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HEALTH & CONSUMERS DIRECTORATE-GENERAL

Directorate G – Veterinary and International Affairs
Unit G5: Veterinary programmes

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REPORT ON THE
**TASK FORCE MEETING OF THE
RABIES SUBGROUP**

29 – 30 November 2012

Zagreb, Croatia

REPORT ON THE TASK FORCE MEETING OF THE RABIES SUBGROUP

OBJECTIVES	Assessment of the rabies situation in Croatia Recommendations for improvement and future actions
DATE OF MEETING	29 – 30 November 2012
LOCATION	Zagreb, Croatia
AGENDA	In Annex
PARTICIPANTS	
Subgroup members	Andrea Höflechner (AT) Franco Mutinelli (IT) Jedrt M. Wernig (SI) Spiros Doudounakis (GR) Thomas Müller (FLI, Federal Research Institute for Animal Health, Wusterhausen, DE) Florence Cliquet (ANSES, Nancy, FR)
Observers	Ivica Sučec, Veterinary Directorate Ivana Lohman Janković, Veterinary Directorate Tomislav Bedeković, Croatian Veterinary Institute
European Commission	Christophe Bertrand (DG SANCO, Head of Unit G5) Panayiotis Demetriou (DG SANCO, Unit G5)

Welcome speech was given by Ivana Lohman Janković, Veterinary Directorate of Croatia.

Introduction of Task Force, its objectives and composition of Rabies subgroup was given by the Commission.

Short information and update of rabies situation and eradication activities of participating MS

Italy: Since 1997 Italy had been free of rabies. ORV has been performed until 2004. In 2008, rabies was detected again in the northeast part of the country. The last case occurred in February 2011. Implementation of ORV: vaccination area of about 30.000 km²; in 2010 – 4 emergency vaccination campaigns were conducted, in 2011 and 2012 - regular ORV campaigns, spring and autumn were performed. In 2013, ORV will be reduced to a vaccination belt (30 km) along the border with Slovenia. However, Italy is prepared for emergency vaccination in case the epidemiological situation requires. Information about surveillance and post vaccination monitoring was provided.

Germany: Rabies free since 2008. Targeted surveillance programme is still in place.

Greece: Greece had been free since 1987. Due to detection of rabies in FYROM, Greece started with a reinforced surveillance programme in 2012. The programme was approved and co-financed by the EU. It consisted of laboratory testing of fox and other susceptible species brains, gathered from 15 higher-risk prefectures in northern part of Greece. In October 2012, the first case was detected in the

north-western part of the country. A fox with strange behavior was killed and sent for laboratory testing. Since, further cases are being detected in the area.

France: Rabies free since 2001. The last case was detected in 1998 though imported dog rabies cases have been detected sporadically.

Austria: The last case was reported in 2003. The country was declared as free of rabies in 2008. ORV has been performed along the border with Slovenia and Italy as the enlargement of the existing vaccination areas in those countries. In 2013 Austria will stop with the ORV activities.

