Vaccination of humans and domestic and wild animals against rabies in Lithuania 2006–2010

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The incidence of rabies and vaccination against this disease in human and animal populations were investigated in Lithuania in 2006–2010. In the last five years, up to 7–10 thousand people in Lithuania suffered from the diseased animals every year. Dogs are the most common domestic animals that humans suffer from (in earlier years, about 70–80% every year); in 2006–2009, 23–30% of human victims were exposed to rabid dogs, whereas in 2010 only 1.92%. The exposed people were vaccinated with inactivated vaccine against rabies Verorab® (Sanofi Pasteur, France) and immunoglobulin Imogam®Rabies (Sanofi Pasteur, France). For about a year (2006–2007) Favirab immunoglobulin (Sanofi Pasteur, France) has been used. This preventive method is applied to 60–70% of people exposed to potentially rabid animals every year. During the time frame of investigation only in 2007 a case of human rabies was reported in a person who had not been preventively vaccinated. That was a man who travelled in India and was bitten by a stray dog.

Dogs, cats and cattle are the most common domestic animals responsible for transmission of human rabies, while red foxes and raccoon dogs are most common in the wild animal group. In the last five years, 9 554 domestic and wild animals were investigated for rabies. Among 2 980 samples taken from domestic animals and 6 574 samples from the wild ones, 16.77% and 35.39%, respectively, were positive. Since 2006 in Lithuania twice a year (in spring and autumn) wild animals are orally vaccinated using “Lysvulpen” vaccine (Bioveta, Czech Republic). In 2006–2010, 3 130 blood serum samples from wild animals were examined for efficiency of rabies oral vaccination (ORV). The greatest number of serologically positive samples was taken from red foxes and raccoon dogs: 57.55% and 50.00%, respectively. Systematic ORV is responsible for the rabies decline in animals. In 2010, rabies was reported in 13 Lithuanian districts: Ignalina, Salčininkai, Zarasai, Širvintos, Molėtai, Vilnius, Varėna, Utena, Kaunas, Raseiniai, Skuodas, Panevėžys and Pasvalys. Vaccination against rabies is the main immunopreventive measure controlling the disease in animals.

Key words: rabies, humans, animals, risk, vaccination

INTRODUCTION

Rabies is a zoonotic viral disease: one of the oldest and most dangerous human and animal diseases. It is transmitted from animal to animal and from animal to humans. Rabies has been already known more than 2000 years ago [1]. The disease occurs almost in all continents of the world: Asia, Africa, Europe and America. Yet there are countries and islands where rabies is not common: Japan, New Zealand, Greece, Portugal and Chile [6]. About 55 000 people all over the world die of this disease every year [9, 10]. The symptoms of rabies occur in humans 30–50 days after being
bitten [25]. The disease is fatal for humans and animals. Vaccination is the most effective preventive measure against rabies which means that timely vaccination and strengthening of immunity can avert the disease.

Rabies is an acute viral disease of warm-blooded animals which affects the central nervous system. The disease is caused by a neurotropic virus of the family Rhabdoviridae of the Lyssavirus genus. It has RNA and is commonly found in the brain of infected humans and wild and domestic animals. Also, in spinal cord, saliva glands and saliva. Transmission of rabies occurs through contact with infected saliva after bites and scratches. The most common sources of human rabies are stray domestic animals, therefore the danger of being infected with rabies virus is real.

Mammal predators (foxes, raccoon dogs, cats, bats, wolves, etc.) are the most common sources of rabies among wild animals. The red fox (Vulpes vulpes) is one of the main carriers of rabies virus accounting for 83% of positive cases [3]. Raccoon dogs (Nyctereutes procyonoides) are the second largest source of rabies in Europe. Their adaptability is higher than that of red foxes. In Lithuania, the population of raccoon dogs has been growing more rapidly than that of red foxes (in 1995 it amounted to 6 100, whereas in 1999 to as many as 18 thousand). Wide distribution of raccoon dogs is responsible for the higher incidence of rabies in Lithuania [30]. Today, raccoons occur not only in Lithuania but also in the neighbouring countries (Russia, Poland, Belarus and Latvia).

