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# Rabies occurrence in red fox and raccoon dog population in Lithuania

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The rabies situation in red fox and raccoon dog in Lithuania in the period 1996–2005 and the virus distribution during the long-term rabies persistence period in these populations, were described. In the period of the present investigation, the number of hunted foxes increased fourfold (4051 animals were hunted in 1996 and 16949 in 2003), while the number of hunted raccoon dogs increased more than eight times (577 in 1996 versus 5215 in 2003), but that had a minimal influence on the real rabies situation in the country: 4404 cases among wild animals were registered in all districts of Lithuania (45% in red fox population and 41% in raccoon dogs). The situation remained most dangerous in the red fox population in which in the last 5 years a 35–68% increasing tendency of rabies cases remained. The number of rabies cases in the raccoon dog population were 10% less, but the tendency was increasing by 78–118%. The highest prevalence of fox rabies cases was registered in the Vilnius and Alytus counties (389 and 317 cases, respectively) and the lowest in the Telšiai and Marijampolė counties (45 and 102). In the red fox and raccoon dog populations, rabies cases were tested during the whole year in ascending order. In the March–May period there were diagnosed 26.9% of foxes and 21.7% of raccoon dogs, versus 18.7% of foxes and 28.1% of raccoon dogs in the October–November period.

**Key words:** Lithuania, rabies, epidemiology, wildlife

## INTRODUCTION

Understanding the spatial epidemiological dynamics of an infectious disease is critical in any attempt predicting its emergence or spread to new geographic regions. Information about host ecology influencing variation in transmission rates between host and pathogen populations often is not readily available and can be very expensive to obtain, especially for those diseases primarily associated with wildlife. The epidemic spread of rabies has proven to be an extremely useful system for exploring a variety of approaches to disease dynamics in different population structure and regions (Smith et al., 2002).

Rabies is a zoonotic disease with an epidemiological complex. Historically mainly reported in dogs, it had virtually disappeared from Central Europe at the turn of the twentieth century. During the last century, important modifications of the epidemiological cycles of rabies in Europe were observed, and the establishment of new epidemiological and biologic investigations revealed evidence of new epidemiological cycles. The main epidemiological cycle of rabies in wildlife animals in Europe is maintained by the red fox (*Vulpes vulpes*) and another by the raccoon dog (*Nystereutes procyonides*). Following the high co-adaptation of the current rabies virus strain to the red fox, and due to fox ecology, no other species play a significant role in maintaining the disease in the infected areas, although many wild (raccoon dog, marten, badger) and domestic (cattle, dog, cat) animals are affected and may transmit the disease (EC, 2002; Bourhy, 2005).

Recently rabies cases of red foxes and the raccoon dogs have been dominating in Lithuania, and risk of rabies transmission for domestic animals is increased. The specific structure of the distribution of rabies cases in different wildlife and domestic species of animals has changed.

The objectives of the present work were to describe the rabies situation in the period 1996–2005 in Lithuania during the long-term rabies persistence period in the red fox and raccoon dog populations.

## MATERIALS AND METHODS

Rabies is a major zoonosis for which diagnostic techniques have been standardized internationally (OIE, 2004).

For the rabies antigen detection the fluorescent antibody (FA) technique is used. The test is based on microscopic examination under ultraviolet light of impression sections of tissue after they have been treated with anti-rabies serum or globulin conjugated with fluorescein isothiocyanate. For the virus isolation, the intracranial inoculation of mice (MIT) and neuroblastoma cells (NA C1300) inoculation tests are used. The sensitivity of virus isolation in neuroblastoma cells is higher than 98% and can reduce the time required for rabies diagnosis from 10 days for the mouse inoculation test to 1–2 days using NA C1300 (WHO, 2004).The information about rabies distribution in different wildlife species in Lithuania during 1996–2005 was based on the annual data summaries of the Lithuanian State Food and Veterinary Service (SFVS) and Multiannual animal diseases status (OIE, 2004). The data from all ten Lithuanian counties were used in this work.

