Epidemiological data of Rabies in Ukraine

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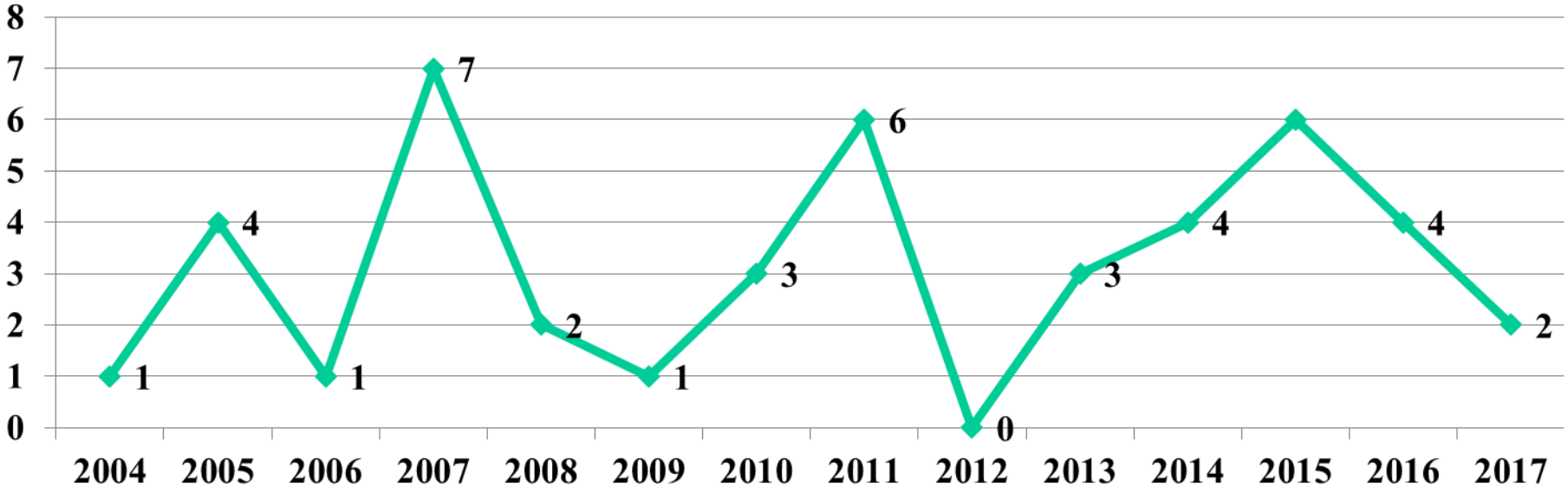
April 24th 2018

HUMAN



Evolution of rabies deaths/year from 2004-2017 for human

HUMAN RABIES IN 2017

1. 66350 of animal bites

1. 2 of human rabies deaths in 2017:

- All cases laboratory confirmed by PCR:

2.Human rabies is a notifiable disease since 2002

3.Prevention of rabies is organized in 976 public hospitals.

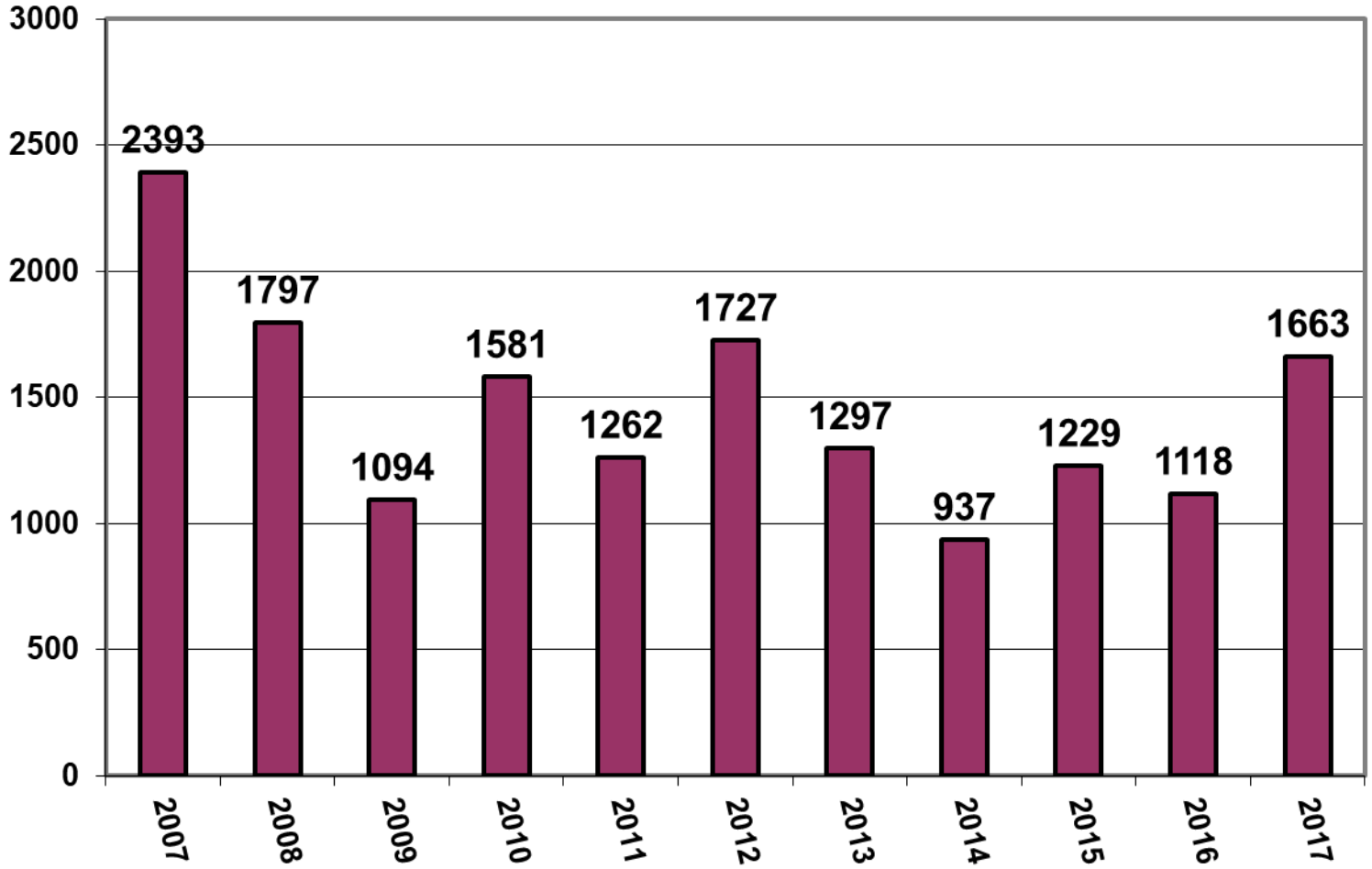
PRE-EXPOSURE PROPHYLAXIS (PrEP)

1. There are not developed and implemented programs for preventive vaccination against rabies in Ukraine.
2. People at risk are pass preventive vaccination: employees of diagnostic laboratories, scientists of institutions.
3. Vaccination schedule: 3-dose IM regimen used (D0, D7, D28)
4. Vaccine used: Verorab (Sanofi Pasteur), KOKAV (Mikrogen), Indirab (Bharat Biotech) 174 people received PrEP.

