COMPARATIVE PATHOMORPHOLOGICAL ANALYSIS OF CHANGES IN DOGS AND CATS CAUSED BY INOCULATION OF A VACCINE STRAIN AND FIELD ISOLATES OF AUJESZKY'S DISEASE VIRUS

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ABSTRACT

The great sensitivity of carnivorous animals to the Aujeszky's disease virus makes them an indicator of the presence or absence of the causative agent on a farm. The massive spread of rodents and especially cats, from porcine industrial farms with reproductive disorders, is the reason to investigate the clinical manifestations and pathomorphological changes induced by the vaccine strain and two terrain uterotropic strains of the Aujeszky's disease virus in dogs and cats. Kittens of 50–60 and dogs at 35–40 days of age were used. It was found that strain Mogila was highly virulent for dogs and cats, and the vaccine strain was more pathogenic for dogs and less pathogenic for cats. Cats had no itching. Pathohistological changes were localised in the nervous and respiratory system. They have the character of nonsuppurative encephalitis and interstitial pneumonia. In the lungs of cats, infected with strain Mogila, a fibrinous-necrotizing inflammatory response was observed.

Key words: dogs, cats, Aujeszky's disease virus.

Introduction

Aujeszky's disease (AD) is a herpes viral infection in animals and affects a wide range of hosts, except humans and tailless primates. In pigs it is a latent infection or unapparent with nervous signs, influenza-like symptoms and abortions. In other mammals the disease is manifested with non-purulent encephalitis and severe pruritus at the place of inoculation and replication of the virus. Conducted large-scale vaccinations of the pigs reduce the losses for the industry, otherwise affected by this disease. The vaccine strain Barta is pathogenic for dogs (Willemse et al., 1977). Lately, a new manifestation of the disease was found. It was characterized by the birth of physically weak and lifeless piglets. Recombinant variants of vaccine and terrain strains of AD virus from the organs of infected pigs were isolated (Christensen and Motovski 1993). There was a decrease in rodent and cat populations observed in pig farms with reported reproductive disorders. The reason was found in the mode of transmission of the infection in carnivores which ate organs from infected pigs (Thomson, 2001; Lazić et al., 2017). Hugoson and Rockborn (1972) noted that rodents and carnivores developed the symptoms and died before the disease was detected in pig farms. Monroe (1989) established that death occurred between 6 and 96 hours after inoculation in experimentally infected dogs. Morphologically a nonsuppurative encephalitis was found in the brainstem. Necrosis, neuropagia, mononuclear clusters in Virchow-Robin spaces, glial nodules glee assemblies and intranuclear inclusions type Cowdry A were seen in brainstem. (Whitley and Nelson, 1980; Quiroga et al., 1998; Zhang et al., 2015). Hagemoser et al. (1980) found diffuse microglial clusters and mononuclear perivascular cuffing in the brain of cats. According to the authors, these signs were observed steadily in the brains and had a diagnostic value. The lungs the alveolar walls were thickened due to the accumulation of mononuclear cells. Hyperaemia and oedema have been reported in dogs experimentally infected with AD virus (Shell et al., 1981; Zhang et al., 2015).
The aim of the study was to clarify the clinical picture and pathological changes in the organs of dogs and cats after inoculation with a vaccine strain and two field isolates of the Aujeszky's disease virus.

Materials and methods

In the experiments 28 mixed-breed dogs (aged 35–40 days) and 16 small kittens (weighing 0.6 ± 0.100 kg, at 50-60 days of age) European shorthair breed, both without antibodies against the AD-virus were used. Animals were dewormed, divided into four groups of seven (n=7) and inoculated subcutaneously with Tissue Culture Cytopathogenic Units 50% (TCCU50) with a dose of 0.5 cm³ (for dogs) and 0.3 cm³ (for cats) as follows: Group I – strain Mogila, group II – strain Stara Zagora II and group III – with MK-35 (gE). The fourth group was used as a control one (unvaccinated). The animals were observed for 15 days. Clinical monitoring was performed daily with regard to body temperature, breathing and general behavioural reactions. On the 15th day after infection, blood serum samples from living animals were obtained, and tested for antibodies against the AD virus.

