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Original article

RABIES IN POLAND IN 2010–2019: A NEW VIRUS RESERVOIR

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Summary

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The paper presents the epizootic situation of rabies in Poland during the last decade. During this period, the annual oral immunisation of free-living foxes was continued, but in recent years it has only covered selected regions. This was due to the decreasing number of virus cases found in free-living foxes, confirmed by 2019, when the virus was found in only one fox in the Lublin Province. The results indicate that despite high costs, oral fox immunisation (ORV) treatments have had the desired effect by eliminating rabies in terrestrial mammals. At the same time, an increasing tendency for virus occurrence in bats was noted during this period. These animals have now become the primary reservoir of the virus in Poland. In addition, in 2019 rabies in bats was found in voivodeships that were considered virus-free, and thus no preventive vaccination of foxes was carried out in them. This confirms the thesis that there are no geographical barriers to the spread of the virus. These elements are quite relevant and are very important in the field of epizootic threat as well as public health.

Key words: bat, fox, oral immunisation, Poland, rabies

INTRODUCTION

Despite various administrative and veterinary activities in the field of virus prevention and control, rabies remains one of the most dangerous zoonoses in the world. According to the World Health Organization, it kills annually about 60,000 people, of which 40% of cases involve children under 15 years of age. For several years, the highest incidence, and thus also mortality, reaching 95% of all deaths in the world as a result of this disease occurred in Africa and Asia, where dogs remain the main reservoir of the virus (Rupprecht *et al.,* 2002; 2004; Knobel *et al.,* 2005; Gliński & Kostro, 2013; Satora *et al.,* 2018).

Rabies is a zoonotic infectious disease of the central nervous system caused by neurotropic viruses of the genus *Lyssavirus*, which, along with other genera, belongs to the *Rhabdoviridae* family within the Mononegavirales order. Despite the fact that in February 2019 the International Committee on Virus Taxonomy (ICTV) updated the taxonomy of the Mononegavirales order, within the

genus *Lyssavirus* there were no changes. It includes 16 classified species, and two more are pending classification (Amarasinghe *et al.,* 2018; Maes *et al.,* 2019).

Poland is a European country where a number of extensive activities related to the eradication of the virus have been carried out for many years both in the natural environment and in pets. However, due to its geographical location, it is undoubtedly a meeting point of regions with intensive and usually effective preventive actions in Western European countries and the lack of such actions or their limited nature in Eastern European countries. Thus, in such conditions, controlling the virus is quite difficult and requires taking decisive, and at the same time high-cost initiatives necessary to eliminate the virus from the environment, and thus remove the epizootic and epidemiological threat (Flis *et al.,* 2018; Flis, 2020).

Due to the fact that in the natural environment the virus occurs almost exclusively in carnivores, until the beginning of the 20th century in Poland dominated the so-called street rabies, where stray dogs and cats were the main source of infection. However, the administrative and practical actions taken to conduct the annual mandatory vaccination and the elimination of stray animals have led to almost complete elimination of the virus in domestic animals. However, soon the successfully eliminated "urban" virus found a new reservoir in free-living foxes and other wild carnivores (Mól, 2004; Smreczak, 2007). The greatest development of this form of the disease occurred in the post-war period, and that is why in 1949 the obligatory immunisation of dogs – the main reservoir and also a vector of the virus, was introduced. In 1948, a total of 3,600 cases of rabies were found in Poland (Mól, 2001). In subsequent years, an upward trend in the number of cases was observed, and dogs were the main reservoir (Mól, 2004). Starting from the mid-1950s, there has been a dynamic increase in wildlife incidence. This was directly related to the epizootic of this disease in the former German Democratic Republic (Serokowa, 1962). In subsequent years, a further increase in rabies cases was observed, mainly in wild animals. In the period 1986–1997 on average, it was about 2.5 thousand cases, of which $^{2}/_{3}$ were found in free-living foxes (Seroka, 1998; Flis, 2019).

