Bull Vet Inst Pulawy 48, 195-200, 2004

RABIES AMONG WILD AND DOMESTIC ANIMALS IN LITHUANIA IN 1993-2002: EPIZOOTIOLOGY, DIAGNOSIS AND VACCINATION

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Received for publication March 01, 2004.

Abstract

The prevalence of rabies in the animal population of Lithuania during the period of 1993-2002 was investigated. The immunofluorescence method and the mouse inoculation test were used to diagnose the disease. The diagnosis was confirmed by standardized methods recommended by the OIE. During these years 3 667 domestic and 4 312 wild animals were examined. Rabies was diagnosed in 35.73% of domestic and in 64.27% of wild animals. The disease was found to be spread in all regions of Lithuania. The largest centers of rabies infection were determined to be in the districts of Lazdijai, Panevėžys, Utena, and Klaipėda. The highest number of rabies cases (242) was recorded in the Lazdijai district. During the period of 1995-2000 the program of oral vaccination of wild foxes was performed. Approximately 919 000 vaccine baits were disseminated. The tetracycline marker was used to check the bait up-take.

Key words: animals, rabies, epidemiology, oral vaccination, Lithuania.

Rabies is an acute infectious viral disease of the central nervous system that affects all warm-blooded wild and domestic animals and humans. The mortality rate is close to 100%. Rabies is caused by a neurotropic RNA virus, which belongs to the *Lyssavirus* genus of the family *Rhabdoviridae*. The virus is found mostly in the brain, spinal cord, saliva glands, and saliva of the affected animals. In the nature, wild animals are reservoirs of the virus and they transmit the disease usually by biting and via saliva rich in the virus. Rarely, transmission by non-salivary routes has been recorded. These include aerosol transmission to humans in the laboratory and in bat-infested caves. Most often, humans contract the disease from stray domestic animals. Animals contract the disease via wounds, when they are bitten by another animal infected with rabies. Most dangerous wounds are those close to the head or wounds that do not bleed. The incubation period is prolonged and highly variable, depending on the quantity of the *inoculum* and the site of the bite, being shorter after a bite near the brain than following a more distant one. The shortest incubation period is 7-8 d, but in most cases it lasts a few weeks. Sometimes, however, symptoms of the disease can appear even after several (11-19) months (1, 6, 9).

Rabies is being detected in many European countries, with the exception of Belgium, Cyprus, Finland, Greece, Iceland, Ireland, Italy, Luxemburg, Norway, Portugal, and Sweden (4). Rabies is also found in Africa, the Near East, many Asian countries and Central and South America.

In Lithuania rabies is known from old times. In 1897 V. Orlovskis established a Pasteur station in Vilnius. It is one of the oldest Pasteur stations. There are no reports of rabies diagnosed in Lithuania for the years 1919-1921; however, it is unlikely that Lithuania was then free of rabies because there were 222 animals diagnosed as being infected in 1922. In 1923, the largest rabies outbreaks occurred in the districts of Kėdainiai, Raseiniai, Kaunas, and Panevėžys. In 1924-1927 and from 1928 to 1931, 261 and 288 cases of rabies in animals were reported, respectively. According to the records, in 1940-1950 mostly cats and dogs were infected. In 1960-1969, a total of 1 094 cases of rabies were diagnosed and domestic animals were infected more often (68%) than wild animals (32%). During the period of 1970-1979, 1 333 cases of rabies were reported, with a similar ratio of domestic *versus* wild animals. During the period from 1980 to 1989, 1 251 cases of rabies were diagnosed. Domestic animals were infected more often (70%) than wild ones (30%) (5). The spread of rabies in the animal population creates a great danger to the public health. Therefore, it is very important to evaluate constantly the epizootic situation, to determine prevalence of rabies in the populations of various animals and investigate patterns of the spread of the disease. This can be only accomplished by using precise diagnostic methods.

Vaccination of cats and dogs against rabies is mandatory in Lithuania. All other domestic animals are vaccinated after exposure to an infected or suspected of infection animal, or in rabies outbreak areas.