POST-EXPOSURE PROPHYLAXIS (PEP) in 2017

1. Vaccination schedule: 6-dose Essen regimen used schedules
2. Vaccine used: Verorab (Sanofi Pasteur), KOKAV (Mikrogen), Indirab (Bharat Biotech)
3. 14215 people received PEP.
4. 2660 received RIG.
5. Who pays for PEP? Central government (human health)

Cases of rabies in Ukraine from 2007 to 2017



- Therefore, an increase in the number of stray animals, the approaching foxes to populated areas, irresponsible owners of domestic animals, especially in suburban areas, in country areas, gardening areas and the outskirts of large cities negatively affects the effectiveness of measures to eradicate rabies in Ukraine.

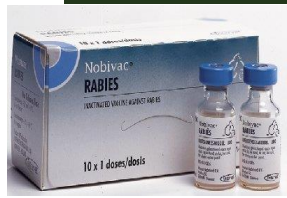
For today :

- unsolved problems of stray and domestic animals in cities, urban-type settlements, villages and adjoining territories (not necessarily hunting);
- regulation of the number of epizootologically important species of wildlife is carried out inappropriately;
- large-scale deratization on the territories of agricultural lands is not conducted.


Rabies

The main specific measures being held in Ukraine

- **Parenteral vaccination of farm and domestic animals**
- **Oral vaccination of wild carnivores**
- **Regulation of the number of red fox in the wild nature**
- **Awareness campaign among the population**



In 2017, more than 5.4 million of animals were vaccinated against rabies in Ukraine

In state institutions of veterinary medicine, vaccination of animals against rabies is carried out free of charge

- The basis for the prevention of people's rabies is the elimination of rabies among animals through their immunization

- **100% coverage of the total number of dogs throughout the country;**
- **cats – in persistently infected areas;**
- **farm animals – in case of outbreak of a disease**

During the last 5 years vaccinations against rabies have been carried out:

2013 – 6,01 million (including 3,98 million of dogs and 1,70 million of cats);

2014 – 5,66 million (including 3,70 million of dogs and 1,65 million of cats);

2015 – 5,19 million (including 3,41 million of dogs and 1,50 million of cats);

2016 – 1,77 million (including 0,99 million of dogs and 0,47 million of cats);

2017 – 5,43 million (including 5,25 million of dogs and 0,15 million of cats).

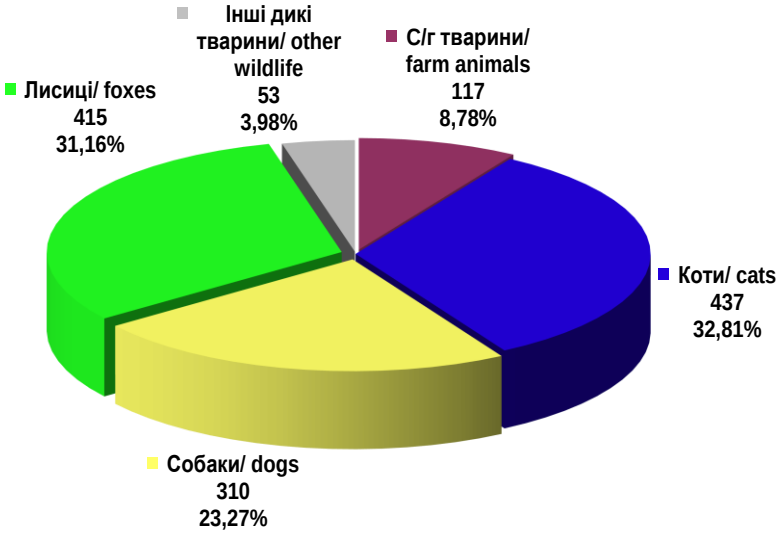
FACTORS

Reasons for the high percentage of incidence among domestic carnivorous animals (dogs and cats) on rabies is the lack of 100% coverage of preventive immunization for the total number of dogs throughout the country and cats - in the areas of stable ill-being

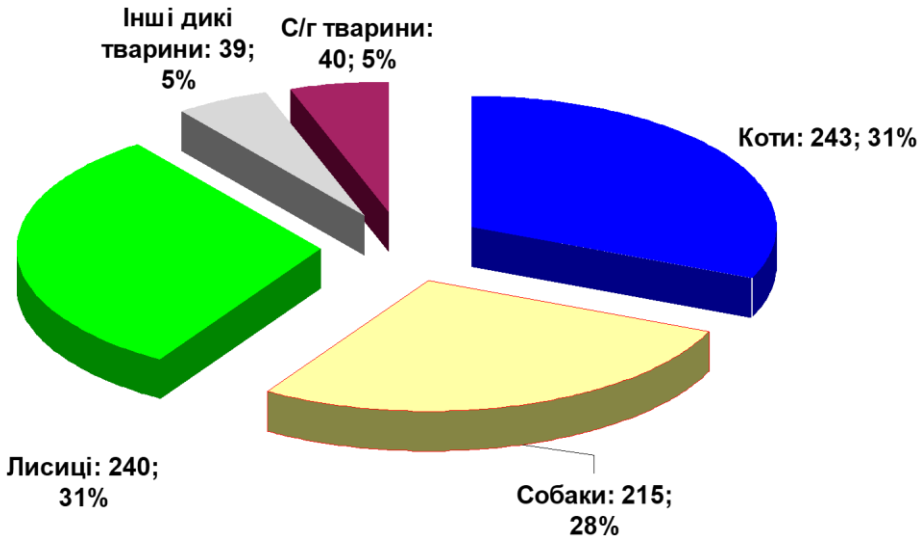
The research found that only 26 (12.9%) dogs were stray, the other 202 (87.1%) - had owners, however, were not preventively vaccinated against rabies.

