

INCIDENCE OF RABIES AND EFFICIENCY OF IMPLEMENTING THE RABIES ORAL VACCINATION PROGRAM IN FOXES IN BISTRITA-NASAUD COUNTY

INCIDENȚA RABIEI ȘI EFICIENȚA IMPLEMENTĂRII PROGRAMULUI DE VACCINARE ANTIRABICĂ ORALĂ A VULPIILOR ÎN JUDEȚUL BISTRIȚA-NĂȘĂUD

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ABSTRACT | REZUMAT

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

Therefore, this study aims to evaluate the effectiveness of the oral vaccine in both fox populations and among other wild animals and domestic animals. Thus, the data from both 1968-1993 and 2000-2007 were analysed, during which time no vaccination was carried out. All the animals processed in the ward of the Bistrita-Nasaud Sanitary Veterinary and Food Safety Laboratory were taken into account. In comparison, to prove the effectiveness of vaccination, all samples from domestic and wild animals were tested, samples received between 2008 and 2017, during which time the annual vaccination of the fox population was performed with vaccine baits.

Key words: foxes, vaccine baits, combating rabies, vaccination

Turbarea este una dintre cele mai periculoase zoonoze, finalizând-se întotdeauna cu moartea indivizilor afectați. Prin implementarea programului de vaccinare antirabică orală a vulpilor se urmărește combaterea turbării în România prin vaccinarea vulpilor, controlul turbării la populațiile de vulpi precum și eradicarea bolii de pe teritoriul țării. Astfel, se realizează vaccinarea antirabică a populației de vulpi cu momeli vaccinale și monitorizarea evoluției turbării în raport cu evoluția programului de aplicare a momelilor vaccinale și rezultatele obținute. Prin urmare, studiul de față își propune să evalueze eficiența aplicării acestor momeli vaccinale atât în cadrul populațiilor de vulpi, cât și în rândul altor animale sălbatice și animalelor domestice. Astfel, s-au analizat datele atât din anii 1968-1993, cât și perioada cuprinsă între anii 2000-2007, timp în care nu s-a aplicat vaccinarea. S-au luat în calcul toate animalele recepționate în cardul Laboratorului Sanitar Veterinar și pentru Siguranța Alimentelor Bistrița-Năsăud. Comparativ, pentru a dovedi eficiența vaccinării, s-au testat toate probele provenite de la animalele domestice și sălbatice, recepționate din 2008 până în 2017, perioadă în care s-a realizat vaccinarea anuală a populației de vulpi cu momeli vaccinale.

Cuvinte cheie: vulpi, momeli vaccinale, combaterea turbării, vaccinare

The surveillance, control and eradication of rabies in Romania, both in domestic and wild animals, is carried out by observing and applying the provisions of Order no. 35/2016. The target animal group is represented by all animals susceptible to rabies virus infection, in risk regions in urban, forestry and rural areas (4, 5, 9, 11).

Laboratory investigations are done in the following

situations:

a) In case of suspicion of disease: examination is performed by direct immunofluorescence test (DIF) or intracerebral inoculation of mice / inoculation on cell cultures, when DIF is negative, in case of human exposure; Confirmation of the disease is performed by DIF examination according to the OIE diagnostic manual.

b) In the case of domestic and wild carnivores found dead: an examination is carried out by DIF with the issuance of an analysis report (3, 10, 11).

The surveillance, control and eradication of rabies in foxes in Romania are based on the observance and application of the Order no. 35 of March 30, 2016 on the approval of the Methodological Norms for the

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application of the Program of actions for surveillance, prevention, control and eradication of animal diseases, those transmissible from animals to humans, animal protection and environmental protection, identification and registration of cattle, pigs, sheep, goats and equidae, as well as the Methodological Norms for the application of the Surveillance and Control Program in the field of food safety, issued by the National Veterinary Health and Food Safety Authority and published in the Monitorul Oficial of Romania no. 303 of April 20, 2016 (9, 10).