Vaccination is the only preventive measure which can avert rabies after exposure to a potentially rabid animal. All domestic animals should be preventively vaccinated against rabies every year. Oral rabies vaccination is the main form of vaccination and preventive measure against rabies [5]. In Lithuania, ORV of wild animals using vaccine "Lysvulpen" (Bioveta, Czech Republic) was started in July, 2006.

ORV is combined with analysis of epidemiological situation of rabies in the country. It helps to determine the trends of incidence of this disease and to reduce the danger of human rabies. Immunological investigation methods are applied, whereas vaccines are used as an effective measure of immunoprophylaxis for eradication of rabies.

The aim of the present study was to analyse epidemiologically the situation with human and animal rabies in Lithuania in 2006–2010, i.e. to determine the number of cases of human rabies and the volume and effect of vaccination of wild and domestic animals.

MATERIALS AND METHODS

Following the guidelines of the Ministry of Health of the Republic of Lithuania, the epidemiological situation with rabies and immunoprophylaxis against it in Lithuania in 2006–2010 were evaluated through investigation of the cases of medical aid to exposed persons and identification of the sources of rabies. The epidemiological analysis of rabies was performed at Lithuanian Centre for Prevention and Control of Communicable Diseases (CCDPC) (since 2008 Centre for Communicable Diseases and AIDS). Also, the data from State Food and Veterinary Service (SFVS), National Veterinary Laboratory (NVL) and (since 2007) National Food and Veterinary Risk Assessment Institute (NFVRAI) were analysed for evaluation of epidemiological situation and efficiency of ORV in Lithuania.

Centre for Communicable Diseases and AIDS (ULAC) is in charge of epidemiological control of rabies in Lithuania to prevent its outset and spread. The epidemiological analysis was performed based on the data provided by CCDPC and later ULAC for 2006–2010 about the number of exposed persons, the source of injuries, immunopreventive measures and the amount of the used vaccine and immunoglobulin against rabies. Following the rules for immunoprophylaxis, medical aid and epidemiological control, vaccination was applied and urgent medical aid was rendered to all persons bitten or slobbered by identified or unidentified animals as well as to persons exposed during veterinary aid to animals, slaughtering of animals or autopsy of sick animals. The persons at risk of exposure due to professional activity were vaccinated preventively.

In 2006–2010, for vaccination against human rabies a purified inactivated rabies vaccine Verorab® (Sanofi Pasteur, France) was intramuscularly injected in the area of humerus. The vaccine is cultured on Vero cells (WISTAR strain RABIES PM/WI 38-1503-3M) approved for exposure prophylaxis. The pre-exposure vaccination regimen includes: initial administration (3 injections on days 0, 7 and 28), the first repeated administration a year later and further repeated administrations every 5 years.

The post-exposure vaccination scheme of adults and children includes the following steps: five 0.5 ml doses of vaccine (on days 0, 3, 7, 14 and 28). Immunoglobulin and vaccine against rabies were used immediately after bites and scratches and exposure to saliva. From 2006 till 2007 immunoglobulin Favirab (Sanofi Pasteur, France) containing fragments of horse immunoglobulin F(ab′)2 against rabies was used. Immunoglobulin is indicated after severe bites to the face, head, neck or arms when a domestic or wild animal, which made the bites, cannot be examined or is potentially rabid. It is used immediately after the exposure at a dose of 40 TV/kg of body weight together with the rabies vaccine yet not later than the eighth day after the administration of the first dose of the vaccine. From 2007 till 2010 immunoglobulin Imogam® Rabies Pasteurized (Sanofi Pasteur, France) containing IgG class human rabies immunoglobulin with minimal titre of 150 TV was used. It is designed for adults and children: one dose of 20 TV/kg of body weight is administered on the same day.
as the first dose of rabies vaccine. If it is possible anatomically, the major part of the preparation should be injected as close to the injured areas as possible. The remaining portion should be injected intramuscularly into the deltoid muscle or into the external part of the thigh in children, opposite to the area of administration of vaccine. When for some reasons the beginning of prophylaxis is delayed, Imogam® Rabies Pasteurized must be administered irrespective of the time span between the exposure and the beginning of prophylaxis before the eighth day after administration of the first dose of the vaccine.

