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HEALTH & CONSUMERS DIRECTORATE-GENERAL
Unit 04 - Veterinary Control Programmes

SANCO/3922/2008

*Programmes for the eradication, control and monitoring of certain
animal diseases and zoonoses*

Eradication programme of Rabies

Approved* for 2009 by Commission Decision 2008/897/EC

Romania

* in accordance with Commission Decision 90/424/EEC

**Programme for Surveillance, Control and Eradication of Rabies in
Romania
2009**

Summary:

1. Identification of the programme
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Identification of the programme

Member State: Romania

Disease: Rabies

Application year: 2009

Reference of this document: National Sanitary Veterinary
and Food Safety Authority
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2. Historical data on the epidemiological evolution of rabies in Romania.

Rabies is a mortal, acute encephalitis of warm blooded animals and humans, caused by a RNA-virus of Lyssavirus Families, which spread mainly by the saliva of diseased animals, as a result of their bites. The disease can also spread by the contamination of wounds of the skin or mucosal membranes with the saliva of the diseased animals. All warm blooded animals are affected.

Rabies has two clinical forms – furious and dumb. Both forms are characterized by signs showing the affection of the central nervous system, behavioral deviation, salivation and the paralysis of the skeletal and pharyngeal muscles. Incubation period for rabies is between 14 days and 6 months, or more. An animal infected by rabies can spread the virus up to 10 days before the appearance of clinical signs. After showing the clinical signs, the animal dies in 10 days.

Rabies is disseminated on the whole globe, except certain countries in which, due to geographical particularities, either the virus never entered or the country became free of the disease, consequently to the application of certain serious combating measures (Luxemburg, Island, Norway).

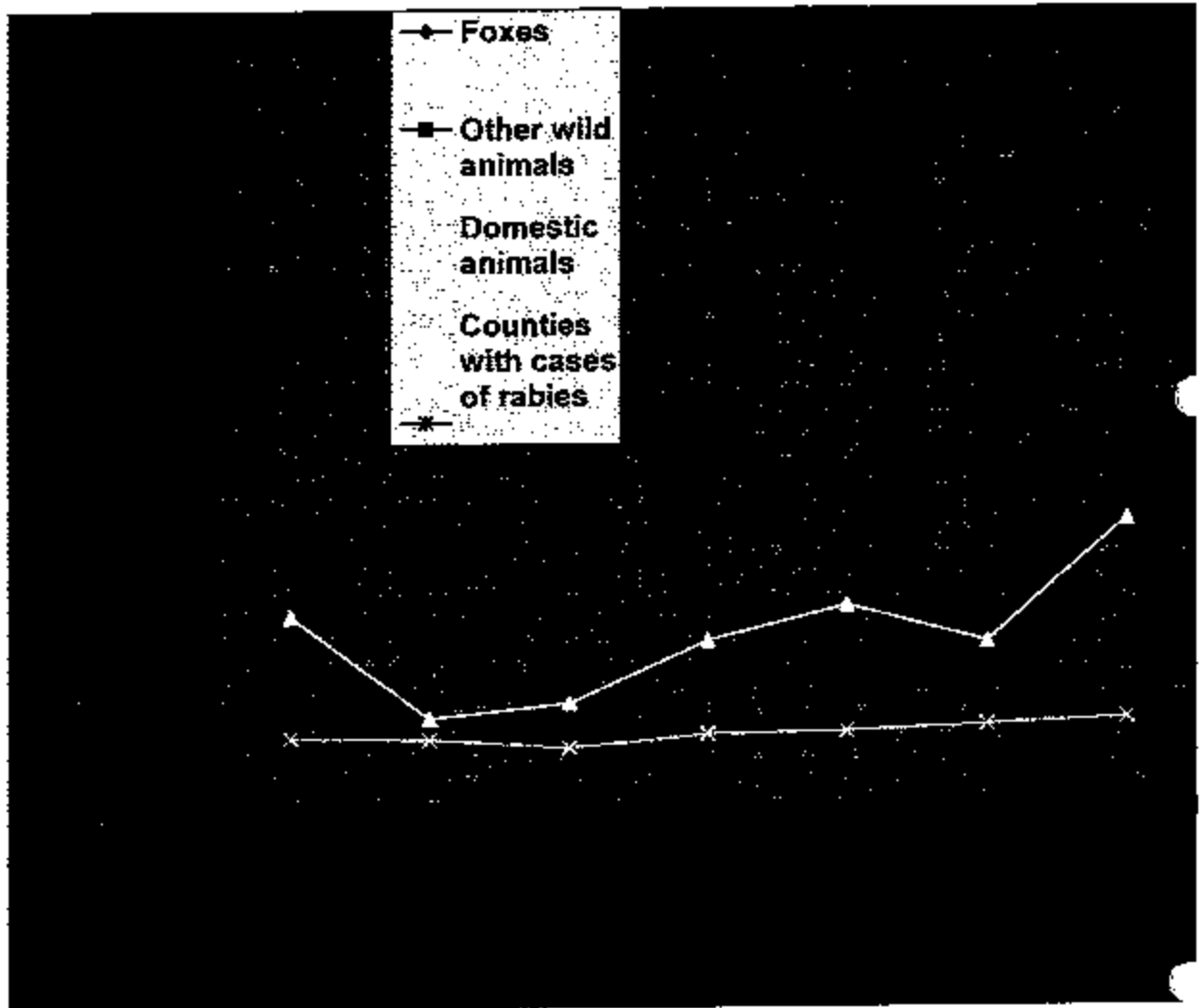
Lately, it was noticed a recrudescence of rabies in different regions of the world due to maintenance of the virus in the population of wild animals.

Romania, in the past, was one of the countries having the highest number of rabies cases from Europe.

Starting with 1950, following the measures applied, including immune- prophylaxis, rabies became preponderantly limited to wild carnivores, especially foxes.

The number of cases in foxes, in comparison with the number of cases in other wild and domestic animals, in an 8 years period (1999-2007), is shown in **Graphic 1**.

Number of rabies cases between 1999-2007 in animals population of Romania



Graphic 1

Foxes population of Romania

Romania has a surface of more than 237 500 km² of which 62346 km² is covered by forests. (Diagram 1).



Diagram 1

In Diagram 2, is represented the percentage of geographical distribution of a over than 60.000 fox population in 2007, distributed per counties. The fox population is distributed in 2151 hunting grounds (managed by the National Forests Administration and the Association of Hunters and Fishers at which these are officially registered (Diagram 2)). From numerical point of view, the fox livestock in Romania, in the last years, is maintained in constant limits, which determine that their density to be under 1 animal per km².

The stock-taking of foxes is done annually in spring, when is also established the cote for fox hunting.

