Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

MINISTRY OF AGRICULTURE, FORESTRY AND WATER MANAGEMENT
Veterinary Directorate

Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

CRIS No: 226-870

Strategic operational multi-annual action plan for eradication, control and monitoring of Rabies in Serbia

October 2010

Project funded by the European Union

MINISTRY OF AGRICULTURE,
AESA –IZSA&M – IZSVe – NVRI – OPERA
FORESTRY AND WATER MANAGEMENT
Veterinary Directorate

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DRAFT

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and monitoring of Rabies in Serbia

October 2010

1. Project Title

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2. Details

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<th>Partner Institution / Beneficiary</th>
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<td>CRIS No:226-870</td>
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List of Abbreviations

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<table>
<thead>
<tr>
<th>Term</th>
<th>Abbreviation</th>
<th>Description</th>
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<td>CSF</td>
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<td>CD</td>
<td>Computer Database</td>
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<td>IPA</td>
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<td>World Animal Health Organization</td>
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<td>PDA</td>
<td>Personal Digital Assistant (handheld computer)</td>
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<td>PRAG</td>
<td>Practical guide to contract procedures for EC external actions</td>
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<td>PVS</td>
<td>OIE Tool for the Evaluation of Performance of Veterinary Services</td>
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<td>RASFF</td>
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<td>Standard Operating Procedure</td>
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<td>Veterinary Information Management System</td>
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<td>WHO</td>
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<td>WTO</td>
<td>World Trade Organisation</td>
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1. MANDATE

The present project is a part of a general programme initiated by DG SANCO: the IPA project “Western Balkan programme for eradication of rabies/classical swine fever” managed by DG ENLARG with implementation sub delegated to DG SANCO. The project encompasses the setting up and execution of surveillance; coordination and exchange of information on the situation and evolution of CSF and rabies programmes in the Western Balkans countries; and regional and international cooperation between the involved national veterinary services.

The Project is assisting the Veterinary Directorate of the Ministry of Agriculture, Forestry and Water Management to control and eradication of Classical swine fever (CSF) and rabies in Serbia in compliance with EU standards. According to ToR the project partners should assess the epidemiological situation and prepare multi annual action plan containing disease control and eradication measures and protocol for disease surveillance.

2. EXECUTIVE SUMMARY

This document entitled: “Strategic operational multi-annual action plan for eradication, control and monitoring of Rabies in Serbia”, sets out the disease control principles have been approved by the Veterinary Directorate at the Ministry of Agriculture Forestry and Water Management. It is based on the Serbian Veterinary Legislation, which is harmonised with the EU Veterinary legislation and in compliance with the recommendations of the OIE Terrestrial Animal Health Code, OIE Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals.

It provides a road map and timeframe for medium and long-term priority activities supported by the Serbian Government for future preparedness and response to Rabies outbreaks.

The strategic plan will be implemented over two time frames: medium term, with a duration of 5 years (from 2010 to 2015) and long term another 5 years (from 2016 to 2020).

- The medium term objective is to reduce the number of rabies cases through progressive control and eradication measures including oral vaccination of foxes and surveillance, reducing the number of stray dogs, as well as preventive measures against re-introduction of disease in disease free areas;

- The long-term objective is to eradicate the disease from Serbia and to maintain the disease free status of the country by all control measures, including vaccination in case of outbreaks, post-vaccination monitoring. Investigations to better understand the transmission dynamics and the role of different wildlife animals in Serbia, and ways to prevent the introduction of rabies in disease free areas. This should include developing of pro-active surveillance programmes and emergency preparedness plans.

The strategy recognises that control and eradication of rabies from Serbia cannot be viewed as only responsibility of the Veterinary Directorate at the Ministry of Agriculture Forestry and Water Management, and that the country must have an integrated national system of plans of all government levels and all non-government sectors to address the threat. It is guided by the following principles:

- Government will use all instruments of national power to address the threat of the disease.

- Districts should have credible preparedness plans to respond to outbreaks within their jurisdictions.

- Non-governmental organizations (NGOs) like hunting associations, animal welfare organizations etc., will play a significant role in the preparedness and implementation of the control and eradication measures.
Individual citizens should be educated about the risk of infection and the protective measures if they or their family members are involved.

The regional and global partnerships will be leveraged to address the threat.

The Strategy addresses both animal and human health. Although the circumstances that connect these environments are very different, the strategic principles remain relevant.

The pillars of the Strategy are:

- Preparedness and Communication: Activities that should be undertaken to ensure preparedness, and the communication of roles and responsibilities to all levels of government, segments of society and individuals.
- Surveillance and Detection: Domestic and international systems that provide continuous “situational awareness”, to ensure the earliest warning possible to protect people and animals.
- Control and eradication: Actions to limit the spread of outbreaks and gradually eradicate the disease.
- Measures to maintain the disease free status of Serbia and people’s awareness in respect to rabies.

Pillar one (Preparedness and communication): Preparedness is the underpinning of the entire spectrum of activities, including surveillance, detection, control and eradication efforts, and clearly communicate expectations to individuals, communities and governments, recognising that all share the responsibility to limit the spread of infection and to eradicate the disease.

Pillar two (Surveillance and detection): Early warning of an rabies outbreak and the ability to closely track the spread of the disease is critical to be able to rapidly employ resources to contain the spread of the virus. An effective surveillance and detection system will save lives by allowing the government to activate the response plans to prevent further cases, enforce additional surveillance mechanisms and initiate vaccine supply and administration.

Pillar three (Control and Eradication): The most effective way to protect country is to keep the infection beyond the borders of Serbia, but it is recognised that if the neighbouring countries are not implementing similar measures the disease can be easily re-introduced in the country. Therefore, control and eradication of rabies at regional level is a more realistic outcome if all the countries in the region have the same approach.

Pillar four (Measures to maintain the disease free status): In contrast to the previous pillars, this one stresses on changing the strategy. In respect of disease, the change is from vaccination to non-vaccination policy with well-organized surveillance and early reporting system. Regarding people’s awareness, the change is from “raising awareness” to “maintaining awareness”.

3. BACKGROUND

For many years the Community has contributed financially towards campaigns for the oral vaccination of foxes in a number of Member States affected by epidemics of wildlife rabies.

The Republic of Serbia is a potential candidate country for EU membership and is in the process of harmonising its legislation, administrative and control systems with that of the EU. The European partnership with Serbia (Council Decision 2008/213/EC of 18 February 2008) identifies a need for Serbia to strengthen its veterinary legislation and controls.

The Stabilisation and Association Agreement (SAA) between Serbia and the EU was ratified by the Serbian Parliament in September 2008 but needs also to be ratified by the EU Member States before it comes into effect. The SAA includes a commitment on development
of co-operation between the parties in all priority areas of the *acquis communautaire* in the field of agriculture as well as in veterinary and phyto-sanitary domains.

Serbia has started to adopt European standards in veterinary policies and legislation. In October 2005 the Serbian Parliament adopted the Law on Veterinary Matters (Official Gazette of RS No. 91/2005).

Serbia is also negotiating and preparing for accession to the World Trade Organisation (WTO) which requires compliance with the agreement on Sanitary and Phytosanitary (SPS) measures.

Most countries in Western Europe are currently free of rabies in. However, the disease is present in Serbia as well as in the neighbouring countries (Bosnia, Bulgaria, Croatia and Romania). The number of cases of rabies in Serbia has been relatively high and was increasing during the last three years. The cases of rabies have been reported from Districts throughout the country.

The table below shows the cases of Rabies in Serbia for the last 10 years (*Source: Rabies Information system of the WHO Collaboration Centre for Rabies Surveillance and Research*)

<table>
<thead>
<tr>
<th>Year</th>
<th>Dog</th>
<th>Cat</th>
<th>Cattle</th>
<th>Sheep &amp; Goat</th>
<th>Swine</th>
<th>Fox</th>
<th>Raccoon</th>
<th>Wolf</th>
<th>Badger</th>
<th>Marten</th>
<th>Other carnivore</th>
<th>Deer</th>
<th>Total</th>
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<td>3</td>
<td>1</td>
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</table>

Fox populations act as a reservoir for rabies presents and thus presents a permanent risk for transmission to humans, either directly or via domestic carnivores. In Western Europe, oral vaccination of foxes has been successfully applied and resulted in eradication of rabies. For an effective control and eradication of rabies in Serbia, oral vaccination of foxes would be necessary in addition to the ongoing compulsory registration and vaccination of domestic animals.

The fox population has been indicatively estimated at more than 60,000. Data about other species with epidemiological importance like badgers, jackals, raccoon dogs, wolfs etc. are needed. According to data of hunting associations the number of jackals in Serbia is increasing. However, this estimation may need to be verified as the Animal Health and Welfare Department within the Veterinary Directorate, which is, among its other tasks, responsible for the wild life surveillance, is understaffed and may lack specialised equipment necessary for surveillance of fox population that is spread widely throughout the country.
3.1 Project overall objective

The overall objective of the project is to improve the animal health in Serbia and surrounding countries in line with EU standards, thus improving the prospects for trade of agricultural products.

3.2 Project purpose

1. To eradicate, control and monitor rabies in foxes in the Republic of Serbia;
2. To eradicate, control and monitor CSF in feral pig population as well as to prevent the spread of CSF from feral pig population to domestic pigs.

The Project partners fully recognise that it cannot achieve these objectives by acting alone. To achieve success, we need to deepen and strengthen the existing collaborative approach, maintaining effective partnerships at all levels. All those with an interest in animal health will have their role to play and responsibilities to fulfil in optimising performance and results. Together we can look forward with renewed confidence to a better targeted and more streamlined approach to all aspects of animal health. There is no pre-described strategy for control and eradication of Rabies, but there are some principals:

- In endemicly infected countries, particular attention must be given to reducing the incidence of rabies.
- In countries with sporadic cases of rabies, intensive efforts to eradicate the disease must be supported, given the current disease situation, this is possible.
- In countries free of the disease but at risk of incursion, rabies preparedness and capacity for early detection and response must be improved.

In order to effectively control the disease, countries should have a complete plan of action and the financial and human resources to implement it under the particular conditions prevailing in the country. A regional approach is also necessary.

Cooperation and collaboration is strongly recommended among regionally grouped countries through greater engagement and commitment from appropriate regional organizations for a harmonized and coordinated approach to control and eradication of Rabies.

This approach focuses on the development of formal long-term and sustainable cooperation and collaboration, taking into account regional specificities, for the development of policies and regulatory frameworks, harmonization of Rabies eradication and control strategies, surveillance and reporting, preparedness and planning. This approach is supported by EU funded projects for control and eradication of Rabies in other Balkan countries and is encouraged by OIE Regional Commission. Strategic initiatives include building of regional capacity and enhancing the role of regional and sub-regional networks for epidemiological and laboratory expertise.

Examples of cross-border re-infections are numerous. They are the result of the immediate juxtaposition of vaccinated areas (where fox populations are increasing) and areas where rabies is endemic. These re-infections can be prevented by synchronising control measures on both sides of political or administrative borders and when this is not possible by maintenance of an immune belt at the border. International cooperation in border areas is essential at all level to achieve effective control programmes. Neighbouring countries should carefully coordinate their activities along the common borders. If field trials reach a country border, local administrative staff from both countries should coordinate their efforts. Oral rabies vaccination generates new epidemiological and ecological concerns within and beyond national borders. For this reason, planning, implementation and evaluation of campaign should be coordinated at both country and international levels. Preliminary contacts should be made with neighbouring countries when oral vaccination policy is decided; these contacts should be maintained through regular regional meetings until the
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4. RABIES AETIOLOGY

Rabies is a zoonotic viral disease, which causes acute encephalitis in domestic and wild mammals. It is transmitted through close contact with saliva from infected animals (i.e. bites, scratches, licks on broken skin and mucous membranes). Once symptoms of the disease develop, rabies is fatal to both humans and other animals. Rabies virus belongs to the order Mononegavirales, viruses with a non-segmented, negative-stranded RNA genome. Within this group, viruses with a distinct "bullet" shape are classified in the Rhabdoviridae family. The genus Lyssavirus includes 7 genotypes: genotype 1 (classical rabies virus), genotype 2 (Lagos bat virus), genotype 3 (Mokola virus), genotype 4 (Duvenhage virus), genotype 5 (European bat lyssavirus 1) genotype 6 (EBL 2), and a newly discovered Australian bat lyssavirus (genotype 7) (Bourhy et al., 1993).

The most widely used test for rabies diagnosis is the direct immuno-fluorescence test (FAT) on acetone-fixed smears of hippocampus, cerebellum or medulla oblongata. Testing of hippocampus and cerebellum sample is compulsory according to WHO. Additional test for virus detection is virus isolation test on mouse neuroblastoma cell culture or molecular RT-PCR test. Virus neutralisation tests, rapid fluorescent focus inhibition tests (RFFIT) and fluorescent antibody virus neutralisation tests (FAVN), are used primarily to evaluate vaccinal antibody responses against rabies virus or detection and determination of rabies antibody titre in blood of foxes or dogs after vaccination (OIE, 2000).

5. THE OCCURRENCE OF RABIES IN EUROPE

Since around 1939, the epizootic of fox rabies spread 1,400 km westwards from Poland, with a 20 to 60 km advance per year, resulting in the infection of several European countries. The farthest west point of spread in France was reached in 1982. Eighty-three percent of the reported cases (Aubert, 1995) were in red foxes (Vulpes vulpes) which is the main reservoir as well as the main vector of the virus. The racoon dog (Nyctereutes procyonoides), an Asian species introduced into western Russia around 1920 has also transmitted the virus in Central Europe and in Finland (10.5% of cases) (Finnegan et al., 2002). Nine human rabies cases have been reported in Europe in 2000 (Müller, 2000). Humans are at risk mainly through exposure to the virus from infected species of domestic animals (cattle, cats and dogs). Imported rabies cases in humans have occurred following infection from dogs in countries where canine rabies is endemic. In 2001 two human cases were recorded in the United Kingdom following infection in The Philippines and Nigeria (Fooks, 2001). A genetically distinct virus within rabies virus type I is found in arctic foxes and this virus has been recognised in outbreaks among non-arctic animals, including the red fox and domestic animals (Nadin-Davis et al., 1993).

In Europe the insectivorous bat rabies cycle is independent from the epidemiological rabies cycle that involves foxes and other terrestrial mammals. Bat virus isolates of European bat lyssaviruses (EBLs) are genetically different from those found in foxes. In bats the infection seems to persist without inducing any clinical symptoms until a stress situation eventually activates the disease in some of them (Rønsholt et al., 1998). Later investigations have strengthened the assumption that EBL may infect most bats in a population without any noticeable clinical symptoms, possibly leaving many of them latently infected (Serra-Cobo et al., 2002; Wellenberg et al., 2002). From 1977 to 2000, 630 bat rabies cases were recorded in free-living insectivorous as well as captive frugivorous bats in Europe, also comprising 3 human cases (Müller, 2000). Experimental infection with a Danish EBL-1a strain indicated that the cat may become infected (Fekadu et al., 1988). Natural infection in sheep has been
observed twice in Denmark (Rønsholt, personal communication) and in 2001 a spill-over of EBL 1 in a stone marten was also reported (Müller et al., 2002).

6. PRINCIPLES OF RABIES ERADICATION

6.1 General considerations

The ultimate goal of any rabies control measure in wildlife is to decrease the rabies incidence and eventually eliminate rabies and hence, to achieve freedom from the disease. Rabies in wildlife can be controlled by both, an intensive reduction of the population density and by mass vaccination of reservoir populations. The final aim of both methods is the disruption of the natural route of infection by reducing the number of susceptible animals. The conventional method included attempts of hormonal sterilisation of foxes, the distribution of poison baits, trapping, digging and destroying of fox cubs at dens, den gassing and intensive culling as well. In fact, generally all these methods were incapable of reducing the fox population below the endemic threshold and maintaining the fox population under this level. Thus, based on many experiences, it was not possible to decrease the rabies incidence effectively by using these means.

Since the idea of a mass vaccination of the reservoir species, the red fox, had been successfully demonstrated for the first time under natural conditions in Switzerland at the end the 1970s, oral vaccination of foxes against rabies (ORV) has become the ultimate concept in rabies control throughout Europe. However, to have a goal and a concept is definitely not enough to reach the rabies free status. The road to success and the key to rabies elimination is the strategy to be applied, in this case the vaccination strategy.

A vaccination strategy is defined as a long-term plan of action designed to achieve a particular goal, in this case rabies elimination. From a managers point of view a vaccination strategy needs to fulfil certain requirements such as (i) state-of-the-art of science and technology, (ii) effectiveness under any condition, (iii) minimal expense but maximal success, (iv) cost-effectiveness and last but not least (v) principles to follow.

The efficiency of a vaccination strategy puts its benefit into relation to its costs with the benefit and costs defined as the decrease of rabies cases and the money spent for ORV, respectively. Only a vaccination strategy where the money spent does result in a significant decrease in rabies cases is considered to be effective whereas the efficacy still can vary considerably from economical point of view. The efficacy of a vaccination strategy can simply be measured using a linear regression model in which the cumulated costs and the number of rabies cases (log transformed) are used as parameters. The slope of the linear regression line can be used as an efficiency index.

The efficiency will be highest if the vaccination strategy follows principles. By definition a principle is a basic generalization that is accepted as true or a basic rule that guides or influences action or decision-making. ORV principles can be defined as rules used by veterinarians to organize the elements of ORV to create a unified vaccination strategy such as (i) the size of the repeatedly vaccinated area, (ii) the timing of vaccination campaigns, (iii) the mode of distribution and number of baits, (iv) the duration of vaccination campaigns and (v) surveillance and monitoring. All rules depend on each other; hence a violation of one rule may put the success of the vaccination strategy at risk.

6.2 Size of repeatedly vaccinated area

Where ever possible, large-scale vaccination is the method of choice installing a coherent vaccination area large enough to cover all observed rabies cases with a certain buffer of at least 50 kilometres between the locations of cases and the edge of the vaccination area. This
area has to be vaccinated continuously for several consecutive vaccination campaigns. There is proven experience from Germany and other European countries that using large-scale vaccination one needs less time to eliminate rabies in contrast to small-scale vaccination. Often most of the decision makers are discouraged or even deterred from this approach because of the high costs for vaccine baits and distribution and therefore, tend to prefer small-scale vaccination. In the end, however, small-scale vaccination results in a lower efficiency of the vaccination strategy, a higher duration of ORV and higher total costs. Countries that changed the strategy from small-scale to large-scale vaccination could suddenly eliminate rabies within a short period of time.