In 2006–2010, ORV was carried out twice a year (in spring and autumn) all over Lithuania. "Lysvulpen" vaccine was used. It is an attenuated SAD (Street Alabama Dufferin) Berne vaccine [15]. For identification of epidemiological situation with rabies in animals and evaluation of ORV in wild animals, the material for laboratory trials was collected from the whole territory of Lithuania. The diagnostic trials were performed by NVL and later by NFVRAI and county SFVS, whereas the efficiency was evaluated by NVL and later by NFVRAI. By the fluorescent antibody test (FAT), 9554 brain samples were examined. In NFVRAI, the investigations of rabies were carried out using standard Office International Epizootic (OIE) [2] and World Health Organization (WHO) [28] methods: direct Fluorescent Antibody Test (FAT) [17, 21].

Direct Fluorescent Antibody Test (FAT) is based on the ability of fluorescent antibodies to bind to homologic antigens and make them visible as fluorescent particles in the ultraviolet part of the spectrum of luminescent microscope [17]. The analysed material – brain tissue – was fixed on the object glass and tagged using fluoresceine isothiocyanate (FITC) antirabic polyclonal antibodies covalently bound to fluorochrome [17]. Polyclonal antibodies of FITC anti-rabies virus manufactured by Bioveta (Czech Republic) were used. Albeit high performance and specific character of FAT, the positive cases were confirmed by isolation of rabies virus in tissue cultures (RTCIT).

Rabies Tissue Culture Infections Test (RTCIT). For this test the following reference material was used: a cell line N2a CCL-131 (Neuroblastoma cells) and for the positive control – rabies virus CVS-11 (ATCC VR 959) (Challenge Virus Standard) [17, 21]. Reference material was provided by the EU Reference Laboratory for Rabies, Anses, Nancy, France. After 72 hours of incubation at 37 °C, the inoculated cells N2a CCL-131 were fixed with acetone 80% and stained with FITC monoclonal antibodies against rabies (Fujirabio, USA). NVL (later NFVRAI) represents an EU certified laboratory which using FAVN method performs antibody titre of animals vaccinated against rabies [4, 21]. The anti-rabies antibody titre, according to OIE directives, must not be below 0.5 TV/ml [4, 17, 21, 28]. The test is performed following the requirements set by Regulation (EC) No 998/2003 of the European Parliament and of the Council on animal health requirements applicable to the non-commercial movement of pets. During five years, 3780 animal blood serum samples were examined using FAVN method.

Fluorescent Antibody Virus Neutralization Test (FAVN). The following reference materials were used for this assay: cell line BHK-21 (Baby Hamster Kidney) (C13) (ATCC CCL-10), rabies virus CVS-11 (ATCC VR 959) (Challenge Virus Standard), positive reference serum 0.5 TV and negative dog blood serum [4, 21]. After 48 hours of incubation at 37 °C, the inoculated cells BHK-21 (C13) were fixed with acetone 80% and stained with FITC monoclonal antibodies against rabies (Fujirabio, USA).

For determining ORV antibody titre in the blood of wild animals, Bio-Rad Platelia™ Rabies II Kit (France) for enzyme-linked immunosorbent analysis ELISA was used. For evaluation of the efficiency of ORV of wild animals (red foxes and raccoon dogs), 3 130 blood samples were examined using ELISA method.