The dead dogs and cats were autopsied and brain and lung samples were taken for histological and virological examination. The materials were fixed in 10% neutralised buffered formalin and Baker formalin, processed by the classic paraffin method and stained with haematoxylin-eosin, by Heidenheim, by Nissl and with phosphoric acid-haematoxylin (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. 17000002, issued to the Faculty of Veterinary Medicine, UF-Sofia) and Ordinances No. 16 and 22 for minimizing suffering of animals during euthanasia.

Results

The dogs, infected with strain Mogila, exhibited clinical signs on the second and the third day after infection, as death occurred within the 9th to the 18th hour after the first clinical manifestation – pruritus. The dogs infected with strain St. Zagora II died 4 to 5 days after infection. Only one dog survived until the end of the experiment. Three of the dogs, infected with strain MK-35 (gE), showed clinical signs on the first day and died after 12–18 hours and the other four dogs – between the 4th and the 8th day. The virological study of histological materials (brain and internal organs), obtained from the infected animals, the applied virus was again isolated. - The virus was reisolated from brain and internal organs of inoculated animals. Only the dogs, infected with strain Mogila, reacted clinically with a short-term rise in body temperature. Animals from all groups refused to eat, shivered, yowled painfully, vomited, and had great salivation, diarrhoea, uncoordinated movements and opisthotonus. Six to eight hours from the beginning of these signs the animals were constantly striving for scratching with the hind leg in the area of the withers and neck. The fur has fallen off in the places where the animal has scratched itself and excoriations on the skin were observed. A less pronounced brain cerebrovascular hyperaemia was observed in the macroscopic study on the CNS. In addition to hyperaemia, in the perivascular space of Virchow-Robin, mononuclear cell accumulation forming cell clusters was observed microscopically. Nodular or diffuse accumulation of microglial cells, most commonly located near the third and fourth brain ventricles and dorsal areas of hemispheres, was detected. The Purkinje cells in the cerebellum showed central tigrolysis and
enlightenment of the nucleus. In the grey matter of medulla oblongata and spinal cord, necrotic changes in ganglion cells were detected. Pycnotic and lytic changes were most often observed, sometimes with signs of neurophagia (Fig. 1A and B). In the cerebellum and hemispheres local reactive micronecrosis was found. The most intense changes were in dogs infected with Mogila strain. The macroscopic examination of lungs revealed hyperaemia and the presence of local dark red, slightly sunken areas, with a size varying form a grain to a one Euro coin. Microscopically, an atelectasis and pronounced hyperaemia were detected. In some areas erythrocytes have been found in the alveolar lumen. Clusters of mononuclear cells and neutrophilic leukocytes were observed in the interstitium, as well as necrotic changes in the bronchial and bronchiolar epithelium (Fig. 2C and D).

The cats, infected with strain Mogila, primarily developed clinical signs and died within the 3rd to 4th day. The death of cats infected with strain St. Zagora II, occurred on the 4th and 5th day, whereas from infected cats with strain MK-35 (gE), one died on the 5th day, and the other three survived the experiment. The cats from the control group did not show clinical signs. A change in body temperature was not detected. The animals had salivation, tachypnea, uncoordinated movements, refused to eat, painfully meowed, turned their heads and anxiously looked at their backs. After this period, the cats became apathetic and hided at corners. Vomiting and pruritus were not observed. Hyperaemia of hemisphere vessels in the brain was found. macroscopically.