6.3 Timing of vaccination campaigns

Based on the observations of the annual rabies incidence which was directly correlated with dynamic the fox population itself, two time points were considered optimal for the distribution of the baits in the field. Hence, baits were distributed twice a year in spring and autumn at the beginning of April and October, respectively. In order to increase the overall vaccination coverage within the fox population attempts were made to reach the juvenile foxes. Hence, an additional vaccination campaign was introduced known as den baiting. Here, baits were directly placed at the entrance of fox dens at the beginning of May in addition to the usual vaccination campaign in spring. Although the bait-uptake (TC marked individuals) in juvenile foxes could be increased using this method it was still speculative if this also resulted in a protective immune response. After all, based on experimental studies it turned out that maternal transferred immunity causes a partially impaired immune response in fox cubs less than 8 weeks old resulting in an insufficient protection against rabies, and hence certain periods during spring were considered less suitable for bait distribution to reach the young foxes. Therefore, den baiting was replaced by summer distribution of baits conducted in June.

6.4 Mode of bait distribution and number of baits

In general, baits can be either distributed hand or by aircraft (fixed-wing aircraft, helicopter). In contrast to fixed-wing aircrafts, the use of helicopters is amplifying the costs for aerial distribution enormously. However, helicopters are more mobile and therefore maybe helpful in cases where fixed-wing aircraft reaches its limits as in fragmented landscapes or difficult topographical terrain (high mountains). Aerial distribution of baits in general, has several advantages over hand distribution of baits as it (i) needs less organisational expenses and man power, (ii) is the method of choice for large-scale vaccination, (iii) allows for accessibility of areas that can hardly be baited by hand (iv) is cost-effective and last but not least (v) guarantees decision makers a check-up of the quality of the measurement. Even in the early days of aerial distribution many things had to be made by hand. Not only that the baits had to be dropped by hand, the crew also had to plot the flight lines on appropriate maps and during the flight the pilots often had to orientate at explicit objects in the field. Nowadays, GPS and satellite navigated and computer-supported fully automatic dropping systems (SURVIS) are used which not only facilitate the work but also help optimizing aerial distribution. SURVIS allows the documentation of the precise location (coordinates) of every single vaccine bait drop. Afterwards, using GIS software the flight-line distance and bait density on the ground can easily be determined. Similarly, non-flying zones or areas with suboptimal bait density can be identified, plotted on maps and forwarded to veterinary authorities to organise complementary hand distribution of baits within an appropriate period.

Despite the advantages of aerial distribution hand distribution of baits is an essential complementary measure in nearly every vaccination campaign. Hand distribution of baits is the preferred method of vaccine release in urban and suburban areas and shall include parks, industrial parks and wasteland, cemeteries, railway property and recreation areas, large residential areas and garden plots. When distributing baits manually, baits should be
uniformly distributed according to a raster model based on prepared maps considering attractive places for target species.

To answer the question how many baits have to be distributed in a vaccination area one needs to consider three points; e.g. fox density, the density of potential bait competitors and the assumption that a 5-10 surplus is needed to effectively interrupt the infectious chain within the fox population. Because there was and still is no efficient method available to determine the wildlife densities on a large scale, typically the size of hunting bags or other statistics are used for this purpose. So, the calculation of the bait density is rather empirically than a real scientific approach. As a result, at the very beginning of ORV in early 1980s 12-15 baits per sq km were regarded sufficient to reach enough foxes to interrupt the infectious chain and at the beginning it worked perfectly. However, over time by eliminating an important mortality factor ORV led to a significant increase of the fox population especially in long-term vaccinated areas. At the same time also densities of wild boars, the main bait competitor of foxes in Europe, increased.

To cope with this problem and to achieve an adaptation of the baiting strategy to the increasing fox density, almost all European countries that faced this problem tried to adapt their baiting strategy accordingly by increasing the bait density. As a result, during the past 25 years, the bait density increased from 12-15 to 18-20 baits per sq km. In recent years, however, an average bait density of about 25-30 baits per sq km and campaign is used, especially in long-term vaccinated areas. In any case, the choice of an appropriate bait density depends at which stage of the ORV program one stands, e.g. initial phase, intermediate phase, final phase.

An essential factor in aerial distribution is an appropriate flight line distance. Again the choice of the appropriate flight line distance depends on the stage of the ORV program and the density of the target population in a given area. Usually, at the beginning of ORV programs a flight line distance of 1 km is sufficient, but may need to be adapted accordingly during the course of ORV, especially in long-term vaccinated areas. Often, in view of increasing wildlife densities the disease manager’s point of view is - ‘Much is more’, and hence, as a logical consequence only the bait density is increased tremendously. However, increasing the bait density by keeping the flight line distance is “Doing the wrong with the highest precision” (Urs Breitenmoser, Switzerland). The solution is a more homogenous distribution of baits in the field to allow every target animal access to the baits. This can be done either (i) by double vaccination, in which in a second campaign baits are distributed about 14 days after the first campaign in the area using perpendicular flight lines (1 km) or (b) by reducing the flight line distance from 1.000 meters to 500 meters. A reduction of the flight line distance less than 500 meters turned out to be ineffective for bait-uptake but increases costs.

6.5 Duration of vaccination campaigns

The relative frequency of rabies elimination in a vaccination area is influenced by the duration of vaccination (years) and the prevailing rabies incidence. Depending on the prevailing rabies incidence the time needed to successfully eliminate rabies may vary with areas having a low rabies incidence needing less time and vice versa. In the process of rabies elimination, the number of rabies cases follows an exponential curve making it difficult to really detect the last rabies case necessary to calculate the duration of vaccination campaigns. In general, however, rabies can be considered eliminated in a vaccination area after 6 years of consecutive vaccination on average. This time period should be considered a minimum time period for long-term planning of ORV campaigns. Strictly speaking this means, if the budgeting of ORV programs is against long odds for 6 years there is a risk of running inefficient.
Demonstrating freedom from rabies under field conditions using diagnostic methods is impossible as there is a detection threshold depending on the quality of the implemented surveillance system. Even in case of intensive surveillance this detection threshold is still existent. From theoretical point of view freedom from rabies in wildlife reservoir species could only be proven if all target animals in a given area would be tested; this of course is practically impossible. Computer modelling showed that rabies can persist on a low level under vaccination for several years. The risk of a re-emergence of rabies after the cessation of ORV, however, was shown to be minimal in case ORV is continued two years after the last rabies case in a vaccination area. Experience from the field supports this theory.

Take home message:
- Rabies can persist on a low level under vaccination for a long period of time! Risk of low level persistence does not depend on duration of vaccination;
- Freedom from rabies can only be proven if vaccination is stopped;
- Recommendation 2 y after last rabies case.

6.6 Surveillance and Monitoring of ORV campaigns

Rabies surveillance and monitoring (follow-up) of oral vaccination campaigns are essential parameters for the evaluation of the success of ORV program and require a sustained, constant and intensive approach. It is of utmost importance to note that from an epidemiological point of view both methods aim at different subpopulations of the reservoir population.

Passive surveillance: Rabies surveillance is the index of ORV and aims at the detection of animal infected with RABV (infected or ill subpopulation). Hence, a risk based sampling scheme should be implemented focussing on so-called indicator animals, e.g. all animals showing clinical rabies symptoms, animals suspected of having rabies, animals found dead and road kills. Also, if necessary animals involved in biting incidents (human exposure) should be tested for rabies. Testing of animals taken from the hunting bag is counterproductive as those animals are typically healthy and therefore, do not provide any clues on the occurrence of rabies in a given area. The chance to detect rabies virus in indicator animals is up to 16 times higher than in animals from the hunting bag. In all instances, negative and positive results shall be recorded. As general rule, rabies surveillance needs to be conducted not only in vaccination areas itself but also in the non-vaccinated areas bordering the vaccination area.

Active surveillance: Surveillance within these areas is of utmost importance in order to detect a possible re-infection of areas already freed from rabies as early as possible. Sample size of eight foxes/100 km² and year is recommended by the EU, whereas the WHO recently recommended reduced sample size of 4 target animals/100 km² and year. Monitoring of ORV programs shall provide information on the herd immunity after vaccination, e.g. how many animals have been successfully vaccinated. This can be done by, the determination of the (i) bait-uptake and the (ii) immunity in foxes in vaccination areas. In contrast to rabies surveillance, monitoring of ORV aims at the 'none-infected' subpopulation, e.g. either being protected due to vaccination or being unprotected (still being susceptible) to the disease. Hence, for those investigations sampling shall focus on animals from the hunting bag.

Concerning the monitoring of ORV no recommendations on the sample size are given. However, if necessary the sample size can easily be calculated using statistical procedures. Ideally, the sample size should be homogenously distributed within the vaccination area to avoid spatial clustering, the latter one running the risk of providing biased information that may not reflect the real situation. Whereas the determination of the bait-uptake (detection of tetracycline specific fluorescence in the teeth or bones) in the target species does not cause bigger problems, the
determination of the level of immunity in the target population is strongly influenced by the sensitivity and specificity of the serological test used and the quality of the sera. As a principle, only good quality sera should be considered for testing. An ideal method for obtaining high quality sera is night shooting of target animals. However, this method is time and labour intensive and is often not practicable. In any case, sera collected from the field shall be pre-treated prior to testing to avoid toxic effects, especially in sera-neutralisation assays.

If data from follow-up investigations of ORV campaigns (bait-uptake, sera-conversion) are being evaluated the age structure of the sample size (juvenile, adult) should be taken into account. In addition, the 95% confidence intervals should be calculated as the true prevalence varies considerably with the sample size investigated. The establishment of an epidemiological unit devoted to ORV and the help of a statistician is strongly recommended.

6.7 Setbacks in ORV

A vaccination strategy can never be a static methodology but needs to be adapted according to the prevailing or changing field conditions. However, once ORV is implemented and the first campaigns have proven to be successful, ORV often runs the risk to get a kind of self-acting process. Such a scenario will inevitably result in setback which in turn prompt the need of checking, improving and adapting the applied vaccination strategy. In the more than 25 years history of ORV almost any European country which used ORV had to face more or less severe setbacks in rabies control. Interestingly, when setbacks occurred, disease managers instantly first questioned the vaccine baits used. The most frequently asked questions were:

- Doesn’t the vaccine work anymore?
- Isn’t the bait attractive anymore?
- Did the temperature stability of the vaccine change?
- Did the residual pathogen of the vaccine change?

Those questions are often the easy way out for the following reason: For oral vaccination of wildlife only registered and licensed vaccine are accepted. To get registration oral rabies virus vaccines have to fulfil strong requirements of the WHO and the European Pharmacopoeia in terms of efficacy, potency and safety. It is a known fact that slight variations in temperature stability and residual pathogen among commercially available attenuated rabies virus vaccines exist; only close tolerance is accepted for registration. In this respect, one should not forget that the successful control and subsequent elimination of wildlife rabies from a great number of countries is solely due to those 'unsafe' vaccines.

**Note: A vaccine works or not!**

Setback analysis from many countries showed, that often too much attention was paid to those questions, instead close examination identified numerous other reasons for failure:

- Violation of principles
- Limited financial resources
- No long-term planning
- No chain of command
- No continuous control
- No cross-border activities
- Small-scale vaccination
- No complementary hand baiting
- Stop of ORV too early
- Inadequate bait distribution
- No epidemiological analysis
- No centre of expertise
- Other priorities (diseases)
- Decreasing awareness
- Deficient surveillance
- Deficient cool-chain of vaccines
- No exchange of information
- Missing motivation of hunters
- No adaptation of vaccination strategy

7. VACCINATION CAMPAIGNS IN EUROPE

In 1989, an increase of rabies cases occurred and produced the highest peak of rabies incidence of recent decades (Figure 1 describes the western limit of the rabies front in 1989 and in 2001). As result of oral rabies vaccination campaigns, the rabies situation in European countries has greatly improved since 1989 (Müller, 2000). A drastic decrease in the rabies incidence has been recorded in most western European countries (Figure 2).

In 1988, Finland experienced an outbreak of rabies in raccoon dogs and foxes, close to the south-eastern border of the country. Field vaccination campaigns started in 1988 using 2 bait-laying a year, and since 1991 a single bait-laying each year in autumn. Thereafter the country has remained free of reported cases of rabies, although the disease remained endemic in Russia and Estonia. Italy carried out vaccination campaigns in the infected areas only when cases were recorded, starting from 1984. The same strategy was successfully pursued during the following years. The Belgian programme covered the entire infected area from 1989 until 1991, with five campaigns in total, leading to an 80% decrease of rabies cases.

Then more restricted campaigns were conducted in 1992, 1993 and 1994 only along the borders of the country. Rabies cases were recorded again from 1994 to 1996, coming from a border residual focus. In 1996, the vaccination strategy was modified and adapted to control rabies re-infection in the presence of a high density fox population. Two aerial vaccinations were carried out during the cold season (November and March: when the fox population density is at its lowest of the year). Control of aerial distribution was intensified by use of GPS (Global Positioning System) and reducing the distance between flight lines to 500 metres and finally baiting density was increased from 15 to 17 baits per km², supplemented by an additional den vaccination. Following this modification of the strategy and a close cross border co-operation with their French counterparts, rabies was efficiently controlled (the last rabies case occurred in July 1999 in a 28 month old cow).

Switzerland proposed the vaccination strategy that has been followed by other European countries, consisting of the compartmentalisation of the infected areas using natural or artificial barriers. The last rabies case was recorded in Switzerland in 1996.

In France, with a peak of more than 4,200 rabid animals in 1989, the strategy consisted of establishing an immunological barrier from the English Channel to the Swiss border, which succeeded in stopping the westward and southward spread of the disease. During the following years, the vaccinated area was shifted towards the borders resulting in a 99.7% decrease in rabies incidence from 1989 to 1996. In France, the last rabies case of vulpine origin was recorded in a cat in December 1998. A part of the success of Belgium, France, Luxembourg and Switzerland in controlling fox rabies is because they developed close co-operation for preventing cross-border contamination and improving their vaccination techniques (Aubert et al., 1994).

In Poland, despite vaccination campaigns, the number of recorded cases in wildlife and domestic animals and the size of the infected area has been increasing since 1999. In the Czech Republic, several vaccination campaigns led to a significant decrease in rabies incidence, although two foci remain, located at the borders with Germany and Poland. To date, seven European countries are reported to be free from rabies following the use of oral

Oral rabies vaccination has, as in other European countries, drastically decreased the rabies incidence in Germany from 10,487 to 83 cases reported in 1983 and 1997 respectively. However, in contrast to the eastern parts of the country, severe setbacks occurred in some areas of the western parts. In 1998 and again in 2000 an increase in the rabies incidence was observed, with the vast majority of cases occurring in Bavaria and Hesse and in North-Rhine Westphalia. Following recent changes in vaccination strategies in Bavaria and North-Rhine Westphalia, no rabies case has been observed since March and June 2001, respectively. In Hesse since July 2000 rabies cases have occurred at a low level in a very small (65 km²) suburban/urban area affecting two adjacent communities (Mühlheim and Offenbach). In March 2002, the disease spread into 2 further adjacent communities. In Saxony, a vaccination belt has been maintained along the border with the Czech Republic and Poland since 1997 to prevent re-infection from rabies-infected areas.

8. FOXES AND RABIES

8.1 Fox biology

General information on fox biology has been published by many authors, see among others Corbet and Harris (1991). The red fox is a medium-sized carnivore family. It is a highly adaptable species with little specific habitat requirements. It is most abundant in fragmentary and diverse habitats, including cities, which offer a wide variety of cover and food.

Foxes live in couples, in territories they may or may not share with a family group (depending on the population density). Size of territory ranges between 40 and 400 ha (Artois et al., 1990). This variation may be related to individual dominance and the availability of food and shelter provided by the habitat. Not all parts of the territory are used with the same frequency and adjacent territories may overlap to a certain degree. Activity is mainly nocturnal and crepuscular (with a less active period in the middle of the night). Foraging behaviour regimens are highly adaptable. Prey species and food sources are very diverse: mammals, birds, insects, earthworms, fruits and scavenged items are important sources. Females have a single oestrus per year and can reproduce from 10 months old (yearlings). In the northern hemisphere, mating occurs from mid-December to mid-February. Gestation lasts 53 days, with a peak of cubs being born in late March. In rural areas mainly, earths are used for litters (earths are infrequently used by foxes during other periods). Mean litter size is four to five, and cubs are born blind and deaf. By 8 weeks, their woolly coat begins to be covered by hairs with a colour and pattern very like that of the adult. They suckle until 4 weeks, and are then progressively weaned on to solids. First emergence above ground is at this age. They eat a large variety of solid items by 5-6 weeks, and become progressively more independent during summer. Dispersal occurs principally in animals 6-11 months old (from August to March). Young males disperse earlier and further. The proportion of dispersers depends on the density of population and possibly on the level of human activity and control. Social instability due to mating and dispersal has been cited as a cause for the peaks of rabies incidence during spring and autumn (Kauker and Zettel, 1963).

8.2. Fox population counting

Hunting statistics are an acceptable indicator for the fox population trends at a regional or national level, provided that the records have been compiled consistently over the years and the hunting pressure has not changed greatly. Although the impact of hunting on the overall fox population is not very well documented, the relationship in Switzerland between the hunting statistics and the fox population is discussed in Breitenmoser et al. (2000). Hunting could also affect the dispersal of animals, although available data are limited. The effect of more intensive fox hunting on the success of a vaccination strategy has not been clearly
elaborated. However, the submission of specimens by hunters aids in the monitoring and surveillance of vaccination campaigns, allowing bait uptake and sera-conversion rates to be measured. More accurate method for measuring of fox population can be applied by trained field ecologists in smaller areas, but such data cannot be extrapolated to a larger area or an entire country. The most commonly used methods are:

- Night counting index: the number of fox sightings per 10 km is recorded driving along a defined distance at a given speed;
- Road kills: fox carcasses are collected on roads according to a standardised protocol;
- Distance sampling is a recently developed “line transect method” similar to night counting and seems to allow a direct evaluation of the population density (Buckland et al., 1993);
- Analysis of the population structure of foxes (age, sex) provides data on the structure, status and dynamics of the population.

8.2.1. Jackal biology

Jackals are in progress in Serbia, especially in north part of country. These animals are often found in great numbers around the walls and ruins of old cities; they live in holes or burrows that they dig in the ground. The presence of jackals and their choice of habitat are determined largely by food abundance, the presence of water and the presence of thick brush where they can conceal themselves from both their prey and enemies. Although the most desert adapted of jackals, they avoid waterless deserts, being found there only on their very edges. In areas with little dense vegetation, jackals reside in low hillocks, where they take refuge in dry channels, caves and abandoned fox dens.