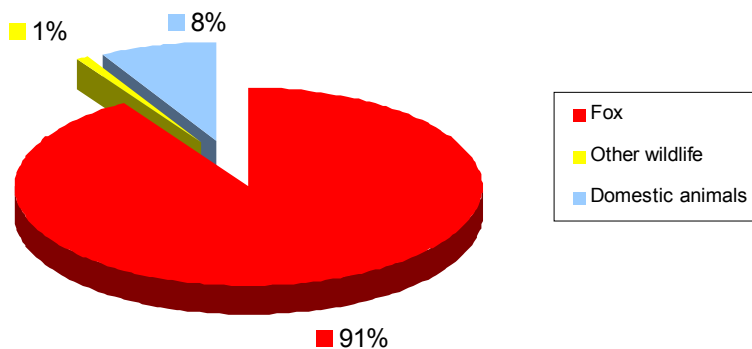
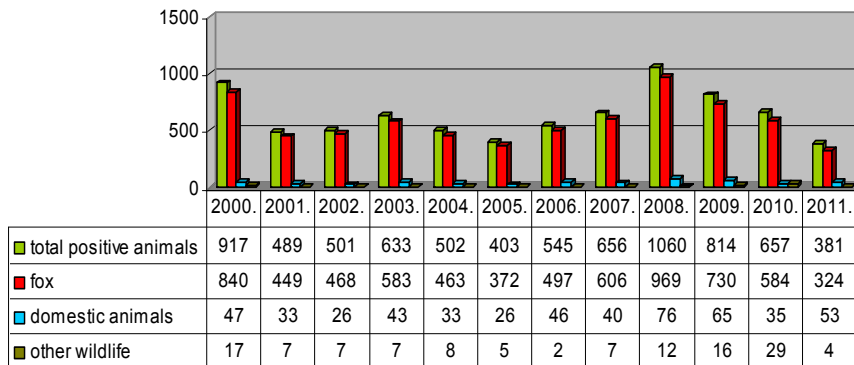
Slovenia: ORV has started in 1988 with manual distribution of baits in a limited area. In 1995, aircraft distribution of baits has started. The number of rabies cases significantly decreased to only few cases per year, all located in the bordering region with Croatia. In 2011, no cases were detected; in 2012 – 2 cases, both in foxes (one of which was vaccine-associated case). Two ORV campaigns have been performed per year. Spring campaign is usually performed in late May and beginning of June while autumn campaign is performed in October and November. In both campaigns a total of 920.000 baits are distributed in a density of 22 – 26 per km². Fuchsoral (SADB19) baits are used.

After this short introduction the hosting country presented situation regarding rabies and ORV activities.

History and epidemiological evolution of rabies in Croatia, present situation

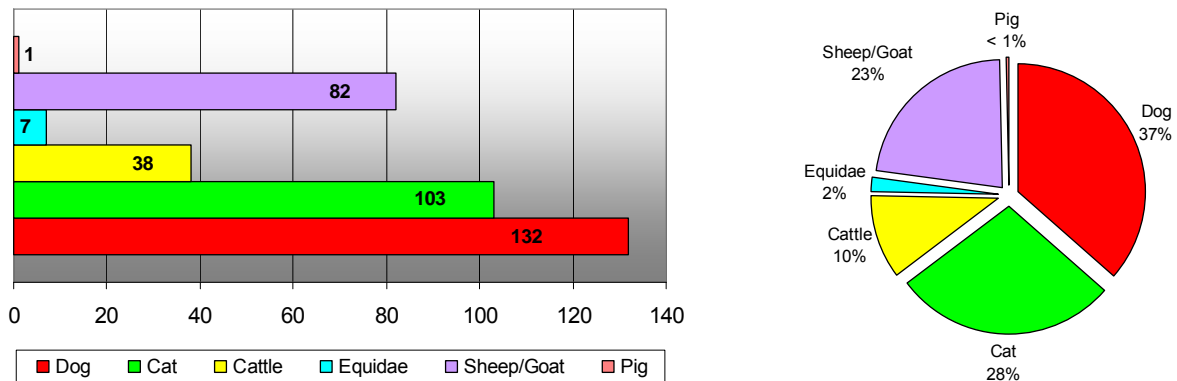
The last case of urban rabies was detected in Croatia in 1967. The first case of sylvatic rabies was detected in 1977 in border municipalities with Hungary and Serbia. Since 1985 rabies has been spread throughout the Croatia territory with the exemption of Adriatic Islands. The red fox represents the main rabies reservoir, since more than 91% of all cases are in red fox.

Picture 1, 2: Total No positive cases wild/domestic animals (time period 2000-2011)



Cases in domestic animals are mainly due to the contact with rabid wildlife. On average domestic rabies cases presents 4 – 8% of all positive cases. Rabies in domestic animals occurs mainly in dogs and cats and pasture animals.

Picture 3, 4: Total n. of rabies cases in domestic animals (time period 2005 – 2011)



Throughout the years the epidemiological situation remained almost unchanged. Annually, 450 – 550 rabies cases were detected. Since 2007, the number of cases has increased to 650 – 850 cases per year. Adriatic islands remained free of rabies.

A legal framework was established which enables implementation of all relevant measures to control rabies.

Monitoring and surveillance programmes for control and eradication of rabies have been systematically conducted on the entire territory of Croatia ever since 1977. Each year programmes are prescribed by the minister with the annual order.

All foxes shot or found dead, and all other wild or domestic animals suspected of being infected with rabies (showing clinical signs, abnormal behavior,...) or were in contact with a rabid animal must be sent for investigation to the laboratory.