Geographical distribution of foxes in Romania, 2007

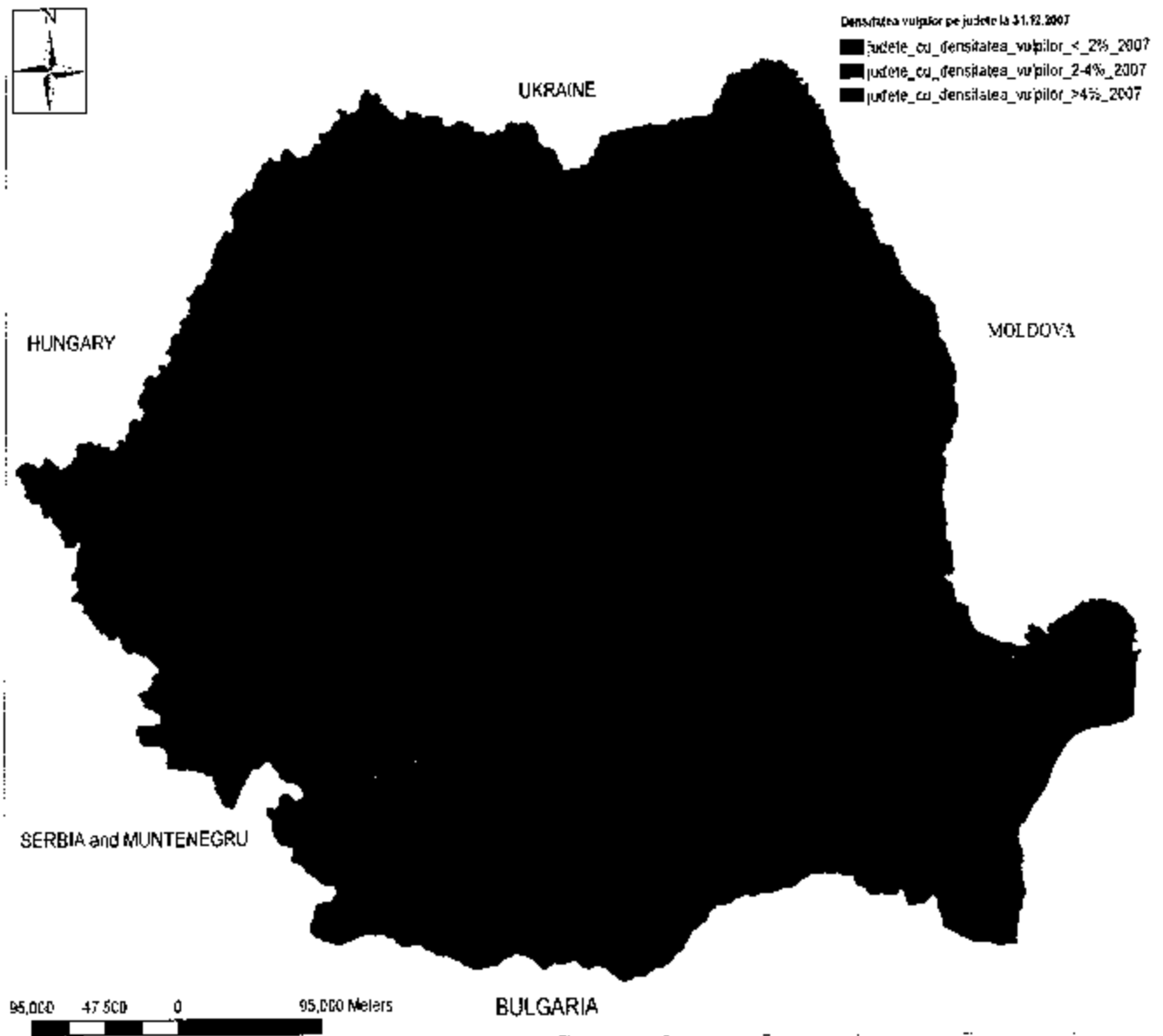
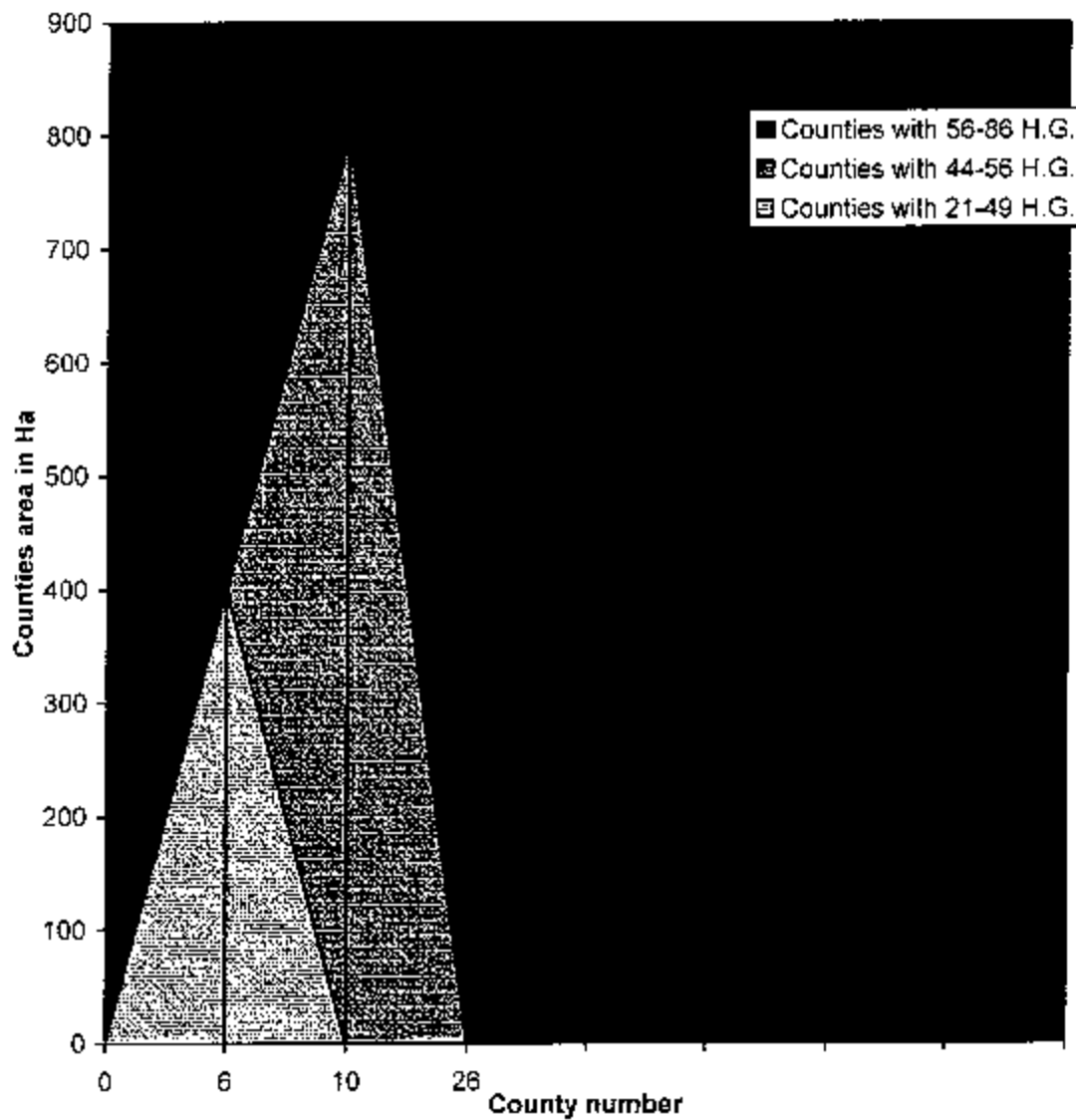


