EUROPEAN COMMISSION
HEALTH AND CONSUMERS DIRECTORATE-GENERAL

Director General

SANCO/10253/2014

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

The programme for the eradication of rabies

Romania

Approved* for 2013 by Commission Decision 2013/766/EU

* in accordance with Council Decision 2009/470/EC

Programme for Surveillance, Control and Eradication of Rabies in Romania
2013

Summary:

Revizia 4 din 30.10.2013
Trimis la CE in data de 30.10.2013

Identification of the programme

Member State:	Romania
Disease:	Rabies
Application year:	2013
Reference of this document:	N.S.V.F.S.A.
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Date of submission to the Commission: 30.04.2012
Revised 1: 31.08.2012
Revised 2 : 19.10.2012
Revised 3: 12.09.2013
Revised 4: 30.10.2013

2. Historical data on the epidemiological evolution of rabies in Romania.

Rabies is mortal, acute encephalitis of warm blooded animals and humans, caused by a RNA-virus of Genus Lyssavirus, 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 6 days and 6 months, or more.

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 (UK, the British Isles, Scandinavia , Spain and Portugal , Luxemburg).

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 is one of the countries having the highest number of rabies cases from Europe, the incidence is especially in foxes and dogs, who are responsible for cases of domestic animals.

In the year 2011 was made the oral vaccination of foxes in 16 counties (Arad, Alba, Bihor, Mureş, Maramureş, Bistriţa Năsăud, Braşov, Cluj, Covasna, Caraş-Severin, Harghita, Hunedoara, Sălaj, Sibiu, Satu Mare,Timiş) in West and center of Romania, which is the entire territory bounded by the Carpathian Mountains. The baits distribution included Hungarian, Serbian and part of Ukrainian border.

This activity included the air distribution of baits (approx. 20 baits/km2) also the manual distribution around localities and areas difficult to reach by plane (approximately 25 bites/km2).

Foxes population of Romania

Romania has a surface of more than 237500 km^2 of which 62346 km^2 is covered by forest (Diagram1).

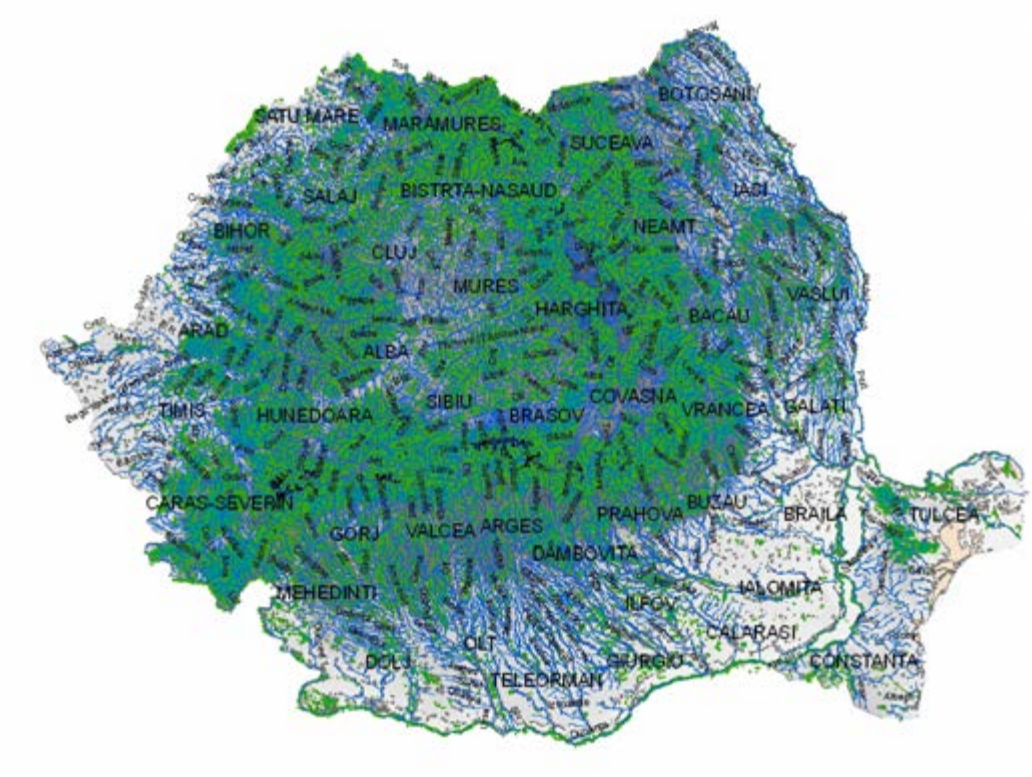


Diagram 1

In Diagram 2, is represented the percentage of geographical distribution of an over than 58570 fox population in 2011, distributed per 42 counties. The fox population is distributed in hunting grounds managed by the National Forests Administration, the Association of Hunters and Fishers and the private managers at which these are officially registered (Graphic 2). From numerical point of view, the fox population in Romania, in the last years, is maintained in constant limits, which determine that their density to be under 1 animal per km^2.

