



UNVEILING THE PATHOGENIC LANDSCAPE: A STUDY ON VIRAL DISEASES IN THE WILD BOARS OF NORTHEAST ROMANIA

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Abstract: This study delves into the prevalence and distribution of viral infectious diseases among wild boars inhabiting northeast Romania. The investigated pathogens include African Swine Fever (ASF), Classical Swine Fever (CSF), Transmissible Gastroenteritis Virus (TGEV), Porcine Respiratory Coronavirus (PRCV), and Aujeszky's Disease (AD). Notably, ASF first surfaced in a backyard holding in Satu-Mare County in July 2017, with persistent outbreaks occurring subsequently, as we are still discovering new cases almost every month. In 2002, CSF outbreaks among domestic pigs prompted strict vaccination prohibitions, placing a spotlight on the region's vulnerability to disease transmission. In October 2007, Romania was declared CSF-free. Iași county is part of the "buffer belt" that consists of 10 km along Romania's border with the neighboring countries of Moldova and Ukraine (EU Commission Decision 2008/855), which means that wild boars are still part of the seroprevalence program for CSF. While PRCV and TGEV are prevalent in domestic pig populations globally, their presence and impact on wild boar populations remain understudied. Recent reports of AD outbreaks in European countries (France and the Czech Republic) raise concerns regarding cross-species transmission and the potential threat posed to wild and domestic pig populations, also to hunting dogs. To comprehensively assess the viral landscape, we conducted serological and molecular assays on samples collected from 80 wild boars. We investigated the presence of the antibodies that were specific to all the aforementioned viruses, the positivity rate being above 0% for two assays: ASF, with 1.25%, and AD with 32.5%. We also looked for the antigens of two of the diseases, ASF and CSF, both with a 0% positivity rate. We also reviewed the existing literature. By elucidating the spectrum of viral strains circulating among wild boars in northeast Romania, this study offers important insights into disease dynamics and potential risks to animal health.

• Introduction

Geographically, Romania is situated in Eastern Europe, bordered by countries such as Hungary, Ukraine, Bulgaria, and Serbia. The country boasts diverse wildlife species, and its biodiversity is notably rich. The wild boar (*Sus scrofa ferus*) holds significant importance as a big-game species in Romania, with an annual hunting bag averaging around 390 pigs last year in our county (Iași) alone. Their presence still raises concerns about the potential role of wild boars as reservoirs for infectious diseases (other than ASF, which is already present) that could end up affecting domestic pigs, but also, when it comes to Aujeszky's disease, cats and dogs.

• Material and method

The samples were collected from animals included in the national surveillance program, according to order 35/2016 and subsequent additions. Thoracic liquid samples were collected from 80 shot wild boars throughout 29 of the 55 hunting areas in Iași county (Fig. 1.). The samples were selected from the 2021/2022 hunting season, the first samples being collected in January 2022 and the last in September 2022.

