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Immunohistochemical diagnostics of porcine reproductive and respiratory syndrome

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Immunohistochemical diagnostics of porcine reproductive and respiratory syndrome

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Abstract. For the diagnostics of porcine reproductive and respiratory syndrome the method of immunohistochemical identification of the virus antigen inorgans and tissues was applied, which provides the reliability of the diagnosis of the disease and confirm the tropism of the virus to cells and tissues of pigs organs. The research was conducted based on own monoclonal antibodies to the virus nucleocapsid protein on experimentally infected pigs, and also on animals during the outbreaks of the disease in pig farms. The results of the immunohistochemical study during experimental infection of pigs with the PRRS virus indicate that the main pathological changes are localized in the lungs and bronchial lymph nodes, followed by accumulation of the virus in the alveolar macrophages and bronchial lymph nodes. The changes detected in other parenchymal organs are not associated with the accumulation of virus in them.

1. Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) causes significant economic impact on the swine industry. The confirmation of the etiological role of PRRS virus in respiratory pathology remains a relevant task [1, 2]. As the disease caused by the PRRS virus belongs to the group of respiratory pathologies, the clinical signs will be similar to the diseases with a different etiological origin. It has been described a co-infection of RRRS and Porcine circovirus diseases (PCV) [1, 3]. The circulation of the PRRS virus in adult livestock animals is asymptomatic, which has a significant effect on the epidemiological situation [4, 5].

It should be noted that despite the well-studied questions about the structure of the causative agent the cytopathology effect of the virus in vivo, the principles of its interaction with the immune cells of the animal, the patterns of its distribution in organs and tissues remain insufficiently studied [2]. Thus, taking into account the epidemiology of PRRS, it is necessary to confirm the etiological role of the virus in the respiratory pathology where it participates in combination with other pathogens every time, because the final diagnosis can be made only by proving the replication of the virus in vivo. In this research the pathomorphological and immunohistochemical studies of porcine reproductive and respiratory syndrome under the experimental conditions and disease outbreaks in the pig farms was carried out.

2. Materials and methods

For the research, blood serum and parenchymal organs from 40 piglets aged from 21 to 240 days, experimentally infected on the VIEV experimental base and naturally infected from pig farms of the Moscow, Smolensk, Rostov and Tyumen regions were used. The virulent strain "West Siberian 13" with 4.66 lg TCID 50/cm3 and reference strain "Lelystad" with infectious activity of 5.0 lg TCID50/cm3 of PRRS virus was used for the infection of piglets. Virus-containing material was inoculated in a dose of 2 cm3 intramuscularly and 3 cm3 intranasally. The control group animals were inoculated with an uninfected MARK-145 cell culture with the same method and dose.

The blood serum of the experimentally infected animals was tested for the presence of the PRRS virus genome using test system based on PCR (Vetbiochem, Russia). For the detection of IgG – antibodies to the PRRS virus the "PRRS – Serotest plus" kit was used (Vetbiochem, Russia). While the PCR was positive, the infectious activity of the virus was determined in the porcine alveolar macrophage cell culture. Detection of IgG antibodies to the PRRS virus in serum was performed in ELISA by the "PRRS-Serotest plus kit" (Vetbiochem, Russia). For the IHC method, own monoclonal antibodies 3h9, 4h7h9, specific to the capsid protein of the PRRS virus were used. The obtained samples of organs were placed in 10% solution of neutral buffered formalin. For cryotomic techniques, native samples delivered to the laboratory on ice no later than 36 hours after sampling were used. For the pathomorphological studies ofobtained material the paraffin and cryotomic techniques were applied for standard, specific, and IHC staining.

3. Results

The experimental infection of piglets with the pathogenic strain "West Siberian 13" of PRRS virus with an infectious activity of 4.66 lg TCID50 / ml showed a 50% mortality of infected piglets with signs of dyspnea and depression during the first 24 hours. At necropsy, haemorrhages in lung tissue and splenomegaly were detected. The most specific pathological changes of PRRS were observed in piglets died after 72 or more hours from the beginning of an experiment which were comparable to pathological changes in organs from spontaneously infected animals. During the histopathologic examination of tissues sections of piglets, dead in the first 24 hours after infection with pathogenic strain "West Siberian 13" of PRRS virus an extensive areas of hemorrhages in the lungs (figure 1); alveoli atelectasis (figure 1 A); emphysema (figure 1 B); obturation of bronchioles by cell conglomerate (figure 1 C); swelling of the parenchyma of the spleen, venous congestion, hemorrhages were detected. In animals died later than 72 hours more obvious histological changes in the lung tissue and lymph nodes (discomplexation of cells in bronchial lymph node) were detected (figure 2).

