

The 1995-2000 ORV program in Lithuania wildlife

DAINIUS ZIENIUS, SAULIUS PETKEVIČIUS, ANTANAS VYŠNIAUSKAS

Veterinary Institute of Lithuanian Veterinary Academy, Instituto 2, Kaišiadorys, LT-56115, Lithuania

Zienius D., Petkevičius S., Vyšniauskas A.

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Summary

The present study investigated the influence of rabies oral vaccination program (ORV) on rabies distribution in wildlife in Lithuania. From 1995 to 2000 on a territory of 10,000 km² in 27 out of 44 districts in Lithuania, 950,000 doses of oral vaccines SAG-1, Lysvulpen, Rabifox were distributed (15-25 baits/km²). The total cost of the rabies prevention campaign in wildlife was €746,731, wherein €685,760 constituted vaccine costs (28.3 €/km²) and €60,971 distribution costs. In 1995-2000 period 189 (59 positive) samples for oral tetracycline markers (OTS) and 138 (75 positive) fox sera samples for IFA were investigated. This comprised 0.023% (OTS) and 0.017% (IFA) of all ORV baits distributed in that period. Before ORV was established, 204 cases of rabies (70 cases in foxes and 25 in raccoon dogs) had been registered in wildlife from 1993 to 1997. In 2000-2003 (after ORV program) had been completed, 2568 cases of rabies in wildlife were registered (678 cases in foxes and 665 – in raccoon dogs), respectively. The results demonstrated that the ORV program in Lithuania was unsuccessful. In the 3 years after vaccinations, an 8.2 fold increase of rabies cases among foxes and 22.3 fold increase of cases among raccoon dog population were registered. The future of such oral vaccinations of wildlife in Lithuania may be seriously questioned. It is necessary to prepare a scientifically grounded long term ORV program in the entire territory of Lithuania.

Keywords: rabies, oral vaccination, Lithuania

Oral vaccination campaign in Europe has led to the elimination of rabies in various regions and has pushed the front of the rabies wave back eastwards. In fact, it is the first example of pathogen elimination from the wild animal population by means of vaccination and not by means of reduction of host population (1).

Oral immunization of foxes with baits containing live virus (SAD Bern or SAD B19) was efficient in many European countries (Switzerland, Germany, Italy, Belgium, Luxembourg, France). From 1983 to 1998 more than 70 million SAD B19 vaccine baits were distributed in 13 European countries as part of wildlife rabies control program (19). As a result the number of rabies cases in vaccination areas has significantly decreased. In 1994 the incidence of rabies cases had shrunk to less than 20% compared to 1983. Further, in 2002 in EU Member States were registered totally only 43 rabies cases (19 cases in Germany and 24 in Austria) (18).

However, the elimination of fox rabies can be hampered for two reasons: the persistence of residual foci in some areas, and the risk of re-infection of zones already freed, which is parallel to an increase in fox population (13). Therefore, different measures to improve the efficacy of fox oral vaccination have been tested in the field: increase of the number of vaccine baits distributed in one campaign (4); vaccination of areas with two passages separated by 15 or 30 days

(5); vaccination of fox cubs by distributing vaccine baits at the entrance of fox dens (21); vaccination during the summer period (9); and need to be optimized in experimental trials on captive foxes.

In 1983-1986 period oral vaccination of a limited number of foxes (277), raccoon dogs (73) and badgers (14) were initiated in Lithuania as a pilot measure to control rabies in wildlife species at seven high-incidence foci of five districts. Vaccines produced from a live modified virus (a Vnukovo derivate (EVMTI-VVMKI-71)) a hamster cell line, adapted ERA derivate), injected in fish pieces or minced meat were used (14, 17). After this campaign in above districts 25-50% reduction of rabies cases were registered (15).

The objectives of the present work were to investigate the influence of rabies oral vaccination programme (ORV) in Lithuania during 1995-2000 on distribution of rabies in wildlife.