Owners of grazing animals are obliged to prevent contact between wild and domestic animals.

In accordance with the annual order rabies vaccination is obligatory for all dogs older than three months. Vaccination of other animals is recommended but not obligatory.

If the epidemiological situation requires so the Minister of Agriculture may also order vaccination of other species of animals against rabies.

Only inactivated monovalent rabies vaccines are prescribed and approved for vaccination of animals.

One of the most important preventive measures for control of rabies and in management of free-roaming dog population is obligatory identification and registration of dogs. All dogs in Croatia have to be marked with microchip. Newborn dogs have to be marked within 90 days from the date of birth and registered in Lysacan-electronic database. Marking of dogs, entering data in Lysacan, vaccination and issuing of prescribed documentation (pet passports or certificates) is obligation of veterinary organizations.

In Lysacan compiles data on:

- Number of dogs vaccinated against rabies and marked
- Dangerous dogs
 - o Castration
- Date of checkout of dog
 - o Reason for the checkout (death, changed owner)
 - o ID of the old passport

- For each vaccinated dog
 - o ID number (microchip and mark) and “personal” data (age, sex, breed)
 - o Number of International health certificate
 - o Owners data (ID, address, phone)
 - o Vaccination (date, previous vaccinations, serial no of the vaccine used, name of vaccine (s) used)

Table 1: No. of newly marked dogs per year/% of marked dog population

Year	No. of marked dogs/% of marked dog population	
	No.	%
2004	1,615	0,58%
2005	21,880	6,89%
2006	51,629	16,07%
2007	84,445	25,51%
2008	118,337	35%
2009	144,646	42 %
2010	179,806	57%
2011	174,150	93%

Picture 5: Since 2009, authorized vets can check Lysacan via GSM



In 2012, the total number of marked dogs was 339.988 which is 98% of total number of vaccinated dogs in Croatia.

All dog's movement (internal or international) have to be accompanied by pet passport.

Free-roaming dog population – control strategy

A legal framework for implementation of all relevant measures in order to manage free-roaming dog population has been put in place. The aims of the free-roaming dog control program are to improve animal health and welfare of the population, to reduce the number of free-roaming dogs, to promote microchip identification and rabies vaccination of dogs, to decrease the risk of zoonotic diseases and prevent possible impact on the environment and illegal trade of dogs. In the programme roles and responsibilities of each stakeholder are clearly defined. With the combination of different control measures the aims of the programme could be achieved. Special provisions for shelters for abandoned animals are in place. Measures implemented in shelters are oriented towards minimizing the risk of rabies spread with animals released from shelters and animal welfare.

Organisation of oral vaccination campaigns in Croatia

First ORV campaigns in Croatia had been performed between 1991 and 1996, in counties in the Northern and Western part of the country, mainly in the bordering region with Slovenia. Altogether 11 campaigns were performed in that time.

Since 2011, Croatia has been using IPA funds for implementation of ORV campaigns. In the frame of IPA 2008, the first four ORV campaigns were performed - spring and autumn campaigns in 2011 and 2012, and under IPA 2010, ORV campaigns in 2013. After the accession of Croatia to the EU, ORV programmes will be submitted for co-financing in the frame of Council Decision 2009/470/EU.

Apart from the purchase and distribution of rabies vaccine baits, also the supply of laboratory equipment, surveillance activities and awareness campaign were funded under IPA.

Initially, vaccination was confined to the 16 counties in the Northern part of the country. The vaccination area represented 60% of the entire territory - 35.000 km² out of 56.542 km². The vaccination area was determined according to the recommendations given by the EU experts and was dependant on the available funds. Thus in 2011, 875.000 vaccine baits were distributed per campaign, with a density of 25 baits per km². Lysvulpen vaccine baits by BIOVETA, Czech Republic were used. Baits were distributed by fixed-winged airplanes in parallel lines with a distance of 500 m. Aerial distribution of the vaccine baits is done by using a fully automated system supported by computer and GPS which allows also recording of flight lines and positions (location) and amount of dropped baits.

The whole stock of vaccine baits for each campaign is stored in a refrigerated truck. The truck is moved from airport to the airport from which distribution is taking place. Trucks used for that purpose are equipped with thermograph which enables the control of temperature and of maintaining of cold chain.