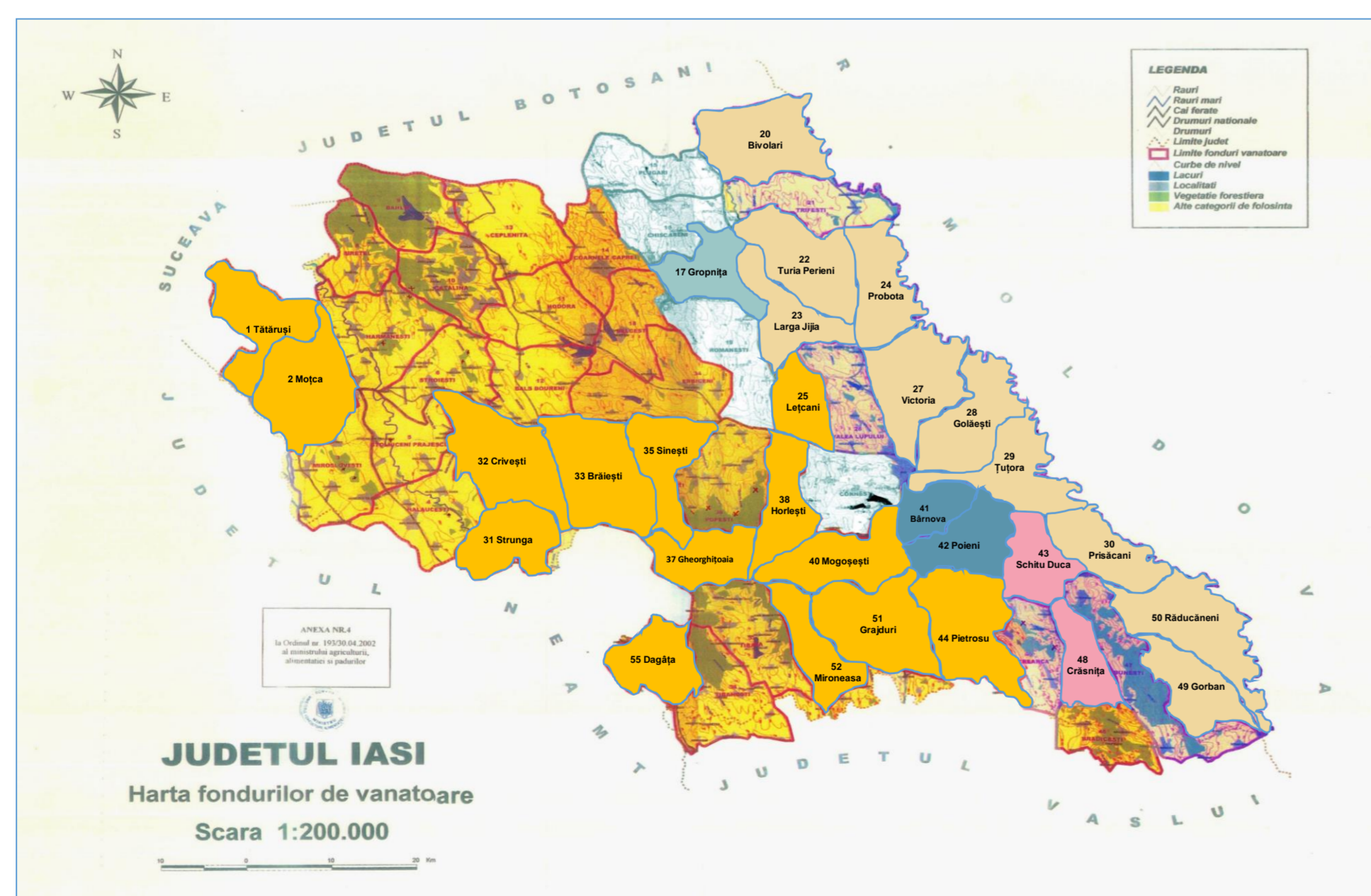


Fig. 1. - The cynegetic map of Iași county's hunting funds with the hunting funds that the samples have been collected from being highlighted; Source: Ministry of Agriculture, Food and Forests Order no. 193/2002 on updating the delimitation of Romania's cynegetic fund in hunting funds.

Molecular investigations against several viral diseases

1. Real-time PCR assay for detecting the ASFV
2. Real-time RT-PCR assay for detecting the CSFV

Serological investigations against several viral diseases

1. Enzyme immunoassay for the detection of antibodies against ASFV
2. Enzyme immunoassay for the detection of antibodies against CSFV
3. Enzyme immunoassay for the detection of antibodies against PRCV/TGEV
4. Enzyme immunoassay for the detection of antibodies against Pseudorabies Virus (PRV)/Aujeszky's Disease (ADV)

• Results and discussions

The results of the molecular assays were negative for both ASFV and CSFV, for all 80 wild boars that were considered for this study. However, the results of our serological endeavor were rather interesting, as can be seen in Table 1. Following the LSVSA's protocols, we sent the sample that was positive for the presence of antibodies anti-ASFV to IDSA, where the detection of anti-African swine fever virus antibodies by immunoperoxidase assay was conducted, in order to confirm our positive result. The IDSA result came out to be positive as well. So, we had a 1.25% (1 of 80, one from 41 Bârnova hunting fund) positivity rate. We were mostly surprised by the results concerning the presence of Aujeszky's disease antibodies in the samples. Having a positive result in 32.5% (26 of 80) of the samples was incredibly interesting.

