PATHOMORPHOLOGICAL STUDIES IN NEWBORN PIGS INDUCED BY INFECTION WITH VACCINAL STRAIN MK 35GE- AND FIELD ISOLATES MOGILA AND ST. ZAGORA OF SUID HERPESVIRUS 1.

PART I. NERVOUS SYSTEM

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ABSTRACT

The purpose of the present study was to perform a comparative morphological analysis of the changes in the central nervous system of new-born pigs prior to colostrum intake. The infection was artificially created by application of a vaccine strain MK 35gE- and two strains, isolated from intrauterine infection cases – Mogila and Stara Zagora II. A restriction profile of the viral strains was made. No neurological signs or 100% mortality were registered in the pigs that were infected with the strains St. Zagora II and MK 35gE-. Pathohistological changes caused by these two strains of the virus in animals CNS were similar and were characterized primarily by reproductive problems. The pathological changes induced by Mogila strain were obvious by presence of alteration, exudation, and proliferation (less pronounced) in the brain.

Key words: newborn pigs, CNS, Suid herpesvirus 1.

Introduction

Aujeszky's disease (AD) is an emerging health problem for all countries. It is caused by Suid herpesvirus 1 /SHV1/ (Virus Taxonomy, 2008; Dee, 2016). McFerran and Dow (1965), Baskerville, et al. (1972, 1973) found differences in viral strains isolated in terms of virulence and tropism to host organisms. The disease is primarily associated with swine and causes high mortality and encephalitis in piglets, severe respiratory signs, and weight loss in fattening pigs and abortion in sows (Wittmann et al., 1980). After a clinical outbreak, the AD virus can cause life-long latent infection in the nerve tissue and tonsils. It can be reactivated by exposing pigs to various stress factors (Rock, 1993). It is known that the administered vaccinations are not capable of completely preventing the onset of latent infection. According to Vannier and Cariotet (1991), the widespread use of vaccines leads to a reduction in nerve symptoms, but the virus persists in pigs. A BUK vaccine strain was isolated from the organs of clinically ill animals, which is evidence that attenuated vaccine strains possess a certain virulence (Christensen et al., 1991). The phenotypic combination was found to be responsible for the emergence of a virulent virus of vaccine origin (Lipskaya et al., 1991). In recent years, there have been massive reproductive disorders such as birth of weak and lifeless piglets that die in the first days after birth. The virus was isolated from the organs of these animals and antibodies in their blood serum were detected prior to colostrum (Motovski, 1991). Through restriction analysis, Christensen and Motovski (1993) prove that these are natural recombinants of wild and vaccine viruses. In the central nervous system, some authors find a nonsuppurative meningoencephalitis, including the presence of glial nodules, degenerative changes of ganglion cells with pronounced neuronophagia and vascular changes (Jovanovic, 1985, Vannier, 1987, Thomson et al., 2001).

The purpose of this study was to perform a comparative morphological analysis of CNS changes in new-born pigs before colostrums intake, as the infection in animals is artificially
provoked by the application of the vaccine strain MK 35gE- and two strains isolated in cases of intrauterine infection – strain Mogila and strain St. Zagora II.

**Materials and methods**

*Experimental animals.* 21 new-born piglets originating from an industrial pig farm were used in the studies. By serological examination of blood samples obtained from the umbilical cord, it was found that the animals did not have antibodies against SHV1.

Experimental set-up. At the time of delivery, pigs were separated from the mothers and raised in isolation. They were divided into three groups of 6 pigs; the control group contained 3 animals. The pigs were fed every 2 h with sterilized milk, yoghurt enriched with vitamins, antibiotics, and glucose. At the 30th hour after the birth, the animals were simultaneously infected by intranasal inoculation of a 0.5 cm³ cell culture virus suspension containing about $5 \times 10^{3.0}$ tissue culture cytopathogenic units of 50% (TCCU₅₀) and oral administration of 1.0 cm³ of the same suspension.