The target animal group is represented by foxes from the forest. The program provides for sampling of foxes shot for post-vaccination assessment. Sampling from shot foxes shall be performed at least 45 days after the distribution of the rabies vaccine, 4 foxes / 100 km² / year are taken for samples (5, 8, 12).

The vaccination of foxes will take place in two campaigns, by air distribution, by plane and / or helicopter, respectively 25 vaccine doses / campaign / km².

Human inhabited areas, waterfalls, highways, national roads will be avoided. Around these areas, as well as in the areas hard to reach by plane, the distribution of vaccine baits will be done manually, at the entrance to the den and on the routes circulated by foxes (7, 8, 14).

Initially, the virologic examination is performed by direct immunofluorescence test (DIF) in all shot foxes, in order to evaluate the rabies vaccination activity in foxes. The direct immunofluorescence test is performed by all Veterinary and Food Safety Laboratories accredited for this method. The second test is an enzyme-linked immunosorbent assay (ELISA) for the determination of post-vaccine rabies antibodies in fox serum / thoracic fluid. Samples will be harvested from foxes hunted for the purpose of assessing the effectiveness of vaccination, at least 45 days after the end of the vaccine distribution. Samples are tested for post-vaccine rabies titer (10,12,13).

Detection of the vaccine marker (tetracycline) in the bones collected from vaccinated foxes, by microscopic examination (UV), is a method based on the fact that the tetracycline present in vaccine baits, once ingested, is deposited in bones and teeth, where it can be highlighted by microscopic examination in ultraviolet light. In sections of bones or teeth, tetracycline deposits appear as yellow circular areas (3, 6, 8, 9).

The aim is to fight rabies in Romania by vaccinating foxes, controlling rabies in fox populations, as well as eradicating the disease in foxes in the country. In order to achieve these objectives, rabies vaccination with baits is carried out in fox populations and the evolution of rabies is monitored in correlation with the oral vaccine application program and the results obtained.

It is also intended to monitor the fox population in the country, to control the application of the vaccina-

tion program and evaluate its effectiveness. All these are done by using the diagnostic methods, by collecting and recording appropriate data, as well as using all the information to combat and eradicate rabies in Romania (12, 13, 14).

The bait vaccination program in foxes runs for a period of 10 years, beginning with 2008, and shall apply to the entire fox population (9).

MATERIALS AND METHODS

The case study was carried out within the Bistrita-Nasaud Sanitary Veterinary and Food Safety Laboratory. For the years 2000-2014, the data were taken from the diagnostic registers, and starting with 2015, all animal samples received in the laboratory were tested, including those with suspected rabies and from foxes shot in order to evaluate vaccination effectiveness. 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 direct immunofluorescence test (DIF) and by intracerebral inoculation test in mice (in case of suspicion of negative DIF).

In the case of foxes shot in order to assess vaccination effectiveness, the brain, thoraco-abdominal fluid and mandible were sampled. Initially, the virologic 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).

RESULTS AND DISCUSSION

The incidence of rabies in Bistrita-Nasaud county between 1968-1993

The epidemiological study includes the span 1968-1993 and the span 2000-2017. The data regarding the causes of rabies between 1968-1993 were obtained from the study of Oltean (4). Thus, between 1968-1993, 102 cases of disease were registered, both in wild animals and in domestic animals. In 1973, 1976-1978, 1983 and 1992, no cases of rabies were registered in the Bistrita-Nasaud county. The forest environment was free of disease in 1975, 1981, 1986, 1987, 1990-1991, and no cases were documented in the urban environment in 1971 and 1989 (1, 2).

There was a variable number of cases in wild animals, over a span of 25 years (1968-1993). Thus, among the foxes, out of the total of 94 samples brought within the Bistrita-Nasaud Veterinary Laboratory, a number of 35 tested positive.