The wide range of occurrence, both in Poland and in many European countries, caused that in the 1960s the concept of oral immunisation of free-living foxes – ORV – was developed. The first field studies on this issue were carried out in Switzerland in 1978, and the following 1983 in Germany (Rupprecht *et al.*, 2004; Flis, 2020). In Poland, oral immunisation campaigns began in 1993. Initially, it covered the voivodeship of the western part of the country, and since 2002 vaccinations have been carried out throughout Poland. The vaccine was administered twice a year, mainly in forest areas or other places where foxes lived. Then, monitoring was carried out to determine the effectiveness of vaccination through research, and initially 8 and later 4 foxes from every 100 km^2 of the area were affected by the immunisation (Flis, 2018; Anonymous, 2019). Beginning with the period of oral immunisation throughout Poland, the number of detected cases of rabies has been significantly reduced in wild animals and has been virtually eliminated in domestic species (Sadkowska-Todys & Łabuńska 2002; Smreczak *et al.,* 2012; Flis & Rataj, 2018).

Oral immunisation of foxes eliminated the virus in many European countries, including Poland, in both wild and domestic terrestrial mammals, but the virus has found a new reservoir – bats. It is worth emphasising that bats have several strains of the virus, which is mainly determined by their geographical location (Kuzmin *et al.,* 2005; Ceballos *et al.,* 2013; Moldal *et al.,* 2017; McElhinney *et al.,* 2018; Nokireki *et al.,* 2018; Picard-Meyer *et al.,* 2019; Smreczak *et al.,* 2020). An analysis of the prevalence of rabies in Europe over the past decade has shown that despite the downward trend, in 2019 bats were diagnosed with the virus in 6 countries, and most cases were found out in Poland and Germany (Flis, 2020).

MATERIALS AND METHODS

Material

The material for work was the data of the Chief Veterinary Inspectorate regarding the occurrence of rabies in Poland, in wild animals, domestic animals and bats in 2010–2019. Data from the results of monitoring studies regarding the effectiveness of immunisation activities were also used. On their basis, the rate of vaccine uptake by foxes and the rate of immunisation of these animals against rabies virus was calculated. The costs of oral fox vaccinations were broken down into vaccine purchases and the costs of laying them out in the field were also presented. Based on these data, a geographical pattern of rabies in the last year of the study period was also prepared.

Research

Monitoring studies consisted of collecting brain tissue, serum and mandibles from shot foxes. The presence of the virus in the collected biological material was confirmed by brain imprint immunofluorescence. On the other hand, tests of the bones of the jaw and the use of seroneutralisation tests – rapid fluorescent focus inhibition test (RFFIT) and enzyme-linked immunosorbent assay (ELISA), allowed the determination of the presence of a marker located in the vaccine bait and the name of the virus antibody from serum isolated from heart clots from shot foxes. In a situation where no rabies cases were found in a given province in two consecutive years, the vaccine was lectured once a year. When no rabies was found in a given voivodeship in 3 consecutive years – then vaccination was stopped (Flis, 2018; Anonymous, 2019).

Analysis

The obtained data regarding the occurrence of the virus in Poland and vaccine collection by foxes and their immunisation were compiled in graphic form. In addition, a geographical pattern of rabies occurrence in the first and the last year of the assessment period was made, to outline the regions with the greatest threats and trends of changes and the current epizootic picture of rabies in Poland, taking into account the specificity of animals.

RESULTS

Rabies in wild and domestic animals

Starting from 2010, the number of rabies cases in Poland, after a dramatic drop in previous years, began to increase rapidly. In 2010, 151 cases of the virus in animals were recorded, and at the same time the virus appeared more and more frequently in bats (Table 1). The increase in virus

Table 1. Number of rabies cases in animals during the last decade

Rabies	Years									
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Total number of cases	151	160	257	204	105	97	22	10	9	11
Total number of cases in bats	6	4	3	8	7	4	6	8	5	10

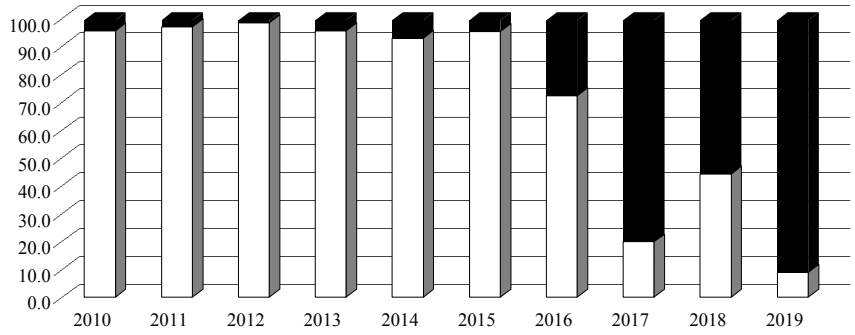

Fig. 1. Occurrence of the virus in bats (blackbars) in all cases (white bars) found during the last decade.

