Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union

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ABSTRACT

Rabies is a notifiable disease in animals in the European Union. Despite the existence of several recommendations made by international organizations for rabies control, surveillance and monitoring of rabies in animals vary greatly between Member States. In this report recommendations are proposed for improving and harmonising rabies surveillance and reporting in animals in Europe. An adequate system of surveillance should be in place in all countries, whatever the rabies status (rabies-free and infected countries). Surveillance should be evenly distributed in time and space and should target animals suspected of having contracted the disease. All countries should report both positive and negative results of rabies diagnosis. For countries involved in oral rabies vaccination programmes (infected as well as rabies-free countries), the monitoring of rabies vaccination, based on investigating hunted animals from vaccinated areas, should be undertaken for assessing the efficacy of these programmes. The standardisation of diagnostic reference techniques and new confirmatory tests (such as Polymerase Chain Reaction) used in European Union is recommended. A national bat rabies surveillance network should be established in all European countries based on the testing of sick, rabies-suspect or dead bats of all bat species for lyssavirus infections. The Rabies Bulletin Europe is recommended as the basis for the reporting scheme of animal rabies in Europe with additional information to improve the existing data collection system and monitoring of rabies trends over time. Veterinary authorities should also report cases regularly to the OIE database interface.

KEY WORDS

Rabies, fox, surveillance, harmonisation, EU, animal, risk, disease, reporting.
SUMMARY

Despite the substantial progress that was made during the 20th century in reducing the burden of rabies, especially in central and eastern Europe, the disease remains endemic in animal populations in many European countries.

The principal reservoirs of classical rabies in Europe are the red fox (*Vulpes vulpes*) and the raccoon dog (*Nyctereutes procyonoides*). In addition, distinct epidemiological cycles occur in certain bat species involving different lyssaviruses. Although classical rabies has been eliminated in many Member States through the implementation of oral rabies vaccination programmes, rabies is still prevalent in wildlife in several eastern Member States and adjacent non-Member States. The majority of the western European countries are now free of classical rabies, with reported rabies restricted to relatively rarer bat cases (European bat lyssaviruses type-1 and -2). Although fox is the principal rabies vector in Europe, the raccoon dog plays a significant role in the epidemiology of rabies in the Baltic countries where numbers of infected raccoon dogs can exceed that of foxes. In large parts of Europe, rabies is being successfully controlled in animals thanks to programmes of oral vaccination.

Different tools exist for the reporting of rabies incidence with different objectives. Sample size, collection procedures and specimen type are only well regulated in Member States with active oral vaccination programmes. Similar to many important diseases of human and animals, a number of reporting systems have been implemented to record individual disease outbreaks. European Community legislation lays down data reporting requirements regarding rabies in animals. Furthermore, for members of the Lyssavirus genus several international organizations monitor cases of rabies and make data available to the general public. RabNet (www.who.int/rabies/rabnet), the Rabies Bulletin in Europe (www.who-rabies-bulletin.org/) and the World Organisation for Animal Health (OIE - www.oie.int/eng/ en_index.htm) all act as reporting systems. These surveillance reports are essential when monitoring the status of countries for presence of the virus. The objective of this project was to reconsider the existing system in place in Europe for the monitoring and reporting of animal rabies. Information on diagnostic methods currently available for rabies are compiled and discussed, particularly focusing on new tools not yet recommended by the World Health Organisation (WHO) and the World Organisation for Animal Health. The current sampling strategy and reporting systems for rabies in terrestrial animals and in bats are reviewed and analysed, identifying possible gaps.

These analyses are used to propose recommendations for a harmonised rabies surveillance system in the European Union:

- an adequate surveillance system should be in place in all countries, whatever the rabies status. Surveillance should be evenly distributed in time and space with both positive and negative results reported;
- rabies surveillance should target animals suspected of having contracted the disease and animals imported from endemic third countries showing clinical signs suggestive of rabies;
- oral vaccination of wildlife requires long-term monitoring. This monitoring is based on investigating hunted animals. A sufficient number should be investigated from all vaccinated areas to follow the World Health Organisation’s recommendations (four animals per 100 km² annually). Blood samples and teeth of animals should be analysed for serology and biomarker examination, respectively, and data (positive and negative results) reported;
- bats found sick, showing clinical signs or abnormal behaviour, dead bats of all indigenous bat species as well as bats involved in contact incidents, e.g. biting or scratching, or animals caught by pets, should be tested for lyssaviruses;
Development of harmonised schemes for the monitoring and reporting of rabies in animals in the European Union

- the Rabies Bulletin Europe is recommended as the basis for the reporting scheme of animal rabies in Member State with additional information reported to both the European Food Safety Authority and the Rabies Bulletin Europe to improve the existing data collection system, such as:
  - imported cases of rabies,
  - details on the vaccination programmes and any animal rabies vaccine-induced cases;
  - surveillance data: including positive and negative results. Rabies-free countries should also report the number of tested suspect animals;
  - bat rabies surveillance: negative and positive tested bats, with the causative lyssavirus genotype (EBLV-1 or EBLV-2) identified; and
- veterinary authorities should also report cases regularly to the OIE database interface.

Finally, recommendations are given for the data required by the European Food Safety Authority to monitor rabies trends over time.
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BACKGROUND

The EU system for the monitoring and collection of information on zoonoses is established by Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents. This Directive requires Member States (MSs) to collect, evaluate and report data on zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks to the European Commission (EC) each year. The monitoring and reporting system used is based on that of MSs, and in a few cases it is harmonised by EU legislation to the extent that the results from the monitoring are directly comparable between MSs.

