PhD THESIS

Epidemiological study of rabies in foxes in Bistrița-Năsăud county and the effectiveness of bait vaccination

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ABSTRACT

Rabies is one of the most dangerous zoonoses that can be transmitted to humans, always ending in the death of affected individuals. The implementation of the oral rabies vaccination program for foxes aims to combat rabies in Romania by vaccinating foxes, controlling rabies among fox populations and eradicating the disease in the country. Thus, the rabies vaccination of the fox population with vaccine baits is carried out and the evolution of the rabies is monitored in relation to the evolution of the vaccine bait application program and the results obtained.

Therefore, the objectives of this paper were:

• evaluating the effectiveness of vaccine baits in both fox populations and other wildlife and domestic animals by:

• analysis of rabies cases diagnosed between 2000-2007, a period in which rabies vaccination was not performed;

• testing for rabies, by laboratory diagnostic methods, of all samples from domestic and wild animals, received from 2008 to 2017, during which the annual vaccination of the fox population with vaccine baits was performed.

This study was carried out within the Bistriţa-Năsăud Veterinary Sanitary and Food Safety Laboratory. For the years 2000-2014, the data were taken from the diagnostic registers within the Sanitary Veterinary and Food Safety Laboratory Bistriţa-Năsăud, and starting with 2015 all the samples of domestic and wild animals received in the laboratory were tested, both those with suspected rabies as well as foxes shot in order to assess the effectiveness of vaccination. Throughout the study, rabies-suspected foxes, those killed, those that came in contact with humans, all wild animals as well as domestic animals suspected of rabies were examined by the direct immunofluorescence test (DIF) and the inoculation test- intracerebral control of mice (in case of suspicion of negative DIF).

In the case of foxes shot to evaluate the effectiveness of vaccination, the brain, thoraco-abdominal fluid and mandible were taken. Initially, the virological examination was performed by the direct immunofluorescence test (DIF), the thoraco-abdominal fluid was examined by the enzyme-linked immunosorbent assay ELISA, and at the dental level the presence or absence of the tetracycline marker was determined by microscopic examination (UV).

The direct immunofluorescence test (DIF) was performed at the Bistriţa-Năsăud Veterinary Sanitary and Food Safety Laboratory, and the thoraco-abdominal and

mandible fluid samples were examined at IDSA Bucharest and at the Suceava Veterinary Sanitary and Food Safety Laboratory. .

This paper entitled "Epidemiological study of rabies in foxes in Bistrița-Năsăud county and the effectiveness of bait vaccination" is structured in two parts: the first part includes bibliographic research and is divided into four chapters, and the second part, which presents its own research, contains eight chapters. The first part (Literature review) presents information related to the epidemiology and pathogenesis of rabies, as well as methods of diagnosis and immunoprophylaxis. **The first chapter** presents data from the literature related to the history of the rabies virus, the etiology of the rabies virus, epidemiology, pathogenesis and clinical symptoms encountered in rabies, both in domestic and wild animals, as well as in bats and humans. Also in the first chapter is described the well-known morphopathological picture present in rabies. **The second chapter** describes the diagnostic methods used in rabies investigations, and **the third chapter** contains data from the literature on rabies immunoprophylaxis, as well as requirements for vaccines and biological rabies products. **The fourth chapter** presents the legislative programs regarding the surveillance, control and eradication of rabies in animals in Romania, the prophylactic rabies vaccination against foxes, as well as data on the oral rabies vaccine-LYSVULPEN.

The second part (Personal contribution) is structured in eight chapters and includes the purpose of this paper (**Chapter 5**), the materials and methods used in research (**Chapter 6**) and the study itself (**Chapters 7** to **10**). **Chapter 11** illustrates the conclusions and recommendations of the research, and the bibliographic references are presented in **Chapter 12**.

Chapter 7 presents the number of samples received and analyzed for rabies, within the Sanitary Veterinary and Food Safety Laboratory Bistrița-Năsăud, in the period 2000-2017. Thus, between the years 2000-2017, a number of 1838 samples, both wild and domestic animals, were received and analyzed within the Sanitary Veterinary and Food Safety Laboratory Bistrița-Năsăud. Out of the total number of samples analyzed in the direction of rabies, a number of 348 positive samples were diagnosed, between 2015-2017 there were no outbreaks of disease.

Regarding foxes, in the period 2000-2017 a number of 1567 foxes were received and analyzed. Both the foxes found dead, the foxes killed by dogs or killed in the household, and those shot as a result of the vaccination campaign were analyzed to determine the effectiveness of the vaccination. Thus, regarding the foxes found dead, in the period 2000-2007, 97 foxes were examined, of which 46 were positive, and in the period 2008-2017, 125 samples were analyzed, of which 54 were positive.

In the case of foxes killed in households, from 2000 to 2007 only two foxes were examined, both testing positive, and from 2008 to 2017, 86 samples were analyzed, of which 59 had a positive result.

Regarding the category of foxes killed by dogs, from 2008 to 2017, a number of 36 samples were analyzed, 25 being positive.

Within the Sanitary Veterinary and Food Safety Laboratory Bistrița-Năsăud, in 2007, 9 shot foxes were analyzed (other than those from the vaccination campaign), 4 tested positive, and from 2008 to 2017 were examined 69 of tests, 15 tested positive.