Pathological material in a leak-proof rigid container (animal heads or brain samples) were taken for investigation by both private (75%) and state veterinarians (25%). The samples were sent to a district state veterinary service and to a regional veterinary laboratory. Brain samples were collected on opening the skull in a necropsy room or by using the retro-orbital route for brain sampling (SFVS, 2000). All rabies-suspected hunted, road-killed and dead animals were included in this investigation. The results of all rabies testing were reported as positive, negative, or equivocal; equivocal results were not considered in these analyses. Data on the wildlife populations and hunting statistics in Lithuania were obtained from the annual reports of the Environment Ministry (EM) and Statistics Department (SD). The rabies epidemiological status and reported cases in different wildlife populations were used to compare the influence of the hunting statistics in different periods of time and in different Lithuanian regions on rabies infection in red fox and raccoon dog populations. The Prism3 program (Graph Pad Software, Inc, San Diego, USA) was used to calculate regression and correlation.

## RESULTS

Analysis of the hunting statistics on Lithuanian wildlife in 1996–2005 showed (Table 1) that 108 479 foxes and 26193 raccoon dogs were hunted in Lithuania. 13339 rabies-suspected samples were investigated in Lithuania during this period and 6679 were rabies-positive, including 4404 cases among wild animals. Rabies in the red fox population comprised 1957 (45%) and in raccoon dogs 1827 (41%) of all the cases. Statistical analysis of linear regression, linear and rank correlation between the number of hunted animals and rabies-positive cases in fox and raccoon dog populations showed that the correlation coefficient (r) in red fox was 0.9547 and the rank correlation (Sp.r) 0.9758 versus 0.8714 and 0.8182 in raccoon dogs. The standard error (SE) was 0.0035 in red fox and 0.01299 in raccoon dog, while the statistical interval of confidence (95% CI) varied from 0.8150 to 0.9859 and within 0.5354–0.9692, respectively.

In 1996–2005, 3784 red fox and raccoon dog rabies cases were diagnosed in all districts of Lithuania, with an average of 37.84 cases in a district per year (Table 2). 15987 red foxes and 4887 raccoon dogs were hunted in Šiauliai and 11129 foxes and 3671 raccoon dogs in Panevėžys counties, but 389 positive fox rabies cases were diagnosed in the Vilnius and 335 raccoon dog cases in the Utena counties. A comparison between fox and raccoon dogs hunting and rabies cases in different Lithuanian regions indicated the standard error (SE) to be 0.0097 for red fox and 0.02350 for raccoon dog, with the statistical interval of confidence (95% CI) between 0.7458 and 0.6584 and within 0.6247–0.7702, respectively. The correlation coefficient (r) in red fox was

Table 1. **Hunting data and rabies epidemiological status in red fox and raccoon dog populations in Lithuania in 1996–2005 (EM, SD, SFVS, 2005)**

| Years | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
|---|---|---|---|---|---|---|---|---|---|---|
| Tested samples | 357 | 436 | 450 | 654 | 1549 | 1631 | 1639 | 1989 | 2123 | 2901 |
| Rabies-positive s. | 108 | 208 | 226 | 364 | 850 | 677 | 933 | 1108 | 553 | 1652 |
| **Red fox** | | | | | | | | | | |
| Hunted (x) | 4051 | 7268 | 7611 | 10504 | 12726 | 12850 | 13018 | 16494 | 9450 | 14052 |
| Rabies-positive (y) | 6 | 46 | 72 | 130 | 272 | 221 | 274 | 388 | 201 | 327 |
| n | SE | r | | r (sq) | | S (y. x) | 95% CI | | Sp. r | p |
| 10 | 0.0035 | 0.9547 | | 0.9115 | | 39.021 | 0.8150–0.9859 | | 0.9758 | <0.001 |
| **Raccoon dog** | | | | | | | | | | |
| Hunted (x) | 577 | 610 | 743 | 1217 | 2311 | 3642 | 4525 | 5215 | 3914 | 3493 |
| Rabies-positive (y) | 6 | 20 | 43 | 125 | 233 | 245 | 318 | 312 | 162 | 363 |
| n | SE | r | | r (sq) | | S (y. x) | 95% CI | | Sp. r | p |
| 10 | 0.01299 | 0.8714 | | 0.7593 | | 68.27 | 0.5354–0.9692 | | 0.8182 | <0.001 |

SE – standard error; r – correlation coefficient; S (y. x) – standard deviation of residuals from lines; 95% CI – confidence interval (lower–upper); Sp. r – Spearman rank correlation.

