

ABSTRACT

Key words: rabies, avian influenza, African swine fever, domestic and wild animals

The doctoral thesis entitled "***Research on the epidemiological and zoonotic impact associated with certain hunting species in the North-Eastern region of Romania***" comprises 200 pages and, in accordance with current academic regulations, is structured into two main parts. The first part, entitled "**The Current State of Knowledge**", to which are added: the table of contents, the introduction, and the list of abbreviations, comprises **54 pages (27%)**, while the second part, "**Personal Contributions**", to which are added: the summary, the related bibliography, and the appendices - the list of figures, tables, and the list of published works - comprises **146 pages (73%)**.

The first part, "**Current State of Knowledge**" is divided into three chapters and provides a synthesis of the specialized literature concerning the bioecology of certain wild mammals and birds of hunting interest (roe deer, wild boar, European hare, red fox, wild birds) in the North-Eastern region of Romania. It also addresses the most significant etiopathological entities affecting game species involved in the transmission of diseases to domestic animals (rabies, avian influenza, and African swine fever), as well as the updated surveillance and control measures for these major diseases, in line with both national and international legislation. A total of 271 bibliographic sources were consulted for this section.

The second part, "**Personal Contributions**" is structured into six chapters, each detailing the results obtained during the doctoral research period. The final chapter presents a synthesis of the main conclusions drawn from the conducted studies.

Chapter IV outlines the "***Purpose, objectives of the doctoral thesis and institutional framework***," briefly describing the scientific context in which the research topic was chosen, as well as the specific objectives pursued throughout the study. The thematic justification stems from the epidemiological context of North-Eastern Romania, where three major pathological entities, avian influenza, rabies, and African swine fever, have evolved concurrently, significantly impacting both wildlife and domestic animal populations from a veterinary public health perspective, and in certain cases, from a zoonotic standpoint.

The simultaneous propagation of these diseases is not an isolated phenomenon, as the region's specific geographical, ecological, and socio-economic factors create a complex epidemiological environment. The presence of migratory corridors, high densities of wild animal populations, cross-border movement, and traditional animal husbandry practices contribute to a favorable setting for the transmission, maintenance, and re-emergence of pathogens with epizootic and zoonotic potential.

The main objective of the thesis was to conduct an in-depth analysis of this regional epidemiological reality, with the aim of providing relevant data to improve surveillance, control, and prevention strategies adapted to local specificities. The present study proposes a multidimensional approach to assessing the epidemiological risks posed by the concurrent evolution of the three major diseases in North-Eastern Romania. The key objectives included the seroepidemiological assessment of these infectious diseases in both wild and domestic animal populations, to determine their prevalence and geographical distribution.

Another important objective was the characterization of the anatomical and clinical lesions associated with these infections, and the confirmation of etiological agents through serological methods (ELISA, direct immunofluorescence-IFD, hemagglutination inhibition assay-IHA), molecular biology techniques (real-time PCR and real-time RT-PCR), and bioassays, correlating clinical data with epizootiological findings.

This stage also aimed to conduct a comparative analysis of how these pathological entities manifest in game fauna (foxes, wild boars, migratory birds) versus domestic animals. Finally, the research sought to develop epizootiological management recommendations to supplement current strategies for disease surveillance, control, and eradication, tailored to the ecological and administrative specificities of the region.

Chapter V, entitled “*Seroepidemiological research on the presence and prevalence of rabies, avian influenza and african swine fever in some wild and domestic animals in North-Eastern Romania*”, presents the results of seroepidemiological studies conducted to determine the prevalence of rabies, avian influenza, and african swine fever in both wild and domestic animal populations.

Subchapter 5.1 presents and discusses data collected between 2018 and 2024 on rabies antibody prevalence in fox populations from the counties of Botoșani, Neamț, Suceava, Iași, and Vaslui. The epidemiological analysis revealed that the efficacy of oral rabies vaccination campaigns varied between counties and fluctuated from year to year, reflecting the influence of ecological, logistical, and institutional factors. The year 2021 marked a peak in antibody prevalence across most counties, likely due to efficient vaccine coverage during the preceding year.

