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Update on oral vaccination of foxes and raccoon dogs against rabies

Scientific Panel on Animal Health and Welfare (AHAW)

Abstract

An assessment of a report issued by the European Commission in 2002 is presented about the oral vaccination of foxes against rabies, as well as additional topics such as the rabies aetiology and its occurrence in Europe, the target species for oral vaccination, i.e. foxes and raccoon dogs, the oral vaccines available and their thermostability, rabies surveillance and monitoring, and the recent international guidelines for rabies surveillance are discussed. Foxes are the only known reservoir for rabies in Europe, and raccoon dogs are important transmitters, while other carnivores play a less important epidemiological role. The demographic expansion of raccoon dogs and their movements after hibernation are risk factors for rabies recurrence. The combined densities of foxes and raccoon dogs, which often share the same habitats, could allow rabies epizootics to persist. The epidemiological role and the pathogenesis of rabies in raccoon dogs and other carnivores must still be clarified. Four vaccines are authorised in the EU and their effectiveness is proven to successfully contribute to rabies elimination. Experimental and field evidence shows that climatic and weather conditions may impact on bait casings and vaccine stability. Data on the stability of vaccine baits under different field conditions should be provided. It is recommended that bait should keep its integrity at the release point. Because of bait stability issues, the season most appropriate for implementing oral rabies vaccination or a heat-stable vaccine should be considered. Rabies surveillance and monitoring of vaccination, based on assessment of bait uptake and seroconversion in host species, are important tools for the evaluation and adjustment of vaccination campaigns, although alternatives to tetracyclines as biomarkers should be developed. Oral immunisation by vaccine baits has proven to be successful in eliminating terrestrial wildlife rabies, but long-term strategy, temporal continuity of vaccination and cross-border cooperation are needed and recommended.

Keywords: rabies, fox, *Vulpes vulpes*, raccoon dog, *Nyctereutes procyonoides*, vaccine, bait

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Summary

In 2002, the former Scientific Committee on Animal Health and Animal Welfare of the European Commission (EC) issued a scientific report on the oral vaccination of foxes against rabies, covering the type of vaccines and baits, the methods of release of vaccine baits, the density of baits and distribution patterns, and the seasonal pattern of releases.

In accordance with the present mandate, the EC asks the European Food Safety Authority (EFSA) i) to update the above-mentioned report and, in particular, to consider if the conclusions and recommendations are still valid; ii) to assess the additional following topics: the new target species (e.g. raccoon dogs), the availability of a new vaccine in the European Union market, the thermostability of the bait casing and the possible impact on stability of the virus strain, and the recent European and international guidelines for rabies surveillance and oral vaccination monitoring.

The following topics are presented and discussed in the present opinion: (i) the rabies aetiology and an update on the phylogeny of the rabies virus and related diagnostic tests; (ii) an update of the occurrence of rabies in animals and humans in Europe up to 2014; (iii) a description of the potential reservoir and transmitter species for sylvatic rabies; (iv) an update on the ecology of foxes and raccoon dogs and the rabies epidemiology in these species, including the interaction dynamics of these two populations; (v) the characteristics of the vaccines available; (vi) the thermostability of the bait casing stability of the virus strain, confirmed by providing the results of experimental studies and the prescriptions of the new version of the European Pharmacopoeia; and (vii) a description of the criteria for rabies surveillance, monitoring of oral vaccination campaigns and the vaccination strategy, in line with the new EC 'Guidelines to design an EU co-financed programme on eradication and control of rabies in wildlife'.

The recent European and international guidelines for rabies surveillance, as requested in the Terms of Reference have been used as a source of data and information for the current work. This includes documents issued by the EC, the EU task force on the eradication of animal disease (subgroup rabies), the World Health Organization (WHO), the World Organisation for Animal Health (OIE) and the European Pharmacopoeia. In particular, the most recent guidelines for rabies surveillance and oral vaccination are the EC guidance document issued in 2015 about the design and implementation of an EU co-financed programme on the elimination and control of rabies in wildlife; the update of the European Pharmacopoeia 'Rabies vaccine (live, oral) for foxes and raccoon dogs' issued in 2014, which includes raccoon dogs as target species; chapter 2.1.13, about rabies, of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual), updated in 2014; and the EFSA external scientific report 'Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union' issued in 2010, which provides recommendations for improving and harmonising rabies surveillance and reporting in animals in Europe. Scientific literature has been also screened, and the databases from Rabies Bulletin Europe and the Animal Disease Notification System (ADNS) has been used to provide updated information on the occurrence of rabies in Europe, both in Member States (MSs) and in neighbouring countries.

The conclusions and recommendations from the previous report issued by the EC in 2002 were comparatively assessed for their validity.

With regard to the host species, it is concluded that foxes are the only known reservoir for rabies in Europe, whereas raccoon dogs are not known to be reservoirs, but they are considered as important transmitters. Other carnivores may be infected by rabies, although their role as virus transmitters is generally less important. Ecological patterns of raccoon dogs may be important in rabies epidemiology, such as their rapid demographic expansion, as a risk factor for rabies recurrence, and the movements of adult raccoon dogs after hibernation in spring and the dispersal of juveniles. If winters are mild, depending on the latitude (e.g. in western Europe), raccoon dogs do not usually hibernate. As regards the interaction of host species, it is concluded that foxes and raccoon dogs often share the same habitats and their home ranges overlap making contacts between species likely. Moreover, their combined densities could lay above the threshold density values for rabies transmission, thus allowing rabies epizootics to persist in a certain area. Epidemiological studies and surveillance systems are needed to clarify the role of raccoon dogs and other carnivores in rabies epidemiology and pathogenesis.

With regard to the vaccines available in the EU, there are currently four vaccines authorised, all of which comply with the prescriptions of the European Pharmacopoeia for efficacy and safety. Each vaccine batch should be tested and approved for titre and stability by an acknowledged quality control scheme in accordance with OIE standards and WHO recommendations. Laboratories involved in the monitoring and evaluation of rabies programmes are advised to monitor the virus titre of batches of rabies vaccine baits before and during release into the field. The effectiveness of the four marketed oral vaccines is proven in the field and epidemiological data demonstrate that the existing vaccines have contributed to the success of rabies control and elimination in several European countries.

As regards the thermostability of bait casings and vaccines, there is experimental and field evidence that weather conditions may have a negative impact on the bait casings and vaccine virus stability; however, conditions are often different from those tested by the producers under laboratory conditions. Therefore, data on the stability of bait under different field conditions of landscape and temperatures, and on the related melting point, should be provided. In relation to thermostability of baits and vaccines, the countries implementing oral rabies vaccination (ORV), in particular those in southern Europe, should conduct vaccination campaigns in seasons when temperatures and/or climatic conditions do not compromise bait and vaccine stability, or should use a heat-stable vaccines. It is recommended that bait casing should keep its integrity when released into the environment, so that it does not melt and the capsule of the vaccine is still fully covered by the casing.

With regard to vaccination strategy and distribution patterns, the recent EC guidelines issued in 2015 provide clear and exhaustive information about bait distribution methods, and the current document is in line with the information provided there. Oral vaccination campaigns should be conducted on a biannual basis, in spring and autumn, taking climatic conditions into account. Autumn vaccination should generally be performed in September or October. Spring distribution should be carried out taking into account the birth time of fox and raccoon dog cubs, and also the possible hibernation of raccoon dogs, in order to increase the efficient access of fox and raccoon dog cubs to baits. Vaccinations at den entrances are recognised as costly and time-consuming, thus they should be applied only after a careful cost–benefit evaluation.

The minimum distributed bait density should not be less than 20 baits/km^2. An increased bait density of up to 25–30 baits/km^2 in combination with reduced flight-line distance (e.g. 300 m) should be considered in cases of setbacks and persisting residual foci.

In cases of reintroduction of rabies from infected neighbouring regions, buffer vaccination zones should be established, taking into account natural barriers. This belt should be at least 50 km beyond the front of a rabies endemic zone, unless natural barriers are present, then it could be reduced to a width of 20 km. In cases of rabies re-emergence in a previously rabies-free area, emergency vaccination needs to be conducted immediately. An emergency vaccination area with a radius of at least 50 km around the outbreak should be established. Manual distribution of baits can be used as a complementary method to aerial distribution, and managed using a case-by-case approach. In general, wherever the distribution system allows flexibility, the pattern of habitat use of host species should be considered.

Rabies surveillance and monitoring of the vaccination effectiveness are important tools for the assessment and adjustment of vaccination campaigns. Harmonised surveillance and monitoring methods should be sought in order to facilitate comparison of results and international cooperation.

Rabies surveillance should include examination of all wild and domestic mammals suspected of being infected by rabies, those found dead, including road kills, as well as those at the origin of human exposure. The viruses isolated from all positive cases in the vaccinated areas should be typed with the currently available molecular methods, in order to distinguish field rabies virus from vaccine associated cases.

Monitoring of vaccination programmes includes assessment of bait uptake through biomarker testing and of seroconversion in host species during vaccination campaigns. The need for a biomarker in vaccine baits and related testing for bait uptake could be reconsidered after the first few years, e.g. after the first two or three years, of ORV campaign implementation, according to the rabies situation in each country and to the overall control strategy in place. Moreover, the use of tetracyclines as biomarkers could potentially give rise to safety issues related to ecotoxicity and antimicrobial resistance, although no risk assessment has ever been performed on these aspects. Therefore, it is recommended that risk assessments are performed on this topic, and alternatives to tetracyclines as biomarkers should be developed. Also, the vaccine capsules inside the baits should be made of biodegradable material, so to avoid environmental contamination.

As a general conclusion, it was confirmed that oral immunisation against rabies by means of vaccine baits has been found to be successful in eliminating terrestrial wildlife rabies in most cases, both in foxes and raccoon dogs, although it requires a long-term strategy, temporal continuity of implementation of the vaccination campaigns and cross-border cooperation. The latter conditions are recommended for the success of the campaigns. For example, several MSs (the Czech Republic, Germany, Italy, Austria, Estonia, Latvia and Lithuania) have acquired or re-acquired the status of rabies-free country since 2003, thanks to oral vaccination against rabies.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

The former Scientific Committee on Animal Health and Animal Welfare issued on 23 October 2002 a scientific report on the oral vaccination of foxes against rabies. The mandate conferred to the Committee included a comparative study of the various fox vaccination protocols implemented, using the following parameters:

1) Type of vaccines and baits
2) Methods of release of vaccine baits
3) Density of baits and distribution patterns
4) Seasonal pattern of the releases

In addition to those parameters the report included a description of the methods of evaluation of the vaccination programmes, such as the determination of bait uptake or fox immunity.

Those methods have been reviewed by a scientific report on the 'Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union' submitted to EFSA and issued on 16 July 2010 in accordance with Article 36 of Regulation (EC) No 178/2002.

The 2002 report had constituted an important supporting document for Member States and the Commission to help implement the most appropriate strategy to control and to achieve the eradication of rabies. Programmes of oral vaccination of wildlife against rabies have been co-funded by the European Union for several years. They have proven to be successful in eradicating the disease from Member States in western, central and north-eastern Europe.

Efforts are now focused in Eastern Europe and the Balkans while worrying re-emergence of rabies is observed in those areas where the disease had virtually disappeared. The following Member States are currently running an annual or multi-annual programme for the eradication of rabies approved in accordance with Commission Implementing Decision 2013/722/EU see Article 11 thereof: Bulgaria, Croatia, Estonia, Finland, Greece, Hungary, Italy, Latvia, Lithuania, Poland, Romania, Slovakia and Slovenia. Considering the above and given that new tools and vaccination strategies have become available to eradicate rabies, the Commission considers it opportune to request EFSA to update the scientific evidence and revise the aforementioned 2002 scientific report.

In view of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA to update the 2002 Report of the former Scientific Committee on Animal Health and Animal Welfare on 'Oral vaccination of foxes against rabies', and in particular to consider if the conclusions and recommendations of this report are still valid.

As regards the update of the comparative study of the vaccination protocols in wildlife, the following additional parameters should be taken into consideration:

- new target species (e.g. raccoon dogs);
- the availability of a third vaccine in the EU market (with the SAD Bern strain) in addition to those available in 2002 that are still used (made of SAG2 strain and SAD B19 strain);
- the thermo stability of the bait casing and possible impact on stability of the virus strain used in relation to different climate conditions and temperature at the releasing point;
- Recent European and international guidelines for rabies surveillance and oral vaccination monitoring to determine the effectiveness of vaccination programmes.

1.2. Interpretation of Terms of Reference

The present opinion provides an update of the report issued in 2002 by the European Commission (EC) Scientific Committee on Animal Health and Animal Welfare (EC, 2002). The Terms of Reference (ToRs) of the 2002 report were to assess the reasons for failures in the implementation of rabies control protocols and identify the corrective actions to bring about the elimination of rabies in the Community as soon as possible. Furthermore, the different protocols of oral vaccination were comparatively assessed, considering (i) the type of vaccines; (ii) the type of baits; (iii) the methods of release of vaccine baits; (iv) the density of baits and distribution patterns; and (v) the seasonal pattern of the releases.

These points are considered and updated in the present opinion, following the structure of the EC report of 2002, which covered the following topics: (i) rabies aetiology; (ii) the occurrence of rabies in Europe up to 2002; (iii) a description of red fox biology; (iv) an assessment of vaccines available in Europe, the bait stability and tools for monitoring vaccination; and (v) the vaccination strategy, including temporal and spatial patterns of baits distribution and issues about host population dynamics. These topics are presented and discussed and new sections have been added to address the Terms of References (ToRs).

Section 3.1 of the present assessment addresses **rabies aetiology** by providing an update on the phylogeny of the rabies virus and related diagnostic tests, while section 3.2 provides an update of the **occurrence of rabies in animals and humans in Europe** up to 2014.

The point in the ToRs about **new host and target species** is addressed in section 3.3, which presents the role of known reservoirs of rabies and of the potential neglected reservoirs in Europe.

An update on the **ecology of foxes and raccoon dogs and the rabies epidemiology in these species** is presented in sections 3.3.1 to 3.3.5, while the **interaction of these two populations** and its effect on rabies spread is discussed in section 3.4.

The availability and the characteristics of a new vaccine (ToR 2) are addressed in section 3.5.1.

The thermostability of the bait casing and possible impact on stability of the virus strain (ToR 3) is addressed in section 3.5.2 by describing the results of experimental studies in the field and the prescriptions of the European Pharmacopoeia in terms of bait and vaccine stability.

Sections 3.5.3 and 3.5.4 provide a description of the criteria for **rabies surveillance and monitoring of oral vaccination campaign**, as outlined in the scientific report submitted to EFSA in 2010, 'Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union' (Cliquet et al., 2010), and in the new EC 'Guidelines to design an EU co-financed programme on eradication and control of rabies in wildlife' (EC, 2015).

Furthermore, the ToR about **recent European and international guidelines for rabies surveillance** is addressed in section 2.1, under 'Data and methodologies', since the section presents the current available guidelines and reference documents about rabies control, on which the assessment of the present opinion is also based.

Finally, it is considered important to recall the difference between eradication and elimination, since in the background of the mandate on the eradication of rabies has been mentioned. If the definition provided by the World Health Organization (WHO) and World Organisation for Animal Health (OIE) is considered (Dowdle, 1998), the following definitions would apply:

- Control: a reduction in the incidence, prevalence, morbidity or mortality of an infectious disease to a locally acceptable level;
- Elimination: the reduction to zero of the incidence of disease or infection in a defined geographical area;
- Eradication: the permanent reduction to zero of the worldwide incidence of infection.

Therefore, considering the achievements of the EU programmes on rabies in some of the Member States (MSs), the latter situation (eradication) would not be the case, rather the opinion deals with the elimination of rabies from certain geographical areas. Further information is provided in recent reviews (Rupprecht et al., 2007; Müller et al., 2015a). Hence, the term 'elimination' will be used throughout the present document.

2. Data and Methodologies

2.1. Sections on rabies aetiology (3.1), wildlife reservoir and transmitters (3.3), interaction of fox and raccoon dogs (3.4)

The screening of the published peer-reviewed scientific literature has been one of the main sources of data for the assessment performed in sections 3.1, 3.3 and 3.4. A protocol of the literature review is presented in Appendix A, which describes the objective of the review and the criteria for the inclusion of studies.

2.2. Section on the occurrence of rabies in Europe and current oral rabies vaccination strategy in different countries (3.2)

The description of the occurrence of rabies in Europe and the update on the current oral rabies vaccination (ORV) strategy in the MSs and neighbouring countries is based on data provided by the EU task force on rabies and by MSs through the control plans submitted to the EC and with direct consultation with the national authorities of the MSs in charge of ORV programmes. The graphs and maps (Figures 1-10) showing the occurrence of rabies in Europe are based on data from the Rabies Bulletin Europe (WHO) and the Animal Disease Notification System (ADNS). Different databases related to different reporting schemes are in place in Europe and at international level (Rabies Bulletin Europe, OIE World Animal Health Information Database (WAHID), MS notification to EC in accordance with Directive 99/2003[1], each one with specific objectives). The main differences in the reporting systems are described in the scientific report submitted to EFSA in 2010 (Cliquet et al., 2010).

2.3. Section on vaccines (3.5.1) and on the thermostability of the baits (3.5.2)

The sections on the efficacy and safety of EU-authorised vaccines and on the thermostability of the bait casings, including their possible impact on the vaccine, is based on the review of the published scientific literature as well as on data from the updated monograph related to oral rabies vaccines of the European Pharmacopoeia (2014).

2.4. Section on rabies surveillance (3.5.3) and monitoring of vaccination (3.6.3) and vaccination strategy (3.6)

A series of core documents issued by the EC, WHO, OIE and other expert committees have been considered and are described in section 2.5. The data from those documents have been used for the sections on rabies surveillance and monitoring (3.5.3 and 3.5.4) and the section on the vaccination strategy (3.6).

2.5. Recent European and international guidelines for rabies surveillance and oral vaccination

All guidelines on rabies surveillance and oral vaccination issued by the EC, WHO and OIE after the 2002 report are described in this section.

2.5.1. Reports of the European Union task force for monitoring animal disease elimination – rabies subgroup

The EU task force on the elimination of animal diseases consists of representatives from MSs and the EC. It monitors disease elimination within the MSs in order to improve the EU co-funded programmes for disease control. It consists of subgroups, which each provide disease-specific technical assistance to MSs. For rabies, the subgroup has produced six reports from meetings held in MSs between 2009

[1] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC

and 2012.[2] The reports contain updates on the rabies disease situations from participating countries and ORV activities. Recommendations relate to the rabies situation in the MSs at the time and the activities undertaken so far under the co-funded programmes. They include recommendations for dealing with specific issues in participating countries as well as general recommendations for ORV implementation, monitoring and surveillance.

2.5.2. European Commission guidelines to design and implement a European Union co-financed programme on eradication and control of rabies in wildlife

The EU co-funded programmes on animal diseases and zoonoses provide financial assistance to participating MSs' animal disease control activities. Countries wishing to participate submit proposed elimination, control and surveillance programmes for which they would require support. The EC provides updated guidelines on these programmes (EC, 2015).

The purpose of the guidelines is to provide guidance to the MSs and the neighbouring countries that assist with the planning and implementation of rabies EU co-funded elimination programmes in wildlife, and associated activities, and the improvement of the effectiveness of such programmes. A further purpose of these guidelines is to accelerate the progress of elimination programmes and stimulate discussion on future strategy in relation to rabies elimination.

2.5.3. Monograph of the European Pharmacopoeia, 'Rabies vaccine (live, oral) for foxes and raccoon dogs'

Monograph 746 of the European Pharmacopoeia, 'Rabies vaccine (live, oral) for foxes and raccoon dogs', has been recently updated and now includes raccoon dogs; tests for safety and immunogenicity for raccoon dogs were added when raccoon dogs became a target species for ORV (European Pharmacopoeia, 2014).

2.5.4. Animal disease elimination, control and surveillance programme indicators

The EC co-funds programmes submitted by MSs on elimination, control and surveillance of animal diseases and zoonoses. The document on 'Animal disease elimination, control and surveillance programmes indicators' (EC, 2012b) of the EC lists and describes disease-specific indicators used to evaluate the results of the implementation of these programmes in MSs.

Indicators are obtained from MSs' annual programme reports, the EU summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks (EFSA and ECDC, 2015) and other data submitted by MSs. Indicators are divided into two categories: (1) 'activities', to verify whether or not planned measures have been implemented; and (2) 'progress', to measure progress with regard to achieving programme objectives.

2.5.5. EFSA external scientific report 'Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union'

The 2010 external report on the 'Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union', published by EFSA (Cliquet et al., 2010), provides recommendations for improving and harmonising rabies surveillance and reporting in animals in Europe. It is a response to the great variation seen between MSs with regard to surveillance and monitoring. The report provides an overview of the disease situation and national level monitoring and reporting in MSs, identifies possible infected animal species suitable for monitoring, and the most suitable diagnostic methods and sampling approaches. It also includes a consideration of how to enhance bat rabies surveillance within the EU, and an animal monitoring and reporting scheme for MSs. The type of information needed for analysis of the disease situation and trends within and between MSs are outlined.

[2] http://ec.europa.eu/dgs/health_food-safety/funding/cff/animal_health/vet_progs_en.htm

2.5.6. Reports of the WHO expert consultations on rabies

The two reports of the WHO expert consultations on rabies from 2005[3] (WHO, 2005) and 2013[4] (WHO, 2013) provide information on rabies in both humans and animals. This includes information on vaccines, prevention of human rabies and management for rabies patients, national programmes for dog rabies control, prevention and control of rabies in wild animals, surveillance, global and regional activities on rabies, regional information exchange and diagnostics.

Since the first WHO expert consultation on rabies in 2005, WHO and its network of collaborating centres on rabies have been advocating the feasibility of rabies elimination regionally and globally, and promoting research into sustainable cost-effective strategies. These joint efforts have begun to break the cycle of rabies neglect, and rabies is becoming recognised as a priority for investment. The last consultation concluded that human dog-transmitted rabies is possible to control, and that it should be possible to achieve regional elimination in the medium term and even global elimination in the long term.

2.5.7. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*,* 2013 (chapter 2.1.13, Rabies)

The 'OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)' provides harmonised, disease-specific information on diagnostic laboratory methods and requirements for vaccine production and control (OIE, 2014).[5] It contains information on diagnostic techniques and laboratory tests, including information on virus identification and sample collection. Requirements for vaccines include those specific to oral vaccines and for their manufacture, authorisation/licensing/registration, efficacy and stability. Chapter 2.1.13 of this manual about rabies was updated in May 2013. It outlines the internationally standardised diagnostic techniques (fluorescent antibody test (FAT), enzyme-linked immunosorbent assay (ELISA), rapid immunodiagnostic tests and virus neutralisation tests) and the requirements for vaccines for both injectable and oral use.

