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Original Scientific Paper

AUJESZKY'S DISEASE IN HUNTING DOG IN THE TERRITORY OF THE REPUBLIC OF SRPSKA

Srđan GLIGORIĆ[1*], Dragan KNEŽEVIĆ[1], Dragan KASAGIĆ[1], Dimitrije GLIŠIĆ[2], Darko MARINKOVIĆ[3]

[1] PI Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka, Bosnia and Herzegovina
[2]Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia
[3] University in Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia
*Corresponding author: Srđan Gligorić, srdjan.gligoric@virs-vb.com

Summary

Aujeszky's disease (pseudorabies) is an acute, contagious, viral disease, primarily of pigs, and then of other species of domestic and wild animals, caused by porcine herpesvirus type 1. In pigs, the infection is enzootic, with a high mortality rate upon the first entry of the virus into the group and is characterized by encephalitis and a high mortality rate in piglets, pneumonia in older pigs, and abortions in sows. The disease occurs sporadically in secondary hosts and is accompanied by a short incubation period and a fatal outcome. Carnivores become infected through direct or indirect contact with pigs and ingestion of raw or undercooked meat and internal organs, and is characterized by neurological disorders in the form of pruritus to self-mutilation.

The paper describes a case of Aujeszky's disease in the territory of the city of Teslić in 2024, in a hunting dog of the Plot breed, which came into direct physical contact with a wild boar during hunting, as well as the carcass of a shot animal. Initial signs of the disease appeared after 24 hours in the form of inappetence, fever and vomiting. After the initial signs, neurological disorders manifested in the form of tilting the head to the side, hypersalivation and pruritus, which progressed to self-mutilation in the head area. Death occurred 36 hours after the onset of neurological disorders. Macroscopic examination revealed lesions on the skin of the head and neck, conjunctival hyperemia, generalized lymphadenopathy, hyperemia of the lungs, meninges and brain. Gastric dilatation with right-sided splenic torsion was also found, most likely as a result of acute shock and pain. Histopathological analysis revealed non-suppurative meningoencephalitis, characterized by discrete perivascular mononuclear infiltrates and perivascular hemorrhages in the cerebrum, gliosis, satellitosis, and neuronophagia in the medulla oblongata, as well as intranuclear eosinophilic inclusions in medulla oblongata neurons. The results of molecular analysis of brain tissue and tissue from skin lesions, using the qPCR method, demonstrated the presence of porcine herpesvirus type 1 nucleic acid in the examined samples.

Sporadic infection of hunting dogs may indicate virus circulation in the wild boar population in the Republic of Srpska.

Key words: Aujeszky's disease, porcine herpesvirus type 1, hunting dog, non-purulent meningoencephalitis.

INTRODUCTION

Aujeszky's disease (pseudorabies) is an acute, contagious, viral disease primarily of pigs, and then of other species of domestic and wild animals. The causative agent is swine herpesvirus type 1 (*Suid herpvirus 1*), a DNA virus from the genus *Varicellovirus*, from the subfamily *Alphaherpesvirinae*, which is included in the family *Herpesviridae* (WOAH, 2018). Aujeszky's disease does not have zoonotic potential (CFSPH, 2017). Due to its strong potential to cause infection of nervous tissue, and on the other hand due to its septicemic-viscerotropic pathological effects on other organ systems, it is defined as a pantropic virus with pronounced neurotropism (Sofrenović and Knežević, 1994). In pigs, the infection is enzootic in nature, with a high mortality rate upon the first entry of the virus into the herd. The manifestation of clinical signs of the disease in pigs depends primarily on the age of the animal, and then on the immune status, farm management and virulence of the pathogen. The disease in pigs is characterized by encephalitis and a high mortality rate in piglets, pneumonia in older pigs and abortions in pregnant sows (Pejsak and Truszczynski, 2006; Koshemetov et al., 2021). However, in domestic and wild pigs, latent infection with localization of the virus in the nerve ganglia can often develop. In this way, conditions for active or passive carriership are created, and domestic and wild pigs represent a reservoir of the virus for secondary hosts (most often sheep, cattle, carnivores) (Milicevic et al., 2016; Sehl and Teifke, 2020). In secondary hosts, the disease occurs sporadically, is characterized by a short incubation period and a fatal outcome. Sheep and cattle become infected when cohabiting with pigs (Deblanc et al., 2019; Ciarello et al., 2022). Carnivores become infected through direct or indirect contact with pigs, or by ingestion of raw or undercooked meat and internal organs. After initial, nonspecific signs of the disease, signs of neurological disorders appear in the form of pruritus, which can progress to self-mutilation. Pruritus in secondary hosts is a manifestation of trigeminal ganglioneuritis, infection of the dorsal root ganglia and paravertebral ganglia. Pigs do not show signs of pruritus (Laval and Enquist, 2020).