Diagram 2

Graphic 2. Repartition of hunting grounds (H.G.) depending on the counties surface



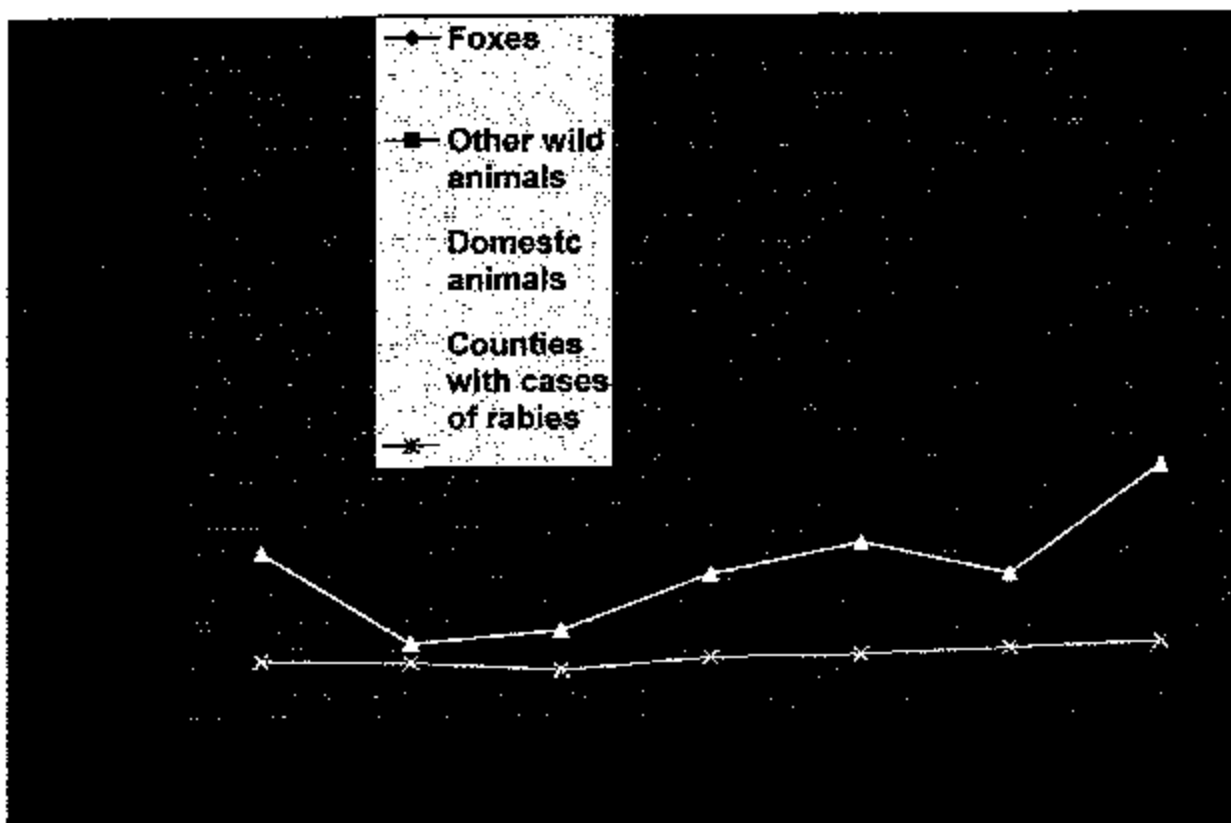
Rabies situation in foxes in Romania

Rabies in foxes living in forests evolves for many years.

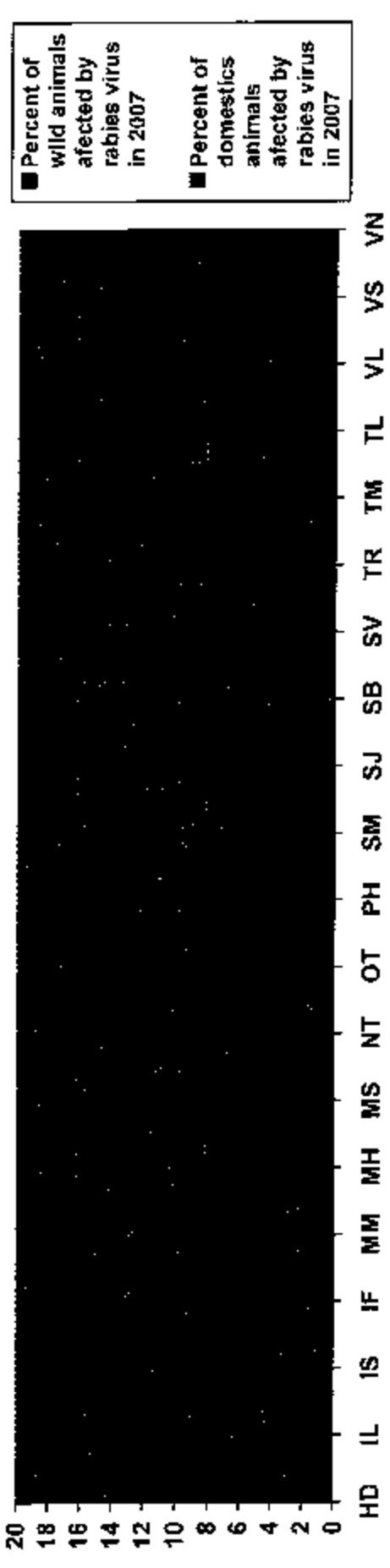
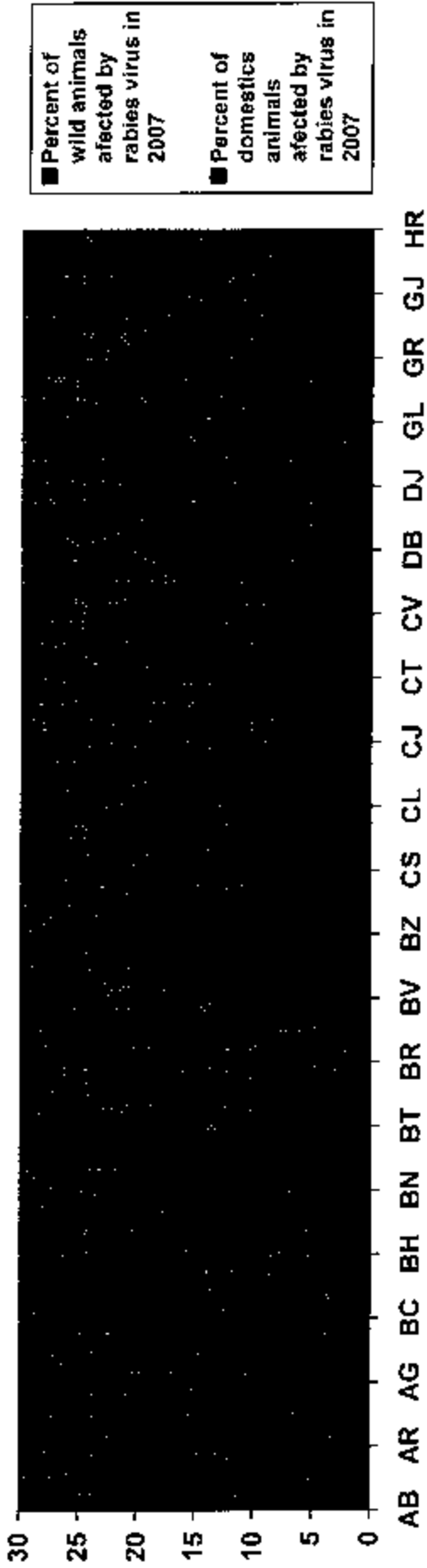
By analysis of Graphic 1, where are presented the number of rabies cases occurred in the last 8 years in domestic and wild animals, it can be appreciated that rabies is an endemic disease with increasing evolutionary tendencies.