Estrus begins in early February and during warm winters in late January. Males take part in the raising of their young, and will dig burrows for them. The gestation period lasts 60-63 days. Litters usually consist of 3-8 pups, which are born with shut eyelids and with soft fur which ranges in color from light grey to dark brown. Jackals mainly hunt hares, small rodents, pheasants, partridges, ducks, coots, moorhens and passerines. They readily eat lizards, snakes, frogs, insects, fish and mollusks.

According the hunters experiences jackals and foxes have competition in nature. During the winter period, they will kill many nutrias and waterfowl. During such times, jackals will surplus kill and cache what they do not eat. Jackals will feed on fruits such as pears.

8.3. Rabies in foxes

Rabies, historically mainly reported in dogs, had virtually disappeared from central Europe at the turn of the twentieth century. However, from the 1940s the disease established itself in the fox population in Eastern Europe and spread inexorably south and west, eventually to encompass almost the whole of Western Europe (Taylor, 1976; Wachendörfer and Frost, 1992). Following the high co-adaptation of the current rabies virus strain to the fox, and due to fox ecology, no other species play a significant role in maintaining the disease in the infected areas, although many domestic and wild mammals (cattle, domestic cats, dogs, badgers, roe deer, racoon dogs etc.), are affected and may transmit the disease. Rabies in foxes is characterised by a highly variable incubation period from 11 days up to 15 months. The median duration probably does not exceed 30 days. Irrespective of the incubation duration, the morbidity phase is short (from zero to 14 days). The rabies virus multiplies in the brain and salivary glands and is transmitted through biting, either as a part of normal behaviour or provoked through a neural disorder. Up to 14 days before the onset of clinical symptoms, foxes incubating rabies have been shown to be able to transmit the disease to healthy individuals. Clinical signs are anorexia and changes in behaviour- the most visible sign is the loss of fear of people, making the foxes more visible, although aggressive behaviour towards people is rare (not more than 2% of human exposures to rabies in contaminated areas). Rabies infection in foxes is considered invariably lethal and there is no report of any fox surviving clinical rabies. When the virus spreads into a rabies free area,
either through contact of an infected fox with healthy neighbouring foxes or contacts with dispersing individuals, the disease may kill most of the resident foxes, lowering the local population density below the threshold value of rabies persistence. However, the area will be re-populated through inward migration of foxes from neighbouring areas and the high reproductive potential of the species is a factor. On a larger scale, a dynamic equilibrium occurs between patches of fox depopulated areas, areas without infected, or with long incubating, foxes, and areas with active foci of infection where foxes rapidly decline in numbers.

Healthy or incubating foxes disperse in all directions, but stay more readily in less fox-populated areas. As a result, rabies persists in a clustered pattern, without eliminating all foxes, although it decreases their overall abundance. These pathogenic and epidemiological features explain how the fox can be: the victim and the reservoir of the disease, and is at the same time the key to rabies control through oral vaccination.

8.4. Rabies control in foxes

The earliest attempts to control the disease in foxes focussed on radical reduction of the fox population. However, in practice it was nearly impossible to reduce the population density below a threshold value where disease transmission would cease (Aubert, 1992). More promising was the vaccination of the main host, using immune territorial foxes as a barrier to the spread of the virus. As Wandeler (1991) aptly wrote: “The wild mammal does not follow an invitation to visit a veterinarian, and there is no owner to bring it there. It has to be lured by some trick to vaccinate itself”. The combined cost-benefit balance of rabies and of fox population reduction (including the costs for culling) versus oral vaccination of foxes (including baits, bait delivery and follow-up to ensure the efficiency of the vaccination) have been compared. In France, the cumulative costs of both strategies remained comparable up to the fourth year. Thereafter, the oral vaccination strategy became more beneficial (Aubert, 1999).

Following early attempts in the early 1960s and field tests in the 1970s, the simplest, most efficient and economic method for vaccinating foxes proved to be industrially manufactured baits, made of an envelope attractive for foxes, containing a capsule or a plastic sachet filled with an attenuated anti-rabies vaccine in liquid form (Schneider et al., 1987). The bait envelope contains 150 mg of tetracycline to mark bait consumers. The bait shall be thoroughly chewed in order to guarantee that the sachet is punctured and the vaccine is released into the mouth of the consumer (see chapter 6.3.1). Hence, foxes and other species may have their teeth and bones marked with tetracycline deposits without necessarily being vaccinated (Kappeler, 1991).

9. ORAL VACCINATION OF FOXES AGAINST RABIES

A comparative study of the various fox vaccination protocols implemented, using the parameters listed below, may allow conclusions on the most appropriate strategy to eradicate rabies as soon as possible from Serbia:

- Type of vaccines
- Type of baits
- Methods of release of vaccine baits
- Density of baits and distribution patterns
- Seasonal pattern of the releases

9.1. Vaccines

It has been shown that immunisation and protection cannot be achieved when inactivated rabies vaccines are given by the oral route. For example, the preliminary results of Atanasiu et al. (1982) suggesting that domestic cats may be immunised using inactivated vaccines
were not confirmed on the red fox, even when enteric coated tablets were used that protected the vaccine against gastric acidic pH (Aubert et al., 1982). Since the use of inactivated vaccines has been demonstrated not to be effective, attenuated vaccines are therefore used. Regarding the use of attenuated vaccines and vaccines derived from the vaccine virus, possible contact of immune-suppressed people with those vaccines is considered and whether infection could become established in those immune-suppressed individuals.

Three categories of oral rabies vaccine should be considered as their origin explains their difference in residual pathogen:

- VRG is a genetically engineered vaccine derived from vaccine virus. It presents no rabies risk to humans and the environment, although an infection with VRG has been reported in the US in a woman who had epidermolytic hyperkeratosis and was 15 weeks pregnant (Rupprecht et al., 2001). She suffered swelling and erythematic but healed ten days later without any antiviral treatment and remained free of symptoms. Her pregnancy was normal and she delivered a healthy child.

SAD-Bern virus strain is a derivate of the SAD virus, which was originally produced on BHK-21 cells at the institute of veterinary microbiology of the University of Bern, Switzerland. The virus may be distinguished from field fox strain using Mabs.

- SAG1 and SAG2 have been made from the SAD Bern strain following one and two successive mutations of the Arginin 333 codon, respectively. Any change in this codon leads to a considerable loss of virus pathogen (Lafay et al., 1994).

- SAD B19 and SAD P5/88 were produced from the SAD Bern strain by attenuation following several passages in cell culture. Another strain termed SAD VA1 has been used in field trials in Germany. However, there is insufficient data available to include this vaccine in the present review.

Modified live virus vaccines to be used for oral vaccination of foxes fulfil all the requirements of the European Pharmacopoeia monographs, e.g. Vaccinum rabiei per orale vivum ad vulpem, European Pharmacopoeia, (2002), and should also take account of WHO recommendations (1989). The main characteristics of vaccines used in EU Table 2.

Table 2. Summary of main characteristics of oral rabies vaccines used in the EU (Data compiled from manufacturers and EMEA)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>VRG</th>
<th>SAG2</th>
<th>SAD B19</th>
<th>SAD P5/88</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proprietary name</td>
<td>Raboral</td>
<td>Rabigen</td>
<td>Fuchoral</td>
<td>Rabifox</td>
</tr>
<tr>
<td>Company</td>
<td>Variabilis</td>
<td>Variabilis</td>
<td>IDT</td>
<td>IDT</td>
</tr>
<tr>
<td>Virus titre</td>
<td>&gt;8 log10 TCID50/dose</td>
<td>&gt;8 log10 TCID50/dose</td>
<td>7 log10 FFU/ml</td>
<td>7 log10 FFU/ml</td>
</tr>
<tr>
<td>Thermotolerance, virus titre</td>
<td>Stable (time and temperature details not available)</td>
<td>0.16 log10 reduction after 2 days at 25°C</td>
<td>0.4 log10 reduction after 7 days at approx. 25°C</td>
<td>0.26 log10 reduction after 7 days at approx. 25°C</td>
</tr>
<tr>
<td>Melting point of bait casing</td>
<td>&gt;50°C</td>
<td>43°C</td>
<td>35°C (new bait casing under development)</td>
<td>35°C (new bait casing under development)</td>
</tr>
<tr>
<td>Non-target species tested</td>
<td>52</td>
<td>approx. 30</td>
<td>approx. 20</td>
<td>approx. 15</td>
</tr>
<tr>
<td>Tested Horizontal transmission</td>
<td>None in foxes (adults and cubs), dogs, cats, cattle, ferrets</td>
<td>None in foxes, may be found in salivary glands of young dogs</td>
<td>None in foxes, rodents, skunks and dogs</td>
<td>None (no information on species)</td>
</tr>
<tr>
<td>No Reversion to virulence after</td>
<td>7 backpassages in mice (intracerebral and footpad), 10 backpassages in vero cell cultures, 1 backpassage in fox</td>
<td>5 backpassages in suckling mice</td>
<td>5 passages in foxes and 10 passages in suckling mice</td>
<td>10 passages in suckling mice</td>
</tr>
<tr>
<td>Lowest protective dose tested</td>
<td>10^7 TCID50/dose</td>
<td>10^6 TCID50/dose</td>
<td>10^6 log10 FFU/ml</td>
<td>10^7 log10 FFU/ml</td>
</tr>
</tbody>
</table>

Note: TCID: tissue culture infective dose; FFU: focus forming units, EMA: European Agency for the Medicinal Products
9.2 Efficacy

Immunisation and protection may be given orally using attenuated vaccines as demonstrated by several experiments in:

- North America: Baer et al. (1971), Black and Lawson (1973)

The level of protection against experimental challenge (percentage of survivals) is correlated with the dose of the vaccine (virus titre measured in mice or cell cultures, dose-effect curve). Numerous vaccination challenge studies have been performed in the laboratory and confirm this observation (for an early review see Blancou et al., 1986).

Oral administration of 10^6 TCID50/ml vaccines SAD Bern has been shown to protect red foxes against challenge. Ingestion of 10^7 TCID50 produced seroconversion in foxes, dogs and cats (WHO, 1996).

When delivering the vaccine to the red fox using a bait, the vaccine should be approximately 10 times more concentrated to obtain the same level of protection as the same vaccine given by direct oral instillation (Blancou et al., 1986).

9.3 Immunity: “booster” effect and maternal immunity

The FAIR project CT 97 – 3515, "Wildlife vaccination against rabies in difficult and emergency situations and its potential impact on the environment", included a task aimed at studying several aspects of fox and fox cub immunity. The following results are summarised from the final report for this task and from other recent scientific papers. Compared with one single oral vaccination (VRG or SAG2), two vaccinations with an interval of 35 days with the same vaccine give no advantage in terms of:

- Antibody level four months later;
- Cell-mediated immune response;
- Protection to challenge, (Lambot et al., 2001).

In addition, a field study has demonstrated that no significant benefit for immunisation of adult and young foxes was obtained by two delayed distributions of baits (Bruyère et al., 2000). On this basis, no immunological reason for performing double vaccination exists.

Four-to-five week old fox cubs are able to respond to varying extents to oral vaccination with VRG or attenuated rabies viruses such as SAG2 and SAD B19, depending on the existence of maternal immunity. When born from non-vaccinated vixens or from vixens vaccinated with VRG, cubs develop a complete protective immunity against mortality from rabies challenge (Blasco et al., 2001). Cubs born from vixens vaccinated with SAG2, and which are vaccinated with SAG2 produce a lower neutralising antibody response after vaccination than cubs vaccinated with VRG. In addition, they are less well protected against challenge (FAIR CT 97-3515, part 1. Müller et al. (2001) with the SAD B19 vaccine, had shown a strong interference between passively and actively acquired immunity. With the SAD B19 vaccine, this interference affected the ability of 7 out of 10 fox cubs to resist the virus challenge. The results indicate that VRG is better able to overcome the effects of maternal immunity than other vaccines.

9.4 Safety

Rabies vaccines have various levels of attenuation and there have been several meetings organised by the WHO aimed at defining the safety and efficacy requirements for the oral rabies vaccines. Safety requirements recommended by the WHO deal with safety in target species (the red fox) and non-target species, such as wild carnivore and rodent species.

Following the discovery that the original SAD Bern strain is highly pathogenic for the baboon by the oral route (Bingham et al., 1992), non-human primates have been added to this list as...
a model for human exposure to vaccines. Table 3 summarises the main results on the residual pathogenicity of these vaccines.

Ingestion of the vaccine SAD-Bern can be fatal 1-5% of some rodent species and in baboons (*Papio cynocephalus*). However, its residual pathogenicity is similar to that of the ERA strain. In foxes that have been artificially immunosuppressed with corticosteroids, the virus can be isolated from the salivary glands 8 -14 days after oral application/introducing (Schneider et al., 1988). This vaccine contains the attenuated rabies virus. If the human is exposed to the vaccine (eyes, mouth, nose, and injured skin) a doctor should be consulted immediately.

Table 3. Summary of the main results from safety trials carried out on target and non-target species using the VRG, SAG2 and SAD B19 vaccines (When no reference is given, results have been drawn from WHO reports 1989-1998)

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Carnivora</th>
<th>Rodents</th>
<th>Immunocompromised mice</th>
<th>Non human Primates</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRG</td>
<td>No pathogenicity</td>
<td>No mortality</td>
<td>No mortality in 10 SCID mice (10^3 TCID50)</td>
<td>No pathogenicity for 10 baboons (10^3 PFU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAG2</td>
<td>No pathogenicity</td>
<td>No mortality</td>
<td>No mortality in 10 SCID mice (10^3 TCID50)</td>
<td>No pathogenicity for 10 baboons (10^3 PFU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAD B19</td>
<td>No pathogenicity in several species</td>
<td>Pathogenic for Skunk at high doses (10^3 FFU)</td>
<td>Up to 6% mortality in several European wild species (Artosi et al., 1992)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TCID: tissue culture infective dose, FFU: focus forming units, PFU: plaque forming units, SCID: severe combined immune-deficient mice

While thermo-stability of oral rabies vaccine viruses is essential to guarantee vaccination success, vaccine viruses that remain stable over a prolonged period of time could also pose a potential safety risk. This is because all presently available commercial oral rabies vaccines, live-modified or recombinant-based, are self-replicating and not completely without risks (Rupprecht et al., 1996). Baits containing oral rabies vaccines that are thermo-stable over a long period of time and that are not consumed by the target species could therefore be considered as potential bio-hazardous waste (Maurer and Guber, 2001). However, generally, most vaccine baits disappear within 7 days following distribution in the field (Brochier et al., 1988; Hadidian et al., 1989).

9.5 Vaccine and bait stability

The FAIR project CT 97 – 3515, "Wildlife vaccination against rabies in difficult and emergency situations and its potential impact on the environment", included a task studying the stability of all the vaccine baits available in the EU. The consortium gathered all the major scientific teams involved in oral vaccination in the EU (Belgium, France, Germany, Italy) and Switzerland. During a multi-site trial conducted in each of the above mentioned countries in spring, summer and autumn 1999. Comparative controlled trial conducted in Italy in summer 2000 single batches of a genetically engineered rabies recombinant vaccine (VRG) and three attenuated rabies virus vaccines (SAG2, SAD B19, SAD P5/88) were tested for 21 days post-delivery under varying local conditions (shade, half-shade, sunlight).

The stability of the vaccine baits in terms of virus titre and physical stability of the bait casing was recorded over a 3-week period in relation to temperature, sunlight and rainfall. At temperatures below 30°C, as reflected in trials during spring and autumn, virus titres in
attenuated vaccines were only slightly reduced during the 3-week observation period, whereas all attenuated vaccines showed a significant loss of titre when exposed to high temperatures (30°C or above). The recombinant VRG vaccine retained a protective titre at all temperatures studied.

Significant differences in bait casing stability were observed between vaccines when exposed to high ambient temperatures (30-35°C) and rainfall. Under such extreme conditions only the VRG bait casing remained stable. The casings of attenuated vaccines disintegrated more or less completely following exposure to high temperature and rain, SAG2 showing an intermediate resistance and the SAD B19 and SAD P5/88 baits being least resistant. The loss of titre in attenuated vaccines at elevated temperatures is presumably aggravated by disintegration of bait casing leading to less physical protection of the vaccine capsule. In the experiments performed in the FAIR project some trials demonstrated contrasting results and it is therefore difficult to draw firm conclusions on the basis of the data. However some relevant stability characteristics were identified:

- The VRG bait was always delivered with a high titre and was highly stable in all trials.
- The SAG2 bait was delivered with a high titre in 6 out of 7 trials. In trials where the mean maximum temperature near the baits did not exceed 30°C, the SAG2 bait remained stable for 21 days in 5 out of those 7 trials.
- The SAD B19 bait was delivered with a high titre in 4 out of 5 trials. In trials where the mean maximum temperature near the baits did not exceed 30°C, the SAD B19 bait remained stable for 21 days in 4 situations out of 9.
- The SAD P5/88 bait was delivered with a high titre in 2 out of 4 trials. This could not be interpreted as mere instability as the titres were even initially below the threshold level and no dramatic decrease indicative of instability was observed.
- Moreover, the SAD B19 bait casing was less stable than the VRG and the SAG2 bait casing when the mean maximum temperature exceeded 30°C.
- The SAD P5/88 vaccine was the least stable of all the vaccines tested in these trials.

Based on data available from the manufacturers and results from the FAIR project, under all conditions VRG appeared to be the most stable.

9.6 Field observations

In practice all of the above-tested vaccines, (VRG, SAD B19, SAG2, SAD P5/88) have been effectively reduce the incidence of rabies in wildlife enlarge European areas (Aubert et al., 1994; Schlüter et al., 1997; Müller, 1997; Müller & Schlüter, 1998; Vos et al., 2000; Breitenmoser et al., 2000; Brochier et al., 1990, 2001).