Difficulties that were observed during implementation of first ORV campaigns were related mainly to the distribution data which had to be submitted to the CA by the contractor. However, data important for regular monitoring of distribution and implementation of corrective actions if necessary were not provided.

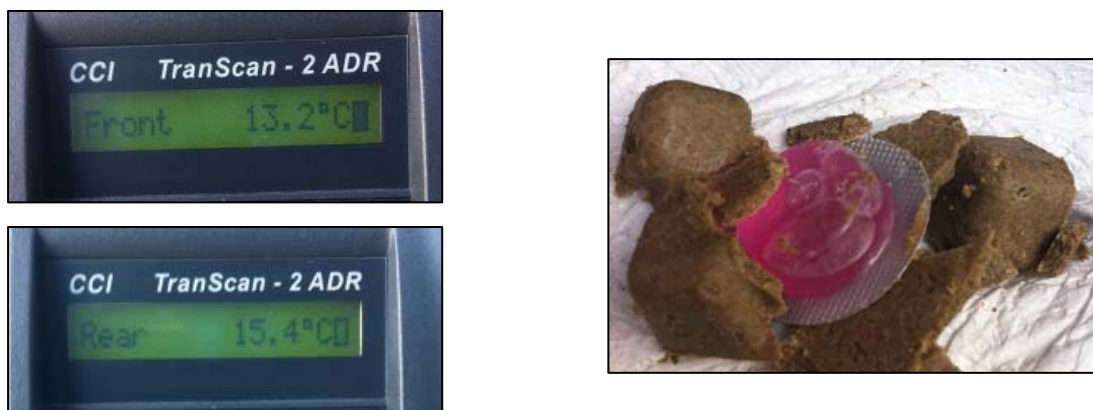
Picture 6: Vaccination area 2011 - 2012



It was foreseen for 2012 to expand the vaccination area to the entire territory. Due to the exceptional circumstances the spring 2012 vaccination area remained the same.

In the frame of the ORV monitoring the vaccine supplier has to provide independent evidence regarding the safety and efficacy of vaccine batches supplied. Additionally, Croatian national veterinary institute (HVI) performs bait titrations and stability and quality tests on each vaccine batch to be used in campaigns. Results of tests performed by HVI before first vaccination campaign in southern part of Croatia (5 counties) that was planned for spring 2012 showed insufficient virus titer in two batches of vaccine baits. Both batches were retrieved to the manufacturer and vaccination has been postponed for the autumn. This proved that the system in place is reliable and is functioning. Inappropriate storage regime and interrupted cold chain were probably one of the reasons for poor quality of vaccine baits.

Pictures 7, 8 and 9: Inappropriate storage regime, poor quality of vaccine baits



For the 2012 autumn campaign, the vaccination area was extended and included the whole mainland of Croatia. The number of baits has increased from 875.000 in previous campaigns to 1.413.550 in autumn campaign. The baiting density remained at 25 baits per km².

Picture 10: Vaccination area autumn 2012



Effectiveness monitoring of OV in Croatia, organisation, implementation and results

To monitor the effectiveness of vaccination campaigns hunters are obliged to submit the whole fox carcasses for the laboratory investigation. There are approximately 1.000 hunting grounds in Croatia and approximately 50.000 hunters. According to the vaccination area the target was set to collect 1.750 samples per campaign, which corresponds to the recommendation of scientific committee on AHAW of testing 8 foxes per 100 km² for monitoring the efficiency of ORV campaigns. A sample size is set for each hunting ground. Each hunting association receives instructions and sampling forms which, duly completed by hunters, have to accompany fox carcasses to the authorised veterinary organisations. The forms are equipped with serial numbers.

