ABSTRACT: The thesis presents the analysis of the oral immunization effectiveness, taking cognizance of wild foxes. The preventive measures conducted 15 years ago have significantly contributed to the substantial decline in the number of verified cases of rabies among wild and domestic animals. Despite the fact, that aforementioned measures have been generating high costs covered by the state's funds, they dramatically reduced the risk of epizootic danger, thus epidemiological risks. Undertaken actions also led to the dynamic growth of population of foxes, which resulted in a kind of disruption of natural ecological system concerning the 'predator – prey' relation, therefore they have indirectly contributed to the deepening of the already ongoing contraction of the basic small animals.

KEY WORDS: fox, rabies, immunization, cost, effectiveness of vaccination
Introduction

One of the most dangerous animal-origin diseases, called zoonoses that accompany the man almost always, is rabies. As an infectious disease caused by RNA virus of the family *Rhabdoviridae*, genus *Lyssavirus*, that occurs in 7 biotypes, it is strongly pathogenic for humans, because it may lead to death due to respiratory failure. In ancient Greece, in the writings of Democritus and Aristotle, there is information about its occurrence and course. However, the first mention of the disorder, that in its symptoms resembled rabies, can be found in the Code of Hammurabi (1700 years BC). All warm-blooded animals are sensitive to infection, which is transmitted through bites of an animal excreting the virus with saliva. Thus, it is defined as a disease with a global reach and plays an important epizootic and epidemiological role (Smreczak 2007; Gliński 2016; Flis 2016).

It is the most common in developing countries in Africa and Asia, where the mortality rate among people reaches up to 96% of cases. In these areas, the access to health care is generally limited, while the estimates of incidence and mortality are not very accurate and usually do not reflect the scale of the problem. The presence of rabies is also quite a serious economic problem. Estimated losses on a global scale, associated with the occurrence of rabies, its diagnosis, prevention, treatment and loss of income due to illness, are estimated at more than 8.5 billion dollars a year (Hampson et al., 2015). Due to the fact of quite significant risk of occurrence and the wide possibilities of the spread of the virus, multidirectional attempts of preventive activities were undertaken for many years. Undoubtedly, the turning point was the second half of the nineteenth century, when the first vaccination of human chewed by a dog with symptoms of rabies, was performed in 1885 (Buczek, 1999; Flis et al. 2017; Smreczak, 2007).

In our country, the first attempts to limit so-called street rabies, were undertaken after the end of World War II. The compulsory vaccination of dogs, that were the primary reservoir for the virus, was introduced then. Within the 8 years, more than 900 thousand dogs were subject to vaccination, each year. During this period, the number of rabies cases decreased from 3682 in 1949 to 65 in 1956 (Stryszak, 1957). Despite the high efficiency of operations, they have proved to be ineffective in the long term. In the second half of the 60s, there was observed yearly increase in the number of ascertained rabies cases, especially in wild animals, mostly foxes. Due to the shift of the virus reservoir towards wild animals (so-called forest rabies) and the lack of effective instruments of prevention, the main methods of containment and eradication of rabies was the creation of protection and surveillance zones. The circles were marked, and prohibition to release domestic
animals was used in subsequent years, especially dogs and cats. In addition, sanitation hunting was organized on these areas. These treatments were not very effective, and the annual number of cases ascertained in animals was high, and some fatalities were reported in humans. Therefore, the pilot program of oral immunization of wild foxes, combined with the screening activities towards efficiency of its use, has been introduced since 1993 in western Poland. Due to the high efficiency, the program has covered all over the country since 2002 (Buczek, 1999; Mól, 2001; Mól, 2004; Flis, 2009; Flis et al., 2017). The effectiveness of oral immunization of foxes is high, since from its inception the number of ascertained cases of rabies in the country, despite the intrinsic fluctuations between years and regions of the country, shows a downward trend (Buczek, 1999; Bombik et al., 2014; Flis, 2016). At the same time, these activities are associated with relatively high costs of purchase and lining the vaccine (Flis et al., 2016).

Prevention efforts conducted over a period of fifteen years, have contributed to a significant reduction in the number of the virus cases in wild animals and virtually eliminated its occurrence in domestic animals. At the same time, they also contributed to a significant increase in the population of free-living foxes. Despite the increasing hunting pressure on this species, during the past four decades, the state of this species population in the country increased by slightly more than 3-fold (Bombik et al., 2014; Flis, 2013, Flis et al., 2016; Flis et al., 2017; Goszczyński et al., 2008; Kamieniarz et al., 2008; Panek, Bresiński, 2002).