2.5.8. The Blueprint for Red Fox Rabies Prevention and Control

The Blueprint for Red Fox Rabies Prevention and Control[6] is both an information platform on fox-mediated rabies and rabies control and a guide to develop country-specific rabies elimination programmes. Developed by international rabies experts and contributors from around the world, the guide makes use of information from international organisations and field data. The Blueprint is intended to be a practical tool for countries wishing to understand rabies prevention and control. The five sections include a discussion of roles and responsibilities; infrastructure, legislative framework, costs and funding; communications plans; and operational activities.

[3] http://www.who.int/rabies/trs931_%2006_05.pdf
[4] http://apps.who.int/iris/bitstream/10665/85346/1/9789240690943_eng.pdf
[5] http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.13_RABIES.pdf
[6] http://foxrabiesblueprint.org

3. Assessment

3.1. Rabies aetiology and related laboratory tests

Rabies is a zoonotic viral disease, which causes acute encephalitis in mammals. It is transmitted through close contact with saliva from infected animals (i.e. bites, scratches, licks on broken skin and mucous membranes), although atypical exposures have also been ascertained (i.e. organ transplantation, meat consumption). Once symptoms of the disease develop, rabies is always fatal. Rabies virus belongs to the order Mononegavirales, which are viruses with a non-segmented, negative-stranded RNA genome. Within this group, those with a distinct 'bullet' shape are classified as belonging to the Rhabdoviridae family. Rabies virus (RABV) is the first of 14 lyssavirus species identified to date (Dietzgen et al., 2011), with additional genetic evidence of a not yet isolated lyssavirus named Lleida bat lyssavirus (LLEBV) (Ceballos et al., 2013). According to their viral genetic distances and antigenic differences, two major phylogroups have been defined. Phylogroup I comprises the following species: RABV, European bat lyssavirus 1 (EBLV-1) and European bat lyssavirus 2 (EBLV-2), Duvenhage virus (DUVV), Australian bat lyssavirus (ABLV), Aravan virus (ARAV), Khujand virus (KHUV), Bokeloh bat lyssavirus (BBLV) and Irkut virus (IRKV). Phylogroup II comprises Lagos bat virus (LBV), Mokola virus (MOKV) and Shimoni bat virus (SHIBV). The remaining viruses, West Caucasian bat virus (WCBV) and Ikoma lyssavirus (IKOV) cannot be included in either of these phylogroups and have been temporarily assigned to putative phylogroups III and IV, respectively.

The reference test for rabies diagnosis is the FAT on acetone-fixed smears of hippocampus, cerebellum or medulla oblongata, followed by the rapid tissue culture isolation test (RTCIT) (WHO, 2013; OIE, 2014). In the last decades, molecular methods have been largely adopted in reference laboratories to trace the origin and type of a confirmed infection (i.e. detecting a vaccine-associated case occurrence or evaluating the phylogeny of the virus responsible for an outbreak), although their use in routine diagnostic investigations is discouraged by international organisations (Fooks et al., 2009).

Reference virus neutralisation tests, i.e. the rapid fluorescent focus inhibition test (RFFIT) and the fluorescent antibody virus neutralisation (FAVN) test, have been mainly used to evaluate rabies post-vaccination immunity (Pastoret et al., 2014). In addition, the performances of several types of ELISA have been evaluated for the assessment of herd immunity elicited by vaccination, especially in wildlife (Servat et al., 2007; Wasniewski et al., 2013).

3.2. The occurrence of rabies in Europe

In this section, an update on the occurrence of rabies in humans and animals, excluding bats, in Europe (MSs and neighbouring countries) is provided. This supports the discussion about the ToR on new target species and vaccination programmes in Europe.

The incidence of rabies in both domestic and wild animals in EU MSs has drastically reduced over the past decades after systematic oral vaccination campaigns, and rabies cases have disappeared in western, northern and most of central Europe. About 83 million euros of EU contributions were needed between 2005 and 2012 for oral vaccination programmes in wildlife in MSs and bordering areas of neighbouring countries, as the vast majority of rabies cases in the EU occur in those areas. This is likely because of the continued presence of sylvatic rabies in neighbouring countries, which continues to feed the endemic cycle in certain areas of the EU. At present, in several neighbouring countries in eastern Europe, rabies remains a serious endemic disease. The recurrence of rabies in some countries highlights the fragility of rabies-free country status and the need for continuous surveillance. Mass vaccination of pets provides a first line of defence to prevent rabies in humans, whereas oral vaccination of foxes and raccoon dogs has proved efficient for the long-term control and elimination of terrestrial rabies. Rabies in pets imported from endemic countries is regularly reported in Europe, highlighting the need for continued vigilance concerning pet movements (EFSA and ECDC, 2015).

3.2.1. Rabies in humans

Generally, very few cases of rabies in humans are reported nowadays in the EU, and most MSs have not had any autochthonous cases[7] for decades. Two cases were reported in Romania in 2007 and 2012 (Figure 1). Autochthonous human cases are still reported in neighbouring countries, such as Belarus (the two last cases were reported to the Rabies Bulletin Europe in 2011 and 2012), Russia (29 cases reported in the last five years, two of them in 2014), Ukraine (the last two cases in 2010), Turkey (the last case in 2012), and Georgia (three cases in 2013 and four in 2014). This steady decrease can be attributed to the disappearance of dog-mediated rabies, the vaccination of domestic animals (both pets and livestock), the reduction of rabies occurrence in wildlife through EU co-funded ORV programmes and the systematic application of post-exposure treatment in cases of contact between humans and suspect animals.

Source: Rabies Bulletin Europe, WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut[8]

Figure 1: Rabies cases in humans reported in the period 2003–2015

3.2.2. Rabies in animals (excluding bats)

The situation in EU MSs

Between 2003 and 2014, the total number of rabies cases at EU level in wild animals (carnivores and ungulates) decreased very significantly, from 3 748 cases (2 500 cases in foxes and 1 000 cases in raccoon dogs) to 215 cases (200 of which were reported in foxes) in the EU-28 countries (Rabies Bulletin Europe, WHO). The reported cases in domestic animals (livestock, dogs, including stray dogs, and cats) dropped from 800 cases to 65 cases in 2014, mostly reported in Romania (41 cases) and Poland (16 cases).

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[7] Disease case contracted in the area where reported.

[8] Rabies Information System of the WHO Collaboration Centre for Rabies Surveillance and Research (http://www.who-rabies-bulletin.org).

The majority of samples from wild and domestic animals tested for rabies are taken based on the suspicion of rabies infection, including animals found dead. In addition, countries carrying out oral vaccination programmes of wildlife monitor the efficiency of vaccination campaigns. Endemic rabies still occurs in foxes, raccoon dogs and other wildlife species in certain eastern parts of the EU, in particular in Romania, with sporadic spillover to domestic animals, mainly dogs and cats (pet and stray) and ruminants, in Poland and in Slovakia in an area bordering Poland, and recurrence has recently been reported in Greece and Hungary (EFSA and ECDC, 2015).

With regard to imported cases to EU countries, 21 cases have been counted since 2003, mostly in dogs. In 2013, three MSs reported imported cases, namely in one cat in France, and in two dogs, one in Spain and one in Germany, all after illegal import from Morocco (EFSA and ECDC, 2015). The last case was detected in May 2015 in a dog illegally imported into France from Algeria.[9]

Situation in EU-neighbouring countries

Russia

Rabies is endemic throughout large parts of the Russia. For the past 30 years, the number of rabies cases has been increasing. In the past 10 years, between 1 406 and 5 503 new rabies cases were reported per year, mostly in the European part of the RF.

In the Kaliningrad region of Russia, the main reservoir of rabies is the red fox; in recent years, 25–70 cases of rabies have been registered annually. Vaccination of pet animals against rabies is compulsory to prevent the spread of the disease to humans and domestic animals. Since the year 2010, the number of infected animals has been decreasing, being 43 in 2010 and 21 in 2011. The last cases were detected in 2012 and since then no further cases have been diagnosed.

In the Leningrad region of Russia, the last case of rabies in wild animals was recorded in 1987. The Karelia Republic of the RF was free from rabies infection between 1954 and 2010 (EC, 2012a).

Belarus

Within the past 10 years, an average of 1 000 rabies cases have been diagnosed annually in Belarus, with peaks in the years 2006 (1 587 cases) and 2011 (1 372 cases). The majority (≈70%) of cases are diagnosed in foxes. These cases are distributed uniformly in the territory, with the exception of eastern areas, where infection pressure seems lower (EC, 2012a).

Ukraine

The analysis of disease dynamics in Ukraine over the last 10 years shows that the number of reported cases in wildlife and in domestic animals has halved, with 469 and 615 cases reported in 2014, in wild and in domestic animals respectively. Of the latter, 498 cases were found in cats and dogs. It is mandatory to immunise the dog population, whereas cats are vaccinated only in endangered areas. Livestock is vaccinated in areas surrounding outbreak sites. The majority of cases in wildlife occur in foxes (EC, 2012a), with 421 cases of rabies in foxes reported in 2014; 36 cases were also recorded in raccoon dogs.

Moldova

In Moldova, the main wild reservoir for rabies is the red fox, with 167 cases being reported since 2003, and 75 being reported in the last five years, to the Rabies Bulletin Europe (WHO). No rabies cases have been reported in other wild carnivores. In domestic animals, 78 cases have been reported in dogs and 39 in cats in the last five years, whereas among livestock, cattle are the main affected host species with 170 cases occurring in the last five years. No human cases have been reported since 2003.

[9] World Animal Health Information Database (WAHID) of OIE, and Animal Disease Notification System (ADNS) of the EU.

Balkan countries

The red fox appears to be the principal wild disease reservoir in Bosnia Herzegovina, Serbia and Montenegro, where, respectively, 341, 692, 163 cases were reported between 2003 and 2015, although with an overall decreasing trend. Rabies cases in domestic animals have been reported in dogs, cats, cattle and small ruminants, although in the last few years the reported incidence has been close to zero. Albania, the Republic of Kosovo[10] and the former Yugoslav Republic of Macedonia (FYROM) reported very little information about rabies to the Rabies Bulletin Europe. In FYROM, the eight rabies isolates reported in 2011/2012 were preceded by a 10-year break (Picard-Meyer et al., 2013). Unlike in northern Europe, the raccoon dog is not a reservoir in this region (Johnson et al., 2008).

Phylogenetic relationships among the virus isolates throughout the Balkan countries provide evidence for cross-border movement of rabies by both wildlife and canine vectors (Johnson et al., 2008). All of the Balkan isolates group within the European/Middle East lineage, with the majority most closely related to east European strains rather than to the west European strains (McElhinney et al., 2011). Similar molecular findings highlight a close genetic relationship between isolates from FYROM, Serbia and Bulgaria, and indicate that wildlife is responsible for rabies movements in the region (Picard-Meyer et al., 2013). The viruses isolates found in the Balkan countries were not phylogenetically linked with rabies foci in Turkey, suggesting that natural barriers, such as the Balkan Mountains, may play a significant role in constraining the southerly spread of rabies in south-east Europe (Johnson et al., 2007).

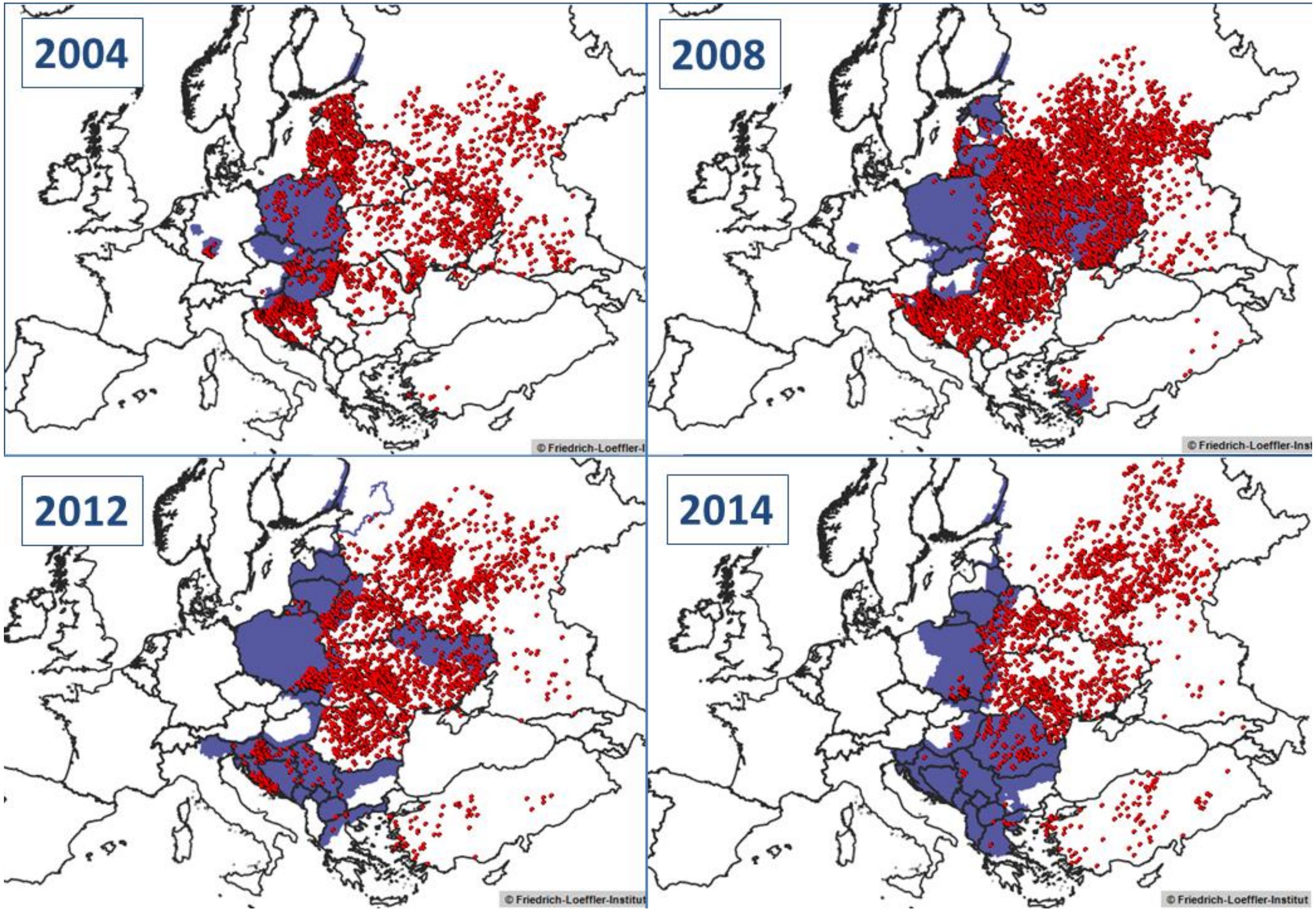
Turkey

In Turkey, the main reservoir is the dog, especially in the urban environment, with an increasing trend in the last five years (76 cases reported in 2010 and 256 cases in 2014). Rabies cases in wildlife species including jackals (*Canis aureus*), wolves (*Canis lupus*) and red foxes (*Vulpes vulpes*) have been reported only infrequently, amounting to approximately 2 % of the total number of cases reported (Rabies Bulletin Europe). Between 1990 and 1999, only 35 rabies cases in wildlife were recorded. At the end of the 1990s in the Aegean region of Turkey, rabies spread rapidly among foxes. Phylogenetic studies suggest that this spread likely resulted from a sustained spillover infection from domestic dogs (Johnson et al., 2003; Vos et al., 2009). As a result, reported cases increased from 10 cases in 2010 to 82 cases in 2014. Among domestic animals, an increasing incidence of rabies is observed in cattle (52 cases reported in 2010 and 302 in 2014).

3.2.3. Spatial and temporal trend of rabies in Europe

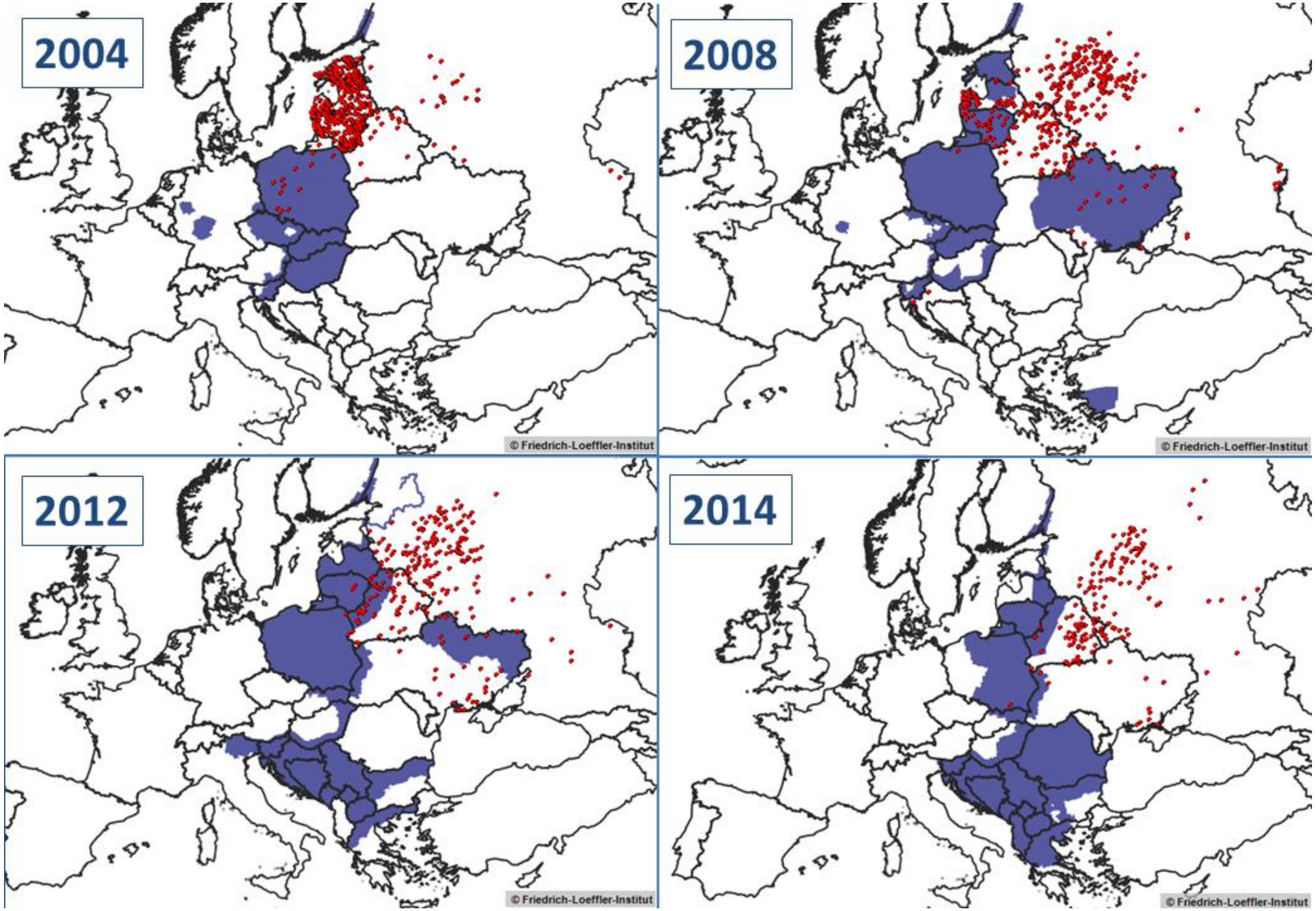
The spatial evolution of rabies cases reported in Europe in foxes and raccoon dogs, according to the Rabies Europe Bulletin, is shown in Figures 2 and 3.