Between 1968 and 1993, a total of 130 samples from dogs were analysed, of which 21 were positive.

25 cases of rabies were diagnosed in cats, out of a total of 66 samples analysed, 7 outbreaks of disease were recorded in cattle out of a total of 27 samples analysed, and 8 positive cases in sheep out of a total of 14 samples brought in diagnosis. The fewest cases of rabies were among pigs, with just one single outbreak in 1968 (1, 2).

The incidence of rabies in Bistrita-Nasaud county between 2000-2017

Between 2000-2017, a number of 1838 samples, from both wild and domestic animals, were received and analysed within the Bistrita-Nasaud Veterinary and Food Safety Laboratory. From the total number of samples tested for rabies, a number of 348 were positive, while no outbreaks of the disease were registered between 2015-2017.

The incidence of rabies in foxes

Over a period of 17 years (2000-2017), a number of 1567 foxes were received and analysed in the Bistrita-Nasaud Veterinary and Food Safety Laboratory.

We analysed both foxes that were found dead, foxes killed by dogs or killed in households and foxes shot following the vaccination campaign, to determine vaccination effectiveness. Thus, regarding the foxes found dead, 97 foxes were examined between 2000-2007, of which 46 tested positive and in the between 2008-2017, 125 samples were analysed, of which 54 tested positive.

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

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

Within the Bistrita-Nasaud Veterinary and Food Safety Laboratory, 9 shot foxes were analysed in 2007 (other than those from the vaccination campaign), of which 4 tested positive, and from 2008 to 2017 a number of 69 samples were examined, of which 15 tested positive. It should be mentioned that, since 2015, there have been no cases of rabies in Bistrita-Nasaud county.

Regarding the *foxes shot* during the vaccination campaign, in the span 2008-2014, a number of 543 foxes were received by the laboratory; their brain was sampled for the direct immunofluorescence test, mandible and blood were also sampled, all in order to assess vaccination effectiveness. Of the 543 foxes tested, 80 were diagnosed with rabies.

In 2008, the first year of vaccination, 28 foxes were shot, from which samples were taken for direct immunofluorescence and the fox's jaws were sent for analysis to the Bucharest Institute of Diagnosis and Animal

Health to determine the vaccine marker (tetracycline). All these diagnostic tests were performed to determine the effectiveness of vaccination. Out of the total of 28 samples analysed, 10 samples were positive. The samples were received in January and February.

In 2009, 71 foxes were shot and the brain, tibia and thoraco-abdominal fluid (blood) were collected. The brain was analysed at LSVSA BN, and the tibia (long bone) and thoraco-abdominal fluid were analysed at IDSA Bucharest. Out of a total of 71 foxes shot, a total of 20 tested positive at direct immunofluorescence; the samples were received at LSVSA BN in January, February, March and December.

In 2010, 30 foxes were analysed, 3 of which tested positive and samples were brought to the laboratory in January and February.

In 2011, a number of 46 foxes were shot and brain, mandible and thoraco-abdominal fluid (blood) were sampled for analysis in order to determine vaccination effectiveness. The samples were received in August, September, October, November and December and out of a total of 46 samples, only 3 were diagnosed positively at the direct immunofluorescence test.

2012 was the first year in which 200 foxes were shot and samples from the brain, mandible and blood were analysed; 12 cases of rabies were diagnosed. The samples were received in January, February and March, November and December.

The number of foxes shot to determine the vaccination effectiveness decreased in 2013, when only a number of 97 foxes were analysed. However, this year saw the highest number of positive cases, with 27 positive samples diagnosed in the direct immunofluorescence test. The samples were brought to the laboratory in the first quarter (January, February, March) and last quarter of the year (September, October, November).

The last year with positive cases of rabies recorded in foxes was 2014. Thus, a number of 71 foxes were shot and the brain, mandible and thoracic fluid were harvested; 5 cases were rabies positive, a significantly lower number than in 2013. The foxes were shot and sent to the Veterinary and Food Safety Lab BN in January, February, March, April and July.