Material and methods

Material for study consisted of data from the Central Veterinary Inspectorate on the preventive vaccination in Poland over the last five years. These vaccinations are carried out twice a year. When no rabies cases are not indicated in a given province for subsequent two years, they are limited to one immunization annually. On the other hand, when the province does not note the rabies cases over subsequent three years, immunization treatments are not carried out until finding the first case in a given area. Vaccine in the form of blisters surrounded by a bait mass, which includes fish meal, coconut oil and paraffin, as well as tetracycline as a biomarker, is discharged from the aircraft. Where conditions do not allow, it is manually distributed. Two vaccines are the most commonly used for vaccination: Fuchsoral (SAD B19) or Lysvulpen (SAD Bern). The number of vaccine doses is dependent on the afforestation degree and terrain sculpture, estimated population of wild animals – especially foxes, and the method of delivery. It is recommended not less than 20 doses per 1 km² area covered by the immunization action...
Collected data included the monitoring results on the number of ascertained rabies cases in wild and domestic animals, as well as costs of vaccination and subsequent monitoring of their effectiveness. This monitoring is conducted on a sample of 8 foxes culled from every 100 km², where an immunization is carried out. Foxes necessary for the analyses are provided by hunters. The research is based on three methods. The first allows to determine the presence of rabies virus in the collected material and is based on immunofluorescence of the brain imprints. Second, checking the presence of a biomarker contained in the vaccine bait confirming its acceptance, is based on the facets of the jaw bone at acquired foxes. And determination of antibody titers of the rabies virus in serum collected from heart blood clots or chest fluid, through the use of serological tests (RFFIT or ELISA), allows for the conclusion that animals are immune. These tests allow to determine the number of animals that are the virus vector and those that have been in contact with the vaccine and acquired the immunity by producing antibodies.

Analysis of the vaccination costs and subsequent monitoring was carried out on the basis of individual groups of expenditure incurred on these projects.

The effectiveness of vaccination in terms of ecology was also assessed. The analysis included issues related to the functioning of fox population in the changing ecological systems.

**Results**

**The presence of rabies in Poland**

In the last five years, rabies was found in both wild and domestic animals. In wild animals, after an increase between the first two years of studies, a gradual decrease in the number of ascertained cases was noted in the following years. It can be confirmed by the equation of the trend line \( y = -22.4x + 202.2 \) and determination coefficient \( R^2 = 0.3825 \). The main reservoir of the virus were wild foxes, at which during the evaluation period were diagnosed almost 86% of cases among wild animals and just over 70% of all findings of rabies in animals in this period (figure 1). In addition, rabies was diagnosed in other species of carnivores and 11 cases in wild ruminants (fallow and roe deer), as well as 26 cases in bats. In the same period in pets, the primary reservoir of the virus were dogs and cats with 2/3 of all diagnosed virus cases (figure 2). Rabies has also been found in 35 cattle, one sheep and one pig. At domestic as well as wild animals, a downward trend in the virus prevalence occurred (\( y = -5.4x + 45.8; R^2 = 0.5178 \)).
Figure 1. Presence of rabies in wild animals in Poland in 2011-2015

Figure 2. Presence of rabies in domestic animals in Poland in 2011-2015

The effectiveness of immunization

In the last five years, the efficacy of the immunization should be considered as high. The vaccine uptake indicator assessed by the presence of biomarker contained in the vaccine bait ranged from 86.04% to 89.09% in the last five years (figure 3). These results confirm that only every 10 fox had no
contact with the vaccine. On the other hand, fox immunization index values assessed by the presence of antibodies in the serum from heart clots and thoracic fluid showed a decreasing tendency of seropositive results in terms of antibodies against the rabies virus. In the first four years of evaluation, the percentage of foxes, with detected antibodies ranged within 75.11-79.49%. In the last year of assessment, when the study was conducted only by ELISA test, this ratio decreased to the level of 54.1%. The results of the last year are clearly different from previous ones, yet it can be determined by the type of the test used, which has higher sensitivity and slightly less specificity. Thus, despite the fact that this test is widely used in most European countries, its use can lead to an increased risk of false negative results. In addition, the cause of decline in immunization of foxes in the last year of the study may be that in some provinces only autumn immunization was carried out in this period, and thus juveniles obtained for testing during summer and early fall, could not have any contact with the vaccine.