MATERIALS AND METHODS

An autopsy of the dog was performed in 2024, with suspicion that the death occurred due to Aujeszky's disease. The material for the autopsy was the caracass of a deceased hunting dog of the Plot breed from the territory of the city of Teslić, aged 19 months, intact bitch, in good condition. After the autopsy, brain and medulla oblongata, lymph nodes, liver and lung tissues were sampled for histopathological analysis. After fixation in 10% neutral buffered formalin, the tissues were dehydrated in a series of alcohols,

enlighten in xylene and, after impregnation in liquid paraffin, embedded in paraffin molds. Tissue sections 3-5 μm thick were stained with hematoxylin and eosin.

A portion of the brain tissue sample was separated for testing for the presence of Aujeszky's disease virus nucleic acid using real-time quantitative polymerase chain reaction (qPCR). The brain sample was homogenized using a mortar and pestle, then mixed 1:10 with phosphate buffer and centrifuged at 4500 rpm for 10 minutes. The resulting supernatant was collected and frozen at -80°C until further analysis. Viral DNA was extracted using the Indical IndiSpin Extraction Kit, according to the manufacturer's instructions. Viral nucleic acid amplification was performed using previously published primers by Ma et al. (2008). For qPCR, a commercial *Luna® Universal Probe qPCR Master Mix* (New England Biolabs, Ipswich, MA, US) was used, and the reaction temperature profile included the following steps: viral DNA denaturation at 95°C for 1 minute (1 cycle), primer binding at 95°C for 15 seconds (50 cycles), and DNA strand synthesis at 60°C for 30 seconds. A reaction mixture with a total volume of 12.5 μl was prepared by adding 2.75 μl *Luna® Universal Probe qPCR Master Mix*, 0.50 μl F primer (10 μM), 0.50 μl R primer (10 μM), 0.25 μl probe (10 μM), and 2.5 μl nucleic acid.

RESULTS

According to the anamnestic and epizootiological data taken from the owner, the bitch came into direct contact with a wild boar during the hunt, and immediately after the hunt she licked the blood from the carcass of the shot wild boar and traces of blood that remained in the environment after the carcass was removed. After 24 hours, the dog showed non-specific signs of illness in the form of vomiting, inappetence, lethargy and fever. After 30-36 hours from the appearance of the first signs of illness, signs of neurological disorders appeared: tilting the head to the side, hypersalivation and pruritus up to self-mutilation in the head area. Neurological disorders intensified over several hours. Death occurred 36 hours after the appearance of neurological signs of illness.

Macroscopic changes determined by external examination of the caracass were characterized by lesions on the scalp in the form of hairless, hyperemic areas on the auricles, lower neck, around the eyes and on the muzzle, and hyperemia of the conjunctiva (Figure 1). After removal of the skin, lymphadenopathy of the submandibular lymph nodes was observed. Internal examination revealed hyperemia and hemorrhages in the lungs, as well as hyperemia of the meninges and initiation of blood vessels in the brain (Figure 2). Mild hyperemia was determined in the liver. Also, discrete dilatation of the stomach and torsion of the spleen to the right side were determined, most likely as a result of acute shock and pain (Figure 3).

Figure 1 A, B: Scalp lesions as a result of self-mutilation; C: Hyperemia of the conjunctiva

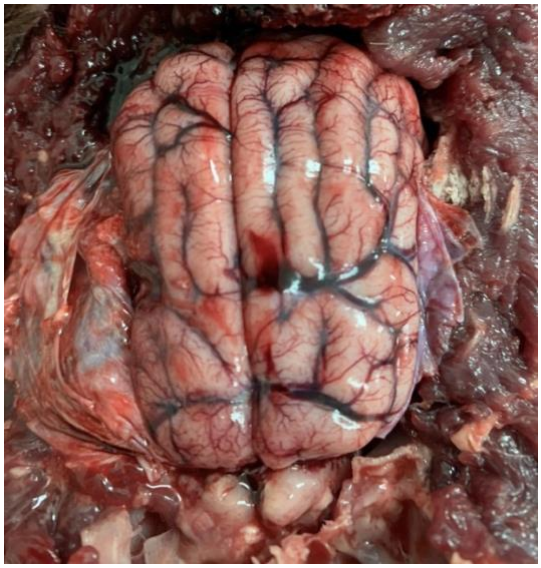

Figure 2 Brain after separation of the dura mater, hyperemia and initiation of blood vessels