In Botoșani County, the most stable and elevated antibody prevalence was recorded throughout the entire study period, with a peak of 61.11% in 2021 and values exceeding 47% in 2022–2023. These results suggest a well-implemented vaccination program and effective post-vaccination monitoring. In Neamț County, an upward trend was observed, peaking in 2021 (60.55%) and maintaining stable values between 31–41% during 2022–2024, indicating the overall success of the vaccination efforts and a satisfactory immune response in the fox population.

In Vaslui County, significant fluctuations were recorded, ranging from highs of 64.81% (2018) and 73% (2021) to lows of 7.14% (2019) and 14.29% (2022), underscoring the need to strengthen the continuity of the vaccination program. In Iași County, a fluctuating trend was noted, with a peak in 2020 (44.3%) followed by sharp declines in 2022 (3.23%) and 2024 (2.25%), highlighting the need to enhance post-vaccination surveillance and reassess vaccination campaign management.

In Suceava County, no rabies antibodies were detected throughout the study period, with the sole exception of 2023 (1.06%), pointing to a significant inefficacy of oral vaccination in that area. Several interrelated factors may account for the absence of vaccine-induced rabies antibodies in foxes from Suceava, including: ineffective bait distribution due to mountainous terrain and dense vegetation; unfavorable climatic conditions that may degrade the vaccine; low bait uptake by foxes, possibly due to competition with other species; poor immune response resulting from the physiological status of the animals; insufficient post-vaccination monitoring; or sample collection occurring too soon after vaccination.

Data on tetracycline prevalence clearly demonstrate the effectiveness of oral vaccine bait uptake in foxes. However, a clear distinction must be made between vaccine administration and actual immunization. Serological evaluation results show that although the prevalence of the tetracycline biomarker is generally high, it does not always correlate with sufficient and protective levels of rabies antibodies.

The high antibody prevalence recorded in most counties and across several years confirms bait ingestion and, by extension, the significant contribution of these campaigns to rabies control and eradication efforts. Continuous monitoring of this biological marker remains essential for adjusting vaccination strategies to the ecological and behavioral specificities of each region.

Subchapter 5.2 presents the results obtained from the detection of avian influenza (AI) antibodies in migratory wild birds (ducks, swans, storks) and domestic birds (ducks, geese, quails). The circulation of avian influenza viruses in Northeastern Romania during the 2018–2024 period was primarily associated with wild birds, particularly wild ducks and swans. Antibodies were consistently detected in this group, whereas positive cases among domestic birds were sporadic or absent.

The highest seroprevalence levels in swans were recorded in Suceava and Neamț counties, especially in the years 2020 and 2023. These peaks may be correlated with the introduction of aggressive HPAI strains (e.g., H5N1), which were confirmed in the same period in Central and Eastern Europe by EFSA and WOA. In Iași County, seropositive cases in wild birds followed a sinusoidal pattern, with detection points in 2018, 2019, 2021, and 2023. In domestic birds, the seroprevalence peak was observed only in 2018, suggesting improvements in biosecurity and reduced exposure to natural vectors.

In Botoșani County, no positive cases were recorded among domestic birds, but results from wild avifauna—particularly ducks and swans—indicate intermittent viral exposure, underlining the need for continued surveillance. In Vaslui County, no positive cases were detected until 2023; however, the confirmation of a single seropositive swan in 2024 underscores the importance of maintaining epidemiological vigilance, even in areas with a historically negative status.

Comparative analysis by bird type revealed a statistically significant difference in seroprevalence between domestic and wild species, with wild birds exhibiting a more than ninefold higher probability of infection. The consistent seroprevalence observed in wild birds in certain counties supports the existence of a natural viral reservoir with potential for seasonal

reactivation, positioning the Northeastern region of Romania as a sentinel ecosystem for the early detection of HPAI strains.

The absence of AI-specific antibodies in domestic birds, despite confirmed viral circulation in wild populations, indicates the effectiveness of biosecurity measures. However, it also highlights the need to correlate serological surveillance data with viral isolation and ecological findings. Continued seroepidemiological surveillance in Northeastern Romania remains essential—particularly during migratory periods—for the prevention and control of avian influenza and for integration into a European early warning system.