Table 2
 Results of the enzymatic immunoassays considered in this study

ELISA assay antibodies against	Positive results
ASFV	1
CSFV	0
PRCV	0
TGEV	0
ADV	26

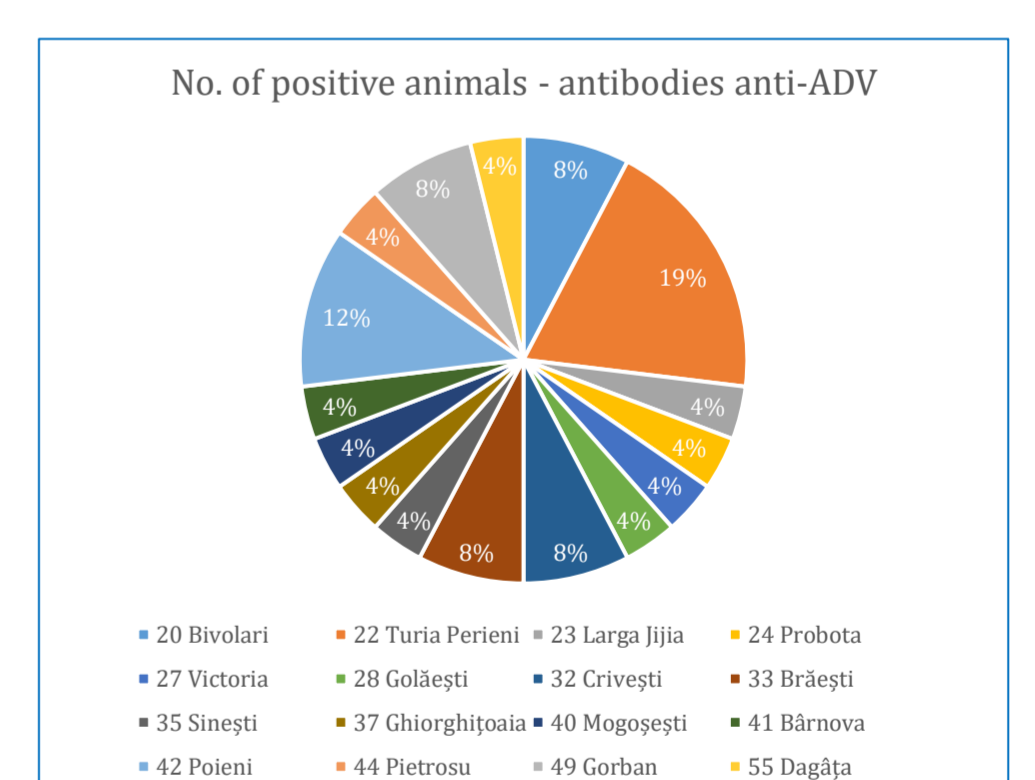


Fig. 2. - Chart depicting the distribution of positive results as a percentage based on the hunting funds of origin for the sampled animals.

We tried to identify if there was a connection between the geographical location of the hunting fund and the positive results for anti-ADV antibodies (Fig. 2.). Judging by the distribution of positive results as a percentage based on the hunting funds of origin for the sampled animals, the highest percentage of positive results was in 22 Turia Perieni, with 19,23% (5 of 26), closely followed by 42 Poieni, with 11,54% (3 of 26).

• Conclusions

In conclusion, while ASFV and CSFV were not detected in the sampled wild boars, the presence of anti-ASFV and especially anti-ADV antibodies highlights the importance of ongoing surveillance and management efforts to monitor and control infectious diseases in wild boar populations. Further research, including viral isolation in cell culture and rabbit inoculation for ADV detection may be needed in order to understand the implications of these findings for wildlife and domestic animal health.