Vaccine and bait stability is, however, an important criterion for the efficacy of oral vaccination programmes. Several field observers provided direct or indirect evidence that field efficacy also depends on the dose of the vaccine (virus titre), on the stability of the vaccine titre, and on the stability of the bait envelope:

- Balbo and Rossi (1988) described oral vaccination in Italy (1984-1987) and observed that vaccine (SAD B19) stability in field conditions proved to be correlated negatively with environmental temperature, in particular when the maximum temperature approached 30°C. They found that the observed lower sera-conversion rate in foxes corresponded with the rapidly decreasing virus titres from vaccines collected on the terrain.
- Thomas et al. (1989) explained abnormal discrepancies between tetracycline and sera-conversion rates observed in some vaccinated areas in Belgium by hypothesising a rapid decrease in vaccine titre in baits in these areas.
- Masson et al. (1996) obtained a better field efficacy for the SAG1 vaccine bait (decrease in rabies incidence) following progress made in ensuring the stability of both the vaccine and the bait casing at higher temperatures. Similar observations were made by the Swiss team when they used the SAG1/2 vaccine (Zanoni et al., 2000).
- Aubert et al. (1994), then Masson et al. (1996) showed constant differences in the efficacy in decreasing rabies incidence by the three vaccine baits distributed in France: the decreasing efficacy order was VRG, SAG1 and SAD B19. These results were observed repeatedly following several vaccination campaigns, which covered very large areas encompassing various milieus and epidemiological conditions: 23,000 km², 37,800 km² and 31,300 km² for the VRG, SAG1 and SAD B19 bait respectively. The same authors observed that the stability of the vaccine and the bait envelope measured in the field varied similarly. The melting point of the bait envelope was shown to be above 50°C, equal to 43°C and below 40°C for the VRG, SAG1 and SAD B19 baits respectively (Masson et al., 1996) (see also Table 2).

9.7 Monitoring of vaccination

The WHO expert committee on Rabies (1992a) states that most field trials with oral vaccination employ three methods of evaluation:

- Testing for the occurrence of a biomarker (usually tetracycline), which is incorporated into the bait, in the target species;
- Examining sera from the target species for rabies virus neutralising antibody;
- Analysing the incidence of rabies in animals before, during and after the oral vaccination programme.

When using attenuated rabies virus vaccines, typing of rabies virus isolates originating from vaccination areas needs to be performed to distinguish vaccine strains from field rabies strains. Freshly collected sera are preferred for virus neutralising antibody titration. Most Western European countries carry out these follow up investigations. In some countries, the titration of the vaccine in baits sampled during bait distribution ("out of the helicopters") is performed as this allows the stability of the vaccines to be checked during the carrying out of vaccination in local field conditions.

Several biases may arise when studying only the evaluation of rabies incidence, because it depends on the collection of animals for diagnosis. The intensity and quality of sampling depend on the motivation of the general public, veterinarians, and the facilities of veterinary authorities of the administrative units of different countries. These conditions may vary between areas and also from year to year. Unfortunately there is no easy way to measure the quality of the sampling. The method of choice for rabies diagnosis is the fluorescent antibody test (WHO, 1996; OIE, 2000).

9.8 Bait uptake

Tetracycline is recommended by the WHO as 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. So far, tetracycline is considered as the best long term post-mortem tissue marker and is the most commonly used. Other biomarkers have been assessed in small-scale field trials: Sulfadimethoxine (broad-spectrum antimicrobial, short-term ante-mortem sera-marker), iophenoxic acid (relatively long-lived, 6-12 weeks, seromarker), and Rhodamine B (ante-mortem external marker). Thus, there is no currently available effective marker which could be used instead of tetracycline.

Determination of tetracycline uptake provides an easy way of monitoring bait uptake and is especially useful when identifying other causes for vaccination failure. The monitoring tetracycline uptake alone leads to overestimating the vaccination efficacy:

- Fluorescence in teeth may be observed without any tetracycline absorption or foxes may find other sources of tetracycline besides vaccine baits (e.g. other possible sources of tetracycline include placental remnants from cows treated with tetracycline for infections associated with retained placentas, or from scavenging fish from fish farms where
tetracycline may be used). Therefore, if tetracycline is proposed to be used as a marker, it is necessary to estimate “the background level” of tetracycline in fox populations before the beginning of an oral vaccination programme.

- Foxes may only consume the attractive casing of the bait and discard the vaccine sachet/vaccine, hence leading to foxes found to be positive for tetracycline but negative for rabies antibody titration.

- Contact between the vaccine suspension and the oropharyngal mucosa may be insufficient for immunisation but sufficient for tetracycline fixation.

The minimum dose of tetracycline per bait sufficient to mark an adult fox has been estimated as equal to 10 to 15 mg tetracycline/kg of fox weight (Cliquet et al., 1995). The tetracycline dose in one bait, greater than or equal to 150 mg is equivalent to 25 mg/kg of adult fox. Considering the weight of young foxes, a fox cub consuming only one fifth of the envelope of one bait (i.e. 8 g) will be shown to be “tetracycline positive”. Therefore, overestimation of vaccination coverage, based on tetracycline as a marker, is probably greater with fox cubs than with adult foxes.

Tetracycline examination facilitates the epidemiological surveillance of rabies in areas freed of the disease and where vaccination is no longer practised. It allows an assessment of the level of animals which are still marked (tetracycline fixation is lifelong).

9.9 Fox immunity

The most efficient and commonly used method to assess the efficacy of rabies oral vaccination campaigns is to measure the antibody response after vaccination in the target species. The methods currently recommended by the WHO and the OIE are the rapid fluorescent focus inhibition test (RFFIT) and the fluorescent antibody virus neutralisation test (FAVN test) (Cliquet et al., 1998; Aubert et al., 2000). However, in continental Europe, fox serum samples collected in the field by hunters, gamekeepers or by technicians are in most cases “body fluids”. The RFFIT, and any other cell culture-based techniques, require a specialised laboratory with fluorescent microscopy and facilities to handle tissue culture and the virulent rabies virus. These tests are sometimes too sensitive to cyto-toxicity that occurs with bad quality samples, possibly leading to false positive results (WHO, 1992a), and they have to be standardised using an appropriate standard as a control.

A simple test (ELISA test) has been developed, and has been used in France since 1992, which is rapid, safe and economical for large scale serological and epidemiological surveys following vaccination programmes. This test can be used to accurately titrate highly contaminated body fluids obtained from animals killed in the field (Cliquet et al., 2000). A European inter laboratory standardisation programme using this ELISA has been carried out recently and demonstrated an almost perfect agreement between four European laboratories (FAIR project CT 97-3515, Cliquet, personal communication). This ELISA test system facilitates serological evaluation of oral vaccination campaigns within European countries.

9.10 Rabies incidence

WHO recommends the examination of at least 8 foxes/100 km2 for rabies each year. Priority needs to be given to examining and testing those animals showing abnormal behaviour suggestive of rabies. Animals found dead, such as road-kills, are also useful sources for rabies diagnosis as these animals can be considered to be suspect animals.

10. VACCINATION STRATEGY

Vaccination programmes are required to be conducted and continuously monitored by a scientific team dedicated to this task. The team needs to be trained in field surveys and use validated laboratory methods for rabies diagnosis, titration of vaccines, evaluation of bait
uptake by the target species, and rabies antibody titration. The whole procedure, including bait distribution in the field, needs to be carefully processed, followed and documented.

10.1. Population dynamics

10.1.1. Introduction

Well-known phenomenon is that after the end of a rabies epizootic in a given area, the local fox population shows a strong increase (Vos, 1995; Wachendörfer et al., 1996; Breitenmoser et al., 2000; Chautan et al., 2000; Aubert et al., 1993). This is experienced as a typical consequence of a rabies vaccination campaign.

The increasing abundance of the vector species also has a considerable impact on the success of an oral vaccination campaign, especially if the control measures have to be applied over several years. Problems of persisting rabies, experienced during the final phase of the rabies epizootic in Switzerland, Belgium and Germany, coincided with a growing fox population, showing the need to adapt the rabies control strategy to the increased fox population. In situations of continued vaccination campaigns, it is crucial to compensate for the higher abundance of the vector species through an adjustment of the vaccine bait distribution. Although this seems to be an obvious recommendation, such an adjustment was not foreseen when rabies control programmes began. As consequence, reliable data on the dynamics of the vector population were usually not gathered and hence not available when the problem arose. The following section summarises the underlying mechanisms, using empirical data or estimations for illustration purposes. The course and the amplitude of a fox population increase can however vary according to local conditions, and it is therefore indispensable to monitor and analyse each local situation carefully.

10.1.2. Dimensions of the increase

Although empirical data are available on trends in fox populations during the course of vaccination campaigns (Breitenmoser et al., 2000) it is also possible to extrapolate models of fox population changes under various circumstances. If a closed population is infected with rabies virus, the population will decrease until the density falls below the threshold value of rabies persistence (Fig. 6). From there, the population will re-increase up to the carrying capacity of the habitat, following a sigmoid shape. The dimensions of the population growth are not precisely known, as there is a lack of reliable census data for fox populations. Usually, the population dynamic is estimated from mortality data, such as the hunting bag or road kills. These data sets indicate that the increase continues for 5–10 years after a population reaches a minimum, and that the amplitude of the increase can be from 4–5 up to 10 fold compared to the minimum. The maximum population density depends on the carrying capacity of the habitat and differs from area to area. The threshold density of rabies persistence (the minimum population density at which the disease can persist) is also influenced by the landscape and topography, but is probably a relatively constant value. In a real situation (i.e. in a non-isolated fox population) and in the absence of rabies control measures, a local increasing population will probably face a re-infection before it reaches the carrying-capacity density again, and will hence fluctuate in the longer term around the threshold value of rabies persistence (Breitenmoser, Personal communication) (Fig. 1).
Figure 1: Course of a fox population increase after a rabies infection (Rab).

The disease disappears when the population density (N) falls below the threshold value of rabies persistence (KR). The population increases in an S-shaped curve until it reaches the carrying capacity of the habitat (K). The population growth is characterised through the amplitude (A) and the duration (P) of the increase. KR is a conjunct of fox population density and contact rate.

10.1.3. Influence of herd immunity and population size on the success of the vaccination campaign

The herd immunity, used as a standard immunological term, is a relative measure of the immunity of a population (fraction of individuals protected against infection), and it does not indicate the absolute numbers of immune or susceptible foxes in the field.

The oral immunisation of foxes against rabies has two goals: (i) to defeat the infection in a given area, and (ii) to prevent the local population from becoming re-infected. The first goal requires the rapid increase in the herd immunity – experience has proven that three vaccination campaigns might be enough to eradicate rabies from a certain region (Masson et al., 1996), whereas the second goal is the maintenance of sufficient herd immunity as long as the infection persists in neighbouring areas.

It is obvious that the second goal needs to take into account the increase in the fox population. Assuming that the oral vaccination of foxes starts when the population is at its lowest (Fig. 2), the herd immunity will increase along with the number of vaccination campaigns but will never cover the entire population. Typical values for the herd immunity, evaluated from tetracycline analyses, ranged from below 50% up to 90% in adult foxes when antibody titration is used these percentages might be 30 to 80% respectively). When the population increases after the start of the vaccination campaigns, the number of susceptible foxes may also increase, as indicated in Fig. 2.

This is not a problem as long as the density of susceptible individuals remains below the threshold density of rabies persistence. However, if this threshold value is exceeded, the population remains susceptible to the disease.
Figure 2: Population growth with oral vaccination of the vector population.

In Figure 2, the population increase follows the sigmoid curve up to the carrying capacity $K$. Due to the vaccination campaigns most foxes are immune against rabies. However, the herd immunity will never be 100%; a certain proportion of the population will always be susceptible to the disease. If the herd immunity is below 100%, the probability of transmission of infection depends on contact rate in the region and transmissibility. As long as the density of the susceptible individuals remains below the threshold density of rabies persistence $K_R$ (situation A), the oral vaccination campaign will still be successful. If the density of the susceptible foxes exceeds $K_R$ (situation B), the disease will persist even if the herd immunity increases.

A high level of herd immunity may give a false feeling of security when the absolute number of non-immunised foxes is high. In other words, the herd immunity required to eliminate rabies or protect a population from re-infection is required to increase along with the population. Once an absence of rabies cases is reached at a certain herd immunity level, that level of herd immunity will need to increase with the increasing fox population in order to prevent a reoccurrence of cases. If, in a given moment, a herd immunity was empirically found to be enough to defeat rabies, a higher herd immunity may be needed to prevent a re-infection of the same population some years later, due to the increase in the population in the intervening period.

10.1.4. Modification of vaccination strategies to account for the fox population increase in prolonged vaccination campaigns

To allow for an adaptation of the rabies control strategy to the increasing fox density, the fox population should be monitored. It is not enough to sample a constant number of foxes in order to determine the herd immunity an indicator for the dynamics of the population is needed. Such indicators can be the hunting bag, road kills, night counting, and line transects. Even if such parameters do not really indicate the absolute number of foxes, they will be satisfactory for the population trend to be followed.

An additional complication is that an increasing population density may also influence the social structure and behaviour and the land tenure system of the fox. Social group composition, dispersal patterns and individual home range size may change. Analysis of the foxes and of an independent control sample in regard to age structure and sex ratio would allow identification of the problem categories and permit adequate measures to be taken.

Problems with re-infections typically occur along administrative borders. This is the result of the immediate proximity of vaccinated, increasing fox populations to areas where rabies is endemic. Sometimes, administrative borders are also barriers to the fox movement (as for example the river Rhine between France and Germany), but very often, they are not. In the latter case, the following points need to be observed in order to avoid continued re-infections:
- To set up large-scale vaccination zones;
- To strictly synchronise all control measures within the zone and across political or administrative borders;
- A vaccination zone to ideally extend up to the next geographical or artificial physical barrier and include the entire infected area.

10.2. Temporal patterns

The annual frequency of vaccination campaigns is required to be considered with reference to the months of baiting for a variety of campaign strategies. Based on experience in previous oral rabies vaccination campaigns, it is considered important that vaccination campaigns continue for a period of at least two years after the last reported case of fox-related rabies.

10.2.1. Regular vaccination campaigns

The classical pattern of two "single" vaccination campaigns per year, carried out in spring and autumn, has been found to be successful whatever the fox population density. This biannual distribution frequency has been used in all European programmes of oral vaccination that resulted in the elimination of rabies (Zanoni et al., 2000; Breitenmoser et al., 2000; Bruyère and Janot, 2000; Brochier et al., 2001; Besch, 2001).

Spring distributions are preferably carried out in May or June in order to increase the efficient access of fox cubs to baits. However, early spring campaigns carried out in March-April (targeting exclusively the adult fox population at its annual lowest density) were also shown to be beneficial in Belgium, Luxembourg, and several German Bundesländer (Brochier et al., 1996, 2001). Where snow is abundant, its melting may degrade the vaccine baits, and in such areas vaccination is preferably performed before the snow starts to melt. Autumn distribution is generally organised in September or October.

In both autumn and spring campaigns, short delayed baiting at intervals ranging from a few days to 3-4 weeks (so-called "double" vaccination strategy), aiming either at inducing an immune booster effect or at increasing the bait uptake rate, is not advisable. However, when vaccination campaigns are initially launched repeated distribution of baits within such a short time interval can be performed. Any effect of such double distribution is probably mediated through increased bait-uptake rate in the fox population by redistributing baits along other flight lines (for targeting foxes that would not have been reached during the first distribution).

10.2.2. Additional vaccination of fox cubs at den entrances

In spring, an additional distribution of vaccine baits at den entrances (targeting fox cubs) may be carried out in focal areas from mid-May to mid-June (Vuillaume et al., 1997). When using rabies modified vaccines, the distribution needs to preferably take place in early-June, because of a potential interference between passive and acquired immunity in fox cubs (Müller et al., 2001; Blasco et al., 2001; Barrat et al., 2001) but only if external maximum temperatures do not exceed 30°C. It should be noted that when directly exposed to the sun, the temperature of baits may be 10-20°C higher than temperatures measured under shelter.

Such distributions can usefully complement the regular spring campaign (Vuillaume et al., 1998; Brochier et al., 2001; Besch, 2001; Breitenmoser, 1995) but due to their organisational burden and associated cost they can only be applied in limited areas in problem situations (residual rabies foci with high fox population density) and in particular habitats.

10.2.3. Emergency vaccination

In cases of re-emergence of rabies in a focus where rabies had been previously eliminated, vaccination needs to be implemented immediately, whatever the period of the year. Such an emergency vaccination might thus be carried out in summer or in winter under unfavourable conditions.
weather conditions that require the use of a highly heat-stable vaccine-bait system such as the VRG (Masson et al., 1999; Pastoret et al., 1996).

In general, vaccination is not advised to be carried out at temperatures below 0°C, because:
- Frozen vaccines do not induce a sufficient immune response and
- The virus titre may decrease caused by freezing-thawing cycles, except for VRG which has been shown to remain stable in such conditions (Pastoret et al., 1996).

Vaccination using attenuated rabies virus vaccines is not recommended during hot weather conditions. At temperatures above 30°C, melting of the bait casing occurs and vaccine titre decreases.

10.2.4. Synchronisation of vaccination campaigns in neighbouring Administrative or political entities

Examples of cross-border re-infections are numerous (Schaarschmidt et al., 2002). They are the result of the immediate juxtaposition of vaccinated areas (where fox populations are increasing) and areas where rabies is endemic. These re-infections can be prevented by synchronising control measures on both sides of political or administrative borders and when this is not possible, by the maintenance of an immune belt at the border (see also “spatial aspects” below).

10.3. Spatial aspects and patterns

10.3.1. Size of a vaccination area - “buffer” zones

The size of the vaccination zone needs to ideally include the entire infected area or be as large as possible (5,000 km² at least) and extend up to natural or artificial barriers such as a motorway, canal, river, stream, lake, or mountains (e.g. in Alpine regions a vaccination zone should include a whole valley). Occasionally, administrative borders may constitute barriers to the movement of foxes, but in most situations vaccination zones need to be defined and vaccination campaigns synchronised across administrative borders.

When considering a “punctual occurrence” of rabies, that is an isolated residual or localised re-infection focus, the size of a vaccination area needs to range between 2,000 and 8,000 km² (radius of 25 to 50 km, respectively, around the site) depending on the landscape and the availability of natural or artificial barriers (Thulke et al., 1999). In the absence of any barrier, the larger radius (50 km) is advisable as the rule. Consequently, to protect a rabies-free area from a neighbouring infected area, the immunological barrier (a buffer zone) along the border with the infected area should be 50 km deep. This distance appears to be the minimum allowing for a sufficient reaction time to expand the zone from one campaign to the next if rabies entered in the border area.

Similarly, the minimum buffer zone (depth of a vaccinated strip in km) ahead of the front wave of the spreading epizootic should be 50 km.

If the endemic area is limited by a natural physical barrier (e.g. a river, lake, etc.), the depth of the buffer zone beyond this barrier depends on the supposed effectiveness of this barrier, the landscape and the expected fox density on both sides of the barrier. Beyond a barrier that may be crossed by foxes (e.g. a river), the minimum distance advisable is 20 km.

If vector species other than the red fox are involved (e.g. raccoon dogs), the buffer zone needs to be enlarged in respect of the maximum movement distance of this species.

