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

Tartu, Estonia  
8-9 November 2010

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OBJECTIVE	To improve animal disease eradication and the cost-benefit ratio of animal disease eradication by producing conclusions, recommendations and specific action proposals
DATE OF MEETING	8-9 November, 2010
VENUE	Tartu, Estonia
AGENDA	In annex
PARTICIPANTS	
Subgroup Members:	Governmental experts from EU Member States: Enel Niin (EE), Miia Kristiina Jakava-Viljanen (FI), Martins Serzants (LV), Vaidotas Kiudulas, (LT), Marczin Smreczak (PL), Dalibor Polak (SK)  Private experts: Florence Cliquet (ANSES, Nancy, France), Thomas Müller (Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Wusterhausen, Germany)
Observers:	Artem Metlin (Russian Federal Centre for Animal Health), Vladimir Kadochnikov Veterinary Service of the Kaliningrad region of the Russian Federation, Mykhaylo Babkin (Ukrainian State Science-Control Institute of Biotechnology and Strains of Microorganisms), Sergii Degtiarenko (Department of Veterinary Medicine of Zaporizka region, Ukraine)
European Commission- Health and Consumers DG (DG SANCO)	James Moynagh, Head of Unit SANCO 04, Panayiotis Demetriou, veterinary administrator.

The welcoming address of the two-day meeting was conducted by Dr. Ago Pärtel, Director General of Veterinary and Food Board of Estonia. Information on the up to date rabies situation of participating countries followed:

**Finland:** The country was declared free of rabies in 1991. The last outbreak of rabies occurred 1988-1989. Since then rabies has been diagnosed in 2 imported animals, one human (infected abroad) and one bat. In south and in south-east Finland blood serum and saliva samples are collected from bats are tested for virus in the framework of a research project. Passive surveillance among domestic and wild animals (~100 samples annually) and active surveillance in the population of foxes and racoon dogs (~500 samples annually) is carried out on yearly bases. Oral vaccination (OV) is regularly implemented in a 5 000 km<sup>2</sup> (4 000 km<sup>2</sup> without lakes) area along the Finnish-Russian south-east border. To evaluate OV efficiency tests for the determination of biomarker presence and level of herd immunity of the target population are carried out in vaccination area.

**Latvia:** In 2010 the whole territory of the country was vaccinated twice with the Fuchsoral vaccine. Since autumn campaign of this year the distance between flight lines was reduced from 1000 m to 500 m , and the number of vaccine baits distributed per km<sup>2</sup> was increased up to 26,5 baits/km<sup>2</sup> in the areas of the country where vaccination was interrupted due to tendering problems. Until November 2010, 16 cases of rabies have been diagnosed, 11 of them in foxes. Both bait uptake and seroconversion rate in target animals are showing ascending curve at the initial results of 2010 OV compared to 2009 spring OV campaign results. The general flight line interval intended to be used in year 2011 is 500 m.

**Lithuania:** Large-scale OV program against rabies has been launched since 2006. The number of rabies cases has shown decreasing trend, being 428 in 2007, 69 in 2008 and 63 in year 2009. Within 10 months in 2010 in total 25 rabies cases have been confirmed. Majority of cases diagnosed in last two years occurred in the bordering area with Belorussia, some cases occasionally occurred in other parts of country. Since the introduction of vaccination campaigns in Kaliningrad no cases in areas bordering its territory have been detected. Results of 2010 OV campaign evaluation show presence of biomarker in ~75% and rabies virus neutralising antibodies in 54% of tested animals. For year 2011 in addition to vaccination in whole country, it is planed to conceive 50 km deep immunological barrier along the Lithuanian –Belorussian border in Belorussian surface.