0.08646 and the Spearman rank correlation (Sp. r) 0.2143, versus 0.1430 and 0.0246 in raccoon dogs.

The regression and correlation statistical analysis of red fox and raccoon dog rabies positive cases (Table 3) in 1996–2005 and in different Lithuanian counties showed that in for the 10-year period the standard error (SE) was 0.1080 and the confidence interval (95% CI) was 0.8261–0.9902; the correlation coefficient (r) during this time was 0.9576 and the rank correlation 0.9515. The comparison between fox and raccoon dog rabies cases in different Lithuanian regions indicated the standard error of 0.2400 and the confidence interval (95% CI) 0.0302–0.7091; the correlation coefficient (r) was 0.6467 and the rank correlation 0.7091.

An increasing tendency of rabies cases in red foxes (Fig.) was diagnosed in April and October (152 and 164 rabies cases, respectively), but in raccoon dogs the number were 116 in May and 213 in November. During the March–May period there were diagnosed 26.9% of fox and 21.7% of raccoon dog rabies cases, versus 18.7% for foxes and 28.1% for raccoon dogs in the October–November period.

## DISCUSSION

In Lithuania, in 1996–2005 the number of hunted red foxes increased four times and of hunted raccoon dogs more than eight times. In the last four years there had been no investigations on red fox and raccoon dog population density in Lithuania. It can be speculated (30–35% of fox and 10–15% of raccoon dog populations statistically can be killed during a hunting season) that

Table 2. **Hunting data and rabies epidemiological status in red fox and raccoon dog populations in different Lithuanian counties in 1996–2005 (EM, SD, SFVS, 2005)**

| Counties | Šiauliai | Panevėžys | Klaipėda | Kaunas | Vilnius | Alytus | Marijampolė | Utena | Telšiai | Tauragė |
|---|---|---|---|---|---|---|---|---|---|---|
| **Red fox** | | | | | | | | | | |
| Hunted (x) | 15987 | 11129 | 9711 | 8882 | 6441 | 5143 | 4997 | 4284 | n/d | n/d |
| Rabies-positive (y) | 202 | 207 | 247 | 125 | 389 | 317 | 102 | 181 | 45 | 159 |
| n | SE | r | | r (sq) | | S (y. x) | 95% CI | | Sp. r | p |
| 8 | 0.0097 | –0.08646 | | 0.007476 | | 102.61 | –0.7458–0.6584 | | 0.2143 | >0.1 |
| **Raccoon dog** | | | | | | | | | | |
| Hunted (x) | 4887 | 3671 | 2881 | 2626 | 1694 | 828 | 920 | 3228 | n/d | n/d |
| Rabies-positive (y) | 180 | 148 | 221 | 215 | 266 | 228 | 63 | 335 | 35 | 142 |
| n | SE | r | | r (sq) | | S (y. x) | 95% CI | | Sp. r | p |
| 8 | 0.02350 | 0.1430 | | 0.02046 | | 86.198 | –0.6247–0.7702 | | 0.02046 | >0.1 |

SE – standard error; r – correlation coefficient; S (y. x) – standard deviation of residuals from lines; 95% CI – confidence interval (lower–upper); Sp. r – Spearman rank correlation.

Table 3. **Correlation in rabies-positive cases between red fox and raccoon dog populations in different Lithuanian counties in 1996–2005 (SFVS, 2005)**

| Years | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
|---|---|---|---|---|---|---|---|---|---|---|
| Red fox (x) | 26 | 46 | 72 | 130 | 272 | 221 | 274 | 388 | 201 | 327 |
| Raccoon dog (y) | 6 | 20 | 43 | 125 | 233 | 245 | 318 | 312 | 162 | 363 |
| n | SE | r | | r (sq) | | S (y. x) | 95% CI | | Sp. r | p |
| 10 | 0.1080 | 0.9576 | | 0.9171 | | 40.068 | 0.8261–0.9902 | | 0.9515 | <0.0001 |

| Counties | Šiauliai | Panevėžys | Klaipėda | Kaunas | Vilnius | Alytus | Marijampolė | Utena | Telšiai | Tauragė |
|---|---|---|---|---|---|---|---|---|---|---|
| Red fox (x) | 202 | 207 | 247 | 125 | 389 | 317 | 102 | 181 | 45 | 159 |
| Raccoon dog (y) | 180 | 148 | 221 | 215 | 266 | 228 | 63 | 335 | 35 | 142 |
| n | SE | r | | r (sq) | | S (y. x) | 95% CI | | Sp. r | p |
| 10 | 0.2400 | 0.6467 | | 0.4194 | | 73.138 | 0.0302–0.9073 | | 0.7091 | <0.01 |

SE – standard error; r – correlation coefficient; S (y. x) – standard deviation of residuals from lines; 95% CI – confidence interval (lower–upper); Sp. r – Spearman rank correlation.