10.3.2. Bait density - distribution pattern

All fox home ranges, whatever their size and shape, need to ideally receive several baits. Therefore, the general principle for the distribution of vaccine baits is as follows:
- all habitats should be treated except heavily urbanised areas and large stretches of water, taking the pattern of fox habitat into consideration;
- baits should be distributed in a regular pattern within a given area.
Concentrations of baits in clusters or along distant lines cannot be relied upon and needs to be avoided. Distances between baits cannot be neither too large nor too short, as otherwise individuals may go unvaccinated or there may be over-baiting. If a regular distribution is applied, a raster model is better than a parallel line model (this at least is valid for double vaccination).

Baits distributed along landscape interruptions such as forest edges, hedgerows, creeks, etc. will more likely be found by a fox than those in the middle of forest or farmland. Furthermore, the increased use of anthropogenic food resources by foxes is required to be considered.

Foxes often visit edges of settlements or parks and the role of other species such as cats and dogs competing for baits needs also to be considered, as well as public awareness and safety issues when distributing baits in urban and suburban areas.

10.3.2.1. Regular vaccination campaigns

According to radio-tracking studies in Western Europe (Artois et al., 1990), the smallest fox home range was 77 ha. In this situation, if vaccines are dropped at regular time intervals, along parallel lines separated by 400 metres, the minimal number of vaccine baits dropped in this fox home range will be 10. If the lines were 1,000 metres apart, this fox home range may receive no bait at all. The relationship between flight-line distances and the spatial arrangement of fox home-ranges is a key factor when considering bait distribution strategy (Thulke et al., 2001).

The distance between flight lines appears more crucial considering that:
- Foxes usually explore only 1/3 to 1/2 of their territory every day (Artois et al., 1990) which gives more opportunity for non-target species to pick up baits before the fox;
- Several foxes may share the same home range when fox density increases;
- In suburban areas, the size of a fox family home range may not exceed 25 ha (Brochier, unpublished data).

When distributing baits manually, baits need to be uniformly distributed according to a raster model. The map is required to be divided into equal plots and every plot should receive at least 1 bait. For a bait density equal to 20 per km², plots will be 223 metres x 223 metres. Inside every plot the place to choose to locate baits will be a forest edge, a bunch of trees in the middle of a meadow, a village boundary, etc.. By using this method, baits can be deposited throughout the landscape giving preference to “fox-lines” (forest edges, hedges, creeks, village boundaries etc.) and fox habitat.

When using the aerial method of bait distribution, flight sectors need to be defined in advance using natural or artificial landscape features. To ensure that most of the fox territories are given at least 1 bait, baits are distributed along parallel flight lines. Based on flight lines 500 metres apart, this entails two flight lines per km and approximately 110 metres distance between baits when the bait density is equal to 18 per km². When increasing the bait density to compensate for an increase in fox population, the bait distribution pattern needs to be reconsidered like the distance between flight lines is advised to be reduced from 500 to 300 metres (i.e. a change from two flight lines per km to three; with 100 metres between baits for a bait density of 30 per km²).

Based on field experience gained of vaccination campaigns in various countries and use of computer simulation models, densities of 18-20 and 20-30 baits per km² are advisable for low and high fox population densities, respectively. Although low and high fox population densities are difficult to precisely define, relative measures of the population dynamics can be used in combination with parameters such as bait uptake rate to modify bait densities appropriately.
10.3.2.2. Optional vaccination campaigns

If considering spring vaccination of fox cubs at dens, in early spring, fox dens need to be located and recorded on detailed maps by appointed and trained people (forest rangers, hunters, gamekeepers). The knowledge on the precise number of active fox dens within a given area is essential.

At the end of May to early June, the dens recorded previously are re-visited by the same persons (wearing gloves) and at least 10 baits are deposited at the den entrances.

Emergency vaccination is required to follow the protocol used in the context of a regular vaccination campaign adapted to the situation of high fox population density: 20-30 baits/km² with 3 flight lines/km².

10.4. Distribution methods and systems

Vaccine baits need to be deposited throughout the fox habitats (i.e. almost everywhere). Unfortunately, foxes do not consume all baits. Baits may remain undiscovered or be taken up by other wild or domestic species, or even be picked up by humans. However, vaccination campaigns carried out during spring and autumn for several years led to the durable elimination of the disease in most of Western Europe (Müller, 1997, Breitenmoser et al., 2000, Bruyère and Janot, 2000, Brochier, 2001). All of the distribution systems used so far have been found to be efficient, provided bait dispersal is properly designed. Each of them has its advantages and disadvantages.

**Manual distribution** allows a very precise and uniform spreading of baits (according to a raster model) and may be used to encourage public involvement and awareness. Furthermore, baits can be hidden (covered with grass, leaves, etc.) to avoid human contact, ingestion by birds and exposure to direct sunlight. However, it requires a thorough organisation and important human resources, qualitatively (competency, motivation) as well as quantitatively, and it is slow. Consequently, one can never be totally assured that baits are distributed everywhere. Any forgotten place (lack of motivation of a single team) can constitute a future rabies focus. Distribution by hand is the preferred system in suburban areas, in combination with an aerial distribution (helicopter) whenever possible.

**Aerial distribution** may be performed either by helicopter or by fixed-wing aircraft flying at 100 -150 m altitude and at a speed of 100-150 km/hr. Precise maps are required to be prepared before flights and followed during flights by a trained, independent person. A GPS (Global Positioning System) may be helpful for reporting the exact distribution pattern (Vos et al., 2001), but cannot replace the thorough work with maps (Breitenmoser and Müller, 1997).

Appointed and trained persons drop baits at a given mean rhythm (according to the ground speed) with more emphasis on fox habitats (hedges, village surroundings, isolated bunch of trees, etc.). An electronic metronome, connected to GPS that allows adjustment in dropping tempo to speed, may be helpful, but the dropper may increase this tempo to favour fox “places”. In Germany, for aerial distribution a satellite navigated and computer supported automatic bait dropping system was developed. The exact location and time of each bait released can be recorded together with all relevant flight details, so that authorities can verify if the achieved bait distribution pattern corresponds with the previously determined baiting strategy (Vos et al., 2001). The delivery by helicopter is fast and allows precise dropping of baits (flexibility in both flight speed and altitude). The above-mentioned spatial pattern of bait distribution (low distances between flight lines) can be performed more easily by helicopter. In addition, the helicopter allows working in less favourable weather conditions.

Therefore, the use of helicopter is advised for the treatment of all habitats (rural, agricultural, mountains, forests, suburban areas and settlements). Delivery by fixed-wing aircraft is the most economical of all distribution systems, but does not allow for a fine distribution of baits according to the fox-habitat in the landscape. Therefore, the use of fixed-wing aircraft is only
advised for the treatment of uniform, large and low density inhabited areas (e.g. large forests, mono-agricultural areas).

10.5. Evaluation of oral rabies vaccination programmes

In all European countries, the infected regions were initially too large to be vaccinated as a whole when oral rabies vaccination commenced. When logically planned, strategies tried to consist of vaccinating a limited part of the infected region every year, and in shifting the vaccinated area whenever possible from the areas freed from rabies to the areas still infected until the elimination of the disease in the whole region. However, in the past there have often been diverse strategies applied with varying success. These strategies mainly differed in the selection and size of the vaccination areas and the continuous treatment of these areas over the course of the vaccination programme. While in some countries vaccination areas were frequently adapted to the concurrent rabies situation (patchwork), others used large scale or overlapping areas or utilised natural barriers (Müller et al., 2001).

These differing strategies seemed to result in variation in the time taken to eradicate rabies (Müller and Schlüter, 1998). To address the reasons for this variation in eradication time, a retrospective evaluation of oral rabies vaccination programmes in European countries was conducted as part of the FAIR project CT 97-3515. Twenty-eight regions, which were involved in an oral rabies vaccination programme between the years 1978 and 2000 were selected in Belgium, Germany, Italy, and Switzerland. They were defined either by administrative units or by natural barriers and their size ranged between 313 and 66,362 km². For each region, the programme was considered from the 1st vaccination campaign to either the eradication of rabies or the end of the year 2000. Rabies was assumed to be eradicated if the disease was not recorded within a two-year surveillance period following the last confirmed case in the area.

To quantify the observed spatial and temporal differences in vaccination strategies, an Area Index (AI) was calculated. This index has been calculated for each region, using the area of the whole region concerned by the oral rabies vaccination programme ($vA_{max}$), the size of the areas successively vaccinated during campaigns at time t ($vA_t$), the number of successive vaccination campaigns ($n$), and the size of the overlapping of vaccinated areas successively from campaign to campaign ($\Phi_t$)

$$AI = \frac{1}{n} \sum_{t}^{n} \frac{vA_t}{vA_{max}} \Phi_t$$

Figure 3: Concept and formula of the area index (AI)

Thus, the AI is a measurement of the proportion of areas repeatedly vaccinated within a region during the observation period, and has assigned values ranging between 0 and 1. A region in which the total area has been vaccinated since the beginning of the programme would be characterised with an AI close to or equal to 1. An AI equal to 0 would indicate that no overlapping of successive vaccinated areas has ever been done. An AI close to 0 would indicate that such overlapping was limited and/or that the proportions of the vaccinated areas over the size of the whole region were systematically small.

There was a large range of AI (from 0.18 to 1) indicating a large variety of strategies in the countries studied (Belgium: 0.56; Switzerland: 0.20-0.98; Germany: 0.13-1; Italy: 0.60-0.92). There was no significant difference in the mean AI between rabies free and regions still
infected at that time. However, when rabies-free regions were divided into two groups by size (above and below 6,000 km²), in both groups the time from the beginning of oral rabies vaccination to eradication of rabies given with the number of campaigns is negatively correlated with the AI. In regions showing a high AI (0.8 - 1), rabies was eradicated within 3-6 campaigns for small regions (<6,000 km²), and 12-15 campaigns for large regions (>6,000 km²). In contrast, regions with a low AI (0.2 – 0.6) required 5-16, and 27-29 vaccination campaigns, respectively (Fig. 4).

![Figure 4: Linear regression of AI vs. number of vaccination campaigns in rabies-free regions](image)

The validity of this approach is confirmed when regions still infected with rabies are considered. It was observed that 3 regions in Germany (all larger than 6,000 km²), which developed a strategy characterised by a low AI (lower than 0.6), were still rabies infected after 30 to 34 campaigns. In these regions, the vaccination plan did not follow a systematic approach, in contrast to the one that had been followed in the eastern part of the same country where the whole region was covered by vaccination during successive campaigns (high AI). In conclusion, these studies illustrate that an AI can explain the variation encountered in dissimilar oral rabies vaccination strategies, i.e. the differences in times taken to eradicate rabies. In order to improve the efficiency of oral rabies vaccination systems in general it is necessary to guarantee a high AI to eradicate rabies in due course. However, the AI cannot take into account the question of re-infection across the border of neighbouring regions. Logically, any correlation between the AI and the number of campaigns required for rabies elimination can only be observed as long as such re-infection can be ruled out.

11. CONCLUSIONS

11.1 General Conclusions

1. In Europe, oral immunisation by means of vaccine baits has been found to be successful in eliminating terrestrial wildlife rabies in most cases. However, the ultimate success of oral rabies vaccination campaigns requires a long-term strategy and cross-border co-operation.

2. Rabies in wildlife was eliminated most efficiently in those countries where the vaccination campaigns were planned on a national level and co-ordinated with neighbouring countries.

3. Thorough surveillance of rabies epizootic and monitoring of the vaccination efficiency (using a tetracycline marker and sera-conversion rates) are important tools for the assessment and adjustment of vaccination campaigns. Standardised surveillance and monitoring methods facilitate international comparison and cooperation.

11.2 Types of vaccines and baits

1. Insufficient stability of some rabies virus vaccines is likely to have been a source of vaccination failure in specific situations (e.g. combination of climatic and meteorological
conditions and areas of high fox population density). Among currently available vaccines, based on available data, the vaccine recombinant vaccine appears to be the most stable.

2. For den vaccination of fox cubs, it is desirable that a vaccine should be able to overcome as much as possible the effects of maternal immunity. A limited number of studies indicate that the vaccine recombinant vaccine is better able to overcome maternal immunity than other vaccines.

11.3 Methods of release of vaccine baits

An appropriate bait delivery system (helicopter, fixed-wing aircraft, or manual) is needed when planning vaccination strategies, in order to achieve optimal bait distribution.

11.4 Bait density and distribution patterns

1. The bait density and bait distribution pattern should take into account habitat and landscape features, species competing for baits and fox population density in order for the baits to be taken up by a sufficient number of individual foxes. Vaccination at den entrances can be used as an additional measure in situations of high fox population density.

2. During prolonged rabies-control measures, the fox population will increase and consequently the herd immunity may become insufficient to control rabies, unless compensated for in the design of the vaccination strategy and by increasing bait density applied.

11.5 Seasonal pattern of the releases

1. Selection of the months when baiting is performed is an important consideration when planning vaccination strategies, in order to ensure access of foxes and fox cubs to baits.

2. Spring distribution is best carried out in April and/or May in order to increase the efficient access of fox cubs to baits. However, early spring campaigns carried out in March-April (targeting exclusively the adult fox population) have also been shown to be beneficial.

12. RECOMMENDATIONS

12.1 General Recommendations

1. Dynamics of the fox population should be monitored during the vaccination campaign in order to compensate for the higher abundance of the vector species through an adaptation of the vaccination strategy. It is most important that vaccination campaigns should be designed in a way to raise herd immunity along with the fox population in order to avoid setbacks in rabies eradication. Monitoring of vaccination programmes should include a sustained, constant and intensive surveillance of (i) the rabies incidence, (ii) bait-uptake and (iii) immunity in foxes during vaccination campaigns. For the surveillance of the rabies incidence in foxes in regions where oral vaccination is carried out, an examination of all foxes suspected of having rabies, those found dead and road kills should be performed.

2. In order to ensure the success of vaccination campaigns, these campaigns should be planned and coordinated across administrative and political borders. Regular contacts and consultations between stakeholders (national veterinary authorities, local veterinary authorities, hunters and the public) are very important for the successful outcome of vaccination campaigns and should be encouraged.

3. Vaccination should be continued for at least two years after the last reported case of rabies.
4. All rabies virus isolates should be typed in areas where attenuated rabies virus vaccines are used, in order to distinguish between vaccine and field virus strains.

5. Serological methods to be used for quantification of the antibody response in foxes following vaccination should be standardised as recommended by the WHO and OIE. The Community Reference Laboratory should take a lead in standardising these methods. Standardised ELISA tests, which are now available, may replace serum-neutralisation tests.

**12.2 Types of Vaccines and Baits**

1. Live rabies vaccines used for oral vaccination of foxes should fulfil the requirements of the European Pharmacopoeia monographs as well as the efficacy and safety recommendations of the WHO. Vaccine titre at batch release should correspond to at least ten times the dose found to completely protect an experimental group (indicative 100% protective dose). The titre 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 titre and stability by an acknowledged quality control scheme according to OIE standards and WHO recommendations. Laboratories involved in the monitoring and evaluation of rabies programmes are advised to monitor the titre of all batches of rabies virus baits before and during release into the field.

2. 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. Vaccine producers and National Laboratories should provide detailed information to the Community Reference Laboratory on the stability of baits to be used in the field. The National Reference Laboratory should perform additional tests or trials if required.

3. The use of tetracycline as a biomarker in the teeth and bones of foxes is recommended to evaluate bait-uptake in target species, until alternative markers without negative biological effects become available.

4. When handling baits and vaccines, storage and transportation conditions and cold-chain requirements should be strictly adhered to.

5. The use of the most stable vaccine should be preferred in situations where high stability is considered important. For the vaccination at dens of cubs born to vaccinated vixens, the vaccine that is best able to overcome the effects of maternal immunity should be used.

**12.3 Methods of release of vaccine baits**

1. The advantages and disadvantages of the distribution systems should be taken into account when vaccination campaigns are planned, and detailed identification and mapping of the vaccinated areas should be performed.

2. The use of helicopters is recommended for the treatment of all habitats (rural, agricultural, mountains, forests, suburban areas etc.). The use of fixed-wing aircraft is only recommended for the treatment of uniform and large areas of low density inhabitation (e.g. large forests, mono-agricultural areas). Distribution by hand is the preferred system in urban and suburban areas, in combination with the use of an aerial distribution whenever possible. Vaccination programmes should include comprehensive training of and provision of information to hunters and pilots. A proposed bait distribution methodology is given in an Annex of the present report, based on the available knowledge and experience.

**12.4 Bait density and distribution pattern**

1. Rabies infected regions should be vaccinated as a whole and campaigns should be repeated until rabies elimination is ascertained (and until any risk of cross-border infection is ruled out). The minimum size of a vaccination area should be 5,000 km². However, in regions
2. In cases of rabies-infected neighbouring regions, the following points should be considered in order to avoid subsequent re-infections:

- Large-scale vaccination and buffer zones should be established with the establishment of immune belts at borders between infected and non-infected regions;
- Control measures within the zone and across national or international borders should be strictly synchronised;
- Vaccination zone should extend up to the next geographical or artificial physical barrier.

3. In case of an isolated residual or re-emerging focus of rabies a vaccination area, with a radius of 25 to 50 km around the site should be applied, depending on natural barriers.

4. To protect infection spreading to a rabies-free area from a neighbouring infected area, the minimum vaccination buffer zone beyond the front of a rabies endemic zone should be 50 km. In case of an existing natural physical barrier, the minimum distance recommended is 20 km. If vector species other than the red fox are involved (e.g. racoon dogs), this buffer zone size should be adjusted to the maximum distance travelled/ranged by that species.

5. Taking topographical factors into account (e.g. urban and suburban areas), all fox home-ranges should be included in vaccination campaigns and wherever the distribution system allows flexibility (e.g. distribution by hand or helicopters), the pattern of fox habitat should be considered.

6. Homogeneous distributions of 18-20 and 20-30 baits per km² are recommended for low and high fox population densities, respectively. For den baiting, at least 10 baits are recommended to be deposited at the main den entrance.

7. When using the aerial method of bait distribution, flight line distance should not exceed 500 metres and when the fox population is high it should be reduced to 300 metres. When distributing baits manually, baits should be uniformly distributed according to a raster model based on prepared maps.

12.5 Seasonal pattern of the releases

1. In general, oral vaccination campaigns should be conducted on a biannual basis, in spring and autumn while taking climatic conditions into account. Autumn vaccination should generally be performed in September or October; spring distribution should be preferably carried out in April and/or May in order to increase the efficient access of fox cubs to baits. Den vaccination should be considered to effectively complement the regular spring campaign.