**Poland:** OV started in confined areas in 1993. Vaccination in the total territory of the country is conducted since 2002. Mainly aerial, in limited areas also manual, vaccination is carried out with two vaccines: Lysvulpen and Fuchsoral. As a result of 2009 OV campaign, presence of biomarker in 87% and seroconversion in 80% of the target species was observed. Rabies –positive cases are typed for rabies virus isolates; all isolated viruses confirmed to be fields strains. Sharp reduction of rabies occurrence has been observed in recent years. In 2009 only 8 cases (6 in terrestrial animals, 2 in bats) were diagnosed. Most cases have occurred along the borders with Belarus and Ukraine. The situation in areas bordering Kaliningrad has significantly improved over the recent years.

In August 2010 a re-emergence of rabies cases occurred in Malopolskie region, bbma district after 7 years freedom of area from rabies. By the end of October 15 rabies cases had been detected. The virus strain responsible is distant to other viruses detected in Poland however the way it was introduced to the area remains unknown for time being. Susceptible domestic animals in the area were vaccinated and an additional OV is planned in the following weeks.

**Slovakia:** The last case of rabies diagnosed in 2006. OV is still performed in most of the territory. In 2010 OV was conducted in ~80% of the surface of Slovakia, in 2011 the vaccination area will be around 30% of total territory of country. In OV area 8 foxes are sampled/100 km<sup>2</sup>, in area without OV, 5 animals are tested per 100km<sup>2</sup>.

**Russia:** Rabies is endemic throughout the Russian territory. Cases are diagnosed also among human population; 8 human deaths have occurred until now in 2010. The number of recorded cases in wildlife and domestic animals has been increasing in 2010 compared to 2009. Disease incidence is higher in the western part of the country and in Moscow region. Vaccination for humans, cats and dogs is available free of charge. EU financed OV programs are running in the Finnish-Russian border and Kaliningrad region.

**Ukraine:** OV programme is implemented via aerial distribution of vaccine baits twice a year since 2007. Due to limited resources, only the areas most affected are vaccinated. A reduction in rabies cases has been observed in 2009. The majority of cases are diagnosed in domestic animals. Vaccine baits used are produced locally, liquid (recombinant vaccine VRG) vaccine is provided by company Merial. In next year it is foreseen to conduct OV in western part of the country.

**Kaliningrad region of the Russian Federation:** OV in the entire territory of Kaliningrad has been carried out once a year in period 2007-2009. Vaccination twice a year, in spring and autumn, is implemented since 2010.. In addition, starting from 2010 the number of samples tested for evaluating the efficiency of vaccination was tripled in accordance with the terms of the grant agreement with the Commission. This has led to a slight increase in the detected rabies cases in 2010. Subsequent years of reinforced sampling are expected to demonstrate the improvement of the epidemiological situation. Population density of foxes and racoon dogs in Kaliningrad is growing (~ 0.36 per km<sup>2</sup>).

**Information from DG Sanco:** Rabies OV programs are running in 11 Member States. All, with exception of 2, of them are bordering with Third Countries. Approved programs are co-financed from Veterinary Fund resources; reimbursement typically covers 50% of costs for carrying out laboratory tests, also for purchasing and distribution of vaccine-baits. In accordance with Commission Decision 2010/732/EU, from present year, the abovementioned costs are reimbursed at the range of 75%. An EU financed plan is running in Kaliningrad since 2007 and a request has been introduced to extend financing after year 2011. Multiannual cooperation exists also between Russia and Finland. Transboundary co-operation on rabies control between the EU and neighbouring countries (Russia, Belarus and Ukraine) should be expanded. Funding for the creation of vaccination belts in the territories of these countries could take place through the co-financed programmes of the neighbouring Member States. 100% costs of vaccine

purchase and vaccine distribution could be covered. For this purpose, agreements should be signed between the Member State(s) and the Third Country. For the current time Slovakia has included the adjacent Ukrainian area into its programme, similar action has been taken by Lithuania concerning contiguous Belarus territories. The Commission adopted a decision approving the financing of rabies vaccination activities outside the territory of these two Member States and is ready to approve similar requests from other Member States bordering Third Countries for 2011 or subsequent years.