Subchapter 5.3 presents data on the detection of african swine fever (ASF) antibodies in wild boars and domestic pigs, collected between 2018 and 2024 from the counties of Botoșani, Suceava, Neamț, Iași, and Vaslui. Over the course of the seven-year period, a total of 17,622 samples were collected and tested for ASF-specific antibodies. The epidemiological situation varied periodically and by county.

In Iași County, 4110 samples were analyzed, of which 354 (8,6%) tested positive. In Botoșani County, 365 out of 2133 samples (17,1%) were positive. In Suceava County, 288 out of 3113 samples (9.3%) tested positive. In Neamț County, only 33 out of 3883 samples (0.8%) showed seropositivity. In Vaslui County, 182 out of 4,383 samples collected from wild boars (4,2%) were seropositive for ASF.

The ASF seroprevalence analysis in wild boars between 2018 and 2024 highlights a heterogeneous epidemiological pattern across Northeastern Romania, with significant differences between counties. During this period, no ASF antibodies were detected in domestic pigs, with the exception of a single outbreak reported in Vaslui County in 2021, when six seropositive cases were confirmed.

Annual seroepidemiological analysis by county revealed the following: Botoșani County consistently reported the highest ASF seroprevalence among wild boars, with a peak of 31.5% in 2021, and persistently high values throughout the 2018-2024 period. In Suceava County, the maximum seroprevalence was recorded in 2019 (28.57%), followed by a gradual decline, suggesting early epidemic activity potentially followed by stabilization. Vaslui County experienced a notable increase in seroprevalence in 2022 (15.76%) and 2023 (14.55%), after lower levels in previous years, which may reflect a recent resurgence in viral circulation. In Iași County, a fluctuating trend was observed, with a peak of 22.73% in 2021, followed by substantial declines in 2022 and 2024, suggesting intermittent virus circulation. Neamț County consistently reported the lowest seroprevalence, never exceeding 3.5% in any year, possibly reflecting limited viral activity or lower wild boar population density.

The year 2021 marked the period with the highest overall seroprevalence at regional level, indicating a possible intensification of ASF transmission in wildlife during that time. The variable dynamics of ASF antibody prevalence reflect the fluctuating epidemiological character of the infection, potentially influenced by factors such as wild boar density and the effectiveness of veterinary surveillance and management strategies.

Statistical analysis using Fisher's exact test confirmed that inter-county differences observed in certain years were not due to random variation, but rather reflect true disparities in disease distribution.

Chapter VI, entitled “*Anatomoclinical research on the diagnosis of rabies, avian influenza and african swine fever in some wild and domestic animals in North-Eastern Romania*”, presents the anatomical and clinical aspects observed in disease outbreaks occurring between 2018 and 2024. The clinical signs and lesion patterns associated with rabies, avian influenza, and African swine fever (ASF) are discussed in three subchapters.

In **subchapter 6.1**, several anatomoclinical aspects observed in foxes and cattle are presented. The choice to discuss the anatomoclinical picture of rabies exclusively in foxes and cattle is based on epidemiological considerations and practical relevance in the context of zoonotic disease surveillance. The fox is the main sylvatic reservoir of the rabies virus in Europe, playing an essential role in maintaining the epizootic chain and transmitting the infection to other species, including domestic animals and humans. Its deviant behavior in the clinical stages of the disease is an important early indicator for identifying virus circulation in the ecosystem.

As for cattle, they are frequently involved in clinical rabies episodes in rural areas, being large species of economic interest and in direct contact with the human population. Although they are not primary hosts, they can present dramatic clinical forms, with obvious neurological conditions. Therefore, focusing on these two species meets a practical need for early recognition of rabies in the context of active and passive surveillance, as well as for preventing the zoonotic risk among veterinary personnel and the general population.

Between 2018 and 2024, by participating in hunting campaigns carried out to control vector populations and regulated through active rabies surveillance programs, an attempt was made to assess the symptomatology of foxes in their natural environment, but the observations were limited and inconclusive. In foxes, observations highlighted the specific behavior of a tame animal, lacking inhibition. The animal had a fixed stare, unequal pupils, and a facial expression suggestive of rage.