[10] This designation is without prejudice to positions on status, and is in line with UNSC 1244 and the ICJ Opinion on the Kosovo declaration of independence



The area in blue is where oral rabies vaccination (ORV) was performed each year. Source: WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut

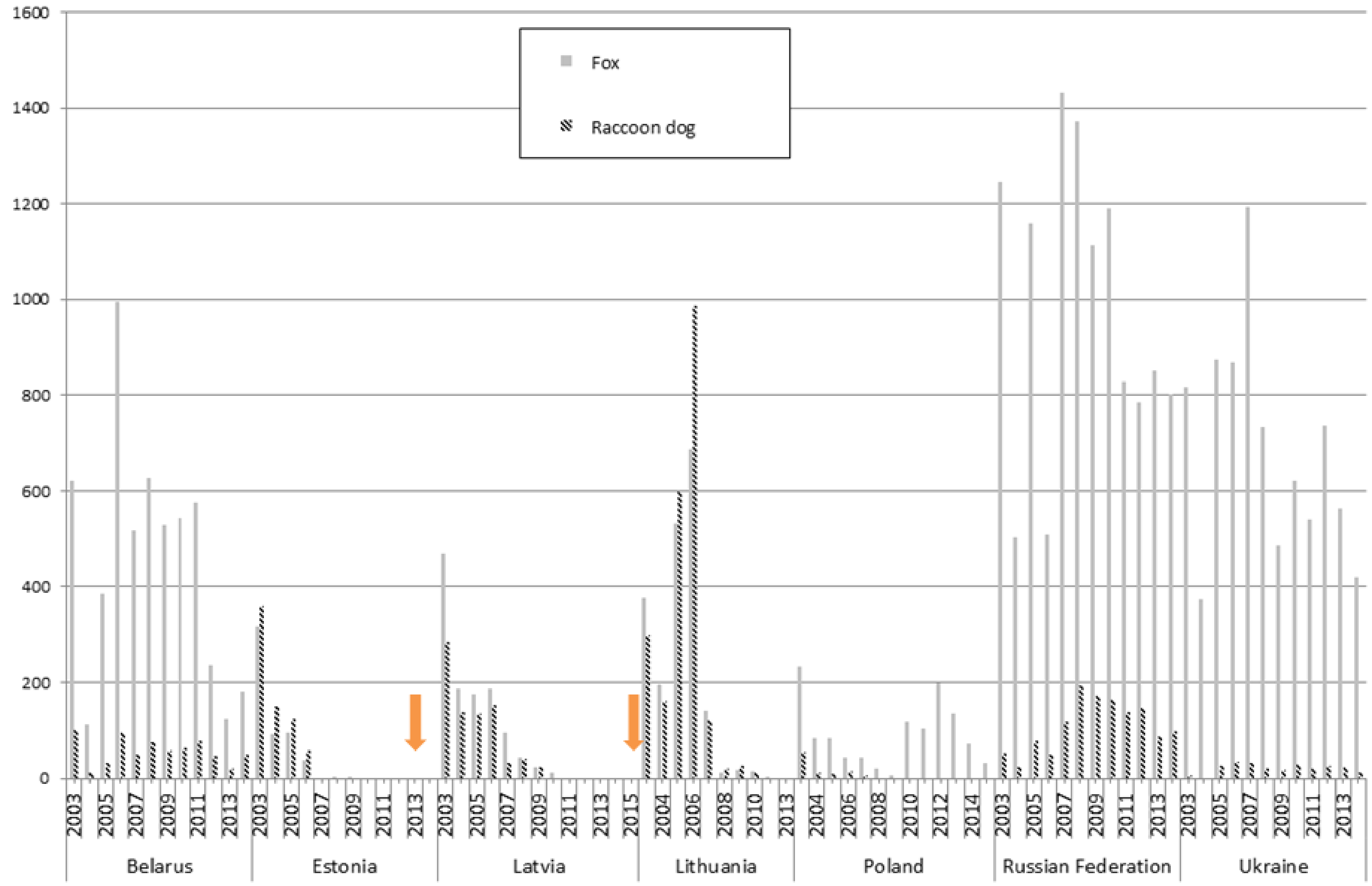
Figure 2: Rabies cases in red foxes notified in 2004, 2008, 2012 and 2014



The area in blue is where ORV was performed in each year. Source: WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut

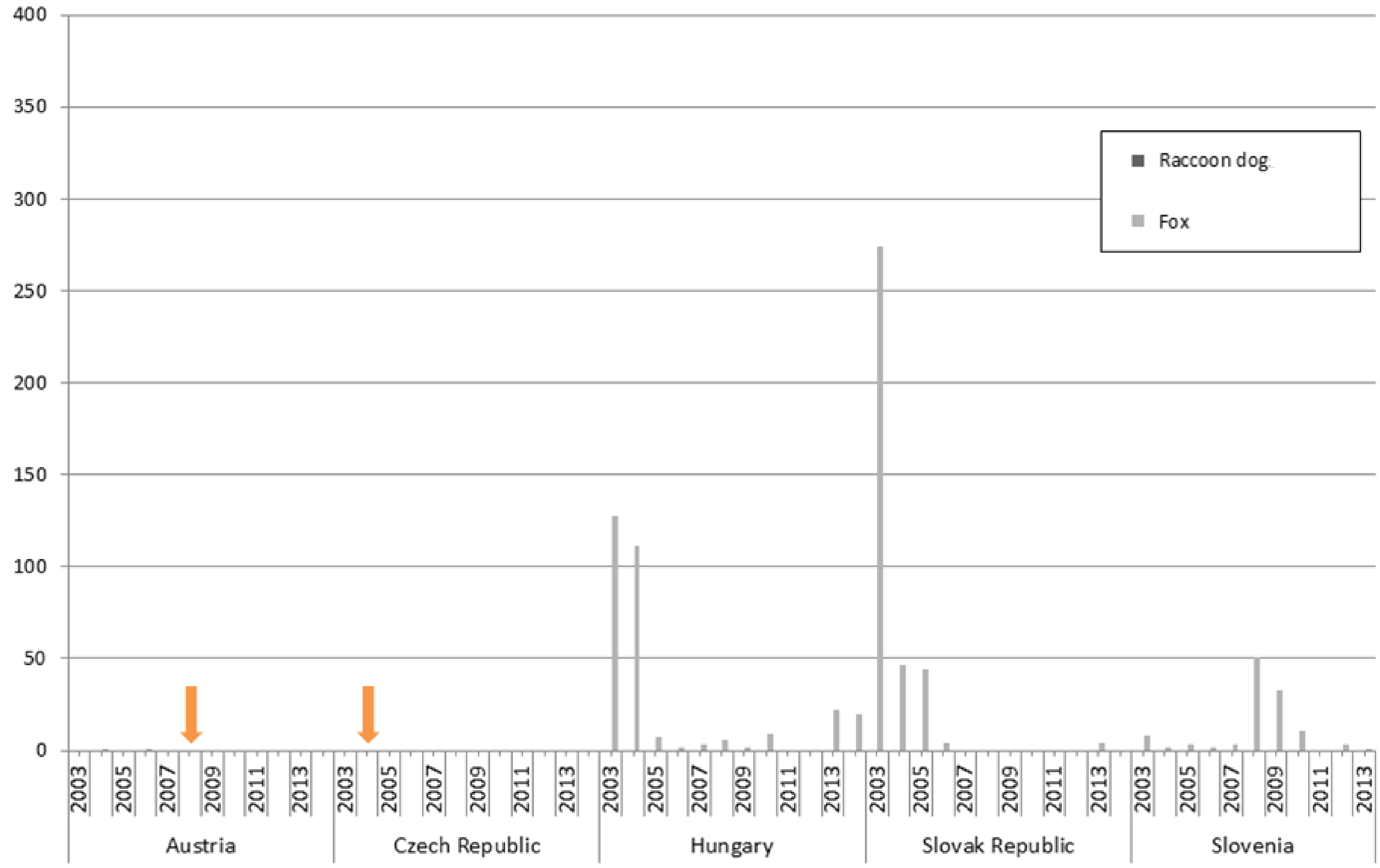
Figure 3: Rabies cases in raccoon dogs notified in 2004, 2008, 2012 and 2014

The temporal evolution of rabies cases reported in foxes and raccoon dogs, by European regions (central, eastern and western Europe, and Balkan countries), is shown in Figures 4, 5, 6 and 7.



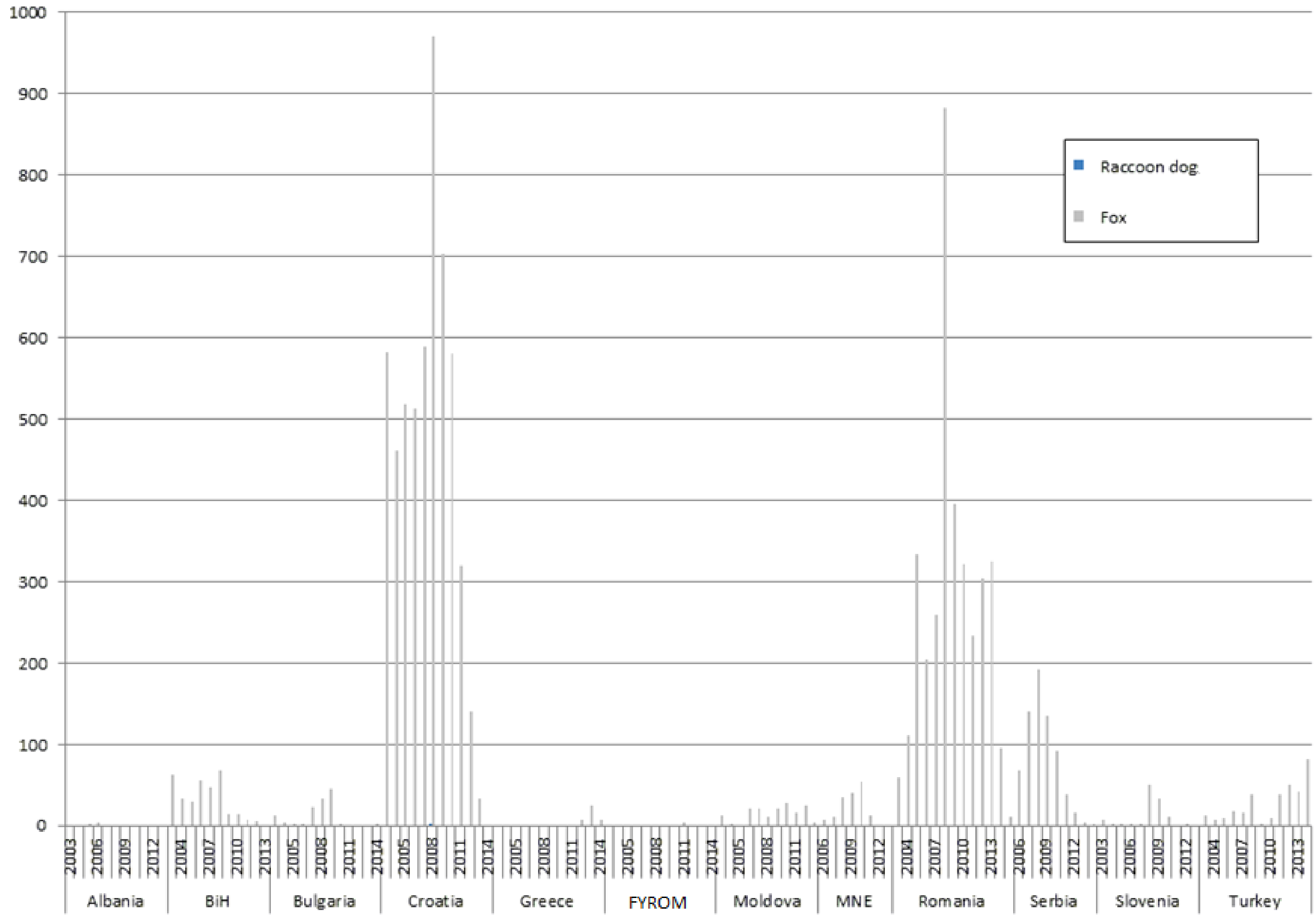
A number of cases are not visible in the graph: Estonia: one case (fox) in 2008, three cases (foxes) in 2009 and one case (raccoon dog) in 2011; Latvia: one case (raccoon dog) in 2010; Lithuania: two cases (raccoon dog) and one case (fox) in 2012. The arrows indicate the year of acquisition of OIE rabies-free status. Data source: WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut

Figure 4: Rabies cases in red foxes and raccoon dogs since 2003 in eastern Europe



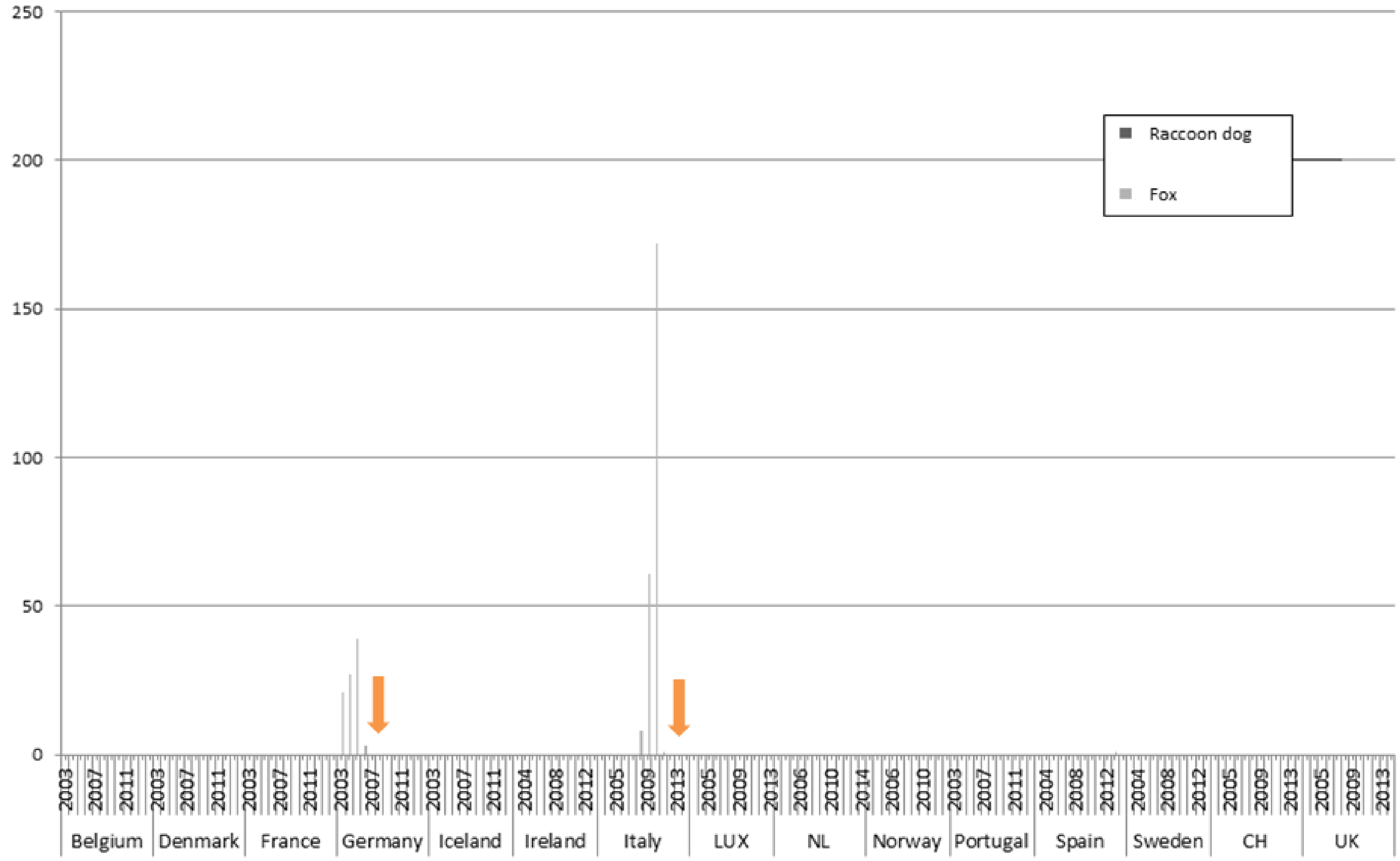
A number of cases are not visible in the graph: Austria: one case (fox) in 2004, one case (fox) in 2006; Slovakia: four cases (foxes) in 2013; Slovenia: three cases in 2012 and one case (fox) in 2013. The arrows indicate the year of acquisition of OIE rabies-free status. Data source: WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut

Figure 5: Rabies cases in red foxes and raccoon dogs since 2003 in central Europe



A number of cases are not visible in the graph: Bosnia Herzegovina (BiH): six cases (foxes) in 2012; Bulgaria: two cases in 2010, one case in 2011 and 2012, two cases in 2014, only in foxes; Croatia: one case (fox) in 2014; FYROM: four cases (foxes) in 2011; Moldova: five cases (foxes) in 2013. Data source: WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut

Figure 6: Rabies cases in red foxes and raccoon dogs since 2003 in the Balkans



The arrows indicate the year of acquisition of OIE rabies-free status. Data source: WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut

Figure 7: Rabies cases in red foxes and raccoon dogs since 2003 in western Europe

For comparison with the data reported to the Rabies Europe Bulletin (WHO), the rabies cases of foxes reported by the MSs to the ADNS system managed by the EC are presented in Figure 8.

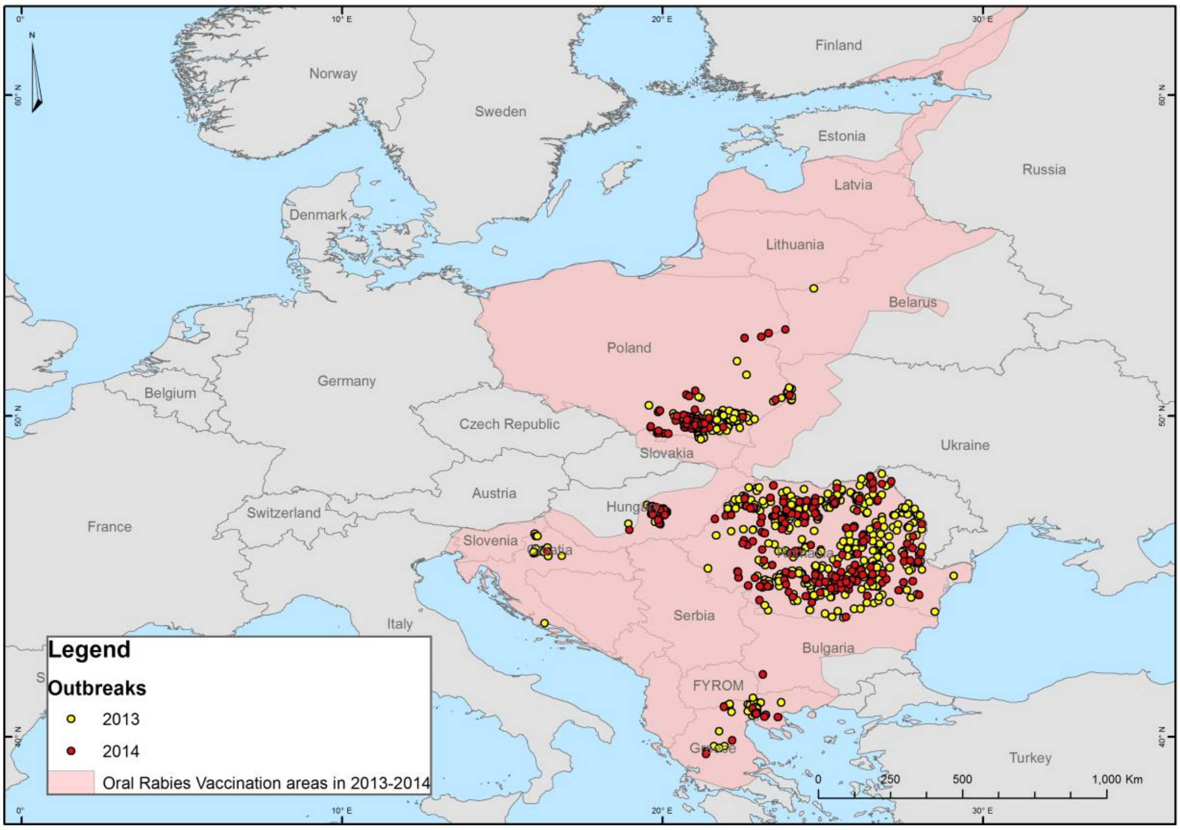
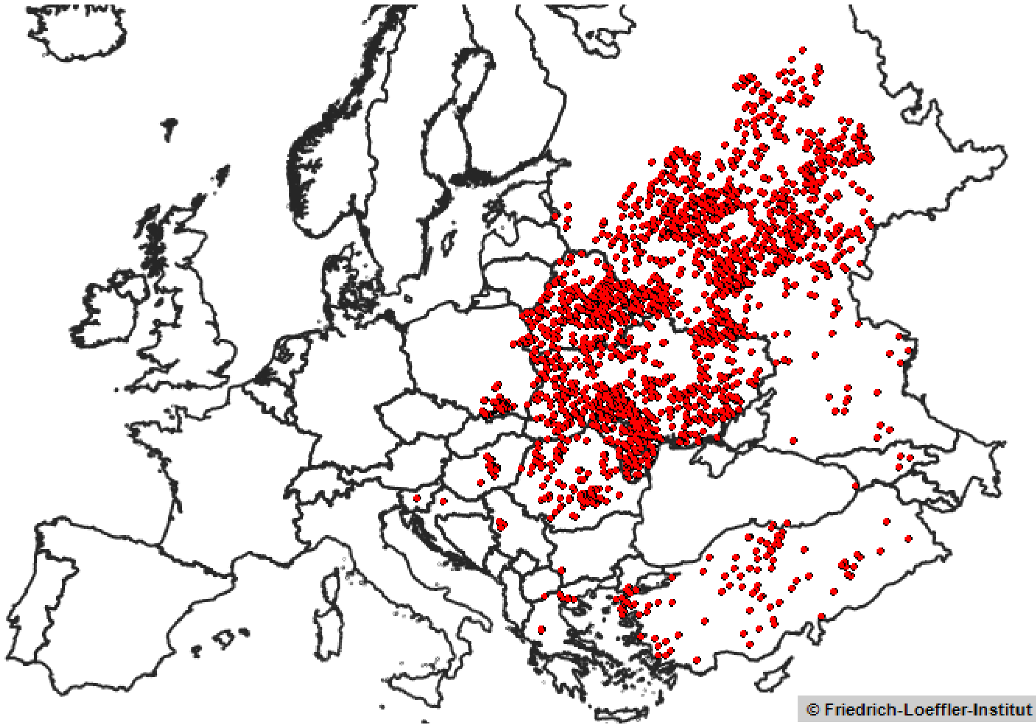


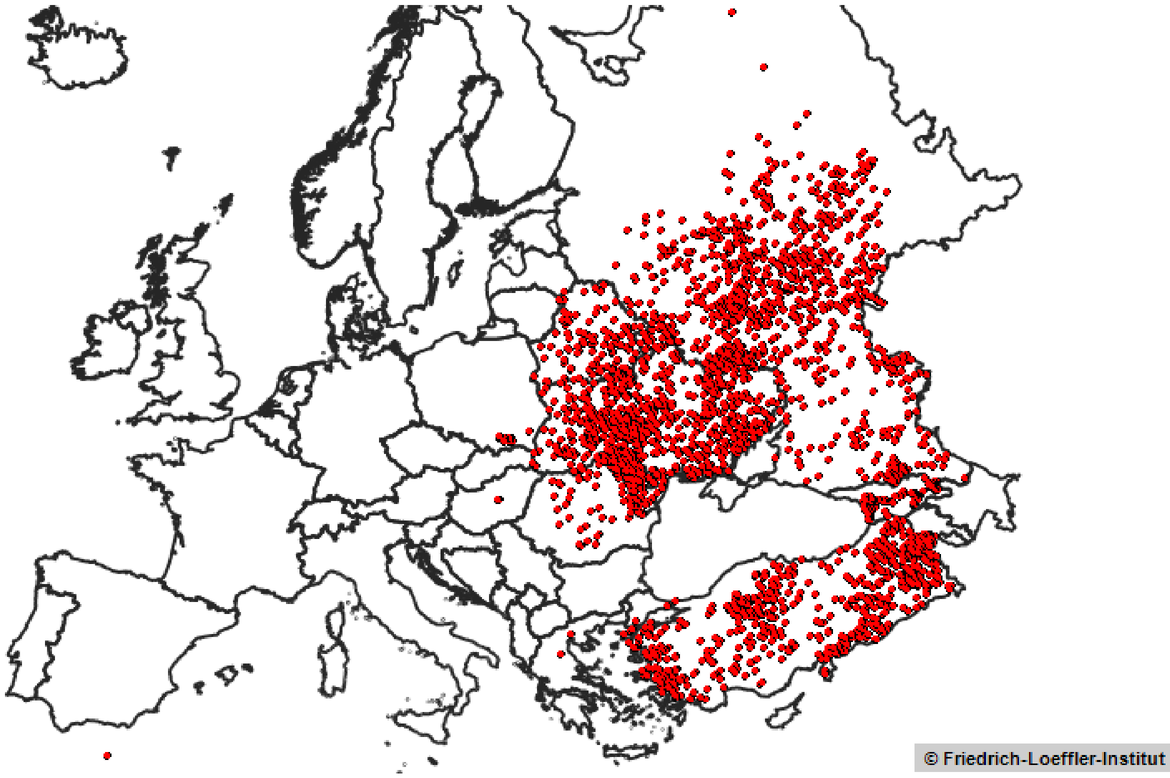
Figure 8: Rabies cases in MSs in red foxes in 2013 and 2014, according to the ADNS database

The current situation of geographical distribution of reported rabies cases in wildlife and domestic animals is displayed in Figures 9 and 10.



