TRADITION AND MODERNITY IN VETERINARY MEDICINE

Online First

Published online: November 26, 2017

PATHOMORPHOLOGICAL CHANGES IN NEWBORN PIGS INDUCED BY INFECTION WITH VACCINAL STRAINS AND FIELD ISOLATES OF SUID HERPES VIRUS 1. PART II. RESPIRATORY SYSTEM

Vasil Manov

University of Forestry, Faculty of Veterinary Medicine,
Sofia, 1756, Bulgaria
e-mail: manov_vet@abv.bg

ABSTRACT

Comparative pathomorphological studies of newborn piglets prior to colostrum intake after infection with a vaccine strain and two uterothropic strains of the Aujeszky's disease strains – Mogila and Zagora II were performed. The studies were conducted with 21 large, well-developed piglets derived from an industrial pig farm, without antibodies to Suid herpesvirus 1. Histological, histochemical and electron microscopic studies have been performed. It was found that strains of the Aujeszky's disease, which differ in virulence and tissue tropism, caused different severity, characteristic clinical signs and pathomorphologic changes in experimental animals. The clinical signs are related to changes in the respiratory and digestive systems, bedsores and apathy. In the lungs of the pigs, infected with strain St.Zagora II and strain MK 35gE$^-$, atelectatic and proliferative changes were observed. Serous - fibrinous pleuritis, fibrinogen necrotic pneumonia and the presence of inclusions type Cowdry A in the epithelial and connective tissue in lungs of the tested animals were inspected.

Key words: newborn pigs, respiratory system, Suid herpesvirus 1

INTRODUCTION

In recent years a new form of Aujeszky's disease (AD) an intrauterine infection in the context of regular vaccinations in pig farms, has been revealed (Motovski, 1991). Viruses that differ in viral genome restriction profile were isolated from the organs of weak and lifeless pigs. These strains have persisted in the pig population in previous periods (Christensen and Motovski, 1993). A new way of disease transmitting was ascertained – airborne over long distances, changing the infection entry pathway concept. Nauwynck and Pensaert (1994) demonstrated that the virus gets into the pulmonary monocytes and are transmitted through the placenta to the fetus, regardless of the presence of a post-vaccination immunity. Morphological changes in the respiratory tract were characterized by the presence of interstitial pneumonia (Shibata et al., 2003), alveolitis, interstitial perivascular edema, and the presence of intranuclear inclusions type Cowdry A (Narita et al., 1990). Ducatelle et al. (1982) established intranuclear inclusions in naturally infected piglets. They have diagnostic value for viral pneumonia typing caused by the AD virus.

The aim of this investigation is to conduct histological, histochemical and electron microscopic studies in the lungs of newborn pigs infected with one vaccine and two uterothropic strains of Aujeszky's disease virus.

MATERIALS AND METHODS

New-born pigs without antibodies against SHV1 (21 pigs, weighing about 1.6 kg) taken from a pig farm were used. The experimental animals were divided into three groups of 6 and one

Online First

Published online: November 26, 2017

control group that contain 3 pigs. At the 30th hour after birth, the animals were infected by intranasal inoculation with a cell culture virus suspension at a dose of 0.5 cm^3 (about 5 x $10^{3.0}$ $TCCU_{50}$) and oral administration of 1.0 cm^3 of the same suspension. The first group was treated with strain Mogila, second and third – with strains St. Zagora II and MK-35 (gE^-), respectively. The control group was untreated (group IV). Twice a day, the temperature response and the general response were monitored. The number of respiratory movements was counted during the study. A 10% organ suspension with chlorine solution was prepared to isolate the AD virus. The euthanized and dead pigs were autopsied. The histological material was processed using the classic paraffin method; the sections were stained with haematoxylin-eosin, by Heidenhain and phosphoricacid-haematoxylin (Dyakov et al., 1989). The materials for electron microscopic studies were fixed 4% glutaraldehyde, post fixed in 2% osmium tetroxide, and included in Durkupan. The sections were stained with uranyl acetate and lead citrate and then examined on a JEOL 1200Ex electron microscope at magnification from 6000 to 20000.

The experiments were carried out according to the requirements of Ordinances No. 25 and 15 of the humane treatment of laboratory and experimental animals (permit No. 17000002, issued to the Faculty of Veterinary Medicine, UF-Sofia) and Ordinances No. 16 and 22 for minimizing the suffering of animals during euthanasia.