Rabies vaccination campaigns in foxes also took place in 2015, 2016 and 2017 and during all these three years there were no cases of rabies. Thus, a number of 600 hunted foxes were received by the lab in order to determine the effectiveness of the rabies vaccination. Samples were taken from the brain and the direct immunofluorescence test was performed, all results being negative. The mandible and thoracic fluid were collected for the determination of post-vaccine rabies antibodies and for the microscopic (UV) detection of the vaccine marker (tetracycline) in the bones collected from vaccinated foxes. These were tested wi-

thin IDSA Bucharest - LNR Rabies and within LSVSA Suceava.

The fox shooting campaign was carried in 2015 throughout the year except in May, June and July.

In 2016 and 2017 the foxes were brought to the laboratory in January and December, July, August and September.

Incidence of rabies in other wild animal species

The incidence of rabies among other wild animals is quite low, which demonstrates that the main reservoir of rabies virus in the wild is the fox. Between 2007-2009, 4 wolf brain samples were tested and all four tested positive, in 2006 and 2009 only 2 cases of ferret rabies were diagnosed and in 2008 a wild cat was diagnosed positive.

Between 2015-2017, a deer brain sample, a lynx brain sample, a bear brain and a squirrel brain were received and tested at LSVSA BN, all of which were diagnosed as negative by the direct immunofluorescence test.

Incidence of rabies in domestic animals

Over a span of 17 years (2000-2017) urban rabies was diagnosed in a number of 56 domestic animals out of a total of 264 samples received and tested within the Laboratory in Bistrita-Nasaud.

Between 2000-2007, a number of 49 dogs were analysed, of which 14 tested positive. Between 2008-2017, 93 dog samples were analysed, of which 14 were diagnosed with rabies. Regarding cats, between the years 2000-2007, a number of 10 samples were received and analysed within the Laboratory in Bistrita - Nasaud, of which 6 were positive and between the years 2008-2017 19 samples were tested and 6 cases were diagnosed with rabies. In the case of both animal species there was an increase in the number of animals tested between 2008-2017, and the number of rabies cases remained the same.

In the case of cattle, 9 samples were analysed between 2000-2007, of which the number of positive cases was 4 and between 2008-2017 a number of 7 positive samples were registered out of a total of 48 tested. Although the number of animals tested increased, the total number of positive cases decreased.

Regarding sheep, before the start of the vaccination period, between the years 2000-2007, 8 samples were received and tested, 2 of which were positive for rabies. It should be noted that although over a period of 10 years (2008-2017) a larger number of sheep were analysed (15 samples), but no cases of disease were diagnosed.

Among the pigs, 8 samples were tested between 2000-2007, and only one case was positive, and between the years 2008-2017, from the 2 samples ana-

lysed, no case of rabies was identified.

It is worth mentioning that even among domestic animals, no positive cases of rabies have been diagnosed since 2015.

Incidence of rabies in foxes on areas

1. Incidence of rabies before vaccination

Between 2000-2007, a number of 52 cases of rabies were registered in foxes, out of a total of 108 analysed samples, in 31 localities. Most cases were diagnosed in Magura Ilvei, a number of 5 cases in 2002, 2003, 2004, 2006 and 2007, in the spring-summer period and one case in winter. Two outbreaks were recorded in 18 localities at intervals of 6 and 4 years, respectively 3, 2 or even 1 year apart. In two of the localities, Sangeorz Bai and Bistrita Bargaului, in 2006 and 2007, respectively, two outbreaks of rabies were diagnosed.

In 2001, there were 9 cases of rabies, followed by 4, 7 and 5 outbreaks, with a peak reached in 2007, when 16 positive samples were recorded.