Cases of rabies in Ukraine in the context of animal species

2016



2017



The epizootic situation on rabies in Ukraine has a number of features, in particular, the evolution of epizootics as a natural-focal, as well as anthropological (urban) type.

In the first case, the reservoir and the source of the infectious agent is a fox, in the second - stray dogs and cats.

Measures taken by the State Service of Ukraine on Food Safety and Consumer Protection:

- **Epizootological monitoring** – is carried out by means of epizootiological examination, timely detection of outbreaks of the disease and analysis of the epizootological situation;
- **Laboratory investigations** – are conducted under suspicion and confirmed rabies in animals using methods that meet international standards;
- **Control of the quality of means of specific prevention** – provides the control of all series of vaccines for oral and parenteral immunization used in Ukraine, in accordance with the recommendations of the OIE and WHO;
- **Conducting explanatory work on the prevention of rabies among the population (sights, booklets, leaflets, posters, television, radio, meetings, interviews, seminars, internet resources, etc.)**

Strategy for oral vaccination of wild carnivores in Ukraine

Oral immunization of wild carnivores against rabies in Ukraine is carried out by representatives of regional bodies and state institutions of the State Service of Ukraine on Food Safety and Consumer Protection

ORAL VACCINE «BROVARABIES V-RG»

V-RG strain is recommended by WHO,OIE and European Commission due to it's safety, efficacy and stability.


Епізоотичний стан по сказу за 2007 рік
Epizootic rabies situation, 2007
Польща
Варшава
Бєларусь
Росія
Київ
Молдова
Кишинів
Румунія
Бухарест
Чорне море
Волинська
Рівненська
Чернігівська
Сумська
Житомирська
Київська
Полтавська
Хмельницька
Львівська
Тернопільська
Харківська
Луганська
Івано-Франківська
Черкаська
Дніпропетровська
Закарпатська
Вінницька
Кіровоградська
Донецька
Чернівецька
Одеська
Миколаївська
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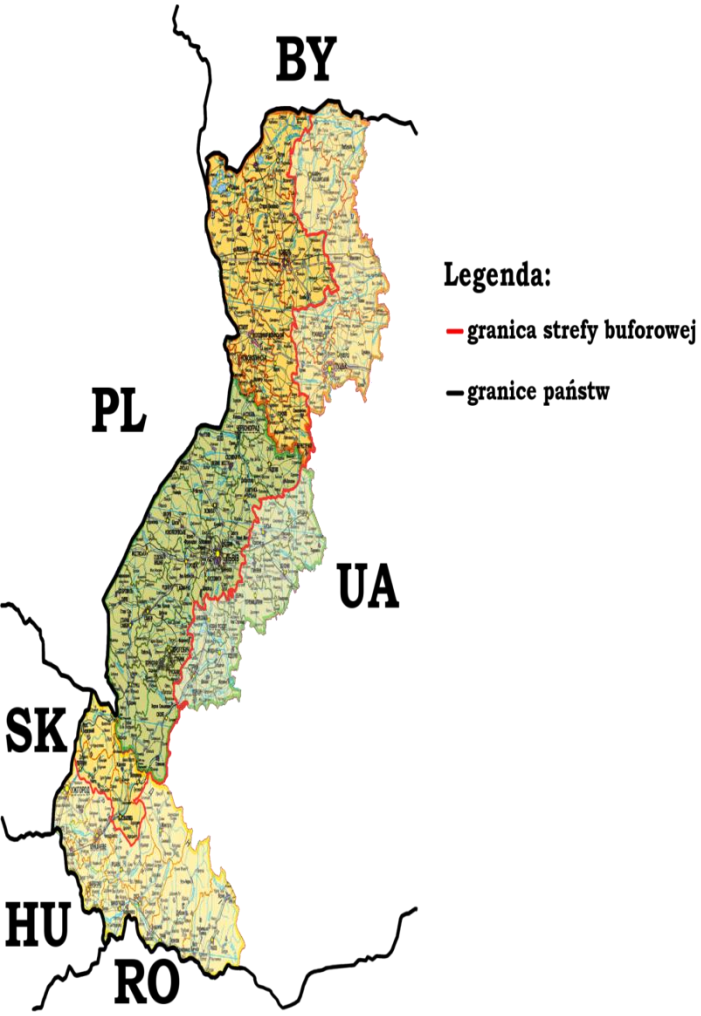


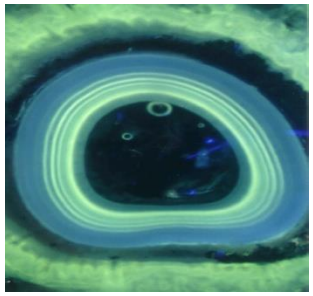
From 2012 annual Agreements between the **Ministry of Agrarian Policy and Food of Ukraine** and the **Ministry of Agriculture and Rural Development of the Republic of Poland** are being implemented.

In 2017, an agreement is being implemented between the Ministry of Agrarian Policy and Food of Ukraine and the **Ministry of Agriculture of Hungary** (an autumn campaign for the oral immunization of wild carnivores in the Volyn, Transcarpathian, Ivano-Frankivsk, Lviv and Rivne regions was carried out).

Negotiations on the signing of the Agreement between **the Ministry of Agrarian Policy and Food of Ukraine and Romania** are ongoing.

Control of the effectiveness of vaccination

- Control of vaccine consumption
- Investigation of fox teeth on the presence of tetracycline
- Investigation of fox blood serum on the presence of virus neutralizing antibodies
- Epizootic surveillance of the vaccination zone



Indicators of the implementation of the Agreements with Poland and Hungary

– consumption of baits with vaccine – 84 %;

– presence of virus neutralizing antibodies – 38 %;

– presence of tetracycline marker – 65 %

Serological evaluation of anti-rabies immunity

Creating a Bank of Serums

Animal blood serum characteristics, investigated in 2009-2016.

Year	Total	The origin of serum					Control serums
		Dogs	Cats	Human	Cattle	Foxes	
2009	340	238	91	2	-	-	9
2010	772	241	74	3	-	429	9
2011	1669	323	107	3	50	1336	11
2012	2508	440	146	5	125	1449	11
2013	1943	442	156	6	15	1310	14
2014	1470	378	109	6	-	963	14
2015	2861	217	84	5	-	2148	14
2016	3498	1108	427	4	-	1945	14
Total	14481	3387	1194	34	190	9580	96

The study of the correlation between the immunogenity of vaccines and the humoral immune response after their administration.