RESULTS

Respiratory movements in pigs of the I^{st} group were of the abdominal type and twice as fast as those of the control group. The respiratory rate in group II animals was slightly increased on the first day post-infection, and then normalized, while group III was slightly increased compared to the control.

Macroscopic changes in the pigs' lungs are presented in Figure 1. The animals in group III had barely visible local redness in the apical fragments, compared to group II where these changes were better expressed. The redness areas were dense, with dark red spots ranging in size from poppy to lentil grains. Medial-lymph nodes were juicy and hyperaemic. In the thoracic cavity in the pigs of I^{st} group there was an increased content of a pale yellow liquid, located predominantly in the right half. The left part of the lung was red, often in the apex, as well as in the ventral lobes, in two of the pigs. The right part of the lung has almost doubled in size, venereal, with a thick meat-like consistency.

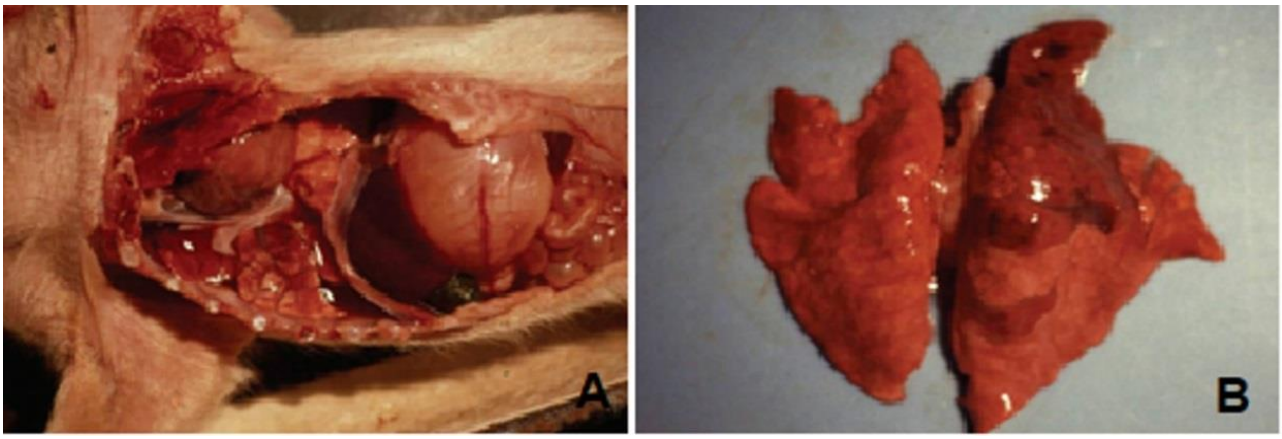

Figure 1: Macroscopic changes in the lungs of pigs infected with Mogila strain. A: Fluid collection in thoracic cavity and lung interstitium B. Pneumonic areas in the right lung.

Online First

Published online: November 26, 2017

The interstitium was filled with fluid and clearly visible. Similar lesions were also found in the dorsal part of the heart and ventral lobe. The microscopic changes in the pigs' lungs of the third group were the thickening of individual alveolar septae based on atelectatic changes, hyperaemia and infiltration with monocytes, lymphocytes and neutrophilic leukocytes. Analogous changes were also demonstrated in the electron microscopic study (Fig. 2A).

Changes in epithelium of bronchi and bronchioles have not been observed. Disintegration and desquamation of epithelial cells and presence of desquamated cilia were noted (Fig. 2B). In pigs of group II, changes were concentrated in alveolar septae which was thickened due to the increased number of mononuclear cells (Fig. 2C). Atelectatic outbreaks were also observed. Although rarely found in individual alveoli, the presence of exudate, clustering of cells with pycnotic and rectic nuclei, as well as alveolar macrophages was visible. Alveolar macrophages were also observed in bronchial and bronchiolar lumen, the epithelium of which was disintegrated and desquamated (Fig. 2D).

