deposition of 48 hours. Colouring on fibrin. H. 100.

5. Conclusion

Pathological changes in tissues with viral diseases are incredibly diverse. However, in spite of a wide range of changes there are also common pathological processes. These formations include the deposition of fibrin, which is not only a sign of the development of the pathological process, but also allows identifying the timing of changes in the tissues by histochemical methods and suggest the time from the beginning of the development of the pathological process in an animal [8]. In our work, we determined the presence of fibrin of different ages. Its appearance in tissues has a pathological effect on the organs of the respiratory tract and lymphatic system, complicating the pathogenesis of the disease. The pathogenesis vector is determined by which of the structures is coagulated fibrin. So, laying on the mucous membranes of the alveoli and trachea causes obturation of the lumen of these structures and makes breathing difficult, the production of pulmonary surfactant, causes impaired function of the apud-system of the lungs. Coagulation of fibrin in the lymph nodes causes impaired lymphatic drainage and concomitant structural changes.

The data obtained may contribute to the differential diagnosis of respiratory pathology in adults, whose PRRS is asymptomatic, or has minor deviations in the clinical picture.

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