

ABSTRACT

This doctoral thesis, entitled “*Molecular epidemiological study of selected viral diseases in dogs from public shelters in North-Eastern Romania*”, addresses a topical issue of major importance for veterinary public health and animal welfare, namely the prevalence and impact of viral infections in the canine population in public shelters. In the global context of increasing numbers of stray dogs and their poor management in many regions, shelters are becoming potential hotbeds for the rapid spread of pathogens, with severe implications for the health of the animals in shelters, the environment and, by extension, human health. The peri-urban and rural areas of north-eastern Romania, characterized by a significant number of stray dogs and heterogeneous kennel management practices, were chosen as the study area, highlighting the need for a thorough understanding of the epidemiological dynamics of viral diseases.

The main objective of this thesis was to evaluate the extent and clinical impact of two major viral diseases - canine parvovirus (CPV) and canine distemper (CDV) - in canine populations in public shelters in northeastern Romania, to identify and genetically characterize the circulating viral strains and to propose an integrated framework for the preventive management and control of these diseases. By identifying risk factors and by analyzing the clinical response to treatment, the work aimed to contribute to the optimization of biosecurity and veterinary public health strategies.

The thesis is structured in two parts. In the first part, general aspects of the most common viral diseases found in shelter dogs are presented, including current data from the specialized literature, clinical, pathophysiological and diagnostic aspects, as well as the legislative and veterinary sanitary context in Romania. This section is intended to provide a scientific basis for the practical directions addressed later in the paper. Viral diseases represent a major veterinary public health problem in canine shelters, not only because of the high transmission rate, but also because of the difficulty of early diagnosis and the limitations in applying standardized treatments. These diseases can lead to significant loss of animal lives, considerable veterinary expenditure, stress for the staff involved and even compromise the reputation of the managing institutions. In addition, infected animals can be a continuing source of pathogen spread in the community, especially when they are adopted before the end of quarantine or treatment periods. For this reason, a good understanding of these diseases is essential for any sustainable strategy to control the stray canine population and protect community health.

The second part of the thesis is dedicated to our own research carried out in shelters in counties located in the north-eastern part of the country. This comprehensive approach included epidemiological, molecular sero-epidemiological and genotypic characterization studies of viral strains. Animals were selected on the basis of clinical manifestations suggestive of viral infections (digestive, respiratory or nervous syndromes). Clinical samples (feces, conjunctival, nasal, conjunctival secretions, whole blood and serum) were collected under strict hygienic conditions and transported to the laboratory for analysis. Diagnosis was established by a combination of methods:

- Rapid immunochromatographic tests: used for initial screening and rapid confirmation of the presence of viral antigens (CPV, CDV).
- ELISA (Enzyme-Linked Immunosorbent Immunosorbent Assay): For the detection of specific antibodies or antigens in serum, providing insight into immune status or active infection.
- Polymerase Chain Reaction (PCR): This advanced molecular method has been fundamental for the accurate detection and identification of viral DNA/RNA, including the differentiation of viral strains. Both conventional RT-PCR and Real-Time PCR were used for increased sensitivity and specificity.

- Genetic sequencing: for the identified viral strains, DNA sequencing allowed genotypic characterization, identification of viral variants, identification of relevant mutations and establishment of phylogenetic relationships with known strains globally. This provided essential information on viral evolution and potential implications for virulence or vaccine efficacy.

In addition to laboratory analysis, detailed clinical observations were made and epidemiological data were collected on the age of the animals, vaccination history (if available), maintenance conditions and general kennel management. The study confirmed an alarmingly high prevalence of viral infections in the analyzed shelters, especially canine parvovirus (CPV) and canine distemper (CDV), highlighting a complex epidemiological dynamic and a constant infectious pressure.

Canine parvovirus disease (CPV) was identified as the most prevalent viral disease in most of the shelters studied, with a particularly high incidence in puppies and young. Cases showed severe symptoms of hemorrhagic gastroenteritis, with mortality rates exceeding 90% in the absence of early intensive treatment. Even with supportive treatment, mortality was significant, reaching 80.55% of severe cases of VPC in one shelter (Bacău). The prevalence of CPV was significant in Iasi, Botoșani and Vaslui counties, reflecting an active viral circulation and an increased vulnerability of the unvaccinated or incompletely vaccinated canine population.

Canine distemper disease (CDV) was also present, predominantly affecting young dogs and manifesting in respiratory, digestive and neurological forms. Co-infections with CPV were frequent, exacerbating clinical severity and complicating therapeutic management. It has been confirmed that young canines represent the most vulnerable category, with extremely high morbidity and mortality rates. For example, in the Botoșani kennel, mortality among puppies reached around 90% in 2019, highlighting the devastating impact of these infections on juvenile populations in shelters.

Molecular analyses revealed genotypic and phenotypic diversity of circulating viral strains, suggesting possible implications for the efficacy of vaccines and treatments used. This genetic variability may at least partially explain vaccine failures reported in certain situations and the need to adapt immunization strategies.

The thesis makes significant contributions to the understanding of the epidemiology and management of viral diseases in dog shelters, providing a detailed perspective on the situation in northeastern Romania. Among the most important contributions are:

- *Mapping the prevalence and impact of viral disease:* This paper provides concrete and up-to-date data on the prevalence of CPV and CDV, highlighting the geographical areas at highest risk and the most affected animal categories. This mapping is essential for efficient resource allocation and the development of targeted intervention strategies.
- *Molecular characterization:* Through genotypic analysis of local viral strains, this thesis contributes to the global database on the evolution and diversity of canine viruses, crucial information for the development of more effective vaccines and improved diagnostic methods.
- *Proposing an operational model of integrated management:* based on biosecurity measures, adapted vaccination, active and passive surveillance, staff training and inter-institutional collaboration.

The tailored management plan model includes strict biosecurity protocols (quarantine measures for newly admitted animals, rigorous sanitation of premises and visitor control), tailored vaccination strategies (complete and timely vaccination of all animals in the shelters, with vaccination schedules adjusted to the local infectious pressure and the types of circulating strains), continuous monitoring (implementation of systematic testing of newly admitted animals and constant monitoring of viral circulation by molecular for early detection of outbreaks and rapid intervention), training and personal involvement through ongoing training of shelter staff on early recognition of symptoms, hygiene protocols and correct treatment, and a plea for increased inter-institutional collaboration (local

authorities, DSVSA, non-governmental organizations, veterinary faculties) to ensure coherent and effective management of the stray dog population and associated risks.

The results of this thesis demonstrate the urgency of adopting proactive and scientifically sound measures for the control of viral diseases in animal housing. By implementing the proposed management framework, morbidity and mortality can be significantly reduced, improving animal welfare and contributing to a safer environment for the community. This work serves as a valuable tool for decision makers in the development and implementation of public policies for the management of stray dogs, with the ultimate goal of sustainable and ethical management of these populations. Future research directions should focus on functional studies of local viral strains, long-term evaluation of the efficacy of proposed management protocols, and exploration of innovative diagnostic and prophylactic solutions.