Table

Antigenic activity of anti-rabies vaccines with different immunogenic activity

Vaccine	Immunogenic activity	The number of blood serum samples	Immune response, %		Titre VNA IU /cm^3
			Serocon-version	Titre VNA ≥0,5 IU/cm^3	
A	1,2 IU/dose	15	90,0	20,0	**0,35±0,11**
B	1,5 IU/dose	25	100,0	48,0	**0,73±0,32**
C	1,8 IU/dose	11	100,0	45,0	**0,62±0,21**
D	3,8 IU/dose	31	100,0	100,0	**10,24±4,12***

Note:

* - the difference is significant relative to the results in groups A, B and C at $p < 0.001$.

Table

Results of the blood serum research of dogs and cats for the availability of VNA during 2009-2016

Year	Dogs			Cats		
	Total	VNA level, IU/cm^3		Total	VNA level, IU/cm^3	
		Above 0,5	Below 0,5		Above 0,5	Below 0,5
2009	238	206	32	91	89	2
2010	241	207	34	74	72	2
2011	323	273	50	107	102	5
2012	440	349	91	146	143	3
2013	442	384	58	156	153	3
2014	378	339	39	109	105	4
2015	217	203	14	84	83	1
2016	1108	1022	86	427	423	4
Total	3387	2983	**404**	1194	1170	**24**

Verification of the ELISA method for detecting antibodies to the rabies virus (Drozze, 2016)

OIE positive standard serum
Run 1

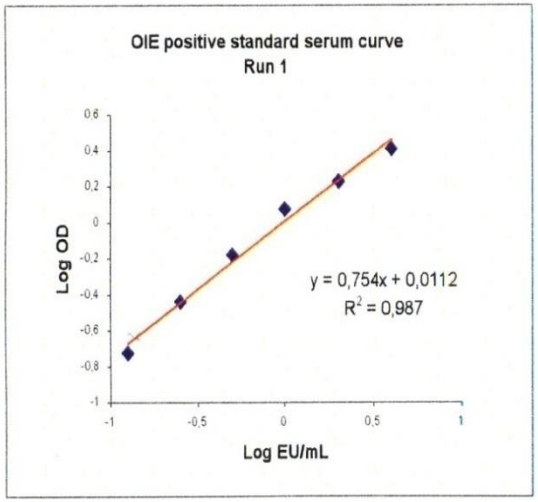
EU / ml	Mean of OD
4,00	2,586
2,00	1,705
1,00	1,199
0,50	0,667
0,25	0,365
0,125	0,189

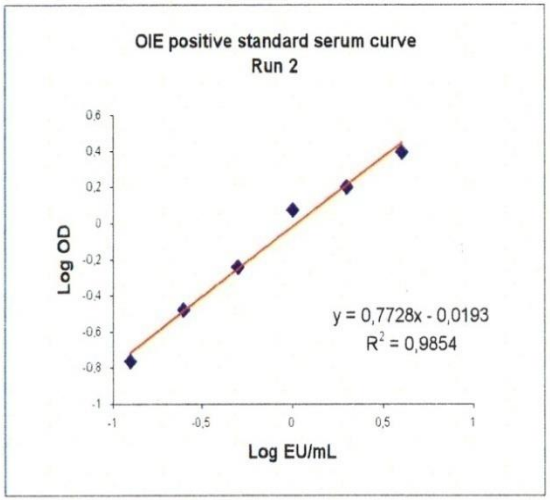
OIE positive standard serum
Run 2

EU / ml	Mean of OD
4,00	2,478
2,00	1,589
1,00	1,191
0,50	0,572
0,25	0,331
0,125	0,173

OIE positive standard serum
Run 3

EU / ml	Mean of OD
4,00	2,405
2,00	1,498
1,00	0,987
0,50	0,506
0,25	0,327
0,125	0,159





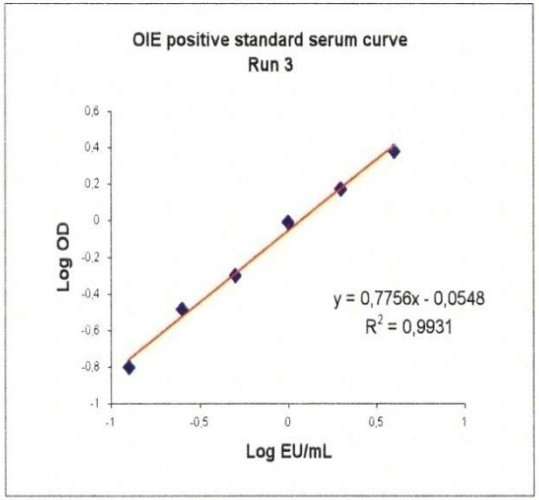


Fig. Quantification by TF-IFA method of standard blood serum of 4 IU / cm3.

Evaluation of immunological response in foxes for oral immunization against rabies

