



Spatial and temporal analysis of rabies and effectiveness of the oral rabies vaccination program in Ukraine

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Rabies has been known as a lethal disease since time immemorial. Nonetheless, modern modeling estimates suggest that this infection is a cause of death of almost 60 thou people around the globe every year. In Ukraine, rabies is also a relevant problem in veterinary and human medicine. In the early 21st century, the epizootic situation regarding this infection was characterized as intense with periodic disease outbreaks. As of now, the most effective modern way to combat rabies is oral vaccination of wild carnivores against rabies. In 2018, a large-scale campaign of vaccination of feral carnivores began in Ukraine. To identify the efficacy of this anti-epizootic event, we used a complex approach that included the use of all the control elements: surveillance of rabies spread, assessment of tetracycline marker, and identification of the immunity to rabies virus in foxes in the area of vaccination. In 2022, due to the Russian military invasion of Ukraine, the oral vaccination of wild animals was not conducted. Therefore, complex assessment of efficacy of vaccination is necessary to monitor achievements of the Veterinary Service of Ukraine in combating this infection. This paper, based on our epizootic, histological, and serological studies, presents the trends of spread of rabies cases in Ukraine, which were confirmed in laboratory studies in 2018–2022. Also, the paper demonstrates the downward dynamics of rabies in all species of the animals. Analysis of the efficacy of the oral vaccination campaign of wild carnivores against rabies revealed its efficiency. At the same time, to achieve the best effect, oral vaccination of wild carnivores against rabies should be carried out twice a year – in spring and autumn. Extending the analysis to include domestic carnivores (dogs and cats) revealed problems in the current system of rabies prophylaxis.

Keywords: spatio-temporal analysis; epizootic situation; oral vaccination of wild carnivores; anti-rabies antibodies; tetracycline marker.

Introduction

Epidemiological studies of the spread of rabies in the European countries, including Ukraine, demonstrated that the reservoir of the virus is in the wild, and that the red fox (*Vulpes vulpes*) is the main vector of rabies (Pastoret et al., 1999; Cliquet et al., 2004). The presence of a rabies reservoir in the population of those animals is what promotes the disease's transmission from foxes to other wild animals, and also from foxes to domestic carnivores (dogs and cats) (Blancou, 1988; Bourhy et al., 1999).

Given the widespread presence of rabies in wild populations, one of the most effective ways to combat this infectious disease is oral vaccination of wild carnivores against rabies – ORV (oral rabies vaccination) (Müller et al., 2015; Maki et al., 2017).

Provision of high efficacy of ORV became possible thanks to highly immune oral anti-rabies vaccines and air transport as a method of delivering vaccine-containing baits. Implementation of large-scale campaigns of oral rabies vaccination, which eradicated fox-caused rabies in countries of Western Europe, significantly reduced the number of new cases of rabies in animals. Elimination of rabies in countries of Western Europe is a good example of proper control of spread of this virus (Brochier et al., 1994;

Bugnon et al., 2004; Matouch et al., 2007; Müller et al., 2012; Bedeković et al., 2018; Čeme et al., 2021; Stankov et al., 2021). That is why, with financial support of the European Union, ORV programs continue to be implemented in many EU and EU-neighboring countries, including Ukraine (Cliquet et al., 2012; Yakobson et al., 2014; Lupulovic et al., 2015; Müller et al., 2015; Robardet et al., 2016; Lojkić et al., 2021).

In our country, rabies is a serious problem in veterinary medicine and the healthcare system in general. Rabies has been consistently reported across all of Ukraine's oblasts and natural-geographic zones. Attempts to oppose it using oral vaccination of wild carnivores in Ukraine have started about 20 years ago. Large-scale (in 18 of 25 oblasts) campaigns were conducted in 2006–2014. Since 2012, treatment has also been provided at the border between Poland and Hungary (Polupan et al., 2019). In 2018, a large-scale campaign of oral rabies immunization of wild carnivores in Ukraine was resumed. In 2018, 7,448,078 doses of vaccine were distributed across an area of 372,403.87 km². The drugs used were the anti-rabies vaccine Orisvak and the recombinant vaccine Brovarabis V-RG, manufactured by the Ukrvetprompostach Ltd.

In 2019, the campaign of oral immunization of wild carnivores covered 465,230.47 km², dispersing 11,630,764 doses of the Orisvak anti-rabi-

es vaccine. In 2020, the area of the vaccination effort accounted for 406,298.74 km², using 10,157,470 doses of Orisvak. In 2021, the area covered was 435,615.28 km², and 10,890,382 doses of Orisvak were used. Yearly campaigns were conducted once a year (March–April) (Lojkić et al., 2021). The bulk of baits with the vaccine were distributed using aerial transport (except the NOTAM zone – Noticeto Missions). Electronic metronome, connected to the global positioning system (GPS Control Distribution Unitgen.4, GPSCDU-4), regulated the frequency of dropping the vaccine taking into the account airplane speed, which ensured even distribution, equaling 25 baits per 1 km² (Müller et al., 2012).

In 2022, oral rabies vaccination of wild carnivores in Ukraine was not conducted due to the Russian invasion. In 2023, ORV in Ukraine was resumed. The spring campaign was carried out in only 4 oblasts in the area of 18,498 km² and the autumn campaign was performed in parts of territories of 18 oblasts, in the area of 120,463.31 km². However, due to the ongoing prohibition of using civilian aerial transport in Ukraine, baits with vaccine were distributed only by the means of ground transport.

Therefore, large-scale ORV in Ukraine requires complex assessment of its efficacy, considering all control elements: surveillance of rabies spread and assessment of tetracycline marker and seroconversion. The conducted analysis of manifestations of rabies enzootic during ORV can be

useful for assessing the tendency of enzootic development and improving the introduced national practical strategies of controlling the disease spread (ORV, parental vaccination, regulation of number of wild animals, combating feral animals, etc.). In this study, we identified the spatio-temporal trends of spread of rabies in Ukraine, based on cases that were laboratory-confirmed in 2018–2022. The results can be used for assessing efficacy of future ORV campaigns in Ukraine.

Materials and methods

Study area. Ukraine is administratively divided into 25 level-1 administrative units: 24 oblasts and 1 autonomous republic. Analysis of rabies cases was conducted throughout Ukraine, except the areas uncontrolled since 2014 as a result of the military conflict (Autonomous Republic of Crimea and southeast parts of Donetsk and Luhansk oblasts), where the Ukrainian authorities cannot monitor rabies and take measures against it. This territory accounted for 43,300 km², that is 7% of territory of Ukraine. Moreover, due to expansion of the conflict to other areas of Ukraine and ruination of laboratories of the State Food Service in 2022, the analysis contains no data on rabies cases in animals of Donetsk, Luhansk, Kherson oblasts, and a part of Zaporizhzhia Oblast in 2022 (Fig. 1).