2. In case of re-emergence of rabies in foxes in an area where rabies has been previously eliminated, vaccination should be implemented immediately, whatever the period of the year, except under extreme climatic conditions which would severely hinder bait and vaccine stability.

12.6. Bait distribution methodology

This methodology is based on the data reviewed, field experience, and the conclusions and recommendations of the report.

1. Aerial distribution of baits

All habitats should be treated except large stretches of water (e.g. lakes, rivers) and motorways and big towns.
The vaccination area should be divided into plots by using natural or artificial landscape features (roads, railway tracks, canals, etc.)

1.1. Low fox population density:
- Bait density: 18-20 baits / km²
- Distribution pattern: linear: 2 flight lines (2 x 10 baits) / km²
- Distance between flight lines: 500 m +/- 100 m
- Distance between each bait (along the same flight line): approx. 100 m
- Vehicle: helicopter
- Flight altitude: 100-150 m
- Flight speed: 100-150 km/h

_Dropping procedure:_ Human: appointed and trained persons drop baits at a given mean rhythm (according to the ground speed) with more emphasis on the most convenient places for the fox (hedges, village surroundings, isolated bunch of trees etc.). An electronic metronome, connected to GPS (Global Positioning System), that allows adjustment of dropping tempo to speed may be a help, but the dropping tempo may be altered to favour more likely fox habitats.

_Control method:_ detailed map or preferably GPS.

1.2. High fox population density:
- Bait density: 20-30 baits / km²
- Distribution pattern: linear: 2 flight lines (e.g. 2 x 13 baits) / km²
- Distance between flight lines: 500 m +/- 100 m
- Distance between baits along the same flight line: 80 m
- Flight altitude: 100-150 m
- Flight speed: 100-150 km/h
- Vehicle: helicopter

_Dropping methodology:_ Human: appointed and trained persons drop baits at a given mean rhythm (according to the ground speed) with more emphasis on the most convenient places for the fox (hedges, village surroundings, isolated bunch of trees etc.). An electronic metronome (connected to GPS that allow to adjust dropping tempo to speed) may be a help, but the dropper has not to stick to this tempo to favour the places where foxes may live.

_Control method:_ detailed map or preferably GPS.

_Comments:_ The above mentioned pattern of bait distribution (low distances between flight lines) can be performed more easily using a helicopter. The use of a helicopter is more adapted to the treatment of low-densely inhabited zones in rural areas. The delivery by helicopter is fast and, unlike fixed-wing aircraft, allows precise dropping of baits (flexibility in both flight speed and altitude). The helicopter allows operation in less favourable weather conditions. Fixed-wing aircraft may be used for the coverage of large uninhabited areas (such as large forested areas) allowing long flight lines.

2. Manual distribution of baits

The manual method of baiting allows a very precise and uniform dispersal of baits but requires a thorough organisation and important human resources, qualitatively (competency, motivation) as well as quantitatively. Therefore, it should be applied for the coverage of small size areas.

2.1. Distribution of baits at fox dens

Vaccination of fox cubs at dens can usefully complement aerial vaccination for the treatment of local residual foci and re-infected areas (especially when fox density is high). It can also be used to supplement manual uniform distribution in suburban areas (see below).

_Methodeology:_

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In early spring, fox dens should be located and recorded on detailed maps by appointed and trained people (forestry rangers, gamekeepers, hunters). At the end of May – early June, previously located dens are visited again by the same persons (bearing gloves) and baits are deposited (generally at least 10) at the den entrances according to their status, whether inhabited or not:

- When occupation indices are present, 10-20 baits are deposited in the close surroundings of the breeding den regardless of the number of entrances (there are often only one or 2 entrances);
- In the absence of occupation indices, 6 baits are deposited in the close surroundings of the den regardless of the number of entrances.

Baits should be hidden (with grass, leaves etc.) to avoid ingestion by birds and exposure to direct sun light.

2.2. Uniform distribution of baits in suburban areas

In such a habitat, up to 4-5 fox family groups can be counted per km2. Consequently the percentage of foxes needed to be immunised to eliminate rabies might theoretically approach 100%. In addition, the high density of competing pets population can significantly affect the rate of bait uptake in foxes. For safety and feasibility reasons, aerial distribution is replaced by manual distribution in such high-densely inhabited areas. However, whenever possible, the combination of several distribution systems (hand-helicopter) is to be recommended. Appearance of foxes in urban area is not frequent, because the presence of stray dogs.

Methodology:

- Hand distributors: appointed and training persons (bearing gloves).
- Bait density: 50 baits / km2
- Dispersal of baits: Raster pattern: uniform but more intensive in predicted fox habitats.
- Baits should be hidden to avoid human contacts, ingestion by birds and exposure to direct sunlight.

Control method: detailed map

13. DESCRIPTION OF THE MULTI-ANNUAL ACTION PLAN

Annual and multi-annual plans: are essential to ensure a coordinated approach towards proper implementation of control and eradication of diseases. Instructions, standards and SOPs for official controls, adopted by the VD will ensure proper application of the plans. SOPs related to: programming and management of activities, including official controls, prevention, control and notification of disease outbreaks, risk analysis, epidemiological surveillance and zoning, inspection and sampling techniques, diagnostic protocols and manuals, disinfection procedures, treatments intended to destroy pathogens, etc will be needed.

Surveillance program for rabies: preparation and implementation of appropriate surveillance on rabies is an essential tool to detect rabies in domestic and wildlife, to monitor disease trends, to facilitate control measures, to support claims for disease free status, to provide data for use in risk analysis for animal and public health and to substantiate the rationale for sanitary measures. Both domestic and wild animals are susceptible to rabies, but it is also a zoonoses. Wildlife disease surveillance presents specific challenges. The impact on public health has to be taken into consideration. These challenges would facilitate multi-sectored approach and involvement of different authorities and stakeholders in designing of surveillance programme.

- Sound surveillance data is essential for successful design and operation of a multi-annual plan. Surveillance may be based on different data source including active or passive, pathogen-specific or general surveillance, structured surveys and non-random data source. In addition, surveillance data should be supported by related information such as: data on the epidemiology of disease, including environmental, host population distribution and climatic...
information, data on animal movements and natural wildlife migration, history of imports, the risk and consequences of disease introduction etc.

- Populations and time frame: ideally, surveillance should be carried out in such a way as to take into account all species susceptible to the disease in the country. Surveillance should be carried out at a frequency that reflects the biology of the infection and susceptible species (e.g. foxes) and the risk of introduction of the virus.

- Epidemiological units and clustering: The relevant epidemiological unit for the surveillance system should be defined to ensure that it is representative of the population. Therefore, it should be chosen taking into account factors such as carriers, reservoirs, vectors, immune status, age, sex and other host criteria. Infection in a country usually clusters rather than being uniformly or randomly distributed through a population. Clustering should be considered in the design of surveillance activities and the statistical analysis of surveillance data.

- Vaccination plan should consider physical conditions of different areas involved (mountainous, flat plains, etc) and program timing of plans with those of bordering countries.

- A cost-benefit analysis: Rabies control and eradication plans are extremely expensive and can be implemented according to alternative technical solutions. A cost-benefit analysis of solutions is an important tool for strategic decision making by central veterinary services.

- Field staff must always be fully and properly informed on the general approach towards Rabies strategies and comprehend underlying logic. It is important to remember that the first level of control is represented by the field inspection level. Seminars and trainings for field staff are essential. Data exchange system should be established/strengthened including, but not limited, to e-mailing and development of web portal and e-learning module.

- Commitment from stakeholders: it is essential to obtain commitment and understanding from farmers, wildlife keepers, hunters and other stakeholders on the importance of a proper and timely notifications and obtain active participation. Appropriate collaboration and communication with public health authorities have to be established.

- Guidelines, manuals and check lists should be widely adopted to facilitate uniform harmonised procedures. Documents must be simple and easy to use. A long and complex check list will risk being completed in an improper manner. A less time consuming (although maybe less complete) check list will be accepted more easily by field staff.

- Data collection and management: The success of a surveillance system is dependent on a reliable process for data collection and management. The process may be based on paper records or computerised. The consistency and quality of data collection and event reporting in a format that facilitates analysis, favourable integrated with VIMS, is critical.

13.1. Territorial data of the Republic of Serbia

The objective of this programme is to ensure eradication of rabies on the territory of Republic of Serbia. It is foreseen this to be achieved by oral vaccination of foxes on the territory of Serbia. This vaccination is to be performed for a period of at least 5 consequent years (2010, 2011, 2012, 2013 and 2014), twice per year in spring and autumn (May-June and September-October).

Table 3: Territorial data of the Republic of Serbia

<table>
<thead>
<tr>
<th>Territory</th>
<th>Territory/Area km²</th>
<th>Regions/ Districts</th>
<th>Municipalities</th>
<th>Settlements</th>
<th>Urban settlements</th>
<th>Other settlements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Republic of Serbia</td>
<td>77.474</td>
<td>24</td>
<td>165</td>
<td>4,720</td>
<td>181</td>
<td>4,539</td>
</tr>
</tbody>
</table>
Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

<table>
<thead>
<tr>
<th>Territory</th>
<th>Territory/Area km²</th>
<th>Forests km²</th>
<th>Agricultural land %</th>
<th>Average territory size of the settlement km²</th>
<th>Estimated Area for vaccination km²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Republic of Serbia</td>
<td>77.474</td>
<td>23.217</td>
<td>64.6</td>
<td>14.2</td>
<td>69.996</td>
</tr>
</tbody>
</table>

Source: Republic statistical administration of the Serbia

The total size of the territory where vaccination will be provided is about 69 000 km² and it comprises territories located within 25 administrative districts (AD), as follows:

Table 4: Calculated vaccination area

<table>
<thead>
<tr>
<th>District</th>
<th>Territory/Area km²</th>
<th>No of settlements</th>
<th>Vaccination area km²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zapadnobacki</td>
<td>2420</td>
<td>37</td>
<td>2217</td>
</tr>
<tr>
<td>Severnobacki</td>
<td>1784</td>
<td>45</td>
<td>1635</td>
</tr>
<tr>
<td>Severnobanatski</td>
<td>2329</td>
<td>50</td>
<td>2134</td>
</tr>
<tr>
<td>Juznobacki</td>
<td>4016</td>
<td>77</td>
<td>3679</td>
</tr>
<tr>
<td>Srednjebanatski</td>
<td>3256</td>
<td>55</td>
<td>2990</td>
</tr>
<tr>
<td>Sremski</td>
<td>3486</td>
<td>109</td>
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<td>Juznobanatski</td>
<td>4245</td>
<td>94</td>
<td>3888</td>
</tr>
<tr>
<td>Macvanski</td>
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<td>228</td>
<td>2994</td>
</tr>
<tr>
<td>Beograd</td>
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<td>166</td>
<td>2952</td>
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<tr>
<td>Podunavski</td>
<td>1248</td>
<td>59</td>
<td>1143</td>
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<tr>
<td>Brancevski</td>
<td>3865</td>
<td>189</td>
<td>1395</td>
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<td>3540</td>
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<td>2267</td>
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<tr>
<td>Zlatiborski</td>
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<td>5624</td>
</tr>
<tr>
<td>Moravicki</td>
<td>3016</td>
<td>206</td>
<td>2762</td>
</tr>
</tbody>
</table>
### 13.2 Characteristic of rabies viruses circulating in the Balkans

The phyla-genetic analysis provides evidence for the movement of rabies-infected hosts across the borders of the countries of the former Yugoslavia. The clustering of three Bulgarian sequences, two foxes and one wolf, with a wolf and a fox from Bosnia and Herzegovina (U42704 & U42706) and two foxes from the Federal Republic of Yugoslavia (U22839 & U42703), is clearly suggestive of movement of rabies across national boundaries by wildlife vectors. No virus sequences are available from Romania although the country reports numerous cases of rabies (Rabies Bulleting Europe, WHO). Further epidemiological studies on samples from different border areas are needed for the regional control and eradication programme. Such a strategy was successfully applied for the elimination of rabies from Switzerland (Wandeler 1988). However, rabies appears to be endemic within a diverse range of reservoir species present within the Balkan region, and this will present further challenges to the development of control strategies in countries such as Serbia.
13.3. Oral vaccination of foxes against rabies

- **The first vaccination campaign** is to be performed in the autumn of 2010 and will cover the whole territory of Serbia (25 administrative districts), the total area being 77,474 km² (Central Serbia – 55,968 and Vojvodina – 21,506). On the territory of these 24 ADs (Central Serbia – 17 and Vojvodina – 7) there are 4,539 settlements (villages and towns) in Central Serbia – 4,124 and in Vojvodina – 415, located on an area of 14,200 km². Thus, the area to be covered by oral vaccination is about 70,000 km². In addition, of this because of emergency vaccination possible manual vaccination in urban area total procurement of vaccine should come about 1,500,000 baits.

- **Next vaccination campaigns** are to be performed twice a year in the spring and autumn of years 2011, 2012, 2013 and spring 2014 overall of the above-mentioned territory.

### 13.4 Numbers of vaccination baits needed a five years period:

#### 13.4.1. First autumn vaccination campaign Year 2010

The dose should be 20 vaccine baits per 1 km². The territory for vaccination shall be the whole Serbia. Total area is 77,474 km². The number of settlements is 4,539 of total area of 14,200 km², which leaves area for vaccination of about 70,000 km². The number of baits is 1,490,839 in 2010.

**Table 5: Number of baits per district needed for the 1st campaign, autumn 2010**

<table>
<thead>
<tr>
<th>District</th>
<th>Territory/Area km²</th>
<th>No of settlements</th>
<th>Vaccination area km²</th>
<th>No of bites per District /20 per km²</th>
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</thead>
<tbody>
<tr>
<td>Zapadnobacki</td>
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<td>37</td>
<td>2.217</td>
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<tr>
<td>Severnobacki</td>
<td>1784</td>
<td>45</td>
<td>1.635</td>
<td>32.701</td>
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</table>
### Table 6: Number of baits per district for 2nd and 3rd campaigns 2011

<table>
<thead>
<tr>
<th>District</th>
<th>Territory/Area km²</th>
<th>No of settlements</th>
<th>Vaccination area km²</th>
<th>No of bites 2nd campaign /23 per km²</th>
<th>No of bites 3rd campaign /23 per km²</th>
</tr>
</thead>
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<tr>
<td>Severnobanatski</td>
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<td>73.577</td>
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<tr>
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<td>109</td>
<td>3.193</td>
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<td>Juznobanatski</td>
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</tr>
<tr>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

### 13.4.2. Year 2011 (spring and autumn vaccination campaigns)

**Second and third vaccination campaigns**: – the dose should be increase to 23 pieces of vaccination baits per 1 km². The total number of unit baits needed for the whole year 2011 will be about 3,220,000 pieces of vaccination baits.
Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

<table>
<thead>
<tr>
<th>Region</th>
<th>Code</th>
<th>Count</th>
<th>Code</th>
<th>Count</th>
</tr>
</thead>
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<td>49.082</td>
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<td>Juznobacki</td>
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<td>84.617</td>
</tr>
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<td>≈ 1.610.000</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>≈ 3.220.000</td>
<td></td>
</tr>
</tbody>
</table>

13.4.3. Year 2012 (spring and autumn vaccination campaigns)

Fourth and fifth vaccination campaigns: – the dose should be again 23 pieces of vaccination baits per 1 km². The total number of baits is 3.220.000 in 2012.
Table 7: Number of baits per district for 4th and 5th campaigns 2012

<table>
<thead>
<tr>
<th>District</th>
<th>Territory/Area km²</th>
<th>No of settlements</th>
<th>Vaccination area km²</th>
<th>No of bites 4th campaign /23 per km²</th>
<th>No of bites 5th campaign /23 per km²</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Rasinski</td>
<td>2668</td>
<td>295</td>
<td>2.444</td>
<td>56.212</td>
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</tr>
<tr>
<td>Zajecarski</td>
<td>3623</td>
<td>173</td>
<td>3.319</td>
<td>76.337</td>
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</tr>
<tr>
<td>Pirotski</td>
<td>2761</td>
<td>214</td>
<td>2.529</td>
<td>58.167</td>
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</tr>
<tr>
<td>Nisavski</td>
<td>2729</td>
<td>285</td>
<td>2.500</td>
<td>57.500</td>
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</tr>
<tr>
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<td>267</td>
<td>2.043</td>
<td>46.989</td>
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<tr>
<td>Jablanicki</td>
<td>2769</td>
<td>336</td>
<td>2.537</td>
<td>58.351</td>
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</tr>
<tr>
<td>Pcinjski</td>
<td>3520</td>
<td>363</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td>≈ 1.610.000</td>
<td>≈ 1.610.000</td>
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</tbody>
</table>
13.4.4. Year 2013 (spring and autumn vaccination campaigns)

Sixth and seventh vaccination campaigns: – the dose should be again 23 pieces of vaccination baits per 1 km². The total number of baits is 3.401.210 in 2013.

Table 8: Number of baits per district for 6th and 7th campaigns 2013

<table>
<thead>
<tr>
<th>District</th>
<th>Territory/Area km²</th>
<th>No of settlements</th>
<th>Vaccination area km²</th>
<th>No of bites 6th campaign /23 per km²</th>
<th>No of bites 7th campaign /23 per km²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zapadnobacki</td>
<td>2420</td>
<td>37</td>
<td>2.217</td>
<td>50.991</td>
<td>50.991</td>
</tr>
<tr>
<td>Severnobacki</td>
<td>1784</td>
<td>45</td>
<td>1.635</td>
<td>37.605</td>
<td>37.605</td>
</tr>
<tr>
<td>Severnidanatski</td>
<td>2329</td>
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<td>2.134</td>
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<tr>
<td>Juznobacki</td>
<td>4016</td>
<td>77</td>
<td>3.679</td>
<td>84.617</td>
<td>84.617</td>
</tr>
<tr>
<td>Srednidanatski</td>
<td>3256</td>
<td>55</td>
<td>2.990</td>
<td>68.770</td>
<td>68.770</td>
</tr>
<tr>
<td>Sremski</td>
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<td>109</td>
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<td>73.439</td>
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</tr>
<tr>
<td>Juzndonatski</td>
<td>4245</td>
<td>94</td>
<td>3.888</td>
<td>89.424</td>
<td>89.424</td>
</tr>
<tr>
<td>Macvanski</td>
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<td>228</td>
<td>2.994</td>
<td>68.862</td>
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<td>Beograd</td>
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</tr>
<tr>
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<td>59</td>
<td>1.143</td>
<td>26.289</td>
<td>26.289</td>
</tr>
<tr>
<td>Braničevski</td>
<td>3865</td>
<td>189</td>
<td>1.395</td>
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<td>Boski</td>
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</tr>
<tr>
<td>Zlatiborski</td>
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</tr>
<tr>
<td>Moravicki</td>
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<td>2.762</td>
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<td>63.526</td>
</tr>
<tr>
<td>Raski</td>
<td>3918</td>
<td>359</td>
<td>3.589</td>
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<td>82.547</td>
</tr>
<tr>
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<tr>
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<td>3520</td>
<td>363</td>
<td>3.224</td>
<td>74.152</td>
<td>74.152</td>
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</tbody>
</table>
13.4.5. Year 2014 (spring and autumn vaccination campaigns)

Eighth and ninth vaccination campaigns: – the dose should be again 23 pieces of vaccination baits per 1 km². The total number of baits is 3.401.210 in 2014.