In cattle, examination was carried out in an advanced stage of the disease, when most neurological symptoms were already installed: head or horn striking, hypersalivation, frequent and guttural mooing accompanied by extreme agitation, slowing or loss of reflexes, with the appearance of tongue protrusion and ptosis accompanied by dysphagia, and the onset of posterior limb paralysis, which led to permanent recumbency.

No anatomopathological examination was performed since the symptoms were suggestive of rabies, and the definitive diagnosis was established and confirmed by **direct immunofluorescence** and **RT-PCR**. Moreover, macroscopic lesions in rabies are often nonspecific in cattle, and the collection of brain tissue for virological confirmation is considered sufficient and a priority in the context of a rapidly progressing zoonotic disease.

In **subchapter 6.2**, the results obtained from the evaluation of 28 swans confirmed with avian influenza are presented and discussed, which manifested with variable frequency the following symptoms: torticollis, balance disorders with abnormal movements in water, marked lethargy, and poor head positioning, indicating neurological involvement. Due to the peracute evolution of the disease, specific macroscopic lesions were rare, but in some cases, cerebral and pulmonary congestion, diffuse hemorrhages in muscles and serosal surfaces, hemorrhagic enteritis, as well as hepatic and splenic congestion were identified. The sensitivity of swans to

H5N1 infection and their ability to shed the virus into the environment until death makes them an important bioindicator in the epidemiological surveillance of avian influenza. Early clinical observations in this species can decisively contribute to the activation of control and prevention measures in aquatic ecosystems.

Subchapter 6.3, aspects related to symptomatology and the lesion profile of African swine fever in wild boars and domestic pigs are presented and discussed. During our study (2018–2024), a significant number of wild boars from the areas of Iași, Botoșani, Suceava, Neamț, and Vaslui counties were examined. Clinical observations in wild boars were difficult to obtain, as symptoms were nonspecific and short-lasting, most likely varying depending on the clinical stage of evolution and the virulence of the viral strain. Lesions in domestic pigs were, in most cases, systemic: severe splenomegaly, systemic hemorrhages (lymph nodes, kidneys, urinary bladder, pericardium), pulmonary congestion and edema, as well as hemorrhages in the gastric mucosa. These lesions, which correspond in most cases to the acute phase of the disease, were essential for rapidly raising the suspicion of ASF and initiating quarantine measures, even if the full lesion picture was not always observed, this being consistent with the disease's stage of evolution and with some post-mortem factors.

During the same period, domestic pigs from commercial and non-commercial holdings (households) in Northeastern Romania, affected by African swine fever, were also examined. In the examined animals, the disease evolved with varied clinical forms, from peracute (sudden death) to chronic forms with mild signs. Post-mortem examinations were carried out on affected farms, in accordance with biosecurity protocols. As in wild boars, necropsy lesions in domestic pigs were identified, including congestive splenomegaly, renal petechiae, nodular granulomatous hepatitis, hemorrhagic lymphadenopathy and severe pulmonary lesions. Cyanosis of the extremities, hemorrhagic enteritis and abortions in pregnant females completed the clinical and pathological picture. The high prevalence of vascular lesions reflects the systemic viral pathogenesis of ASF, and their identification has major diagnostic value in active outbreaks. The anatomoclinical data obtained through necropsy examinations significantly complete the epidemiological picture of outbreaks, offering essential information for active surveillance, disease control, and on-site confirmation of ASF diagnosis, especially in areas with mixed virus circulation between wildlife and non-commercial farms.

Chapter VII, entitled *"Confirmation of the diagnosis of the three major diseases identified in some wild and domestic animals in North-Eastern Romania"*, includes data and their analysis, highlighting the importance of a rapid and standardized diagnosis for diseases such as rabies, avian influenza, and African swine fever, which circulate in game areas. The diagnosis of these infectious diseases constitutes an essential component within strategic mechanisms for epidemiological surveillance, prevention, and control, with major impact on both animal health and public health. The data were structured and analyzed in three subchapters.