The association between the number of rabies cases in fox population and in domestic animals population is sustained by *Graphic 3*. In *Diagram 3* is presented the percent of domestic and wild animals infected with the rabic virus, distributed per counties, in 2006. In the last years, as an immuno-prophylactic measure, in limited areas, the oral vaccination of foxes was practiced manually, depending on the epidemiological situation, but having inconclusive results.

It must be added, that the Danube Delta is a particular area, protected under the Administration of Biosphere's Reservation, where beside the fox population and other wild animals, are also living domestic animals in a semi wild condition.



Graphic 1



Graphic 3

Rabies prevalence in foxes, Romania 2007

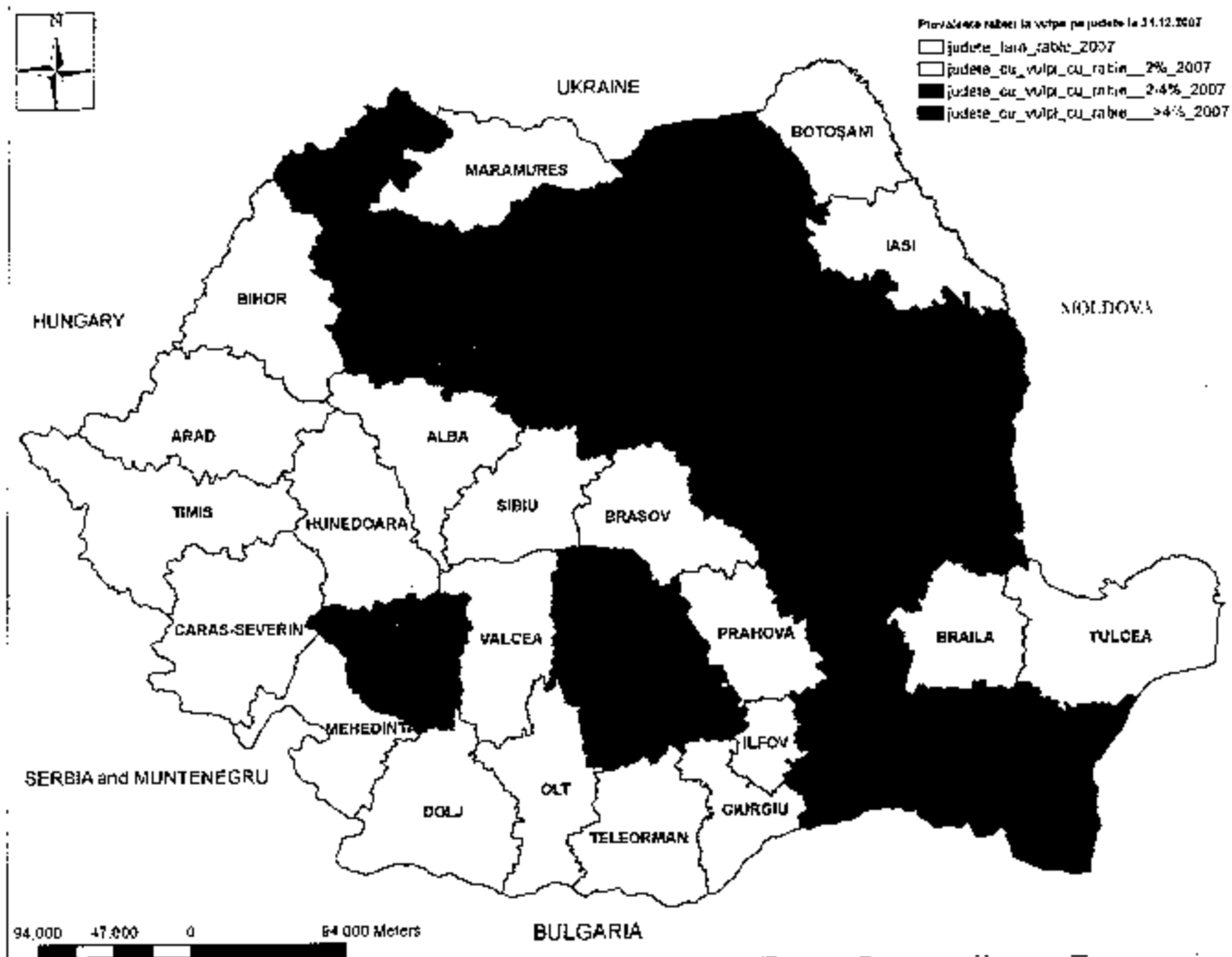


Diagram nr. 3

Rabies situation in wild animals, other than foxes

Species of domestic animals affected by rabies in Romania, in the last 8 years are presented in Table 1.

Against the situation presented, it can be appreciated that rabies evolves sporadically in the population of wild animals, other than foxes, its occurrence not being dependent of the existence of infected foxes in the relevant area.

Number of rabies cases in wild animals, other than foxes in Romania 1999-2007

Year/Species	1999	2000	2001	2002	2003	2004	2005	2006	2007
Cat	2	1	1	1	1	3	3	6	1
Ferret	1	1		1	1		3		1
Badger	1							6	0
Jackal		1					1	2	1
Brock			2	5		2			1
Otter			1						1
Musk			1						0
Weasel					1				0
Fallow-Deer					1				0
Marten						1	1		2
Bear						1	1		1
Deer						1			0
Boar							1		0
Linx							1		0
Wolf			1		3	1	1	1	2
Others								5	
Total	4	3	6	7	7	9	12	20	10
Foxes	18	51	237	65	79	115	269	203	322

Table 1

Situation of rabies in domestic animals in Romania

Species of domestic animals in which rabies cases were registered on the territory of Romania, in the last 8 years, are presented in Table 2.

Rabies cases registered in domestic animals within 1999-2007

Animals/Species	1999	2000	2001	2002	2003	2004	2005	2006	2007
Dogs	9	18	45	18	17	33	35	27	47
Cats	8	4	13	11	12	18	31	19	36
Bovine	8	5	16	5	12	14	17	19	32
Horses				3	1	4	4	3	6
Sheeps	4	1	5	1	5	5	1	1	1
Goats							1	2	6
Pigs	1	1	5	2		1		2	4
Buffalos							1		0
Asinine							1		0
Total	30	29	84	40	47	75	91	73	132

Table 2

Among these, most rabies cases were registered in the dog population, but an important number of cases were also registered in the feline and bovine populations. (Diagram 4.)