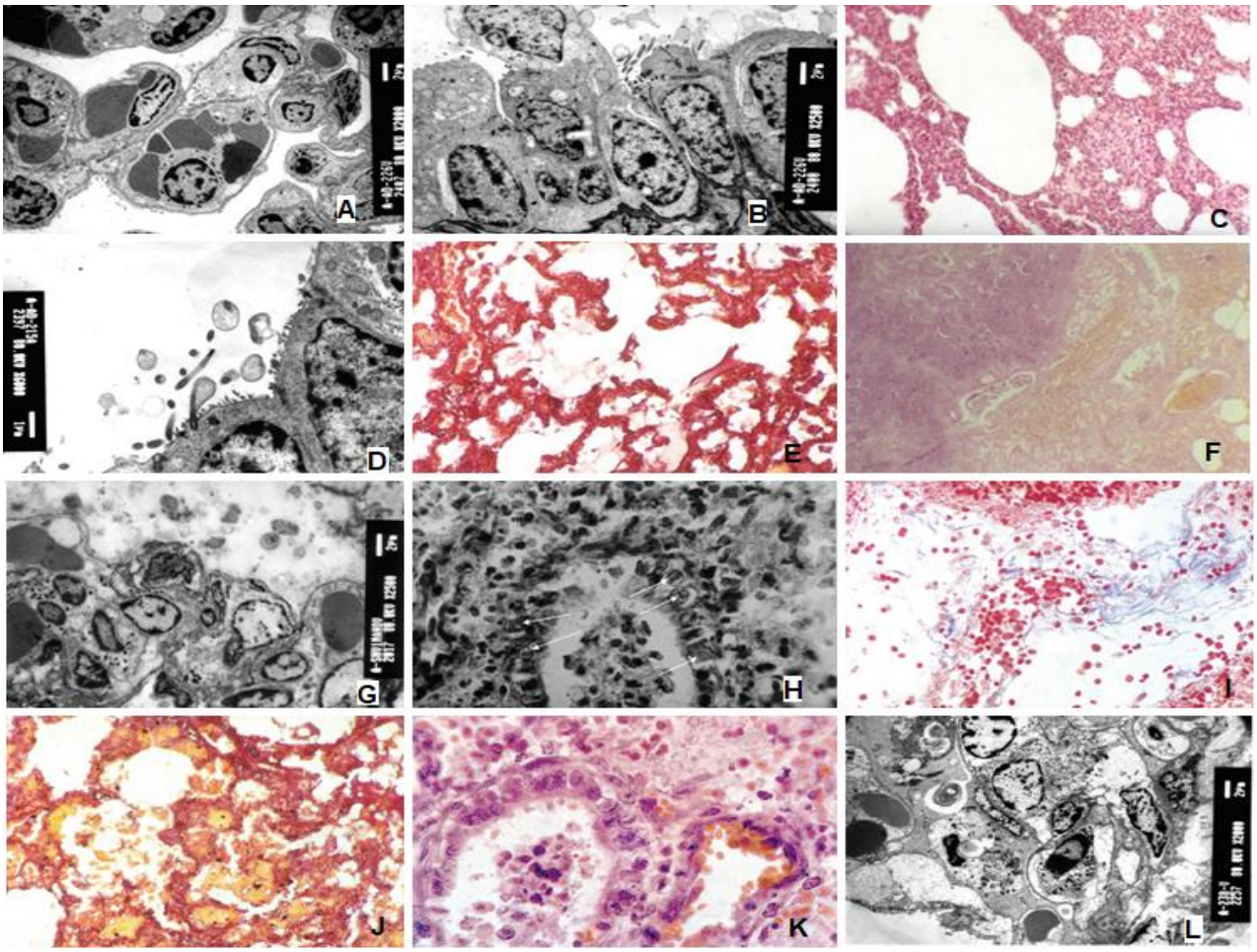

Figure 2: Light-microscopic and ultrastructural changes in the lungs of new-born pigs infected with the AD virus. **A:** Thickened alveolar septae due to hyperaemia and infiltration with mononuclear cells, lymphocytes and neutrophil leukocytes in a pig infected with MK-35 strain (Uracil acetate-lead citrate stain line; = 2µm). **B:** Bronchial epithelial cells with loss of cilia in pigs infected with MK-35 strain (Uracil acetate-lead citrate stain; line = 2µm). **C:** Atelectasis and accumulation of cellular elements in alveolar septae in pigs infected with strain St. Zagora II. (H&E stain. Magnification X25) **D:** Bronchiolar epithelial cells with truncated and disintegrated cilia in pigs infected with strain St. Zagora II (Uracil acetate-lead citrate stain; line = 1µm). **E:** Staining by PAH, negative for fibrin, in a pig infected with St. Zagora II strain (Magnification X10). **F:** Fibrinogen necrotic pneumonia in pigs infected with Mogila strain (H&E stain. Magnification X4). **G:** A strongly thickened alveolar wall infiltrated with lymphocytes, mononuclear and