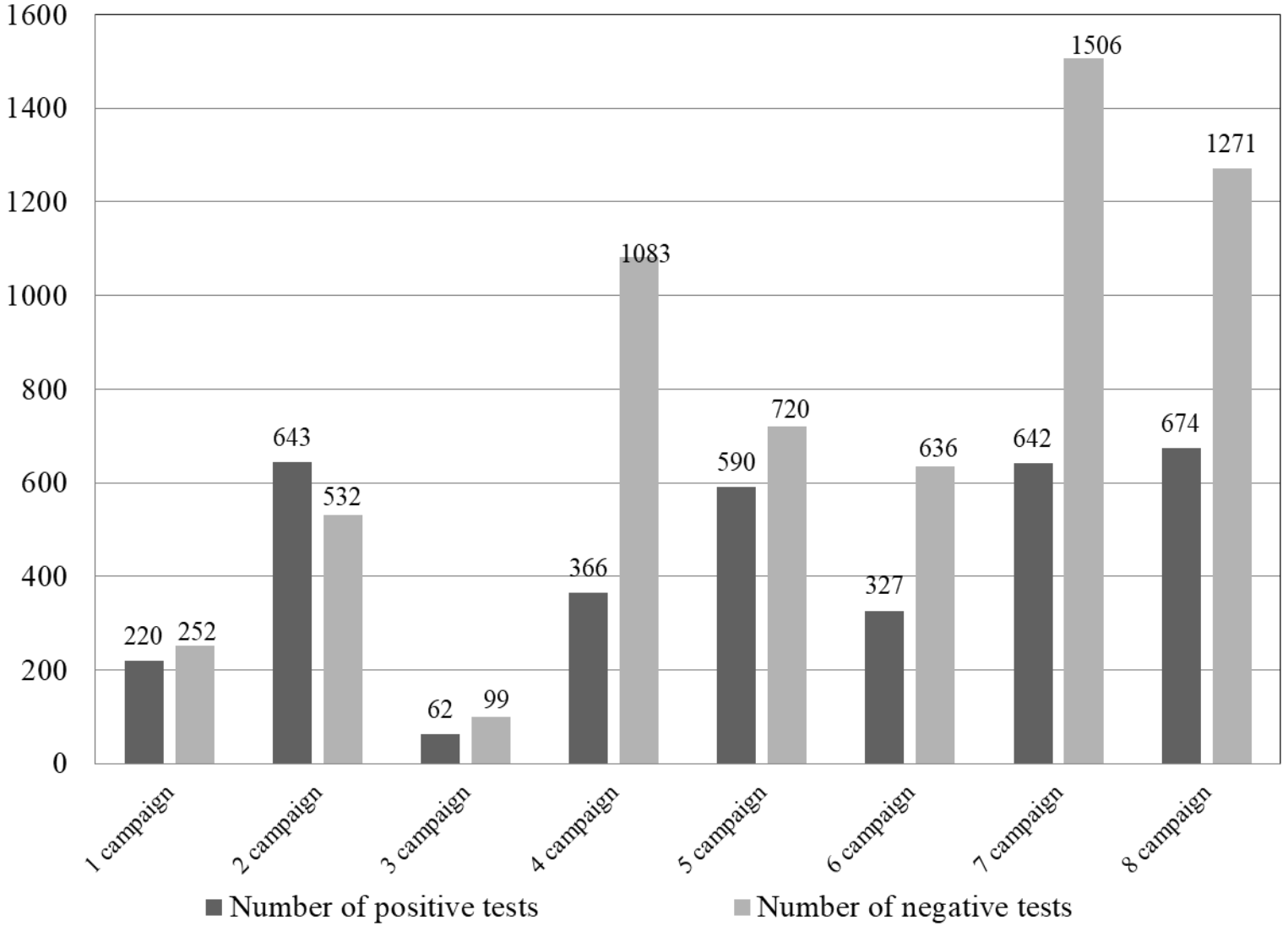


Fig. 8 Number of investigated samples with positive and negative results.

Evaluation of population immunity

The calculation of the vaccination effectiveness was carried out according to the formula:

$$\text{Vaccination efficiency} = \frac{\text{Number of immune animals}}{\text{Number of investigated animals}} \times 100$$

The protective level of specific antibodies (> 0.5 IU / cm^3) in foxes was detected in 1334 blood sera from 9580 examined in 2006-2014, which was **13.92%** of the total number of investigated samples (from 4.3% to 21.3% in different campaigns)

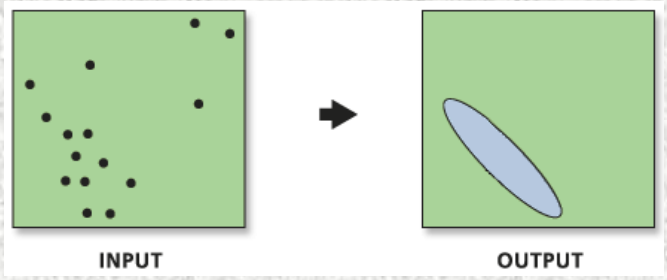
Scientific achievements of rabies team

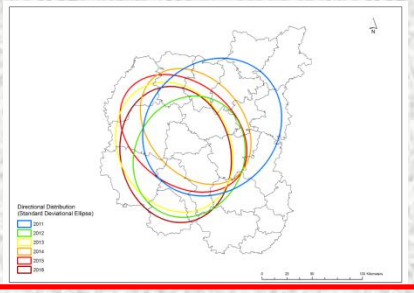
24.04.2018

Directions:

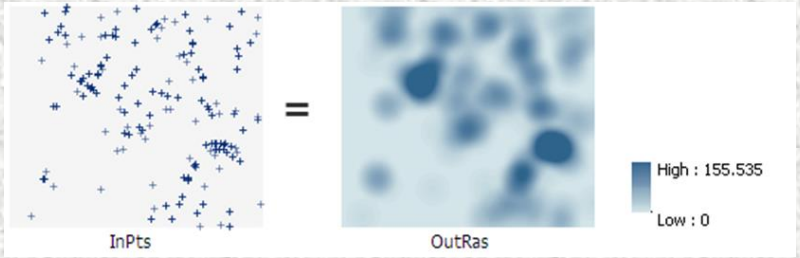
1. Monitoring of epizootic situation with rabies in Ukraine;
2. Implementation of geographic information systems (GIS) for studying the regional epizootology of rabies;
3. Molecular-genetic research of Lissavirus in Ukraine;
4. Development of means of immunofluorescence diagnosis of rabies;
5. Investigation of potential possibilities of obtaining diagnostic preparations on the basis of anti-rabies Ig Y birds;
6. Development of the method and analysis of the effectiveness of oral immunization of dogs against rabies.

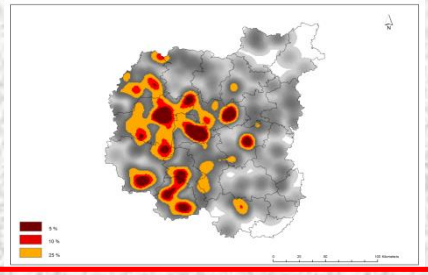
2. GIS-analysis of the epizootic situation



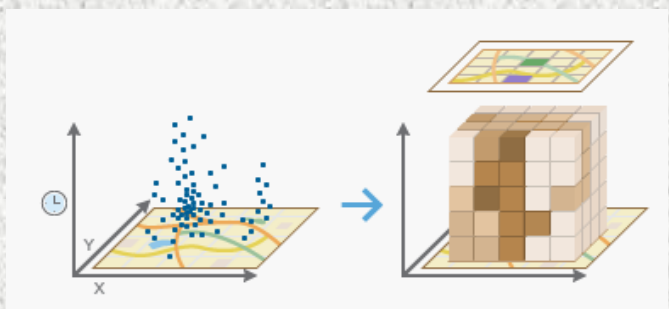


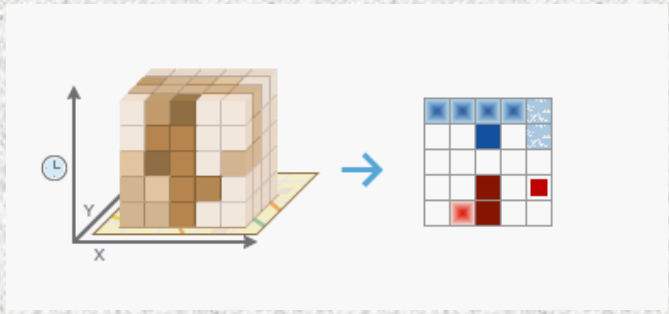
Directional Distribution (Standard Deviational Ellipse)