Efficacy of rabies oral vaccination indirect (ELISA). Bio-Rad Platelia™ Rabies II ELISA Kit (France) was used for the assay of IgG rabies virus glycoprotein in animal blood. The assay was performed strictly to the manufacturer’s instructions. Additionally, positive reference serum 0.5 TV/ml and positive reference serum 0.4 TV/ml were used. The control sera were used following the guidelines of OIE and WHO [21, 28]. The method is based on the indirect ELISA [23]. The tested blood and the reference positive controls were poured into the wells of glycoprotein-covered microplate. The results are expressed in optical density units, read from the microplate by an automatic microplate analyser Elx808 (manufactured by Bio-Tek, USA) within band length 450 mm.

RESULTS

In 2007, Bernhard Nocht Institute for Tropical Medicine in Germany confirmed one case of human rabies. The experts from Vilnius Public Health Centre identified 10 family members and 13 members of medical staff who were in contact with the infected person. All these persons were vaccinated against rabies with Verorab®.

On January 5, 2007, ULAC received information from Republican Hospital of Infectious Diseases and Tuberculosis about the death of a person potentially infected with rabies. During the epidemiological examination it was determined that in October–November, 2006 the aforementioned man travelled in India. At the beginning of October he was bitten by an unknown dog. The man applied for medical aid in India yet there is no detailed information about what kind of aid was rendered to him. According to the relatives of the diseased, some “preparation” was injected. On his
return home, the man did not apply for medical aid in Lithuania and did not inform his family members about his being bitten by a dog. A month after the trip, the man felt sick and was hospitalized in the intensive therapy unit. The situation soon became critical and at the beginning of January the man died. His family objected to autopsy and the body was returned to them on January 5, 2007. The diagnosis of human rabies was proved after his saliva sample had been sent to the laboratory of Bernhard Nocht Institute for Tropical Medicine in Hamburg (Germany), and the obtained result confirmed a positive rabies case by polymerase chain reaction (PCR).

Except for the fatal case of human rabies, reported in 2007, the morbidity from this disease was not very high in the last 50 years. In the time frame under consideration (1960–2010), 12 fatal cases of human rabies were reported. Dogs and foxes were the main source of infection (Table 1).

Analysis of the epidemiological situation with human rabies showed that in 2006–2010 from 6,923 to 10,790 persons exposed to various domestic and wild animals applied to health care institutions (Table 2).

In 2006 the epidemiology of rabies was unfavourable because animal sources were reported over the territory of Lithuania, representing a serious hazard for human health. In 2006, 10,790 persons exposed to domestic and wild animals applied to medical institutions. In the following years, the numbers were: 2007 – 8,591, 2008 – 7,451, 2009 – 6,923, 2010 – 6,980 (Table 2). The frequency of rabies in adults exceeded that in children.

In 2006–2010, 23,565 adults and 10,190 children injured by healthy, potentially rabid and rabid animals were reported. Adults and children most often suffer from dogs. Stray dogs or dogs neglected by their owners attacked 10,015 children and 20,485 adults. Rabid dogs bit 911 persons who received timely medical aid by administration of vaccine against rabies. Cats bit and scratched 6,055 persons. 461 person was injured by rabid cats, 3,314 by healthy cats and 2,279 by unknown cats. Rural population usually suffer from cattle and their get. In 2006–2010, medical aid was requested by 1,017 persons living in rural areas, including 844 persons injured by rabid cattle, 146 injured by healthy cattle and 27 by unknown cattle. These persons were timely vaccinated with anti-rabies vaccine and immunoglobulin. In 2006–2010, 2,421 person exposed to wild animal, applied for medical aid: 1,519 were injured by rabid wild animals, 158 by healthy wild animals and 744 by unknown wild animals.

In 2006–2010, anti-rabies vaccine and immunoglobulin were administered to 25,176 adults and children (61.80%) and immunoglobulin was administered to 116 (0.46%) persons (Fig. 1).