Fig. 1. Study area with indication of Level 1 administrative units (oblasts) of Ukraine: shaded – the territory where no observations have been conducted since February 2022; the territory of the country is divided by a wide water barrier – the Dniro River

Surveillance data. The data on rabies cases in animals in Ukraine in 2018–2022 was collected by the state regional laboratories of the State Service on Food Safety. In laboratories, all the samples of the animals’ brain were analyzed for presence of the rabies antigen using the DFA method (direct fluorescent antibody test) (WOAH, 2024), a part of DFA-negative samples (in case of animal contact with humans) was studied using MIT (mouse inoculation test). During 2018–2022, regional laboratories of the State Service on Food Safety received 75,691 samples of animal brains for detection of the rabies antigen. The highest number of samples (57,085) was from foxes.

The bulk of the samples from foxes (53,860 samples of the brain, or 94.35%) came as a result of active surveillance (shooting foxes) as the

element of controlling ORV-campaign efficiency. The rest of the samples of the brain of wild and domestic animals (21,831) were obtained through passive surveillance of rabies, i.e. sent to the regional laboratories by veterinary workers.

The analysis does not present 40 cases of rabies that were identified by various regional laboratories in 2018–2022 but could not be geographically localized. The data for the statistical and spatio-temporal analyses of rabies cases included species of animals, administrative oblast where the case was found, coordinates of the settlement (or accurate coordinates if there was information with an address where the infected animal was found) where the samples were received from, and date of admission of the brain samples to the laboratory.

Descriptive statistical analysis. The data was classified into four categories: domestic carnivores (cats, dogs), foxes, other wild animals, and other domestic animals (Table 1). We designated foxes separately because they are the reservoir of rabies in the wild and the target animal of ORV (Blancou, 1988; Bourhy et al., 1999; Pastoret et al., 1999). Also, we classified domestic cats and dogs in a separate group because they are the animals to which rabies cases in people in Ukraine are linked the most. All other species are less significant in spreading the disease, with rabies occurring in single cases. Data on the cases in each administrative oblast in Ukraine are given in Table 2.

Data regarding sampling of domestic carnivores and foxes are presented on maps in Figures 2 and 3. We developed epidemical curves for each group of animals, reflecting the monthly number of positive cases (Fig. 4).

To analyze for presence of temporal trend in data on each group, we used the Seasonal Mann-Kendall trend test, realized in the Kendall pack in R (McLeod, A. I. (2022). Kendall: Kendall rank correlation and Mann-Kendall trend test. R package version 2.2.1. <https://CRAN.R-project.org>).

For each group of animals, we calculated the percentage of found positive results by years by dividing the number of positive samples by the total number of samples (Table 3). Exact binomial confidence intervals for percentage of positive samples was calculated using the function `binom.exact` from the pack `epitools` 0.5–10.1 (RCoreTeam, 2019) in the environment for statistical calculations R 4.1.0 (<https://desktop.arcgis.com/en/arcmap/10.4/tools/space-time-pattern-mining-toolbox/emerginghot-spots.htm>).

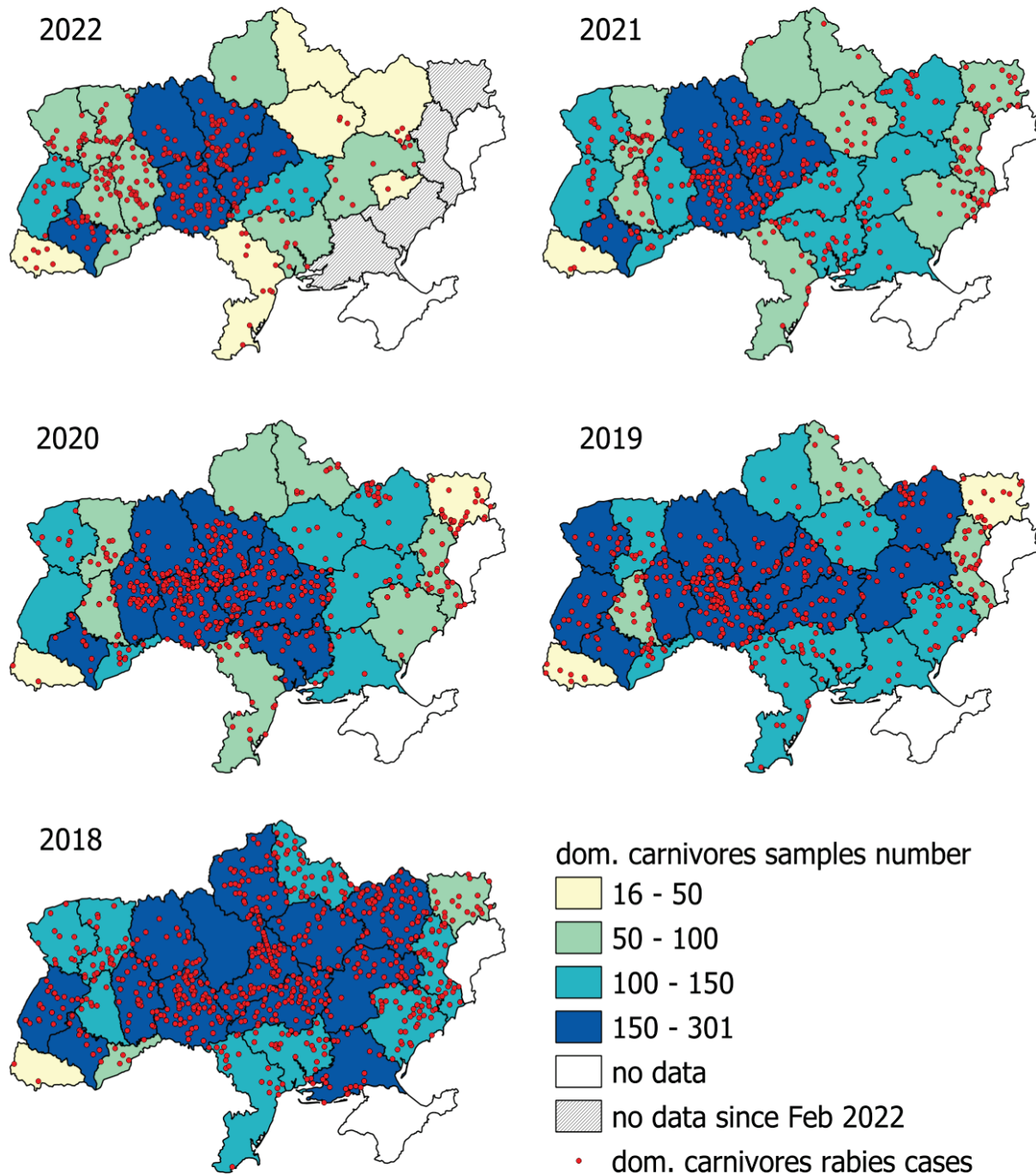


Fig. 2. Distribution of samples from domestic carnivores by oblast and year: red dots indicate rabies cases in domestic carnivores

Each group of animals was analyzed for difference in percentage of positive samples in 2018–2021 using the Pearson’s chi-squared test (χ^2) from the R 4.1.0 Stats Package (R Core Team, 2019). For paired comparisons of percentages of positive samples between years, we used the pair-

wise proportion test with correlation of P-values using Bonferroni’s method, implemented in the function `pairwise.prop.test` in the R Stats Package. We carried out no statistical tests to compare the 2022 data to the previous years because the area available for surveillance had reduced.