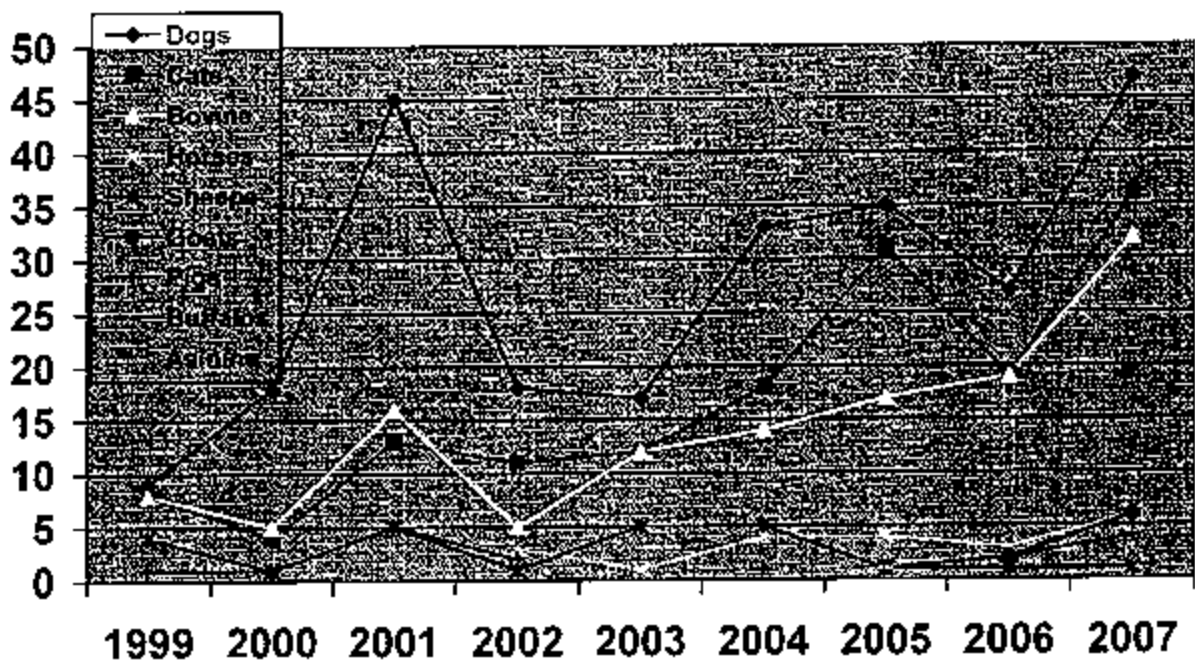


Diagram 4

3. Description of the programme

The Programme for Monitoring, Control and Eradication of Rabies will rule on the entire territory of Romania and it will apply to the entire population of foxes.

Its objectives will take into account that:

- rabies evolves in the territory of Romania both in the wild animals population, particularly in foxes and in domestic animals population;
- rabies has an endemic evolution in foxes and sporadic in other animals;
- lately we assist to the growth of the number of counties in which rabies was diagnosed, so as in 2006, out of 42 counties, the disease was diagnosed in 33;
- most of rabies cases in domestic animals were registered in dogs and cats; the situation is not adventitious if we take into account that there is a high number of dogs and cats without owners;
- the Danube Delta having a particular biotope, where wild animals cohabit with domestic animals, can be regionalized;

The objectives of the programme comprise:

- **Control of rabies in fox population in Romania**
- **Surveillance of rabies in fox population in Romania**
- **Monitoring of effectiveness vaccination in fox population in Romania**
- **Eradication of rabies in Romania**

Actions undertaken for fulfillment of the objectives:

- Vaccination of the entire fox livestock
- Monitoring of evolution of rabies correlated with the plan for application of vaccination and the results obtained
- Control of the application of vaccination baits and evaluation of its effectiveness
- Collection of data, their proper registration, their statistic and informatics procession and their presentation in proper forms in order to be used in the practice of combating and eradication of rabies in Romania.

The implementation of the programme shall be done by the Sanitary Veterinary Services in collaboration with services from the frame of the Ministry of Agriculture, Food and Rural Development based on a strategy, under the co-ordination of NSVFSA and will take into account of:

- The current epidemiological situation referring to the way of transmission of rabies and the geographical distribution of the areas affected by rabies.
- Existence of the fox population as the main vector of rabies in the low ground, hills, sub-mountain areas, close to and even in the neighborhood of populated areas.
- Carrying out of a division in areas depending on the natural or artificial barriers
- The degree of spread in the territory, categories of animals likely to be infected and really infected
- Transmissibility to humans

4. Measures provided in the submitted programme

4.1 Duration of the programme: 10 years

First year: 2007

Last year: 2016

4.1.1 Combating and control measures for 2009:

4.1.2 Vaccination

The vaccination area for 2009 will be all country, in the whole territory, in over than 237.000 km² surface.

The whole oral vaccination surface, for 2009 year, will be more than 237.000 km².

Romania will use the classic vaccination scheme, with two annual campaigns, in spring and summer. Oral vaccination of foxes in spring will be carried out depending on the weather, using 4.750.000 pieces of baits containing vaccine.

The vaccination will be repeated in the autumn, taking into account the weather, when another 4.750.000 pieces of bait will be distributed in the same territory.

For the manual distribution, SVFSD in co-operation with the territorial forest ranges will deliver the pieces of bait containing vaccine to the hunting associations and foresters of the hunting grounds.

Sanitary veterinary inspectors will check and supervise the stocking and distributing process of the pieces of baits containing vaccine. Concessionaire vets will take part at the distribution process of pieces of bait containing vaccine, as well as at the surveillance of the correctness au the actions, establishing details for identification of the vaccinated areas. Due to the fact that the effectiveness of the vaccination is conditioned by the modality of stocking and distribution of the pieces of bait containing vaccine, is absolutely necessary, that all the staff to be trained on the rules referring to stocking and

distribution of the pieces of bait, sampling and sending of samples in order to be investigated to the specialized laboratories. The assessment of this effectiveness will be carried out by determination of the immune status, of the vaccine marker in the fox population after vaccination and of the rabies virus incidence.

Samples will be taken at **30** days after each vaccination campaign.

4.2 Central Authorities in charge with the control and co-ordination of competent services for the application of the programme:

The main institutions implicated in the application of the programme for control, monitoring and eradication of rabies are: NSVFSA, County Sanitary Veterinary and Food Safety Divisions, Institute for Diagnosis and Animal Health, National Administration of Forests, District Forest Ranges Associations of Hunters and Fishers of Romania.

Distribution of the pieces of bait containing vaccine must be done by the District Forestry Services.

Institute for Diagnosis and Animal Health co-ordinates and administrate the testing capacity of the county laboratories, the application of diagnosis methods, the trainings required by the application of new techniques and together with the CSVFSD collect data regarding the laboratory tests.

4.3. Geographical and administrative areas in which the programme will be applied in 2009:

The Programme will rule on in all the country, taking into account the whole territory.

It will be taken into account the entire surface of approximately 237.000 km², being distributed 20 vaccine-doses/km². The surface covered by forests of the above mentioned a vaccination area is over 62000 km².