The aims of the study were:

1) to analyse the epizootic status of rabies infections in domestic and wild animals (1993-2002),
2) to determine regional specificity of the spread of the disease,
3) to evaluate the efficacy of diagnostic and preventive methods, including vaccination programs used in Lithuania.

Material and Methods

Rationale for the study. In order to evaluate epizootic situation of rabies during the period of 1993-2002, the data from the State Food and Veterinary Service of the Lithuanian Republic, the National Veterinary Laboratory, as well as data from district and regional veterinary laboratories were analysed. The field samples were collected from various regions of Lithuania. Diagnostic tests were performed at the National Veterinary Laboratory and at 10 regional veterinary service laboratories. The following criteria for the collection of samples were used: (a) an animal suspected of having rabies, i.e. showing clinical symptoms (changed behaviour, aggression, increased irritability and sensitivity to external irritants, paresis, paralysis, *etc*.), (b) a dead animal. The whole corpse or the head of the dead animal were sent to laboratory for testing. Samples were delivered according to strict sanitary and personal hygiene requirements, packaged in tightly sealed containers and provided with accurately filled out accompanying documents.

Diagnostic procedures. The immunofluorescence method and the mouse inoculation test (3, 11) were used to diagnose the disease. Positive diagnosis of rabies was confirmed using standardized methods, recommended by the OIE (3), identifying the rabies virus or its components (11).

Immunofluorescence method. The direct immu-nofluorescent test was performed using a commercial diagnostic kit ("Bioveta", Brno**,** Czech Republic). Brain impressions were prepared from the Ammon's horn (hippocampus), cerebellum and medulla oblongata. The slides were dried, fixed by heat, and incubated with conjugate in a humid chamber at 37˚C for 30 min. Afterwards the slides were briefly rinsed in phosphate buffer solution (PBS), and washed three times in PBS for 10 min each and rinsed with distilled water. The preparations were mounted in buffered 80% glycerin, pH 7.4, and analysed by epifluorescence microscopy. The following controls were used: antirabies positive conjugate + negative preparation and negative conjugate + positive preparation. However, despite high specificity and sensitivity of the immunofluorescence method, the mouse inoculation test was used in all negative cases in order to assure proper recognition.

Mouse inoculation test. This test was used to confirm the negative results obtained using immunofluorescence method. Three-four week old (12-14 g) BALB/c mice were inoculated intracerebrally with 0.05 ml of 20% homogenized brain suspension in PBS, pH 7.4, with gentamycin and penicillin, prepared from the Ammon's horn, cerebellum or brain cortex. Inoculated mice were observed for 28 d. The brain of each dead mouse was tested for rabies by the immunofluorescence method. After 28 d. all surviving mice as well as dead mice showing negative results by the immunofluorescent test confirmed the negative diagnosis.

Vaccines. The following tetracycline marker rabies vaccines were used: SAG-1 (Virbac, France), Lysvulpen (Bioveta, Czech Republic), and Rabifox (Germany).

Area of vaccination. Oral vaccination program for wild foxes started in 1993 in Panevėžys, Pakruojis, and Joniškis districts. In 1996, spring vaccination was carried out in the area of 4 000 sq. km of 13 northern regions of Lithuania. A hundred thousand doses of the vaccine were distributed manually. In one district (Biržai), the vaccine was disseminated from an airplane. In 1997, vaccination campaigns were performed twice, i.e. in spring (May) and in autumn (October-November) – total of 200 000 baits were distributed covering 4 338 sq. km area of 22 districts. In 1998, the oral vaccine was distributed in the area of 6 375 sq. km encompassing 26 districts of the northern and western parts of Lithuania. Again total of 200 000 baits were disseminated. In 1999-2000, spring and autumn vaccinations were also carried out in 29 districts. From 1995 to 2000, approximately 919 000 vaccine baits were disseminated manually or from airplanes. In 2001-2002, the vaccination of foxes was not performed.

