

AUJESZKY'S DISEASE AND AFRICAN SWINE FEVER IN THE POMORAVLJE AND ŠUMADIJA DISTRICTS

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Abstract

Wild boars are important reservoirs of Aujeszky's disease virus and African swine fever (ASF), making the animals a key factor in the spread of these diseases to domestic pig populations. One of the most common routes of virus transmission among wild boars is sexual contact, particularly during the close contact associated with mating, while migration and interactions with human activities further facilitate the spread of infections. The seroprevalence of Aujeszky's disease varies across Europe, ranging from 0% to over 50%, depending on the region. In Serbia, the first case of ASF in domestic pigs was confirmed in 2019, and in wild boars in 2020 in border areas. The aim of this study was to determine the presence of Aujeszky's disease virus and ASF in the wild boar population in central Serbia, specifically within the epizootiological units of Pomoravlje and Šumadija. The research was conducted using molecular and serological methods to detect the viruses and specific antibodies. Additionally, the spatial and temporal distributions of these diseases in the observed area were analyzed. For the purposes of this study, samples from wild boars of different sexes and ages

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were analyzed. The samples were collected during the hunting season from August to November 2023 in the territories of the Pomoravlje and Šumadija districts. A total of 90 samples were examined, with blood sera used for serological analysis and organs (spleen and kidney) from the same animals used for molecular analysis. Serological analysis of the examined samples revealed a high concentration of antibodies against Aujeszky's disease virus in wild boars from both Pomoravlje and Šumadija. Regarding ASF virus, positive serological findings in the wild boars were recorded only in Pomoravlje, while all samples from Šumadija tested negative. Based on the total number of samples examined (n=90), the seroprevalence of Aujeszky's disease was determined to be 26.7%, while that of ASF was 3.3%. Molecular analysis of wild boar organs did not confirm the presence of viral genetic material for either Aujeszky's disease or ASF. Serological findings indicate ongoing circulation of these viruses in wild boars and a potential risk to domestic pigs. Continuous monitoring is, therefore, essential for early detection and effective disease prevention.

Keywords: African swine fever (ASF), Aujeszky's disease, wild boars, Pomoravlje, Šumadija

INTRODUCTION

Wild boars serve in nature as reservoirs for many diseases, including Aujeszky's disease, also known as pseudorabies, and African swine fever (ASF). They can transmit diseases to other wild boars as well as to domestic pigs. Wild boars most commonly spread the viruses among themselves through direct or indirect contact (via feces, saliva, blood, or mating). Research has shown that the oronasal and sexual routes are the most common modes of transmission. The oronasal route is more often associated with females that live in groups, while transmission through mating is attributed to males (Lari et al., 2006). Wild boars frequently come into contact with human activities, which also facilitates the spread of viruses in this way (Cukor et al., 2020; Denstedt et al., 2021). Given that these animals are capable of migrating over long distances, the virus can spread rapidly to new areas.

Although the clinical form of Aujeszky's disease is rare in wild boars, seroprevalence in Europe ranges from 0% to over 50%. For example, in Spain, the seroconversion rate ranged from 0.8% to 44% (Vicente et al., 2005; Closa-Sebastià et al., 2011), in France it was 3.5% (Arias et al., 2000), while in Italy it ranged between 30% and 51% (Montagnaro et al., 2010; Lari et al., 2006). In Switzerland, 1.5% seroprevalence was recorded (Köppel et al., 2007), 55% in Croatia (Zupancic et al., 2002), 11% in Poland (Szweda et al., 1998), 31% in Slovenia (Vengust et al., 2006), and 32% in Russia (Kukushkin et al., 2009). In contrast, in countries with a small number of wild boars, such as the Netherlands and Sweden, where the wild boar populations have only recently begun to increase, no seroconversion has been recorded (Elbers et al., 2000).

ASF has been detected in many European countries since its introduction into Georgia in 2007. In Serbia, the first case of ASF in the domestic pig population was confirmed in 2019. In early 2020, ASF was detected in wild boars in open hunting grounds in the

southeastern region near the country's border with Romania and Bulgaria (Prodanov-Radulović et al., 2023).

The aim of this study was to determine the presence of Aujeszky's disease and ASF in central Serbia, specifically within the epizootiological units of Pomoravlje and Šumadija. These districts were selected because they represent the epizootiological units of the Jagodina Veterinary Specialist Institute. These districts have not been previously studied despite their significant epizootiological importance; they border neighboring districts where ASF is already widely distributed. Therefore, it was important to obtain data from these areas to provide a more complete understanding of the distribution and risk of disease spread. Data on Aujeszky's disease in wild boars from the Pomoravlje and Šumadija districts were lacking, so the aim of this study was to update and expand national information on this disease by particularly focusing on these districts. This involved testing wild boar samples from these districts using molecular and serological methods to detect the viruses and specific antibodies against Aujeszky's disease and ASF, as well as analyzing the spatial distribution of these diseases.