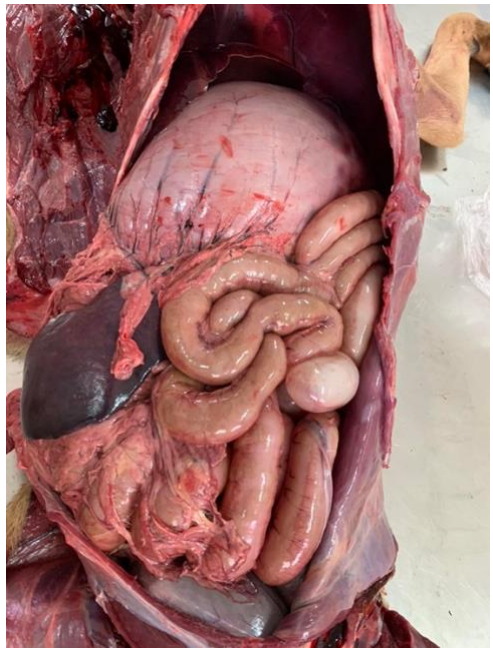

Figure 3 Discreet dilatation of the stomach and torsion of the spleen to the right side

Histopathological examination revealed non-purulent meningoencephalitis with accompanying phenomena in the brain and medulla oblongata. Changes in the brain were characterized by the finding of a discrete perivascular mononuclear infiltrate with lymphocytes and histiocytes, and perivascular hemorrhages (Figure 4). Changes in the medulla oblongata were characterized by the finding of satellitosis and neuronophagy, focal accumulation of glial cells, degeneration and necrosis of neurons (Figure 5). The presence of intranuclear eosinophilic inclusions was found in the neurons of the medulla oblongata (Figure 6). Changes found in other organs consisted of lymphocyte depletion in the germinal centers of the lymph nodes, hyperemia, edema and emphysema of the lungs, and moderate liver hyperemia and discretely expressed cloudy swelling of hepatocytes.

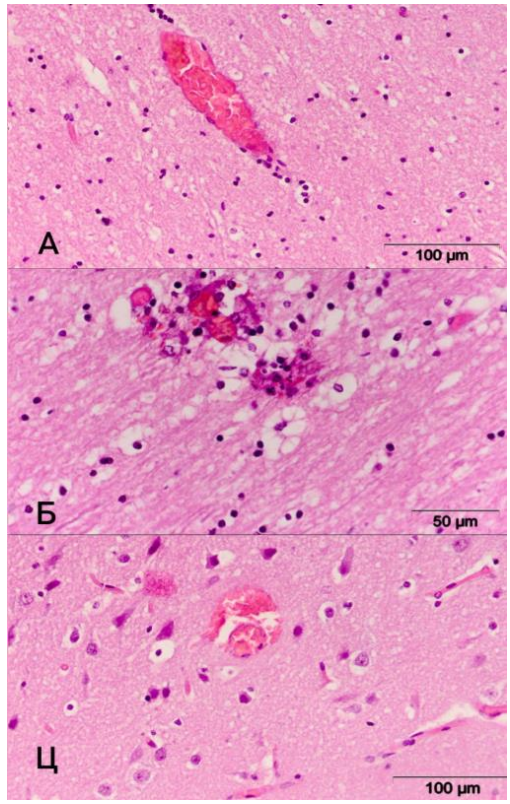

Figure 4 Brain
A, B: Discrete perivascular mononuclear infiltrates, HE × 400, HE × 600; C: Perivascular bleeding, HE × 400

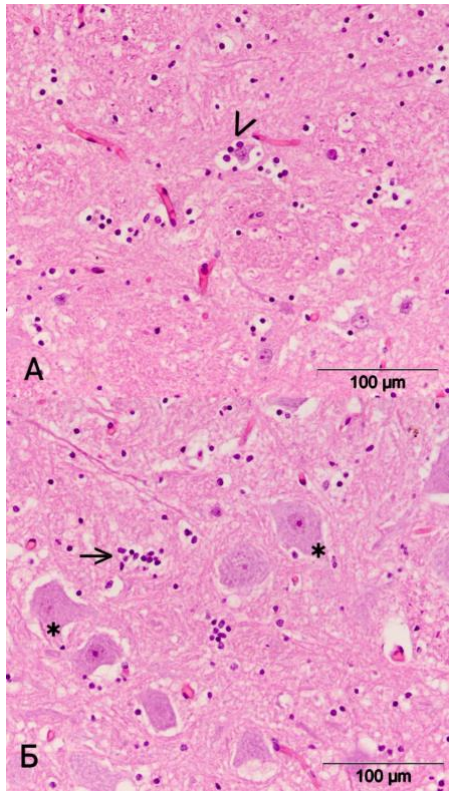

Figure 5 Medulla oblongata
A: neuronophagia (arrowhead), HE × 400; B: focal accumulation of glial cells (arrow), degeneration and necrosis of neurons (asterisk), HE × 400