Material and methods

The vaccination area involved 9,800 km² at 27 of 44 districts of Lithuania, where totally 19,500 foxes were counted. The vaccination area was chosen because of natural barrier of Baltic Sea and typical red fox area of habitation (hedges, village surroundings, isolated bunch of trees etc.) combined with a high incidence of rabies. There were 5 counties and 25 districts towns in vaccination area and the human population consisted of 1,865,000 inhabitants.

The following oral rabies vaccines were used: SAG-1 (Virbac®, France) vaccine as a third SAD Bern derivative following one and two successive mutation of the Arginin 333 codon and cultivated on BSR/BHK cells under monoclonal antibody pressure (vaccine titre – min $8 \log_{10}$ TCID₅₀/dose); Lysvulpen (Bioveta®, Check Republic) vaccine SAD Bern strain obtained from first attenuated ERA vaccine strain after passaging on canine and bovine kidney cells (vaccine titre – min $7 \log_{10}$ FFU/ml); Rabifox (IDT) vaccine as a SAD Bern passage on two different BSR – subclones of BHK cells (vaccine titre – min $7 \log_{10}$ FFU/ml). All vaccines baits were in original containers dispensed into blisters, plastic capsules sealed with an aluminium foil and covered with a bait substance including tetracycline as a vaccination indicator.

ORV programme in Lithuanian wildlife was organized according to the Lithuanian National Rabies prevention programme (1995-2000). Baits were deposited at every km² (at least 1 bite in every 200 meters). The campaign was carried out twice per year – in spring (March-April) and in autumn (October-November). Baits with tetracycline marked vaccine were distributed by aircraft, private hunters, and partially by game wardens and forest rangers. The aircraft over flied every km² twice (distance between flight lines – 500 m) at an altitude of less 200 metres and at the maximum speed of 150 km/h, with perfect ground visibility, the entire area to be treated being indicated on the map given to the pilot. The baits were dropped by means of special equipment by technicians, previously trained by a specialist from the Lithuanian State Food and Veterinary Service.

The rabies distribution in different wildlife species in Lithuania during the 1993-2003 period, and the information regarding biomarkers and serology investigations were based on the annual data summaries of the Lithuanian SFVS. Data about the wildlife populations and hunting statistics were obtained from the annual reports of the Lithuanian Ministries of Agriculture and Environment.

The ORV program monitoring was based on three methods of evaluation: testing of the occurrence of biomarker (usually tetracycline); examining of sera of the target animals for rabies virus neutralizing Ab; analysing the incidence of rabies in wildlife before, during and after ORV program (16). The Tetracycline biomarkers and rabies antibodies (IFA) were detected at the Pulawy National Veterinary Research Institute – PNVRI (Poland).

Results and discussion

During the period of 1995-2003 the number of hunted wildlife in Lithuania increased with minimal influence on real rabies situation in the country. During 1993-1997 the numbers of rabies cases in wildlife were relatively stable, but afterwards the situation changed dramatically (fig. 1). From 1993 to 1997 it was registered only 204 rabies cases, whereas in 2000-2003

Tab. 1. Rabies cases and incidence in ORV area in Lithuania wildlife

Years	Rabies cases	Density (cases/km ²)	Vaccination area (km ²)	Number of baits (per km ²)	Cost of vaccination (€) (vaccine/distribution)
Before the ORV program					
1992	12	0.0012	-	-	-
1993	17	0.0017	-	-	-
1994	15	0.0015	-	-	-
During the ORV program					
1995	21	0.0021	970	19.000 (20)	19.760/1449,3
1996	23	0.0023	5631	200.000 (20-25)	208.000/6188,4
1997	51	0.0051	5509	200.000 (20-25)	208.000/7536,2
1998	82	0.0084	6149	170.000 (15-20)	78.200/13.043,5
1999	168	0.0171	6534	170.000 (15-20)	122.400/13.913,1
2000	347	0.0354	9796	200.000 (15-20)	132.000/18.840,5
After the ORV program					
2001	316	0.0322	-	-	-
2002	419	0.0427	-	-	-
2003	495	0.0505	-	-	-

period – 2,568 cases of rabies were diagnosed (an increase on 12 fold). The most dangerous situation was registered in fox and raccoon dog populations: during the last 5 years, cases of rabies in fox population have increased on 9,7 times compared to the situation in 1993-1997; and in raccoon dog population the rabies situation was even worse, cases of rabies have increased on 26,6 times compared to the period from 1993 to 1997.