On day 1 four presentations were made by the host concerning rabies in Estonia:

### 1. Epidemiological evolution of rabies in Estonia, present situation

Rabies has been a serious threat to human and animal health in Estonian territory for centuries. In the 18- 19 century, the main source of rabies in Estonian areas were stray dogs. Arising from compulsory vaccination of pets from 1953, also extermination of stray animals, urban rabies was eradicated. From 1960 to 1967 no case of disease was reported. Sylvatic rabies invaded Estonia from year 1968 and spread rapidly all over the country including islands. Main reservoirs of the disease were red foxes and racoon dogs. The average number of positive cases has varied usually from 150 – 300 per year.

Distribution of rabies cases in years 1968-2009 can be followed by chart 1.

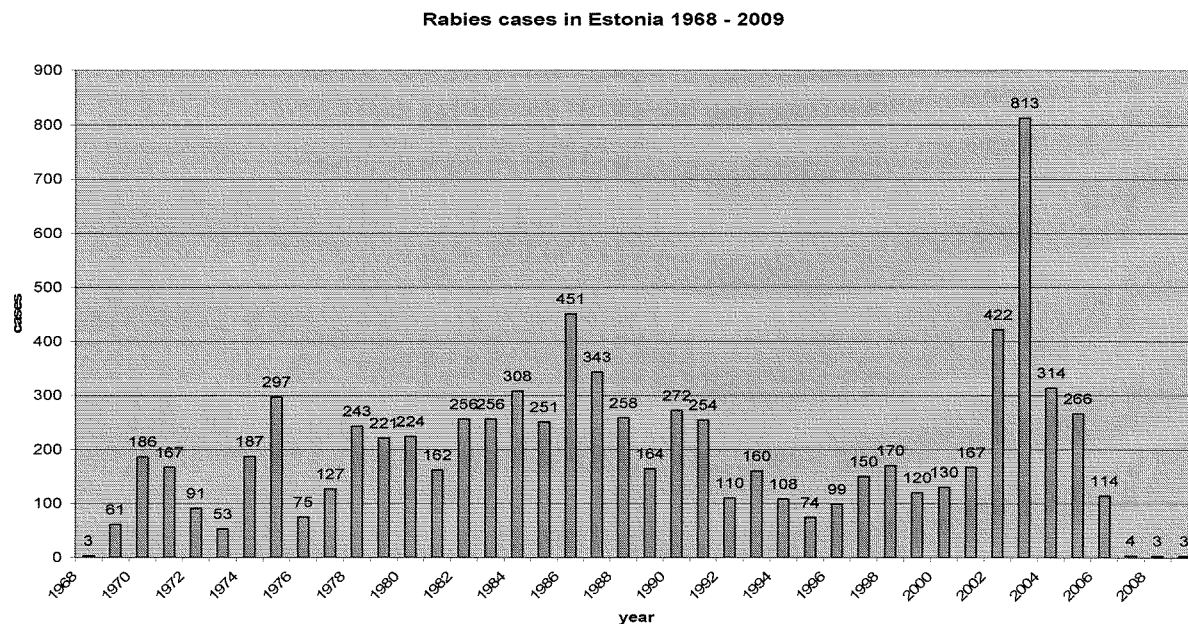


Chart 1 Rabies cases from 1968 to 2009

The structure of rabies infections across species has been relatively stable over these years. During 1968–2009 farm animals accounted for 6-7%, dogs and cats for 9–23% and wild animals for 71–84% of all the cases of illness. In years 1968-2001 red foxes have composed majority of rabies cases, but year-by-year number of racoon dogs infected with rabies has aggravated, composing around 50% of all rabies infections from year 2002 until 2006. Number of rabies infected animals has been highest in 2003- 813 cases of with 315 cases in foxes and 362 cases in racoon dogs. Since year 2006, a sudden decrease of rabies cases could be observed, due to start of OV campaigns in total territory of country. In 2007 four positive cases of rabies infection were diagnosed, in 2008 in winter-spring period three rabies-positive animals were found. From March 2008 until now, the only rabies cases occurred have been three rabid foxes found in summer 2009 in very close surrounding (less, than 5 km) of Estonian –Russian Federation land border in south-east.