In 2006–2007 rabies epidemiology was very widespread, therefore, in 2008–2009 the situation remained rather un-

### Table 1. Reported cases of human rabies in Lithuania in 1960–2010

<table>
<thead>
<tr>
<th>Region</th>
<th>Years</th>
<th>Number of cases, n</th>
<th>Source of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vilnius city</td>
<td>1960</td>
<td>1</td>
<td>Dog</td>
</tr>
<tr>
<td>Kaišiadorys district</td>
<td>1962</td>
<td>1</td>
<td>Fox</td>
</tr>
<tr>
<td>Švenčionys district</td>
<td>1965</td>
<td>1</td>
<td>Raccoon dog</td>
</tr>
<tr>
<td>Kėdainiai district</td>
<td>1972</td>
<td>1</td>
<td>Badger</td>
</tr>
<tr>
<td>Trakai district</td>
<td>1979</td>
<td>1</td>
<td>Fox</td>
</tr>
<tr>
<td>Joniškis district</td>
<td>1992</td>
<td>1</td>
<td>Raccoon dog</td>
</tr>
<tr>
<td>Trakai district</td>
<td>1992</td>
<td>1</td>
<td>Dog</td>
</tr>
<tr>
<td>Trakai district</td>
<td>1993</td>
<td>1</td>
<td>Cat, dog</td>
</tr>
<tr>
<td>Kėdainiai district</td>
<td>1997</td>
<td>1</td>
<td>Fox</td>
</tr>
<tr>
<td>Pasvalys district</td>
<td>2000</td>
<td>1</td>
<td>Fox (Raccoon dog?)</td>
</tr>
<tr>
<td>Prienai district</td>
<td>2004</td>
<td>1</td>
<td>Not found</td>
</tr>
<tr>
<td>Vilnius city</td>
<td>2007</td>
<td>1</td>
<td>Dog (in the territory of India)</td>
</tr>
</tbody>
</table>

### Table 2. Distribution of exposed persons according to the source animal species in 2006–2010

<table>
<thead>
<tr>
<th>Source of infection</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>7,152</td>
<td>6,562</td>
<td>5,821</td>
<td>5,430</td>
<td>5,535</td>
</tr>
<tr>
<td>Cats</td>
<td>1,458</td>
<td>1,293</td>
<td>1,139</td>
<td>1,054</td>
<td>1,111</td>
</tr>
<tr>
<td>Rats</td>
<td>63</td>
<td>74</td>
<td>70</td>
<td>58</td>
<td>38</td>
</tr>
<tr>
<td>Cattle</td>
<td>550</td>
<td>212</td>
<td>187</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>Other domestic animals</td>
<td>175</td>
<td>46</td>
<td>66</td>
<td>85</td>
<td>67</td>
</tr>
<tr>
<td>Other wild animals</td>
<td>1,392</td>
<td>404</td>
<td>168</td>
<td>246</td>
<td>211</td>
</tr>
<tr>
<td>Total</td>
<td>10,790</td>
<td>8,591</td>
<td>7,451</td>
<td>6,923</td>
<td>6,980</td>
</tr>
</tbody>
</table>
favourable. In the last five years, 9,554 brain samples were examined for rabies in wild and domestic animals. 2,827 samples turned out to be FAT positive (29.58%). Analysis of rabies incidence in wild and domestic animals showed a yearly decline in the number of rabid animals (Fig. 2).

Occurrence of rabies in wild animals was determined by investigation of red foxes and raccoon dogs as the most common hosts of rabies (Fig. 3).

The highest rates of rabid domestic animals (cats, dogs and cattle) were determined in 2006 – 346 (29.10%). The
incidence of rabies in wild animals in the same time span was 1 883 (73.10%) (Fig. 3). In the following years, the incidence of reported rabies was: 15.14% in 2007, 4.74% in 2008, 4.79% in 2009 and only 0.74% in 2010 (Fig. 2). Analysis of the results obtained in the last five years by FAT and RTCIT assays showed that wild animals were the main source of rabies in Lithuania. In 2006, rabid raccoon dogs accounted for 89.40% and rabid red foxes for 79.95% of cases (Fig. 4). In 2007, the number of reported cases of rabies declined: raccoon dogs – 37.77% and red foxes – 32.19%. In the following years, the numbers were: 6.81% and 3.29% in 2008, 8.88% and 5.09% in 2009 and 2.88% and 3.80% in 2010 (Fig. 4).