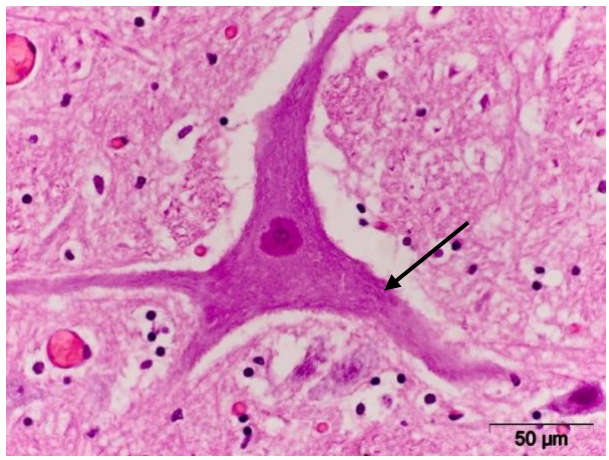

Figure 6 Medulla oblongata – intranuclear eosinophilic inclusions (arrow), HE × 600

The qPCR method detected Aujeszky's disease virus (porcine herpesvirus type 1) DNA in brain tissue samples and skin lesions.

DISCUSSION

Wild boars are a numerically significant game species on the Balkan Peninsula, and thanks to their reproductive potential, they can be classified as one of the more invasive wild species. Rich forests, as well as the availability of water sources, provide this game with both a refuge and an inexhaustible source of food, which explains their large numbers. Hunting is a traditional activity in the Republic of Srpska, and wild boars are a frequently hunted game species. Hunting dogs, mainly hounds, are traditionally used in wild boar hunting, so during this activity, contact between dogs and wild boars (chasing and barking) is inevitable. After shooting, dogs often come into contact with the carcass of the shot animal, but also with its meat and internal organs. Aujeszky's disease is sporadically present in the population of domestic and wild boars on the territory of the Republic of Srpska. In the period from 2019 to February 2024, 44 outbreaks were recorded, i.e. the disease was confirmed in 209 domestic pigs and 3 dogs. The disease is present in all surrounding countries (Černe et al., 2023; Milicevic et al., 2016; Lazić et al., 2017), as well as in Europe (Cay and Letellier, 2009; Ciarello et al., 2022). Ciarello et al. (2022) report that there is a high prevalence of the disease on pig farms in Sicily, and that cases of infection in cattle have been recorded. In Sicily, Aujeszky's disease rarely occurs in dogs, and it has been described in two hunting dogs. In these cases, the dogs consumed uncooked meat of suspected wild boar after hunting. In the period 1995-2022, Freuling et al. (2023) detected Aujeszky's disease in 35 dogs and 3 foxes in Germany. In most dogs, the manifestation of signs of the disease was epidemiologically associated with the consumption of undercooked wild boar meat or contact with wild boar. Dogs, like other carnivores, become infected with the virus through direct or indirect contact with pigs and the ingestion of raw or undercooked meat and internal organs. The sporadic occurrence of the disease, as well as the short period of clinical signs, are the reasons why Aujeszky's disease in carnivores is often undiagnosed.

Cano-Terriza et al. (2019) report that direct contact poses a potential risk of infection in hunting dogs. They describe the exposure of hunting dogs to Aujeszky's disease virus in a highly endemic area for this virus in wild boars in Spain. Ciarello et al. (2022) report pruritus to the point of self-mutilation as the dominant neurological symptom. Sehl and Teifke (2020) report non-suppurative encephalitis as the dominant histopathological finding in nervous tissue. They also describe histopathological findings in the liver and lungs, characterized by necrosis, hemorrhage, and inflammation.

In the period 2006-2020, cases of Aujeszky's disease in hunting dogs were described in Slovenia, and the dominant histopathological finding in these cases was the presence of non-purulent meningoencephalitis (Černe et al., 2003). Numerous authors also detect non-purulent encephalitis with vasculitis as a consistent histopathological

finding in Aujeszky's disease (Freuling et al., 2023). Cay and Letellier (2009), having confirmed Aujeszky's disease in two hunting dogs in Belgium by virus isolation and the application of the Real Time PCR (RT-PCR) method, state that characteristic neurological signs of the disease appeared in the dogs after hunting. In Italy, Aujeszky's disease was detected in a seven-year-old hunting dog that had been in contact with wild boar blood (Pizzurro et al., 2016). Porcine herpes virus type 1 was proven using RT-PCR and isolation on rabbit kidney cell culture (RK13).

CONCLUSION

Based on the epizootiological history, clinical picture and histopathological findings, Aujeszky's disease was suspected, and based on the results of molecular testing, the presence of Aujeszky's disease virus in a hunting dog was confirmed. Sporadic infection of hunting dogs indicates the circulation of the virus in the wild boar population in the Republic of Srpska. Accordingly, thermal processing of meat and internal organs of wild boars is mandatory.

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Conflict of interest statement: The authors declare that there is no conflict of interest.

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