Subchapter 7.1 presents the methods and discussion of the results obtained from the testing of brain samples collected from 3,612 foxes and 27 cattle, using the direct immunofluorescence technique (IFD) and 43 diagnostic confirmations through mouse

inoculation bioassay. The samples were analyzed in the Sanitary-Veterinary Laboratories of the counties included in the study during the period 2018–2024.

Since there are no pathognomonic lesions specific to rabies, establishing the diagnosis is only possible within a specialized laboratory. The analysis of rabies cases confirmed by IFD and bioassay in the period 2018–2024 indicates significant differences between the monitored counties. The annual evolution of positive cases shows an ascending trend in Botoșani, Iași, and Suceava counties, with a clear peak in 2023, which may suggest an intensification of virus circulation or increased surveillance efficiency. In Neamț, the number of positive cases recorded a descending slope, while in Vaslui, they remained at a low but constant level.

The distribution by species (domestic and wild) indicated a predominance of cases in wild fauna in Botoșani and Neamț, while Suceava and Vaslui had a higher share of cases in domestic animals, an aspect that highlights the epidemiological risk. The high confirmation rate through bioassay in some counties confirms the complementary role of this method in strengthening the diagnosis. However, the data cannot be correlated with those from testing by immunofluorescence, due to the fact that the bioassay is not used simultaneously with IFD, but only in cases where it provides inconclusive or negative results, even in the presence of clear clinical suspicions. This practice limits the possibility of making a direct comparison between the two methods in terms of their sensitivity and specificity.

Subchapter 7.2- in this subchapter presents and discusses the results regarding the role and accuracy of the Real-Time PCR method in confirming the diagnosis of avian influenza in swans. The identification of the avian influenza virus genome by PCR techniques complements classical virus isolation methods in establishing a definitive diagnosis and consists in identifying the viral RNA in the acute phase of infection by amplifying the matrix protein gene. Between 2018 and 2024, 197 tissue samples collected from swans located in Northeastern Romania were tested. In total, 197 swans were tested, of which 119 (60.40%) were positive for the H5N1 virus genome. This percentage indicates significant virus circulation in certain periods and areas. The temporal distribution shows a notable increase in virus activity in 2022 and 2023, when most testing was conducted, and the highest number of positive cases was recorded. In 2022, 42 swans were examined, of which 34 were confirmed by RT-PCR (Neamț), and in 2023, 148 swans were tested, with 75 confirmed in the counties of Neamț, Botoșani, and Suceava. From a geographical perspective, Neamț County was the most consistent in RT-PCR surveillance, with testing conducted each year between 2018 and 2023. This consistency in sampling and diagnosis enabled the early identification of outbreaks and supported an appropriate epidemiological response. Botoșani County, although present only in 2023, recorded the highest positivity rate for the 19 swans tested. In Suceava, viral activity was confirmed in 2023 and 2024, with 47 and 10 positive RT-PCR cases, respectively, out of 110 tested specimens. In contrast, in the counties of Iași and Vaslui, no suspicions of avian influenza in swans were reported, or they were very rare (only one case in Vaslui). The limitations of this dataset are evident: testing was not performed systematically in every year or county, and some areas (e.g., Iași, Vaslui) are underrepresented. Nevertheless, the results support the integration of swans as ecological bioindicators in active avian influenza surveillance programs.