neutrophilic leukocytes in a pig infected with Mogila strain. The alveolar lumen contains lamellar corpuscles of disintegrated type II pneumocytes (Uracil acetate-lead citrate stain; line = 2μm). **H:** Neurotic changes in the bronchial wall and presence of desquamated cells in the lumen in a pig infected with Mogila strain. Many of the epithelial cells have Cowdry A type nuclear inclusions (H&E stain. Magnification X40). **I:** Fibrinous exudate. Azan and Heidenheim staining in a pig infected with Mogila strain (Azan stain. Magnification X25). **J:** Staining by PAH, positive for fibrin, in a pig infected with Mogila strain (Magnification X25). **K:** Necrotic changes in bronchioles and presence of Cowdry A intranuclear inclusions in epithelium of a pig infected with Mogila strain (H&E stain. Magnification X10). **L:** Strongly thickened intralobular interstitium, infiltrated with mononuclear and neutrophilic leukocytes in pigs infected with Mogila strain (Uracil acetate-lead citrate stain; line = 2μm).

Permanent finding was also the hyperplasia of the pulmonary blood vessels. The presence of fibrin in the lungs of pigs of groups II and III was not detected after staining of the samples by Azan and with phosphoric acid-haematoxylin (Fig .2E). The histological lesions in the pigs of Ist group were fibrinous-necrotic pneumonia type (Fig. 2F). Necrotic changes were detected in alveolar septae and blood vessels, with bronchi and bronchioles being most severely affected. Their epithelium was totally or partially disintegrated, desquammed and fallen into the lumen, forming cellular cylinders. Disintegrated pneumocytes were observed on electron microscopy (Fig. 2G). In the same structures, fibrinous exudate, alveolar macrophages, erythrocytes and mononuclear cells were found. Necrotic changes in epithelial cells of bronchi and bronchiole were predominantly of caryotoxicity, plasmo-resection and plasmopycnosis type (Fig. 2H). Bacterial-induced dystrophy in the epithelial cells was detected. There was an oedema that also exposes the peribronchial tissue in the lamina propria. Alveolar septae were necrotized, and alveoli collapsed and filled with fibrinous exudate (Fig. 2I and G).

Among the finely intertwined fibrils in the alveolar lumen necrotizing cells and nuclear epithelial cells of the alveolar septae, alveolar macrophages, lymphocytes, histiocytes, multiple erythrocytes and single leukocytes were visible. This is why the lung structure was completely disrupted. The interstitium was thickened as a result of its filling with an exudate in which cellular elements and fibrin fibres were observed. Accumulations of mononuclear cells in the individual lung and alveolar septa sites was found. The elastic and collagenous fibres of the alveolar and interstitial blood vessels in some areas were lysed with bleeding clearly visible. Blood vessels were filled with erythrocytes, among which many nuclear cellular elements were found. In the epithelial cells of the bronchi, bronchioles and the alveolar wall, the adjacent lysed areas, as well as in the fibroblast cells, acidophilic Cowdry A - intranuclear inclusions with a light peripheral halo were found (Fig. 2K). By an electron microscopic study it was demonstrated that the alveolar septa thickening were due not only to mononuclear cell elements but also to the involvement of leukocytes that could not be recognized in the histological examination (Fig. 2L).

DISCUSSION

Various macroscopic changes in the lungs of experimental animals were observed. While group III animals had barely noticeable redness in the apical parts, the second group had dense, dark red lesions ranging from poppy to lentil grain. The most severe changes occurred in pigs infected intranasal and orally with the Mogila strain. They were characterized by an exudate in the right half of the chest cavity, where the right lung part was enlarged almost twicely and with well-visible interlobular septae. There was also large flushing in the left pulmonary lobe. A similar finding was found by Vannier (1987), according to whom this lesion was only caused by a highly virulent viral strain. The microscopic changes in lungs of pigs in group III were atelectatic and proliferative, which corresponded to the results of Vesselinova (1982) and Narita et al. (1990). The authors believe that mildly virulent strains were pneumotropic and caused interstitial pneumonia. The lesions in the interstitium could occur in a haematogenic way. The histological changes found in