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, 2011

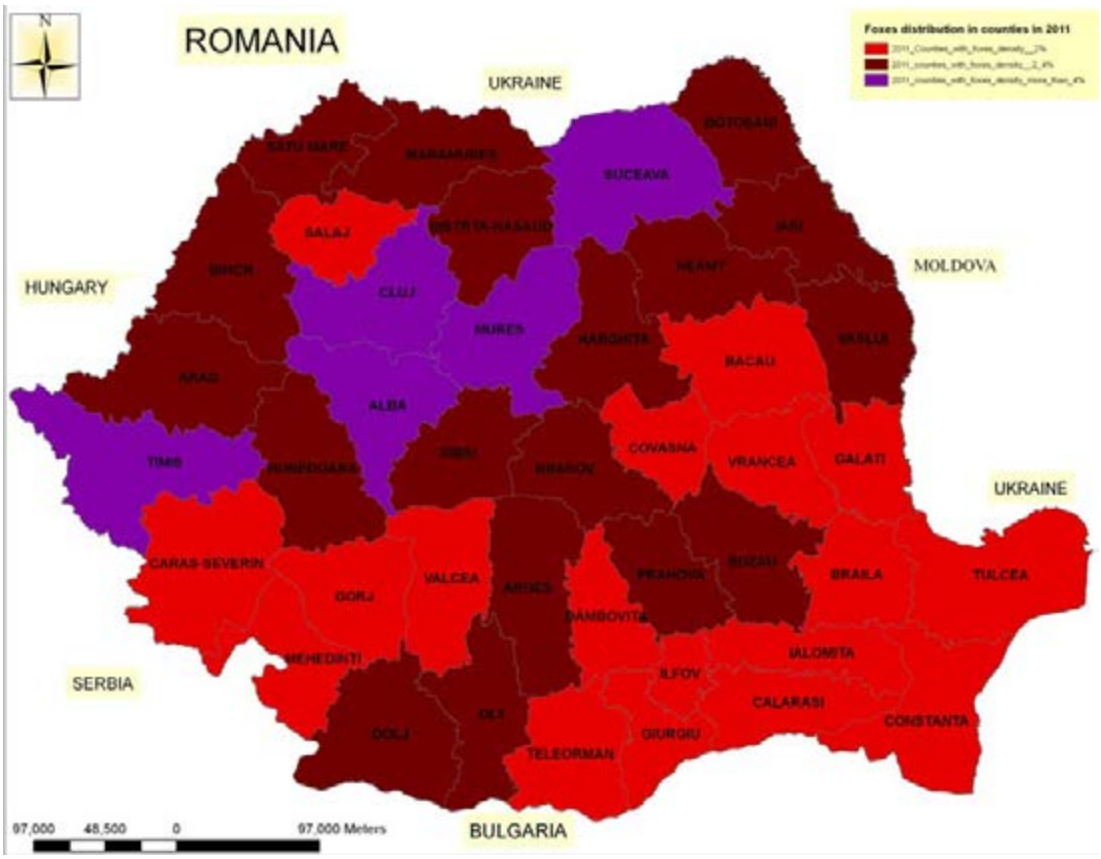
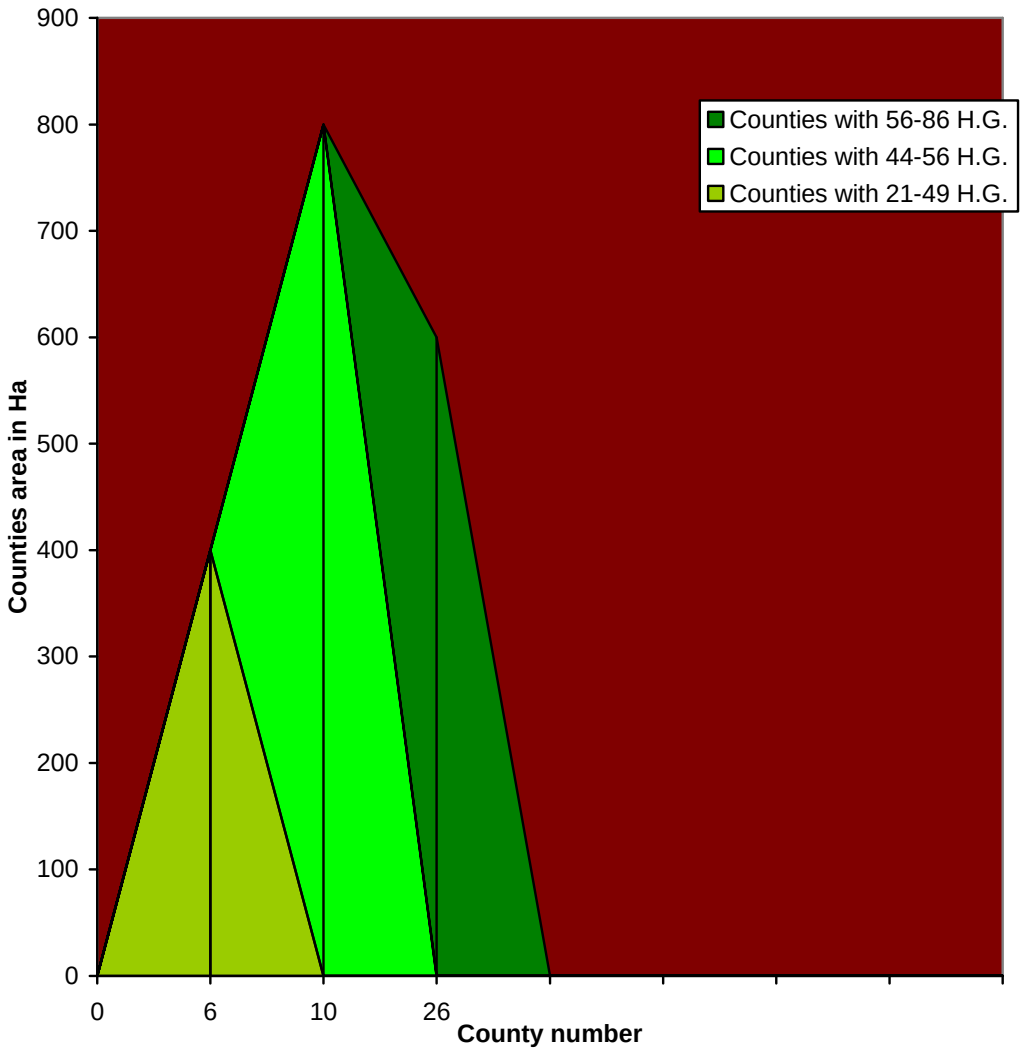


Diagram 2

Graphic 1. Repartition of hunting grounds (H.G.) depending on the counties surface



- Hunting grounds (H.G.)

Rabies situation in Romania

The prevalence of rabies , especially in silvatic reservoir, is a high risk for the most important zoonosis in Romania.

Evolution of endemic rabies situation in Romania is periodically: years with a *boom* of cases and years of epidemiologicaly regression .

It must be added, that the Danube Delta is a particular area, protected under the Administration of Biosphera's Reservation, where beside the fox population and other wild animals, are also living domestic animals in a semi wild condition. The veterinarian central service aims to draw up a special program for Danube Delta after study of the existing situation.

Against the situation presented, it can be appreciated that rabies evolutes sporadically also in the population of wild animals, other than foxes.

The incidence is especially in dogs and foxes, which are responsible for cases of domestic animals.

Manly of dogs positives cases was in rural area. The transmission was wild to domestic (wild animals-dogs).

Rabies prevalence in foxes, Romania 2011

Diagram 3

Number of animal tested and number of rabies cases for domestic and wild animals in 2011:

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3. Description of the programme

For 2013, the Programme of Monitoring, Control and Eradication of rabies will rule on the entire territory of Romania and it will be applied to the entire population of foxes.

Concerning the vaccination strategy adopted to the domestic animals, will be vaccinated dogs and cats from backyards and also emergency vaccination will be done for domestic animals only in the outbreaks.

To reducing the risk in wild populations, it will be considered also the wild dog populations in rural areas complied to fox vaccination program.

Its objectives will take into account that:
- rabies develops in Romania both in animal population wildlife, especially in foxes, wild dogs and also in domestic animals population;
- rabies develops endemically in foxes and dogs and occasionally in other animals;
- most cases of rabies in domestic animals have been recorded in dogs and cats. The situation is not casual if we consider that Romania has a very large number of stray dogs and cats;
- The Danube Delta, a unique biotope where wild animals live together with livestock, must have a special program which is under study for the moment.

The objectives of the programme will comprise:

- surveillance the prevalence of rabies in wild animal populations
- control of rabies in fox population in Romania
- monitoring of rabies in fox population in Romania
- vaccination effectiveness
- surveillance the prevalence of rabies in domestic animals population, included dogs and cats

Actions undertaken for the fulfillment of the objectives:

- oral vaccination of foxes by airplane distribution in order to obtain an territory free of rabies;
- creating vaccination barriers around localities by the manual vaccination in foxes in order to decrease the prevalence of rabies in dogs and cats
- monitoring of evolution of rabies correlated with the plan for application of vaccination and the results obtained;
- control of the application of vaccination plans 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;
- compulsory vaccination of dogs and cats;
- identification and registration dogs and cats;
- control of the population of stray dogs and cats;
- monitoring movements of animals involved Regulation (EC) No 998/2003 on the animal health requirements applicable to the non-commercial movement of pet animals and amending Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports into the Community of

animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules, monitoring the pets commercial movement.