cases was associated with the rapid outbreak of the epidemic in the Lesser Poland Voivodeship, where in 2010, slightly more than 78% of all rabies cases in Poland were found. The upward trend continued until 2012. In subsequent years, the annual downward trend was recorded until 2017, when only 10 cases of rabies were found in Poland. In the next two years, the number of cases found was similar. At the same time, annually a virus was found in bats with an increasing share of this species as a virus reservoir (Fig. 1). In the years 2010–2015, the percentage of rabies cases recorded in bats was at an average level of 3.7%. In 2016, it increased to 27.3%, and in 2017 to 80.0%. In 2019, this percentage was 90.9%.

Although rabies virus in bats has been found for many years, in the period from 2002, when the action of oral immunisation covered the whole of Poland, the share of this group of animals as a virus reservoir was not significant, as it was from 2 to 9 cases per year, which in association in the number of cases in foxes represented a low percentage of participation. However, the assessment of the number of cases of rabies found annually in the long term ($y=0.1218x+4.2876$) indicates a slow but growing trend of the share of these animals as a potential source of infection. Thus, along with the radical decline in virus statements in foxes, the bat becomes the species constituting its primary reservoir. In addition, in 2019, apart the provinces of eastern Poland, rabies in bats were also recorded in Masovia region, Kuyavia region and most cases in Greater Poland,

where a year earlier the virus was found only in 2 bats. It is worth emphasising that these are regions in which currently no immunisation activities are carried out, and some of them were described as virus-free.

Vaccination effectiveness

Studies on vaccine uptake by free-living foxes indicate that over the past decade this rate has remained at a level close to 90% (Fig. 2). In turn, during the same period there was quite a significant variation in immunisation of foxes. The results of the monitoring studies carried out indicate that the immunisation rate ranged from 50.05% to 85.90%. The average value of this indicator over a ten-year period was 65.26%.

Vaccination costs

The analysis of the costs of annual preventive vaccinations showed their considerable diversity in individual years (Table 2). It depended mainly on how many voivodeships these actions were

Table 2. Costs for oral immunisation of free foxes over the past decade*

Year	Cost of purchase	% overheads	Lecture cost	% overheads	Totality
2010	6,431,744.85	65.0	3,470,860.65	35.0	9,902,605.50
2011	7,342,742.62	63.9	4,148,825.41	36.1	11,491,568.03
2012	7,915,538.68	60.0	5,278,018.78	40.0	13,193,557.46
2013	7,368,673.83	63.8	4,176,727.70	36.2	11,545,401.52
2014	5,572,362.82	68.7	2,535,524.90	31.3	8,107,887.71
2015	2,528,158.91	50.2	2,507,792.54	49.8	5,035,951.45
2016	2,019,417.31	32.0	4,297,206.09	68.0	6,316,623.40
2017	1,692,553.58	30.2	3,919,723.04	69.8	5,612,276.61
2018	2,914,724.63	43.8	3,733,072.71	56.2	6,647,797.34
2019	2,384,144.28	43.7	3,076,674.95	56.3	5,460,819.23

* The average euro price of 4.0 PLN was used for the calculations.