Moreover, in the parenchyma of the liver, the focal accumulations of lymphoid cells and hemorrhagic glomerulonephritis in kidneys were observed. It was not possible to reproduce the specific pathological lesions in experimentally infected piglets with reference strain "Lelystad" of PRRS virus (infectious activity of 5.0 lg TCID50/ml). Only loss of appetite and lethargy were seen. The pathomorphological examination revealed the infiltration of stroma of lung and bronchial lymph nodes by cells of the lymphoid series. In other organs the histoarchitectonics disorders were observed. The natural infected piglets showed similar pathologic and pathomorphological changes to those in experimental infected with the pathogenic strain "West Siberian 13" of PRRS virus characterizing by lymphocytic infiltration of the interalveolar septa, with effusion of a small amount of fibrin on the mucous membrane of the alveoli, necrosis of the bronchial mucosal membrane. In the bronchial lymph nodes, areas of hemorrhages, structural discomplexation of the cortical layer of cells, and the deposition of hemosiderin in the medulla were revealed. The immunohistochemical examination showed the presence of PRRS virus antigen in the affected organs and tissues, which gives direct confirmation of the etiology of pathological changes. The positive IHC reaction was determined by specific staining of peroxidase chromogen in brick color. On the figures 3 and 4 the sections of lung and bronchial lymph node with a positive reaction on IHC (B, C) in comparison with the control histological section (A) are presented. On these figures, positively responsive areas of tissue are clearly visible. The additional staining of cells nuclei with Meyer's hematoxylin provides the determination of the primary differentiation of cells.

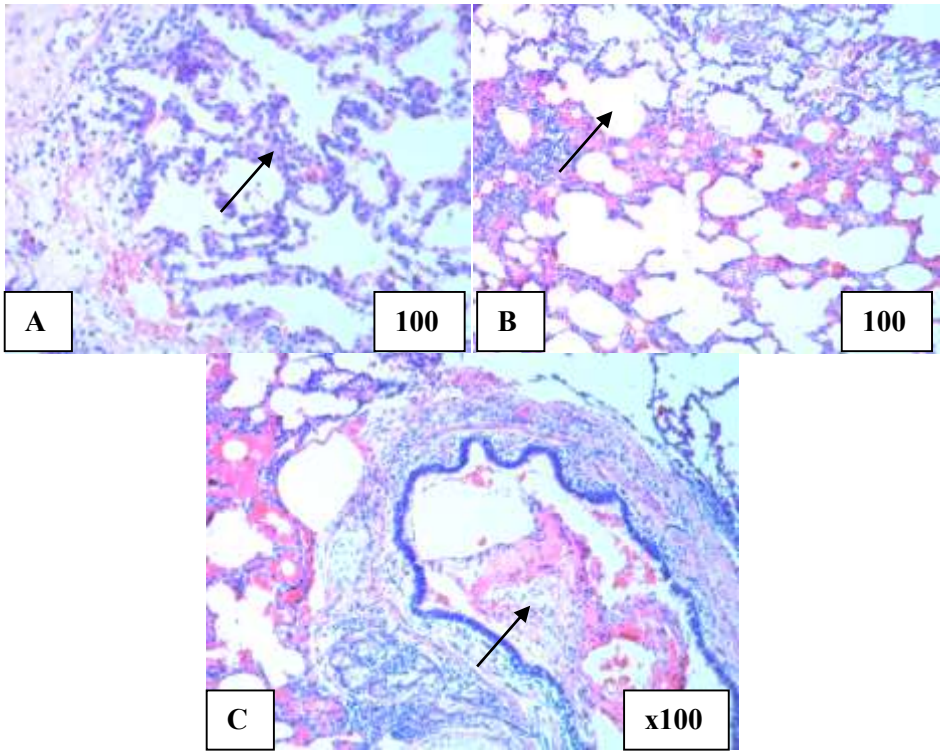

Figure 1. Lung section. Hematoxylin and eosin stained sections: A alveoli atelectasis; B emphysema; C obturation of bronchioles.

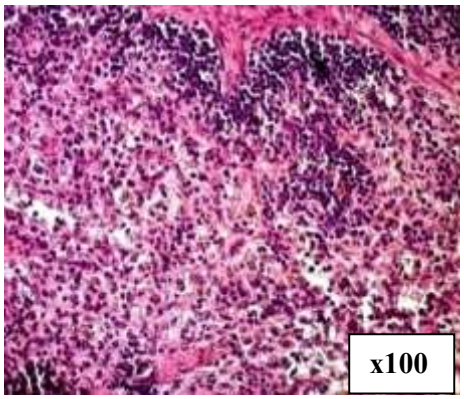

Figure 2. Bronchial lymph node. Hematoxylin and eosin stained sections. Discomplexation of cells.