From 1947 to 1955, one to eight persons died of rabies every year. Also rabies caused death of humans at a rate of one case per year, in years 1984-1986. Last case of rabies in humans was registered in 1986. Costs of post-exposure treatment and in some occasion's also prophylactic vaccination of humans are covered by State Budget.

Legal bases of rabies prevention are laid down by Regulation of Minister of Agriculture No 67 and State Program of Monitoring and Surveillance of Infectious Diseases approved annually by director general of Veterinary and Food Board.

In Estonia vaccination of dogs and cats against rabies is compulsory since 1953 for the time being. The State Budget covers the cost of the vaccine and vaccination procedure. Vaccination of farm animals in pasturelands is recommended. Parenteral vaccination procedures can be conducted by licensed veterinarians, sampling and other necessary procedures in case of rabies suspicion are carried out by authorized veterinarian or veterinary officials. According to existing data 107 000- 138 000 animals, commonly dogs and cats, have been vaccinated annually between 2002 and 2009. For decades as a rule, animals were revaccinated once a year, but due to improvement of rabies situation in recent years, since 20.07.09, it is allowed to make booster vaccination in accordance with instructions described in product information sheet of vaccine used, but interval between vaccinations can not be longer, than 24 months have passed from last vaccination.

Number of animals tested for virus in consequence of rabies suspicion has varied between 231-1400 in years 2000-2009.

## **2. Management of stray animals in Estonia**

Animal Protection Act and the Regulation of Government of Estonia No. 130 (04/16/2002) "Procedure for the capture and keeping of Stray Animals, for the identification of on their Owners, and for the Killing of Stray Animals." give the legal basis for management of vagabond animals in Estonia.

Estonia's territory is divided into counties (15), rural municipalities (226 local government units: 33 urban and 193 rural municipalities) and cities. Local governments shall organise the capture, keeping and euthanasia of stray animals, also destruction of animal carcasses within their territories.

In Estonia there are 10 shelters for stray animals. Animals placed in shelters will immediately be inspected by veterinarian. The animals of different sexes and various types, as well as the sick or injured animals in shelters are kept separate. The interval from starting the verification the owner of the animal until execution must be at least two weeks. Dogs and cats are vaccinated against rabies and micro chipped before granting to new owner, who should bear costs of abovementioned procedures.

### **3. Organisation of oral vaccination campaigns in Estonia**

Wild animals were not vaccinated against rabies in Estonia until year 2005, with exception of the vaccination trial in Vormsi island (92 km<sup>2</sup>), when small-scale OV was carried out via manual distribution of baits.

In autumn 2005, the first large scale oral vaccination campaign of wildlife was carried out in the Northern part of the country (25 540 km<sup>2</sup>) from the west to the Eastern border, including islands. A continuous line using the roads, coast of the lake Peipsi and the river Narva were used as the borders of the vaccination area. Around half of million baits were distributed in the vaccination area by three planes, as a general rule 20 baits per square kilometre.

Estonian territory is 45 226 km<sup>2</sup>, if to exclude water-lands and cities ~43 000 km<sup>2</sup> surface is suitable for bait dropping. The country has common borders with Russia in the east (343 km) and Latvia in the south (339 km). The country is flanked by the Baltic Sea in the West and Gulf of Finland in the North.