Oral vaccination of foxes will be carry out in two vaccination campaigns, in spring and autumn, by planes, with 20 baits/campaign/km2, and a distance between flight lines 1000 m, up to 1000 meters altitude. Air distribution of baits will be adjusted with manual distribution, where the plane can not act in areas with significant surface water, and around localities with rabies cases, the vaccination will be done manually.

After each campaign, 45 days later from baits administration, will be performed hunting for vaccine efficiency, shooting at most 4 foxes/2campaign/100km2.

For monitoring the vaccination campaign, samples harvest from shooting foxes will be tested for post vaccination rabies antibodies and tetracyclines presence.

Rabies surveillance will be carrying out on samples from indicator animals (suspected, found dead - FAT).

4. Measures provided in the submitted programme

4.1 Duration of the programme: 10 years

First year: 2011

- Control
- Testing
- Vaccination

Last year: 2021

- Eradication
- Testing

4.2 Organizing, surveillance and the role of all stakeholders involved in the programme

The main institutions implicated in the application of the programme for control, monitoring and eradication of rabies are:

National Sanitary Veterinary and Food Safety Authority (NSVFSA), County Sanitary Veterinary and Food Safety Directorates (CSVFSA), Institute for Diagnosis and Animal Health (IDAH), Ministry of Environment, National Administration of Forests, District Forest Ranges, Associations of Hunters and Fishers of Romania, Institute for Control of Biological Products and Medicines for Veterinary Use .

National Sanitary Veterinary and Food Safety Authority

The tasks of the central sanitary veterinary authority responsible are to supervise and coordinate the departments which implement the programme.

NSVFSA is also responsible for assuring funds to cover the needs created by implementation of the programme.

At county level, responsible for the programme implementation are all the County Sanitary Veterinary and Food Safety Directorates.

NSVFSA, invested as Central Units for acquisition of services in view of foxes vaccination is responsible for organizing the tender and for monitoring and evaluation of vaccination efficacy, as well as the activity conducted by the society selected as winner of tender.

CSVFSD verify the transport and vaccine storage conditions, monitors the vaccine circulation within the territory, and controls the training of personnel in charge with vaccination.

Institute for Diagnosis and Animal Health

The main responsibilities are:

- Coordinate and administrate the testing capacity of the county laboratories, the training of personnel to apply the diagnosis methods;
- Coordinates the diagnostic activity for rabies;
- Draw up the epidemiological reports, based on the interpretation of the results regarding rabies;
- National Reference Laboratory rabies use the diagnostic methods in accordance with OIE Manual: Fluorescent antibody test, bio sample on mice, the test on the determination of postvaccinal antibodies against rabies by ELISA, Tetracycline determination.
- Being the National Reference Laboratory (NLR), cooperates with Community Reference Laboratory for rabies for typing and sub-typing of wild strain rabies viruses.

Institute for Control of Biological Products and Medicines for Veterinary Use

The main responsibilities are:

- Authorizes, for commercialization, biological products used for immunization against rabies in Romania;
- Performs the quality control of all vaccine batches against rabies, accordingly with European Pharmacopoeia, OIE Diagnostic Manual and the SCAHAW report, for the oral vaccination of foxes against rabies, adopted on 23 October 2002;
- Provides consultancy regarding biological products used for the immunization against rabies in Romania;

Ministry of Environment manages of National Administration of Forests, Associations of Ranges, Hunters and Fishermen of Romania.

National Administration of Forests

The main responsibilities are:

- Assures the maintenance of foxes population in reasonable limits within areas, by performing the seasonal hunting approved as a supplement to the already approved hunting quota; approve supplementary hunting quota outside the legal hunting season in the scope of sustaining the present Programme;
- Assuring, by the personnel from hunting ground, the achievement of sampling and transmission of the samples for accomplishing of laboratory surveillance for the diagnosis of rabies, according with the approved sampling programme and for the evaluation of post vaccinated immunization;
- Assures the functioning of the system for collecting, transport and neutralizing of cadavers.

National Administration of Forests estimates each year the fox population and establishes the annual the number of foxes proposed to be hunted (hunting quota).

Associations of Ranges, Hunters and Fishermen of Romania

The main responsibilities are:

- Monitoring and evaluate the density of foxes population from Romania's hunting grounds;
- Monitoring and control the implementation of measures which are incumbent on the administrators of hunting grounds;

- Cooperates with CSVFSD for the implementation of the programme;

Associations of Rangers, Hunters and Fishermen of Romania organizes the hunting sessions following to the vaccination campaigns, assure the transport of the samples harvested by the personnel in charge with sampling at the level of CSFSD.

4.3 Descriptions and delimitation of geographical and administrative areas in which the programme is being implemented:

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 km^2 in 2 campaigns being distributed approx. 25 vaccine-baits/$km^{2.}$ The surface covered by forests of the above mentioned a vaccination area is 62346 km^2.