Traceability of vaccination. Analysis of the tetracycline marker was performed, in order to determine the traceability of oral vaccination: Sixty micron thick cuts from the mandible of the hunted foxes were evaluated under a luminescence microscope.

Results

Between 1993 and 2002, a total of 7 979 samples were tested for rabies and 3 696 positive cases of the disease (46.32%) were diagnosed (Table 1).

Animal rabies was found to be widespread in all regions of Lithuania (Fig. 1). The majority of rabies cases were found in Lazdijai, Panevėžys, Klaipėda, Tauragė, Utena and Šilutė districts. The lowest number of cases – in Mažeikiai, Akmenė, Kaišiadorys, Jonava, and Trakai districts. Based on the number of reported rabies cases, all districts of Lithuania were divided into 3 groups: group I: 1-60 cases, group II: 61-120 cases, group III: more than 120 cases. The following districts comprise group I: Akmenė, Anykščiai, Jonava, Kaišiadorys, Kretinga, Kupiškis, Mažeikiai, Molėtai, Plungė, Rokiškis, Skuodas, Šakiai, Šalčininkai, Šilalė, Švenčionys, Telšiai, Trakai, Varėna, Vilkaviškis, and Zarasai.

Table 1
Rabies infection rates of domestic and wild animals in Lithuania (1993-2002)

Animal	Number of animals tested	Positive (%)
Cattle	1 219	718 (58.9)
Cats	906	319 (35.21)
Dogs	1 394	238 (17.07)
Horses	64	26 (41.18)
Other domestic animals	84	18 (21.43)
Foxes	1 997	1 084 (54.28)
Raccoon dogs	1 461	1 019 (69.75)
Martens	366	168 (45.9)
Polecats	218	58 (26.6)
Other wild animals	270	48 (17.77)
Total	7 979	3 696 (46.32)

Table 2
Traceability of oral vaccination of foxes (1995-2000)

Index	Year					
	1995	1996	1997	1998	1999	2000
Number of vaccination campaigns per year	1	2	2	2	2	2
Bait set-up methods (manual–M, from airplane–A)	M	M/A	M/A	M/A	M/A	M/A
Vaccine type	SAG-1	SAG-1	SAG-1	Lysvulpen	SAG-1, Rabifox	Rabifox
Vaccination area (sq. km)	940	4 000	4 338	6 375	15 000	12 000
Total number of baits	19 000	200 000	200 000	100 000	200 000	200 000
Analysis of tetracycline markers: number/positive (%)	11/5 (45.45%)	17/7 (41.18%)	N.D.	76/25 (32.89%)	26/8 (30.77%)	189/45 (23.81%)

N.D. – not determined.

The following districts were assigned to group II: Alytus, Biržai, Joniškis, Jurbarkas, Kaunas, Kelmė, Kėdainiai, Marijampolė, Pasvalys, Prienai, Radviliškis, Raseiniai, Širvintos, Ukmergė, and Vilnius. The following districts fall into group III: Ignalina, Klaipėda, Lazdijai, Pakruojis, Panevėžys, Šiauliai, Šilutė, Tauragė, and Utena (Fig. 1). In the first group, 901 positive cases of rabies were diagnosed, which comprises 24.39% of all rabies cases diagnosed in Lithuania. In the second group, 1 404 positive rabies cases were diagnosed, which make up 37.95% of all rabies cases diagnosed in Lithuania. In the third group – 1 391 positive rabies cases or 37.66% of all positive rabies cases. This group shows a bit smaller infection percentage than the second group; however, the average of rabies infections for this group are 154.56 cases. The analysis of the data collected shows that the majority of animals infected with rabies are located in the Eastern (Utena, Ignalina), Southern (Lazdijai), Southwestern (Tauragė, Šilutė, Klaipėda), and Northern (Joniškis, Panevėžys, Šiauliai) parts of Lithuania. The borders of these districts, with the exception of Panevėžys, Šiauliai and Klaipėda, are shared with neighboring foreign countries, i.e. Poland, Belarus, Russia (Kaliningrad region), and Latvia (Fig. 1). The data show also that in 1993-2002 rabies infections in wild animal population comprised 64.27% of all reported cases, while the number of rabies cases in domestic animals was lower (35.73%) (Fig. 2). The percentage of rabies infection in domestic and in wild animals in 1993-2002 is provided in Fig. 3. The number of positive cases of rabies among foxes, raccoon dogs, cattle, cats and dogs is shown in Fig. 4. Details of vaccination results are presented in Table 2.