Figure 3. Effectiveness indicators [%] of oral immunization of free-living foxes
The costs of vaccination and monitoring

During the last five years, the costs associated with the oral immunization of foxes decreased (figure 4). This was due to the decrease in the number of provinces, where vaccinations were carried out, as well as in some of them, the actions were performed only once a year. In the first three years of the study, the average annual costs of carrying out the action were slightly higher than 48 million PLN. In 2010, they decreased to 32.4 million PLN, while in the last year, they amounted to 20.1 million PLN. Overall, in the group of expenses incurred to carry out the immunization, slightly more than 60% are expenses related to the purchase of vaccines.

![Figure 4. Costs [% and PLN] of purchase and distribution of vaccine during the study](image)

Second, no less important group of costs necessary for the annual preventive measures, are those related to monitoring. These include the payment for foxes provided by hunters for testing, as well as costs of carrying out various tests and laboratory analyses by the veterinary hygiene centers. During the study period, the overall rate of these costs decreased slightly more than 1.5-fold (figure 5). On average, they were shaped at 2.7 million PLN annually. In this group of expenditures for monitoring, the share of costs associated with laboratory tests ranged from 71.4% to 80.3%. The remaining group consisted of costs for paying for foxes delivered for testing. Overall,
during the five-year study period, the total cost of monitoring the effectiveness of vaccination amounted to 13.5 million PLN and in the general pool of all costs associated with immunization, they accounted for less than 7%.

Figure 5. Costs of biological material and laboratory tests [% and PLN] in the five-year study period

Ecological effects

Evaluation of the vaccination effectiveness through the ecological lens reveals that conducted operations significantly affected the dynamics of fox population. This was due to the fact that through the immunization of animals, the basic factor of mortality in this species, which until recently was rabies, was eliminated. In the past five years, the size of fox population remained at a high level exceeding 200 thousand individuals (figure 6). This resulted in the enhancement of hunting pressure on this species. The intensification of culling assessed by increasing value of the indicator of hunting exploitation of that species by 17.4%, limited further growth, and even contributed to a slight decline. This is supported by the equation of the trend line and determination coefficient \((y = -2.57x + 213.83; R^2 = 0.6667)\). Despite of this, persistently high numerical states of this species negatively affect the
functioning of many populations of small animals. Predation by foxes is mentioned as the primary factor contributing to ongoing for a number of years, recourse of hare, partridges and pheasants population.

![Graph showing dynamics of abundance and hunting acquiring of foxes in Poland during the study period](image)

**Figure 6.** The dynamics of abundance and hunting acquiring of foxes in Poland during the study period*

* – according to data GUS

61,3** – indicator of hunting population exploitation

**Conclusions**

Conducted research and analysis made it possible to formulate the following statements and conclusions:

1. Oral immunization of wild foxes against rabies is characterized by high efficiency, which is confirmed by the continued decline in the number of annually ascertained cases of rabies in wild and domestic animals. This is also confirmed by indicators of the vaccine uptake and the immunization level of foxes annually examined in the framework of the monitoring.

2. Analysis of fairly high cost of vaccination policy, which is covered by the state budget, suggests that its level depends on the size of area they cover.
and the number of actions held on an annual basis. The highest component of these costs are related to the purchase of vaccines and their distribution. On the other hand, costs of monitoring tests accounted for only 7% of the overall incurred costs to carry out the immunization.

3. Analysis of the vaccination effectiveness in ecological terms suggests that protective immunization effectively eliminated the virus from environment. The negative aspect is an excessive increase in the number of foxes, which resulted from the elimination of recently basic mortality factor, which was the rabies virus. In turn, the high numerical states of foxes contribute to increased predation on small animals that for many years has been experiencing a population regression.

4. The oral immunization of foxes performed for fifteen years, has contributed to the decrease in epizootic and thus epidemiological risk, and also contributed indirectly to undermined the ecological system predator-victim. In this situation, the only solution seems to be to continue the initiated, annual intensification of fox acquiring by culling.

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