Figure 1: A: Hemisphere of a dog infected with strain Stara Zagora II. Diffuse accumulation of microglial cells near the fourth brain ventricle (H&E stain, Magnification X25). B: Medulla oblongata of a dog infected with strain MK-35(gE). Necrotic changes – pycnosis and lysis (H&E stain, Magnification X25). C: Spinal cord of a dog infected with strain Stara Zagora II. Necrotic changes in ganglion cells (pycnosis) (Nissl stain, Magnification X100). D: Lung of a dog infected with strain Mogila. Atelectatic and hyperaemic-haemorrhagic changes (H&E stain, Magnification X25).
Histologically, mononuclear cell clusters around the vessels of the meninges and brain parenchyma were detected. In the ganglion cells of the medulla oblongata and spinal cord, lytic and pycnotic changes as well as eccentric deployment of the nucleus were identified. In some of the damaged neurons an aggregation of microglial cells with manifestations of neurophagia was found. Glial accumulations were observed among the brain parenchyma, most often localised in the ventral parts of the brain, close to the fourth brain ventricle. In some cases, the clusters consisted of 4-5 cells, while in others there was an accumulation of a significant number of cells. In the cats infected with strain Mogila the most pronounced changes were found, and the worst - for those infected with MK-35 (gE-) (Fig. 2A and B). In the lungs focal redness and atelectatic zones, mainly in the cardiac and apical lobes, were observed. Microscopically, hyperaemia, atelectasis and slight septal thickening due to mononuclear cell clusters were observed. In cats infected with strain Mogila, fluid accumulation in the interstitial tissue and pulmonary parenchyma was established. Fibrin fibres were present in the exudate after staining by Azan (Fig. 2C). Necrotic changes and accumulation of mononuclear cells in the submucosa were found in the bronchial and bronchiolar epithelium (Fig. 2D).

Discussion

The clinical signs and symptoms in dogs and kittens that we identified were similar to those described by other authors in the same animal species and ruminants (Muller and al., 2003). The
Aujeszky's disease virus was isolated from all organs of the dead animals. AD was manifested in dogs and cats with anxiety, great salivation, tachypnea, diarrhoea, food refusal, uncoordinated movements and lack of aggressiveness towards people. The absence of the later clinical sign was proved by all authors who studied AD. Only in 2.6% of cases Monroe (1989) found aggression but he could not explain this fact. In our studies it was found that the change of body temperature was recorded only in dogs, infected with Mogila strain. This could be a result of increased muscle contractions due to tremor and uncoordinated movements. Picking, falling fur and skin excircations we detected only in dogs. According to Hagemoser et al. (1980) this clinical sign was not proven in kittens. The presence of all other symptoms was an indicator that in both species the pathogenesis of the disease was the same. In our experiments we found that death occurred earlier in dogs and two-month kittens, infected with strain Mogila. Strain MK-35 (gE) exhibited stronger virulence in dogs and weaker in cats, causing death in only one of the experimental animals on the 5th day. Bartha strain (similar to the vaccine strain used by us) caused infection and death in dogs without picking (Willemse и сътр., 1977). The results obtained correlate with data by Gaskell and Bennett (1999) who well pronounced connection between clinical manifestation of the disease and the virulence of the strains.

In both species the pathohistological changes were mainly localized in the nervous system and lungs. Microscopic changes in the brain were visible by mononuclear infiltration in the parenchyma and neurophagia. Whitley and Nelson (1980) found intranuclear inclusions Cowdry A in dogs’ brains. Thomson et al. (2001) described intranuclear eosinophilic and basophilic inclusions in the brain. On the opposite, we did not prove these changes in the nuclei of the nerve cells. In the lung we observed hyperaemia, atelectasis and weak thickening of the alveolar walls as a result of mononuclear clusters. The results of Shell et al. (1981) were similar. They detected bronchial oedema and hyperaemia in experimentally infected dogs. In the cats infected with strain Mogila the presence of fibrin in the exudate and necrosis in the pulmonary parenchyma was detected. Lesions of this type have not been described in the literature until now.

Conclusion

The clinical signs in puppies and kittens infected with one vaccine and two Aujeszky's disease virus isolates were anorexia, tremor, shivering, uncoordinated movements, painful yowling, salivation, vomiting, and lack of aggressiveness toward humans. Pruritus before death was observed only in dogs. Pathohistological change of the nervous system in dogs and cats, inoculated with different strains of AD virus, was nonsuppurative meningoencephalitis. In the respiratory tract atelecstatic and hemodynamic changes were observed. In cats, infected with strain Mogila, fibrinous-necrotizing pneumonia was detected. The strains Mogila and St. Zagora II were found highly virulent for dogs and cats, as Mogila caused the most striking CNS and respiratory changes. In dogs strain MK-35 (gE) was more pathogenic than in cats. The ability feline organism to develop an immune response, after consumption of internal pig organs, containing Aujeszky's disease virus, makes cats inappropriate as an indicator for the presence or absence of virus on farms.

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References