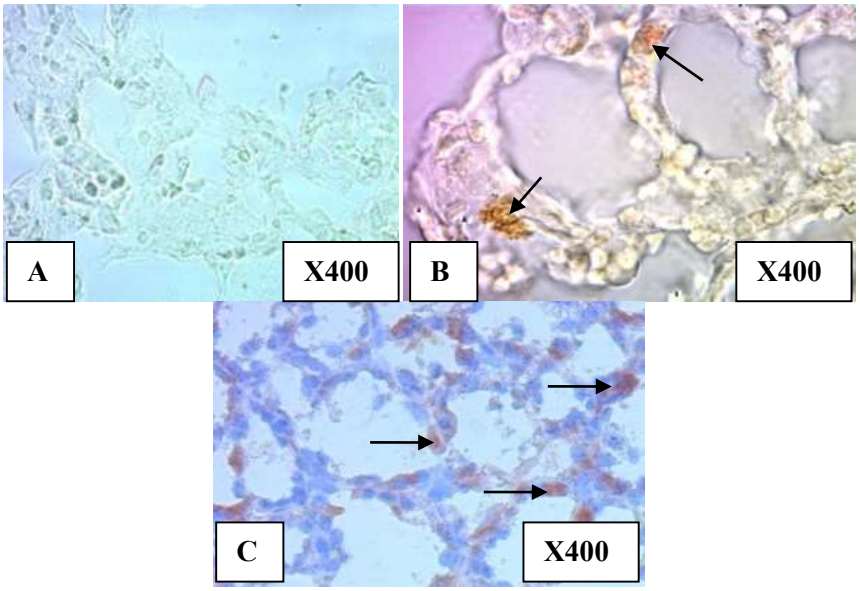

Figure 3. Lung section: A. Negative control, without nuclei repainting; B. Positive IHC reaction on the section without repainting the nuclei with Mayer's hematoxylin; C. Positive IHC reaction on the section with Mayer's hematoxylin.

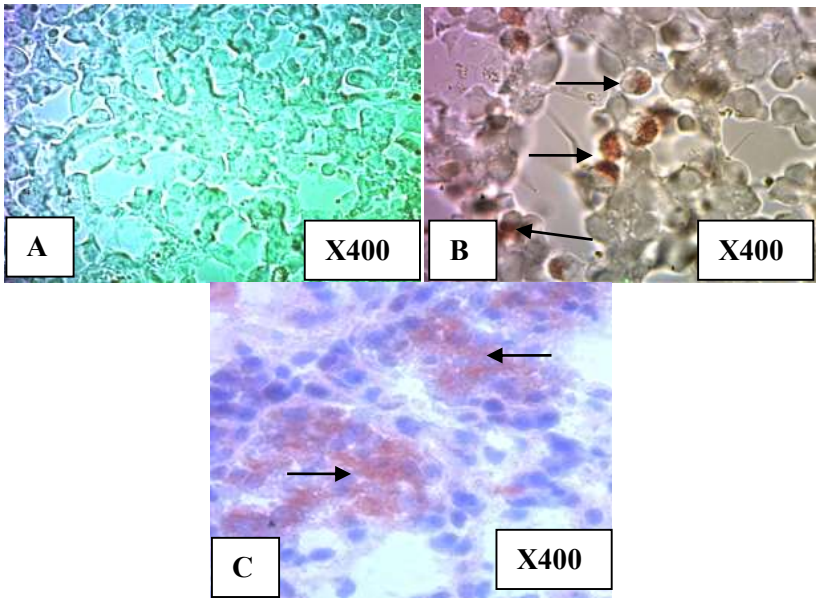

Figure 4. The section of the bronchial lymph node: A. Negative control, without over-coloring of the nuclei; B. Positive IHC reaction on the section without repainting the nuclei with Mayer's hematoxylin; C. Positive IHC reaction on the section with the Meyer hematoxylin.

4. Conclusion

The immunohistochemical method have expanded and clarified the data of pathological examination of pigs' organs during natural and experimental infection with PRRS virus. It has been identified the histomorphological changes in the parenchyma and stroma of the lungs, bronchial lymph nodes and

other parenchymal organs during experimental infection and natural infection. The dynamic of dissemination of causative agent in the organism of pigs is notified. The immunohistochemical diagnostic method has been developed based on the domestic 4h7h9 monoclonal antibodies specific for the capsid protein of PRRS virus, which allows detecting the virus antigen in organs and tissues of pigs when infected with European and North American virus types. Immunohistochemical diagnostics can be used as confirmation of these pathological-morphological studies and used as an additional method of PRRS diagnosis. This method is an important part of complex diagnostics, which determine the etiological role of the PRRS virus and gives the basis for choosing the strategy of immunospecific prevention.

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