**Viral strains.** The following viral strains were used: Mogila strain isolated from the new-born lifeless pig brain with AD symptoms from an enzootic AD pig farm in a period of missed vaccination against the disease at a 9th passage level, $10^{5.0}$ titre, TCCU₅₀ (the virus restriction profile is characteristic for Hermann's genotype I and was modified in place 8'−14' and 12'Bam HI fragments); Strain Stara Zagora II was isolated from the liver of a new-born lifeless piglet. The piglet did not show clinical symptoms of AD and has not been fed with colostrum. The piglet was born from a clinically healthy pig in an enzootic pig farm with regular vaccinations against the disease at a 9th passage level, $10^{5.0}$ titre TCCU₅₀ (the virus restriction profile was characteristic for genotype I and the genome was modified in place 5'−14 Bam HI fragments) (Figure 1), MK-35 (gE⁻) attenuated vaccine strain.

Group I animals were treated with Mogila strain, group II with St. Zagora II and the group III with strainMK-35 (gE⁻) (control group IV was untreated). The temperature status was monitored daily as well as the number of respiratory movements and behavioural responses. A 10% organ suspension that includes tonsils, lung, liver, brain and spinal cord was prepared to isolate the AD virus. After centrifugation at 1200 x g for 20 min, the chicken embryonic fibroblasts cell cultures were infected with the supernatant, followed by the appearance and development of a cytopathic effect.

**Histological, histochemical and electron microscopic analysis.** Euthanized and dead pigs were autopsied by taking material from the brain and spinal cord. The materials were processed by the classic paraffin method and stained with haematoxylin-eosin and Nissl (Dyakov et al., 1989). 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. 1700002, issued to the Faculty of Veterinary Medicine, UF-Sofia) and Ordinances No. 16 and 22 for minimizing the suffering of animals during euthanasia.
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Figure 1: Electrophoretic profile of isolates of virus of AD.
Results

Clinical status tracking results indicated that group I pigs ate meagrely or temporarily refused to take food at the 28th hour post-infection. Two of the animals died on the 3rd day and the other four on the 4th day. 10-14 hours before the death of all animals apathy and anorexia were observed. During this period the pigs lay down, buried in the bedding. Breathing was of accelerated abdominal type. One of the animals of group II was euthanized on the 4th day in a state of agony, the other one – on the 7th day. Two of the pigs died on the 5th and 6th day after the infection and the last two of the group survived the experiment. From Group III, one of the pigs died on the 3rd day and two pigs died on the 4th day. The animals from groups II and III vomited and had a variable appetite during the first two days of study onset and 6-8 hours before death. No breathing changes were identified. Pigs from the control group did not show any variation in the clinical signs and behavioural responses. One of the pigs was euthanized on the 4th and two more on the 7th day after the infection. Animals from group I and II responded with a rise in body temperature on the first day after the infection, which was better pronounced in the animals from Group I. After the 3rd day and especially 1-2 days before death hypothermia was observed. The applied strain of AD virus was reisolated in virological examination of organs from the dead or euthanized animals. Upon opening of the cranial cavity, meningeal blood vessel hyperaemia (Figure 2) was observed.

Figure 2: Brain of a pig infected with Mogila strain. Hyperaemia A: Hemisphere B: Basal part

The changes were most pronounced in pigs from group I, whereas in the animals from group III and IV, changes were not observed or were poorly pronounced. Pathohistological changes in the CNS are presented on Fig. 3. They were manifested with blood vessel hyperaemia in the meninges and the middle basal hemisphere area of pigs from group III. Around some vessels in the Virchow-Robin spaces a cluster of mononuclear cells and single neutrophilic leukocytes were found. In the quadrigeminal plate perivascular oedema and swelling of intimal endothelial cells were found. A cluster of glia cells was also found, that was nodular or diffused, located predominantly near the ependymal cells of the lateral brain ventricles (including the nasal and the olfactorial bulbus). Alteration changes were found in ganglion cells and neuronophagia manifestation was also present. In some brain regions, the Purkinje cells showed central tigrolysis. The nuclei became pale, blood vessel hyperaemia and perivascular lymphohistiocytic proliferates were also reported. In the grey matter of the spinal cord, necrotic changes such as tygrolysis and tigropyknosis of the neurons were observed. Around some of the altered ganglion cells microglial cell satellitosis with neuronophagia was found.