It is worth mentioning that, since 2015, there have been no cases of rabies in Bistrița-Năsăud County.

Regarding the foxes shot during the vaccination campaign, between 2008-2014, a number of 543 shot foxes were received in the laboratory, from which the brain was harvested for the direct immunofluorescence test, the mandible and the blood, all to determine the effectiveness of vaccination. Of the 543 foxes, 80 were positive.

The vaccination campaign for foxes with vaccine baits began in 2008. Thus, from 2008 to 2017, 233 cases of rabies were registered out of a total of 1459 fox samples received and analyzed within LSVSA Bistrița-Năsăud. There were both foxes shot during vaccination campaigns and foxes found dead, killed by dogs or killed in households. It is worth mentioning that, since 2015, no rabies-positive foxes have been diagnosed, which proves that vaccinating foxes has proven to be a solution in reducing the number of outbreaks of rabies in Bistrița-Năsăud County.

Most outbreaks of rabies have been confirmed in Sânmihaiul de Câmpie, 12 cases. Within the Rebra hunting ground, 12 outbreaks of disease were also confirmed. 11 positive cases of rabies were registered in Galați Bistrița, Chiochis and Bistrița.

Chapter 8 details the incidence of rabies in wildlife species other than foxes. Over a period of 17 years (2000-2017), the incidence of rabies among wild animals is quite low, which shows that the main rabies virus reservoir in the wild is the fox. Between 2007-2009, 4 wolf brain samples were tested, 3 samples tested positive, in 2006 and 2009, 2 cases of ferret rabies were diagnosed, and in 2008 a wild cat was positively diagnosed.

The epidemiological study of rabies in domestic animals is illustrated in **Chapter 9**. Thus, over a period of 17 years, from 2000 to 2017, 264 brain strains from domestic animals were tested, with a total of 56 outbreaks of rabies. As for the affected localities, in the period 2000-2017, rabies in domestic animals occurred in 25 localities, most cases being recorded in dogs, followed by cats. Between 2000 and

2014, a total of 10 outbreaks of rabies were diagnosed in Bistrița, followed by Milaș with 5 cases of rabies and Ilva Mare, Lechința and Nimigea with 4 outbreaks.

Chapter 10 compares rabies in both domestic and wild animals from 2000-2017 to the period 1968-1993. It also shows the incidence of rabies according to the localities where most cases of wild and domestic diseases have been recorded.

Therefore, the conclusions of this study are presented in **Chapter 11** as follows:

o In the period 2000-2017, within the LSVSA BN, a number of 1567 foxes were received and analyzed for rabies. In the period 2000-2007, 99 foxes were examined, of which 48 tested positive, and in the period 2008-2017, 247 foxes were tested, 138 tested positive. No case of rabies has been reported since 2015. Regarding the foxes shot during the vaccination campaign, in the period 2008-2014, within the LSVSA BN were received a number of 543 foxes, of which 80 being tested positive.

o In the period 2000-2007 there were a higher number of rabies cases, respectively 52, compared to the period between 1968-1993, but also the number of samples analyzed was higher, respectively 108 samples, which represents 48%. On the first place is Măgura Ilvei with a total of 5 cases out of the 5 cases received and analyzed within LSVSA BN.

o Regarding the research years, in 2007 most cases of the disease were diagnosed, 16 positive samples out of a total of 35 samples analyzed.

o Following the start of the vaccination campaign, in the period 2008-2017, a percentage of 16% of positive cases were diagnosed out of the total samples analyzed. No rabies cases have been diagnosed since 2015, which demonstrates the effectiveness of the vaccination program.

o In the period 2008-2015 there were a much higher number of outbreaks of rabies in the localities compared to the period 2000-2007. In Sânmihaiul de Câmpie and within the Rebra hunting ground, 5% of rabies cases were confirmed out of the total analyzed samples, as well as in Galații Bistriței, Chiochiș and Bistrița. It is worth mentioning that in the first two (Galații Bistriței and Chiochiș) cases of rabies were confirmed every year for 6 consecutive years, from 2008 to 2013.

o Regarding the incidence of rabies in other species of wild animals, over a period of 17 years (2000-2017) 11 brain samples were tested from wild animals other than foxes, being diagnosed with 6 cases of rabies. The small number of outbreaks shows that the main source of rabies virus is the fox. As in the case of foxes, since 2015 there have been no confirmed cases.

o Regarding the incidence of rabies in localities, before the start of rabies vaccination (2000-2007), the number of rabies cases is higher compared to the period after vaccination (2008-2017), both for dogs and cats. Among both species there is an

increase in the number of animals tested in the period 2008-2017, the number of cases of the disease remaining the same. Among pigs, there was only one outbreak in 2000. It

should be noted that no positive cases of rabies have been diagnosed among domestic animals since 2015.

Therefore, a gradual decrease in the number of outbreaks of rabies can be observed, and the lack of cases of disease from 2015 until now demonstrates the effectiveness of vaccination of foxes. The continued application of the successive vaccination of foxes and domestic dogs, as well as the mandatory testing for rabies, of both suspicious evidence and foxes shot following the vaccination campaign, will lead to the eradication of rabies and the achievement of a "rabies-free" status.