Discussion

Our results indicate a very uneven distribution of rabies infections and show that during 1993-2002 rabies was registered in the entire territory of Lithuania. The majority of rabies was registered in the north, north-east and south-west parts of Lithuania (in 1991-2000 – in the north and north-west areas) (10). This distribution of the disease is dependent on various conditions and factors such as dimensions of wild animal population (especially foxes and raccoon dogs), forestation of the area, and in part, on the spread of rabies in neighbouring foreign countries.

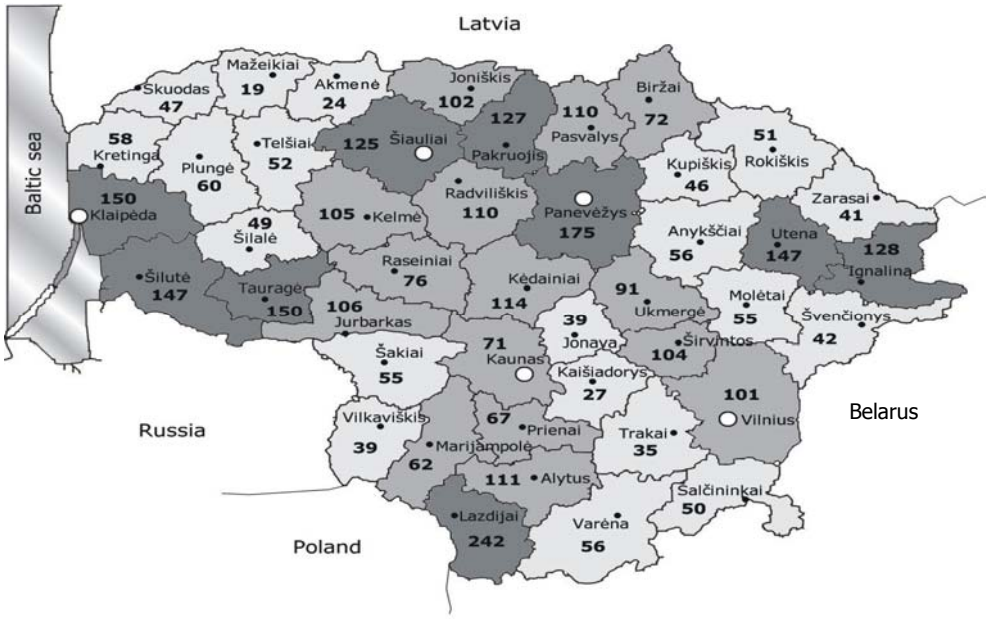

Fig. 1. Number of positive cases of rabies registered in diferent districts of Lithuania from 1993 to 2002. Colour intensity shows: light – 1-60 cases; medium-dark – 61-120 cases; dark – over 120 cases.