Emerging hot spot analysis. To assess the spatio-temporal trends of spread of rabies cases, we used the tool Emerging Hot Spot Analysis (EHSA) from the tool pack Space Time Pattern Mining Tools in the software ESRI ArcMap 10.4.

To conduct EHSA, the data on disease cases were grouped in bins of a selected size, which spatially covered the monitored area, and together with the temporal component of data we constructed a spatio-temporal cube. The chosen spatial size of the bin's side equaled 14 km – the average distance of rabies threat, estimated inferring from the potential threat of rabies spread in epidemically significant animals (foxes, dogs, cats, raccoon dogs, wolves, and mustelids) and their share in the rabies-morbidity structure. The temporal size of the bin equaled 2 months – the average du-

ration of the rabies incubation period. Grouping in time was carried out in the direction from the latest (31/12/2022) to the first date (1/1/2018) of the surveillance period – the Time step alignment parameter. Thus, the developed cube had 30 time steps – five years each, divided into six two-month steps.

To detect statistically significant clustering of events in the time space – clusters of high and low values (hot and cold spots), EHSA uses the spatio-temporal realization of the Getis-Ord G_i^* statistics, which considers each bin in the context of neighboring bins in space and time. Local mean value of a bin and its neighbors is compared to the mean of all cube bins; when such a difference is too great to be random, the result is statistically significant.

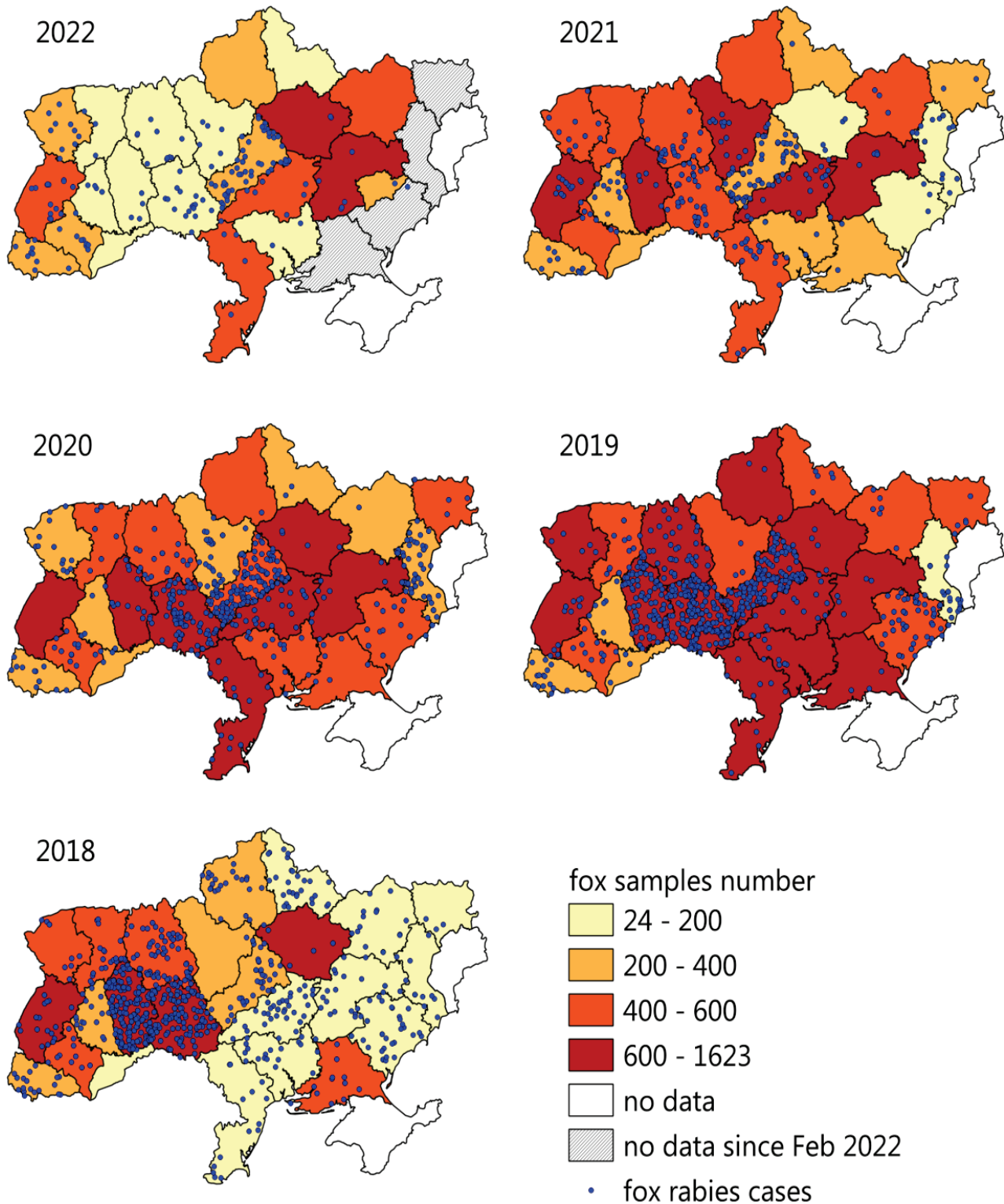


Fig. 3. Distribution of fox samples by oblast and year: blue dots indicate rabies cases in fox

Table 1

Number of samples tested positive by species 2018–2022 and percentage of positives in groups of animals for each year