Data source: WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut

Figure 9: Rabies cases in wildlife in 2014



Data source: WHO Collaboration Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut

Figure 10: Rabies cases in domestic animals in 2014

3.2.4. Vaccination campaigns in Europe

In this section, an update on the ORV implementation in the MSs and neighbouring countries is provided, by considering the starting date, bait distribution density, and tests used for surveillance and monitoring.

Greece

Until 2012, Greece had been considered rabies free since 1987. Because of the detection of rabies in FYROM at the end of 2011, in a very close proximity to the Greek borders, Greece reinforced a pre-existing passive surveillance programme in 2012 and a wild fox was subsequently found to test positive for rabies in the north-western part of the country in October 2012, with other cases detected in wild and domestic animals. An EU co-financed surveillance and vaccination programme was initiated in autumn 2013. ORV targeting foxes and other carnivores, such as jackals and wolves, also considered as potential vectors or transmitters is being conducted in 24 regional units of central and northern Greece with baits distributed by aircraft. To date, only two campaigns have taken place because of delays in the tendering procedures, the first between October and December 2013, and the second between November 2014 and January 2015. The vaccine baits were dropped along parallel flight paths 500 m apart. The density of baits distributed was estimated to be over 20 baits/km^2 (25 baits/km^2 on average for the first campaign and 22 baits/km^2 for the second).

Active surveillance is conducted within vaccination areas and passive surveillance is conducted throughout the country. Tests include FATs, real-time reverse transcription-polymerase chain reactions (RT–PCRs), conventional RT–PCRs, FAVN tests, ELISAs, biomarker detection and age determination. Sequencing analysis is also being performed to differentiate between wild and vaccine virus strains.

Hungary

The occurrence of rabies in Hungary has progressively decreased since the introduction of wildlife ORV in 1992, initially at experimental level, then in the western part of the country (Transdanubia) between 1996 and 2000, and in the whole country between 2004 and 2007. Since 2008, ORV has been conducted twice a year in a 50-km-wide buffer zone along the borders of Slovenia, Croatia, Serbia, Romania and Ukraine, and in specific risk areas in eastern and northern regions using at least 20 baits/km^2. A designated number of foxes must be submitted by hunters for monitoring purposes from the vaccination areas, and tested using FAT, ELISA and biomarker testing. Foxes, jackals and any other species submitted in the framework of the passive surveillance programme from the whole territory of the country are tested using FAT and virus isolation.

Romania

Romania is one of the countries with the highest number of rabies cases in Europe. ORV targeting foxes has been carried out since 2011, with the programme involving aerial spring and autumn campaigns by using baits distributed at a density of 20 baits/km^2 and also by manual distribution. In 2012, because of political changes and legal and economic issues, the spring campaign was not performed and the manual distribution did not take place in autumn. Between 2014 and 2017, a new multiannual programme is in force which should guarantee a density of bait distribution of 25 baits/km^2 by aerial and manual distribution. The whole territory of Romania is included in this vaccination programme including areas along the borders of neighbouring Hungary, Serbia, Ukraine, Moldova and Bulgaria. Foxes caught 45 days after vaccination campaigns are used for monitoring purposes. Tests include FAT, ELISA for the seroconversion, biomarker testing and molecular tests for identification and characterisation of specific rabies viruses.

Lithuania

Large-scale ORV has been implemented in Lithuania since 2006 aiming to eliminate rabies within the country and to prevent introduction from neighbouring countries. Since 2009, the EU co-financed programme includes biannual ORV bait distribution within the whole of Lithuania and along the border of the Kaliningrad region of the RF and, since 2011, along the border of Belarus. The campaigns target foxes and raccoon dogs with baits aerially distributed in March/April and October/November with a density of 20–22 baits/km^2. For surveillance and monitoring, FAT, virus isolation, PCR, virus typing and ELISAs are used, as well as luminescent microscopy on mandibles for determining bait uptake. Mandibula and serum samples from hunted foxes and raccoon dogs, as well as suspected animals found dead, are used. Lithuania self-declared, to the OIE, its status of freedom from rabies in 2015.

Estonia

As a result of the wildlife ORV campaign conducted twice a year between 2006 and 2010 by distributing live vaccine baits with a density of 20 baits/km^2, and covering the entire area of Estonia, the number of rabies cases has rapidly decreased. The last indigenous case of rabies in the country occurred in 2011. Between 2009 and 2011, only four rabies cases in foxes and raccoon dogs were found, close to the Estonian–RF border in the south-east parts of the country. Starting from the year 2011, ORV has been restricted to buffer zones with a depth of 20–50 km along the areas bordering infected countries. The ORV programme covers the north-eastern, south-eastern and southern borders of Estonia. Since 2011, despite the intensified surveillance and testing of all rabies-suspected wild and domestic animals, no rabies cases have been found, and in spring 2013, Estonia self-declared the recovery of its rabies-free status to the OIE. The ORV campaign is evaluated by passive and active surveillance; it involves laboratory testing by FAT, PCR, RTCIT and ELISA for immunisation testing.

Croatia

Rabies has spread throughout Croatia since the detection of the first wildlife rabies case in 1977. Between 2007 and 2010, there was an increase in reported positive cases in both wild and domestic animals. Foxes are considered the most important rabies reservoir and vector in Croatia. After ORV targeting foxes was introduced in 2011, the number of positive animal cases decreased. The current multiannual ORV scheme covers the whole of Croatia and involves spring and autumn bait drops, with a density of 25 baits/km^2. Monitoring is based on testing fox carcasses submitted by hunters. Hunters are obligated to submit animals for testing. Monitoring involves the use of FAT, PCR, ELISA, modified FAVN test, biomarker detection test, bait titration and age determination.

Slovenia

Slovenia has conducted aerial distribution of baits targeting wildlife since 1995. This has led to a significant decrease in the number of sylvatic rabies cases. A multiannual ORV campaign has therefore been maintained, with two ORV campaigns performed per year, in spring (May/June) and autumn (October/November) with a density of 22–26 vaccine baits/km^2. The programme initially covered the whole territory of Slovenia, with border vaccination conducted depending on the epidemiological situation in neighbouring countries. Monitoring involves the testing of indicator animals, with hunters obligated to submit foxes. The numbers tested follow the recommendation of annually testing four foxes/100 km^2. Tests include FAT, ELISA, bait titration and testing for biomarkers.

Slovakia

After implementation of an improved ORV programme in 2000 based on aerial distribution of vaccine baits, Slovakia did not reported any rabies cases between 2006 and 2012. From 2010, the vaccination area was reduced and ORV activities were restricted to eastern regions of the country bordering infected areas in neighbouring countries. Although vaccination has decreased the number of rabies cases, it has also contributed to a rise in fox density. In 2013, three new cases in foxes were detected along the Polish border. The current campaigns cover the entire territory of the Slovak Republic, except for the areas bordering the Czech Republic and Austria and part of the area bordering Hungary. They involve annual aerial and manual bait distribution in spring and autumn. Monitoring involves testing of randomly hunted foxes and raccoon dogs after vaccination. Tests used include ELISAs, FAVN tests, RT–PCRs, restriction fragment length polymorphism (RFLP) tests and tests for biomarkers.

Poland

Poland started ORVs in 1993; however, only the western part of the country was covered by the vaccinations until 2001. ORV covered the whole territory of Poland between 2002 and 2013. This has reduced the number of positive cases. However, since 2010, rabies has re-emerged in some regions, such as the Malopolskie region in south-eastern Poland, resulting in reinfection of adjacent areas in Slovakia. In 2011, rabies also re-emerged in the Warminsko-Mazurskie region bordering the Kaliningrad Oblast of the RF. In this region, there are no natural barriers to the movement of wild animals. Because of sudden re-emergence of rabies, ORV has been intensified in high-risk areas.

In Poland, vaccination is carried out twice a year in most areas, with singular campaigns possible depending on the epidemiological situation in the area. The minimum bait density applied is 20 baits/km^2, whilst in high-risk areas the bait density is increased to 30 baits/km^2. Specific vaccination buffer zones are employed in Ukraine along the border with Poland. This buffer zone is included in the Polish rabies elimination programme. Since 2014, Poland has been carefully reducing the areas covered by vaccination as a result of the improved situation in some parts of the country.

Hunted foxes are used for surveillance and monitoring of vaccination within the vaccination areas in Poland. Tests include immunological (FAT) and serological (RFFIT/ELISA) tests and testing for biomarker presence.

Latvia

The introduction of country-wide wildlife ORV in 2005 has led to a significant decrease in reported rabies cases in Latvia. Baits are aerially distributed in the spring and autumn with a density of 22–25 baits/km^2, targeting foxes and raccoon dogs. Since 2014, ORV campaigns have focused on vaccine distribution in the eastern part of the country and its borders to prevent introduction from neighbouring Belarus and the RF. This EC co-financed programme also includes complementary vaccination activities within Belarus but not, so far, in the neighbouring areas in the RF. A defined number of foxes and raccoon dogs are collected within the vaccination area for testing the ORV efficiency by ELISAs (seroprevalence) and luminescent microscopy on mandibles for determining bait uptake. Passive surveillance is still in place in the whole territory of Latvia. Latvia self-declared its rabies-free status to OIE in December 2014.

Italy

In 1997, Italy was declared a rabies-free country, but the ORV campaigns were still carried out until 2004. In late 2008, however, rabies was detected again in the north-east part of the country, in an area bordering Slovenia. In 2009, despite the implementation of ORV campaigns targeting foxes, the infection spread westwards. In 2010, four emergency vaccination campaigns were implemented, followed by ordinary campaigns performed twice a year (spring and autumn) in 2011 and 2012. The vaccination area covered about 30 000 km^2. The last case registered in a fox dates back to February 2011, and two years later Italy re-acquired its rabies-free status. Nevertheless, Italy is under the permanent threat of disease re-introduction, with foxes considered as the main vector. Preventative campaigns are still undertaken in a reduced 20 km belt along the Slovenian border. Intentionally killed or dead foxes found within the vaccination area are tested for biomarkers and samples analysed using FAT, virus isolation and PCR.

Finland

In south-eastern Finland, from April 1988 to February 1989, a total of 66 virologically verified rabies cases were recorded (48 raccoon dogs, 12 foxes, two badgers, two domestic cats, one dog and one dairy bull). ORV in the outbreak area was started in 1988 and Finland was declared free of rabies again in 1991. To prevent incursions of rabies from the RF, Finland has been implementing ORV in the south-east areas along the border since 1991. The earlier strategy involved the distribution of 20 vaccine baits/km^2 twice a year. Because of the high density of raccoon dogs compared with foxes, the former are considered the main rabies vectors. Between 2003 and 2010, rabies vaccine baits were also distributed by hand on the area adjacent to the Russian territory once per year. In 2011, a new agreement with the Leningrad region of the RF was concluded, including EU co-financed ORV of wildlife in the RF areas along the border. The Karelian Republic of the RF and Finland also signed an agreement to create and maintain a rabies buffer zone in the northern area of Karelia bordering Finland. In 2012, baits were aerially distributed along a 40-km-wide and 350-km-long zone along the south-eastern border with Russia, with no cases detected. Buffer vaccination will continue along the south-eastern Russian border with baits distributed once a year during the autumn. Hunted animals and wild animals found dead in the field are used for surveillance and monitoring purposes. Tests include RFFIT, ELISA, FAT, RTCIT, PCR and biomarker testing.

Bulgaria

Most cases are reported from northern Bulgaria. The Balkan Mountains, which divide Bulgaria into northern and southern regions, were considered a natural barrier. Foxes are considered the main wildlife reservoirs. Between 1992 and 2013, the disease was detected in 477 animals – 276 were wild animals of which 235 were foxes. In 2007, for the first time since 1997, cases of rabies were reported in south-western Bulgaria. The first ORV campaign was implemented in 2009. The current programme involves multiannual vaccination targeting foxes in the northern and eastern regions of Bulgaria. Two campaigns are conducted per year: spring (March–May) and autumn (October–November), by using a distribution density of 20 baits/km^2. Monitoring involves the use of immunofluorescence test, ELISAs and biomarker detection tests conducted on samples collected from whole fox carcasses submitted by hunters within the vaccination areas, as well as passive surveillance within the whole country of suspected animals.

3.2.5. Vaccination programmes in the neighbouring countries

Russia

A rabies programme co-financed by the EU has been implemented in the entire territory of Kaliningrad since autumn 2007. In the years 2007–2009, ORV was implemented once a year. Since 2010, ORV campaigns have been conducted in spring and autumn. Since 2009, the distribution of baits has principally been carried out from air. The number of baits distributed is 25 baits/km^2 and the distance between parallel flight lines is 500 m. Between 2007 and 2011, the vaccine uptake rate ranged from 40 to 65 %, while the seroconversion rate ranged from 37 to 47 %.

In the Leningrad region, to prevent the introduction of rabies from infected bordering RF regions, ORV is conducted in zones along the administrative borders. In addition, ORV has been implemented along the Finnish border since 2003. ORV is conducted in the region via manual distribution of vaccines at a density of 20–25 baits/km^2. Rabies surveillance is based on investigation of suspect animals. For the monitoring of ORV campaigns, 136 and 63 target animals were tested in 2010 and 2011, respectively. The resulting bait uptake rate was at 70–80 % (EC, 2012a).

Belarus

A five-year plan (2012–2016) for rabies control has been elaborated and approved by the Belarusian government. The plan includes ORV, systematic preventative vaccination of pet animals, wild carnivore and stray animal population control, assuring emergency post-exposure prophylaxis for humans involved in animal bite accidents and enhancing awareness among the population. ORV started to be implemented in 2011 with EU financial support provided through the Lithuanian rabies programme. The monitoring of ORV in 2011 revealed a bait uptake of 59 % after the spring campaign and 58 % after the autumn campaign. Seroconversion rates were 30 % in the spring and 40 % in the autumn. In 2012, 58 890 km^2 of Belarusian territory bordering EU MSs (Latvia, Lithuania) was covered by the aerial distribution of 1.4 million vaccine baits (EC, 2012a).

Ukraine

ORV has been conducted twice a year since 2006 in limited areas, where pressure of infection is highest. The bait matrix used is produced locally, while the oral vaccine is imported. In June 2012, an agreement was signed between Poland and Ukraine on the implementation of ORV along the border, funded under the EU approved Polish programme. Vaccine is distributed by aircraft, with a 1-km distance between flight lines and a vaccine bait density of 25 baits/km^2 (EC, 2012a).

Balkan countries

The EU has provided technical assistance and supplies for the elimination, monitoring and control of rabies in several pre-accession countries through pre-accession (Instrument for Pre-accession Assistance (IPA)) funds. Since 2010, multiannual ORV programmes for eliminating fox rabies have been launched in six Western Balkan countries, starting in Kosovo and Serbia in 2010, and followed by FYROM, Montenegro and Bosnia Herzegovina in 2011, then Albania in 2013 (Yakobson et al., 2014).

In Serbia in 2000, a small experimental project of oral vaccination of foxes took place in a small area in South Banat, where fox and wolf packs live. In 2002, an oral vaccination campaign was realised by aerially distributing 20 000 baits in the north of Vojvodina, in an area of 500 km², near the Hungarian border. For 18 months, no rabies cases were reported in this area, while previously there were 14–16 cases annually (Šinković, 2010). In 2010, the Veterinary Directorate started a multiannual project of ORV of foxes and other wild carnivores, in support of a long-term programme of elimination of rabies in Serbia, co-funded by the EU (financed by IPA). Oral vaccination has been conducted twice a year, in spring and autumn, by delivering 23 vaccine baits/km^2 all over the country, with a distance of 500 m between flight lines. Additionally, vaccination of high-risk suburban areas of Belgrade was carried out with 30 baits/km^2. Monitoring of the effectiveness of oral vaccination campaigns has been carried out continuously since 2011 and was based on (i) post-mortem laboratory examination of brain tissue of target animals (foxes, jackals and other carnivores) by FAT; (ii) detection of antibodies against rabies virus in serum samples by ELISA; and (iii) detection of the tetracycline biomarkers in the mandibles for the evaluation of vaccine bait uptake. The monitoring plan covers approximately 2 500 animals per year (four target animals/100 km^2 to be tested). The number of brain samples confirmed as rabies positive has decreased from 177 in 2010, to 73 in 2011, 27 in 2012, eight in 2013 and three in 2014 (Rabies Bulletin Europe). The efficiency of the EC-funded oral vaccination of foxes conducted in Serbia was evaluated by Lupulovic et al. (2015).

In Kosovo, between 2010 and 2014, with the exception of the spring campaign in 2012, ORV campaigns were conducted twice a year, in the spring and autumn. Flight-line distance was 500 m, while in the 5-km border belt (2 000 km^2), a 1 000-m flight-line distance was used. The bait density used was 30 baits/km^2 until 2011, then 25 baits/km^2 was applied, and 250 000 baits were released for each campaign. The implementation of ORV has been monitored by FAT (Yakobson et al., 2014).

In FYROM, the last case of human rabies was recorded in 1976. Between December 2000 and March 2012, nine rabies cases were recorded in animals, of which four were foxes, four were wolves and one was a domestic cat. Virus identification was performed by the National Reference Laboratory (NRL) for rabies on brain smears and brain tissue by using WHO/OIE reference methods (direct antibody test, rabies tissue culture infection test) and conventional PCR.

The programme for oral vaccination of foxes against rabies in FYROM started in 2011. Two vaccination campaigns have been performed biannually (spring and autumn) over five consecutive years or for at least two years after the last confirmed rabies case, taking into consideration the epidemiological situation in the neighbouring countries. In 2012, only the autumn campaign was performed. The aerial distribution of the Street Alabama Dufferin (SAD) B19 vaccine strain was performed using three fixed-wing aircrafts equipped with an automatic dropping system. The distance between dropping lines was 500–600 m. A total of 500 000 baits per campaign were dropped, covering the whole territory of the country excluding water surfaces and dense urban settlements, in order to achieve a vaccine bait density of 21 baits/ km^2. Monitoring of oral vaccination was done by testing seroconversion rate by ELISA and bait uptake (by tetracycline examination). Two animals/100 km^2 were sampled within all vaccinated areas after each campaign. In 2014, more than 90 % of foxes were positive for tetracycline and 48 % were positive for antibodies.

Turkey

The increasing number of reported rabies cases in foxes, the increasing numbers of cases in domestic cattle and a peak in the winter period suggested that an independent endemic fox-mediated cycle of rabies in the Aegean region located in the western part of Turkey (36 847 km²) had been established (Ün et al., 2012). The situation has prompted the Turkish government to introduce oral vaccination against rabies of foxes in this region with financial and technical support from the EU. The first ORV campaign targeted at foxes in Turkey was conducted between 2008 and 2010. A total of approximately 610 000 baits were aerially distributed per campaign in winter at a density of 18 baits/km² with flight-line distances 1 000 m apart. Five hundred foxes were sampled from the vaccinated area and tested for rabies (FAT) and the presence of rabies virus-neutralising antibodies (RFFIT).

3.2.6. Concluding remarks

Since 2003, as a result of ORV programmes, the Czech Republic, Germany, Italy, Austria, Estonia, Latvia and Lithuania have acquired or re-acquired the status of rabies-free country. In Estonia, Latvia and Lithuania, progress has been particularly rapid, with the number of rabies cases reported annually in raccoon dogs decreasing steadily over several years from several hundred to zero. In these countries, a vaccination belt is being maintained to reduce the risk of re-introduction of rabies from neighbouring countries.

In some countries, the persistence of sylvatic rabies is attributed to the temporal discontinuity of the implementation of the ORV campaigns, i.e. when the recommended biannual campaigns are not consistently performed.

3.3. Wildlife potential reservoirs of rabies virus

In Europe and Asia, the role of rabies reservoir is played mainly by red foxes (*Vulpes vulpes*).

Raccoon dogs[11] (*Nyctereutes procyonoides*) act as rabies transmitters but their role as reservoirs is still unclear. Raccoon dog is a novel invasive species which was originally introduced as hunting game to the European part of the former Soviet Union between 1929 and 1953 and has colonised large parts of eastern and central Europe. It is the second most frequently reported species to be infected in eastern and central Europe, but the available ORV campaigns developed for foxes appear also to be efficacious in raccoon dogs (Jacevičiene et al., 2008; Niin et al., 2008; Cliquet et al., 2012; Olsevskis et al., 2012; Müller et al., 2015b).

The role of several other potential rabies reservoir species has been recently reviewed by Müller et al. (Müller et al., 2015b). These species are present in Europe and are expanding their range, which partially overlaps with areas currently infected with fox rabies and raccoon dogs. The sympatric distribution[12] of different transmitters or potential reservoirs is a risk factor for establishment or for enhancing the sylvatic rabies cycle. Rabies has been detected in golden jackals[13] (*Canis aureus*), mostly present, within the EU, in Bulgaria and Romania, although the role of this species in maintaining virus transmission has still to be clarified. ORV campaigns developed for foxes appear to also be highly efficacious in this species. A similar role is played by wolves, which are known to have re-colonised many parts of central and western Europe, where they are widely dispersed. They are not considered a reservoir species, but spillover infections from reservoir species can occur (Müller et al., 2014).

Another species of relevance is the small Indian mongoose[14] (*Herpestes auropunctatus*), an invasive species which is now present in Croatia, Bosnia and Montenegro, and is known to be a rabies reservoir on several Caribbean islands (Müller et al., 2014). Another mongoose species is the Egyptian mongoose (*Herpestes ichneumon*), but it occurs only in the western part of the Iberian Peninsula and, thus, it is unlikely that it could become infected since it is far from areas where sylvatic rabies is present (eastern Europe).

A further potential rabies reservoir species in Europe is the raccoon[15] (*Procyon lotor*), which is the most frequently reported rabies-infected species in the USA. The raccoon species is established in Germany and is expanding its range. In some (semi-)urban areas, there is a high population density of raccoons, reaching 100 head/km^2, and this is a risk factor for the establishment of an independent transmission cycle.

In the north polar regions of Eurasia, Arctic foxes[16] (*Alopex lagopus*) are considered to be another rabies reservoir. There is circumpolar transmission of the arctic variant of RABV by Arctic foxes and, although the region is currently considered free of red-fox-mediated rabies, the increasing temperatures may allow red foxes and raccoon dogs to migrate further north into tundra regions,

[11] http://en.wikipedia.org/wiki/Raccoon_dog
[12] Two species or populations are considered sympatric when they exist in the same geographic area without interbreeding.
[13] http://en.wikipedia.org/wiki/Golden_jackal
[14] http://en.wikipedia.org/wiki/Herpestes_javanicus
[15] http://en.wikipedia.org/wiki/Raccoon
[16] http://en.wikipedia.org/wiki/Arctic_fox

possibly spreading red-fox-mediated rabies over the established vaccination belt at the Finnish–Russian border (Müller et al., 2014).