From the year 2006 until 2010 OV program co-financed by the EU and also from the Estonian state budget comprising total territory of the Republic has been enforced. Main principles of the OV have been similar at the frames of all campaigns. Vaccine used in the frames of all campaigns carried out is Rabigen SAG2, a single-strain live modified rabies virus vaccine. 20 baits per sq km are dropped in the frames of one campaign. OV has been carried out twice a year, in spring (May, early June) and autumn (September, October). Distribution of baits is exclusively carried out using small fixed-wing aircrafts type Cessna-127, dropping performed at regular time by skilled staff manually through the constructed special tube inside the plane. Territory is divided into vaccination squares, which average area is 240 sq km. Interval between dropping lines is 600 m, altitude of plane form ground 100- 150 m, average flight speed 160 - 180 km/h. Distribution of vaccine baits is stopped while flying above the buildings, in area of water (lakes, rivers, deep swamps etc), in area of roads, active domestic animals pastures. Navigation tool used for navigation is GPS Garmin 196, which also allows recording of flight track and make offprint afterwards. Refrigerated lorry stays at the airport for whole campaign to assure proper maintenance of cold-change of baits. PR campaign is

contrived before and at the time of OV campaign to inform publicity about vaccination activities.

Strict control of vaccination procedures is settled. Representative of VFB presides over vaccination for whole campaign long. Special letter of guidance is laid down for staff to carry out vaccine-dropping. The aerial bait distribution is checked by bait distribution records (in electronic and paper format). GPS system is used for recording of flight tracks (journey, co-ordinates, periodicity of flight routes, speed, and altitude from ground). Database of flights contains "vaccination book" where date, planes sign, weather conditions, temperature, airfield(s) in use, number of area, surface in km<sup>2</sup>, number of vaccines, batch number, name of pilot and technician, start and end of flight, start and end of vaccination, flight time, vaccination time and remarks if applicable are laid down. Intense superintendence is ongoing over storage and transportation of vaccine baits, including physical checks and storage-room temperature outprints in every 4 hours. Prior the campaigns from every vaccine batch in use 10 baits are sent to Community Reference Laboratory to ascertain existence of proper vaccine titre. Before start of OV, results proving sufficient quality of baits should be available.

#### **4. Control of OV campaigns in Estonia, plans for the future**

For controlling the efficacy of OV of foxes and racoon dogs against rabies three main methods are used: investigation of all rabies-suspected cases to verify virus prevalence, detection of tetracycline marker by testing the teeth of target population, titration of rabies antibodies to identify seroconversion rate.

All rabies-suspected cases should be notified to veterinarians and relevant samples collected and submitted to the Veterinary and Food Laboratory (VFL). Surveillance of rabies-suspected cases in year 2006 has demonstrated, that from all rabies cases diagnosed in Estonian territory until end of May, 10,3% occurred in area vaccinated in autumn 2005. In II semester 2006 (primary OV in total territory of Estonia took place in April-May 2006), 12 rabies cases were diagnosed. Number of rabies-suspected animals ranked between 373-231 in years 2006-2009, number of positive cases has been 4 in 2007 and 3 in 2008. In summer 2009 3 virus-positive foxes were discovered in short-range of Estonian –Russian border. Starting from March 2008 until nowadays, these have been the only infected animals found. Additionally in 2008 3461 brain samples from target animals collected by hunters in the frames of OV efficiency control have been tested for rabies virus with negative result. In 2009 1/2 of all foxes and racoon dogs brain samples collected (1756 in total), have also undergone virus testing with negative result. 177 of them were indicator animals (animals found dead and road kills.).

To control bait-uptake, jay samples from approximately 4 foxes and 4 racoon dogs per 100 km<sup>2</sup> of vaccinated area are collected by Estonian Hunters Association. Number of serum-samples collected for confirmation of immunisation level is at least from 4 animals/100km<sup>2</sup>. Samples are uniformly divided between 15 counties. Collection of material for these investigations starts in July OV year and lasts until March of next year.



Identification of age-class of animals investigated is realized. Results of the bait-uptake level investigations in years 2005-2009 are summarized in Table 1.