Geographical delimitations by natural barriers in Romania

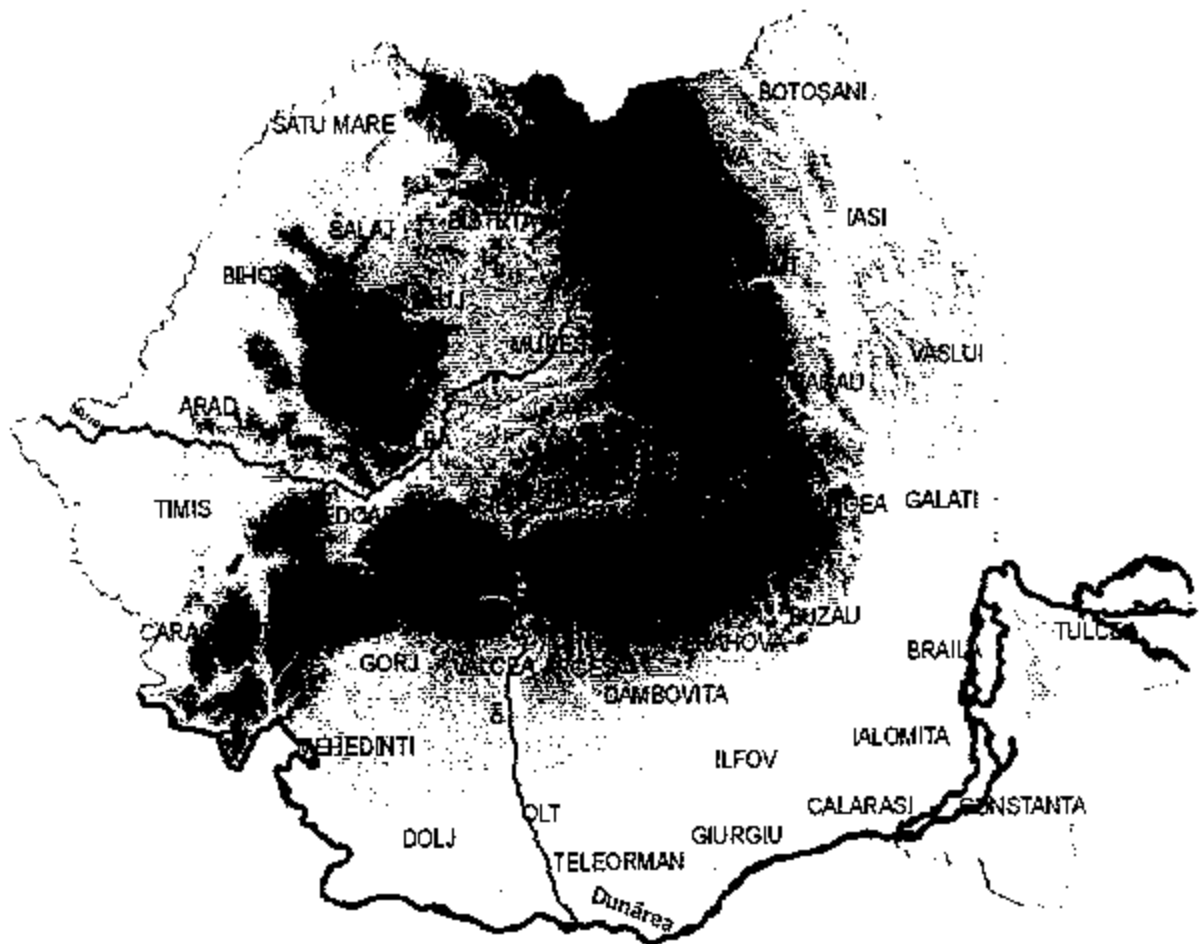


Diagram no. 5

- ○ Carpathian Mountains Chain
- ○ Rivers

Vaccination area for 2009

The vaccination area for 2009 will be all country, in the whole territory, in over than 237.000 km² surface.

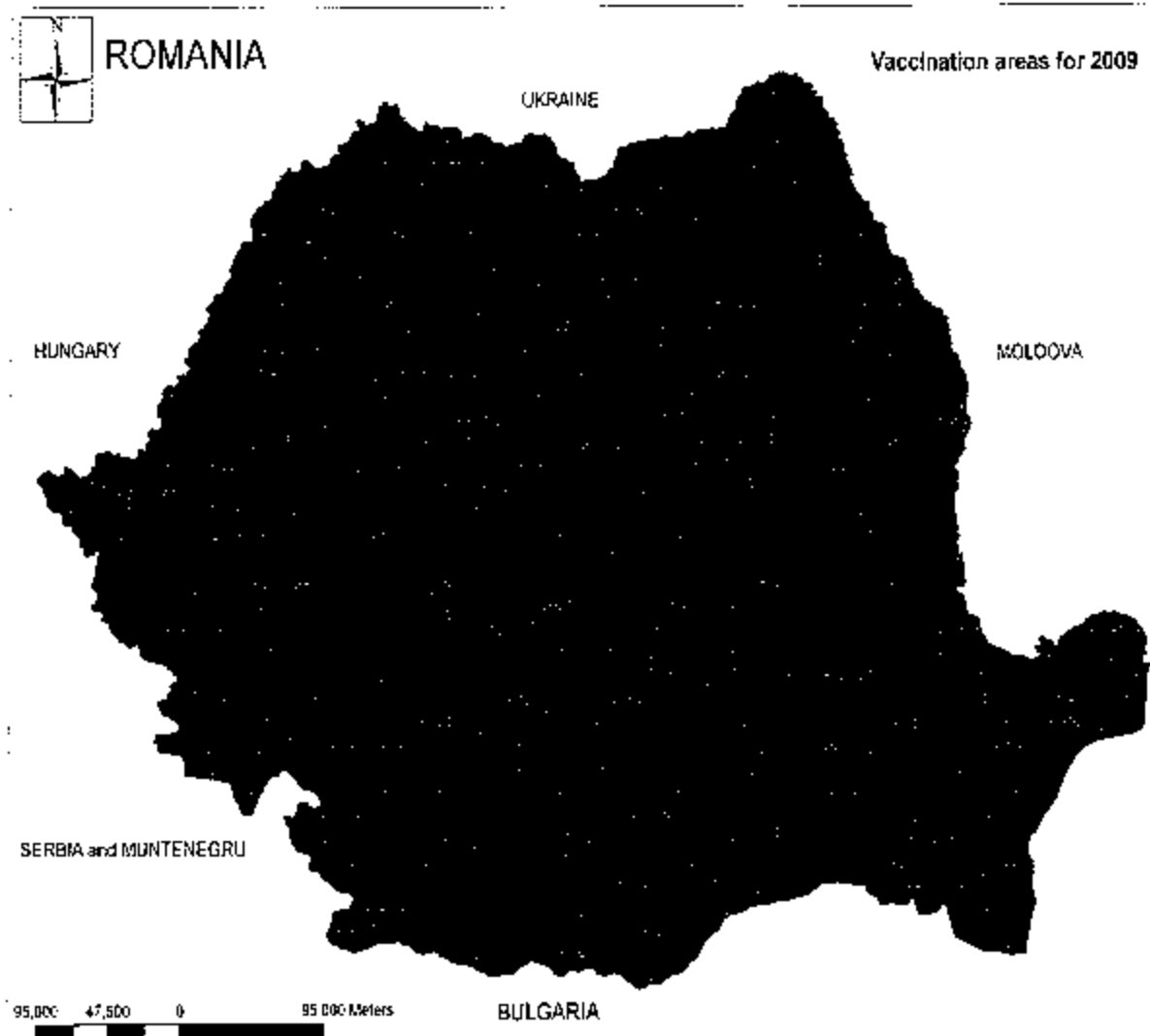


Diagram no. 6

4.4. Measures applied in the framework of programme:

Rabies prophylaxis under legislative aspects in Romania is regulated by the following Orders and Laws:

- **ORDER No. 156 of 27 December 1999 for the approval of the Sanitary veterinary norm on the announcement, declaration and notification of certain transmissible animal diseases (31982L0894)**
- **ORDER No. 107 of 26 October 2005 for the approval of the Sanitary Veterinary Norm regarding the notification of animal diseases, with all subsequent amendments, for the official transposition of Council Directive 82/894/EEC on the notification of animal diseases within the Community.**
- **ORDER No. 642 of 23 December 2002 for the approval of the sanitary veterinary norm on the recognition of vaccines against rabies (31994D0275)**
- **ORDER No. 6 of 7 January 2003 on the approval of the sanitary veterinary norm designating the institution responsible to establish the criteria necessary for the standardization of serological tests with the scope of monitoring of the effectiveness of vaccines against rabies (32000D0258)**
- **ORDER No. 14 of 24 January 2005 for the approval of the sanitary veterinary norm on the official recognition of laboratories authorized to check the effectiveness of vaccination against rabies in certain domestic carnivores (32004D0233)**

According to the abovementioned rules, in Romania, the vaccination and registration of dogs and cats is compulsory.

Emergency vaccination of all domestic animals having contact with infected suspected animals is also compulsory.

5. Description of costs and benefits:

Costs of the programme

Costs related to this programme are listed in point no.8. Financial sources are necessary to cover expenses on:

- acquisition of bait containing vaccines
- stocking of bait containing vaccines
- aerial and manual distribution of bait containing vaccines
- acquisition of diagnosis reagents
- disposable materials and special equipment
- transport expenses
- control expenses
- other costs

Benefits of the programme

The effective completion of the programme for control and monitoring of rabies in Romania will reduce the spreading chances of rabies in wild and domestic animal population, eliminating the risk of rabies transmission to humans and allowing our country to grant the free of rabies status.

6. Data on the epidemiological evolution in the last five years

6.1. Evolution of the disease:

Data referring to the evolution of rabies in Romania in the last 8 years, were presented in Chapter 2.

6.6. Data regarding the number of foxes in Romania

6.6.1. Estimation of fox population:

Estimation method:

Year: 2007

Annually, the State Forestry Services of Romania, by specific methods, estimates the fox population and set up the hunting cote. The counting of foxes is carried out in winter and early spring by the identification of sets, direct observations on certain areas and holdings and blind running.

Hunting of foxes takes place during the whole year, but the main part takes place in the winter.

The national birth rate increment is 100-150% and the hunting cote is 25000-30000 of the total of 61 000 foxes.

Counties	Number/Area Hunting Grounds (km ²)	Foxes number
ALBA	54/5872	2333
ARAD	74/7431	1980
ARGES	51/6419	1274
BACAU	57/6113	923
BIHOR	69/7005	1668
BISTRITA-NASAUD	39/4968	874
BOTOSANI	49/4491	1586
BRAILA	50/4630	584
BRASOV	44/5099	1586
BUZAU	58/5736	1202
CARAS-SEVERIN	76/8274	1791
CALARASI	49/4837	800
CLUJ	56/5994	2915
CONSTANTA	56/6583	538
COVASNA	34/3704	871

DAMBOVITA	41/3685	1126
DOLJ	77/6931	1095
GALATI	44/4183	290
GIURGIU	43/4274	489
GORJ	44/4972	611
HARGHITA	45/5939	1661
HUNEDOARA	70/6764	2004
IALOMITA	44/4244	464
IASI	55/5188	1467
ILFOV	22/1577	250
MARAMURES	54/5857	1435
MEHEDINTI	40/4817	772
MURES	64/6398	2847
NEAMT	51/5359	1259
OLT	57/4920	680
PRAHOVA	43/4112	1161
SATUMARE	49/3978	1798
SALAJ	33/3541	1285
SIBIU	46/5217	2050
SUCEAVA	71/7862	2995
TELEORMAN	62/5630	811
TIMIS	86/8272	2558
TULCEA	54/5987	871
VALCEA	47/5292	1012
VASLUI	49/4776	1048
VRANCEA	44/4543	673
TOTAL	2151/221.474 km²	60.838

Table 3

6.6.2. Serological tests used for the detection of rabies and the immune status in foxes before and after vaccination:

The serological and virological tests used are in conformity with the standard manuals for the OIE diagnosis tests.

i) *Fluorescent antibody test*

The most widely used test for rabies diagnosis is the FAT, which is recommended by both WHO and OIE. This test may be used directly on a smear, and can also be used to confirm the presence of rabies antigen in cell culture or in brain tissue of mice that have been inoculated for diagnosis. The FAT gives reliable results on fresh specimens within a few hours in more than 95-99% of cases. The sensitivity of the FAT depends on the specimen (the degree of autolysis and how comprehensively the brain is sampled, on the

type of lyssavirus and on the proficiency of the diagnostic staff. Sensitivity may be lower in samples from vaccinated animals due to localisation of antigen, which is confined to the brainstem. For direct rabies diagnosis, smears prepared from a composite sample of brain tissue, that includes the brain stem, are fixed in high-grade cold acetone and then stained with a drop of specific conjugate. Anti-rabies fluorescent conjugates may be prepared in the laboratory. Those available commercially are either polyclonal conjugates specific to the entire virus or specific to the rabies nucleocapsid protein, or they may be prepared from a mix of different MAbs. In the FAT, the specific aggregates of nucleocapsid protein are identified by their fluorescence. The specificity and sensitivity of these anti-rabies fluorescent conjugates for locally predominant virus variants should be checked before use.

The FAT may be applied to glycerol-preserved specimens. If the specimen has been preserved in a formalin solution, the FAT may be used only after the specimen has been treated with a proteolytic enzyme. However, the FAT on formalin-fixed and digested samples is always less reliable and more cumbersome than when performed on fresh tissue.

ii) Enzyme-linked immunosorbent assay

Commercial kits are available for indirect ELISA that allow a qualitative detection of rabies antibodies in individual fox samples following vaccination. In accordance with the WHO recommendations, 0.5 IU per ml rabies antibodies is the minimum measurable antibody titre considered to represent a level of immunity that correlates with the ability to protect against rabies infection. The ELISA provides a rapid test that does not require handling of live rabies virus, to determine if vaccinated foxes have sero-converted. The sensitivity and specificity of any kit used should be determined by comparison with virus neutralisation methods.

iii) RT-PCR

Due to the high level of sensitivity and specificity, RT-PCR can be used to typing the isolated field strains.

iv) Another test-

Tetracycline determination

Tetracycline is a marker of bait uptake and provides a life-long marking of bones and teeth that is easily detected on post-mortem. It is innocuous for both target and non-target species and is very stable when incorporated into baits.