MATERIALS AND METHODS

Materials

For the purposes of this study, samples were collected from wild boars originating from the Pomoravlje and Šumadija districts of central Serbia (Figure 1). A total of 90 samples were collected during the regular hunting season from August to November 2023, of which 84 originated from the Pomoravlje district and 6 from the Šumadija district. Of the samples, 50% ($n = 45$) were from male animals and 50% ($n = 45$) from female animals, representing individuals of different ages (Table 1). Blood sera from wild boars were used for serological diagnostics, while organs (spleen and kidney) from the same animals were used for molecular diagnostics. Samples were collected immediately after hunting or during storage in cold chambers. The hunters held valid licenses, and culling was conducted in accordance with applicable legal regulations and hunting rules during the regular hunting season, which lasted from November 2022 to November 2023. Humane euthanasia was not required, as all animals had already been killed at the time of sample collection. Sampling was performed within the framework of official surveillance of wild boars for classical swine fever and ASF, in accordance with applicable Serbian legislation, and animals were not hunted exclusively for research purposes.

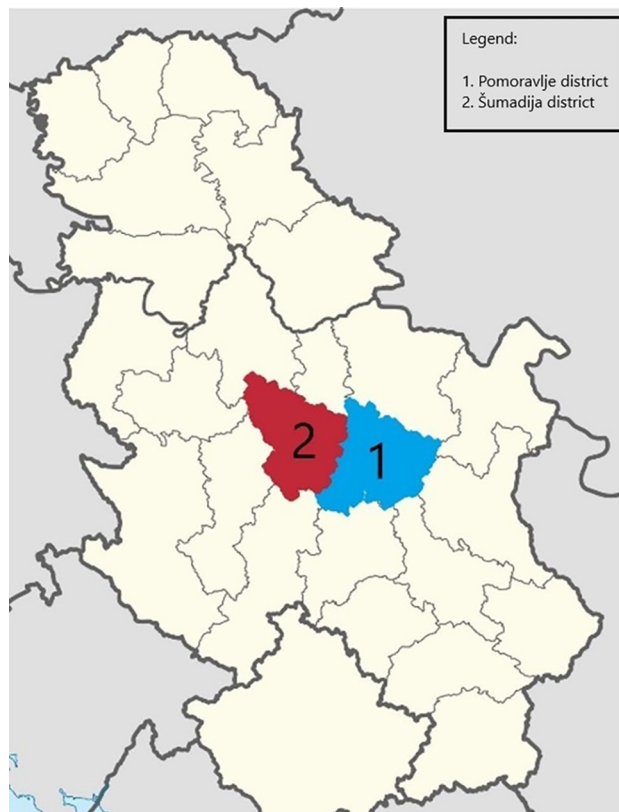


Figure 1. Districts from which the samples for testing were collected (Pomoravlje and Šumadija)

Table 1. Structure of the examined group of wild boars by month of sampling, sex and age

MONTH	SEX		AGE			
	MALE	FEMALE	0-6 MONTHS	6-18 MONTHS	1.5-2.5 YEARS	MORE THAN 2.5 YEARS
AUGUST	1	4	1	2	1	1
SEPTEMBER	17	17	7	10	4	13
OCTOBER	14	9	6	9	3	5
NOVEMBER	13	15	10	5	3	10

Methods

Serological and molecular diagnostics of aujeszky's disease and african swine fever

For serological diagnosis of Aujeszky's disease, the commercial INGEZIM ADV TOTAL ELISA kit (11.ADV.K1; INMUNOLOGIA Y GENETICA APLICADA, S.A., Spain) was used, which is an indirect ELISA containing purified viral antigen from American and European virus strains. For ASF, the INGEZIM PPA COMPAC ELISA kit (11.PPA.K3; INMUNOLOGIA Y GENETICA APLICADA, S.A., Spain) was employed. This blocking ELISA uses the purified VP72 viral protein, the main structural protein of the ASF virus, as the antigen. The ELISA procedures were performed in accordance with the manufacturer's protocols.

To detect the DNA genome of Aujeszky's disease and ASF viruses, a Real-Time PCR test was used (WOAH, Chapter 3.9.1). DNA was extracted using the commercial IndiSpin Pathogen Kit (Fisher Scientific). Commercial reaction mixtures and primers were used for amplification and detection of genome fragments specific to the viruses being tested (Table 2).