Year	% of positives by species		% of positive in average
	Fox	Raccoon dog	
2005	74	73	73
2006	87	84	85
2007	85	83	84
2008	93	88	90
2009	91	86	88

*Table 1.* Percentage of tetracycline positive animals in years 2005-2009

Humoral response is evaluated by using Bio-Rad ELISA test, result is reported as positive if antibody level higher than 0,5 EU/ml is detected. On average, the seroconversion rate found to be similar among foxes and racoon dogs. Information about serum samples investigation results in last four OV periods are summarized in Table 2.

Year	% of positives by species		% of positive in average
	Fox	Raccoon dog	
2006	55	55	55
2007	34	38	36
2008	51	54	53
2009	48	47	48

*Table 2.* Result of the investigations of seroconversion rate in years 2006-2009

All positive rabies cases occurred in OV area since the beginning of eradication program have undergone in EU reference laboratory investigations to detect virus genotype.

Results obtained proved, that no vaccine-induced rabies case has occurred. All positive animals were infected with classical rabies virus (genotype 1), wild rabies strains present in Estonia.

In the phone of OV data (hunting bag, winter track index, index in abundance, age structure of hunted animals; changes in abovementioned indicators) concerning population dynamics of foxes and racoon dogs show increase of both populations.

Taking into account favourable rabies situation achieved and potential threat of re-infection from rabies-infected areas, it is decided in year 2011 to establish buffer-zones (~9400 km<sup>2</sup>) with neighbouring countries wherewith Estonia is bordering with land (Russia, Latvia) and conduct OV twice a year only in these buffer areas to maintain sufficient level of immunity among wild racoon dogs and foxes.

Continuous surveillance and monitoring for rabies will be carried out in Estonian territory. OV efficiency control will follow in vaccinated areas.

In case re-emerging rabies cases will appear, OV around the site of event will be officiated.

On day two of the meeting participants had the possibility to tour in Estonian Veterinary and Food Laboratory (VFL) Central Laboratory building in Tartu. Most of diagnostic work for rabies is carried out in these facilities.

Representative of the laboratory made a lecture concerning rabies testing in Estonia.

VFL has central laboratory and three departments: in Tallinn, Rakvere and Kuressaare. All laboratories of the VFL are accredited by the Estonian Accreditation Centre according to EVS-EN ISO/IEC 17025:2006.

Rabies investigations are carried out predominantly in Tartu, in Tallinn VFL's diagnostic department testing of rabies-suspected animals originating from northern part of country is performed.

Rabies virus is detected in the brain using the immunofluorescence test (IFT). In case IFT gives positive result, test report will be forwarded immediately to Veterinary and Food Board. Bioassay in cell culture and polymerase-chain reaction (PCR) are used to confirm, or rule out, the disease on FAT-negative samples in incident with a known contact to human or an unvaccinated domestic animal exposure. In case IFT gives negative result on animal without any contact, test report will be issued. Central laboratory offers services to private sector, including testing of travelling pets by FAVN. All investigations of OV efficiency control (detection of tetracycline bio-marker in teeth, determination of animal age, detection of rabies post-vaccination antibodies by Bio-Rad ELISA and viral antigen from brain tissue) is done in VFL Central Laboratory.

After brief discussion, the subgroup laid down the following conclusions, recommendations and observations:

### **Conclusions on the Estonian program**

Estonia has implemented a successful oral vaccination program for the elimination of wildlife rabies since autumn 2005.

The planning, organisation and elaboration of oral rabies vaccination campaigns comply with the recommendations of the 2002 EC report "The oral vaccination of foxes against rabies". Monitoring and surveillance activities have been correctly carried out and confirm the excellent results of the program.

The laboratory at the headquarters in Tartu has good facilities, it is sufficiently equipped and is working in close cooperation with the veterinary authorities and field services.

With the exception of the areas adjacent to the south-eastern borders with Russia, rabies cases have not been detected in the Estonian territory for almost 3 years. Therefore, the decision to reduce the area where oral vaccination is applied only to higher risk areas in future campaigns is considered justified.