Species	2018 n(%)	2019 n(%)	2020 n(%)	2021 n(%)	2022 n(%)	Total n(%)
Domestic carnivores	876 (47.4)	487 (34.3)	573 (46.7)	422 (55.0)	347 (59.3)	2705 (46.3)
Cat	462	276	292	230	184	1444
Dog	414	211	281	192	163	1261
Fox	737 (39.9)	811 (57.1)	552 (45.0)	277 (36.1)	179 (30.6)	2556 (43.7)
Other domestic	160 (8.7)	82 (5.8)	78 (6.4)	41 (5.3)	47 (8.0)	408 (7.0)
Bovines	128	71	63	34	38	334
Wild goats	29	9	12	7	7	64
Horses	2	2	2	–	2	8
Swine	–	–	1	–	–	1
Rabbits	1	–	–	–	–	1
Other wild	74 (4.0)	41 (2.9)	23 (1.9)	27 (3.5)	12 (2.1)	177 (3.0)
Squirrels	2	–	1	4	–	7
Beavers	2	–	–	2	–	4
Badgers	6	5	4	1	1	17
Otters	–	–	–	1	–	1
Wolves	8	6	–	1	–	15
Deer	1	–	–	–	–	1
Raccoon dogs	31	12	6	3	1	53
Hares	–	2	–	–	–	2
Hedgehogs	–	1	–	–	–	1
Wild boars	–	–	–	–	1	1
Bats	2	1	–	–	–	3
Roe deers	1	–	1	–	–	2
Martens	14	6	3	3	7	33
Weasels	–	–	1	–	–	1
Mice	–	–	–	–	1	1
Muskrats	1	–	–	–	–	1
Polecats	1	–	2	2	–	5
Hamsters	–	3	–	–	–	3
Jackals	3	4	4	9	–	20
Rats	2	1	1	1	1	6
Total	1,847	1,421	1,226	767	585	5,846

Table 2

Rabies cases by oblast, 2018–2022

Oblast	2018	2019	2020	2021	2022
Vinnytsia	263	346	226	107	81
Volyn	23	20	24	26	25
Dnipropetrovsk	86	24	23	22	14
Donetsk	57	75	102	56	no data
Zhytomyr	99	68	52	45	39
Zakarpattia	21	26	16	15	16
Zaporizhzhia	106	115	46	15	5
Ivano-Frankivsk	23	20	25	18	27
Kyiv	50	19	114	66	55
Kirovohrad	152	63	82	43	36
Luhansk	41	24	35	29	no data
Lviv	40	27	15	20	40
Mykolaiv	59	35	44	34	10
Odesa	46	37	46	43	15
Poltava	43	20	15	21	5
Rivne	52	27	17	30	30
Sumy	81	19	11	3	0
Temopil	26	27	15	32	29
Kharkiv	109	37	30	26	5
Kherson	46	12	15	10	no data
Khmelnyskyi	213	122	62	16	67
Cherkasy	116	221	197	84	81
Chemivtsi	6	25	10	5	4
Chemihiv	89	12	4	1	1

Using the Mann-Kendall trend test, EHSA detects statistically significant time trends in results of Getis-Ord G^* statistics, assessing where z -values of hot and cold spots increase or decrease with time. For this study, we did not specify the neighboring distance, but relied on the internal calculations of the tool which it makes from the data and extent of the study. In our research, this distance equaled 67.7 km. By default, contiguity in time was chosen as a value equal to the previous time interval.

We were interested in identifying where and with what trend rabies cases in 2018–2022 (when large-scale ORV campaigns were carried out) could be clustered. According to the estimates, the EHSA tool classifies each location to one of 18 kinds of trends. In our case, there were 3 (Table 4, Fig. 5). Description of the complete set of trends can be found on the website of this tool (<https://desktop.arcgis.com/en/arcmap/10.4/tools/space-time-pattern-mining-toolbox/emerginghotspots.htm>).

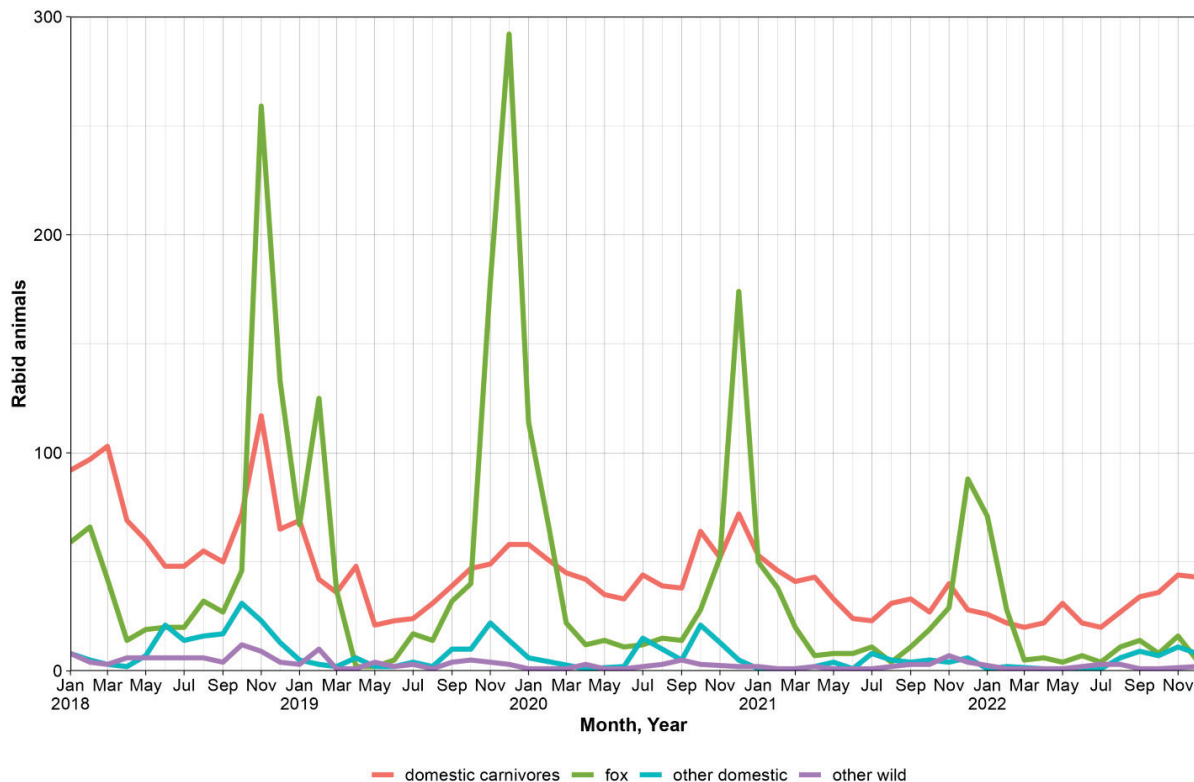
**Fig. 4.** Rabies cases in animal groups by month in 2018–2022