Geographical delimitations by natural barriers in Romania

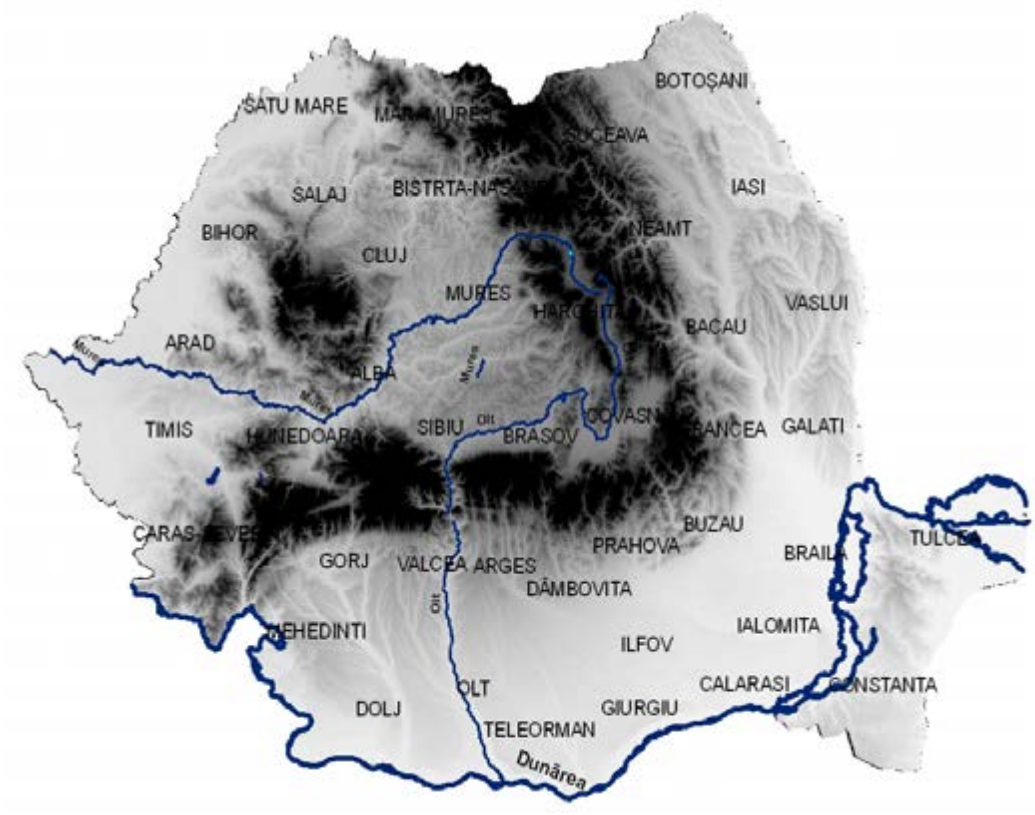

Diagram 5

- **Carpathian Mountains Chain**
- **Rivers**

Rabies vaccination area for 2013

The vaccination area for 2013 will be the whole territory of the country, in 237.000 km^2 surface providing that national legal base and running the still existing contracts tendered in 2011, especially concerning the distribution by airplane.

Diagram no. 6

4.4 Description of the measures provided by this programme

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

- NSVFSA President Order No. 29/2008 for the approval of the sanitary veterinary norm regarding general measures for preventing and control of rabies in domestic and wild animals
- Government Decision No. 55/2008 for the approval of the programme for surveillance, control and eradication rabies in foxes providing air vaccination in 16 counties and who will be replaced by another government Decision that will form the national legal rabies for vaccination in all country.

The Surveillance, control, and monitoring of domestic animals and wild animals for rabies makes the objective "The programme for the actions of surveillance, prevention and control of animal diseases, of those transmissible from animals to man, for protection of animals and environment" which is carried out yearly by the National Sanitary Veterinary and for Food Safety

Authority; this programme is suplimented, everytime it is necessary, with epidemiological and risk analysis.

Accordingly with the above mentioned rules, in Romania, the vaccination and registration of domestic dogs and cats is compulsory.

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

4.4.1 Disease notification

Rabies is a notificable disease in Romania, in accordance with the NSVFSA President Order No.79/2008.

The obligativity of disease notification comes to the free practice empowered practitioners which notify the official veterinarian about the rabies suspicions in the field. Rabies suspicion is notified from the field to SVFSD, and samples are sent to the county sanitary veterinary laboratory accredited and authorized for diagnosis.

The official vet responsible with animal health from county SVFSD, notifies the suspicion by a rapid communication mean to the director of Animal Health and Welfare Directorate from NSVFSA and also by using a notification report form, to NSVFSA all suspected cases of rabies.

Following to laboratory confirmation of rabies, the county SVFSD and of the Bucharest Municipality, will notify, using a notification report form, to NSVFSA all confirmed cases of rabies.

If rabies is confirmed in a domestic animal, the owner is also notified and a complete file is issued in view of applying the control measures, if necessary.

The situation concerning rabies cases is notified twice on a year to OIE and quarterly to the European Institute for Rabies Control.

4.4.2. Target animals and animal population

The target animals of this programme are the foxes. The population of the foxes for the year 2011 is estimated at 58570 animals (see Table no. 2 above).

4.4.3. Identification of animals and registration of holdings - not applied

4.4.4. Qualifications of animals and herds - not applied

4.4.5. Rules on the movement of animals - not applied

4.4.6 Serological and virological 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 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 localization of antigen, which is confined to the brainstem. For direct rabies diagnosis, smears prepared from a composite sample of brain tissue, which 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 nucleocapisid 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 proteolytical enzyme. However, the FAT on formalin-fixed and digested samples is always less reliable and more cumbersome than when performed on fresh tissue.

You find in the chapter 7, subchapter 7.1.1. the estimation number of the animals that will be checked by FAT.

In 2011 during the fox rabies eradication program 1372 foxes were shot and checked by FAT. 51 out of 1372 were positive.

Based on high rabies incidence in Romania, and because some foxes have no clinical/nervous symptoms when there are shot, we decided to check all of them by FAT.

FAT negative samples are sent to NRL for rabies for tetracycline marker and antibodies detection by ELISA, and FAT positive samples are sent to NRL to genotype them and differentiation between wild and vaccinated strains.

ii) Enzyme-linked immunosorbent assay

Commercial kits are available for indirect ELISA that allows a qualitative detection of rabies antibodies in individual fox samples following vaccination. The ELISA provides a rapid test that does not require handling of live rabies virus, to determine if vaccinated foxes have sera-converted. Whereas the recommendations regarding the sampling fraction of foxes for the detection of antibodies is not provided in UE normative acts, 8480 animals have been proposed for examination in 2013 year.

iii) 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 by direct U.V. microscopic examination of sections of bones and teeth provides an easy way of monitoring bait uptake and is especially useful when identifying other causes for vaccination failure.

iv) Another test:

Direct sequencing of nucleoprotein gene, partial cds (coding sequence)
Materials and method:

Biological material is represented by brain homogenate, confirmed by standard method (direct imunofluorescence). They are selected for geographical distribution in order to cover as much as possible from Romanian territory or from oral fox vaccination territory.

All samples are initially homogenized using MagNa Lyzer instrument (*Roche Applied Science*), centrifuged and supernatant is collected.

RNA extraction is performed using commercially available kits, such as RNEasy Mini Kit (*Qiagen*) or High Pure RNA Isolation Kit (*Roche Applied Science*), according to manufacturer specifications. Briefly, 200µl supernatant from the previously step was used as starting material, with the elution of RNA in 50µl final volume.

Revers-transcription and amplification: the protocol is a conventional RT-PCR with primers targeting the nucleoprotein gene, generating an amplification product of 606bp (base pairs, *Heaton et al.*). All reactions are performed using "*single tube*" technique, with commercially available kits – One Step RT-PCR kit (*Qiagen*) or equivalent according to the manufacturer specification, with a recommended final concentration for each reagent.

Electrophoresis is conducted in a 1X TBE buffer with 2% agarose gel stained with ethidium bromide (all *Bio Rad Laboratoires*) and the results were visualized on GelDoc instrument (*Bio Rad Laboratoires*).

Gel isolation and purification: we use commercial kits, according to manufacturer recommendation, followed by fragments quantification with Biophotometer (*Eppendorf*).

Direct sequencing is performed with BigDye Terminator Cycle Sequencing Kit version *1.1* or *3.1*, one of the PCR primers and capillary electrophoresis on 3130 Genetic Analyzer (both *Applied Bioscience*).

Results obtained are blasted against GenBank and personal database to confirm rabies virus. For alignment, we used *on-line* available softwares (*Bio Edit Sequence Alignment Editor*, *Clustal W*), resulting in a final fragment of 322bp (base pairs).