Picture 11: Sampling form and laboratory protocol

Uzorakovanje odstreljenih lisica u okviru kontrolisanih uslova orasne vakcinacije lisica protiv bjesnoće

MINISTARSTVO POLJOPRIVREDE, RIBARSTVA I RURALNOG RAZVOJA
Uprava za veterinarstvo

OBRAZAC ZA DOSTAVU LISICA NA LABORATORIJSKO PRETRAŽIVANJE

Serijski broj obrascu: **INS 00040**

PODACI O UZORKOVANJU

Ime: **J. J. 1118 Sveta Janka**

Naziv kmetstva: **1118 Sveta Janka**

Županija: **Zagrebačka**

PODACI O UZORKU

Lokacija uzorkovanja (preko kojega u kojem je odstreljena lisica): **1118 Sveta Janka**

Udaljenost mjesta odstrela lisice od naselja (označiti): Mlada od godinu dana Starija od godinu dana

300-500 metara od naselja 500-1000 metara od naselja više od 1000 metara od naselja

Datum odstrela: **01.08.2011**

Datum dostave lisice u ovlaštenu veterinarsku organizaciju: **01.08.2011**

PODACI O POSILJATELJU LISICE U LABORATORIJU (ispunjava ovlaštena veterinarska organizacija)

Naziv ovlaštene veterinarske organizacije: **VETERINARSKA STANICA JASTREBRSKO**

Adresa: **10450 JASTREBRSKO MB. 3118788**

Županija: **Zagrebačka**

Datum slanja lisice u laboratorij: **02.08.2011**

Uzorak poslan u Hrvatski veterinarski institut (označiti): Zavod Križevci Zavod Rijeka Zavod Vinkovci

HVI - Zagreb

Naknada za odstreljenu lisicu biti će isplaćena samo u slučaju kada je na pretragu dostavljena kešina naznačenog zdrave lisice.

Ugovore o odstreljivosti lisice koje su pokazivale simptome bolesti dostavljaju se uz Obrazac propisan Naredbom o mjerama zaštite životinja od zaraznih i nametničkih bolesti i njihovom financiranju u 2011. godini.

Kopija za Upravu za veterinarstvo

HRVATSKI VETERINARSKI INSTITUT ZAGREB

17025-HAA
10000 Zagreb Savska cesta 143
Telefon: 01/ 6123 666 Telefaks: 01/ 6190 841
ured@vinst.hr

Zagreb, 17.10.2011

Jedinstveni broj uzorka: 41806/2011

Kupac: Veterinarska stanica Jastrebarsko d.o.o., Trešnjevka 61, 10450 Jastrebarsko
Vlasnik: nije navedeno
Proizvođač: **1118 Sveta Janka**

Posrednik: **1118 Sveta Janka**

Vez. podaci iz popratne dokumentacije: **OVL 00040 od 01.08.2011.**

Datum i vrijeme prijema uzorka: 09.08.2011. Temperatura uzorka na prijemu: nije mjereno

Podaci o uzorkovanju: Datum i vrijeme uzorkovanja: 01.08.2011. Temperatura uzorka na uzorkovanju: nije nave.
Metoda: nije navedeno Mjesto: nije navedeno
Organizacija: nije navedeno Osoba: nije navedeno

Izvešće o rezultatima pretraživanja: Z-41806/2011

Laboratorijski broj uzorka: Z-IV-1-1352/2011 Datum i sat završetka pretraživanja: 10.08.2011 13:02
Datum i sat početka pretraživanja: 10.08.2011 09:56

Odjel za patološku morfologiju
LABORATORIJ ZA OPĆU PATOLOGIJU

REZULTATI PRETRAŽIVANJA

Uzorak: LEŠINA LISICE	Količina: 1		
Vrsta pretraživanja: Patološka OVL			
Parametar pretraživanja	Oznaka metode	Količina pretraga	Količina pozitivnih
Kontrola OVL	SOP Z-IV-1-46	1	

* označava metodu koja je akreditirana

Svi podaci o korištenim metodama pretraživanja mogu se dobiti u laboratoriju

Nalaz: Uzorkovanje mišćanog tkiva, mišćnog tkiva i zuba lisice radi pretrage na bjesnoću i kontrole orasne vakcinacije lisica provedeno je u Laboratoriju za opću patologiju.

odjel laboratorija: dr. sc. Zoran Lipič

Predstojnik odjela: dr. sc. Dražen Oraić

GP 29 obrazac 01 - Revizija 04 - Datum: 18.11.2009. Izvešće o rezultatima pretraživanja: Z-41806/2011 Stranica 1 od 2
Nije dopušteno pretraživanje ovog uzorka na bilo koji način, bez prethodnog odobrenja laboratorija. Ispisano predviđenoj stranicama koje nisu stvarno pretraženo, kao ni korištenje oznaka i znakova Hrvatskog veterinarskog instituta i njegovih podružnica u promatranju surta.

