Introduction

Rabies as a disease still represents a threat to humans and animals, and as zoonosis has to be reported and subjected to official control. Under the current law, dogs over three months old should be compulsory vaccinated and boosted every 12 months. The introduction of the rules reduced significantly the incidence of the disease in these animals (9). However, rabies virus has found a new effective host, and from the middle of the 50’s the red fox has become the main infected species (5). Systematic increase in the number of rabies cases in foxes in 1970’s and the lack of effectiveness of conventional methods of fox rabies control forced the course of action aiming at the disruption of the natural route of disease transmission by reducing the fox population density below a certain threshold, until oral rabies vaccination (ORV) of foxes against rabies has been introduced. ORV was carried out for the first time in 1993 in a limited area of Western Poland and in 2002 the whole area of the country was covered with vaccination. With the enlargement of the area of ORV campaign, the number of rabies cases decreased systematically in subsequent years (4, 6, 7). However, achieving total eradication of rabies, using ORV was more complicated than originally expected.

One of the crucial elements of ORV is analysis of the current rabies epidemiological situation on the territory of vaccine distribution. Based on epidemiological analysis correction/modification of ORV campaigns can be made.

The objective of this study was to describe and analyse epidemiological data regarding rabies incidence and recent trends of rabies in domestic and wild animals in Poland in 2009 and 2010 with visualisation of geographical distribution of rabies outbreaks.

Material and Methods

Analysis and evaluation of epidemiological situation of rabies in Poland in 2009 and 2010 were done on the basis of monthly reports sent to the National Veterinary Research Institute (NVRI) in Pulawy from regional laboratories.

Rabies diagnosis was performed in the veterinary regional laboratories (1, 2). Samples for the diagnosis have been collected from suspected animals (indicator animals) showing clinical symptoms of rabies, animals found dead, road kills, and animals, which bit or scratched humans. As a separate group, foxes shot for monitoring of ORV effectiveness, have been analysed for the presence of rabies virus antigen in the brain.

The unified FAT procedure was applied in veterinary regional laboratories authorised for rabies diagnosis. This involves impressions from different parts of the brain (cortex, cerebellum, Amon’s horn, and medulla oblongata) on microscope slides. The impressions were air-dried, fixed in cold acetone for 30 min or flame fixed. After the fixation, the smears were labelled with FITC conjugate (Bio-Rad) diluted according to manufacturer instruction, followed by incubation at 37°C for 30 min in a humid chamber. Then the slides were washed twice in PBS without Mg and Ca ions, rinsed in distilled water, dried, covered with buffered glycerine (pH>8.5), and coverslips. Positive and negative controls were prepared for each FAT run. Slides were examined in a standard incident light fluorescence microscope fitted with mercury vapour lamp at 200x to 400x magnification.
In case of negative FAT and human exposure, virus isolation in cell culture or mouse inoculation test were done to verify the result of antigen detection.

All FAT positive samples were checked for the presence of vaccine strains of rabies virus (SAD B19, SAD Bern) using the RFLP and sequencing. RT-PCR was performed according to previously described methods (3, 8). Based on the results of the sequencing, phylogenetic analysis of the rabies virus isolates was performed (10).

**Results**

In 2009, 24,859 samples from different animal species were sent to the regional laboratories to check the presence of rabies virus antigen. 24,112 out of 24,859 samples were suitable for rabies diagnosis.

3,130 samples have come from indicator animals and 20,982 from shot foxes provided for monitoring of the efficacy of ORV. In 2009, six positive cases were recorded in wildlife and two cases were found in bats. None of the cases was diagnosed in domestic animals. Among wildlife all cases were diagnosed in foxes. Two cases were diagnosed in monitoring foxes (0.01%).

In 2009, most of the cases except bat rabies were located in Eastern part of Poland on the territory of voivodeships bordering with Ukraine and Belarus (Rzeszow, Lublin, and Bialystok) (Fig. 1).

![Fig. 1. Distribution of rabies cases in Poland in 2009](image)

In 2010, 25,950 samples from different species of animals were sent to the regional laboratories to check the presence of rabies virus antigen. 25,081 samples fulfilled the conditions for rabies diagnosis. 3,424 samples came from indicator animals and 21,667 from ORV monitoring foxes. Rabies has been diagnosed in 151 animals. One hundred and
thirty-nine cases out of 3,424 samples (4.05%) were diagnosed in indicator animals, whereas 12 out of 21,667 samples (0.05%) rabies cases were found in monitoring foxes. Twenty-two (0.64%) cases were recorded in domestic animals, 123 (3.59%) in wildlife, and six cases (0.18%) were found in bats.

The highest number of rabies cases in domestic animals was diagnosed in: cats - eight cases (5.3%), dogs - seven cases (4.63%), cattle - four cases (2.65%), sheep - two cases (1.3%), and in horse - one case (0.67%). In wildlife, rabies most frequently has been diagnosed in foxes with 117 cases giving 77.5% in relation to the total number of rabies cases. Two cases have been found in stone marten (1.3%) and one case in raccoon dog, badger, and roe deer (0.7%), respectively. Six cases (3.97%) were recorded in bats.

Twelve cases out of 25,081 samples were diagnosed in monitoring foxes (0.05%). Analysis of the data collected in 2010 showed that the majority of animals infected with rabies virus has been located in the Krakow voivodeship and this was 118 cases. Other cases were located mainly in two voivodeships Rzeszow (13) and Lublin (12) in the zone directly bordering with Ukraine and Belarus. In Rzeszow voivodeship, three cases were located at the border with Krakow voivodeship probably as a result of passing rabid animals from one voivodeship to another (Fig.2).