Table 2. Primers used for the detection of genome fragments of viruses causing Aujeszky's disease and African swine fever

Virus	Primers	Region
Aujeszky's disease	MAD gB FOR 5'-ACCAACGACACCTACACCAAG-3'	gB
	MAD gB REV 5'-CCTCCTCGACGATGCAGTTG-3'	
African swine fever	5'-CTGCTCATGGTATCAATCTTATCGA-C 5'-GATACCACAAGATC(AG)GCCGT-3'	VP72

The preparation of the PCR reaction mixture and the performance of the test for Aujeszky's disease were carried out according to the protocol described by Wernike et al. (2014). The PCR reaction mixture for ASF was prepared according to the manufacturer's instructions (Brilliant III Ultra-Fast QPCR Master Mix, Agilent Technologies).

RESULTS

Of the 90 blood sera examined, specific antibodies against Aujeszky's disease virus were found in 24 individuals (26.7%). Most of the seropositive wild boars (n=22) were from the Pomoravlje district, while only two wild boars were from the Šumadija district (Figures 2 and 3). Additionally, of the 90 sera examined, specific antibodies against the ASF virus were detected in 17 individuals (18.9%), while the reaction was suspicious

in four individuals (4.4%). All seropositive animals originated from the Pomoravlje district. After obtaining these results, the suspicious and positive sera were sent for further testing to the National Reference Laboratory, Scientific Institute for Veterinary Medicine of Serbia (NIVS). Upon completion of the testing, NIVS confirmed the presence of antibodies to ASF in three wild boars (Figure 4). Based on these results, the seroprevalence of ASF in this area was 3.3%.

In some animals younger than 6 months of age, the presence of antibodies against Aujeszky's disease virus was detected in the blood serum (females, n=3; males, n=1). Ten wild boars with these antibodies belonged to the group of animals aged 6 to 18 months (females, n=8; males, n=2), while the same number of wild boars were in the group of animals older than 2.5 years (females, n=7; males, n=3). Wild boars aged 1.5 to 2.5 years were negative for this disease.

Antibodies against ASF virus were detected in the blood serum of five animals younger than 6 months of age (females, n=2; males, n=3). Two wild boars belonged to the group of animals aged 6 to 18 months (females, n=1; males, n=1), while one wild boar was in the group aged 1.5 to 2.5 years (male, n=1). Nine ASF-seropositive wild boars were older than 2.5 years. Four animals showed a suspicious reaction on the ELISA test.

By Real-Time PCR test, genome fragments of Aujeszky's disease and ASF viruses were not detected in the organ samples from the wild boars.

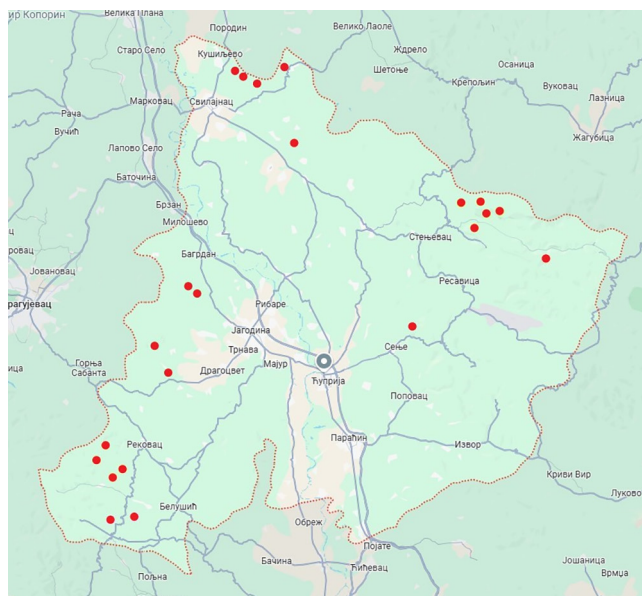


Figure 2. Geographical representation of the locations of seropositive animals for Aujeszky's disease in Pomoravlje (n=22)

DISCUSSION

Studies have shown that wild boars serve as reservoirs for the Aujeszky's disease virus (Herrmann et al., 1984). This disease is present in wild boars in Serbia, with significant seroprevalence, particularly in the eastern parts of the country. According to data from Lazic et al. (2015), the average seroprevalence in wild boars is 21.13%, with the highest percentage, reaching 46.86%, recorded in older individuals (over 2.5 years).