Table 9: Number of baits per district needed for the 8th and 9th campaigns 2014

<table>
<thead>
<tr>
<th>District</th>
<th>Territory/Area km²</th>
<th>No of settlements</th>
<th>Vaccination area km²</th>
<th>No of bites 8th campaign /23 per km²</th>
<th>No of bites 9th campaign /23 per km²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zapadnobacki</td>
<td>2420</td>
<td>37</td>
<td>2.217</td>
<td>50.991</td>
<td>50.991</td>
</tr>
<tr>
<td>Severnobacki</td>
<td>1784</td>
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<td>1.635</td>
<td>37.605</td>
<td>37.605</td>
</tr>
<tr>
<td>Severnobanatski</td>
<td>2329</td>
<td>50</td>
<td>2.134</td>
<td>49.082</td>
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</tr>
<tr>
<td>Juznobacki</td>
<td>4016</td>
<td>77</td>
<td>3.679</td>
<td>84.617</td>
<td>84.617</td>
</tr>
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<td>Srednjebanatski</td>
<td>3256</td>
<td>55</td>
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<td>68.770</td>
</tr>
<tr>
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<td>3.193</td>
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</tr>
<tr>
<td>Juznobanatski</td>
<td>4245</td>
<td>94</td>
<td>3.888</td>
<td>89.424</td>
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</tr>
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<td>Macvanski</td>
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<td>1.143</td>
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<td>Borski</td>
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<td>3.540</td>
<td>81.420</td>
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<td>Zlatiborski</td>
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<td>214</td>
<td>2.529</td>
<td>58.167</td>
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</table>
### Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

<table>
<thead>
<tr>
<th>District</th>
<th>Territory/Area km²</th>
<th>No of settlements</th>
<th>Vaccination area km²</th>
<th>No of bites 8th campaign /23 per km²</th>
<th>No of bites 9th campaign /23 per km²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisavski</td>
<td>2729</td>
<td>285</td>
<td>2.500</td>
<td>57.500</td>
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<tr>
<td>Additional vaccination around settlements /If needed/</td>
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</tr>
<tr>
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<td>≈ 1.700.605</td>
<td>≈ 1.700.605</td>
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</tbody>
</table>

#### 13.4.6. Year 2015 (spring and autumn vaccination campaigns)

**Last vaccination campaign:** — the dose should be 23 pieces of vaccination baits per 1 km². The territory for vaccination shall be the whole Serbia. The total number of baits is 3,401,210 in 2015.

**Table 10: Number of baits per district for 10th and 11th campaigns, 2015**

<table>
<thead>
<tr>
<th>District</th>
<th>Territory/Area km²</th>
<th>No of settlements</th>
<th>Vaccination area km²</th>
<th>No of bites 8th campaign /23 per km²</th>
<th>No of bites 9th campaign /23 per km²</th>
</tr>
</thead>
<tbody>
<tr>
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<td>59</td>
<td>1.143</td>
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<td>Branicevski</td>
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<td>129.352</td>
</tr>
</tbody>
</table>
The oral vaccination shall be performed by strain vaccine that is derivative of the SAD strain and that is stable to high ambient temperatures, since the vaccination periods (April-May and September-October) the temperatures in Serbia are relatively high.

13.4.7. Additional vaccination around suburbs of settlements in each campaign

Well known is that the number of foxes in the suburbs is greatly increased, so we decided to additional vaccination with these suburbs of the largest cities in Serbia.

Around 18 the biggest settlements in Serbia additional vaccination shall be done. Path of aircraft or helicopters should be done in circle around settlement.

The spread of baits from the airplanes or helicopters shall be done by special equipment. Therefore, the airplanes or helicopters shall be equipped with a system for spread of baits connected with GPS system in order to ensure the exact distribution depending on the speed and height of the fly and keep automatic record of the quantity and location of the spread baits. Baits shall be dropped in line around settlements started very close to urban area and next line shall spread out of first for 500 m. The aircraft should fly in the lines around the city, making the nine rounds and each one must be away for 500 m from the previous

Table 11: Cities with the calculated number of baits for the vaccination in suburbs area

<table>
<thead>
<tr>
<th>Name of city</th>
<th>Nine circles around settlement (km)</th>
<th>Number of additional baits per settlements</th>
</tr>
</thead>
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</tr>
<tr>
<td>NOVI SAD</td>
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<tr>
<td>PANČEVO</td>
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<tr>
<td>BEOGRAD</td>
<td>957</td>
<td>13398</td>
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<tr>
<td>SMEDEREVO</td>
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<td>4731</td>
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</table>
POŻAREVAC 282 3943
KOVIN 282 3943
VALJEVO 282 3943
JAGODINA 225 3155
KRAGUJEVAC 619 8670
KRALJEVO 282 3943
ČAČAK 338 4731
UŽICE 282 3943
KRUŠEVAC 338 4731
NOVI PAZAR 282 3943
NIŠ 450 6307
LESKOVAC 225 3155

Remark:
After the completion of this 5-years Program there should be a new vaccination program developed on the basis of the analysis of the results achieved through this 5-years program. The options for such further development are three, as follows:
- Continuing the vaccination in the whole of Serbia;
- Continuing the vaccination in certain individual administrative districts or regions;
- Continuing the vaccination only within some zones (strips) alongside the land borders between Serbia and some neighbour countries.

14. PROCEDURE FOR ADMINISTRATING THE ORAL VACCINE

14.1 Vaccine characteristics

Vaccine for oral vaccination of foxes against rabies contain attenuated live rabies virus and should meet the following requirements:
- To be licensed for use minimum in the one of relevant national authorities EU countries or licensed for use by the European Agency for medicinal products (EMEA) as vaccine for oral vaccination of foxes;
- To be produced by using virus strain “SAD” or his derivates;
- To contain protectors preserving the virus in the baits in environment for minimum of 7 days;
- To induce immunity in vaccinated (target) animals for at least one year;
- The vaccine shall not be residual virulence for animal and humans; (vaccine should not be pathogenic for animals and humans) lowest residual pathogenicity for target and nontarget species;
- The vaccine baits shall contain marker (tetracycline) allowing to identify the vaccinated foxes;
- The vaccine shall induce immunity in vaccinated animals not later than 30 days after intake the vaccine baits and the antibody-titre against rabies shall not be lower than 0,5UI/ml
- The vaccine will allow differentiation of vaccine strain from wild (field) virus;
- Each batch of vaccine shall have expired date minimum 12 months from the date of delivery.
- The vaccine doses for foxes shall be included in appropriate baits;
- The vaccine baits shall be adjusted (adapted) for spread by airplane (therefore they shall be solid and not to break into pieces when fall down to the earth);
- Each ampoule (blister) should be labelled by batch number, expire date, name of product and with security alert
- The instructions for use of the vaccines should be in Serbian language.

14.2. Check the virus titre in the vaccine

Control quality of the vaccine

Each vaccine should be checked for titre of rabies virus at the time of reception and after distribution in the field. Samples must be taken randomly and should be titrated in order to assess the titre and stability of the viral suspension.

The vaccine should be supplied 20-30 days before been placed. Each batch of vaccine should be tested to check the virus titre. The tests will be carried out in the National scientific Veterinary Institute of the Republic of Serbia, Belgrade. Virus titre should be determinate in TCID50/ml (tissue culture infective doses) by virus titration test or in FFU/ml (fluorescent focus unit/ml) depending of the type of vaccine. Virus titration test should be done in 96 well micro-titre plates. Ten baits from each batch of vaccine should be sent to the laboratory for testing. Virus titre is determinate in pool of ten vaccine baits. For this purpose vaccine solutions should be prepared from all baits and than pooled. Ten-fold dilution should be prepared in 10 tubes. Each dilution should be transferred to wells of micro-pale in 8 repeated. 50 µl of BHK21 cell culture suspension (containing 200.000 - 400000 cells/ml) should be added to all wells of the micro-titre plate. After the incubation of 3 – 4 days at 37 °C in 5% CO2 atmosphere, cells should be fixed with 80% cold acetone for 20 minutes, than micro-plates should be rinsed with PBS and stained with FITC conjugate against rabies virus. The stained with FITC cells should be incubated at 37 °C for 60 minutes, than washed three times. The results should be read by using fluorescent microscope. Titre of virus is calculated by Reed-Munch methodology.

Vaccine baits should be stored all the time in chillers, at temperature of -20ºC and temperature of ambient should be checked at least twice per day. The day before being placed, the baits will be delivered to the places where they will be loaded on the airplanes/helicopters, or from where hunters are to be supplied with them for their spreading /placing/. Places for distribution of vaccines (airport) should be equipped with suitable refrigerators. If the uploaded baits are not distributed during the same day (for some reason like for example bad water), they can be stored at temperature of +1 to +8 ºC, but not longer than 5 days.

14.3. Plan of rabies vaccine baits distribution

After import vaccine for oral vaccination of foxes, 10 baits of each batch should be sent to Veterinary institute Beograd and should be tested to virus titre.

14.4. Import and storage of vaccine baits

1. Vaccine baits shall be delivered in Serbia in 2010 before 15.08.2010 for first ORV campaign and second before 15.03.2011 for second ORV campaign.
2. The vaccine baits will be stored in storage facilities of the supplier at temperature and other conditions specified by the vaccine producer. The storage facility should be equipped with device that is able to record the temperature and other parameters (if any) specified by the producer and keep record on those parameters for each day of the storage.
3. Each batch of vaccine should be tested in the Veterinary institute Beograd (where) to titre of virus.
14.5. Place and time of delivery

1. The vaccine shall be spread in two rounds first in September /October 2010 and second in April/May 2011.
2. Each batch of vaccine shall have expired date minimum 12 months from the date of delivery.
3. The vaccine shall be delivered in storage facilities of the supplier, where it will be stored in temperature specified by the producer until the end of the vaccination campaign.
4. Storage facility shall be prepared also at the airport (-20C cooling trucks or freezers for a quantity of baits needed for one day flight activity at every airport)
5. Transport of vaccines from store facility to the airport using cooling trucks (-20C)
6. 13.6. Implementation of the vaccination
7. Vaccination teams shall be trained in advance. Training shall be organised and paid by contractor.
8. The spread of the vaccine shall be done according to preliminary prepared time schedule.
9. Transportation of the vaccine from the storage facility to the airport, where it will be loaded to the airplanes or helicopters shall be done by using cooling trucks that are able to maintain the necessary temperature and conditions (parameters) as prescribed by the producer of the vaccine.

14.6. Mode of bait distribution

1. In the case of aerial distribution, the vaccine is distributed in a frozen condition
2. The airplanes or helicopters shall be equipped with GPS system and supported by a computer program that will allows control the ejection of bait (number, time, location) which will be used during the spread of the baits.
3. The spread of baits from the airplanes or helicopters shall be done by special equipment. Therefore, the airplanes or helicopters shall be equipped with a system for spread of baits connected with GPS system in order to ensure the exact distribution depending on the speed and height of the fly and keep automatic record of the quantity and location of the spread baits.
4. The flight and distribution itself is monitored by a computer application with automatic GPS position recording, date and time of distribution of individual vaccine bait.
5. The airplanes or helicopters shall fly horizontally with an average distance between the flying lines (corridors) 500 m +/- 100 m, the baits will be dropped with the average distance between each other of 100 m +/- 50 m from height between 100 and 300 m.
6. After each flight information shall be submitted to the contractor about the number of distributed baits, the linear kilometres passed and the territory covered. The pilot and the responsible person on behalf of the shall sign a protocol for the completed vaccination, which is prepared by the Veterinary directorate of Serbia.
7. The electronic records containing data about the number of distributed vaccines and covered places from device for spread of vaccine baits shall be given to the contractor at the end of each working day. The information on the course of distribution shall possible to be downloaded from the computer database after each flight or whole campaign. There is a possibility to store data on distribution in each control unit computer in the plane) that is equipped with a slot for memory card. There shall be a date, time and position of each vaccine bait recorded on the memory card
8. Aerial distribution of baits by aircrafts, (fixed-wing aircraft) or helicopters. Baits shouldn’t be distributed in settlements by aircrafts. Pilots should stop with distribution above settlements. Rejects of baits halt to implement 100 meters before the village, courtyard and continues over 100 meters after transiting of settlement. Distribution of baits surrounding villages and cities with helicopters are recommended.
14.7. Duration of vaccination campaigns

Each vaccination campaign should be complete within 45 days considered from the commencing day, decided by the Veterinary directorate prior to signing of the contract. This time period should be extended in the case of manual distribution of baits and for the emergency vaccination activities.

15. RABIES SURVEILLANCE AND MONITORING ACTIVITIES

Activities on oral vaccination of foxes in Serbia shall be controlled and coordinated by Veterinary directorate Republica Serbia (VD).

VD shall set up a Crises management unit for oral vaccination of foxes, which consists of representatives of VD, epidemiologists from Veterinary university, experts in laboratory diagnostic, health services, hunting organizations and operators of bait-laying.

VD in cooperation with project team shall perform education for pilots, hunters and other stakeholders. The pre-exposure vaccination against rabies is recommended for persons to be in direct contact with baits. VD in cooperation with project team shall prepare and implement general public awareness campaign.

Before each start of ORV campaign VD shall organise meeting of Crises management unit and they shall review the work plan of ORV, as well as after the end of ORV campaign. Operator of bait-laying shall give the report of ORV campaign and also Veterinary institute in Belgrade shall give report about post-ORV surveillance activities to Crises management unit.

VD shall control the work of contractors laying baits daily, and periodicaly take the samples of bait before take-off and after landing aircraft and send them in an investigation. Laboratory control will be effected in the Veterinary Institutes in Republic of Serbia.

VD in cooperation with project team and hunters associations shall organise education and informative meetings for veterinarians and hunters in the field talking about oral vaccinations of foxes.
15.1. Hunter organisations

Each hunter organisation should collect foxes and send them to the nearest veterinary services. Collection of samples should be performed compulsory according to the legislation. VD - Serbian Ministry for Agriculture, Forestry and Water Management in cooperation with project team shall prepare and publish in official gazette regulation, that each hunters organisation is obligate to deliver 8 foxes and/or jackals/100 km² area to veterinary service. Each hunting organization must shoot appropriate number of foxes and or jackals, to achieve an appropriate quota 8 foxes and/or jackals per 100 km². Foxes should be shot with buckshot, put carcase in a water-proof bag of PVC, complete shoot form and take it to the nearest veterinary station. Hunters should bring the carcases to the authorized veterinary station in 24 h.
Hunters involved in the vaccination and surveillance campaigns will need to be equipped with personal protective equipment, cartridges for hunting. They will need special trainings on rabies disease, and disinfection of the personal protective equipment, preparation of accompanying documentation (form) that needs to be submitted to the veterinary service together with shot foxes.

15.2. Veterinary organisations and human contact with suspected animals

The veterinary organisations in the vaccinated area in Serbia will receive the fox carcases from hunters together with the Delivery document (form). Veterinarians shall confirm the receipt of fox carcase by signing the form, recording data into the data base and to store the carcases at refrigerator at temperature 1 – 10 °C for maximum 72 hours, for longer time at >-15 C, until they are transported to the competent regional veterinary institute.

Carcasses of foxes and other animals which have been in direct contact with the human should be delivered/sent to the Pasteur Institute in Novi Sad, with respect of case notification flow presented in Figure 6.

Figure 6: Case notification flow of human contact with suspected animals
15.3. Monitoring of oral vaccination of foxes

According to WHO and OIE recommendation as well as to Scientific Committee on Animal Health and Animal Welfare of European Commission monitoring is one of the crucial elements of oral vaccination. Monitoring after field trials employs following methods of evaluation:

- Testing for the presence of biomarker in the target species (tetracycline), which is incorporated into the bait – bait uptake;
- Checking sera from foxes for the presence of rabies virus neutralizing antibody – to check protection/herd immunity;
- Age determination;
- Analyzing of epidemiological situation on rabies during running the oral vaccination programme.

Additionally if attenuated rabies vaccine strain is used in the field typing of rabies virus isolates from ORV area to distinguish vaccine strain from field rabies strain is necessary. Only this methods together gives the full picture how the oral vaccination campaigns are effective and allow us to estimate them and make the correction of the strategy of ORV in the future.

Monitoring of the effectiveness of ORV is carried out using following methods:

1. Fluorescent antibody test (FAT) - for detection of viral antigen (rabies virus) in the smears from different parts of brain;
2. Fluorescent antibody virus-neutralisation (FAVN) or ELISA test- for titration of antibodies in fox serums/body fluids proving the presence of antibodies after vaccination;
3. Examination of fox bones for the presence of tetracycline (TC) as a biomarker of the vaccine uptake.
4. Age determination test for discrimination between young and adult foxes.
5. Virus typing and differentiation of field and vaccine rabies virus strains.

Laboratory control of the effectiveness of oral vaccination will be effected in the National Reference Laboratory - Scientific Veterinary Institute in Belgrade, Scientific Veterinary Institute Novi Sad and Veterinary Institute Kraljevo.

Samples from foxes killed in the vaccinated area, shall be used for laboratory control of the efficiency (efficacy) of the vaccination programme (8 foxes per 100 km2 should be collected). The 12 Veterinary Institutes will collect the carcasses from those Veterinary Stations twice a week after the vaccination campaigns. When fox carcases are brought into the institutes, they should be stored in refrigerators until necropsy is carried out. The institutes will collect blood or body liquid samples as well as lower jaws and brains from foxes during necropsy. For this purpose the 12 veterinary institutes will need transport vehicles (12 pick-up with cooling compartments) as well as refrigerators with sufficient capacity for storage of carcases and samples. They will need also appropriate instruments for necropsy and protective clothes. Staff should be trained for carrying out necropsy according to the specific requirements for rabies.