Other medium-sized carnivores, such as badgers (*Meles meles*), may play a role in the epidemiology of the disease because in certain areas this species may have frequent contact with foxes and raccoon dogs, and has been shown to become frequently infected during epidemics (Westerling, 1991; Nyberg et al., 1992; Nouvellet et al., 2013).

In addition to these terrestrial reservoirs, several species of bats are also reservoirs for lyssaviruses, involving distinct epidemiological cycles. The European bat population is known to harbour a number of lyssaviruses that are sporadically detected thanks to national surveillance networks involving bat biologists and bat handlers. At the moment, the risk of rabies being transmitted by bats to non-flying mammals is considered is low in the continent, although the implementation of harmonised surveillance programmes are needed, in order to detect early any possible jump in host species of lyssaviruses circulating in bats (Dacheux et al., 2009; Cliquet et al., 2010; Schatz et al., 2013).

In the following sections, special focus will be placed on the two most important host species in Europe, foxes and raccoon dogs.

3.3.1. Fox biology and ecology

General information on red fox (*Vulpes vulpes*) biology has been extensively published by many authors (Macdonald and Reynolds, 2004). The red fox is a highly adaptable species with few specific habitat requirements, but it is most abundant in fragmentary and diverse habitats, including urban and suburban areas where its density has increased during recent decades (Contesse et al., 2004; Harris and Baker, 2006; Holmala and Kauhala, 2009). Here, red fox population density can reach 30 foxes/km^2 (Macdonald and Reynolds, 2004). An increase in fox numbers (estimated from fox hunting bags), probably resulting from successful rabies control by vaccination, has been widely reported in mainland Europe.

Territory size ranges from 40 to 700 ha, and sometimes is > 1 000 ha in a barren environment, and correlates negatively with population density (Holmala and Kauhala, 2006). Peripheral parts of adjacent territories may slightly overlap and, as territories are defended against other foxes, fights between foxes may occur. These facts suggest that rabies may spread easily between adjacent territories, especially in a high-density population.

In the northern hemisphere, mating occurs from mid-December to March. Most cubs are born in late March, but in April or May in northern Europe. Mean litter size ranges from four to five. First emergence above ground is at the age of four weeks and cubs become progressively more independent during summer. Dispersal occurs principally at the age of 6–12 months, from August to March (EC, 2002).

The mean dispersal distances in south-east Finland were 21–29 km, with the maximum distance being > 130 km (Kauhala et al., 2006); dispersal distances of almost 400 km are known (Macdonald and Reynolds, 2004). The proportion of dispersers depends on the population density and possibly on the level of human activity and control. Dispersal distances correlate positively with territory size, i.e. in areas where territories are large and population density is low foxes disperse far, which increases the probability that rabies will leap to new areas (Trewhella et al., 1988; Kauhala et al., 2006). Even one long-distance disperser per 14 000 km^2 may be enough to explain the pattern of rabies spread observed (Jeltsch et al., 1997). Hunting may decrease population density and create vacant territories and, therefore, promote dispersal (immigration to the area) and even rabies spread.

3.3.2. Rabies in foxes

The red fox has been extensively studied as a reservoir for rabies. The incubation period ranges from four days to more than 15 months, but clinical signs mostly appear between 2 and 12 weeks. Morbidity periods are usually of two to three days, but may also range from less than 1 to over 14 days (Wandeler, 1980; Aubert, 1992a). Signs may vary but commonly include anorexia, restlessness, hyperactivity, ataxia and aggression. Virus may be excreted through the saliva concomitant with or as long as one month before the onset of clinical signs.

The European epidemic of rabies spread at an annual rate of 20–60 km. Occasionally, the advancing front can increase up to 100 km in one year, this pattern probably representing unidentified sources or undetected series of infections via normal dispersal (Macdonald and Bacon, 1982; Fusaro et al., 2013). Major topographical barriers, such as mountains, wide rivers or large lakes may hinder the movement of the rabies front (Macdonald and Bacon, 1982).

When rabies penetrates a new area, there is a peak in the number of reported cases. The percentage of all the infected cases is likely to be as low as 2–10 % (Pastoret and Brochier, 1999). The prevalence may vary according to the fox status, i.e. it may reach 11 % in dead foxes during the epidemic peak and less than 1 % in living foxes collected from the field within the framework of a post-vaccination efficacy assessment (Nouvellet et al., 2013). About 60–80 % of the resident foxes perish (Voigt et al., 1985). When the number of foxes naturally decreases, the disease enters a silent phase, which normally lasts two to three years or until the local population density climbs above a threshold value. Over a wider area, these cycles are out of synchrony.

Peaks of rabies incidence observed in foxes may be explained either by density or by frequency-dependent transmission, when the stochastic and heterogeneous structures of real populations are accounted for (Morters et al., 2013). Considering the fox population density, the threshold level for rabies spread has been estimated to be 0.63 foxes/km^2 in central Europe and 0.25–0.3 foxes/km^2 in the arid steppes of south and west Siberia (Holmala and Kauhala, 2006, and related references). In most parts of Europe, fox density is high enough to account for a rabies epizootic (Holmala and Kauhala, 2006).

3.3.3. Raccoon dog biology and ecology

The raccoon dog (*Nyctereutes procyonoides*) is an alien species in Europe. It was introduced into the European parts of the former Soviet Union during the first half of the 20th century (Lavrov, 1971; Helle and Kauhala, 1991). General information on raccoon dog biology has been published by some authors in the original distribution area of south-east Russia (Judin, 1977), in the introduced area of the former Soviet Union (Nasimovič and Isakov, 1985) and in other parts of Europe (Kauhala and Kowalczyk, 2011; Kauhala and Kowalczyk, 2012). The distribution area of raccoon dogs in Europe is reported in Figure 11.

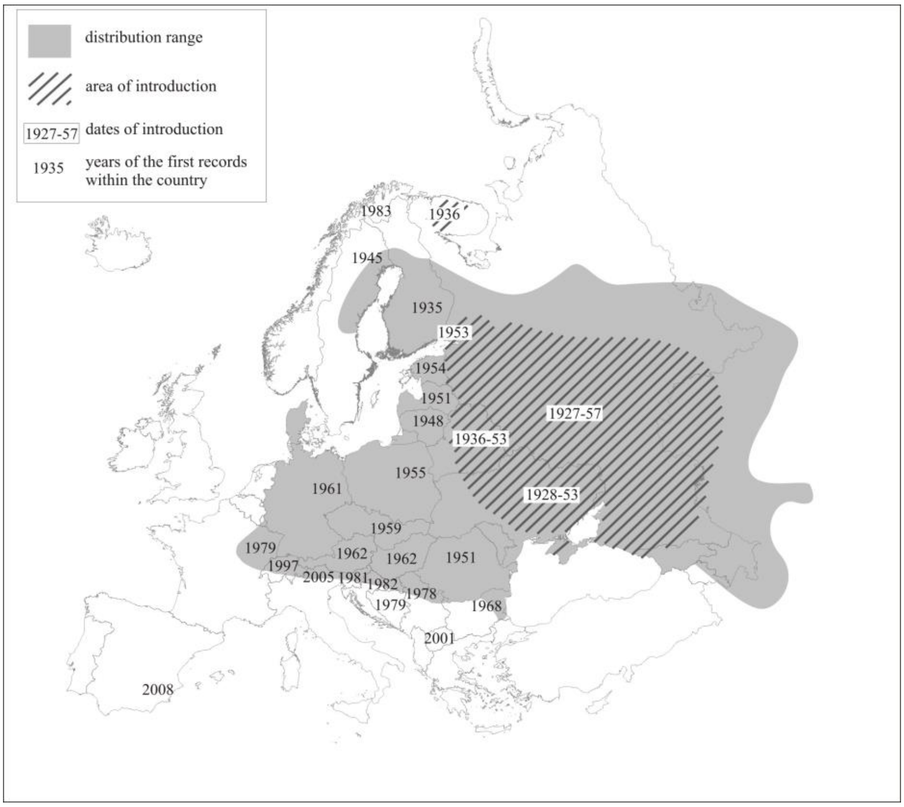


Figure 11: Distribution area of raccoon dogs in Europe (Kauhala and Kowalczyk, 2011)

The raccoon dog is a medium-sized carnivore of the Canidae family. It is the only member of the family which hibernates in areas where winters are harsh. In Finland, the hibernation period usually lasts from November to March, but raccoon dogs may be active even in mid-winter if the weather is mild. They usually stay in their dens when the temperature is below –10 °C, the snow depth is more than 35 cm and the day length is less than seven hours (Kauhala et al., 2007). They may use badger (*Meles meles*) setts and even hibernate together with badgers. In summer, raccoon dogs weigh about 5 kg but almost double their weight during autumn because they gather large fat reserves before hibernation (Kauhala, 1993).

The raccoon dog is a highly adaptable species with a very high reproductive potential (Helle and Kauhala, 1995) and extremely omnivorous diet (Sutor et al., 2010). It is currently the most common medium-sized carnivore in many areas in northern Europe and its numbers are also increasing rapidly in central and western Europe (Drygala et al., 2008). It is most abundant in a landscape which is a mosaic of small patches of deciduous and mixed forests, gardens, meadows and fields, and also often occurs in wetlands (Nasimovič and Isakov, 1985; Kauhala and Auttila, 2010; Kauhala et al., 2010). It prefers areas with sparse canopy but abundant undergrowth where food and shelter are easy to find. Raccoon dog density is low in coniferous forests.

Raccoon dogs are monogamous and live in pairs in their home ranges. They also move and rest together all year round (Kauhala, 1993). Home-range size varies from 100 to 700 ha in southern Finland (Kauhala, 1993; Kauhala et al., 2006; Kauhala and Auttila, 2010), to about 200 ha in northern Germany (Drygala et al., 2008) and 500 ha in Białowieża, Poland (Jedrzejewska and Jedrzejewski, 1998). Home-range size is related to the availability of food provided by the habitat (Kauhala and Auttila, 2010): in a rich habitat with abundant food resources, home ranges are small and population density is high. Population densities vary from 0.3 to 2.0 adult raccoon dogs/km^2 in Europe (Kauhala and Kowalczyk, 2011). Territory density varies from 0.14 to 1.0/km^2. Threshold territory density for rabies epizootics has been estimated to be 0.59–0.69/km^2, depending on whether raccoon dogs hibernate or not (Singer et al., 2009). Threshold densities are lowest when raccoon dogs do not hibernate. The threshold densities may, however, vary between landscapes.

Not all parts of the territory are used with the same frequency: in south-east Finland, the core area where the animals spend 50 % of their time constitutes only 18 % of the total home range, and 35 % of the locations are from the borders of the home range (Kauhala et al., 2006). Overlap between home ranges of adjacent raccoon dog pairs is smallest during the pup-rearing season, but peripheral parts of adjacent territories may overlap to some extent in autumn (Kauhala et al., 1993; Kauhala et al., 2006). These facts suggest that rabies may easily spread between adjacent territories, especially when population densities are high in autumn.

Raccoon dogs are active mainly during dark hours. However, particularly in summer, female raccoon dogs also forage during daytime. They need a large amount of energy to able nurse their large litters (up to 12 pups). Males stay in the den with pups and do not carry food to the den (Yamamoto, 1987; Kauhala et al., 1998a). Raccoon dogs are opportunistic omnivores and prey species and food sources are very diverse: mammals (mainly rodents and shrews), frogs, some birds, insects, fruits, berries and scavenged items are important food sources (Ivanova, 1962; Kauhala et al., 1998b; Sutor et al., 2010). Their diet varies with habitat: the more diverse the habitat, the more diverse is the diet (Kauhala and Ihalainen, 2014). The food niche of the raccoon dog is nearly twice as wide as that of the fox (*Vulpes vulpes*).

Females have a single oestrus per year and can reproduce for the first time at the age of one year: 66 % of one-year-old females give birth (Helle and Kauhala, 1995). The proportion of reproducing females increases with age. Mating occurs from February to April, and the youngest females come into oestrus later than older ones. The breeding season is also affected by climate and is later in northern areas than in the more southern areas. Gestation lasts about 61–63 days, with most pups being born in May. Breeding dens, often old badger setts, are used (Kowalczyk et al., 2008). The litter can also be under buildings, in a hollow tree, under tree trunks and sometimes even under a fir tree (Kowalczyk and Zalewski, 2011). Mean litter size is from 8 to 10 pups in Europe, and it is highest among middle-aged females (Helle and Kauhala, 1995; Kowalczyk et al., 2009). The first emergence above ground is at the age of four weeks and pups start to eat a large variety of solid items soon after that. They start to move around at the age of two months and become progressively more independent during summer.

The annual mortality rate of the population is high, being about 80% (88–89 % for juveniles and 51–54 % for adults) in a stable population (Helle and Kauhala, 1993). About 50 % of the juveniles die during their first summer. The mortality rate is lowest at the age of two to four years. The maximum life span is seven to eight years, but can be, exceptionally, 10 years.

Dispersal occurs principally at the age of four to six months (from August to November). In autumn before dispersal, home ranges of juveniles are larger than those of adults. Young males usually disperse a little further than females. The mean dispersal distances in south-east Finland were 14 km and 19 km for males and females, respectively (Kauhala et al., 2006). Nine per cent of juveniles wander > 40 km, and the maximum distance observed in south Finland was 145 km (Kauhala et al., 1993) and 91 km in north Germany (Drygala et al., 2010). In a stable population, only juveniles disperse, but in a colonising population adults also wander, sometimes hundreds of kilometres (Mikkola, 2011). Hunting may decrease population density and create vacant territories and therefore promote dispersal (immigration to the area). Movements after hibernation and dispersal of young have been suggested as a cause for the seasonal peaks of rabies incidence during spring and autumn (Reinius, 1992; Holmala and Kauhala, 2006).

3.3.4. Rabies in raccoon dogs

Historically, the red fox has been considered as the only rabies reservoir species in Europe. However, since the end of the 1990s, raccoon dogs have become the second most affected wild carnivore species after the red fox in north-east Europe, in particular in Baltic countries and Poland (Vanaga et al., 2003; Niin et al., 2008; Singer et al., 2009; Smreczak et al., 2009; Zienius et al., 2011). In Finland, the raccoon dog is considered to have been the primary rabies virus vector and victim during the epizootic in 1988–1989 (Westerling et al., 2004); however, in Finland, rabies was eliminated in 1991 (Sihvonen, 2001).

In the period from 1990 to 2014, rabies has been confirmed in 4 743 raccoon dogs (4.3 % of all rabies cases in wildlife) in eight countries of Europe (Rabies Bulletin Europe, WHO).

According to the results of studies carried out in Lithuania, the largest proportion of rabies cases (28.1%) in the raccoon dog population is registered in October–November; another seasonal rabies peak in raccoon dogs has been observed in the March–May period (21.7 %) (Zienius et al., 2007).

The results of an experimental study in which different doses of raccoon dog-adapted rabies virus were used to challenge raccoon dogs (n = 11) revealed that the susceptibility of raccoon dogs to a particular rabies virus was significantly higher than the susceptibility of foxes. On average, the incubation period was 22 days; however, a negative relationship between the length of the incubation period and the challenge dose was observed. During the clinical period, the raccoon dogs were agitated and aggressive, and the virus was detected in their salivary glands more frequently than in the salivary glands of foxes (Botvinikin et al., 1983). Other experimental studies were performed for the evaluation of three oral vaccines in raccoon dogs (Schuster et al., 2001; Cliquet et al., 2006; Cliquet et al., 2008a). In the first study, which was conducted on a vaccine strain that is no longer used (Schuster et al., 2001), all raccoon dogs (n = 5) succumbed to rabies, on average, 11.2 days post infection (range: 10–13 days) after challenge with the coyote rabies virus strain. In the second study (Cliquet et al., 2006), raccoon dogs (n = 11) used as a control group were challenged with rabies virus from naturally infected coyote. All raccoon dogs died between 15 and 19 days (17 days on average ± 1.4 days) after challenge. First signs were recorded nine days after challenge and were sometimes preceded by strange behaviours without clinical signs. The most frequent signs were anorexia, excitement, balance problems and prostration. In the third study, raccoon dogs (n = 9) used as a control group were challenged with rabies virus from naturally infected coyote. All raccoon dogs died on average 18.9 days after challenge (Cliquet et al., 2008a; Cliquet et al., 2008b).

The rapidly increasing raccoon dog population in Europe strongly increases the risk of rabies recurrence in many countries (Singer et al., 2009; Sutor et al., 2014). Control strategies should thus be developed to cope with the new situation. Oral vaccination appears to be an effective control measure of rabies in raccoon dogs: the safety, immunogenicity and efficacy of oral rabies vaccine baits were experimentally evaluated in raccoon dogs showing a good protection (Cliquet et al., 2006; Cliquet et al., 2008a). The field experience, e.g. the rapid decrease in rabies cases reported in raccoon dogs in the Baltic countries after ORV implementation, also demonstrates that oral vaccination is effective in eliminating the disease in this species.

Nevertheless, in order to gain a better understanding about rabies infection in raccoon dogs and in other host species, in terms of, for example, the length of the incubation period and mortality, more studies on rabies pathogenesis in these species are needed.

3.3.5. Fox and raccoon dog population counting

The knowledge of fox and raccoon dog population densities could be helpful when starting the ORV campaigns, although not a prerequisite for ORV. Moreover, the available methods for population estimation (EC, 2002) are not fully reliable.

Maximum population density can be, however, estimated from territory size (determined using, for example, telemetry) in species, such as the red fox and the raccoon dog, which are territorial with mainly non-overlapping territories. Because foxes and raccoon dogs usually live in pairs we can assess that there are two adults in each territory. If the whole area is covered by territories, the maximum adult density can be calculated according to the formula: 'Density (adults/ha) = 200/territory size (ha)'. In areas where density is very high, there can be more than two adult foxes per territory, but there will always be areas without territories or territories with single animals. These facts counteract each other. In summer and autumn, the density is higher because of the juveniles.

3.4. Interaction of foxes and raccoon dogs

The habitat use and how it is shared by different host species should be taken into account in order to understand the dynamics of infectious diseases, in this case rabies, and when planning bait vaccinations against rabies. Studies modelling rabies dynamics in a population with two vector species are available (Singer et al., 2009), but these results should be confirmed by field evidence.

3.4.1. Densities

The transmission rate of rabies (the average number of susceptible animals infected by each rabid animal) is determined by population density, home range overlap, activity and habitat use of vector species (Wandeler, 1980; Holmala and Kauhala, 2006; Singer et al., 2009). As an explanatory example, a radio-tracking study from south-east Finland (Kauhala et al., 2006) indicated that adult fox density was 0.35/km^2 and fox territory density was 0.15/km^2 (two adults in one territory). Adult raccoon dog density was 0.51/km^2 and raccoon dog territory density was 0.26/km^2. The combined densities of the two species were > 0.85 adults/km^2 and > 0.40 territories/km^2. Threshold densities, i.e. the lowest density where there is a 50 % risk for rabies epizootic, have been calculated by Singer et al. (2009). The threshold adult density with two vector species was estimated to 0.52–0.64/km^2, and the threshold territory density[17] to be 0.26–0.32/km^2. The lower limits are applicable when raccoon dogs do not hibernate and they may transmit rabies during winter. The upper limits apply when raccoon dogs do hibernate and die of rabies in their dens during winter. The joint densities of the two species were, in both scenarios above the threshold values and thus high enough for a rabies epizootic to persist in the area. Furthermore, population densities in autumn (adults + juveniles) are much higher than the densities given above because of the high reproductive potential of both species (Helle and Kauhala, 1995; Kauhala, 1996).

In Germany, the hunting bag of raccoon dogs increased from almost zero in 1994/1995 to 35 000 in 2008/2009, and raccoon dog density is currently about 1.0 adult/km^2 (Drygala et al., 2008; Sutor et al., 2014). In Poland, the adult density is 0.17–0.7/km^2 (Jedrzejewska and Jedrzejewski, 1998; Goszczyński, 1999; Kowalczyk et al., 2008). Especially in Germany, raccoon dogs pose a high risk and should be taken into account when planning vaccinations, because territory density would be about 0.5/km^2, close to the minimum threshold value (0.59) for raccoon dogs on their own. Together with foxes, the joint territory density and the risk for a rabies epizootic is high. The situation in eastern Poland is similar to that in south-east Finland (fox density 0.3/km^2).

[17] Threshold adult density (density of adult individuals) is about twice threshold territory density (density of territories), because there are usually two adult animals, a male and a female, in one territory, and adjacent territories usually do not overlap.

3.4.2. Overlap of home ranges and activity

In south-east Finland, near the Russian border, one study reported that the mean home range of raccoon dogs was 390 ha (and 260 ha in summer) and that of foxes was 660 ha (Kauhala et al., 2006). There was a high level of overlap between the home ranges of the two species. Both raccoon dogs and foxes are mainly active during dark hours and their nightly routes cross each other frequently, indicating that individuals may have frequent contact (Kauhala and Holmala, 2006). During hibernation of raccoon dogs, the probability of contact is lower. In western Europe, they do not, however, hibernate. In addition, in southern Finland, they may be active during mild winters and, even when they hibernate, they may change their den several times during winter (Kauhala and Holmala, 2006). Thus, the probability of contacts between healthy animals is high, and probably even higher if one of them has rabies, because rabies may cause changes in behaviour (Westerling, 1991).

Home ranges of raccoon dogs and foxes also overlapped with those of other medium-sized carnivores, such as the badger (*Meles meles*) and free-roaming domestic cats (Kauhala et al., 2006). In the case of rabies epizootics, individuals would easily transmit rabies both within and between species. The role of the badger as a vector of rabies may be greater than previously assumed because badgers have been shown to have large home ranges which overlap the home ranges of several other medium-sized carnivores (Kauhala et al., 2006).

3.4.3. Habitat use

Raccoon dogs favour a landscape which is a small-scale mosaic of meadows with tall undergrowth, small patches of deciduous and mixed forests, fields and gardens (Holmala and Kauhala, 2009; Kauhala et al., 2010). Foxes are opportunistic and occur almost everywhere, also in urban areas, villages, gardens and agricultural landscapes (Macdonald and Reynolds, 2004). Foxes and raccoon dogs often move in the same habitats and thus use the same parts of their overlapping home ranges, making contacts between species likely (Kauhala and Holmala, 2006). Both species frequently visit gardens, and there is a risk that they are also in contact with cats and dogs and even humans. Furthermore, badgers and raccoon dogs frequently use the same habitat patches, which further increases the risk of contacts (Holmala and Kauhala, 2009; Kauhala and Auttila, 2010).

Foxes can dig their own dens, but they also use dens of other species, especially old badger setts. Raccoon dogs also frequently use badger setts (Kowalczyk et al., 2008). Raccoon dogs and badgers may even hibernate in the same dens (Kauhala and Holmala, 2006). The use of the same dens increases the probability of contacts between species.