Dendrograms were obtained also using available software (*Mega Software version 4.0*), using algorithmic Neighbor-Joining bootstrap test of phylogeny, with the following conditions: 3000 to 5000 replicates, Kimura 2 parameter.

In the period 2011-2012 the samples subjected to phylogenetic investigations are selected according to geographycal distribution in order to cover all the romanian territory – therefore not all positive samples are tested, but only some of them that are relevant for spatial distribution.

All samples selected for phylogenetic investigations are sended to IDAH (NRL for rabies) and they are processed as descibed above.

So far, all analyzed samples belong to the wild virus cathegory, with high degree of genetic diversity (at least six lineages) - http://www.ncbi.nlm.nih.gov/pubmed/20178821. For quality assessment, NRL for rabies participate to the Ring Trials organised by EURL, including phylogenetic analysis. During the two 2011 vaccination campaigns were not submitted positive cases samples to the EURL.

All the positive cases (samples) detected in vaccinated areas will be differentiated from the vaccine strain. The samples will be sent to IDAH (NRL for rabies) and they will be processed as described above.

4.4.7 Vaccines used and vaccination schemes

Live rabies vaccines used for oral vaccination of foxes should fulfill the requirements of the European Pharmacopoeia monographs as well as the efficacy and safety recommendations of the WHO. Vaccine titer at batch release should correspond to at least ten times the dose found to completely protect an experimental group (indicative 100% protective dose). The titer of the final vaccine in the bait should not fall below the indicative 100% protective dose following exposure to 25°C for seven days. Each vaccine batch should be tested and approved for titer and stability and by an acknowledged quality control scheme according to European Pharmacopoeia, OIE standards, WHO recommendations and the SCAHAW report., for the oral vaccination of foxes against rabies, adopted on 23 October 2002.

The proof compliance about vaccine titer shall be demonstrated in certificates issued by official quality control laboratories recognized by EDQM (European Directorate for Quality Medicine).

Laboratories involved in the rabies surveillance programme, monitor titre during release into the field and the stability of the vaccine virus titer during release into the field, for all batches of vaccines received.

The melting point of the bait casing should be above 40°C to ensure that the capsule of the vaccine is still covered if exposed to such temperatures in the field.

The Community Reference Laboratory should perform additional tests or trials if required.

The use of tetracycline as a biomarker in the teeth and bones of foxes is recommended to evaluate bait-uptake in target species.

The vaccines against rabies which follow to be used in vaccination campaigns against rabies in foxes are presented as vaccine bites, administered by plane on the most important surface from the area established for vaccination, and manually, in zones where the administration by plane is not possible.

The used vaccines need to be immunogenic, harmless and produced for the main susceptible species at rabies, as well as to be used in most of the vaccination campaigns established during the year, no matter the weather conditions.

The oral vaccination of foxes is made by distributing with plane or helicopter with a density of vaccine baits (25 baits/km2), on smooth surfaces or in case of area where the access is burdened, up to 1000 meters altitude; around the localities vaccination will be done by manually distribution (25 baites/km2), by the managers of the hunting grounds, being assisted by the official vets. In areas with significant surface water, the vaccination will be done manually.

In order to be appropriate for use in Romania, the vaccines against rabies need to be authorized for commercialization in our country.

The authorization for commercialization is obtained in accordance with the NSVFSA Order no. 187/2007 regarding The Code of veterinary medical products, published in the Official Journal of Romania Part I, No. 804 bis/26.XI.2007 or in accordance with the Regulation 726/2004/EC for establishing the community procedures concerning authorization and surveillance of medicines for humans and for founding of an European Agency for medicines, published in J.O. Nr. L 136, 30.04.2004.

The authorization conditions for vaccine against rabies are:

- To contain live attenuated vaccine strains;
- To be intended for oral immunization of foxes;
- To be able to be distributed by plane/helicopter;

At delivery, every vaccine series need to be accompanied by the Official Analysis report, in accordance with the request of EDQM (European Directorate for Quality Medicine).

The number of vaccination campaigns, the vaccination scheme and the way in which vaccination is effectively done are described in the 3-rd chapter: “The description of the programme” and 7.3.2. “Data on the vaccination programme in foxes”

Responsible for the vaccination campaigns is General Sanitary Veterinary Directorate, at central level, and county sanitary veterinary directorates, at local level.

For the vaccination of livestock (dogs, cats and other domestic animal), the vaccine it is used in accordance with national and EU legislation.

Vaccination of the domestic carnivore (dogs and cats) – each animal must be vaccinated against rabies from the age of the three months with yearly revaccination according with the NSVFSA

President Order No.29/2008 for the approval of the sanitary veterinary norm regarding general measures for preventing and control of rabies in domestic and wild animals and Commission Decision 94/275/EC on recognizing rabies vaccines.

Prophylactic vaccination of dogs and cats in backyards and dogs from the sheepfold with inactivated vaccine is made by organizing mass vaccination campaigns, annual autumn-winter period, followed by completing vaccination.

Vaccination of domestic animals in the outbreak is done according to the national legislation in force.

4.4.8. Information and assessment on bio-security measures management and infrastructure) in place in the holdings involved- not applied

4.4.9. Measures in case of a positive result

When a rabies cases is confirmed in domestic or wild animals, are applied specific control measures, in accordance with the NSVFSA President Order No. 29/2008.

For these cases is applied the following procedure:

A. Measures applied in case of rabies confirmation in animals from a holding, locality, zone

After rabies confirmation, the county SVFSD acts as follows:

a) perform the epidemiological enquire ;
b) establishes the protection and the surveillance zones;
c) issues the control plan with deadlines and responsibilities;

The control measures in the protection zone include:

- drawing up the epidemiological maps;
- killing of carnivores which were bitted or scratched by sick animals, if they were not vaccinated against rabies, or if they have less than 21 days since first vaccination,
- isolation by the rest of the animals of the vaccinated carnivores which have been bitted or scratched by the sick animal;
- placement under observation of all animals from that holding for 14 days, beginning with the contact moment ;
- killing of all animals from that holding, in case when they manifest clinical signs in this period of time; animals which did not manifest clinical signs of rabies, are released from observation;
-inspection of the carnivores from the protection zone which have been bitten or scratched by the sick animal are made by the free practice empowered, for 14 days, and, if they don't show clinical signs are released from the observation;
- interdiction of animal movement for animal which were under observation.

The control measures in the surveillance zones include:

- a census for all dogs and cats;
- vaccination of dogs and cats with inactivated vaccine;
- surveillance and movement control of dogs and cats.

B. **Measures applied in the hunting founds, in case when rabies is confirmed in wild animals**

When rabies is confirmed, the county SVFSD and that of Bucharest Municipality take the following measures:

a) perform the epidemiological enquire ;
b) establishes and declare the infected area ;
c) Ask to the managers of the hunting founds to evaluate the wild animal population, especially of the foxes;
d) released the control measures plan with deadlines and responsibilities;
e) release and implement a vaccination programme for foxes ;
f) ask for organization of hunting campaigns for foxes, without using hunting dogs ;
g) order the banning of skinning wild animals killed or found dead.