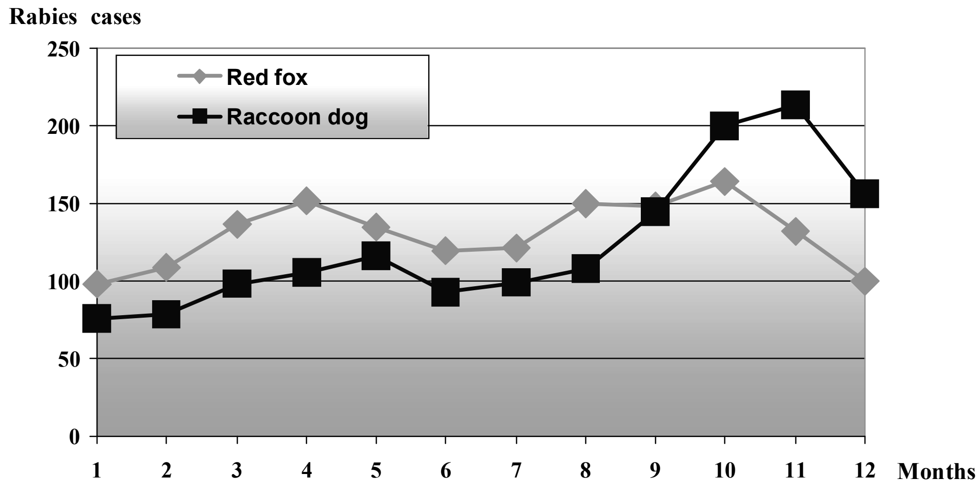


**Figure.** Seasonal dynamics of rabies cases in fox and raccoon dog populations in Lithuania, 2001–2005 (SFVS, 2005)

the fox population was growing very fast three times in 1996–2005), but the increasing tendency in the raccoon dog population was three times higher than in red fox. Hunting statistics are an acceptable indicator for the fox population trends at a regional or national level, provided that the records have been compiled consistently over the years and the hunting pressure has not changed greatly. Although the impact of hunting on the overall population is not very well documented, hunting could also affect the dispersal of animals (Breitenmoser et al., 2000). More accurate methods for measuring fox populations can be applied by a trained field ecologist in smaller areas, but such data cannot be extrapolated to a large area or an entire country. The most commonly used methods are the night counting index, road kills, line transect (EC, 2002).

In Lithuania, during the period 1996–2005 increasing tendencies in hunted foxes and raccoon dogs were very active, but they had only a minimal influence on real rabies situation in the country. In 1996–2000, laboratory-diagnosed cases prevailed among wildlife, reaching 570 in red fox and 433 in raccoon dog, while for the last three years the numbers are 916 and 837, respectively. In the last 5 years in the red fox population the 35–68% increasing tendency remained; the rabies cases in the raccoon dog population were 10% less, but with a 78–118% increasing tendency. Statistically, the correlation between hunted animals and rabies-positive cases, the same among red fox and raccoon dog in different years, shows a positive tendency because of the fact that in 1996–2005 more wildlife rabies cases were diagnosed and hunted animals showed a growing tendency (except 2004). The results were within a 95% confidence interval and considered very significant ($p < 0.001$). However, the number of specimens presented by hunters helps in the monitoring and surveillance of rabies epidemiology in different regions. The total number of submissions for rabies diagnosis has increased more than six times since 1996. This is probably related to the increased number of animals with suspected rabies and a higher public awareness of the danger to animals and humans (Mačiulskis et al., 2005). Recently in Lithuania raccoon dogs have become the most important wildlife infected with rabies, and in 2001–2002 there were more rabies cases in raccoon dogs than in foxes (Mačiulskis et al., 2005). The arrival of the omnivorous raccoon dog further complicates the control of red fox rabies in Eastern Europe. There is evidence that, during their winter hibernation, raccoon dogs can incubate rabies viruses and cause the disease to persist from one season to the next in geographical areas where fox densities are so low that rabies might otherwise die out (Finnegan et al., 2002).