3.4.4. Dispersal

The dispersal of host species largely determines the spread of rabies from one locality to another. Rabies may spread fastest where population densities are low because dispersal distances tend to correlate negatively with population density (Trewhella et al., 1988; Holmala and Kauhala, 2006). Juvenile raccoon dogs disperse in autumn, mainly between August and October. Foxes usually disperse at the age of 6–12 months, i.e. between August and March. Both species also move a lot in late winter/spring: raccoon dogs are active after hibernation in spring and foxes may still be dispersing, and males are on the move because of the mating season. The seasonal peaks in the movements of host species should be taken account when planning vaccinations.

3.5. Oral vaccination of foxes and raccoon dogs against rabies

ORV of foxes and/or raccoon dogs is the most effective method of eliminating terrestrial rabies in wildlife (EC, 2015).

The strategy of ORV initially applied to red fox proved to be effective in raccoon dog populations in several countries (Finland, Baltic countries and Poland) where raccoon dog populations play a significant role in rabies epidemiology. The first successful field trial to adapt a 'fox strategy' for elimination of rabies in wildlife in areas with a significant raccoon dog population was carried out in Finland in 1988. The results of this field trial did not show significant differences between raccoon dogs and red foxes in seroconversion rate or in bait uptake (Nyberg et al., 1992). Later, the ORV was successfully used in the Baltic region and Poland.

The scope of the monograph of the European Pharmacopoeia 'Rabies vaccine (live, oral) for foxes and raccoon dogs' (European Pharmacopoeia, 2014) has been extended to raccoon dogs and tests for safety and immunogenicity for raccoon dogs have been added where raccoon dogs are a target species. With regard to the choice of vaccine virus, the characterisation of the virus strain by gene sequencing has been added, which is an essential tool for characterising virus strains and is of great utility in differentiating between adapted and pathogenic rabies virus strains.

3.5.1. Vaccines

The anti-rabies vaccines used for wildlife immunisation are based on live replication-competent vaccine viruses. The liquid vaccine is incorporated in a capsule or a plastic sachet contained in a bait delivered by the oral route throughout fox and raccoon dog habitats. The bait casing is adapted to the target species with regard to taste (appetence and smell), size and texture, and contains a biomarker (generally tetracycline) to mark the teeth and bones of bait consumers.

All rabies vaccines in use in Europe are derivatives of the original SAD strain, isolated from a naturally infected dog in the USA in 1935. This strain was then passaged *in vivo* in mouse brain cells (ERA strain). Currently, four vaccine strains are authorised in the European market:

- The SAD Bern vaccine was adapted from the ERA strain after various *in vitro* passages in BHK cells. This strain, which is considered to be the ancestor strain of all available vaccines, was provided to other European laboratories in the 1980s for further vaccine development.
- The SAD B19 vaccine was developed from SAD *in vitro* selections using cloned baby hamster kidney (BHK21) cells.
- The SAG2 vaccine (Street Alabama Gif) was selected from the SAD Bern strain after two successive *in vitro* mutations of the Arginine 333 codon by using specific anti-rabies glycoprotein-neutralising monoclonal antibodies.
- The V-RG vaccine (Vaccinia Recombinant Glycoprotein) is a vaccinia virus (Copenhagen strain) recombinant coding for the rabies glycoprotein gene from the ERA strain.

Another vaccine strain, named SAD P5/88, derived from the SAD Bern strain, that was produced in Germany (Kintscher et al., 1990) is not included in the present assessment as the marketing of this strain has been discontinued in recent years.

Until recently, there was little information on the genetic basis of the different SAD-derivative strains used for oral vaccine production for understanding their attenuation. With the development of full-genome sequencing and next-generation sequencing methods, it has been possible to characterise the viral vaccine strains and the genetic composition of the SAD B19, SAD Bern and SAG2 vaccines (Geue et al., 2008; Cliquet et al., 2013; Picard-Meyer et al., 2014). The study undertaken by Geue et al. in 2008 demonstrated that the majority of the currently used commercial attenuated rabies virus vaccines appear to be derived from SAD B19, rather than from SAD Bern, and that two SAD vaccine strains appear to consist of mixed genomic sequences.

It is hypothesised that natural genetic evolution of the original strain due to serial cell culture passages leading to strain subpopulations or a possible change or replacement of the master seed virus and/or the working seed virus could explain the results obtained for SAD B19 and SAD Bern, respectively. Such results should be considered and interpreted carefully by the regulation authorities prior to any extrapolation regarding efficacy and safety issues. These data clearly underline the necessity for vaccine producers to strictly adhere to the requirements of the European Directorate for the Quality of Medicines (EDQM),[18] WHO (WHO, 2013) and OIE (OIE, 2014) to guarantee the identity and the stability of the vaccine strains (in particular the master seed virus) used during the production process.

In the EU, rabies vaccines used for oral vaccination need to be licensed or registered and to comply with the requirements of the European Pharmacopoeia monograph (European Pharmacopoeia, 2014) and with national regulations for veterinary biologicals, with particular regard to efficacy, safety and potency of the vaccine virus and to genetic strain stability.

[18] www.edqm.eu

The authorised vaccine preparations in the EU are Raboral by Merial (V-RG strain), Rabigen by Virbac (SAG2 strain), Fuchsoral by IDT (SAD B19 strain) and Lysvulpen by Bioveta (two dominant sub-populations of SAD Bern- and SAD B19-'like' viruses[19]).

Since the 2002 report (EC, 2002), a new vaccine (Lysvulpen) has been put on the market, and Rabifox (SAD P5/88 vaccine) has been removed from the market.

The main characteristics of vaccines provided by the manufacturers are reported in the EC (2002) report. With regard to the Lysvulpen vaccine, the characteristics of the vaccine reported by the producer are available online.[20]

Efficacy

The efficacy assessment of oral rabies vaccines is carried out during the production process under controlled laboratory conditions in which a challenge test is carried out on experimental animals of the target species previously vaccinated by the oral route with the virus titre corresponding to the minimum releasing titre. The European Pharmacopoeia (2014) monograph stipulates that efficacy is tested using the vaccine field dose on a minimum of 35 target animals (25 vaccinees and 10 controls) that are all challenged at least 180 days after vaccination.

Faced with the increase of rabies cases in raccoon dogs at the beginning of the 2000s and the involvement of northern European countries in ORV programmes, specific investigations to assess the efficacy and also the bait acceptance of available oral vaccines for raccoon dogs have been conducted under controlled conditions (Cliquet et al., 2006; Cliquet et al., 2008a). These studies demonstrated the satisfactory appetence of Rabigen SAG2 and Raboral V-RG vaccines in experimental animals and their ability to produce specific humoral responses against rabies allowing animals to survive a severe rabies challenge. Another study conducted with SAD P5/88 (Schuster et al., 2001), in which caged raccoon dogs were vaccinated by direct instillation in the oral cavity, showed satisfactory results with regard to the protection of animals. On the basis of these experimental results, it is concluded that the current attenuated and recombinant marketed vaccines are efficient and appetent for raccoon dogs as well as for foxes as previously demonstrated (EC, 2002).

The effectiveness of the four marketed oral vaccines is also largely proven in the field, and epidemiological data have demonstrated that the existing vaccines have contributed substantially to the success of rabies control and elimination in several European countries (Matouch et al., 2006; Mulatti et al., 2011; Zienius et al., 2011; Cliquet et al., 2012; Müller et al., 2012; Freuling et al., 2013; Mulatti et al., 2013). However, very few analyses have been published regarding the cost-effectiveness of ORV programmes for achieving rabies elimination (Cliquet et al., 2012).

Immunity: 'booster' effect and maternal immunity

The information reported in the previous EC report issued in 2002 about the booster effect and maternal immunity in foxes is still valid (EC, 2002). In fact, as regards the booster effect of a second vaccination, there is evidence that no immunological reason for performing such a double vaccination during an ORV campaign exists.

With regard to maternal immunity of fox cubs, experimental studies showed that interference between passively and actively acquired immunity may occur in cubs vaccinated orally with live vaccines. This interference appeared to be much lower when recombinant vaccines were used.

However, the parameter dealing with maternal immunity has never been concretely considered when planning and implementing ORV, since young suckling foxes (e.g. up to eight weeks old) are not able to consume baits (Cliquet et al., 2005); therefore, it is of no practical relevance during ORV implementation for the vaccination of fox and raccoon dog juveniles.

No experimental studies relating to the effect of a booster vaccination and to the impact of maternal immunity have been undertaken on raccoon dogs.

[19] http://www.uskvbl.cz/en/authorisation-a-approval/marketing-authorisation-of-vmps/list-of-vmps/authorised-by-national-and-mrdc-procedures?letter=L
[20] http://www.bioveta.eu/en/products/veterinary-products/lysvulpen-por-ad-us-vet-1.html

Safety

The safety of oral vaccines is of major importance since they are live replication-competent vaccines to be delivered in large-scale field areas located near the human environment. The WHO has established recommendations dealing with safety testing in target (red fox and raccoon dog) and non-target species, in particular in carnivore and rodent species and also in non-human primates. The European Pharmacopoeia (2014) monograph stipulates that safety is tested using the vaccine field dose on 20 target animals and a dose containing 10 times the vaccine maximum dose on 10 target and 10 non-target species and to observe the animals for at least 180 days. Molecular characterisation of rabies isolates from animals sampled in vaccinated areas should be considered as part of any ORV programme, in order to distinguish field rabies virus from vaccine-associated cases (De Benedictis et al., 2013; WHO, 2013).

Some modified-live rabies virus oral vaccines may have residual pathogenicity, depending on the level of attenuation of the viral strain (WHO, 2013), as the successive selections from the original strain may produce hazardous and uncontrolled results, and variants may remain pathogenic both in target and non-target species (Vrzal, 2013; WHO, 2013). A double mutation in a portion of the genome (codon 333) whose integrity is required for pathogenicity by the oral route allowed the selection of a highly attenuated virus (Mähl et al., 2014). The recombinant vaccine (V-RG) cannot exhibit residual rabies pathogenicity because it contains only single non-virulent gene products; the already attenuated Copenhagen strain was even more attenuated after the replacement of the thymidine kinase gene by the complementary DNA (cDNA) of the rabies glycoprotein conferring rabies immunity (Kieny et al., 1984). Nevertheless, recently, two human vaccinia infections after contact with a raccoon rabies vaccine bait have been described in the USA, both in persons trying to remove baits from the mouths of dogs (Rupprecht et al., 2001; CDC, 2009).

Recently, six vaccine-induced rabies cases in red foxes in vaccinated areas in Germany (four cases in 2001, 2002, 2004 and 2005) and Austria (two cases in 2004 and 2006) (Vanek et al., 2004; Müller et al., 2009) were caused by SAD B19 and SADP5/88 virus strains (adaptation of SAD Bern to the BHK21 clone). Salivary glands of positive foxes were not investigated. In Canada, nine ERA-BHK21 vaccine-associated cases were recorded during rabies surveillance following ORV in four red foxes, two raccoons, two striped skunks and one bovine calf (Fehlner-Gardiner et al., 2008). Only one animal was tested for salivary excretion and was negative. More recently, a paper demonstrated that a vaccine-associated virus might have been present in the brain tissue and also in the salivary glands of a naturally infected fox in an area vaccinated with the SAD B19 vaccine in Slovenia (Hostnik et al., 2014).

The published safety data regarding the new vaccine preparation on the EU market (Lysvulpen vaccine) are related to a study on 10 rhesus macaque monkeys. Each animal was vaccinated orally with 2.0 mL of a 10^9 tissue culture infectious dose ($TCID_{50}$) of a Bio-10-SAD Bern strain and monitored for 90 days by clinical observation and rabies neutralising antibody determination. Brain tissue samples were analysed after euthanasia and were all negative for rabies virus. All animals developed an immunological response post vaccination (Vrzal, 2013).

Another potential safety issue is the ecotoxicity and the potential antimicrobial resistance (AMR) induced by the tetracyclines used as biomarkers in the baits that are spread in the environment. It is known that, because of their extensive use in veterinary medicine, tetracyclines are found in different ecological compartments, they can be released in active forms into the environment via urine and faeces from humans and animals and thus they can (i) promote the development of antibiotic-resistant microorganisms; (ii) inhibit the growth of terrestrial and aquatic species; and (iii) cause endocrine disruption of aquatic species (Daghrir and Drogui, 2013). Although some research groups reported background tetracycline content in teeth samples collected in areas where no ORV has ever been performed (unpublished data), no full risk assessment has been performed so far to evaluate the ecotoxicological and AMR risk of tetracycline use with vaccine baits.

Another aspect linked to environmental safety is related to the plastic capsules inside the baits containing the vaccine liquid, which in all vaccines available on the market are presently still made of non-biodegradable plastic. It should be ensured that, in future, these capsules are made of biodegradable material, to avoid environmental contamination.

3.5.2. Vaccine and bait stability

The physical resistance and stability of the bait matrix, as well as the stability of the vaccine titre under different conditions, have been poorly investigated, although some data from the producers and from different research groups can be found in the literature (Vos and Neubert, 2002; Maciulskis et al., 2008; Hostnik et al., 2011). Different approaches, such as laboratory versus field conditions, and different protocols for testing vaccine titre have been applied. Indeed, no standardised protocol is available for testing vaccine bait resistance to open-air exposure.

The bait stability has been added to the scope of the monograph of the European Pharmacopoeia (European Pharmacopoeia, 2014) in order to increase awareness regarding this unique aspect of this vaccine (vaccine incorporated in bait to attract the target species) and its unique method of administration (vaccine released in the fox and/or raccoon dog habitat and taken up actively by the target animals). The European Pharmacopoeia monograph recommendation relating to bait stability requires two tests consisting of an incubation of the baits at 25 °C for five days for further vaccine virus titration (the virus titre must be at least the minimum virus titre stated on the label) and heating them at 40 °C for one hour (the bait should remain in its original shape and adhere to the vaccine container).

It should be noted that the bait in an ambient air temperature of 40 °C (in the obscurity of an electric oven) receives the energy from the ambient air by conduction (contact) only. By contrast, when exposed to the sun, it also receives energy by radiation, provoking a significant increase in the temperature near the bait, explaining why experimental and field results may show different results (Maciulskis et al., 2008).

Vaccine baits are distributed by aerial means onto the ground and remain for a certain time in the field exposed to different local conditions of landscape (grass or solid ground) and to different climatic conditions of temperature and humidity (sunlight, shade, half-shade and/or rainfall) before they are consumed by wild animals. Therefore, the bait casing should remain intact when exposed to the prevailing climatic conditions (no melting or degradation of bait) until consumption. Most bait uptake studies have demonstrated that more than 50 % of vaccine baits disappear within one week and more than 80 % are consumed by one to three weeks after distribution (Artois et al., 1987; Brochier et al., 1987; Frisch et al., 1987; Balbo and Rossi, 1988; Nyberg et al., 1992). In a recent study, at least 80 % of the baits had disappeared within one week of distribution, independent of the season (spring or autumn) (Vos et al., 2004). Moreover, foxes usually need three nights to search their entire territory for food (Artois et al., 1990); therefore, baits should ideally remain stable for at least three days under any weather conditions.

The lack of stability of vaccine baits could compromise the effectiveness of vaccination campaigns. This possibility arose in 1988 and 1989, when discrepancies between bait uptake and seroconversion rates in foxes after ORV campaigns were identified (Balbo and Rossi, 1988). Indeed, the effectiveness of a vaccine depends not only on the virus titre (all oral rabies vaccines being live viruses), but also on the integrity of the bait casing, as animals do not consume vaccine baits when the appetent casing of the bait has melted.

Studies demonstrated the negative impact of temperature on the viral titre and on bait matrix integrity (Bingham et al., 1999; Lawson and Bachmann, 2001; Vos and Neubert, 2002; Maciulskis et al., 2008).

The published data showed that the V-RG vaccine bait is highly thermostable, even if exposed to high temperatures (30–35 °C) (Masson et al., 1999; EC, 2002). However, in a recent study, it was shown that field microenvironments can considerably influence the stability of even the V-RG strain. Vaccine titres decreased an average of $10^{3.3}$ $TCID_{50}$/mL within four weeks in open-field or forest–edge microenvironments. The greatest reduction in vaccine titre was observed in the first two weeks of field exposure ($10^{2.2}$ $TCID_{50}$/mL) (Hermann et al., 2011).

Titre losses were observed in all attenuated vaccines with different levels of decrease depending on the vaccines (Hostnik et al., 2011; Mähl et al., 2014). The study of Hostnik et al. (2011) compared two different oral vaccines, A and B, tested in similar conditions with initial titres of $10^{7.5}$ $TCID_{50}$/mL and $10^{7.6}$ $TCID_{50}$/mL, respectively, exposed in open field conditions (direct sunlight) for six days (maximum temperature of 46.3 °C and minimum temperature of 10.5 °C). After 24 hours, the bait matrix of vaccines A and B was partially (vaccine A) or completely (vaccine B) disintegrated, with the vaccine capsule visible in both cases. Within 24 hours, the vaccine titre declined to $10^{2.6}$ $TCID_{50}$/mL for vaccine A and to $10^{1.7}$ $TCID_{50}$/mL for vaccine B, and the following day to $10^{0.7}$ $TCID_{50}$/mL (vaccine A) and no virus at all (vaccine B).

The results of the study undertaken by Maciulskis et al. (2008) showed similar data using the SAD Bern strain (Lysvulpen) with a viral titre decrease of 2 log in baits exposed to temperatures of 40 °C in the field. This study reported also differences in bait stability observed after exposure to ambient temperatures. Baits were more stable in a forest environment than on the edge of a forest. Deformation and disintegration of bait casing occurred with some baits at the beginning of the trial. Rainy weather was detrimental to bait casing stability, making it soft and sticky 24 hours after bait deposition in the field. Vaccine titres were stable for nine days in the forest and six days on the edge of a forest in the spring; however, the results of the study make it possible to predict that vaccine titres may have decreased rapidly in the autumn. Weather conditions, such as sunlight and rain, led to the deformation and disintegration of bait casing and loss of vaccine titre, showing that experimental results about bait stability tested in the laboratory conditions do not always correspond to bait stability in the field (Maciulskis et al., 2008).

3.5.3. Rabies surveillance

Disease surveillance is the continuous investigation of a given population to detect the occurrence of a disease to be controlled, which can include testing parts of the population, as defined in the scientific report submitted to EFSA in 2010 'Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union' (Cliquet et al., 2010). For rabies, the objective is the detection of infected animals.

As defined in the new EC guidelines on how to design a programme for the elimination and control of rabies in wildlife (EC, 2015), rabies surveillance is the key tool for (i) assessing the rabies situation in a given country, (ii) planning, implementing, improving the performance and evaluating the success of any rabies elimination programme, and (iii) certifying freedom of disease.

Therefore, adequate rabies surveillance should be in place in any country irrespective of the rabies status, i.e. in infected and rabies-free countries, and it should be conducted continuously in time and space with both positive and negative results being reported (Cliquet et al., 2010). Moreover, based on the virus pathogenesis and the characteristic course of disease, passive rabies surveillance should be targeted towards animals showing abnormal behaviour suggestive of rabies, animals to which human cases have been exposed, animals imported from endemic third countries that show clinical signs suggestive of rabies and animals found dead including road kills (the so-called 'indicator animals'; EC, 2015).

Assessment of rabies incidence (surveillance) in the vaccinated areas should be carried out by analysing any suspect animal[21] (wild or domestic) for rabies. The viruses isolated from all positive cases in the vaccinated areas should be typed, in order to distinguish field rabies virus from vaccine-associated cases.

Finally the surveillance should be based on laboratory tests, by using standards prescribed by OIE (OIE, 2014).

3.5.4. Monitoring of the effectiveness of the oral vaccination programmes

The monitoring is the follow-up of ORV campaigns against rabies and aims to evaluate the effectiveness of ORV programmes, as defined in the scientific report submitted to EFSA in 2010 'Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union' (Cliquet et al., 2010). It is closely linked to rabies surveillance and both are key elements of ORV programmes. The sampling schemes for monitoring and surveillance are different. Sampling for rabies surveillance targets animals suspected of having contracted the disease and animals imported from endemic third countries showing clinical signs suggestive of rabies. In contrast, sampling for ORV monitoring targets animals hunted homogeneously in the vaccinated areas. The

[21] Autochthonous or imported animals (domestic or wild) showing clinical signs of rabies or abnormal behaviour suggestive of rabies, animals found dead, animals to which humans have been exposed (bites, scratches or licking of wounds, etc.) and road kill animals (only for rabies-endemic countries). These animals are used for rabies surveillance. This definition concerns infected and rabies-free countries (Cliquet et al., 2010).

WHO's recommendation is to sample four animals/100 km² annually. Therefore, monitoring applies to only countries with implemented ORV programmes.

The monitoring of ORV is currently based on:

- the assessment of bait uptake by determining the occurrence of the biomarker (usually tetracycline) which is incorporated into the baits; the sampling for the determination of the biomarker in bones or teeth of target animals (foxes and raccoon dogs) should be performed as explained in the scientific report submitted to EFSA in 2010 (Cliquet et al., 2010);
- the assessment of immunisation rates by testing for rabies antibodies in the target species (foxes and raccoon dogs) sampled in vaccinated areas (Cliquet et al., 2010; EC, 2012b; WHO, 2013; EC, 2015)

The monitoring focuses on animals that are specifically killed and sampled for the purpose of bait uptake and serological analysis after ORV campaigns. Those animals are, in most cases, healthy animals, i.e. animals that are not suspected of being infected with rabies.

In most European countries, the animal sampling scheme involves hunter associations. Depending on the epidemiological situation, target species to be sampled for bait uptake and serological analysis are foxes only or a mix of foxes and raccoon dogs. An adequate sample size should be obtained and the current recommendation (Cliquet et al., 2010; WHO, 2013) is to target four animals per 100 km^2 and per year, i.e. sampling of two animals per 100 km^2 in vaccinated areas after the spring campaign and two animals per 100 km^2 in vaccinated areas after the autumn campaign.

Bait uptake

Biomarkers are indicators commonly used in wildlife and disease management programmes. Tetracycline is the most commonly used biomarker and is an innocuous calciphilic post-mortem marker deposited in the calcification zones of teeth and bone tissues, and can be observed using epifluorescence microscopy (Milch et al., 1958). Determination of tetracycline in field target species sampled in rabies-vaccinated areas is an easy way for assessing bait uptake.

The overall annual proportion of tetracycline-positive results is generally significantly higher in adults than in juveniles, whatever the species considered (Cliquet et al., 2006; Zienius et al., 2011; Cliquet et al., 2012). Determining age can help establish whether bait consumption occurred during the current or previous campaigns.