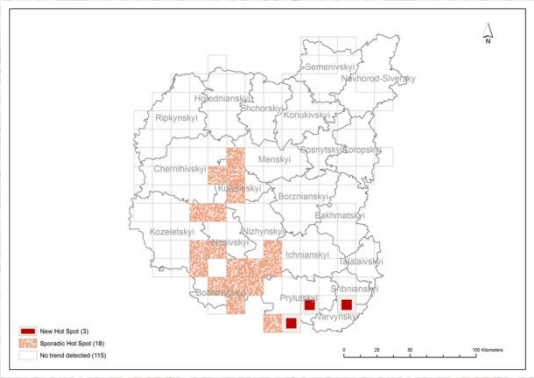




-Kernel Density Estimation







Emerging Hotspot Analysis

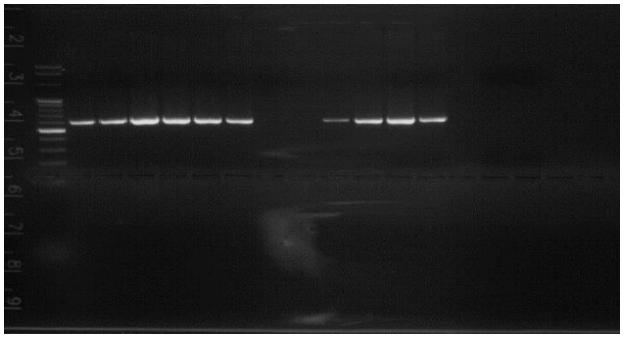
3. Molecular genetic studies of Lyssaviruses in Ukraine

One Step RT-PCR (Invitrogen)

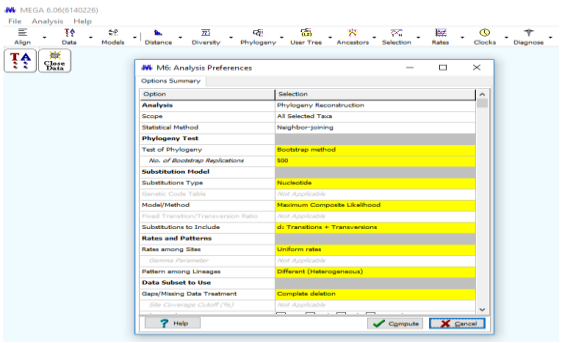
	(µl)		(µl)
2 x reaction mix	12,5	15	187,5
JW6DPL (20uM)	1	15	15
JW12 (20uM)	1	15	15
Mix enzymów Superscript III/Platinium Taq	1	15	15
Woda PCR	7	15	105
	22,5		337,5

22,5 µl miksu + 2,5 µl RNA

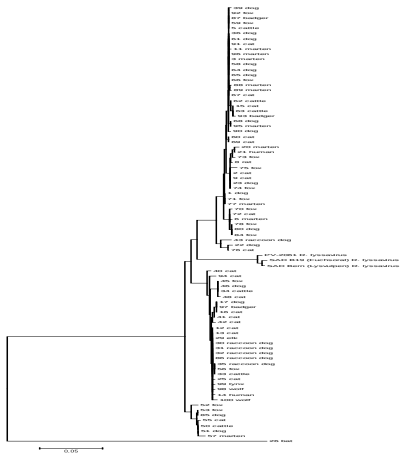
22		50	30 min
23		95	15 min
24		95	30 sc
26		49	30 sc
27		72	1 min
32		72	10 min
35			
37			
38			
43			
K + RNA (gt1)			
K. + RT-PCR			

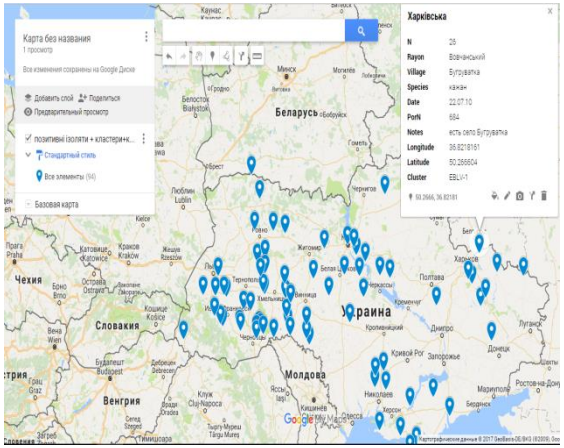


Selection of specific primers, development of ZT-PCR production protocol, Hemi-nested PCR, PCR in real time



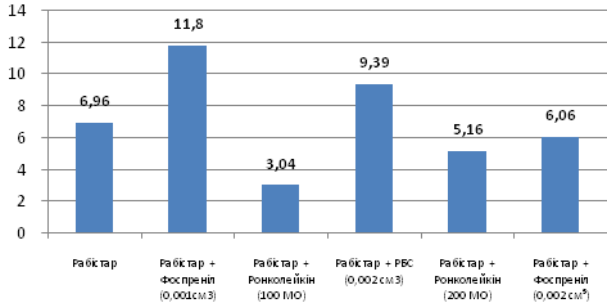
Sequencing phylogenetic and biostatistic analysis





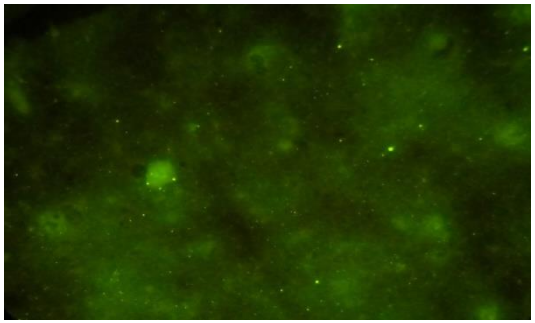
Detection of new genetic variants, clusters and their geographic confinement