Herpesviruses, including the Aujeszky's disease virus, often cause latent infections. In domestic pigs, the virus is most commonly found in the trigeminal ganglia, while in wild boars, it is localized in the sacral ganglia (Spickler, 2010). Diagnostics are performed by analyzing various samples – nasal swabs, oropharyngeal fluid, and tonsils are used for live animals, while tonsils, brain, spleen, and lungs are examined in deceased animals (Fonseca et al., 2012; Ruiz-Fons et al., 2007). In the current study, blood serum, spleen, and kidney samples were analyzed. Serological results showed that 26.7% of wild boars (n=24) were seropositive, indicating the presence of the virus in the population. However, molecular diagnostics did not detect the virus in any organ sample, including those from seropositive individuals. These results suggest that the disease in the hunted wild boars was in a latent phase, with the virus most likely localized in the neural ganglia (WOAH, 2018).

The analysis of Aujeszky's disease virus-seropositive wild boars showed that females predominated over males (75% and 25%, respectively). Previous studies have indicated differences in disease prevalence between sexes (Ruiz-Fons et al., 2007; Ruiz-Fons, 2012). Males, who have more contact with different wild boar groups during mating, are at a higher risk of infection. On the other hand, females, which live in stable groups, often make close contacts, which may increase the likelihood of virus transmission (Gortazar et al., 2009).

In addition to sex, the age of the animals plays a significant role in seroprevalence. Studies have shown that the percentage of infected pigs increases with age due to longer exposure to the virus and a higher number of contacts (Meier et al., 2015). In the current study, seropositive wild boars were between 6 and 18 months old, as well as older than 2.5 years.

The presence of Aujeszky's disease in the wild boar population poses a challenge for countries attempting to eradicate the disease in domestic pigs. Studies have shown that the density of the wild boar population directly affects the seroprevalence of the disease (Ruiz-Fons et al., 2008). Additionally, contact between wild and domestic pigs can contribute to the spread of the infection, especially in areas where extensive pig farming is common, such as Pomoravlje and Šumadija.

ASF has been present in Pomoravlje for more than two years. The first case of ASF in wild boars in Pomoravlje was confirmed in December 2022, when hunters found the carcass of a deceased pig in the municipality of Despotovac. Based on the spread of the disease and the timeline, it can be assumed that ASF has been present in this

region longer than the initially officially confirmed cases. The main issues in the rapid detection of the disease include irregular and inefficient field searches by hunters, delays in reporting suspected cases, and inadequate management of the wild boar population.

During the current study, blood serum, spleen, and kidney samples from wild boars were analyzed using serological and molecular methods. Serological testing revealed that 3.3% of wild boars ($n=3$) were seropositive for ASF virus. The RT-PCR method did not detect the virus genome in any of the organs. This is not an unusual situation, as similar findings have been reported in other European countries where ASF is present. After research conducted in Lithuania in 2018, up to 21.6% of wild boars in some municipalities were seropositive, while PCR tests were negative for the presence of ASF virus (Pautienius et al., 2020). Similar results were obtained during the monitoring of the epizootiological situation in Poland from 2014 to 2020, where wild boars were also identified as seropositive but PCR negative for ASF (Piotr Frant et al., 2022). Such data indicate that the presence of seropositive but PCR negative wild boars is not unusual in regions with long-term circulation of this disease.

ASF virus-seropositive animals can theoretically be considered potential carriers of the virus, but their actual epidemiological role is still under investigation. While some authors believe these animals could contribute to the spread of the infection (Arias & Sanchez-Vizcaino, 2002), others suggest that they do not shed the virus in significant quantities and are not a long-term source of infection (Schulz et al., 2019; Petrov et al., 2018). It is important to note that serological results in piglets younger than six months are not reliable, as it is not possible to determine with certainty whether the ASF antibodies are the result of infection or passive immunity. On the other hand, seropositive results in older pigs can indicate a previous infection and recovered disease.

As the ASF virus persists in the wild boar population, disease control becomes a challenge. Reducing the wild boar population through hunting and traps, and establishing disease-free zones, may be key strategies in limiting the spread of the virus. Successful control requires cooperation between relevant authorities, hunters, and local communities, along with continuous monitoring and timely responses to new cases.

CONCLUSION

Aujeszky's disease is a contagious and economically significant disease in pigs. Pigs are the only species that survive the infection and serve as the main reservoir of the disease. In the wild boar population, the disease can be triggered by stress factors, such as unfavorable environmental conditions or human activities.

ASF is an extremely contagious disease that affects all age groups of pigs and is characterized by high mortality. Wild boars are a key factor in its spread, as they can

transmit the virus and serve as reservoirs of infection. Controlling ASF in the wild boar population requires complex strategies, including regulated hunting and continuous monitoring.