Subchapter 7.3 - in this subchapter presents the results of tests performed using Real-Time PCR to confirm African swine fever in wild boars and domestic pigs from North-Eastern Romania. The results highlight a regionally variable epidemiological dynamic, supported both by the accuracy of the RT-PCR method in confirming viral infection and by the peculiarities of virus circulation in the territory. The epidemiological analysis of the evolution of positive ASF cases in North-Eastern Romania (2019–2023) highlights a regional dynamic of cases, most of which originated from wild boars. Samples from domestic pigs were significantly fewer, with sporadic occurrence of active outbreaks. Regarding the trend in the evolution of positive cases, a progressive and sustained increase is observed for the counties of Iași, Botoșani, and Vaslui. In Iași County, the upward trend was pronounced, from 2 cases in 2019 to 327 cases in 2023, suggesting persistent transmission in wild fauna, probably maintained by wild boar populations and favorable environmental conditions (forested habitat, border area). Like wise, in Botoșani County, positive cases followed an ascending pattern, reaching 212 cases in 2023. A similar situation was observed in Vaslui County, where a clear increase was noted, from 9 cases in 2019 to 141 RT-PCR confirmed cases in 2023. In Suceava County, after a peak of 10 cases in 2020, the evolution fluctuated: 37 cases in 2021, 22 cases in 2022, and 34 cases in 2024. The same low prevalence was recorded in Neamț County, where 2 positive cases were reported in 2019, rising to 20 in 2021, following an ascending trend to 30 cases in 2022 and 29 in 2023. This upward trajectory of ASF cases confirmed by RT-PCR is most likely influenced by the expansion of wild boar habitats, implicitly linked to cross-border mobility. The epidemiological profile of counties with lower prevalence of positive cases, such as Suceava and Neamț, indicates a latent but controlled viral presence in the region, suggesting the effectiveness of biosecurity measures and rapid intervention in isolated outbreaks.

Chapter VIII, entitled "*Measures regarding the strategy for surveillance, control and eradication of rabies, avian influenza and african swine fever in North-East Romania*", is structured into three subchapters.

Subchapter 8.1 - in this subchapter presents the surveillance, control, and eradication strategies for rabies in foxes and domestic animals, as legislated and implemented in Romania, alongside related opinions and recommendations. The European and national legislative framework regarding rabies is well established; however, its application requires more effective adaptation to local realities and the specific characteristics of wildlife, particularly in areas with difficult access. Oral vaccination of foxes remains the main pillar in the control of sylvatic rabies, but its efficacy must be systematically monitored through increased frequency of post-vaccination evaluations and seroprevalence analysis. Passive surveillance depends largely on the involvement of citizens and private veterinary practitioners, justifying the introduction of incentive mechanisms and accessible digital platforms for the rapid reporting of suspicions. Interinstitutional collaboration remains a critical condition for successful rabies control, and the integration of the veterinarian into the post-exposure emergency response circuit, alongside human physicians, is vital for timely and effective interventions and for avoiding excessive or delayed treatments.

Subchapter 8.2 - in this section presents the strategies for surveillance, control, and eradication of avian influenza in Romania, along with certain opinions and recommendations

aimed at improving monitoring, in light of the study conducted between 2018 and 2024. The surveillance of avian influenza in Romania is well structured legislatively, but practical implementation requires strengthening in the field, especially in ecologically vulnerable areas frequently traversed by migratory birds. Active surveillance of wild birds remains insufficiently calibrated to field conditions, necessitating the expansion of collaboration with local institutions and informal networks for the prompt reporting of suspected cases. Commercial farms require stricter and more frequent audits regarding biosecurity, as these continue to be involved in the emergence of outbreaks despite the implementation of standard measures. The lack of effective feedback to farmers reduces community participation in the surveillance system, highlighting the need for direct and transparent communication tools to foster local awareness and responsibility.

Subchapter 8.3 - in this section presents the surveillance, control, and eradication strategies for African swine fever (ASF) in wild boars and domestic pigs. Combined surveillance (active and passive) is essential for the early detection and control of ASF outbreaks, particularly given the virus's circulation among wild boars, which act as a persistent natural reservoir. Reducing wild boar population density below the critical threshold and the prompt management of carcasses are fundamental strategic measures, but they remain insufficiently implemented in practice due to difficult terrain access and a lack of technological resources. Biosecurity measures in commercial farms and traditional households must be adapted and applied differentially, depending on local risk and type of livestock operation, to prevent the introduction and spread of the virus. The success of the eradication strategy depends on interinstitutional collaboration and involvement of rural communities, requiring investments in veterinary education, incentives for hunters, and the integration of modern technologies for the detection and neutralization of infection sources.

Chapter IX- Final Conclusion, presents the final conclusions, formulated as a synthesis of the most important results obtained throughout the study. In total, 24 final conclusions were stated.