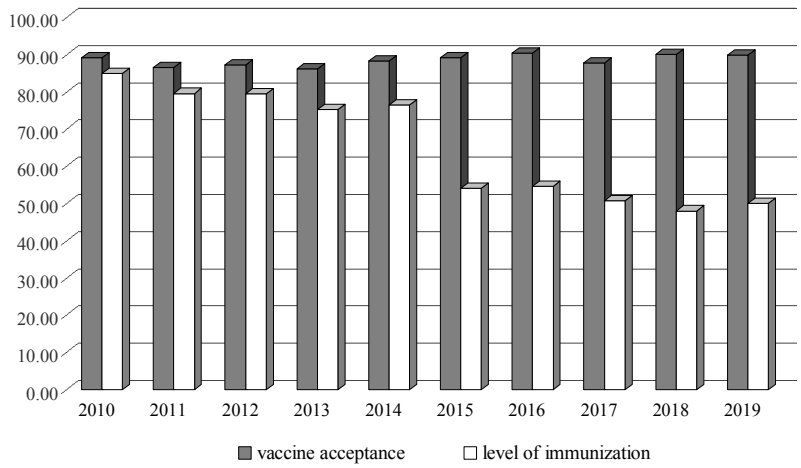

Fig. 2. Vaccination received by free-living foxes and an indicator of their immunisation against the virus during the last decade.

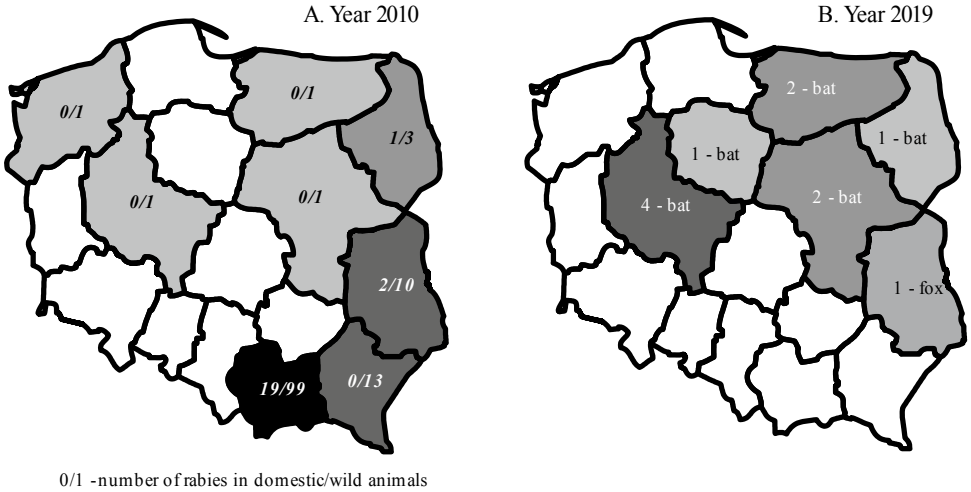

Fig. 3. Geographical pattern of rabies in Poland.

carried out, as well as whether one or two rounds of vaccination took place. The average annual total for Poland was slightly above 8.3 million EUR. In the period of the analysed decade, these costs showed a downward trend, which was conditioned by the elimination of the virus in individual voivodeships, and thus the reduction in the number of vaccinations or complete abandonment of immunisation. On average, 52% of the cost was the purchase of a vaccine. However, a certain pattern can be seen that with the decrease in the number of vaccinations carried out, the cost proportions have changed. Purchase of vaccines has fallen significantly in overhead costs, and distribution costs increased in the field.

Geographical pattern of rabies in Poland

In 2010, rabies was found in both wild and domestic animals. In domestic animals, there were 22 cases in total, most of which in cats (36.4%) and dogs (31.8%). The virus was also found in 4 cattle, 1 horse and 2 sheep. In wild animals, the basic reservoir of the virus remained free-living foxes, which were diagnosed in a total of 117 cases, which was just over 90% of all cases found in wild animals. During this period, the virus was diagnosed in 8 voivodeships, with particular intensity in the Greater Poland Voivodeship (Fig. 3A). Due to the decrease in virus findings in individual voivodeships in 2019, oral immunisation was carried out only in 8 voivodeships, with 4 in them, only in selected regions of those voivodeships. During this period, out of 11 cases of rabies, 10 of them concerned bats. In addition, rabies was found in 6 voivodeships (Fig. 3B), of which in three of them no immunisation was carried out, and in the fourth only in selected areas. The only case of rabies in a fox occurred in the Lublin Voivodeship.

DISCUSSION

Oral immunisation of free-living foxes in Poland, conducted comprehensively since 2002, has eliminated rabies in both wild and domestic animals. Despite the high costs of this type of preventive measures,

which is also confirmed by reports from other countries, they were very effective, which is demonstrated by the results presented as well as the results obtained in other countries (Freuling *et al.,* 2013; Cliquet *et al.,* 2014; Flis *et al.,* 2018; Sartore *et al.,* 2018; Flis, 2019; 2020).