Table 3
Rabies cases by species group, 2018–2022

Species group	Year	Positive	Samples	Percentage of positives	95% CI
Domestic carnivores	2018	876	4,202	20.85	19.63–22.11
	2019	487	3,666	13.28	12.20–14.43
	2020	573	3,198	17.92	16.60–19.29
	2021	422	2,916	14.47	13.21–15.80
Fox	2018	737	8,904	8.28	7.71–8.87
	2019	811	16,861	4.81	4.49–5.14
	2020	552	13,963	3.95	3.64–4.29
	2021	277	11,192	2.47	2.20–2.78
Other domestic animals	2022	179	6,165	2.90	2.50–3.35
	2018	160	247	64.78	58.47–70.73
	2019	82	121	67.77	58.67–75.98
	2020	78	110	70.91	61.48–79.18
Other wild animals	2021	41	104	39.42	29.98–49.49
	2022	47	94	50.00	39.51–60.49
	2018	74	507	14.60	11.64–17.97
	2019	41	444	9.23	6.71–12.32
Other wild animals	2020	23	395	5.82	3.73–8.61
	2021	27	355	7.61	5.07–10.87
	2022	12	184	6.52	3.41–11.11

In addition to standard visualization of the EHSA results, we visualized the spatio-temporal cube in the form of series of 2-month time steps, which comprised the temporal size of a cube bin (Fig. 6). For this purpose, we generated shape file from the data of spatio-temporal cube using the tool Visualize Space Time Cube in 3D in ESRI ArcMap 10.4. The obtained shape file was used for visualization in R using the ggplot2 library (Wickham, 2016).

Data of ORV surveillance. Presence of a biomarker was identified by studying histological sections in UV spectrum under a luminescent microscope. The materials for the study were histological sections of the fangs of animals shot on hunting grounds no less than 30 days after the end of oral vaccination campaign of wild carnivores against rabies.

For the virus antigen, the serums were analyzed using ELISA. To analyze the 2018 ORV campaign, we used the diagnostic sets BioPro Rabies Elisa Ab and Platelia Rabies II BioRad, and to assess the seroconversion against the background of the ORV 2019–2021 campaigns, we used only BioPro Rabies Elisa Ab. When using the test system BioPro Rabies Elisa Ab, positive samples were considered serums with threshold values $\geq 70\%$ blocking. Serums of fox blood, when analyzed using the test system Platelia Rabies II BioRad, were considered positive in case of seroconversion ≥ 0.125 IU/cm³.

Results

Descriptive statistical analysis. During 2018–2022, in Ukraine, 5,846 brain samples of animals were considered positive for rabies in DFA (2018 – 1,847; 2019 – 1,421; 2020 – 1,226; 2021 – 767; 2022 – 585).

The numbers of samples that came from passive surveillance of rabies in domestic carnivores (Fig. 2) and active surveillance of foxes (Fig. 3) varied across oblasts and years.


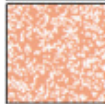
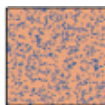
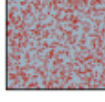
Rabies was laboratory confirmed in 28 kinds of animals: 6 kinds of domestic and agricultural animals (bovine cattle, goats, horses, cats, swine, dogs) and 22 kinds of wild animals (squirrels, beavers, badgers, otters, wolves, wild goat, raccoon dogs, hares, hedgehogs, wild boars, bats, roe deer, rabbits, martens, weasels, foxes, mice, muskrats, polecats, hamsters, jackals, rats) (Table 1). The highest number of positive samples was from domestic carnivores – 2,705 (46.3% of all positive samples in all groups) and foxes – 2,556 (43.7%). There were 408 (7.0%) positive samples from other domestic animals and 177 (3.0%) from other wild animals.

For 2018–2021, there was observed a seasonal downward trend of rabies cases in each group of animals: domestic carnivores ($\tau = -0.574$, $P < 0.001$), foxes ($\tau = -0.583$, $P < 0.001$), and other domestic ($\tau = -0.582$, $P < 0.001$) and other wild animals ($\tau = -0.591$, $P < 0.001$).

Notable seasonality was observed on the epidemic curve for foxes, which was related to the active surveillance of foxes conducted from October to December (Fig. 4). The highest peaks occurred in November–

February in foxes, in October–February in domestic carnivores, and in October–December in other domestic animals. Percentage of positive samples from foxes varied significantly by years ($\chi^2 = 398.37$, $df = 3$, $P < 0.001$). The highest percentage of positive samples from foxes (8.28%) was found in 2018 (the first year of conducting large-scale ORV campaign). In the following years, rabies was declining each year: 4.81% in 2019, 3.95% in 2020, and 2.47% in 2021. Paired comparisons revealed that percentage of positive samples from foxes decreased significantly each year, with a lower percentage than in the previous year ($P < 0.001$). In 2022, the percentage of positive fox samples that came from the controlled area was 2.90%. Percentage of positive samples from domestic carnivores varied significantly by years ($\chi^2 = 95.49$, $df = 3$, $P < 0.001$). The highest percentage of positive samples in this group (20.85%) was observed in 2018. In the following years, this parameter equaled: 13.28% in 2019, 17.92% in 2020, and 14.47% in 2021. Paired comparisons revealed that percentage of positive samples from domestic carnivores in 2019 was much lower than in 2018 ($P < 0.001$), and in 2021 it was much lower than in 2018 and 2020 ($P < 0.001$). In 2022, positive samples from domestic carnivores accounted for 16.82%.

Table 4
Interpretation of legend of Emerging Hot Spot Analysis

Pattern visualization	Pattern name	Definition
	No Pattern Detected	Does not fall into any of the hot or cold spot patterns defined below.
	Sporadic Hot Spot	A statistically significant hot spot for the final time-step interval with a history of also being an on-again and off-again hot spot. Less than 90 percent of the time-step intervals have been statistically significant hot spots and none of the time-step intervals have been statistically significant cold spots.
	Oscillating Hot Spot	A statistically significant hot spot for the final time-step interval that has a history of also being a statistically significant cold spot during a prior time step. Less than 90 percent of the time-step intervals have been statistically significant hot spots.
	Oscillating Cold Spot	A statistically significant cold spot for the final time-step interval that has a history of also being a statistically significant hot spot during a prior time step. Less than 90 percent of the time-step intervals have been statistically significant cold spots.

Percentage of positive samples from other domestic animals significantly varied by years ($\chi^2 = 28.73$, $df = 3$, $P < 0.001$), equaling 64.78% in 2018, 67.77% in 2019, and 70.91% in 2020. Compared to previous year, significant decline in positive samples was observed only in 2021 – 39.42% ($P < 0.001$). In 2022, the positive samples from other domestic animals amounted to 50.00%. In the group of other wild animals, percentage of positive samples significantly varied by years ($\chi^2 = 22.54$, $df = 3$, $P < 0.001$). Paired comparisons indicated significant decrease ($P < 0.001$) in the percentage of positive samples in 2020 (5.82%), as compared with 2018 (14.6%). The positive samples accounted for 9.23% in 2019, 7.61% in 2021, and 6.52% in 2022.