In 2006–2010, vaccination of pets (cats, dogs and ferrets) against rabies over the territory of Lithuania was carried out on a stringent basis. All other domestic animals were vaccinated after exposure or at suspicion of being rabid. In 2006–2010, FAVN assays were performed within the directive on non-commercial movement of pets in the EU member states. The number of examined pets (cats, dogs and ferrets) amounted to 3 780. In 3 575 cases, the determined titre after vaccination against rabies was >0.5 TV/ml (Table 3).

For evaluation of vaccination efficiency of wild animals against rabies in 2006–2010, blood samples of 1 066 raccoon dogs and 2 064 red foxes were examined. 533 blood samples of raccoons and 1188 blood samples of foxes turned out to be positive. Based on the data obtained by IFA method, the immune response intensity in wild animals was determined and the obtained data analysed. The determined ORV efficiency in serologically positive raccoon dogs and red foxes was 54.98% (Fig. 4).

Based on ORV data for 2006–2010, it was determined that serologically positive red foxes accounted for 57.55% and raccoon dogs for 50.00%. In 2009, vaccination efficiency among foxes and raccoon dogs was statistically different, whereas in 2010 the difference was negligible (Fig. 4).

**DISCUSSION**

Notwithstanding the preventive measures against rabies, people should avoid contacts with animals unavailable for testing or animals displaying noticeable signs of behavioural changes. The early symptoms of human rabies bear unspecific character: fever, shiver, headache and general complaint [22, 24]. Later symptoms bear neurological character: anxiety, agitation, insomnia, uneasiness, partial paralysis, hallucinations, excessive production of saliva, laboured swallowing, and aggression. Someone with rabies eventually die a few days after the development of symptoms [7, 8]. After the development of initial symp-
toms of rabies doctors are helpless and the disease is almost always fatal. Rabies in Lithuania is diagnosed only by tests of brain tissue. Human rabies can be prevented only through vaccination and a onetime dose of immunoglobulin. Verorab® used in Lithuania is of high efficiency and its by-side reactions are by far lower than those of all previously used vaccines. Following WHO guide, Verorab® is used as a post-exposure prevention measure by intramuscular administration into the deltoid region. The vaccine has been already used for 20 years in 100 countries and no adverse effects were observed [26]. A person after a contact with a wild or domestic animal, unavailable for examination, should immediately get a full post-exposure treatment. Yet based on the vaccination results in 2006–2010, a conclusion can be made that many people tend to discontinue the course of treatment.

Analysis of vaccination of humans and wild and domestic animals in Lithuania proved to be an effective preventive measure. In 2006–2010, there were no fatal cases of human rabies in vaccinated persons, though the number of persons exposed to animals amounted to 33 thousand. This is another proof that post-exposure prophylaxis shows no contraindications. Even possible unwelcome post-vaccinal reactions are minor in comparison with the danger of rabies [28]. According to WHO recommendations, the post-vaccination antibody titre 0.5 TV/ml is a sufficient limit preventing from the outset of rabies [28, 29]. The same value of the antibody titre is recommended in vaccinated domestic and wild animals [14].

In five years (2006–2010) rabies in wild and domestic animals was reported all over Lithuania. Yet the distribution of rabies in wild and domestic animals varied in different regions and different seasons. In 2006–2010, the highest number of rabies cases in wild animals was reported in February, August and October. In 2006, the highest number of rabies in domestic animals occurred in June and October, in 2007 – February and August. In 2008–2009, a slight increase in the incidence of rabies in domestic animals was observed in March, July, September and October. In 2006–2007, the highest incidence of rabies in wild animals was observed in winter and spring and in August–October. In 2009–2010, the incidence of rabies in wild animals bore no seasonal character. In the last two years, rabies was reported only in the eastern Lithuanian districts. In 2010, rabies was reported in 13 Lithuanian districts. The greatest number of rabies sources – 23 – was identified in the eastern Lithuanian districts bordering with Belarus. Rabies virus supposedly was imported into Lithuania from the neighbouring country where vaccination of wild animals against rabies did not take place. Based on the FAT results, we may state that the incidence of rabies has declined due to annual ORV. We may also assume that rivers act as a natural barrier for rabies in the territory of Lithuania. Presumably, the geographical limits of rabies may be drawn along the Nemunas River in the Lithuanian–Polish border zone and the Neris and Šventoji rivers in the Lithuanian–Latvian border zone [12].