4.4.10. Compensation scheme for owners of slaughtered and killed animals

Rabies is included on the list of the disease for which the government assures the compensation of farmer's losses in case of appliance of the control measures

Compensation for the killing of infected animals and animals which represent sources of contamination and also compensation for animals killed or affected in some other way in the process of killing on the infected premises are covered in Government Decision (GD) No. 1214/2009 with subsequent amendments.

This GD is under modifying now, having regard that the compensation of losses for owner will be done in the future by NSVFSA founds, and not from Ministry of the Agriculture, Forestry and Rural Developments founds as so far.

Government Decision No. 1214/2009 specifies the beneficiaries of the compensation (under art. 4), the method of compensation and the source of the funds for disease control operations and describes the basis for this calculation (covered in art. 4 and Appendix no. 2).

Appendix no. 1 of Government Decision No. 1214/2009 presents the list of diseases for the eradication of which compensation payments are granted.

The compensation will be paid to the owner, by the market value, for the animals killed on suspicion, following, as well as for animal by-products and materials which have been seized and destroyed (direct expenses).

4.4.11 Control of the implementations of the programme and reporting

The control of implementing the programme is made by the NSVFSA by the Directorate for Checks and Border Inspection Post (BIP) Coordination, in accordance with the provisions of the National Programme for Checks, approved through NSVFSA President Order.

At the level of county SVFSD, the control is performed by sanitary veterinary official officers from the service for checks, in collaboration with the official veterinarian from animal health service and sanitary veterinary zonal office who draw up reports concerning the fulfill of the programme. These reports about surveillance shall be sent towards the central veterinary authority and to the Institute for Diagnosis and Animal Health.

5. 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 during the last five years

6.1. Evolution of the disease - not applied

6.2. Stratified data on surveillance and laboratory tests

All serological and virological tests are performed according to the diagnostic manual of the EU

Description of the used serological tests:

1. ELISA test for antibody detection.

Description of the used virological tests:

2. Florescent antibody test

6.2.1. Stratified data on surveillance and laboratory tests

Years: 2007-2011 Desease: Rabies Species **: Foxes**

Romania	Serological tests		Virological tests	
Year	Number of samples tested	Number of positive samples	Number of samples tested	Number of positive samples
2007	Not applied	Not applied	823	321
2008	17	2	964	951
2009	275	25	1173	404
2010	30	0	989	319
2011	770	102	2084	238

Years: 2007-2011 Desease: Rabies Species : **Other wild animals**

Romania Year	Serological tests		Virological tests	
	Number of samples tested	Number of positive samples	Number of samples tested	Number of positive samples
2007	Not applied	Not applied	58	10
2008	Not applied	Not applied	67	26
2009	Not applied	Not applied	48	17
2010	Not applied	Not applied	48	19
2011	Not applied	Not applied	36	9

Years: 2007-2011 Desease: Rabies Species : **Dogs**

Romania Year	Serological tests		Virological tests	
	Number of samples tested	Number of positive samples	Number of samples tested	Number of positive samples
2007	Not applied	Not applied	269	47
2008	Not applied	Not applied	396	43
2009	Not applied	Not applied	287	38
2010	Not applied	Not applied	215	46
2011	Not applied	Not applied	270	40

Years: 2007-2010 Desease: Rabies Species : **Cats**

Romania Year	Serological tests		Virological tests	
	Number of samples tested	Number of positive samples	Number of samples tested	Number of positive samples
2007	Not applied	Not applied	84	36
2008	Not applied	Not applied	157	60
2009	Not applied	Not applied	97	29
2010	Not applied	Not applied	67	25
2011	Not applied	Not applied	92	19

Years: 2007-2011 Desease: Rabies Species : **Other domestic animals**

Romania Year	Serological tests		Virological tests	
	Number of samples tested	Number of positive samples	Number of samples tested	Number of positive samples
2007	Not applied	Not applied	430	49
2008	Not applied	Not applied	470	58
2009	Not applied	Not applied	353	48
2010	Not applied	Not applied	261	65
2011	Not applied	Not applied	200	35

6.3. Data on infection (one row per year)

Years: 2007– 2011 Disease: Rabies Animal Species: **Domestic animals**

Romania[(b)]	Number of herds infected	Number of animals infected
2007	132	132
2008	161	161
2009	93	115
2010	100	136
2011	77	94

Years: 2007 – 2011 Disease: Rabies Animal Species: **Foxes**

Romania[(b)]	Number of herds infected	Number of animals infected
2007	Not applied	321
2008	Not applied	951
2009	Not applied	404
2010	Not applied	319
2011	Not applied	238

6.4. Data on the status of herds at the end of each year - not applied

6.5. Data on vaccination programmemes - not applied

6.6. Data regarding the number of foxes in Romania

6.6.1. Estimation of fox population: 2011

Estimation method:

Annually, the State Forestry Services of Romania, by specific methods, estimates the fox population and set up the hunting quota. 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.

Year: 2011

Nr.	Counties	Hunting grounds(*km2*)	Foxes number
1	ALBA	5873	2930
2	ARAD	7431	1964
3	ARGES	6419	2054
4	BACAU	6125	936
5	BIHOR	7006	1719
6	BISTRITA-NASAUD	4969	1248
7	BRASOV	4491	2165
8	BRAILA	4630	476
9	BOTOSANI	4492	1877
10	BUZAU	5736	1469
11	CARAS-SEVERIN	8274	1066
12	CALARASI	4837	619
13	CLUJ	5994	2701
14	CONSTANTA	6583	368
15	COVASNA	3704	725
16	DAMBOVITA	3686	943

17	DOLJ	6931	1522
18	GALATI	4183	418
19	GIURGIU	3274	341
20	GORJ	4972	423
21	HARGHITA	5939	1421
22	HUNEDOARA	6764	2193
23	IALOMITA	4244	545
24	IASI	5188	1575
25	ILFOV	1577	273
26	MARAMURES	5857	1459
27	MEHEDINTI	4817	768
28	MURES	6398	4650
29	NEAMT	5359	1638
30	OLT	4920	1300
31	PRAHOVA	4112	1503
32	SALAJ	3541	1135
33	SATU-MARE	3978	1325
34	SIBIU	5217	1815
35	SUCEAVA	7862	3221
36	TELEORMAN	5631	819
37	TIMIS	8272	2840
38	TULCEA	5987	1001
39	VASLUI	4776	1425
40	VALCEA	5292	985
41	VRANCEA	4543	625
	TOTAL	**219.884**	**58.570**

Table no. 2

6.6.2. Monitoring of wildlife

Years: 2007 – 2011 Disease: rabies Animal Species: **Foxes**

Description of the used serological tests:

1. ELISA for antibody detection.

Description of the used virological tests:

2. Florescent Antibody Imunofluorescent

Other tests:

3. Tetracycline detection

All serological and virological tests are performed according to the diagnostic manual of the EU

Romania	**Serological tests (ELISA)**		**Virological tests (FAT)**		**Tetracycline detection**	
	Number of samples tested	**Number of positive samples**	**Number of samples tested**	**Number of positive samples**	**Number of samples tested**	**Number of positive samples**
2007	Not applied	Not applied	823	321	Not applied	Not applied
2008	17	2	964	951	20	3
2009	275	25	1173	404	275	18
2010	30	0	989	319	30	0
2011	770	102	2084	236	982	269

6.6.3 Data on the vaccination programme in foxes

Year: 2007-2011 Disease: Rabies Animal Species: **Foxes**

In the year 2011 was made the oral vaccination of foxes in 16 counties (Arad, Alba, Bihor, Mureş, Maramureş, Bistriţa Năsăud, Braşov, Cluj, Covasna, Caraş-Severin, Harghita, Hunedoara, Sălaj, Sibiu, Satu Mare,Timiş) in West and center of Romania, which is the entire territory bounded by the Carpathian Mountains. The baits distribution included Hungarian, Serbian and part of Ukrainian border.