According to the 1995-2000 Lithuanian National Rabies prevention program, ORV was organized in Central and North-western regions of Lithuania (fig. 2, 3). In 1995 first oral vaccination against rabies in wildlife was conducted in the area of 940 km² in Panevėžys, Pakruojis and Joniškis districts. In these areas 19,000 SAG-1 vaccine baits containing tetracycline marker were distributed. Continuously, in 1996 a spring vaccination campaign was arranged in the area

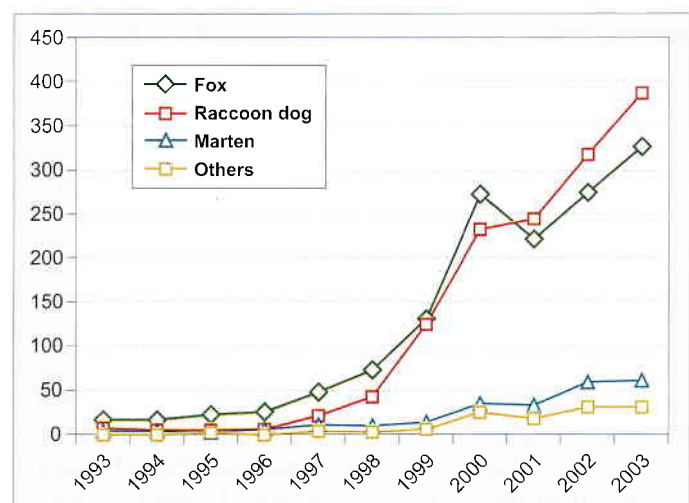


Fig. 1. Rabies cases in wildlife in Lithuania 1993-2003

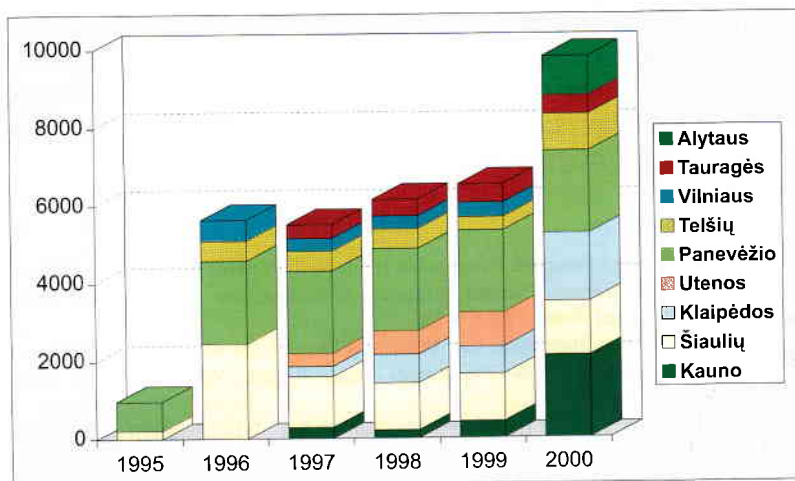


Fig. 2. ORV area in different Lithuania counties (km²) in 1995-2000 period

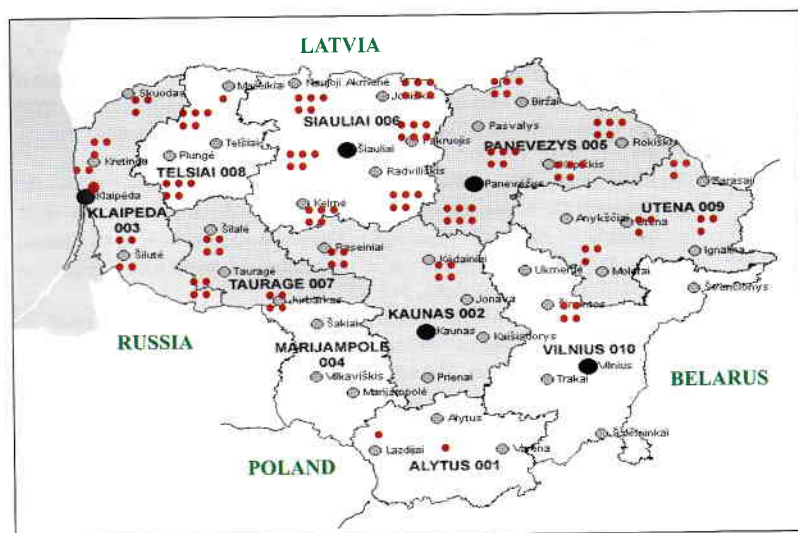


Fig. 3. ORV in Lithuania wildlife in period of 1995-2000

of 4,000 km² in 13 districts of northern Lithuania, when 200,000 doses of rabies vaccine were used (20-25 baits per km²). The baits were placed by hand in forests and bushes near densest. The distribution by aircraft was used in Biržai district. In 1997 two vaccination campaigns in spring (May) and autumn (October-November) in an area of 5349 km² in 22 districts of Lithuania were performed and 200,000 SAG-1 vaccine baits (20-25 per km²). Afterwards, in 1998 the vaccination campaign was arranged in an area of 6375 km² in 26 districts of northern and western parts of Lithuania. During that period 170,000 baits of a new type rabies vaccine Lysvulpen (Bioveta) were