For each submitted sample hunters receive the incentive of 15 €. The number of samples collected and tested and results are shown in table 2. Laboratory results are linked to the submission forms and samples and thus provide for the traceability of positive cases.

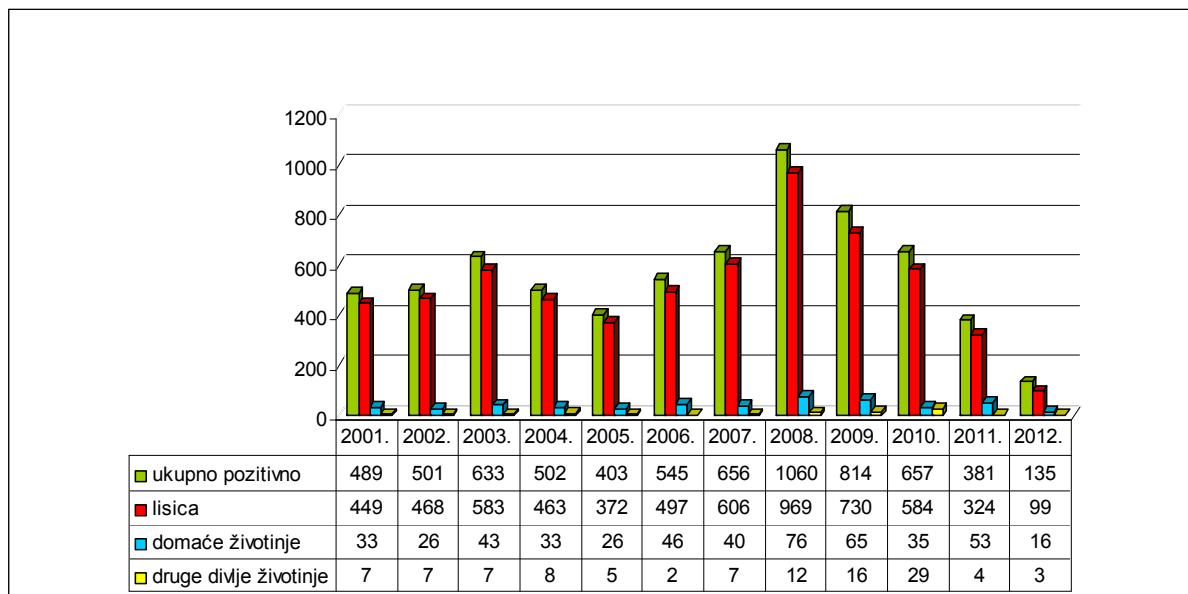
Table 2: Results of ORV monitoring

Test performed	ORV CAMPAIGNS					
	1st - Spring 2011	%	2nd - Autumn 2011	%	3rd - Spring 2012*	%
Planned No. of samples	1270		1678		1270	
No. of collected samples	740	58%	938	56%	425	33%
FAT/tested	740		938		422	
FAT/positive	22	3%	7	0,7%	2	0,5%
ELISA/tested	ELISA Biorad 682		ELISA BioPro 883		ELISA BioPro 402	
ELISA/positive	24	3,5%	418	47%	62	15,5%
IIP/tested	22		7		2	
IIP/positive	22		7		1	
OTC/tested	740		932		424	
OTC/positive	184	25%	684	73%	96	23%
Age determination	740		932		424	
< 1 year	438	59%	314	34%	253	60%
RT-PCR/tested	22		7		2	
RT-PCR/positive	22		7		2	
RFFIT/tested	660		835		374	
RFFIT/positive	115	17%	271	32%	62	17%
VI/tested	22		7		2	
VI/positive	22		7		2	

*Until December 31, 2012

The first results of ORV monitoring showed that bait uptake was approximately 52 % for 2011 and around 20% after the spring campaign in 2012, while the results of seroconversion were approximately 20%. Rather low bait up-take and seroconversion rates could be associated with very high proportion of young foxes tested. The number of positive cases in 2012 decreased significantly.