Investigation of the rabies epidemiological situation in Lithuanian wildlife in 2004 indicated a significant reduction of cases (60–92%) and a threefold increase in 2005 versus 2004: 1108 rabies positive cases in 2003 (1989 tested), 553 cases in 2004 (2123 tested) and 1652 cases in 2005 of 3206 tested. This can be associated with the fluctuation of rabies as natural infection activity. Fluctuations in rabies incidence among red foxes are influenced by the density of the population in a locality, which varies because of the heterogeneous nature of the environment (Childs et al., 2000; Chautan et al., 2000). If a closed population is infected with rabies virus, the population will decrease until the density falls below the threshold value of rabies persistence (the minimum population density at which the disease can be transmitted). From there, the population will re-increase up to the carrying capacity of the habitat, following a sigmoid shape (EC, 2002). In a real situation (in a non-isolated fox population), a local increasing population will probably face a re-infection before it reaches the carrying-capacity density again, and will hence fluctuate in the longer term around the threshold value of rabies persistence (Breitenmoser et al., 2000).

Over the period 1996–2005, the highest prevalence of fox rabies cases was registered in Vilnius and Alytus counties (389 and 317 cases) and of raccoon dog rabies in Utena and Vilnius counties (335 and 228 cases, respectively), whereas the fox and raccoon dog hunting statistics were highest in Šiauliai and Panevėžys counties (15987 and 11129 hunted foxes, 4887 and 3671 raccoon dogs). Rabies cases in fox and raccoon dog in the counties were not directly connected with hunting statistics, therefore the linear and range correlation between the hunted animals and rabies-positive cases in red fox and raccoon dog in different Lithuanian counties was considered not significant ($p > 0.1$). Seven of 10 statistical results were beyond the 95% confidence interval and the correlation coefficient in the red fox group was negative. The population immunity and population structure in different regions and climatic conditions play one of the most important roles in rabies transmission. The epidemiological status of rabies in the population can be different: simultaneously there are susceptible, infected, infectious and immune foxes. The "infected" fox gets "infectious" after a negative exponential distribution with a minimum of two weeks and an effective mean of 3.5 weeks. During the following infectious period of one week, a fox can transmit the disease. It is assumed that infected cubs will die of rabies, but they can only transmit the infection if their incubation period ends after the dispersal period (Vos, 2003; Eisinger et al., 2005). For the spread of fox rabies under natural conditions, contacts between aggressive territory owners and impassive rabid intruders are the most likely scenario (Vos, 2003). Moreover, infected fox can transmit the virus to territory members during non-aggressive social contacts; non-bite exposure across mucous membrane is less efficient and rarely results in disease (Rupprecht et al., 2002). However, territoriality seems to play a key role in the spatial propagation of rabies. Every year 29% of the territory holders died in an undisturbed fox population (no hunting and infectious diseases) (Mulder, 2000).

The rabies incidence in Lithuania, as well as in Europe, shows a clear seasonal pattern. In the red fox and raccoon dog populations, rabies cases were tested during the whole year in ascending order, but with the endemic remission. The growing tendency in the red fox population can be identified in March/April and in October/November and in the raccoon dog population in April/May and October/November. The number of wild animal rabies cases increases in spring when foxes and raccoon dogs breed. Most fox cubs are born in early spring (March to April) and during this time up to 60% of the fox population are cubs. They are most likely infected by adult territory members, but are not involved in the maintenance of the chain of rabies infection and can be considered as the dead-end host (Vos, 2003). The raccoon dog breeding season is 1–2 months later, therefore the increase of rabies cases was observed in May (Gordon et al., 2004). After the mating season and when the vixens have selected a suitable den, territorial stability returns and territorial behavior is less pronounced than during the rest of the year. This behavior is reflected in the annual low in rabies number during late spring and early summer months (Dobson et al., 1996). The number of rabies cases already starts to increase in summer, thus considering a mean incubation period of 2–4 weeks. An increasing number of foxes becomes infected in late spring to early summer, when the movements of the cubs are still limited to the direct surroundings of the den (White et al., 1995; Niewold et al., 1999; Mulder, 2000). The overall increase in rabies incidence in autumn has often been linked to the onset of the dispersal season of the juveniles. In autumn, 25–30% of rabies cases are diagnosed in juveniles. However, in Europe most juvenile do not disperse over large distances (Vos, 2003; Goszcynski, 2002). Late autumn is the period of adult activity. During this time fat reserve is built up to overcome the food shortage encountered during the winter months. The confrontation between territory (food) owners and intruders during this time could be responsible for initiating the increase after the annual low in rabies incidence (Masson et al., 1999; Meek, Saunders, 2000). In the raccoon dog population, the increase of rabies incidence in autumn was initiated by top activity of all population (adults and the dispersal season of juveniles). During this time raccoon dogs are more active in feeding process before the winter hibernation period and have more contacts with rabid wildlife animals including active foxes in the same range (Gordon et al., 2004).