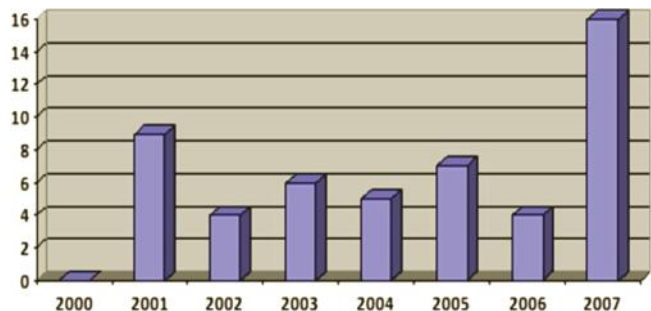


Fig. 1. The number of positive cases of rabies among foxes starting from 2000 to 2007

2. Incidence of rabies after vaccination

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 analysed within the Laboratory in Bistrita-Nasaud.

The samples came from 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 vaccination to be a solution in reducing the number of outbreaks of rabies in Bistrita-Nasaud county.

Most outbreaks of rabies were confirmed in Sanmihaiul de Campie, 12 total cases of which 7 recorded in 2008, followed by one case in 2009 and then one case in 2011 and 2013 and two cases in 2012. As for seasons, March and summer months predominate.

Within the Rebra hunting fund, 12 outbreaks of disease were also confirmed, a first case in 2009, followed by another 11 outbreaks in 2012, 2013, 2014.

In 2013, a number of 8 rabies cases were recorded, which is the highest number of cases recorded in a single year.

Eleven positive cases of rabies were registered in Galatii Bistritei, Chiochis and Bistrita. In the first two localities, cases of rabies were identified every year for six consecutive years, and in the last settlement, outbreaks of disease were diagnosed three years in a row. There were also localities with seven cases of disease diagnosed for two or three years in a row.

Speaking about landforms, Galatii Bistritei, Chiochis and Bistrita are situated in a hill area, Rebra hunting fund is placed in the forest area and Sanmihaiul de Campie is in the plain area.

In 2008, the highest number of cases of rabies were recorded, namely 63 cases, followed by 2009 and 2013 with a number of 40 and 47 outbreaks.

Although 2013 was a year with 47 registered cases, in 2014 the cases decreased to 12 and this was also the last year in which positive cases of rabies was diagnosed.

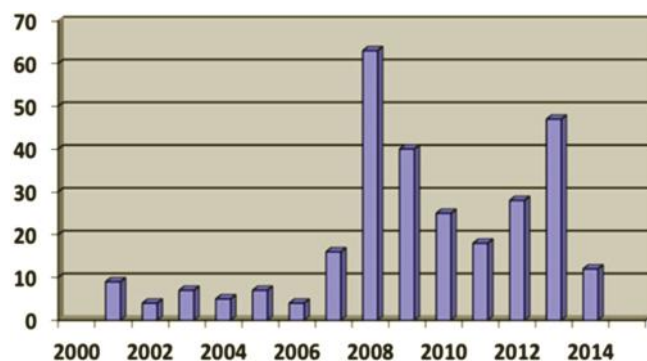


Fig. 2. Number of positive rabies cases in foxes, from 2000 to 2014

CONCLUSIONS

The results show an increase in the number of samples examined among foxes, between 2008-2014 compared to 2000-2007, even if, at the same time, the number of positive cases increased.

On the other hand, in the case of foxes shot to assess the effectiveness of vaccination, there is a decrease in rabies while the number of foxes shot increased. Thus, from 2008 to 2017, 1459 foxes were examined, and 233 cases were registered from 2008 to 2014, but no case of rabies was diagnosed from 2015 to 2017. With regard to domestic animals, the number of subjects tested for rabies increased between 2008 and 2017, but the positive cases are declined compared to 2000-2007. Therefore, it is worth nothing that, both among wild animals and domestic animals, no cases of rabies have been registered since 2015, which highlights the effectiveness of the rabies oral vaccination program in foxes.

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