This activity included the air distribution of baits (approx. 20 baits/km2) also the manual distribution around localities and areas difficult to reach by plane (approximately 25 ites/km2). To 45 days after the end of the vaccination campaign started the shooting of foxes with the hunting associations. Pay for a fox shot is 50 Ron; they are transported to the departmental laboratory.

The oral vaccination of foxes was made with the baits containing the strain SAD Bern. In one bait there is one vaccination virus dose (1.8 ml) closed in aluminium-plastic blister. Round, dark brown bait is made of feed mixture attractive for foxes- strongly fish smell.

The vaccine is used in several Member States with consistent results.

In the year 2011, following the spring vaccination campaign, the mandibles of 982 foxes were tested for the presence of tetracycline. There were 269 tetracycline positive results. Chest

cavity fluid was available for 770 foxes and 102 were positive for rabies antibodies. The shooting foxes campaign, to determine the effectiveness of vaccination continued in 2012, following the autumn vaccination campaign. The mandibles of 1808 foxes were tested for the presence of tetracycline, 681 samples with positive results for tetracycline. Chest cavity fluid was available for 1363 foxes and 278 were positive for rabies antibodies.

In 2011 the hunting funds were privatized. This has led to an increased number of hunting territory managers involved in the shooting of foxes to assess efficacy of the vaccination campaign. For this reason the number of foxes tested to determine the effectiveness of vaccination is low.

In the year 2012:

Due to the political and legislative changes that took place in Romania, the legal basis for approving the oral vaccination of foxes in all territory was not approved until 1st of June, 2012. Therefore, in Romania the spring vaccination campaign of fox's rabies was not performed.

In August 2012 the legal basis has been approved for to implement the oral vaccination of foxes in all territory.

The aerial autumn campaign was not applied because of refusal of the provider of the aerial distribution, results in judicial conflict between the two parts of the contract.

- in November-December only vaccination of foxes by manual distribution was performed, with 80475 baits in 41 counties (aprox. 3350 km²)- with a density of 25 baits / km² :
  - 58.680 baits for national vaccination
  - 21.795 baits for emergency vaccination in counties AG, DB, PH, VL

7. Objectives

7.1. Targets related to testing

7.1.1. Target on diagnostic test:

***Disease*: Rabies**

Animal species: foxes

Region:	**Type of test:**	**Target population:**	**Type of sample:**	**objective:**	**Number of planned tests**
41 counties	1. F.A.T	Suspect foxes	brain	surveillance	1385
		Shot foxes	brain	surveillance	8480
	2. E.L.I.S.A	Foxes	Serum (loracal liquid or blood)	Monitoring of campaigns	8480
	3. Detection tetracycline test	Foxes	teeth and mandibles	Monitoring of the tetracycline marker presence	8480
	4. Direct sequencing of nucleoprotein gene, partial cds (coding sequence)*	Foxes	brain	Differentiation wild strain - vaccinal strain	300

	5. Vaccine titre**	-	Vaccine batch	Testing of vaccine	80 tests (40 estimated batches)
TOTAL					**27205**

*****according to the methodology of work from NLR rabies
**Institute for Control of Veterinary Biological Products and Medicines

Estemated number of fox samples/county

No.	County	Virologycal Tests		Serologycal tests for foxes shot		Others for foxes shot	
		1.F.A.T.*		2. E.L.I.S.A(antibody level)		3. Tetracycline detection	
		Sample no.	Positive cases	Sample no.	Positive cases	Sample no.	Positive cases

1	ALBA	320		236		236	
2	ARAD	330		298		298	
3	ARGES	350		258		258	
4	BACAU	290		240		240	
5	BIHOR	320		280		280	
6	BISTRITA-NASAUD	250		200		200	
7	BOTOSANI	230		180		180	
8	BRAILA	170		150		150	
9	BRASOV	220		180		180	
10	BUZAU	270		230		230	
11	CARAS-SEVERIN	350		330		330	
12	CALARASI	225		194		194	
13	CLUJ	300		240		240	
14	COSTANTA	130		100		100	
15	COVASNA	200		150		150	
16	DAMBOVITA	190		148		148	
17	DOLJ	230		278		278	
18	GALATI	140		120		120	
19	GIURGIU	150		130		130	
20	GORJ	130		150		150	
21	HARGHITA	275		238		238	
22	HUNEDOARA	315		270		270	
23	IALOMITA	190		170		170	
24	IASI	250		208		208	
25	ILFOV	70		64		64	
26	MARAMURES	275		234		234	
27	MEHEDINTI	210		192		192	
28	MURES	300		256		256	
29	NEAMT	250		214		214	

Region :	**Type of test:**	**Target population:**	**Type of sample:**	**objective:**	**Number of planned tests**
30	OLT	215	198		198
31	PRAHOVA	210	164		164
32	SATU-MARE	210	160		160
33	SALAJ	175	142		142
34	SIBIU	250	210		210
35	SUCEAVA	400	316		316
36	TELEORMAN	220	200		200
37	TIMIS	350	330		330
38	TULCEA	255	240		240
39	VASLUI	220	192		192
40	VALCEA	230	210		210
41	VRANCEA	200	180		180
	TOTAL	**9865**	**8480**		**8480**

***(suspected foxes and foxes shot to determine the effectiveness of vaccination)**

***Disease*: Rabies** **Animal species: Domestic animals**

Romania	1. F.A.T.	Other wild animals	brain	Surveillance	300
	2. Direct sequencing of nucleoprotein gene, partial cds (coding sequence)*	Other wild animals	brain	Differentiation wild strain-vaccinal strain	50
Region :	**Type of test:**	**Target population:**	**Type of sample:**	**objective:**	**Number of planned tests**
Romania	1. F.A.T.	Dogs	brain	Surveillance	400
		Cats	brain	Surveillance	175
		TOTAL			**350**
		Other domestic animals	brain	Surveillance	425
	2. Direct sequencing of nucleoprotein gene, partial cds (coding sequence)*	Dogs	brain	Differentiation wild strain-vaccinal strain	70
		Cats	brain	Differentiation wild strain-vaccinal strain	50
		Other domestic animals	brain	Differentiation wild strain-vaccinal strain	60
Total					**180**

*according to the methodology of work from NLR rabies

***Disease*: Rabies** **Animal species: Other wild animals**

Tests for direct sequencing of nucleoprotein gene, partial cds (coding sequence) were estimated according to the positive cases in 2011.