**Fig. 2.** Distribution of rabies cases in Poland in 2010.

Rabies cases in bats were found in five voivodeships (Szczecin, Poznan, Olsztyn, Warszawa, Bialystok).

RFLP analysis showed that all cases of rabies were caused by field strain of rabies virus, which in phylogenetic analysis revealed the highest homology with the rabies virus circulating in Ukraine and Romania (data not shown).
Discussion

As a result of ORV, the rabies incidence drastically decreased during the past 20 years from 3,084 rabies cases in 1992 to eight cases in 2009, the lowest number of rabies cases ever reported in Poland (Fig. 3).

![Fig. 3. Number of rabies cases after introduction ORV in Poland.](image)

Rabies situation in Rzeszow and Lublin voivodeships is simply caused by a classical cross-border problem. In the case of these areas, the increasing rabies incidence in the neighbouring regions of Ukraine and Belarus resulted in permanent re-infection of the Polish site along the common borders. This situation forced the veterinary authority to safeguard the territory by maintaining a vaccination belt in those border areas. In 2009, 21 rabies cases less were diagnosed in comparison with the previous year 2008. No case of rabies was reported in domestic animals in 2009.

In 2010, an increase in rabies incidence was observed with 151 rabies cases recorded, exceeding the level reported in 2004. The reason for such a dramatic change in the situation of rabies epizootic was due to outbreaks of rabies in Krakow voivodeship. From August 12, 2010, till the end of the year, 118 rabies cases were diagnosed in different species of animals. However, vast majority of cases were recorded in the red fox – 94 in total. The outbreak involved 13 districts in Krakow voivodeship with the following number of affected animals: 94 foxes, six cats, six dogs, four cattle, three stone martens, one badger, two sheep, and one horse. Of the 13 districts where rabies occurred, two recorded the highest number of rabies (77%).

Because of the residual pathogenicity of the vaccine strains, a possible spill over the vaccine strain, being a source of the epidemic, was investigated. However, results of the study performed using RFLP method and phylogenetic analysis have confirmed that all isolates were classified as the field strains of rabies virus.

ORV has been conducted in this area from 1997 with the use of vaccines Fuchsoral (SAD B19) and Lysvulpen (SAD Bern). The last documented rabies case was diagnosed in 2004 in cat illegally imported from Ukraine. Since then, no cases have been reported.

Phylogenetic analysis of isolates from the outbreak shows their high similarity with the strains circulating in Ukraine and Romania, which may suggest the possible source of
origin of the rabies virus in the outbreak. This could have been caused by illegal import of pet animals.

To reduce the infection pressure in the area of rabies outbreak and to limit the spread of rabies among animals, as well as to the neighbouring districts and provinces it was decided to perform additional vaccination campaign on the territory of the outbreak and 50 km around the outbreak in November the same year. The campaign was carried out on the area of 5,029 km² with 119,520 doses of vaccine distributed by plane. On the territory of 1,636 km² (most infected area) distances between flight lines were reduced from 1 km to 0.5 km. This action (additional immunity barrier) resulted in stopping the spread of rabies from animal to animal and to adjoining areas. The epidemiological study carried out to date indicates that the number of cases in this area successively decreases. However, attainment of the state before the outbreak requires time and constant supervision of rabies in this region. The first case of rabies in the area was reported in fox. Striking is the fact of spreading of rabies mainly among foxes in this area, whereas the study of the efficacy of ORV campaigns in previous years indicated a high level of uptake of the vaccine and the high level of immunity in fox population. Analysis of the epidemiological situation in this area indicates that the weather conditions (heavy rains and floods) may have contributed to a weaker acceptance of the vaccine baits because of their limited availability. Therefore, the percentage of foxes immunised properly after the spring ORV campaign in 2010 in this area could be lower than usually, and this could have probably been the reason of the lower population immunity in foxes especially among young foxes.

Outbreak of rabies in the province where the disease was not recorded for the last seven years raises the question of the source/origin of the virus. The virus could come from the area where the last case of rabies has been recorded. However, the distance from that point (around 150 km to the East) and no traces of transmission largely exclude this hypothesis.

Possibility of illegal import of animals from other countries should also be taken into consideration. This hypothesis is supported by the results of phylogenetic analysis showing high homology of isolates from outbreak of rabies with isolates from Ukraine and Romania.

As in previous years, cases of rabies in bats were recorded. With the decline in the number of rabies cases in terrestrial animals, epidemiological importance of bat as a reservoir in the coming years will probably be growing. Therefore, increased attention is needed for supervision of rabies in bats and more information should be passed to people about rabies in bats.

Favourable decreasing trend of rabies in 2009 was stopped by the outbreak of rabies in Krakow voivodeship in 2010. This resulted in a dramatic increase in the number of cases of rabies. It should be expected that after the application of emergency vaccination in the zone of outbreak and increasing the number of vaccines per km² bordering areas with Krakow voivodeship, epizootic situation will improve and return to the state as before the outbreak. To achieve this condition strict supervision of all elements of the ORV and surveillance of rabies is necessary.

The presented epidemiological situation of rabies shows how difficult is the problem of rabies control in animals as well as management of the campaign leading to the reduction of rabies incidence among animals. Many factors that were not taken into account in this paper may significantly influence the results of the ORV campaigns. Therefore, it is necessary to analyse and take rapid action to eliminate the unfavourable influences or minimise the effects of their influence.

References