used. Continuously, in 1999 170,000 SAG-1 vaccine baits in the area of 7000 km² in 22 districts were used. Two-vaccination campaigns (April-May and October-November) were carried out. In 2000, the Rabifox (Dessau) oral rabies vaccine was used in an area of 8000 km² in 20 districts of Lithuania, when 200,000 baits (15-20 per km²) were distributed in two-vaccination campaigns. The total cost of the 1995-2000 rabies prevention campaign in Lithuanian wildlife was 746.730,9 € (685.760 € for vaccines and 60.971 € for their distribution). The cost

per 1 km² was of the order of 28.3 €. Because of lack of funds in 2001-2004 oral vaccination of wildlife in Lithuania was discontinued.

1995-2000 ORV programme completely covered Šiauliai and Panevėžys, partially – Vilnius, Telšiai and Tauragė counties. In 22 districts vaccine baits were distributed only 1-3 times during the spring or autumn. The intake of vaccines intake was very variable. In the most cases baits were eaten and bitten-foil capsules containing no vaccines discarded. This occurred for 85% of vaccine baits, the remaining 15% baits were found untouched, unbitten and even unmoved capsules, evidently foxes had just observed.

In 1995-2000, when the ORV programme was performed, there were investigated 189 (59 positive) samples for OTS markers and 138 (75 positive) fox sera samples for IFA. It comprised 0.023% (OTS) and 0.017% (IFA) of all vaccines baits distributed in that period.