When analyzing laboratory results, it is essential to consider the characteristics of the virus and the host, as well as the current epidemiological situation. Serological testing of 90 serum samples, collected from wild boars between August and November 2023, revealed a high seroprevalence of antibodies against Aujeszky's disease virus in the Pomoravlje and Šumadija regions. In the case of ASF, the results showed higher seroprevalence in Pomoravlje, while wild boars from Šumadija tested negative for serum antibodies against the virus. Molecular analyses of organs from trapped wild boars did not confirm the presence of Aujeszky's disease or ASF viruses. These findings provide updated data on Aujeszky's disease and ASF in central Serbia and contribute to national surveillance and control strategies. They also indicate the ongoing circulation of these viruses in wild boar populations and the potential risk to domestic pigs. Therefore, continuous monitoring of wild boars is essential for early detection and effective prevention of disease spread, enabling timely interventions to protect domestic swine populations.

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Authors' contributions

AK: writing, original draft preparation and interpretation of results (together with SR), AK, DM and ĐI: Sample collection, serological and molecular analysis (together with BD3), AK, SN, VI, and BD2: Study design, RS: Conceptualisation, supervision, and writing-review and editing.

Competing interests


The authors declare that they have no competing interests.

Ethical statement

Hunters held valid licenses, and culling was carried out in accordance with applicable legal regulations and hunting rules during the regular hunting season (November 2022–November 2023). Humane euthanasia was not required, as the animals had already been killed prior to sampling. Samples were collected within the framework of official surveillance for classical swine fever and African swine fever, in compliance with Serbian legislation, and animals were not hunted specifically for research purposes.

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AUJECKIJEVA BOLEST I AFRIČKA KUGA SVINJA NA PODRUČJU POMORAVSKOG I ŠUMADIJSKOG OKRUGA

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Kratak sadržaj

Divlje svinje predstavljaju važne rezervoare virusa Aujeckijeve bolesti i afričke svinjske kuge (ASF), što ih čini ključnim faktorom u širenju ovih bolesti na populaciju domaćih svinja. Jedan od najčešćih puteva prenosa virusa među divljim svinjama jeste seksualni kontakt tokom parenja, dok migracije i interakcije sa ljudskim aktivnostima dodatno doprinose širenju infekcija. Seroprevalencija Aujeckijeve bolesti varira širom Evrope, u rasponu od 0% do preko 50%, zavisno od regiona. U Srbiji je prvi slučaj ASF kod domaćih svinja potvrđen 2019. godine, a kod divljih svinja 2020. godine u pograničnim oblastima. Cilj ovog istraživanja bio je da se utvrdi prisutnost virusa Aujeckijeve bolesti i ASF u populaciji divljih svinja u centralnoj Srbiji, tačnije u epizootiološkim jedinicama Pomoravlje i Šumadija. Istraživanje je sprovedeno primenom molekularnih i seroloških metoda radi detekcije virusa i specifičnih antitela. Takođe je analizirana prostorna i vremenska distribucija pojave ovih bolesti na posmatranom području. Za potrebe ovog istraživanja analizirani su uzorci divljih svinja različitog pola i starosti, prikupljeni tokom lovne sezone od avgusta do novembra 2023. godine na teritoriji Pomoravskog i Šumadijskog okruga. Ukupno je ispitano 90 uzoraka, pri čemu su za serološke analize korišćeni krvni serumi, a za molekularne analize organi (slezina i bubreg) istih životinja. Serološka analiza ispitanih uzoraka pokazala je visoku koncentraciju antitela na Aujeckijevu bolest kod divljih svinja u Pomoravlju i Šumadiji. Kada je u pitanju ASF, pozitivni serološki nalazi su zabeleženi samo u Pomoravlju, dok su svi uzorci iz Šumadije bili negativni. Na osnovu ukupnog broja ispitanih uzoraka (n=90), seroprevalenca Aujeckijeve bolesti iznosila je 26,7%, dok je seroprevalenca ASF iznosila 3,3%. Molekularne analize organa divljih svinja nisu potvrdile prisustvo genetskog materijala virusa ni Aujeckijeve bolesti, ni afričke kuge svinja. Serološki nalazi ukazuju na kontinuiranu cirkulaciju ovih virusa u populaciji divljih svinja i potencijalni rizik za domaće svinje. Stoga je kontinuirani monitoring od ključnog značaja za rano otkrivanje i efikasnu prevenciju bolesti.

Ključne reči: afrička kuga, Aujecki, divlje svinje, Pomoravlje, Šumadija