Emerging hot spot analysis. Emerging Hot Spot Analysis revealed spatio-temporal trends in clustering of rabies cases in Ukraine in 2018–2022 (Fig. 5). Trends were seen in 185 of 1,799 analyzed locations: 92 locations of hot and 93 locations of cold trend. In 1,614 locations with detected rabies, we found no statistically significant spatio-temporal trend (Not Emerging). Of the locations identified to the hot clustering trend, 49 in Vinnytsia and 6 in Khmelnytskyi oblasts were classified as Sporadic Hot Spot, 3 locations in Vinnytsia and 34 in Khmelnytskyi and Temopil oblasts – as Oscillating Hot Spot.

All 93 cold-trend locations were classified as Oscillating Cold Spot. This trend was observed mostly on the left bank of the Dnipro, in Poltava, Kharkiv, Luhansk, Donetsk, and Zaporizhzhia oblasts. On the right bank, Oscillating Cold Spot was found in Cherkasy and Kirovohrad oblasts.

Starting from 2019, the area of hot clusters decreased each year (Fig. 6). Notable seasonality and hottest clustering were characteristic for the two first and two last months of each year. In summer months, clustering was the smallest.

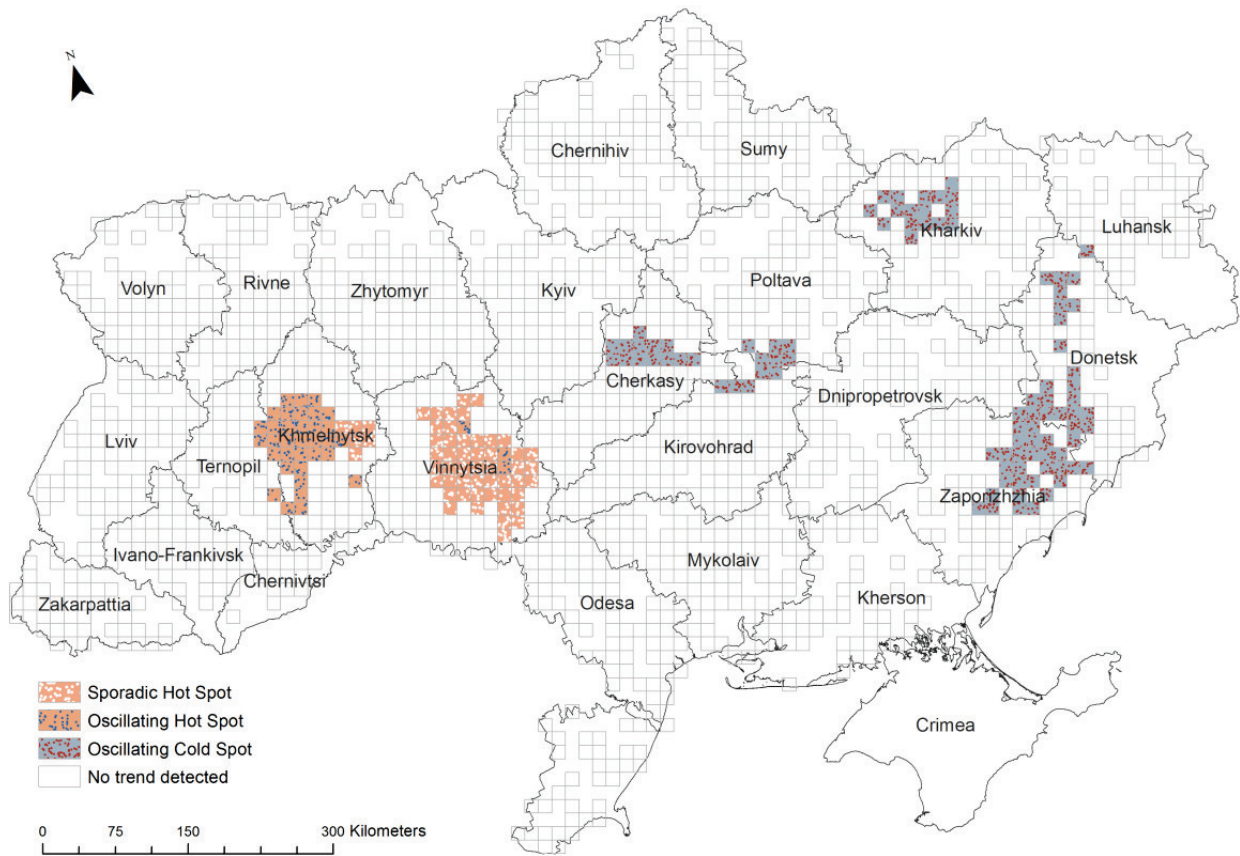


Fig. 5. Spatio-temporal trends in the clustering of rabies cases in Ukraine 2018–2022 identified by Emerging Hot Spot Analysis tool