7.1.2. Targets on testing herds and animals- not applied

7.2. Targets on qualification of herds and animals- not applied

7.3. Targets on vaccination or treatment

7.3.1. Targets on vaccination or treatment - not applied

7.3.2 Data on the vaccination programme in foxes

Year: 2013 **Disease: Rabies**

Vaccination by aerial distribution

Nr.	Counties	Km^2 (estimated)	Aerial distribution		
			Number of doses (baits)/km^2	Number of campaigns	Total number of doses (baits) /county (estimated)
1	ALBA	5873	20	2	234.880
2	ARAD	7431	20	2	297.240
3	ARGES	6419	20	2	256.760
4	BACAU	6125	20	2	244.520
5	BIHOR	7006	20	2	280.200
6	BISTRITA-NASAUD	4969	20	2	198.720
7	BRASOV	4491	20	2	179.640
8	BRAILA	4630	20	2	185.200
9	BOTOSANI	4492	20	2	203.960
10	BUZAU	5736	20	2	229.440
11	CARAS-SEVERIN	8274	20	2	330.960
12	CALARASI	4837	20	2	193.480
13	CLUJ	5994	20	2	239.760
14	CONSTANTA	6583	20	2	263.320
15	COVASNA	3704	20	2	148.160
16	DAMBOVITA	3686	20	2	147.400
17	DOLJ	6931	20	2	277.240
18	GALATI	4183	20	2	167.320
19	GIURGIU	3274	20	2	170.960
20	GORJ	4972	20	2	198.880
21	HARGHITA	5939	20	2	237.560
22	HUNEDOARA	6764	20	2	270.560
23	IALOMITA	4244	20	2	169.760
24	IASI	5188	20	2	207.520

25	ILFOV	1577	20	2	63.080
26	MARAMURES	5857	20	2	234.280
27	MEHEDINTI	4817	20	2	192.680
28	MURES	6398	20	2	255.920
29	NEAMT	5359	20	2	214.360
30	OLT	4920	20	2	196.800
31	PRAHOVA	4112	20	2	164.480
32	SALAJ	3541	20	2	159.120
33	SATU-MARE	3978	20	2	141.640
34	SIBIU	5217	20	2	208.680
35	SUCEAVA	7862	20	2	314.480
36	TELEORMAN	5631	20	2	225.200
37	TIMIS	8272	20	2	330.880
38	TULCEA	5987	20	2	239.480
39	VASLUI	4776	20	2	211.680
40	VALCEA	5292	20	2	191.040
41	VRANCEA	4543	20	2	181.720
	TOTAL	**219.884**	**20**	**2**	**8.860.000**

Vaccination by manual distribution

No	Counties	Km^2	Manual distribution		
			Number of doses (baits)/km^2	Number of campaigns	Total number of doses (baits) /county
1	ALBA	5873	25	2	3110
2	ARAD	7431	25	2	3940
3	ARGES	6419	25	2	3400
4	BACAU	6125	25	2	3240

5	BIHOR	7006	25	2	3710
6	BISTRITA-NASAUD	4969	25	2	2640
7	BRASOV	4491	25	2	2380
8	BRAILA	4630	25	2	2460
9	BOTOSANI	4492	25	2	2700
10	BUZAU	5736	25	2	3040
11	CARAS-SEVERIN	8274	25	2	4390
12	CALARASI	4837	25	2	2560
13	CLUJ	5994	25	2	3180
14	CONSTANTA	6583	25	2	3490
15	COVASNA	3704	25	2	1964
16	DAMBOVITA	3686	25	2	1950
17	DOLJ	6931	25	2	3680
18	GALATI	4183	25	2	2220
19	GIURGIU	3274	25	2	2270
20	GORJ	4972	25	2	2640
21	HARGHITA	5939	25	2	3150
22	HUNEDOARA	6764	25	2	3590
23	IALOMITA	4244	25	2	2250
24	IASI	5188	25	2	2750
25	ILFOV	1577	25	2	840
26	MARAMURES	5857	25	2	3106
27	MEHEDINTI	4817	25	2	2550
28	MURES	6398	25	2	3400
29	NEAMT	5359	25	2	2840
30	OLT	4920	25	2	2610

31	PRAHOVA	4112	25	2	2180
32	SALAJ	3541	25	2	2110
33	SATU-MARE	3978	25	2	1880
34	SIBIU	5217	25	2	2770
35	SUCEAVA	7862	25	2	4170
36	TELEORMAN	5631	25	2	2990
37	TIMIS	8272	25	2	4380
38	TULCEA	5987	25	2	3180
39	VASLUI	4776	25	2	2800
40	VALCEA	5292	25	2	2530
41	VRANCEA	4543	25	2	2410
	TOTAL	**219.884**	25	**2**	117.450

The manually distribution is made around localities (25 momeli/km2), by the managers of the hunting areas with the official veterinarian.

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/no)
1. Tests					
1.1. Cost of analyses	FAT	9865	18	177570	yes
	ELISA	8480	11	93280	yes
	Detection tetracycline test	8480	8	67840	yes

	Direct sequencing nucleoprotein gene	530	130	68900	yes
	Vaccine titre	240 (6 baits/batch)	80	19200	yes
1.2. Cost of wild animals sampled and transpoted		12605	12	151260	yes
1.3. Other costs					
2. Vaccination					
2.1. Buying of baits		8.977.450	0.24	2666796	yes
2.2.Distribution costs	Manual	117.450 doses	0.20	23490	yes
	Aerial	8.860.000 doses/219884 km^2	0.03	265800	yes
2.3. Vaccination of livestock					
cattle	Administration of rabies vaccine	3000	1.30	3900	yes
	Parenteral vaccine costs	3000	0.7	2100	yes
equine	Administration of rabies vaccine	2150	1.30	2795	yes
	Parenteral vaccine costs	2150	0.7	1505	yes
Sheep/goats	Administration of rabies vaccine	3000	0.7	2100	yes
	Parenteral vaccine costs	3000	0.7	2100	yes
pigs	Administration of rabies vaccine	2000	2.45	4900	yes
	Parenteral vaccine costs	2000	0.7	1400	yes
2.3. Administrative costs				30000	yes
2.4.Control costs				30000	yes
2.5.Storage costs				10000	yes
3. Sacrification and destruction					
3.1.Compensation					

of animal					
3.2.Sample transport costs					
3.3.Distruction expenses					
3.4.Losses in case of slaughtering					
4. Cleaning and disinfection					
5.Remuneration					
6. Disposable materials and special equipments					
7. Other costs					
Total				**3.624.936**	**yes**

Intocmit 27.04.2012: dr. Cristina Stoica
Modificat 1 29.08.2012
Modificat 2: 19.10.2012
Modificat 3: 12.09.2013