Before ORV in Lithuania, the stability of “Lysvulpen” attenuated vaccine under unfavourable atmospheric conditions before use was evaluated. The titre of the tested vaccine set by the manufacturer was TCID50 tissue culture infective dose. 100 baits containing vaccine were manually scattered over in spring and autumn in forests and forest outskirts. Air temperature, precipitation and sunlight were registered on a daily basis. The stability of the bait envelope was also observed [13]. In field conditions, the stability of baits containing vaccine is a very important factor for ORV against rabies. It was experimentally proved that the efficiency of oral vaccine under field conditions depends on the dose, virus titre stability and bait envelope [13]. The bait envelope was found to be more stable in shadow areas than in the sunlight. Precipitation caused softening and eventual melting of bait envelope [13]. Under dry air conditions, vaccine titre remained stable, whereas under humid air conditions it reduced. In the conclusions it is emphasized that high air humidity may reduce the effectiveness of ORV [13].

A survey of statistical data for 2006 showed that the occurrence of rabies in wild animals increased (Fig. 2). Yet, already in 2007–2008 the incidence of rabies in wild animals declined (Fig. 3). This can be explained by immunogenicity of “Lysvulpen” vaccine and efficiency of ORV. We have determined that the epidemiology of rabies in red foxes and raccoon dogs depends on the size of population and, presumably, on the immunity status. In 2008–2010, the level of infection with rabies in wild animals remained comparatively stable. According to the results on ORV, reported by other authors for 1997–2003, the number of infected red foxes was increasing every year. In 2001–2003, ORV did not take place [18]. Many red foxes and raccoon dogs were not vaccinated that was presumably the cause of the increase of rabies incidence. Successful ORV of wild animals depends on a few factors: well-defined vaccination site and time, mode of vaccination, the number of baits, vaccine properties and the effectiveness of vaccination programme [19].

The investigation carried out in 2006–2010 confirmed that oral immunization programme is an effective preventive measure against rabies in wild animals. The effectiveness of vaccination is measured in qualitative indices of the immunity status, i.e. the number of animals that acquired immunity after vaccination. The immunity response of red foxes and raccoon dogs intensifies with every vaccination. The ideally planned ORV programme may increase the immunity response to 50–90% when serum antibody titre is 30–80% (0.5 TV/ml) [31]. The strengthening of immunity
increases the population; that means the increased number of infection-susceptible animals, especially among the young in summer [32]. In Lithuania, oral revaccination of wild animals against rabies is carried out twice a year in spring and autumn. The autumn vaccination is targeted at adult and young (up to one year of age) animals. The spring vaccination is targeted at a smaller number of adult and young animal populations. European raccoon dogs breed in May as their pregnancy lasts longer than that of red foxes [11]. Red foxes breed from May 15 to April 15 [20]. Also, feeding differences between red fox and raccoon dog young ones have been observed: red foxes take food with teeth [16] whereas raccoon dogs do not take food [11]. The young raccoon dogs may remain unvaccinated and potentially act as sources of rabies.

Generalizing the obtained results, we may conclude that human and animal populations in Lithuania are not safe with regard to rabies, however, rabies incidence has been decreasing every year. Modern rabies vaccines used in Lithuania are very efficient and safe, therefore at the slightest suspicion of rabies infection a post-exposure vaccination must be applied. In our opinion, oral vaccination of wild animals has brought positive results in eradication of this dangerous communicable disease.

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