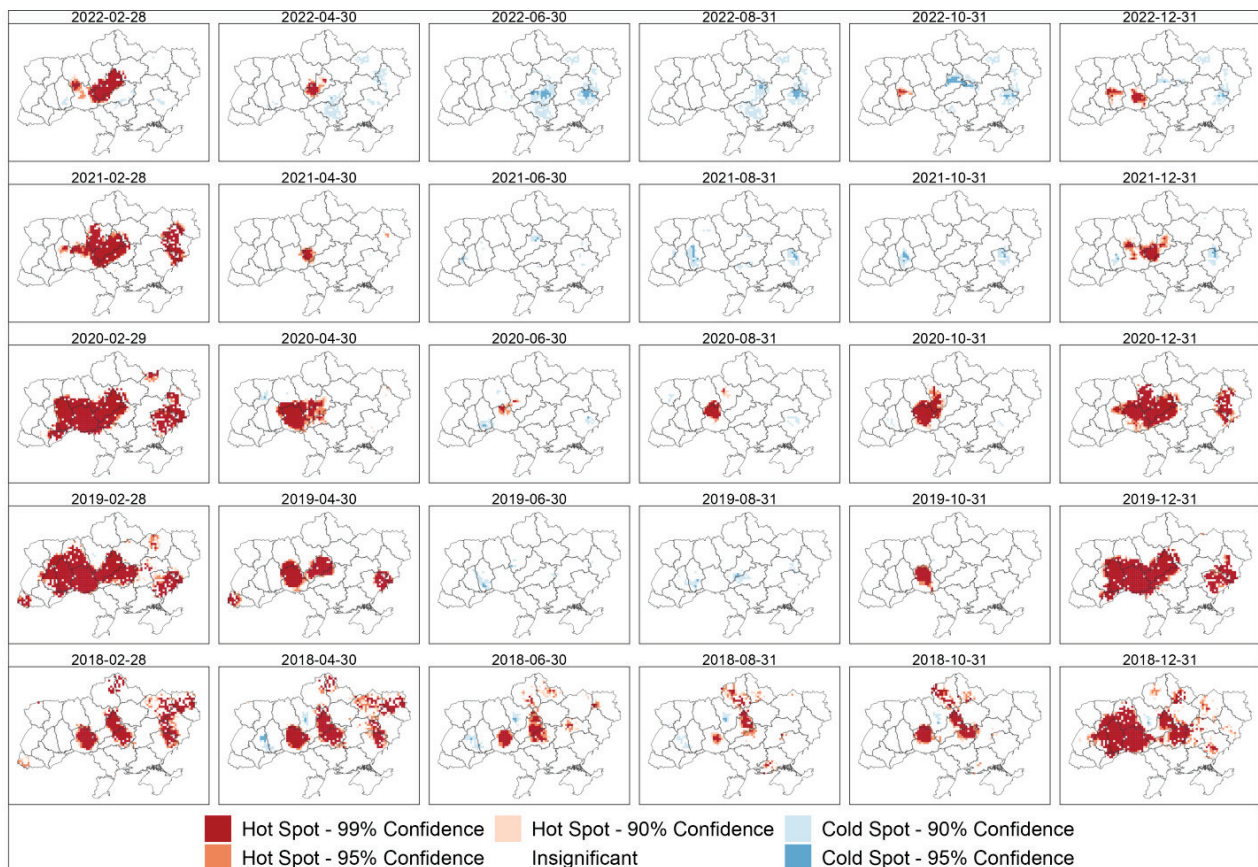


Fig. 6. Spatio-temporal cube from emerging hot spot analysis (time step is 2 months) demonstrating hot and cold spots of rabies cases on each time slice

Cold clusters had the largest area in 2021 and 2022, especially in summer months.

ORV surveillance data analysis. To analyze the ORV campaigns conducted in Ukraine in 2018–2021, 41,926 samples, including 20,112 positive, were studied in laboratory for presence of tetracycline biomarker in fox teeth (Table 5).

Table 5
Results of studies of fox teeth for presence of tetracycline marker after 2018–2021 ORV campaigns

Year	Positive	Samples	Percentage of positive	95% CI
2018	3,582	7,675	46.67	45.56–47.79
2019	6,603	13,178	50.11	49.25–50.96
2020	4,461	10,148	43.96	43.00–44.93
2021	5,466	10,925	50.03	49.09–50.97

Each year of all four ORV campaigns, the percentage of positive results ranged 43.96–50.11%. For serological assessment of effectiveness of four autumn ORV campaigns (2018–2021), 32,314 blood serums of foxes were analyzed for presence of antibodies against the rabies virus using ELISA (Table 6). After 2018 ORV campaign, there were found 37.65% of animals with antibodies against rabies. In 2019, the share of animals with anti-rabies immunity was 24.36%. It accounted for 18.63% in 2020 and 15.12% in 2021.

Table 6
Seroconversion in foxes after 2018–2019 ORV campaigns

Year	Positive	Samples	Percentage of positives	95% BCI
2018	2,081	5,527	37.65	36.38–38.94
2019	2,169	8,904	24.36	23.48–25.26
2020	1,632	8,759	18.63	17.83–19.46
2021	1,380	9,124	15.12	14.40–15.87

Discussion

The large-scale and long ORV campaigns, conducted in the 1990s and in the first decade of the 21st century, had a considerable effect on the spread of rabies in Europe, freeing many European countries from this infection (Müller et al., 2015; Maki et al., 2017).

The trend of rabies in Ukraine has been downward: 1,847 cases were diagnosed in 2018; 1,421 in 2019; 1,226 in 2020; 767 in 2021; and 585 in 2022 (Table 1). That is, over 5 years, the number of laboratory-confirmed cases of rabies in Ukraine became 3 times lower. Such a result has been almost certainly achieved by the large-scale ORV campaigns.

First of all, decline in cases was seen in the main target species of ORV and reservoir of rabies in the wild – foxes. The number of cases in those animals decreased from 737 in 2018 to 179 in 2022.

Rabies in domestic carnivores (dogs and cats) decreased as well. Considering the fact that there occurred no changes in the principles and extents of prophylaxis anti-rabies events in Ukraine in 2018–2022, we may assume that decline in rabies in those animals was also the result of ORV, achieved through reducing the prevalence of the virus in the reservoir – foxes, and thus decreased inter-species transmission of the virus during contacts. In many European countries, ORV not only did decrease the number of cases of rabies in wild animals but completely eradicated rabies in all species of animals (Cliquet et al., 2004; Müller et al., 2015).

Other than decline in rabies in the three main epidemically significant groups of carnivores (foxes, dogs, and cats), there was found decrease in the rabies cases in raccoon dogs, which in our opinion is also the result of ORV. Despite its rarity in Ukraine (compared with foxes) and lack of consideration as a rabies reservoir, this animal was also a target of ORV efforts in Baltic countries (Cliquet et al., 2012; Robardet et al., 2016).

Territorially, the highest number of rabies cases in animals in the studied region was found in Vinnytsia, Cherkasy, and Khmelnytskyi oblasts, which are located geographically close to each other on the right bank of the Dnipro, in the forest-steppe zone (Fig. 1). In total, 37.67% of all the rabies cases in 2018–2022 were found in those three oblasts (Table 2). Significant epizootics of rabies in those oblasts also occurred in the previous years (until 2018), and the downward tendency in cases during 2018–2022 is clearly seen (Makovska et al., 2021).

Moreover, according to surveillance of rabies epizootic at the beginning of the large-scale ORV campaigns in 2018, prevalence in foxes equaled 8.28%. Later on, this parameter decreased to 2.47–2.90% in 2021–2022 (Table 5). Considering that 94.35% of the brain samples came from clinically healthy shot foxes (active surveillance), the prevalence of rabies in the fox population most likely corresponded to actual share of rabies-infected foxes (Polupan et al., 2019). Percentage of positive cases in other wild animals is probably also close to the actual, because includes animals that had been shot by hunters (wolves, jackals, and raccoon dogs) and corpses that had been found and delivered to veterinary laboratories for analyses. Percentage of positive cases in domestic animals, both carnivores and agricultural, did not correspond to the actual prevalence of rabies because samples from those animals came to laboratories already with suspicion of the disease, often with pathognomonic features of rabies prior to animals' death.

A large number of rabies cases were recorded annually in domestic carnivores (dogs and cats), which – despite decline during the surveillance period – indicates insufficient level of anti-rabies prophylactic events in dogs and cats, conducted by veterinary services, as confirmed by other studies (Polupan et al., 2017, 2019; Makovska et al., 2021).