Table 3: Occurrence of rabies (2001 – 2012)



Croatian Veterinary Institute, its tasks, rabies related investigations in NRL

Croatian Veterinary Institute in Zagreb is appointed as NRL for rabies. Routine diagnostics for rabies surveillance (FAT) is performed in Zagreb and in four regional units of HVI. FAT is also ISO/IEC 17025:0007 accredited method. Regional units are also responsible for collection of monitoring samples and submission of those samples to the NRL in Zagreb where other rabies related investigations are performed.

In the NRL three veterinarians, one molecular biologist and two technicians are employed. During 2008 – 2010, laboratory was newly equipped and in 2012 microbiology safety cabinet, incubator and microscope were purchased from IPA funds.

NRL performs FAT for rabies surveillance, FAVN test, and a number of tests for monitoring of ORV: FAT, neutralisation test, RT-PCR, virus isolation, indirect immunoperoxidase test, ELISA, age and OTC determination, baits titration.

HVI has developed a new test for detection of specific antibodies from the muscle juice of the foxes in order to improve diagnostic techniques. With the new test cytotoxicity on the cells can be easily prevented. Laboratory staff regularly participates in trainings organised by EURL for rabies

Laboratory staff regularly participates in trainings organised by EU and OIE Reference laboratories for rabies.

In addition presentations of rabies experts of EURL for Rabies and WHO Collaborating Centre for Rabies were given:

- Dr. Florence Cliquet, ANSES Nancy, France: [Veterinary Authority quality controls on the delivery and handling of vaccines and on the implementation of oral vaccine distribution](#)
- Dr. Thomas Müller, FLI Insel Riems, Germany: [Surveillance for rabies – Best practices and challenges](#)

CONCLUSIONS AND RECOMMENDATIONS OF THE TASK FORCE

Conclusions

1. Croatia made an important step towards rabies elimination by implementing ORV programme in 2011 funded under the EU Instrument for Pre-accession Assistance. Since then, four ORV campaigns have been carried out, the last one covering the entire territory of Croatia.
2. Funding has been ensured through IPA also for 2013 and from 2014 onwards it will be passed to the EU Veterinary Fund.
3. A notable decrease in rabies incidence has been observed thanks to the ORV, which resulted also in an improvement of the epidemiological situation in neighbouring member states.
4. Croatian authorities have established solid legal framework for the control of rabies following international guidelines, and collaboration between central/regional authorities and stakeholders.
5. Good collaboration has been established with the neighbouring countries on the coordination and exchange of information on ORV activities and epidemiological situation.
6. An adequate system of rabies surveillance and ORV monitoring has been put in place. In addition, quality controls on the delivery and handling of vaccine baits have been implemented and enabled for detection of deficiencies.
7. A data base supported system of identification, registration and rabies vaccination of dogs has been successfully applied in recent years.
8. Free-roaming dog management is based on the national legal framework which follows international guidelines (OIE).
9. The national veterinary institute (NRL for rabies) in Zagreb has appropriate facilities, trained staff, is adequately equipped and is working in close cooperation with CA and regional labs.
10. The NRL participates in international proficiency testing and inter-laboratory comparison tests as organized by the EU-RL for rabies. Furthermore, the NRL has established close collaboration with WHO CCs and OIE-RLs for rabies as well with NRLs of neighbouring countries.
11. The NRL conducts research on diagnostic aspects including optimization of serological techniques in the frame of rabies surveillance and ORV monitoring and publishes results in peer-reviewed international scientific journals.

Recommendations

1. The waiting period for the release of free-roaming dogs of unknown health status from shelters, following vaccination for rabies and subsequent serological testing should be established on a national level based on a risk assessment taking into account occasionally long incubation period for rabies.
2. Priority should be given to testing of adult foxes in the frame of monitoring ORV campaigns.
3. Stratified epidemiological analysis of ORV monitoring data is strongly encouraged.
4. Bait distribution data (geo-coordinates of distributed baits) should be provided on a daily basis by the contractor responsible for distribution in order to allow real-time evaluation

of vaccination coverage during each campaign, so that corrective actions can be taken in due time, if necessary.

5. Frequent exchange of information, coordination of program and appropriate communication should be continued with the neighbouring countries.
6. Immediate notification of rejected vaccine batches should be sent to the EC.