Laboratory staff shall be vaccinated against the rabies and trained for carrying out necropsy and diagnostic tests. According to WHO recommendation the scheme of preventive immunisation of exposed staff includes three injections, e.g. at days 0, 7, and 28. The serological evaluation of immunisation is made 1–3 weeks after the last injection, and checked every 6 months in the case of laboratory workers or every 2 years for other diagnosticians.
Booster vaccination must be given when the titre falls below 0.5 International Units (IU) per ml. In the absence of serological monitoring, the vaccination regimen should consist of a booster vaccination at 1 year and thereafter every 1–3 years.

**Blood and liquid body collection:**

Fox carcase should be placed on its back with the feet up for at least 6 hours so that the doctor can collect as much as possible body liquid from abdominal and thoracic cavity. During necropsy carcase should be placed on its back and abdominal and thoracic cavity should be opened. The heart is opened first. Blood is collected by syringe. From 3 to 5 ml blood sample is needed. The sample should be transferred into a tube (10 ml) with thread stopper and should be marked with protocol number. When collection of blood from the heart is not successful, body liquid from thoracic and abdomen should be taken. Samples should be frozen and stored at temperature less than -15°C.

**Lower jaw /mandible/ collection:**

It is necessary to withdraw the entire lower jaw. Sample should be placed in a cooling box, identified with a protocol number and relevant accompany document.

**Methods for detection of rabies virus antigen or rabies virus genome**

Rabies virus is a member of *Rhabdoviridae* family. All warm-blooded animals and humans are susceptible to infection. Soon after clinical symptoms appear the infected animal die. The diagnostics of rabies is based on detection of rabies virus in brain samples of dead or killed animal. In accordance with WHO and OIE recommendations laboratory diagnosis can be performed by using three kinds of procedure:

1. Fluorescent antibody test (FAT)
2. Rabies tissue-culture infection test (RTCIT)
3. Mouse inoculation test (MIT)

In the case of FAT-negative results with human exposure or FAT- inconclusive results such tests should be confirmed using other recommended techniques – isolation of rabies virus.

The detection of viral RNA by molecular techniques, e.g. RT-PCR, nested and hemi-nested RT-PCR, real-time PCR, is currently not recommended or approved for routine post mortem diagnosis of rabies (WHO, 2005), but is now well developed and used routinely in most laboratories working on rabies. Although not recommended for routine diagnosis, those molecular techniques can provide useful information on viral types and rabies epidemiology and could complement recommended techniques.

FAT - fluorescent antibody test - is a rapid and sensitive method for diagnosing rabies infection in animals and humans, allowing specific and highly sensitive detection of the rabies antigen. FAT is the gold standard test for rabies diagnosis. The technique is based on impressions or smears made from brain samples, tissue fixation, mostly in cold acetone, and staining with fluorescein isothiocyanate-labelled polyclonal or monoclonal anti-rabies antibodies.

Virus isolation (VI) can be performed on cells or upon intracranial inoculation of mice using RTCIT and the MIT.

RTCIT - Rabies tissue-culture infection test implies the isolation of rabies virus in a cell culture monolayer, e.g. mouse neuroblastoma cells, by subsequent visualisation by FAT. Murine-neuroblastoma cells are more susceptible to field isolates of rabies virus than other cell lines tested such as cattle brain cells, chicken embryo fibroblasts, Vero cells, baby hamster kidney cells 21 (BHK-21). Because RTCIT is sensitive and specific as MIT and avoid
the use the live animals this test should replace the mouse inoculation test if the laboratory has possibility to introduce it.

MIT- Mouse inoculation test it is a sensitive and robust technique. Laboratory mice are inoculated intracerebrally with supernatant of a brain suspension and observed for up to 30 days after inoculation. In mice rabies induces clinical signs that are relatively typical but it should be confirmed with FAT.

All methods for rabies diagnosis are described in detail in the OIE Manual on Diagnostic Tests and Vaccines (OIE 2008) and in Laboratory techniques in rabies (WHO 1996).

**Method for antibody detection against rabies virus in blood or body liquid samples**

**Virus neutralization test (VN)**

The main application of serology (virus neutralization test) is to determine responses to vaccination, either in domestic animals prior to international travel, or in wildlife populations following oral immunisation of rabies reservoirs. For follow-up investigations in oral vaccination campaigns, virus neutralisation (VN) tests in cell culture are preferred. For the detection of the level of seroconversion in vaccinated foxes RFFIT (Rapid Fluorescent Focus Inhibition Test) or FAVN test can be used.

The FAVN is a classical virus neutralisation test and is the method of choice for determining the levels of antibody to rabies virus in serum.

In the FAVN test, multiple serial dilutions of test serum are incubated with defined amount of the CVS strain of rabies virus. After the serum virus incubation, BHK-21 cells are added to the virus-serum mixture and the test plate is incubated. Sera which contain antibodies to the virus are able to neutralise the aliquot of virus used in the test, thus preventing infection of the cells when they are added to the plate. Where high concentrations of antibody to the virus are present in the serum sample, virus neutralisation will occur even at high serum dilutions. Conversely, where little or no antibody to the virus is present in the test sample, it will be unable to neutralise the aliquot of infectious virus at the first dilution used in the test. The result of the test is the point at which the serum sample has been diluted such that it is no longer able to neutralise all the virus in the test. this dilution, or its log equivalent, is reported as the titre of the serum tested. Because the rabies virus causes little or no cytopathic effect, the cells are stained with anti rabies immunofluorescence conjugate and the test is then read by examining each well for the presence of viral infection in the cell monolayer. A $D_{50}$ endpoint titre (i.e. where 50% of the wells at that serum dilution show the presence of virus) is calculated for each test serum and the control sera and after titre calculation it is converted to a value in IU/ml by comparison to a standard serum of known titre.

**ELISA (Enzyme Linked Immune Sorbent Assay)**

Commercial kits are available for ELISA test that allow a qualitative detection of rabies antibodies in animal serum 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 animals have sero-converted.

**Bait uptake**

Tetracycline is recommended by the WHO as a marker of bait uptake and provides a life-long marking of bones and teeth that is easily detected on post-mortem. Tetracycline is considered as the best long term post-mortem tissue marker and is the most commonly used. Determination of tetracycline uptake provides an easy way of monitoring bait uptake and is especially useful when identifying other causes for vaccination failure.
Age determination

Age determination is a method used for differentiation between young and adult foxes introduced for efficacy control of ORV.

Virus typing

Indirect immune-fluorescent method

Because some of the oral rabies vaccines contain modified live virus (MLV vaccines) rabies-positive cases should be analysed in greater detail by virus typing to clarify further epidemiological issues. In rabies vaccination areas virus typing is done to exclude the occurrence of vaccine-induced rabies. Therefore, a more detailed testing of rabies-positive cases from vaccination areas is mandatory. This test is based on the detection of rabies inclusion in cytoplasm of infected tissues (Negri bodies) by means of the panel of specific murine monoclonal anti-rabies antibodies (Mab). Subsequently, binding of the Mabs is made visible by incubation with an FITC anti-mouse conjugate. RFLP method can also be used for discrimination procedure.

Provisional numbers of tests for control of vaccination efficiency for a five years period:

<table>
<thead>
<tr>
<th>Analysis Type</th>
<th>Number of samples</th>
<th>Technological time</th>
<th>Price per sample (EUR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Titration of rabies virus in baits</td>
<td>2 x 30 =60</td>
<td>8 hours/sample</td>
</tr>
<tr>
<td>2</td>
<td>FAT – direct immunofluorescent test for rabies virus detection</td>
<td>6000</td>
<td>3 hours/sample</td>
</tr>
<tr>
<td></td>
<td>Necropsy of foxes and taking the brain, law jaw(mandible) and blood</td>
<td>6000</td>
<td>1 hour/sample</td>
</tr>
<tr>
<td>3</td>
<td>FAVN test (fluorescent virus neutralisation test) or ELISA for antibody detection</td>
<td>2000</td>
<td>2 hours/sample</td>
</tr>
<tr>
<td>4</td>
<td>Age determination test</td>
<td>6000</td>
<td>20 minutes/sample</td>
</tr>
<tr>
<td>5</td>
<td>Biomarker (tetracycline) determination test</td>
<td>6000</td>
<td>20 minute/sample</td>
</tr>
<tr>
<td>6</td>
<td>RT-PCR test (only positive samples from FAT)</td>
<td>100</td>
<td>4 hours/sample</td>
</tr>
<tr>
<td>7</td>
<td>Genotyping by sequencing</td>
<td>100</td>
<td>4 hours/sample</td>
</tr>
</tbody>
</table>

16. DESIGNATION OF THE CENTRAL AUTHORITY CHARGED WITH SUPERVISING AND COORDINATING THE DEPARTMENTS RESPONSIBLE FOR IMPLEMENTING THE PROGRAMME

The implementation of the whole vaccination Programme on national level is to be steered by the National Veterinary Service of Serbia at the Ministry of Agriculture and in particular by its Animal Health and Welfare Department. All the campaigns are to be organised and performed in close cooperation with:
- Ministry of Health and its district units;
- Ministry of Interior and its district units;
- Ministry of Environment and ecology and its district units;
- National Forestry Administration at the MAFWM;
- Hunting associations and other wildlife and forestry authorities in Serbia and its district and local units;
- Local bodies of the executive authorities – district, municipality and settlement level;
- Veterinary organisations – public and private practitioners.

On administrative districts’ level vaccination campaigns are to be organised and steered by the District Veterinary Inspection Offices in their quality of district units within the organisation structure of the Veterinary Services in cooperation with the local units of all the other aforementioned central and local governments’ institutions.

Establishing public awareness of the Programme objectives and specifics:
- Making all central institutions and organisations involved in its implementation well informed about the Programme for oral vaccination of foxes in Serbia;
- Making all the regional (administrative district) units of the National Veterinary Service well informed and trained in the specifics of the Programme for oral vaccination of foxes in Serbia, in order to ensure that these will properly and effectively organise and steer it on the spot;
- Making all district and local units of the aforementioned central institutions and organisations involved in its implementation well informed about the Programme for oral vaccination of foxes in Serbia;
- Creating public awareness in the population through the local media for mass information, the local cable TV networks, radio broadcasting stations and direct meetings with the public;
- Preparing awareness brochures, posters and others alike that are to be placed on public places and alongside roads.

16.1 Administrative structures responsible for control and eradication of Rabies in RS

The Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management has responsibilities for the safety of food of animal origin, animal health, animal reproduction, animal feed, veterinary medicines, their residues and animal welfare. The Director of the Veterinary Directorate is the Chief Veterinary Officer who is appointed by the Government of the Republic of Serbia.

The Veterinary Directorate currently has 5 Departments (Animal Health and Welfare; Veterinary Public Health; International Trade and Co-operation; Veterinary Inspection and Veterinary Border Inspection), as well as 4 horizontal service units (Legal and General Affairs; Finance and Administration, Veterinary Services and Internal Audit).

25 District Veterinary Inspection Offices report to the Head of Department for Veterinary Inspection. District Veterinary Inspectors supervise the Local Veterinary Inspectors which are responsible for official controls of holdings and approved establishments as well as for the notification of contagious diseases. Each Local Veterinary Inspector covers one or more (up to three) municipalities.

Veterinary stations (staffed with a minimum of three veterinarians) and veterinary practices (208) which were privatized during the course of the past few years, may be authorised to carry out some official duties on behalf of the Veterinary Directorate, such are animal health monitoring, issuing of animal health certificates, identification of animals and support for the registration of holdings. Local Veterinary Inspectors are obliged to conduct regular checks of those veterinary stations and veterinary practices and report the findings to the Veterinary Directorate.
16.2 Stakeholders involved in the control and eradication of Rabies in Serbia

*Ministry of Health, Ministry of Environment, Hunting Associations, Faculty of Veterinary Medicine, Veterinary Institutes etc.*

The currently used laboratory premises in Belgrade Institute are in compliance with BSL-2 standards and need to be upgraded. There are no clearly detached clean and dirty zones and physical barriers between them. There are no changing rooms or shower facilities at the entrance. There are no decontamination facilities for heat or chemical treatment of the waste water or liquids. There are no facilities for decontamination of infected solid materials between the clean and dirty zone like double ended door autoclave or disinfection fumigation chamber. The existing autoclave is in the contaminated area. There is no incinerator. The Laboratory has a contract with the the rendering plant where the infected materials are destroyed.

The proposed facilities for upgrading to BSL-2, are in old building with solid constructions that could be adjusted for the purpose. The upgraded laboratories will include laboratory rooms, a necropsy room and a room for small laboratory animals.

17. LEGAL OBLIGATIONS

17.1. Regarding disease notification:

The Veterinary Legislation regarding prophylaxis and control of rabies in animals should include the following obligations:

1. Owners of dogs and cats, mayors of municipalities and town-councils, veterinary authorities and private veterinary practitioners shall have the following obligations:
   
   1. owners of dogs and cats shall:
      
      h) isolate the rabies suspect animals in closed premise and to immediately inform thereof the veterinary service of the settlement concerned;
      
      j) in case of death of a dog or cat, for which there has been suspicion that this death could be result of rabies, the owner concerned shall keep the carcass and immediately inform thereof the veterinary service of the settlement.
   
   3. official veterinary authorities and private practitioners shall:
      
      a) carry out vaccination against rabies by inactivated vaccine;
      
      c) issue veterinary health-books to the dogs presented by their owners, in which all prophylactic and diagnostic activities must be recorded;
      
      d) place identification marks or microchip
      
      e) carry out monitoring on dogs and cats considered as being rabies suspect animals;
      
      h) carry out, together with municipal authorities, veterinary, informational and explanatory activities to ensure compliance with veterinary-sanitary requirements to keeping dogs and cats.

2. Persons that have observed changes in the behaviour of wild animals, such as loss of orientation in environment, loss of sense of fear from human beings, entering in settlements or unusual aggressiveness shall kill the animal concerned, if possible and without entering in direct contact with it.

   - Persons referred to in Paragraph 1 shall immediately notify the nearest veterinary service regardless of whether they have managed to kill the animal or not.

3. Persons that have found carcass of dead wild animals shall immediately inform thereof the nearest veterinary service.
- The carcasses referred to in Paragraph 1 shall be buried together with their hides and skins after being sampled for laboratory testing.

17.2 Measures in case of a positive result:

1. In case of laboratory confirmation of rabies the Veterinary authorities shall undertake the following measures;

   1. Notify the disease;
   2. Together with the local bodies of Ministry of Health (Regional Inspectorate for Control and Protection of Public Health = RICPPH) perform epizootological and epidemiological inquiry;
   3. Order for killing of the sick animal(s) concerned;
   4. Take sample material for laboratory testing;
   5. Order for destruction /disposal/ together with their hides and skins of all carcasses of the animals killed or dead due to rabies, which must be done in rendering plant or by burial;
   6. Order for carrying out mandatory /compulsory/ vaccination against rabies of all dogs, cats and domestic animals going to pasture in the settlement affected or in part of it;
   7. Impose a ban on movement of animals referred to in Item 7 to other settlements;
   8. Together with the RICPPH inform through the mass media the public about the case(s) of rabies that have occurred.

2. The local body of the National Forestry Administration together with the local units of the Union of Hunters and Anglers in Serbia shall organise shooting of stray dogs and wild carnivorous animals found in areas around the settlement affected.

17.3 Measures regarding different animals and herds

Control procedures and in particular rules on the movement of animals liable to be affected or contaminated by a given disease and the regular inspection of the holdings or areas concerned:

1. In case of laboratory confirmation of rabies in animals, the Veterinary authorities shall undertake the following measures:

   - impose a ban on movements of rabies susceptible animals from the settlement affected to any other settlement;

2. These restrictive measures may be ceased at least 30 days after the last rabies case confirmed.

17.4 Measures regarding control (testing, vaccination) of the disease

1. Science-and-research and laboratory veterinary activity may be effected only in accredited institutes, high schools and laboratories.

2. Laboratory activity related to the official veterinary control shall be carried out in laboratories registered in the Veterinary Service in accordance with internationally recognised methods.

   (a) In case of necessity, the Chief Veterinary Officer may sign contract with accredited laboratory, which is not involved within the VS organisation structure, in order to ensure that activities referred to in Paragraph 1 would be properly effected.

3. The Minister of Agriculture and Forestry shall be responsible for the approval of the list of national reference laboratories included in the VS organisation structure in accordance with the proposal Chief Veterinary Officer

The strategy of monitoring (surveillance) involves:

1. The reception of vaccination baits by foxes by testing the presence of tetracycline in their bone marrow;
2. The presence of rabies virus antibodies in blood samples taken from vaccinated foxes.
19. TARGETS ON VACCINATION OR TREATMENT

The first vaccination round (in the Autumn of 2010) is intended to put the start of the implementation of the Program and to gain the necessary experience by the veterinary services on the spot and by the other institutions and organizations, which are to take part in its implementation. The Program is to be performed on the whole territory of Serbia with overall duration of no less than 5 years /10 vaccination campaigns/, in order to ensure eradication of rabies from the whole territory of the whole country.

20. REFERENCES


Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia


Annex 1. Map of registered rabies cases in foxes in Serbia

Source: WHO Rabies Bulletin

Source: per WHO Rabies Bulletin
Annex 3. Map of registered rabies cases in domestic animals in Serbia

*Source: WHO Rabies Bulletin*
Annex 4. The Delivery document (form) for fox carcases

THE ACCOMPANYING FORM TO SEND FOXES IN THE INVESTIGATION TO RABIES

Hunter's name and surname: ________________________________
Address: ________________________________ Tel: ____________
Species: ____________________________ Date of sending/finding: ________________
Age: more as one year □ less than one year □

Trapping / finding (to encircle)
1. Regular hunting
2. Fox has strange behaviour
3. Fox has been hit by car
4. Animal was in contact with dog
   bite YES NO
5. Animal was in contact with other domestic animal
   with which: ________________________________
   bite: YES NO
6. Animal was in contact with human
   bite: YES NO
7. Others: ________________________________

Location of trapping/finding:
Name of hunter organisation: ________________________________
Nearest village: ________________ name of community: ________________
Coordinate: X __________ Y __________

I delivered the carcase of fox together with form to Veterinary service: ________________________________
Date: ___________ hour: ________________ Hunter's signature: ________________

__________________________
COMPLETED BY THE VETERINARIAN

Name of ve. ambulance: ________________________________ Date: ________________
No. of protocol: ________________________________ Community: ________________________________
Assanosis: ________________________________
Stamp: ________________________________ Veterinary's signature: ________________________________