Determination of tetracycline uptake provides an easy way of monitoring bait uptake and is especially useful when identifying other causes for vaccination failure.

Whereas the recommendations regarding the sampling fraction of foxes for the detection of antibodies is not provided in UE normative acts, 3050 animals have been proposed for examination.

The incidence of the rabies virus 8 samples/100 km² from brain-prints by FAT will be used. Priority needs to be given to examining and testing those animals showing abnormal behavior suggestive of rabies

No.	County	Virological Tests		Serological tests		Others	
		Sample no.	Positive cases	Sample no.	Positive cases	Sample no.	Positive cases
		1.F.A.T.		2. E.L.I.S.A F.A.V.N (antibody level)		3. U.V (tetracycline marker)	
1	ALBA	400		200	-	200	
2	ARAD	500		250		250	
3	ARGES	300		150		150	
4	BACAU	400		150		200	
5	BIHOR	300		150		150	
6	BISTRITA-NASAUD	400		200		200	
7	BOTOSANI	400		150		200	
8	BRAILA	400		150		200	
9	BRASOV	400		200		200	
10	BUZAU	400		150		200	
11	CARAS-SEVERIN	400		200		200	
12	CALARASI	400		150		200	
13	CLUJ	500		250		250	
14	COSTANTA	100		50		50	
15	COVASNA	200		100		100	
16	DAMBOVITA	200		50		100	
17	DOLJ	500		200		250	
18	GALATI	150		50		100	
19	GIURGIU	150		50		50	
20	GORJ	200		100		100	
21	HARGHITA	400		200		200	
22	HUNEDOARA	300		150		150	
23	IALOMITA	200		100		100	
24	IASI	400		150		200	
25	ILFOV	100		50		100	
26	MARAMURES	300		150		150	
27	MEHEDINTI	250		100		150	
28	MURES	400		200		200	
29	NEAMT	300		100		150	
30	OLT	250		100		100	
31	PRAHOVA	300		150		150	
32	SATU-MARE	300		150		150	
33	SALAJ	300		150		150	
34	SIBIU	50		50		50	

35	SUCEAVA	600	200	300
36	TELEORMAN	300	100	150
37	TIMIS	400	200	200
38	TULCEA	150	100	100
39	VASLUI	400	200	200
40	VALCEA	300	100	150
41	VRANCEA	250	100	150
	Total	5800	3050	3050

Table 4

6.6.3. Data on the vaccination programme in foxes

Vaccination program will carry out in all the country taking into account the all surface of Romania, distributing 20 baits/campaign/ km². Romania will use the classic vaccination scheme, with two annual campaigns, in spring and summer. Oral vaccination of foxes in spring will be carried out depending on the weather, using 4.750.000 pieces of baits containing vaccine.

The vaccination will be repeated in the autumn, taking into account the weather also, when another 4.750.000 pieces of bait will be distributed in the same territory. In both campaigns, the main distribution of baits will be aerial by plains or helicopters. Will be avoid locality territory, water surface, high way and so on where the distribution will be manual

For the manual distribution, SVFSD in co-operation with the territorial forest ranges will deliver the pieces of bait containing vaccine to the hunting associations and foresters of the hunting grounds.

Disease: Rabies

Year: 2009

Nr.	County	Km ²	Foxes number	Vaccination programme		
				Baites number /km ²	Campaignes no.	Total no. of bytes /counties
1	ALBA	5872	2333	20	2	220.000
2	ARAD	7431	1980	20	2	300.000
3	ARGES	6419	1274	20	2	260.000

4	BACAU	6113	923	20	2	250.000
5	BIHOR	7005	1668	20	2	280.000
6	BISTRITA- NASAUD	4968	874	20	2	200000
7	BRASOV	4491	1586	20	2	50.000
8	BRAILA	4630	584	20	2	160.000
9	BOTOSANI	5099	1586	20	2	200.000
10	BUZAU	5736	1202	20	2	240.000
11	CARAS- SEVERIN	8274	1791	20	2	330.000
12	CALARASI	4837	800	20	2	200.000
13	CLUJ	5994	2915	20	2	240.000
14	CONSTANTA	6583	538	20	2	250.000
15	COVASNA	3704	871	20	2	120.000
16	DAMBOVITA	3685	1126	20	2	140.000
17	DOLJ	6931	1095	20	2	280.000
18	GALATI	4183	290	20	2	160.000
19	GIURGIU	4274	489	20	2	160.000
20	GORJ	4972	611	20	2	200.000
21	HARGHITA	5939	1661	20	2	240.000
22	HUNEDOARA	6764	2004	20	2	280.000
23	IALOMITA	4244	464	20	2	160.000
24	IASI	5188	1467	20	2	200.000
25	ILFOV	1577	250	20	2	60.000
26	MARAMURES	5857	1435	20	2	240.000
27	MEHEDINTI	4817	772	20	2	180.000
28	MURES	6398	2847	20	2	270.000
29	NEAMT	5359	1259	20	2	200.000

30	OLT	4920	680	20	2	200.000
31	PRAHOVA	4112	1161	20	2	160.000
32	SALAJ	3978	1798	20	2	150000
33	SATU-MARE	3541	1285	20	2	160.000
34	SIBIU	5217	2050	20	2	200.000
35	SUCEAVA	7862	2995	20	2	300.000
36	TELEORMAN	5630	811	20	2	220.000
37	TIMIS	8272	2558	20	2	320.000
38	TULCEA	5987	871	20	2	250.000
39	VASLUI	5292	1012	20	2	200.000
40	VALCEA	4776	1048	20	2	180.000
41	VRANCEA	4543	673	20	2	180.000
	TOTAL	221.474	60838	20	2	8.130.000

8. Detailed assessment of programme's costs:

Costs related to the following measures:	Task books	Number of doses (pieces of bait)/sample	Unitary cost in EURO	Total amount in EURO	Community finances required (yes)
1. Tests					
1.1. Cost of analyses	FAT	12350	20	247.000	123.000
	FAVN	5650	53	161.650	299.450
	UV	6600	10	660.000	330.000
1.2. Cost of sampling		12350	6	74.100	37.050
1.3. Other costs					
2. Vaccination					

2.1. Buying of vaccines		9.500.000	0.82	7.790.000	3.895.000
2.2. Distribution expenses	manual	50.000 doses	10 euro/km ²	500.000	250.000
2.3 Distribution expenses	aerial	9.000.000 doses/100.000 km ²	0.38	3.420.000	1.710.000
2.4. Administrative expenses				100.000	
2.5. Control expenses				100.000	
2.6. Storage expenses				50.000	
3. Scarification and destruction					
3.1. Transport expenses				50.000	
3.2. Distruction expenses				10.000	
4. Cleaning and disinfection					
5. Remuneration				144.000	
6. Training				40.000	
6. Disposable materials and special equipments				150.000	
7. Other costs				100.000	
Total				14.256.750	6.644.500

Table 6