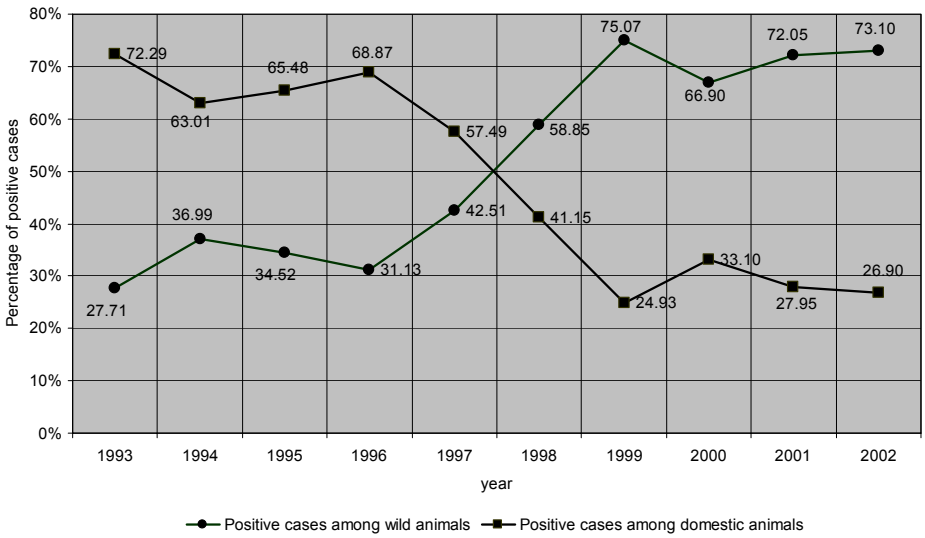

Fig. 2. The spread of rabies among wild and domestic animals in Lithuania (1993-2002).

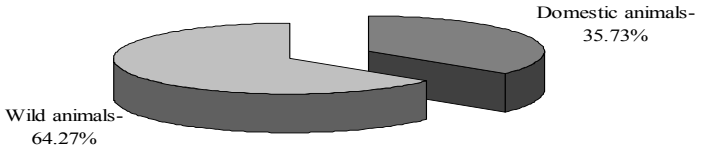

Fig. 3. Percentage of rabies infection in wild and in domestic animals in Lithuania (1993-2002).

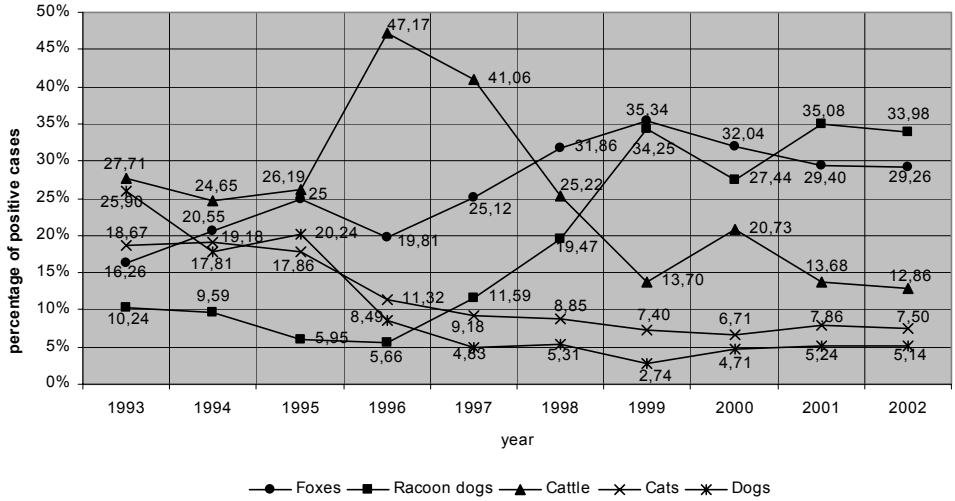

Fig. 4. Dynamics of rabies infection among foxes, raccoon dogs, cattle, cats and dogs (1993-2002).

In Latvia, the prevalence of rabies among wild and domestic animals was not as widespread as in Lithuania. In Latvia, however, the largest number of rabies in 1995-2000 was registered in fox population. During that time rabies were reported in 23 out of 26 districts of Latvia. Rabies was mostly registered in the regions of Kuldinas, Ventspilis, Saldus, and Luizas, as well as in the central regions of Latvia (Dobelė, Jelgava, Bauskė, and Riga). In Poland, during 1995-2000, the largest number of rabies cases was registered in the eastern and north-eastern parts of the country. In Belarus, during 1995-2000, 824 cases of rabies were registered, mostly in fox and racoon dog populations. Therefore, it may be concluded that these two species of wild animals play very important role in the spread of the disease, including other European countries such as Poland, Latvia, Belarus (10).