Online First

Published online: November 26, 2017

pigs of group II were similar to those described in MK-35 (gE-), but more pronounced. Necrotic changes in the alveolar and bronchial wall were also observed. In group I the microscopic picture was fibrinous-necrotic pneumonia. Numerous authors only proved necrotic changes so far (Klimkë, 1991; Narita et al., 1990, Dee, 2016).), which were considered a result of an aerogenic infection (Ducatelle et al., 1982). Narita et al. (1993) also observed fibrin in necrotic debris, which corresponds to our results. Acidophilic Cowdry A type intranucleus inclusions were demonstrated in epithelial and fibroblast cells of bronchus and bronchiolus only in pigs inoculated with the Mogila strain. The results of Ducatelle et al. (1982) in naturally infected young pigs are similar. In their view, this histological picture is of diagnostic value for determining the type of the viral pneumonia caused by the AD virus.

CONCLUSION

Pathohistological changes in neonatal pigs without colostrum intake, caused by Stara Zagora II strain were characterized by proliferative lung changes, similar to those caused by the vaccine strain MK-35 (gE-). Microscopic lesions in the respiratory tract caused by Mogila strain in new-born pigs were characterized by the presence of serous fibrinogen pleuritis, fibrinous-necrotic pneumonia, and the presence of intranuclear Cowdry A type inclusions in epithelial and connective tissue lung cells.

ACKNOWLEDGEMENTS:

I should like to thanks Prof. A.Motovski, NDRVMI –Sofia, Prof. I. Zarkov, Trakia University, Prof. Marin Aleksandrov – BAS, Assist. Prof. Neli Grozeva - Trakia University.

REFERENCES

1. Christensen, L. S., Motovski A. Molecular epizootological studies of Bulgarian isolates of Augeszky disease virus. Eight Congress of the Microbiologist in Bulgaria, 21-23 October 1993, Varna, 7/602.
2. Dee, SA. (2016). Overview of pseudorabies. In: IKahn CM, Line S, Aiello SE, editors. The Merck veterinary manual [online]. Whitehouse Station, NJ: Merck and Co; Available at: http//www.merckvetmanual.com/nervoussystem/pseudorabies/overview-of-pseudorabies.
3. Ducatelle, R., Coussement W., Hoorens J. (1982). Immunoperoxidase study of Aujeszky`s disease in pigs. Res. Vet. Sci. 32, 3, 294-302.
4. Dyakov, L., Lozanov L., Angelov A., Stoykov D. Practicum in Veterinary Medicine Pathology. Zemizdat, Sofia, 1989.
5. Klimke, A. (1991). Diagnosis of Aujeszky`s disease in pigs: analgsis of case records, histological findings and immunochisological findings, I nahgural. Dissertation, Tierozztliche Hochschule, Hanover, Germany.
6. Motovski, A., Peev Y., Dyakov I., Florova I.. Studies on the role of the Aujeszky's disease virus in porcine reproductive disorders. Jubilee Scientific Session on the 90th Anniversary of CRVMI, Sofia, 14-20 IX 1991.
7. Narita, M., Imada T., Haritani M. (1990). Comparative pathology of HPCD pigs infected with wild type and ara-T-resistant strains of Aujeszky`s disease virus. J. Comp. Phatol., 102, 1, 63-69.

Online First

Published online: November 26, 2017

8. Narita, M., Kawashima K., Matsuura S., Uchimura A., Miura Y. (1993). Pneumonia in pigs inoculated with pseudorabies virus by means of a bronchoscope. J. Comp. Pathol., 109, 4, 335-344.
9. Nauwynck, H., Pensaert M. (1994). Interactions of Aujeszky`s disease virus and porcine blood mononuclear cells in vivo and in vitro. Acta. Vet. Hung., 42, 2-3, 301-308.
10. Shibata, I., Yazawa S., Ono M., Okuda Y. (2003). Experimental dual infection of specific pathogen-free pigs with porcine reproductive and respiratory syndrome virus and pseudorabies virus. J. Vet. Med. B. Infect. Dis. Vet. Public. Health. 50, 1, 14-19.
11. Vannier, P. (1987). Le virus de la maladie D`Aujeszky et les affecions respiratoires du Porc. Rec. Med.Vet., 163, 4, 407-417.
12. Veselinova, A. (1982). Pathomorphological, cytological and immunomorphological studies of Aujeszky's disease in pigs. Doctor's thesis, C. 1-366.