So far, rabies remains an epidemiological and economical problem in Lithuania. Controlling rabies needs a close cooperation of medical, veterinary and ecologist services, as well as careful information of the human society on the sources and pathways of rabies.

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## PASIUTLIGĖS EPIZOOTOLOGIJA LIETUVOS LAPIŲ IR USŪRINIŲ ŠUNŲ POPULIACIJOJE

S a n t r a u k a

Aprašoma pasiutligės situacija 1996–2005 metais lapių ir usūrinių šunų populiacijose, esant gamtinei pasiutligės persistentinei infekcijai. Per tyrimo laikotarpį sumedžiotų lapių padaugėjo 4 kartus (4051 gyvūnas sumedžiotas 1996 m. medžioklės sezoną, tačiau 2003 m. sezoną – 16949), o usūrinių šunų – 8 kartus (577 – 1996 m., 5215 – 2003 m. medžioklės sezoną), tačiau tai neturėjo įtakos pasiutligės epidemiologinei situacijai Lietuvoje. Per tyrimo laikotarpį Respublikoje buvo nustatyti 4404 pasiutligės atvejai tarp laukinių gyvūnų (45% lapių ir 41% usūrinių šunų populiacijose). Lapės išlieka pasiutligės vektoriumi, o epizootinė situacija ypač neramina: per pastaruosius 5 metus pasiutligės atvejų daugėjimo tendencija sudarė 35–68% skirtinguose regionuose, tuo tarpu usūrinių šunų pasiutligė buvo diagnozuojama 10% mažiau atvejų, bet daugėjimo tendencija šioje populiacijoje siekė 78–110%. 1996–2005 m. pasiutligė diagnozuota visoje Lietuvos teritorijoje: daugiausia Vilniaus ir Alytaus apskrityse (389 ir 317 atvejų), mažiausiai – Telšių ir Marijampolės (45 ir 102 atvejai). Pasiutligė diagnozuojama visais metų laikais, tačiau šios gamtinės infekcijos sezoninis aktyvumas buvo stebimas pavasarį ir rudenį: kovą–gegužę nustatyta 26,9% lapių ir 21,7% usūrinių šunų, o spalį–lapkritį – 18,7% lapių ir 28,1% usūrinių šunų pasiutligės atvejų. Teigiamas koreliacijos koeficientas nustatytas tarp sumedžiotų ir teigiamų pasiutligės atvejų tiek lapių, tiek usūrinių šunų populiacijose. Koreliacija tarp pasiutligės atvejų skirtingose Lietuvos apskrityse nenustatyta. Pasiutligės virusų perdavimo gamtiniuose židiniuose dinamikoje didžiulį vaidmenį vaidina populiacijos struktūriniai pokyčiai per metus. Perdavimo veiksniai identifikuojami visose amžiaus grupėse, tačiau ypač daug dėmesio skiriama lapiukų migracijai į naujas teritorijas ir patinų teritorinėms kovoms. Usūrinių šunų pasiutligės plitimui turi įtakos ypatingos šių gyvūnų prisitaikymo savybės, viruso persistentinės fazės ilgėjimas dėl žiemos miego ir rudeninės prieauglio migracijos sutapimas su aktyviu visos populiacijos mitybos periodu prieš žiemos miegą.

**Raktažodžiai:** Lietuva, pasiutligė, epizootologija, lapė, usūrinis šuo