Both age and tetracycline determination techniques have been widely used in Europe since the first oral vaccination campaign in Switzerland in 1978. A review of the analyses related to the monitoring of ORV in EU was conducted in 2010 showing high discrepancies in Europe with regard to the observed levels of tetracycline, which ranged from 12 to 91 % (Robardet and Cliquet, 2011). Since then, proficiency tests have been organised by the EU Reference Laboratories (EURL) on a biannual basis, with recommendations proposed for standardising the method throughout European laboratories (Robardet et al., 2012). Subsequently, a significant improvement has been achieved, as shown by harmonisation of the results obtained by the national reference laboratories.

The results are commonly interpreted in terms of the percentage of (positive animals)/(total animals tested) by species and ORV campaigns (and also by animal age whenever feasible), reflecting a herd response to bait palatability and consumption at a certain time in a certain area.

ORV monitoring using tetracycline examination alone may lead to an overestimate of vaccination effectiveness, for the following reasons:

- Fluorescence in teeth may be observed without any tetracycline absorption, or foxes and raccoon dogs may find other sources of tetracycline besides vaccine baits. Therefore, it is necessary to estimate 'the background level' of tetracycline in fox and raccoon dog populations before the beginning of an oral vaccination programme.
- Foxes and raccoon dogs may consume the bait casing and (i) discard the vaccine capsule, or (ii) not develop an immune response (low or no vaccine titre or immunosuppression), hence leading to animals found to be positive for tetracycline but negative for rabies antibody titration.

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

Fox immunity/raccoon dog immunity

The effectiveness of ORV campaigns is usually assessed by the analysis of specific rabies antibodies in foxes and raccoon dogs collected in the vaccinated areas. Rabies antibody determination has been extensively demonstrated as a good indicator of protection against rabies (Aubert, 1992b).

The samples are usually taken by hunters and experience in Europe has shown the difficulties of collecting blood samples on freshly killed animals. The entire animal cadavers are often brought to the veterinary services or to the laboratory without blood having previously been sampled. The logistics and equipment may be insufficient for ensuring proper storage and the regular and rapid shipment to the testing laboratory. The long delay between animal shooting and cadaver sampling explains that blood samples collected are, in most cases, 'body fluids' which are cytotoxic for the cells used for quantitation of the specific antibodies (De Benedictis et al., 2012; Bedekovic et al., 2013; Wasniewski et al., 2014b). This could lead to biased serological interpretations of ORV effectiveness as a result of the weak performances (in terms of sensitivity and specificity) of certain serological tests using serum samples of poor quality.

A simple alternative has recently been proposed consisting of the use of filter paper to collect blood samples from foxes and raccoon dogs directly in the field for the titration of rabies antibodies by ELISA (Wasniewski et al., 2014a).

The most common techniques used for rabies antibody detection are virus neutralisation tests on cell cultures (RFFIT) (Smith et al., 1973) and the FAVN test (Cliquet et al., 1998), which are the reference methods recommended by WHO and OIE (OIE, 2014). They are well validated and standardised, but require highly trained staff and sophisticated laboratories with a high containment level, as workers need to handle live virus. Since they are based on cell cultures, they are sensitive to cytotoxic products (WHO, 1994) and contaminating agents present in samples. In addition, these methods are not suitable for large-scale screening of field samples.

Since the end of the 1970s, alternative techniques have been developed using either cell cultures (Hostnik and Grom, 1997; Bedekovic et al., 2013) or direct or indirect ELISAs (Wasniewski et al., 2014b) and are currently in use for ORV monitoring (Zienus et al., 2011). Different commercial ELISAs have also been evaluated using field samples from red foxes (Servat et al., 2007; Mojzis et al., 2008; Knoop et al., 2010; De Benedictis et al., 2012; Wasniewski et al., 2013) and raccoon dogs (Wasniewski et al., 2013).

The results are commonly interpreted in terms of the percentage of (positive animals)/(total animals tested) by species and by campaign (and by animal age whenever feasible), allowing estimation of whether or not herd immunity has been achieved at a certain time in a certain area.

Serological testing of target animals has been widely used in Europe and, in 2003, a first inter-laboratory test using an ELISA revealed the need for harmonised protocols in the laboratories (Cliquet et al., 2003), in order to better compare serological results and seroprevalence. A review of the analysis related to the monitoring of ORV in the EU was conducted in 2010 and showed that 73 % of the laboratories use an ELISA (two different commercial kits), while 18 % and 9 % of laboratories use the RFFIT or the FAVN test, respectively (Robardet and Cliquet, 2011). Considering the number of different tests in use in Europe, and since different ELISAs are commercially available with various levels of reliability (Wasniewski et al., 2013), proficiency tests should be regularly organised to assess the performances of existing methods used in the laboratories (in terms of analytical specificity and sensitivity) with field samples received for monitoring. The sampling schemes used in the countries involved in ORV are also variable (hunters may directly sample blood from the freshly shot animals or, in most cases, bring animal cadavers to the veterinary services).

Standardisation of the sampling schemes and the serological testing methods would be beneficial for comparing data among EU countries for a more reliable evaluation of the effectiveness of ORV programmes.

The importance of ORV monitoring

Biomarker testing is effective at monitoring bait uptake, being particularly useful at the beginning of ORV campaigns (e.g. the first two to three years of implementation). After that, the need for the biomarker in vaccine baits and related testing for bait uptake could be reconsidered, according to the rabies situation in each country and to the overall control strategy in place. However, the bait uptake does not measure herd immunity. If the rabies incidence decreases (documented by rabies surveillance) and there is an immune response in target species, this would indicate that ORV campaigns are effective. Monitoring for rabies antibodies is an additional approach providing insights on both bait uptake and herd immunity.

3.6. Vaccination strategy

ORV of foxes and/or raccoon dogs is the most effective method for eliminating terrestrial rabies in wildlife. Proper planning, design and implementation of vaccination programmes, as well as coordination of programmes between neighbouring regions or countries, are important to their success. Large-scale and long-lasting ORV strategies are needed for the elimination of rabies.

New guidelines for the design of EU co-financed programmes on the elimination and control of rabies in wildlife have been published (EC, 2015). This section aims to update the information provided about the oral vaccination of foxes in the EC report issued in 2002 (EC, 2002) with regard to both foxes and raccoon dogs and follows the new EC guidelines.

3.6.1. Temporal patterns

When planning and implementing ORV campaigns, special emphasis should be placed on the duration and timing of campaigns, the biology of foxes and raccoon dogs, the determination of vaccination areas, bait densities and flight-line distances, and the monitoring of campaigns. The most effective strategy is a long-term and large-scale approach, with two vaccination campaigns per year and parallel flight distances of 500 m. Based on experience in earlier campaigns, vaccination campaigns should continue for at least two years after the last confirmed case of rabies (EC, 2015).

Regular vaccination campaigns

The classical pattern of two vaccination campaigns per year, carried out in spring and autumn, has been found to be successful whatever the target population density may be. This biannual distribution frequency has been used in all of the European programmes of oral vaccination that resulted in the elimination of rabies (Breitenmoser et al., 2000; Bruyere et al., 2000; Zanoni et al., 2000; Cliquet et al., 2012; Mulatti et al., 2013).

As regards fox vaccination, early spring campaigns carried out in March/April (instead of May/June) have been shown to be beneficial in several contexts, such as Belgium, Luxembourg, Germany and, more recently, Lithuania and Italy (Brochier et al., 1996; Zienius et al., 2011; Müller et al., 2012; Mulatti et al., 2013). Autumn distribution is generally organised in September or October, but field experiences have shown the effectiveness of campaigns implemented later, in November/December (Zienius et al., 2011; Müller et al., 2012; Mulatti et al., 2013).

Regional and local climate should be considered before planning ORV campaigns, noting that vaccination efficacy would eventually be affected by either snow melting, bait freezing or bait melting. Thus, in southern Europe, where temperatures may rapidly increase in late spring, either an earlier distribution of baits or the use of a heat-stable vaccine strain is required. Conversely, in northern regions and mountainous areas, freezing could be reached early in autumn; therefore, bait distribution should be carried out as early as possible in these areas.

Extraordinary vaccination by manual distribution

Manual distribution of vaccine baits, complementary to ordinary vaccination campaigns, could be an effective tool to eliminate rabies in limited contexts (e.g. at den entrances for cub vaccination, in urban areas or where aerial distribution is not possible). The same is valid for extraordinary vaccinations in cases where there is a high risk of rabies introduction from neighbouring areas.

However, in both situations, this approach is costly and time consuming and should only be applied after a careful cost–benefit evaluation.

Emergency vaccination

In cases of the re-emergence of rabies in a free area, emergency vaccination needs to be conducted immediately. An emergency vaccination area with a radius of at least 50 km around the outbreak should be established. Short interval baiting of the emergency vaccination area should be considered at the beginning of the operation (EC, 2015).

Emergency vaccination has proved to be effective, regardless of the period of the year in which it was implemented and of the vaccine bait used, although a heat-stable vaccine would be favourable (Mulatti et al., 2013). The general assumption that vaccination should not be carried out at temperatures below 0 °C has been partly overcome by recent successful field experiences, which demonstrate that the freezing point can be passed over in case of identified residual foci at higher altitudes (Capello et al., 2010; Mulatti et al., 2013). In the case of outbreaks occurring in hot seasons, an emergency vaccination campaign using thermostable vaccines is recommended. At temperatures above, for example, 30 °C, melting of the bait casing occurs and vaccine titres decrease, depending on the quality of the vaccine bait.

Vaccination buffer zones in rabies-free areas

To prevent the infection of rabies-free areas from a neighbouring infected area/country, the establishment of an ORV buffer zone along the border with the infected area/country should be considered. The ORV buffer zone should be 50 km wide, but the depth of the ORV buffer zone may be reduced to 20 km if there is an artificial or natural barrier that limits the movements of foxes and/or raccoon dogs. Derogations to the recommended vaccination standards (two vaccinations/year and parallel flight distance of 500 m) may be acceptable if infection pressure in the non-free area and the density of foxes and/or raccoon dogs are considered and properly assessed. The ORV buffer zone should also be established in neighbouring infected area(s). ORV should continue as long as rabies infection persists in neighbouring area(s) (EC, 2015).

The information provided in the EC report in 2002 about synchronisation of vaccination campaigns in neighbouring administrative or political entities is still valid (EC, 2002).

3.6.2. Spatial aspects and patterns

Vaccination areas

The vaccination area should be as large as possible (5 000 km^2 at least) and, preferably, should include the entire endemic area. ORV campaigns in rabies-free areas should be designed in such a way that the area covers a 50 km belt ahead of the rabies front (EC, 2015).

Distribution pattern and timing

The best distribution method is aerial distribution of baits using fixed-winged aircraft or helicopters. Manual distribution of baits should only be used as a complementary method to aerial distribution. The distribution pattern of baits should take into account habitat and landscape features. The classical flight pattern consists of parallel flight lines set approximately 500 m apart. Deviation from parallel flight lines can be used if the requested bait density/km^2 is guaranteed. The use of global positioning systems (GPS), computer-supported recording of flight routes and coordinates of bait droppings during aerial distribution are required.

ORV campaigns for foxes and/or raccoon dogs should be carried out biannually. Climatic conditions and the biological cycles of foxes and raccoon dogs should be considered in order to decide the best timing for spring and autumn vaccination campaigns (EC, 2015).

Bait density

The minimum bait density distributed should not be less than 20 baits/km^2. An increased bait density of up to 25–30 baits/km^2, in combination with reduced flight-line distances (300 m), should be considered in cases of high population densities, setbacks and persisting residual foci (EC, 2015).

3.6.3. Evaluation of oral rabies vaccination programmes

Laboratory determination and recording of rabies prevalence (rabies surveillance), bait uptake and seroconversion (monitoring of ORV campaigns) should be complemented by epidemiological analysis as a fundamental basis for proper planning, implementation and evaluation of ORV programmes. Any animal disease elimination programme should be as effective and cost-efficient as possible. This will allow the authorities to react swiftly in cases of emergencies, setbacks or stagnating epidemiological rabies situations, by adapting the vaccination strategy accordingly. A continuous collection of data about rabies incidence (surveillance) in the whole country and ORV-related monitoring data and recording in a country-specific computerised rabies database, as well as the establishment of an epidemiological unit devoted to this task, are prerequisites. Basic precepts and the principles underlying the primary methods of analysis of epidemiological data among other quantitative analysis approaches, stratified analysis, sensitivity analysis, and the estimation of trends in effect should be followed. Further information and issues to consider for proper evaluation of ORV programmes are described in the present document in the section 2.5.

4. Conclusions

4.1. Assessment of the conclusions from the European Commission report issued in 2002

Table 1 below reports the assessment of the validity of the conclusions of the report 'The oral vaccination of foxes against rabies' issued by the Scientific Committee on Animal Health and Animal Welfare of the EC in 2002.

Table 1: The conclusions from the EC report issued in 2002 (left column), and their endorsement or the new modified version (right column)

Conclusions from the report issued by the EC in 2002	Endorsement or new version of the conclusions
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 ORV campaigns requires a long-term strategy and cross-border cooperation	In Europe, oral immunisation by means of vaccine baits has been found to be successful in eliminating terrestrial wildlife rabies in most cases. The analysis of the dynamics of rabies cases and vaccination areas confirms that ORV is an efficient tool for rabies control in wildlife, including areas with both foxes and raccoon dogs. However, the ultimate success of ORV campaigns requires both a long-term strategy and cross-border cooperation
2. Rabies in wildlife was eliminated most efficiently in those countries where the vaccination campaigns were planned on a national level and coordinated with neighbouring countries	This conclusion is still valid
3. Thorough surveillance of rabies epizootics and monitoring of the vaccination efficiency (using a tetracycline marker and seroconversion rates) are important tools for the assessment and adjustment of vaccination campaigns. Standardised surveillance and monitoring methods facilitate international comparison and cooperation	Thorough surveillance of rabies and monitoring of the vaccination efficiency (using a tetracycline marker and seroconversion rates) are important tools for the assessment and adjustment of vaccination campaigns. Harmonised surveillance and monitoring methods facilitate comparison of results and international cooperation
Types of vaccines and baits	
4. Insufficient stability of some rabies virus vaccines is likely to have been a source of vaccination failure in specific situations (e.g. a combination of climatic and meteorological conditions and areas of high fox population density). Among currently available vaccines, based on available data, the vaccinia recombinant vaccine appears to be the most stable	There is experimental and field evidence that weather conditions may have a negative impact on the stability of certain vaccines (bait casings and vaccine virus stability). Results under laboratory conditions are generally more favourable, as reported by vaccine manufacturers
5. For the den vaccination of fox cubs, it is desirable that a vaccine should be able to overcome, as far as possible, the effects of maternal immunity. A limited number of studies indicate that the vaccinia recombinant vaccine is better able to overcome maternal immunity than other vaccines	The presence of maternal immunity at the time of vaccination tends to slow (but not prevent) the establishment of active immunity. Furthermore, young suckling foxes and raccoon dogs (i.e. up to eight weeks old) are not able to consume baits. Therefore, maternal immunity is of no practical relevance for the vaccination of fox and raccoon dog juveniles

Conclusions from the report issued by the EC in 2002	Endorsement or new version of the conclusions
Methods of release of vaccine baits	
6. An appropriate bait delivery system (helicopter, fixed-wing aircraft or manual) is needed when planning vaccination strategies, in order to achieve optimal bait distribution	An appropriate bait delivery system (helicopter, fixed-wing aircraft or manual) is needed when planning vaccination strategies, in order to achieve optimal bait distribution. Moreover, the use of GPS, computer-supported recording of daily flight routes and encrypted data of coordinates of bait droppings during aerial distribution are necessary for quality control and detailed analysis of data. Manual distribution of baits can be used as a complementary method in limited areas and in certain circumstances (e.g. in urban areas) and an equivalent recording of bait placements should be used
Bait density and distribution patterns	
7. The bait density and bait distribution pattern should take into account habitat and landscape features, the 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	To ensure sufficient bait uptake, bait distribution and density should take account fox and raccoon dog biology, the habitat and landscape features, the species competing for baits and the target population density. Based on field experience, vaccination at den entrances does not appear cost-effective
8. 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	This conclusion is no longer valid. An increased bait density of up to 25–30 baits/km^2 in combination with reduced flight-line distance (e.g. 300 m) should be considered in the case of setbacks and persisting residual foci of rabies
Seasonal pattern of the releases	
9. 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	Selection of the months when baiting is performed is an important consideration when planning vaccination strategies, in order to ensure bait access for target species, including adult foxes and raccoon dogs and juveniles
10. Spring distribution is best 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) have also been shown to be beneficial	Bait distribution during spring is best conducted after considering each of the following ecological factors: time of birth of fox and raccoon dog pups in spring; climate, so to avoid, for example, high temperatures in southern Europe and snow coverage in northern Europe; and hibernation of raccoon dogs, thus avoiding distributing baits when animals are still hibernated

4.2. Conclusions from the current assessment

The conclusion points from the current assessment are the following:

- **Laboratory test for rabies**
  - Reliable molecular epidemiological methods are available for virus characterisation and differentiation of field and vaccine strains.
  - New serological tests (e.g. ELISA) are available for the assessment of vaccination effectiveness.
- **Occurrence of rabies and ORV**
  - Since 2003, as a result of the ORV programme, the Czech Republic, Germany, Italy, Austria, Estonia, Latvia and Lithuania have acquired or re-acquired the status of rabies-free country. In Estonia, Latvia and Lithuania, progress has been particularly rapid, with the number of rabies cases reported annually in raccoon dogs decreasing steadily over several years from several hundred to zero. In these countries, a vaccination belt is being maintained to reduce the risk of re-introduction of rabies from neighbouring countries.

- In some countries, the persistence of sylvatic rabies is attributed to the temporal discontinuity of the implementation of the ORV campaigns, i.e. when the recommended biannual campaigns are not consistently performed.
- Very few cases of rabies in humans are reported nowadays in the EU, and most MSs have no autochthonous cases. This is possibly linked to the disappearance of dog-mediated rabies, to the vaccination of domestic animals, to the ORV programmes in wildlife and to the systematic application of post-exposure treatment in cases of contact between humans and suspect animals.

- **New target species (e.g. raccoon dogs)**
  - Foxes are the only known reservoir of rabies virus in Europe.
  - Raccoon dogs are not known to be reservoirs for rabies in Europe, but are considered important transmitters, in particular where raccoon dog density is high.
  - The raccoon dog population is expanding rapidly throughout northern and central Europe. This species is the second most commonly rabies-affected wild carnivore in north-east Europe after the red fox, and hence a contributor to the risk of rabies recurrence in countries within its range.
  - The movements of adult raccoon dogs after hibernation in late winter/spring and the dispersal of juveniles (peaking between August and October) are temporally related to the two seasonal peaks of rabies incidence in raccoon dogs. In western Europe, raccoon dogs usually do not hibernate.
  - Foxes and raccoon dogs often share the same habitats and their home ranges overlap making contacts between species likely. The collective density of these two species may exceed the threshold value for rabies transmission contributing to the persistence of rabies in certain areas.
  - Other carnivores may be infected by rabies. However, on the basis of the available data, their role as virus transmitters is generally less important than that of foxes and raccoon dogs.
  - There is limited scientific knowledge about the pathogenesis of rabies in either raccoon dogs or other potential host species.
  - Knowledge of fox and raccoon dog population densities will be helpful when commencing the ORV campaigns, but such knowledge is not a prerequisite.

- **Oral vaccines available and authorised in the EU**
  - The vaccines authorised in the EU for oral vaccination are Raboral (V-RG strain), Rabigen (SAG2 strain), Fuchsoral (SAD B19 strain) and a new vaccine called Lysvulpen (two dominant sub-populations of SAD Bern and SAD B19-'like' viruses).
  - The efficacy of the existing vaccines is one of the factors that has contributed substantially to the success of rabies control and elimination in several European countries.
  - Very few vaccine-associated cases of rabies in the field have been reported and published (a few in foxes and one each in a stone marten and a badger).
  - There is no evidence of human safety issues related to oral rabies vaccines.
  - Biomarker testing is particularly useful at the beginning of ORV campaigns (e.g. the first two to three years of implementation) to monitor bait uptake. Afterwards, the need for the biomarker in vaccine baits and related testing for bait uptake could be reconsidered, depending on the rabies situation in each country and on the overall control strategy in place.
  - Potential safety issues could be related to the ecotoxicity and potential antimicrobial resistance (AMR) induced by tetracyclines, which are used as biomarkers in the vaccine baits. However, no formal risk assessment has ever been performed.

- **Thermostability of the bait casing and possible impact on stability of the virus strain used in relation to different climate conditions and temperature at the releasing point**
  - There is experimental and field evidence that weather or climatic conditions may have negative impacts on bait and vaccine virus stability for certain vaccine baits.
  - Because of different field conditions (e.g. exposure to sunlight), the thermostability tested in the field may be lower than that tested in the laboratory. As a consequence, thermostability values from laboratory tests cannot readily be extrapolated to performance under field conditions.
- **Recent European and international guidelines for rabies surveillance and oral vaccination**
  - Among the most recent European and international guidelines for rabies surveillance and oral vaccination, the following are noteworthy
    - The EC guidance document issued in 2015 which provides guidance about the design and implementation of an EU co-financed programme to eliminate and control rabies in wildlife;
    - The update of the European Pharmacopoeia 'Rabies vaccine (live, oral) for foxes and raccoon dogs' issued in 2014, which includes raccoon dogs as a target species;
    - Chapter 2.1.13 about rabies in the OIE 'Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)', updated in 2014, which provides harmonised, disease-specific information on diagnostic laboratory methods and requirements for vaccine production and control;
    - The EFSA external scientific report 'Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union' issued in 2010, which provides recommendations for improving and harmonising rabies surveillance and reporting in animals in Europe.

5. Recommendations

5.1. Assessment of the recommendations from the European Commission report issued in 2002

Table 2 below reports the assessment of the validity of the recommendations of the report 'The oral vaccination of foxes against rabies' issued by the Scientific Committee on Animal Health and Animal Welfare of the EC.

Table 2: The recommendations from the EC report issued 2002 (left column), and their endorsement or the new modified version (right column).