The results of monitoring studies in countries where oral immunisation procedures were used show high level of seroconversion in foxes, often exceeding 50%. Also the biomarker rate of tetracycline indicating vaccine intake is high and reaches up 90%. Studies conducted in Italy in 2009–2016 have shown the fox vaccine to be in the range of 70.1% to 95.5% (Sartore *et al.,* 2018). In Greece, in turn, the seropositivity of foxes, where vaccinations were carried out, was 60% (Korou *et al.,* 2016), while seropositivity in Croatia in 2011–2012 ranged from 11.24 to 35.64% and the vaccination rate – from 24.86 to 84.62% (Bedeković *et al.,* 2018). In the Baltic States, the seropositivity rate, depending on the location and time of the study, ranged from 30.0% to 73.0%, and the average level of vaccine intake was 80.0% (Robardet *et al.,* 2016).

In earlier years, rabies in Poland was found mainly in the eastern and southern voivodeships of the country, which was associated in particular with the lack or low effectiveness of vaccination of free-living foxes in Ukraine (De Benedictis *et al.,* 2008). Although vaccination covered the entire region along the Polish border in the years 2012–2013 in Ukraine, this did not significantly affect the occurrence of rabies in the eastern regions of Poland, especially in the Subcarpathia Voivodeship, directly bordering Ukraine (Flis, 2015; Polupan *et al.,* 2019). Of all European countries, except for the Russian Federation, Ukraine is characterised by the highest incidence of rabies in Europe, which is why there are quite wide, cross-border possibilities of virus transmission to Poland (Polupan *et al.,* 2019; Flis, 2020).

Currently rabies has been virtually eliminated in terrestrial mammals, both wild and domestic. However, the increasing number of bat cases confirms that the virus is still present in the environment. Depending on the geographical location, bats have been found to produce rabies. Most often it is the European bat lyssvirus 1 (EBLV-1) strain, which is found especially in the late moth bat (*Eptesicus serotinus*) and the desert moth (*Eptesicus isabellinus*). In addition, several other strains have been described that have been confirmed in bats in various European countries (Kuzmin *et al.,* 2005; Ceballos *et al.,* 2013; Moldal *et al.,* 2017; McElhinney *et al.,* 2018; Nokireki *et al.,* 2018; Picard-Meyer *et al.,* 2019; Smreczak *et al.,* 2020). Quite important in this respect is the fact that the clinical picture of rabies acquired from a bat differs from its picture in the case of its transmission from an infected dog, and thus its diagnosis is more difficult and the occurrence of the virus may be overlooked (Begeman *et al.,* 2018).

The described situation is worrying because despite the fact that after successful immunisation many European countries declared rabies free status, it appeared again at different time intervals. This confirms the constant presence of the virus in the natural environment and the absence of any geographical barriers to the spread of the virus to new areas (De Benedictis *et al.,* 2008; Tsiodras *et al.,* 2013; Cliquet *et al.,* 2014; Stahl *et al.,* 2014; Korou *et al.,* 2016; Sartore *et al.,* 2018).

CONCLUSION

The presented situation of the epizootic state of rabies in animals in Poland over the past decade is a confirmation that the preventive action of oral immunisation of free-living foxes (ORV) brought the expected effect. Despite the high costs of these activities, they led to the elimination of the virus in the population of domestic and practically wild animals, except for bats. At the same time, it should be noted that there has been a specific shift of the virus reservoir to bats. This condition creates further opportunities for the virus to spread to new areas, especially since there are currently no preventive measures to control it in this group of animals. Although the number of cases found in this species is small, these mammals, being a new reservoir of the virus, are also becoming its important vector, and thus this situation may increase the epizootic threat. It also carries an increase in epidemiological risk, especially due to the non-specific clinical picture of rabies acquired from bats. Due to the fact that the virus reappears in countries that have a rabies-free status, it seems necessary to constantly monitor the virus in wild and domestic animals. It is also necessary to initiate work on developing preventive measures for bats, especially since the virus in this group of animals is also found in countries bordering Poland (Ukraine, Germany).

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