### **Recommendations**

Estonia should reinforce its efforts to establish cooperation and/or partnership on rabies with the Russian Federation with the objective of creating an EU-financed vaccination belt along the adjoining Russian territory for mutual benefit so that both the risk of disease reintroductions is minimized.

Efforts should be made to increase number of animals submitted for rabies diagnosis (suspect cases, indicator animals) in the whole country, more particularly along the borders with Russia and Latvia to be able to react swiftly in case of an emergency, e.g. reintroduction of rabies.

The sample size for the monitoring of ORV campaigns, e.g. bait-uptake, virus investigations in hunted animals, can be reduced in future campaigns. Sampling could be restricted to certain reference areas in the vaccination belt along the common borders with neighbouring countries.

The achieved immunity in the target wildlife population in Estonia is probably underestimated. To solve this problem, it is suggested to reduce the positivity threshold to the serological test used or to use other commercially available serological tests.

### **General observations of the subgroup**

The drastic reduction of rabies levels in all Baltic Member States now makes it more important to instigate activities at the borders with Third Countries. In this respect building partnership between MS and neighbouring non-EU countries is highly desirable. The recent financing decision for Member State programmes in 2011 introduces this possibility. The TF notes that both Ukraine and Belarus are open to participate.

Transboundary cooperation on rabies oral vaccination has quickly delivered favourable signals in the case of the Kaliningrad region of the Russian Federation, where neighbouring Member States report a virtual absence of rabies in their areas bordering its territory. It is important that the work in Kaliningrad continues without interruption.

The experience in southern Poland with the re-emergence of the rabies through the introduction of non-indigenous rabies virus variants, perhaps by illegal animal movements, shows the usefulness of maintaining surveillance awareness and should put all MSs on alert to the possible reintroduction of rabies into previously cleared areas. The TF strongly suggests that Poland considering an emergency vaccination using reduced intervals and adjusting the vaccination strategy accordingly, e.g. reduction of flight lines, in an area around the outbreak.

Annex

**Task Force Rabies subgroup meeting  
08.-09.11.2010, Tartu, Estonia  
AGENDA**

<b>Day 1 – 8 November 2010</b>	<b>Venue of meeting- Hotel London</b>
10:00 – 10:15	Welcome - host Short introduction and distribution of tasks - European Commission
10:15 – 11:30	Short information /update by the participating MS and TC on their rabies situation and eradication activities – participating countries, 5-10 min. per country
11:30 – 11:45	Coffee break
11:45 – 12:15	Epidemiological evolution of rabies in Estonia, present situation- host
12:15-12:30	Management of stray animals in Estonia- host
12:30 – 13:00	Questions and answers, observations, discussion- subgroup
13:00-14:30	Lunch
14:30-15:00	Organisation of oral vaccination campaigns in Estonia- host
15:00-15:15	Questions and answers, observations, discussion- subgroup
15:15-15:45	Control of OV campaigns in Estonia, plans for the future- host
15:45-16:00	Coffee break
16:00-17:00	Discussion and exchange of views on the implementation of oral vaccination in the RF areas bordering MS
17:00-17:45	Observations concerning presentations of day 1, general discussion, preliminary recommendations and conclusions
17:45	End of day 1

19.00- guided tour in Tartu

20.00 - Dinner

<b>Day 2 – 9 November 2010</b>	<b>Venue of meeting- Central laboratory of Veterinary and Food Laboratory (NRL)</b>
09:30 – 10:15	Presentation of tasks of Veterinary and Food Laboratory, rabies-related investigations in VFL
10:15 – 10:45	Visiting VFL facilities
10:45 – 11:00	Coffee break
11:00 – 11:30	Questions and answers, general discussion and exchange of experience - subgroup
11:30-12:30	Conclusions and recommendations of subgroup on the Estonian rabies program- subgroup
12:30 – 12:45	Future cooperation and activities of TF in 2010- European Commission
12:45	Close of meeting