4. Development of means of immunofluorescence diagnosis of rabies



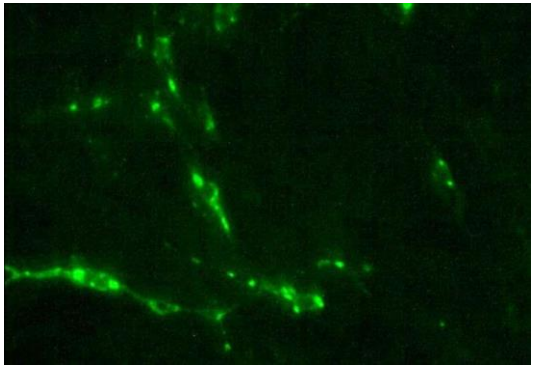
Доба введення	Спосіб імунізації (об'єм/кількість введень)		Фоспренил, см³
	Внутрішньошкірно, см³	Внутрішньом'язово, см³	
0	0,5/5	0,5/1	0,125
21	0,5/5	0,5/1	0,125
35	0,5/5	0,5/1	-
49	0,5/5	0,5/1	0,125
63 (Відбір крові)	–	–	–

Development of the scheme of hyperimmunization of animals with an assessment of the suitability of various immunomodulators



		Кількість досліджених референтних зразків	
		позитивних (60)	негативних (5)
Результати тестування	Кількість позитивних зразків	58 TP	0 FP
	Кількість негативних зразків	2 FN	5 TN
		Діагностична чутливість DSe TP/(TP + FN) 58 / (58 + 2) x 100% = 96,6%	Діагностична специфічність DSp TN/(TN + FP) 5 / (5 + 0) x 100% =100%

Investigation of FITC-conjugate sensitivity and specificity



Середовище	Титр антирабічних антитіл		
	до ліофілізації, МО/см³	після ліофілізації	
		абс., МО/см³	З-я ак-ті, %
№ 1.	11,7 в РН 12,5 в ТФ-ІФА	9,6	17,9-23,2
№ 2.		10,2	12,8-18,4
№ 3.		9,8	16,2-21,6
№ 4.		8,6	26,5-31,2
№ 5.		8,2	29,9-34,4
№ 6.		8,5	27,4-32,0

Development of the branch standard sample of anti-rabies immunoglobulin and its validation

5. Investigation of the potential of obtaining diagnostic drugs on the basis of anti-rabbit Ig Y birds

Immunization of quagmire with antigen of rabies virus.

The following antigens of the rabies virus + complete (first vaccination) and incomplete (second and third vaccination) Freund's adjuvant was used for the preparation of the vaccine for immunization:

- Sample 1 - strain G 52 Wistar (IndiRab vaccine, Bharat Biotech International Limited, India);

- Sample 2 - strain CVS-11 (obtained in laboratory conditions, the titre of the virus 6.23 ± 0.11 TKID50 / 0.05 cm3).

Чутливість та специфічність антирабічних глобулінів отриманих на основі Ig Y з перепелиних яєць / Ветеринарна біотехнологія. – 2016. – № 29. – С. 196-204.

5. Investigation of the potential of diagnostic drugs based on anti-rabbit Ig Y in poultry

Investigation of smears-imprints of the brain of white mice previously infected with rabies virus.

For this purpose, samples of anti-rabies Ig Y after labeled FITC and purification were investigated in PPIF.

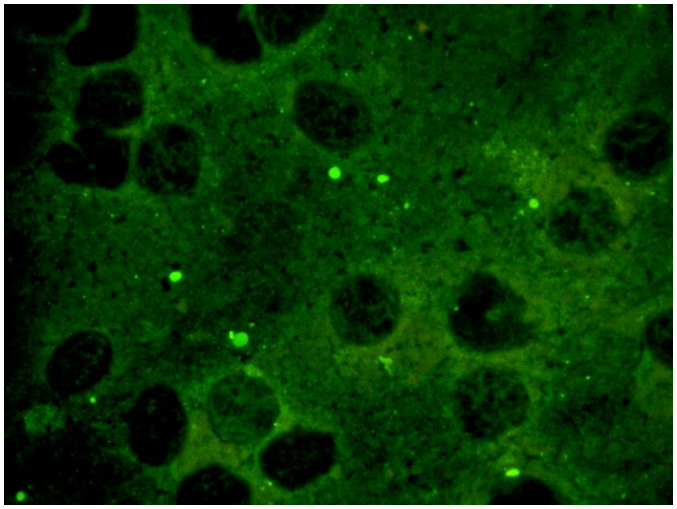


Рис. 2. Positive to rabies material (smear of FITC-Ig Y CVS-11 paints, work dilution 1: 2).

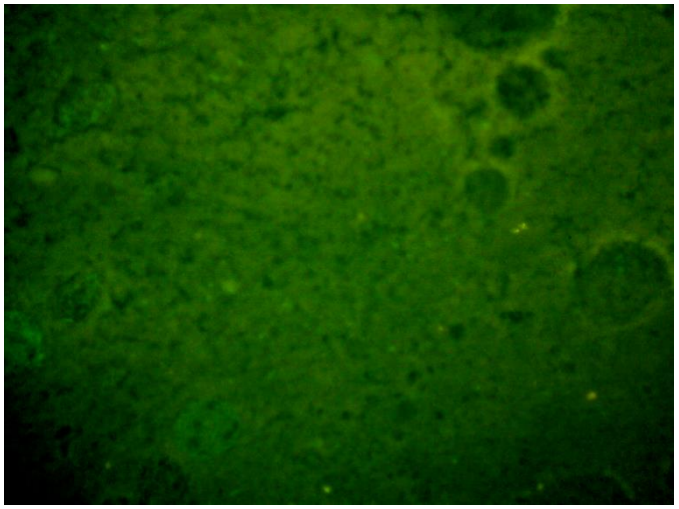


Рис. 3 Negative for rabies material (smear of FITC-Ig Y CVS-11 paints, work dilution 1: 2).

Consequently, the research in PPIF samples of anti-rabies Ig Y labeled FITC showed the specificity of the obtained Ig Y

Чутливість та специфічність антирабічних глобулінів отриманих на основі Ig Y з перепелиних яєць / Ветеринарна біотехнологія. – 2016. – № 29. – С. 196-204.

6. Development of the method and analysis of the effectiveness of oral immunization of dogs against rabies.

In veterinary medicine, where parenteral immunization is the main way to fight dog rabies, since 1985, WHO has been helping to research dogs' populations and target them with immunization in Africa, Asia, and Latin America. Recognizing the inadequacy of the parenteral pathway for eliminating rabies among dogs, WHO stimulates oral vaccination (OV) studies and the development of safer and more effective vaccines and lures for this event..