Recommendations from the 2002 EC report	Assessment of the validity of the recommendations from the 2002 report
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 are designed in a way that achieves 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, and those found dead, including road kills, should be performed	Vaccination campaigns should be designed to achieve herd immunity in the fox and raccoon dog populations, in order to avoid setbacks in rabies elimination. Monitoring of vaccination programmes should include on-going surveillance of (i) rabies incidence in all relevant host species, (ii) bait uptake and (iii) immunity in target species during vaccination campaigns. Surveillance to estimate rabies incidence should include an examination of all wild and domestic mammals suspected of being infected by rabies and those found dead, including road kills, should be performed, as well as those linked to human exposure
2. In order to ensure the success of vaccination campaigns, 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 critical to the successful outcome of vaccination campaigns	This recommendation is still valid
3. Vaccination should be continued for at least two years after the last reported case of rabies	This recommendation is still valid
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	This recommendation is still valid
5. Serological methods to be used for quantification of the antibody response in foxes after vaccination should be standardised, as recommended by the WHO and OIE. The Community Reference Laboratory should take a lead in standardising these methods. Standardised ELISAs, which are now available, may replace serum neutralisation tests	This recommendation is still valid
Types of vaccines and baits	

Recommendations from the 2002 EC report	Assessment of the validity of the recommendations from the 2002 report
6. 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 10 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 after exposure to temperatures of 25 °C for seven days. Each vaccine batch should be tested and approved for titre and stability by an acknowledged quality control scheme in accordance with 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	Live rabies vaccines used for oral vaccination of foxes and raccoon dogs should fulfil the requirements of the updated European Pharmacopoeia monographs, as well as the efficacy and safety recommendations of the WHO. Each vaccine batch should be tested and approved for titre and stability by an acknowledged quality control programme in accordance with OIE standards and WHO recommendations. Laboratories involved in the monitoring and evaluation of rabies programmes are advised to monitor the virus titre of batches of rabies vaccine baits before and during the campaign.
7. 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 Community Reference Laboratory should perform additional tests or trials if required	The integrity of bait casings should be maintained after the distribution of baits into the environment, so that it does not melt and the capsule of the vaccine is still fully covered by the casing
8. 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	The use of a biomarker in the vaccine in the teeth and/or bones of foxes and raccoon dogs is recommended to evaluate the rate of bait uptake in target species. Alternatives to tetracyclines should be developed for use as biomarkers
9. When handling baits and vaccines, storage and transportation conditions and cold-chain requirements should be strictly adhered to	This recommendation is still valid
10. 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 females, the vaccine that is best able to overcome the effects of maternal immunity should be used	The second part of this recommendation is no longer valid because young suckling cubs are not able to consume baits. Therefore, consideration of maternal immunity is of no practical relevance during ORV implementation
Methods of release of vaccine baits	
11. 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	This recommendation is not needed, since the recent EC guidelines (EC, 2015) already provide clear and exhaustive information about bait distribution
12. 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 recommended-only for the treatment of uniform and large areas of low density inhabitation (e.g. large forests and 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 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	This recommendation is not needed, because the recent EC guidelines (EC, 2015) already provide clear and exhaustive information about bait distribution methods
Bait density and distribution pattern	

Recommendations from the 2002 EC report	Assessment of the validity of the recommendations from the 2002 report
13. Rabies-infected areas 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 too large to be vaccinated as a whole, parts of these regions should be vaccinated repeatedly until rabies elimination is ascertained. Newly vaccinated areas should overlap the previously vaccinated ones to prevent reinfection of rabies-free areas	This recommendation is not needed, because the recent EC guidelines (EC, 2015) already provide clear and exhaustive information about bait distribution methods
14. In cases of rabies-infected neighbouring regions, the following points should be considered in order to avoid subsequent reinfections: • 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 • a vaccination zone should extend up to the next geographical or artificial physical barrier	In the case of reintroduction of rabies from infected neighbouring regions, buffer vaccination zones should be established, taking into account natural barriers. Control measures within the zone and across national or international borders should be coordinated
15. In the case of an isolated residual or re-emerging focus of rabies, a vaccination area with a radius of 25–50 km around the site should be applied, depending on natural barriers	This recommendation is still valid
16. 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 the 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. raccoon dogs), this buffer zone size should be adjusted to the distance travelled/ranged by that species	To prevent the spread of infection 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 the case of an existing natural physical barrier, the minimum distance recommended is 20 km
17. 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	Taking topographical factors into account (e.g. urban and suburban areas), all fox and raccoon dog home ranges should be included in vaccination campaigns. Wherever the distribution system allows flexibility (e.g. distribution by hand or helicopters), the pattern of habitat use by host species should be considered
18. Homogeneous distributions of 18–20 and 20–30 baits/km^2 are recommended for low and high fox population densities, respectively. For den baiting, it is recommended that at least 10 baits are deposited at the main den entrance	The minimum bait density distributed should not be less than 20 baits/km^2. An increased bait density of up to 25–30 baits/km^2 in combination with a reduced flight-line distance (e.g. 300 m) should be considered in the case of setbacks and persisting residual foci
19. When using the aerial method of bait distribution, flight-line distance should not exceed 500 m and when the fox population is high it should be reduced to 300 m. When distributing baits manually, baits should be uniformly distributed according to a raster model based on prepared maps	The classical flight pattern should consist of parallel flight lines set approximately 500 m apart. Alternative flight lines can be used if the requested bait density per km^2 is guaranteed. The use of GPS, computer-supported recording of flight routes and coordinates of bait droppings during aerial distribution are required
Seasonal pattern of the releases	

Recommendations from the 2002 EC report	Assessment of the validity of the recommendations from the 2002 report
20. 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 May or June in order to increase the efficient access of fox and raccoon dog cubs to baits. Den vaccination should be considered to effectively complement the regular spring campaign	In general, oral vaccination campaigns should be conducted on a biannual basis, in spring and autumn, after taking into account climatic conditions. Autumn vaccination should generally be performed in September or October. Spring distribution should be carried out taking into account the birth time of fox and raccoon dog pups in spring and also the possible hibernation of raccoon dogs. This is in order to increase the efficient access of fox and raccoon dog cubs to baits. Den vaccinations are recognised as costly and time consuming, thus they should be applied only after a careful risk–benefit evaluation
21. In the 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	In the case of re-emergence of rabies in a free area, emergency vaccination needs to be conducted immediately. An emergency vaccination area with a radius of at least 50 km around the outbreak should be established

5.2. Recommendations from the current assessment

The recommendations from the current assessment are the following:

- **Rabies aetiology**
  - Research on lyssavirus phylogeny should continue in order to clarify rabies epidemiology and to identify new variants of rabies virus and new species of lyssavirus.
- **Occurrence of rabies and ORV**
  - Sylvatic rabies can be reintroduced in areas where it has been eliminated, especially if these areas are close to endemic ones from which reinfection may occur. Vaccination belts neighbouring endemic areas should be conducted on an on-going basis to reduce the risk of re-introduction.
  - When implementing ORV campaigns, the temporal continuity of these should be guaranteed until complete elimination.
- **New target species**
  - Epidemiological studies and surveillance systems are needed to clarify the role of raccoon dogs, badgers and other carnivores in maintaining rabies cycles.
  - The adaptation of the virus to species other than foxes, in terms of length of incubation period and mortality, should be clarified by further studies on the rabies pathogenesis in different host species.
  - Vaccinations should be carried twice a year in order to cover the two seasonal peaks of rabies incidence in foxes and raccoon dogs.
  - Hunting may decrease population density and create vacant territories, thereby promoting dispersal (immigration to the area) and rabies spread. Therefore, there should be no culling of adult foxes or raccoon dogs in their permanent territories during or after ORV programmes have been conducted.
- **Vaccines available and authorised in the EU**
  - A full risk assessment is needed to evaluate the potential ecotoxicological risks and the risks for AMR deriving from the use of tetracycline as a biomarker in oral rabies vaccine baits.

  - The vaccine capsule inside the baits should be made of biodegradable material, to minimise environmental contamination.

- **The thermostability of the bait casing and possible impacts on the stability of the virus strain used in relation to different climate conditions and temperatures at the releasing point**
  - Vaccine producers should provide data on the stability of baits under different field conditions of landscape and temperatures, and on the related melting point.
  - Countries implementing ORV, in particular those in southern Europe, should either use a heat-stable vaccine or conduct vaccination campaigns in seasons when temperatures and/or climatic conditions do not compromise bait and vaccine stability.
  - When needed, field trials on bait and vaccine virus stability should be performed under specific field conditions.

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Glossary

Rabies surveillance	Generally, surveillance means the continuous investigation of a given population to detect the occurrence of a disease to be controlled, which can include testing parts of the population. For rabies, the objective is the detection of infected animals. Rabies surveillance is based on laboratory investigations for rabies diagnosis on suspected domestic and wild animals (found dead or suspected of having the disease) to detect the occurrence of the disease throughout an entire country. Such a system needs to be established irrespective of the rabies status, i.e. in infected and rabies-free countries
ORV monitoring	This term is used for the follow-up of ORV campaigns against rabies. It is based on laboratory investigations of hunted wild animals sampled homogeneously in vaccinated areas for analysing bait consumption (biomarker examination) and herd immunity (rabies serology). This applies only to countries that have implemented ORV programmes
Indicator animal	An animal suspected of having rabies (see definition of 'suspect animal')
Threshold density	The lowest density where there is a 50 % risk for rabies epizootic
Autochthonous case	Disease case contracted in the area where reported
Sympatric species	Two species or populations are considered sympatric when they exist in the same geographical area without interbreeding
Threshold adult density and threshold territory density	Threshold adult density (density of adult individuals) is about twice as much as threshold territory density (density of territories), because there are usually two adult animals, a male and a female, in one territory, and adjacent territories usually do not overlap
Suspect animal	Autochthonous or imported animals (domestic or wild) showing clinical signs of rabies or abnormal behaviour suggestive of rabies, animals found dead, animals to which humans have been exposed (by bites, scratches or licking of wounds, etc.) and road kill animals (only for rabies-endemic countries). These animals are used for rabies surveillance. This definition concerns infected and rabies-free countries

Abbreviations

AMR	Antimicrobial Resistance
BHK	Baby Hamster Kidney
EBLV	European Bat Lyssavirus
EDQM	European Directorate for the Quality Of Medicines
ELISA	Enzyme-Linked Immunosorbent Assay
EURL	EU Reference Laboratory
FAT	Fluorescent Antibody Test
FAVN	Fluorescent Antibody Virus Neutralisation Test
GPS	Global Positioning Systems
NRL	National Reference Laboratory
ORV	Oral Rabies Vaccination
RABV	Rabies Virus
RFFIT	Rapid Fluorescent Focus Inhibition Test ()
RTCIT	Rapid Tissue Culture Isolation Test
SAD	Street Alabama Dufferin
SAG	Street Alabama Gif
TCID	Tissue Culture Infectious Dose

Appendix A – Protocol for the literature review on oral vaccination of foxes against rabies

Background

This literature search protocol was used in the context of the EFSA mandate on oral vaccination of foxes against rabies.

Objective of the literature search

A literature search of studies will be used for updating the scientific report issued by the Scientific Committee on Animal Health and Animal Welfare in 2002 on the oral vaccination of foxes against rabies. The mandate conferred to the committee included a comparative study of the various fox vaccination protocols implemented, using the following parameters: type of vaccines, type of baits, methods of release of vaccine baits, density of baits and distribution patterns, seasonal pattern of the releases, and methods of evaluation of the vaccination programmes.

The task will include the following steps:

- an extensive literature search for potentially relevant citations;
- relevance screening to be used to select the citations identified by the literature search, based on titles, abstracts and full text;
- data categorisation to describe and appraise the information provided in the relevant citations.

As this task involves an extensive literature search, followed by screening for relevance and data characterisation, the research questions will be broad in scope rather than focused as appropriate to a systematic review. The review questions are broken down into key elements using the PIT conceptual model, as outlined below:

- **population of interest (P)**
  - the populations of interest are foxes and raccoon dogs in Europe;
- **intervention of interest (I)**
  - the intervention of interest is oral vaccination with baits against rabies;
- **target (T)**
  - the target is the rabies virus.

Objective/review question

The objective of this literature review is to identify, evaluate and synthesise the evidence on oral vaccination with baits against rabies in red foxes and raccoon dogs.

To achieve this objective, the review question is described below.

Review question:

What is the performance of different type of vaccines, type of baits, methods of release of vaccine baits, density of baits and distribution patterns, seasonal pattern of the releases, methods of evaluation of the vaccination programmes for oral vaccination of foxes and raccoon dogs against rabies in Europe?

Eligibility criteria for study selection

A literature review was conducted in accordance with the framework for review question issuing Population, Intervention, Target (P-I-T) with the inclusion criteria listed in Table 3.

Table 3: Eligibility criteria

Parameter	Criteria
Time	From 1980 onwards
Language	Only literature published in English will be reviewed
Publication type	Primary research studies (i.e. studies generating new data), reviews and reports from governmental bodies
Study design	No specific type of study design will be used to include/exclude relevant studies
Study characteristics	No exclusion will be based on study characteristics
Population	Wild foxes and raccoon dogs **Comment/Explanation**: Farmed animals will be excluded. Raccoons (*Procyon lotor*) will be excluded
Intervention	Studies must report on aspects of oral vaccination with baits against rabies. All the articles that do not report this intervention are to be excluded
Target	Rabies virus
Outcome	Performance of the vaccines, types of baits, methods of release of vaccine baits, density of baits and distribution patterns, seasonal pattern of the releases, methods of evaluation of the vaccination programmes for oral vaccination of foxes and raccoon dogs against rabies in Europe

Identifying research evidence

The literature search will aim to identify studies on oral vaccination with baits against rabies.

Information sources

Search strategies included the use of electronic search engines for bibliographic databases. Review manuscripts (i.e. secondary research studies) were included in the review.

Because of time constraints, only studies in peer-reviewed journals and conference proceedings were included. Informally reported or unpublished data were not collated.

Two databases were searched:

- Web of Science
- Scopus.

Experts consider that these databases are sufficient for the objective of this systematic review.

Search terms

The following search strategy is proposed:

Population: fox or foxes or Vulpes or 'red fox' or 'raccoon dogs' or 'Nyctereutes procyonoides' or procyonoides

Intervention: vaccin* OR bait* OR immun*

Target: rabies OR Lyssavirus OR 'rabies virus'

Search strings

Web of Science

fox OR foxes OR *Vulpes* OR 'red fox' OR 'raccoon dog*' OR '*Nyctereutes procyonoides* OR *procyonoides*

AND

Vaccin* OR immun* OR bait*

AND

rabies *OR Lyssavirus**

P-I-T string

searched for: TOPIC: (fox OR foxes OR Vulpes OR red fox OR raccoon dog* OR Nyctereutes procyonoides OR procyonoides) *AND* **TOPIC:** (Vaccin* OR immun* OR bait*) *AND* **TOPIC:** (rabies OR Lyssavirus OR rabies virus)

Refined by: RESEARCH AREAS: (INFECTIOUS DISEASES OR VETERINARY SCIENCES OR ZOOLOGY OR ENVIRONMENTAL SCIENCES ECOLOGY OR IMMUNOLOGY) AND **DOCUMENT TYPES:** (ARTICLE OR REVIEW) AND **LANGUAGES:** (ENGLISH) AND **COUNTRIES/TERRITORIES:** (FRANCE OR BULGARIA OR GERMANY OR WEST GERMANY OR SLOVAKIA OR ENGLAND OR PORTUGAL OR BELGIUM OR ITALY OR ESTONIA OR MACEDONIA OR SLOVENIA OR CZECH REPUBLIC OR SWITZERLAND OR SPAIN OR POLAND OR HUNGARY OR FINLAND OR NETHERLANDS OR LUXEMBOURG OR UK OR CROATIA OR NORWAY OR LITHUANIA OR SWEDEN OR AUSTRIA OR SCOTLAND) AND **DOCUMENT TYPES:** (ARTICLE OR REVIEW OR MEETING OR BOOK) AND **DOCUMENT TYPES:** (ARTICLE OR REVIEW OR MEETING OR BOOK)

Timespan: 1990–2015.

Scopus

(TITLE-ABS-KEY (**fox** OR **foxes** OR **vulpes** OR (**red fox**) OR (**raccoon dog***) OR (**nyctereutes procyonoides**) OR **procyonoides**) AND TITLE-ABS-KEY (**vaccin*** OR **immun*** OR **bait***) AND TITLE-ABS-KEY (**rabies** OR **lyssavirus***)) AND SUBJAREA (**mult** OR **agri** OR **bioc** OR **immu** OR **neur** OR **phar** OR **mult** OR **medi** OR **nurs** OR **vete** OR **dent** OR **heal** OR **mult** OR **ceng** OR CHEM OR **comp** OR **eart** OR **ener** OR **engi** OR **envi** OR **mate** OR **math** OR **phys**) AND PUBYEAR > **1989** AND (LIMIT-TO (AFFILCOUNTRY , **'France'**) OR LIMIT-TO (AFFILCOUNTRY , **'Germany'**) OR LIMIT-TO (AFFILCOUNTRY , **'Poland'**) OR LIMIT-TO (AFFILCOUNTRY , **'United Kingdom'**) OR LIMIT-TO (AFFILCOUNTRY , **'Belgium'**) OR LIMIT-TO (AFFILCOUNTRY , **'Italy'**) OR LIMIT-TO (AFFILCOUNTRY , **'Switzerland'**) OR LIMIT-TO (AFFILCOUNTRY , **'Slovenia'**) OR LIMIT-TO (AFFILCOUNTRY , **'Hungary'**) OR LIMIT-TO (AFFILCOUNTRY , **'Finland'**) OR LIMIT-TO (AFFILCOUNTRY , **'Lithuania'**) OR LIMIT-TO (AFFILCOUNTRY , **'Estonia'**) OR LIMIT-TO (AFFILCOUNTRY , **'Slovakia'**) OR LIMIT-TO (AFFILCOUNTRY , **'Bulgaria'**) OR LIMIT-TO (AFFILCOUNTRY , **'Czech Republic'**) OR LIMIT-TO (AFFILCOUNTRY , **'Norway'**) OR LIMIT-TO (AFFILCOUNTRY , **'Austria'**) OR LIMIT-TO (AFFILCOUNTRY , **'Greece'**) OR LIMIT-TO (AFFILCOUNTRY , **'Netherlands'**) OR LIMIT-TO (AFFILCOUNTRY , **'Romania'**) OR LIMIT-TO (AFFILCOUNTRY , **'Bosnia and Herzegovina'**) OR LIMIT-TO (AFFILCOUNTRY , **'Croatia'**))

A scoping search identified:

- 258 papers from Web of Science
- 278 papers from Scopus.

A database of the electronic search results will be created with EndNote X5 (Thomson Reuters). Duplicate citations will be deleted using the automated function or manually when required.

Study selection

Study selection was conducted in two stages for 305 papers, in accordance with the PIT criteria:

5) Initial screening of titles and abstracts against inclusion criteria to exclude obviously irrelevant studies was performed. Excluded studies were recorded. Studies that are relevant or unclear went to the second step.
6) Full screening of full papers identified as possibly relevant was performed.

Studies were independently examined by two reviewers to identify papers to include in the literature review. Divergences between reviewers were resolved by consensus after discussion. Reviewers were blinded to author names, institutions and publication titles.

A flow chart of information used through the different phases of a systematic review is described in Figure 12.

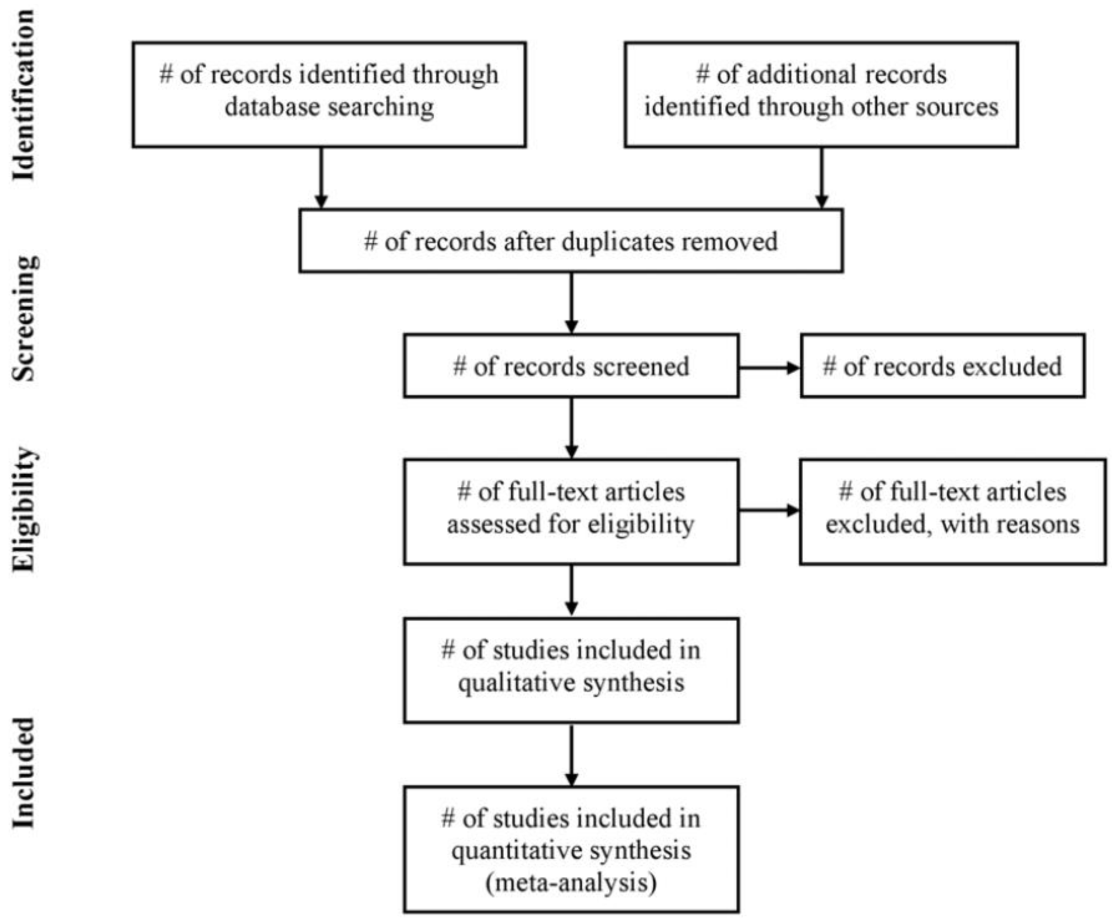

Figure 12: Flow chart illustration of the selection process of the studies for a systematic review (Moher et al., 2009)

Data extraction and approach for exploring and coping with heterogeneity

Data extraction process

For each review question, data will be extracted on:

- the PIT elements.

Data extractions were harmonised across the different studies from which the data were extracted (e.g. measurement unit and data coding).

Selected data were extracted independently by two researchers directly from the full-length articles to structured tables containing all the descriptive variables and relevant outcomes. Divergences between reviewers were resolved by consensus or by a third reviewer, if necessary. Primary study authors were not contacted for missing or ambiguous data.

Extracted outcome data were summarised in a standardised tabular format (see Table 4).

Table 4: Standardised tabular format of extracted outcome data

Reference	Time period	Geographical area	Type of vaccines	Population	Objective	Type of baits	Methods of release of vaccine baits	Density of baits and distribution patterns	Seasonal pattern of the releases	Methods of evaluation of the vaccination programmes