Observation and registration of fox rabies over last 20 years suggests, that the disease spread from the Northwest and from the South towards the Central and Eastern parts of the Europe (6). The red fox remained the main victim and transmitter of rabies in this region followed by raccoon dogs. It should be mentioned, that in raccoon dogs infection level with rabies in 2000-2003 increased more markedly compared to foxes: 1093 rabies cases in foxes and 1183 – in raccoon dogs, respectively. In decade from 1993 to 2003, 1,420 rabies cases in foxes and 1,400 in raccoon dogs were registered, and it was majority (44.6% in foxes and

40.0% in raccoon dogs) of all registered wildlife rabies cases in Lithuania. These two wildlife species can migrate and transmit rabies infection through the borders of Lithuania to neighbouring countries such as Poland, Belarus, Russia and Latvia. During the 5 years period before the initiation of the ORV program in Lithuania wildlife, the rabies were diagnosed more often in foxes (70 cases) compared to the raccoon dogs (25 cases), consequently the 1995-2000 ORV program in wildlife was targeting to the red fox population.

According recommendations for rabies control (16) vaccination campaigns in wildlife are required twice per year in spring and autumn until rabies in vaccination area will not be detected for over two years period. In Lithuania ORV campaigns were performed in April-May and October-November. The traditional early spring campaign seems very attractive, because of the overall fox density and number of adult non-territorial foxes have reached its lowest level, the territories are relatively stable and the adults have sufficient time to develop an immune reaction before the period of enhanced risk of transmission in late spring and early summer (20). Early spring campaigns car-

ried out in March-April (targeting exclusively the adult fox population at its annual lowest density) were also shown to be beneficial in Belgium, Luxembourg and several parts of Germany (3). In spring, around 60% of the fox population are cubs, but at the time the animals become independent this has been reduced to 40% (20). In spring cubs are also the most difficult group to vaccinate. After spring campaigns, bait uptake ranges between 22 and 52% of cubs but reaches 70-80% of adults. After autumn campaigns, bait uptake reaches 70-80% of both adult and young foxes. The increase in the number of young unvaccinated foxes is the one of the main reason of the persistence of rabies (2). When using rabies modified vaccines, the preferable distribution time is early-June, because of a potential interference between passive and acquired immunity in fox cubs (2, 12). After vaccination in April or early May (as in Lithuanian ORV program) a large number of young foxes could stay unprotected during summer and early autumn. Therefore, for vaccination of young foxes in spring it's necessary to distribute baits before the end of May (19). Early baiting can induce the origin of a residual rabies focus with the vaccination area (12). Countries considering oral vaccination campaigns, but that do not have the financial resources to distribute baits on bi-annual basis, could implement a single annual campaign preferable in late autumn and early winter (20). For example, in Texas (USA) and Ontario (Canada) single annual vaccination campaigns in late autumn have been extremely effective (7, 8).

In Lithuanian ORV program vaccine baits were distributed by hand (56%) and by aircraft (44%). During the 1980s, baits were predominantly distributed by hands with assistance of local hunters and forest rangers. Baits distribution by hunters is difficult to organize over several years, this method is rather expensive and more difficult to input into practice. For the other hand, the immunization of fox cubs is more successful if vaccine baits are manually distributed around fox dens entrance (21). Many countries distribute baits by aircrafts. The baits density also differs from country to country, ranging from 13 to 25 baits per km², but in areas with a high population density of foxes 30 baits per km² are recommended (10). Rabies has been eradicated in areas where over 75% of fox population was immunized by oral vaccination during several years (11).

The results demonstrated that from 1998 to 2000 ORV program in Lithuania was unsuccessful, because in 3 years after vaccination 8.2 fold increased rabies cases among fox and 22.3 fold among raccoon dogs. The number of investigations of OTS markers and serology by IFA were extremely low to evaluate the protection level. The future of such oral vaccination of wildlife in Lithuania may be seriously questioned. It is necessary to study the real quantity and quality of fox/raccoon dog population and relationships between animal population density and the minimum density

of vaccine/baits; to investigate the essential level of heard immunity; to eliminate rabies under various environmental circumstances and optimal bait distribution techniques; to prepare the scientific grounded long term ORV program in all territory of Lithuania.

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Author's address: Dr. Dainius Zienius, Instituto 2, LT-56115, Kaišiadorys, Lithuania; e-mail: dainzien@yahoo.com, lvi@org.ktu.lt