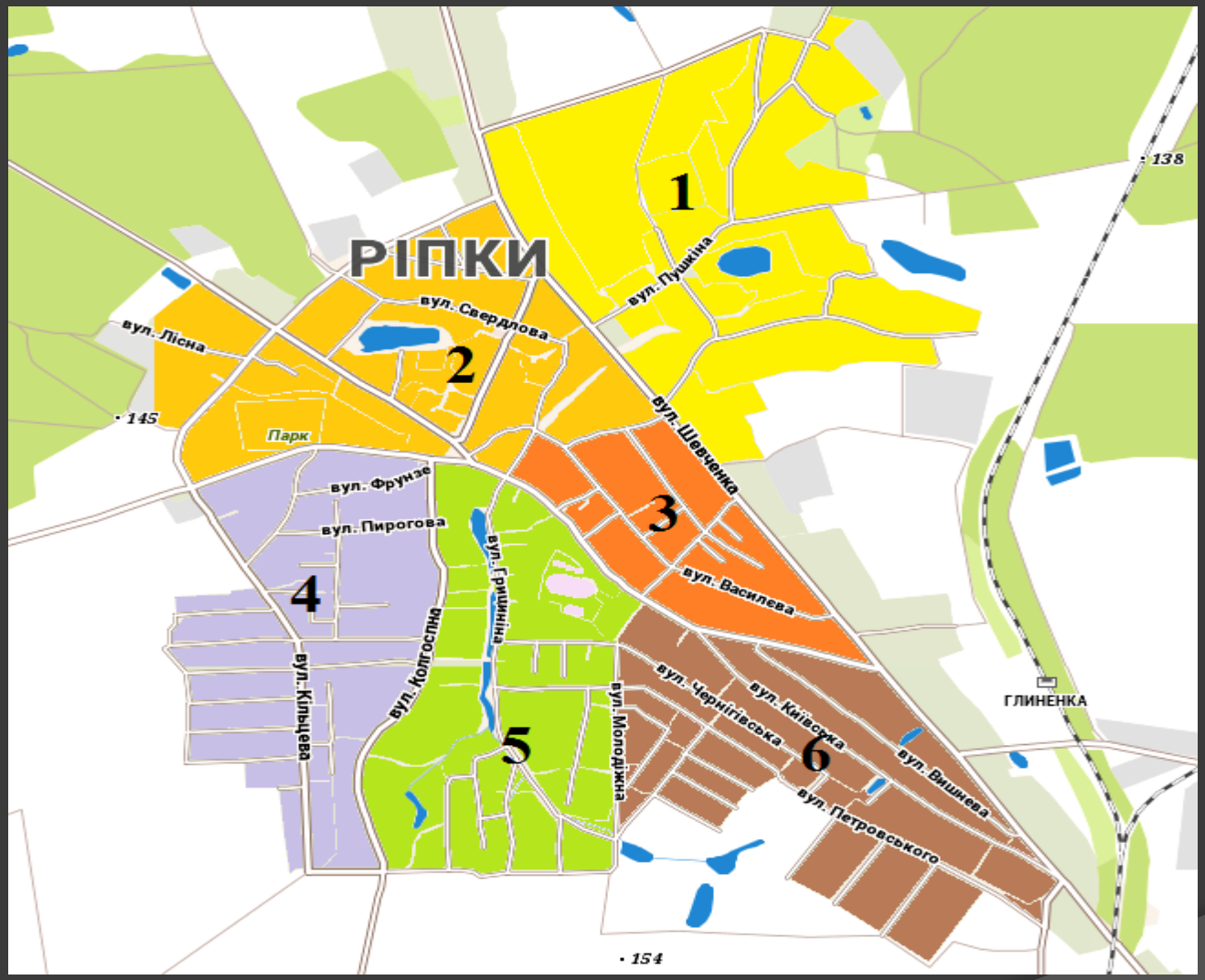
Taking into account the recommendations of the Zoonoses and Veterinary Public Health Unit of WHO, we conducted research on the method of oral vaccination of dogs against rabies

The level of anti-rabies VNA after immunization of dogs with an anti-rabbit vaccine for oral immunization of carnivores "Brovarabis V-RG"

№ п/п	Групи тварин (кількість доз вакцини)	Титр антирабічних антитіл, МО/см^3			
		0-а доба	14-а доба	30-а доба	90-а доба
1	1 доза	0	**1,87**	**1,88**	**1,15**
2	2 дози з інтервалом 7 днів	0	**3,95**	**7,63**	**6,48**
3	2 дози одноразово	0	**5,51**	**5,35**	**4,85**
4	5 доз (протягом 14 днів)	0	н/д	**93,10**	**53,58**

Ефективність пероральної імунізації собак проти сказу вакциною «Броварабіс V-RG» / Ветеринарна біотехнологія. – 2017. – № 30. – С. 41-47.

Evaluation of the population of domestic dogs



The world-renowned methodology used, developed by the WSPA and approved by the ICAM Coalition,

Field trials of the method of oral immunization of homeless dogs against rabies using the recombinant vaccine "Brovarabis V-RG"

50 doses of "Brovarabis V-RG" vaccine were used, which were spread out in 10 vaccine sites in sectors numbered 2, 3 and 4 in Ripky.

The control was carried out by taking into account the consumption of vaccine in certain areas after 24 hours.

In total, 32 doses of the vaccine **(36% of consumption**) were detected, which were taken and placed in the refrigerator at a temperature of 4 ± 2 °.
After 7 days, the re-expansion of the 50th doses in the same places was carried out.
After 24 hours, the consumption of the vaccine with the vaccine was 26 doses, that is, **consumption was 48%.**
In total, it can be assumed that 42% of the vaccine "Brovarabis V-RG" was double-folded after consumption of the vaccine.

The level of antibodies to the rabies virus in blood serum of stray dogs in the Ripky after a double expansion with a vaccine "Browarabis V-RG"

№ тварини	Титр антирабічних антитіл, МО/см^3	
	Сектор № 2	Сектор № 3
1	0	0
2	0	0
3	**2,38**	0
4	0	**3,37**
5	0	0
6	**1,37**	0
Сероконверсія, %	**25,0**	

The conducted researches demonstrated the principle possibility of using the method of oral vaccination against rabies of stray dogs in the territory of settlements of Ukraine.


Thank you for attention