Besides decline in the number of positive samples from all the groups of studied domestic carnivores, foxes, and other domestic and wild animals, the laboratories tended to receive fewer samples for analysis from domestic carnivores and other domestic and wild animals from passive surveillance. This could have also been achieved by the rabies vaccination effort through reduction of inter-species transmission of the pathogen from foxes to other animals, although no significant confirmation of this hypothesis was found.

Analysis of seasonality of the rabies morbidity in animals revealed significant spikes in November–December each year. The highest peak in rabies was associated with increase in cases found in foxes. Perhaps, such a growth was based on the two factors. The first is related to foxes' ecology – young animals usually leave their birth places in spring to inhabit new areas (Cavallini, 1996). They have thus more contacts with other foxes and wild and domestic animals, promoting rabies spread. The other likely factor is significant increase in receiving brain samples of foxes from active surveillance. Thirty days after autumn ORV campaign, there were organized mass events of shooting foxes, taking place between November–December. That is why we may assume that notable spikes in morbidity have been of both natural (ecological) and anthropogenic causes.

Another pattern discovered through analysis of rabies-morbidity of animals was gradual decline not only in recorded cases, but also in morbidity peaks, which confirms presence of an immune population in a certain amount of virus-reservoir animals. Significant outbreaks of any infection, including rabies, are possible only in presence of a large number of susceptible non-immunized animals, as seen in recurrent outbreaks in some European countries following ORV (Smreczak et al., 2023; Robardet et al., 2023).

In 2018–2022, rabies epizootic was largely spread in all natural geographic zones of Ukraine, the largest hot-spot areas being in central and eastern Ukraine (Fig. 6). With time, those zones had been reducing until in November–December 2022 hot clusters were observed only in Vinnytsia, Khmelnytskyi, and Ternopil oblasts. Hot clusters of cases disappeared earlier in oblasts on the left bank of the Dnipro. In 2022, the area had the largest zones of cold clusters.

Decrease in the area of hot clusters of rabies in Ukraine in 2018–2022 indicates effectiveness of ORV campaigns in Ukraine. Rapid reduction of the hot-cluster area in late 2018 corresponded to increased record of rabies cases (Fig. 6). This actually occurred as a result of increased analysis of samples of the fox brain from active surveillance in order to control ORV and detect infected animals, for after only one ORV campaign was the share of immunized animals most likely insignificant.

The clustering trend had distinct spatial boundaries; hot clusters were in the central and western oblasts (Vinnytsia, Khmelnytskyi oblasts, and a part of Ternopil Oblast), whereas the cold clusters were in the eastern oblasts. In Vinnytsia Oblast, there was mostly the Sporadic Hot Spot trend. This trend means presence of hot cluster in the recent time interval of the study (November–December 2022), and also presence of hot and absence of cold clusters in the previous intervals (Fig. 5). In Khmelnytskyi

and Ternopil oblasts, Oscillating Hot Spot prevailed, indicating an outbreak of rabies in those territories in November–December 2022, though in the previous months there were observed one or several cold clusters.

Of the cold-cluster trends, only Oscillating Cold Spot was observed. This suggests alternating presence of two types of clusters in each of those locations over the years and cold cluster in the last two-month interval (November–December 2022). Locations with such a trend were mostly in oblasts on the left bank of the Dnipro (Poltava, Kharkiv, Donetsk, Zaporizhzhia), and also Cherkasy and Kirovohrad oblasts on the right bank.

Along with surveillance of rabies enzootic in Ukraine, ORV efficiency was also analyzed according to tetracycline marker in the foxes' teeth and seroconversion (Tables 5 and 6).

Percentage of tetracycline-marker-positive samples of fox teeth in all four ORV campaigns ranged 43.96–50.11%. However, many European countries achieved better results of ORV according to this parameter (Matouch et al., 2007; Cliquet et al., 2012; Müller et al., 2015).

The conducted serological assessment of ORV efficacy revealed conflicting results. In 2018, 37.65% positive animals were found. However, in the following years the percentage of positive samples of blood serum was lower: 24.36%, 18.63%, and 15.12%. This could be associated with differences in interpretations of the results of anti-rabies activity in fox blood serum by the test systems BioPro Rabies Elisa Ab and Platelia Rabies II Bio-Rad, identified in 2018 in two laboratories. In our opinion, this led to exaggerated assessment of anti-rabies activity in blood serum of foxes of eastern and central oblasts that year.

In many European countries, assessment of ORV efficacy according to seroconversion in foxes found the percentage of positive samples ranging 40–50% (Robardet et al., 2016; Maki et al., 2017). However, considering that oral rabies vaccination of carnivores in Ukraine was carried out in 2018–2021 only once a year, the obtained share of positive samples of fox blood at the level of 15.12–24.36%, in our opinion, was a quite predictable result.

Therefore, a complex analysis of ORV efficiency, conducted in 2018–2021, proved this event to be effective, though to achieve a proper result, that is formation of necessary anti-rabies population immunity, campaigns should be carried out twice a year – in spring and autumn, similarly to how ORV campaigns were conducted in many European countries (Müller et al., 2012, 2015; Robardet et al., 2016; Maki et al., 2017).

Unfortunately, despite the fact that veterinary laboratories in a number of war-affected oblast did not cease operation (Kyiv, Zhytomyr, Chernihiv, Sumy, Poltava, Kharkiv, Dnipropetrovsk, Zaporizhzhia, and Mykolaiv) and sent us their reports, some cases have probably not been included in our research due to problems with collecting and delivering brain samples to the laboratories. Thus, some amount of positive samples was likely unrecorded. Analysis of rabies in 2018–2022 suggests that decline in rabies enzootic as of late 2022 is the best result achieved by the Veterinary Service of Ukraine in the 21st century so far. In the coming years, the situation with rabies in Ukraine can deteriorate due to absence of rabies control in a certain territory of Ukraine due to military actions, no ORVs in 2022, and decrease in ORV extent in the following years. Those factors will almost certainly have a negative impact on rabies enzootic in Ukraine.

Conclusion

Spatio-temporal assessment of rabies enzootic in Ukraine in 2018–2022 demonstrated the dynamics of its decline in all species of animals. However, analysis revealed insufficient efficiency of the conducted ORV campaigns. To achieve best effect, ORV should be performed twice a year: in spring and autumn. Inclusion of domestic carnivores (dogs and cats) also indicated the problem in the existing system of rabies prophylaxis in those animals. In the future, we will continue to study spatio-temporal tendencies of manifestations of rabies enzootic in Ukraine in order to identify scales of to which military actions in Ukraine had exacerbated rabies enzootic. Also, we will assess efficacy of anti-rabies prophylaxis events conducted by the Veterinary Service of Ukraine to improve the existing system of rabies control.

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