In the domestic animal group, the largest number of rabies was detected in cattle, whereas dogs and cats were less infected. It can be surmised that livestock has more opportunities to come in contact with the rabid animals, especially while grazing near forests or shrubberies. In dogs and cats rabies often spreads when they come in contact with wild animals. There is especially a great danger when dogs and cats are kept loose in farmsteads near forests what obviously increases contacts with wild animals. Clearly, stray cats and dogs significantly contribute to incidences and spread of rabies; a serious and organized effort should be made to reduce population of stray animals.

Although test-methods used to diagnose rabies in Lithuania are sufficiently specific and reliable, other rabies detection methods should be developed and applied in order to identify rabies virus genotypes, to improve the efficacy of vaccines, and to investigate specificity evolution relationships of rabies virus genotypes and the disease.

It is obvious that vaccination is a very effective preventive tool to stabilize the spread of rabies among domestic animals. We have determined that during 1993-1999 rabies infections in dogs and cats remained stable but in 2000-2002 the incidence of rabies infection has risen. This increase may be explained by the fact that during the same period (2000-2002), rabies infections among the fox population also rose dramatically. Therefore, it may be concluded that the risk of cats and dogs coming in contact with wild fauna also increased; however, it was also observed that the levels of rabies infection in cats and dogs do not fluctuate markedly and remain rather stable. So it may be concluded that the mandatory vaccination program of cats and dogs is a very effective preventive method against the rabies virus. Analysis of wild fox oral vaccination program calls our attention at two time periods: 1993-1996 when the rabies infections among foxes remained stable; and 1997-2002, when the infection curve in foxes became steeper yearly. It appears that oral vaccination program of foxes during 1995- 2000 has protected a part of the animal population from rabies, and the decrease in rabies cases resulted in an increase in the survival, leading to the growth of fox population. This might explain the sudden and sharp increase in rabies cases in the later years – there was an increase in fox population with likely a larger part of animals remaining unvaccinated, and in addition, no vaccination was performed in 2001-2002.

Acknowledgments: The authors thank Dr habil. Gediminas A. Biziulevičius for careful and critical reviewing the manuscript, Dr Mykolas Mauricas for useful comments, Mr Algis Dranseika for interpretation of some results obtained and Dr Vitolis Vengris for assistance in preparing of the manuscript.

References

1. Bagdonas J., Citvaras V., Nekrašienė N., Liutkevičienė V.: Rabies in Lithuania. Epizootological problems. Agric Sci 2001, **3**, 69-74.

2. Gylys L., Chomel B.B., Gardner I.A.: Epidemiological surveillance of rabies in Lithuania from 1986 to 1996. Rev Sci Tech Off Int Epiz 1998, **17**, 691-698.
3. Office International des Epizooties (OIE). In: *Manual of Standards for Diagnostic Tests and Vaccines*. *Rabies*. Chapter 2.2.5. Brussels 2000.
4. Rabies Bull Eur **26**, 2002.
5. State Food and Veterinary Service of the Republic of Lithuania (SFVS). Rabies situation and oral vaccination of foxes in Lithuania. Rabies Annual Reports, Brussels 2001.
6. State Food and Veterinary Service of the Republic of Lithuania (SFVS). Rabies Annual Reports. Vilnius. Lithuania 1993-2002.
7. Siurin B. N., Samuilenko A. J., Solovjov B. V., Fomina N. V.: Animal Viral Diseases, VNITIBP, Moscow 1998, pp. 300-318.
8. Taršis A., Dranseika A., Kuznecovas N.: Rabies. Ministry of Agriculture of the Republic of Lithuania. Vilnius 1989.
9. Zienius D., Bagdonas J., Dranseika A.: Epidemiological situation of rabies in Lithuania from 1990 to 2000. Vet Microbiol 2003, **93**, 91-100.
10. World Health Organization (WHO). Expert Committee on Rabies. Eighth Report. WHO Technical Report Series No. 824, Geneva 1992.

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