The observed morphological changes in the CNS of the experimental animals in group II were similar in nature to those of the pigs in group III, but clearly distinguished by their intensity and localization. Brain blood vessels in experimental animals were less extensively dilated and endothelial cells were rounded and jutted into the lumen. These changes were also found in the cornu ammonis, quadrigeminal plate, middle commissure, olfactory bulb, medulla and the hemispheres. A perivascular oedema was also found in the quadrigeminal plate. Diffuse clusters of glia in the brain parenchyma were observed in the caudal nucleus, fornix, medulla, and the rostral part of the brain near the lateral ventricles. Focal nodules of glia were found near the lateral ventricle as well as under the ependymal cells and near the blood vessels of olfactory bulb, and the fourth cerebral ventricle. In the brain parenchyma in the cornu ammonis area and the posterior dorsal part of the hemisphere, clusters of microglial cells were observed. Necrotic changes in the ganglion cells were found in the cerebral cortex of the medulla and the spinal cord. Local reactive micronecrosis was found in the medulla and the hemispheres. Hyperaemia, haemorrhages and lymphohistiocytic perivascular
clusters, as well as dystrophic changes in the Purkinje cells, were found in the cerebellum. The cerebral blood vessel hyperaemia in group I was well pronounced and was concentrated in the cornu ammonis area, middle ventral part of the hemispheres, medulla, and to lesser extend in the middle commissure the dorsal part of the hemispheres, and the quadrigeminal plate. The meninges were saturated with serous exudate and infiltrated with histiocytes and lymphocytes. The activation of the vascular endothelium was best manifested in the cornu ammonis area, middle commissure near the third ventricle, where perivascular oedema was also found. In the basal parts of the hemispheres and the cornu ammonis part of the blood vessels were surrounded by histiocytes and single lymphocytes. In stratum pyramidale of cornu ammonis, the anterior and middle ventral regions of the hemispheres, middle commissure, and olfactory bulb, single glial nodes and clusters of microglial cells were observed around necrotic neurons with satellitosis manifestation. Part of the neurons in the medulla was wrinkled, pyknotic, and intensely blue-coloured, as in other parts lysed and pale. In the rostral dorsal and caudal basal part of the hemispheres, as well as near the aqueduct, we found a reactive micronecrosis with karyopyknosis manifestation.

Discussion

During the new-born pigs experiment, two field isolates of the AD virus were selected – Mogila strain and St. Zagora II strain, that differed in the genome restriction profile. The results showed that genetic differences between them also correspond to differences in the degree of virulence. The pathohistological changes in the groups of experimental animals indicated that they differed in both type of changes, intensity and localization. In pig brains, that were infected with Mogila strain and that died on the third and fourth day, there was a limited size and count of glia proliferates in the hemispheres. The study results are similar to those of Vesselinova (1970), according to which such a pathological picture is characteristic for animals that suffered for longer (6-9 days). The observed CNS changes caused by strain MK-35 (gE-) and isolate St. Zagora II are predominantly proliferative. Veselinova (1970) and Thomson et al. (2001) found the same changes and proved that by infecting pigs with virulent strains of AD the encephalitis lesions have strongly pronounced alterative and exudative manifestations, the same that we observed in animals infected with the Mogila strain. The finding of Cowdry A intranuclear inclusions had a different diagnostic value. Their presence is perceived as a characteristic feature of the AD virus strain that circulates in the herd. Some authors found mass inclusions in different tissues after experimental infection (Ducatelle et al., 1982; Jovanovic, 1985, and Vannier, 1987), while other authors rarely proved or did not prove at all the presence of inclusions in naturally occurring AD cases (Olander et al., 1966). We did not find this type of inclusion during the histological examinations of the experimental animals CNS.

Conclusion

MK-35 (gE-), Stara Zagora II, and Mogila viral strains differed in the genome restriction profile and had different virulence, tissue tropism, and cause a variety of clinical signs and morphological changes in new-born pigs. Pathohistological changes in animals caused by Stara Zagora II strain were characterized by productive changes in the CNS and were similar to changes induced by the MK-35 (gE-) vaccine strain, while brain changes in the same animals caused by
Pathomorphological studies in newborn pigs induced by infection with Mogila strain were characterized by predominantly alternative, exudative, and less pronounced proliferative changes.

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References