According to the Directive, MSs have to send their zoonoses report to the EC on an annual basis by 31 May. The EC is asked to submit this information to the European Food Safety Authority (EFSA), who is responsible for examining the data and for publishing the Community Summary Report (CSR) from the results. The report is prepared by EFSA in close collaboration with the European Centre for Disease Prevention and Control (ECDC) and EFSA’s Zoonoses Collaboration Centre. In practice MSs report the information on zoonotic agents in animals and food through a web-based reporting application run by EFSA.

It should be noted that data on zoonoses cases in humans are provided through the Community networks for the epidemiological surveillance and control of communicable diseases established under Decision No 2119/98/EC and coordinated by ECDC.

According to Directive 2003/99/EC, the reporting of information on rabies takes place on the basis of the epidemiological situation in the country, which means that MSs should report the information if this zoonotic agent is considered to be of importance in their country. For the reporting year 2006, 24 MSs provided information of rabies in animals.

In the CSR on zoonoses the information received from MSs is analysed and summarised specifically to identify trends in the occurrence of the zoonotic agents and the sources of human infections. As there are currently no detailed harmonised rules or recommendations for reporting and monitoring rabies, the data obtained is often difficult to analyse and interpret at Community level.

EFSA’s Scientific Panels on Biological Hazards (BIOHAZ) and on Animal Health and Welfare (AHAW) have issued two opinions on the Review of the CSRs on zoonoses, zoonotic agents and antimicrobial resistance in the European Union in 2004 and 2005. In these opinions the panels give some recommendations on improving the monitoring and reporting of rabies. The panels also stated that there is a need for a common strategy on data collection, monitoring and reporting as well as for improvement in the harmonisation of definitions, in order to improve the usefulness of the data presented in the CSR.

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TERMS OF REFERENCE

The objective is to obtain proposals for the development of harmonised monitoring and reporting schemes for rabies in animals under the Directive 2003/99/EC. The schemes shall be applicable in all MSs and in compliance with relevant Community legislation.

The harmonised monitoring and reporting schemes shall, in particular, specify:

• the animal species, which should be monitored and the study populations (subgroups of the population) to be targeted. The animal species may cover wildlife, domestic and pet animals;
• the stage when sampling should take place;
• the sampling strategy (the procedure on how to select the samples) and the sample size (the number of samples to be collected);
• the type of specimen to be taken and the sampling techniques to be used;
• the diagnostic and analytical methods to be used;
• the information to be collected at national level and possibly at regional level; and
• the information to be reported.

The rationale for the specifications chosen in the monitoring and reporting schemes must be given. When developing the schemes, the following shall be taken into account: the public health and animal health needs, the feasibility and cost-effectiveness of the schemes, different MS situations, existing Community legislation as well as the scientific advice of EFSA’s scientific panels as well EFSA’s guidance documents.

The schemes shall also include suggestions for the analyses of data at national and Community levels, and, in particular, indicate where the following of trends over the reporting years would be useful and where spatial analyses would be applicable.

3 For example: Guidance from Task Force on Zoonoses Data Collection on good practices for design of field surveys, The EFSA Journal (2006) 93, 1-29.
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INTRODUCTION AND OBJECTIVES

INTRODUCTION

Rabies is a worldwide viral zoonosis caused by lyssaviruses of the family rhabdoviridae. The genus Lyssavirus is subdivided into different virus species also referred to as genotypes (Bourhy et al., 1993, World Health Organisation (WHO), 2005; International Committee on Taxonomy of Viruses (ICTV), 2009). Mainly domestic and wild carnivores as well as bats act as reservoirs for classical rabies virus (RABV, genotype 1). Except for Mokola virus (MOKV, genotype 3) bats have also been identified as reservoirs for all other genotypes.

The principal reservoir and vector of classical rabies in Europe is the red fox (Vulpes vulpes). Fox-mediated sylvatic rabies is predominately responsible for rabies transmission to other wildlife species and to domestic animals. The raccoon dog (Nyctereutes procyonoides) is also a major wildlife species infected with rabies in eastern and northern Europe (Vanaga et al., 2003; Maciulskis et al., 2006; Niin et al., 2008). The rabies situation in Europe is diverse. While the disease is still prevalent in wildlife in several eastern MSs and adjacent non-MSs, in large parts of Europe sylvatic rabies has been eliminated thanks to the implementation of oral rabies vaccination (ORV) programmes for foxes (Cliquet and Aubert, 2004; Matouch et al., 2006). Some southern and insular Mediterranean countries were never affected by the fox epizootic. Other countries have a long history of freedom from the disease, e.g. the United Kingdom and Sweden.

Distinct epidemiological cycles occur in certain European bat species involving at least two different lyssavirus species (EBLV-1 and EBLV-2 belonging to genotypes 5 and 6, respectively) depending upon geographical areas. EBLV-1 seems to have a specific association with the serotine bat (mainly Eptesicus serotinus and Eptesicus isabellinus in the southern part of the Iberian peninsula) while the EBLV-2 virus is more commonly associated with myotis species (Myotis daubentonii and Myotis dasycneme).

European countries occasionally record cases of rabies in illegally imported dogs and cats as well as in other domestic animals (Barrat, 2006; Metlin et al., 2006). The risk associated with importing pets depends upon geographical location as well as patterns of tourism and exchange practices of countries.

In 2008, a total of 1,407 animal rabies cases confirmed by laboratory testing were reported in 27 MSs and three human cases of rabies acquired outside the EU were reported by MSs (compiled from the Rabies Bulletin Europe (RBE), www.who-rabies-bulletin.org/).

Rabies is a notifiable disease in all European countries. The diagnosis of rabies can only be obtained by laboratory investigations on dead animals (WHO, 2005). Rabies diagnosis is the basis for rabies surveillance and for elaboration of measures in animal populations. Reliable tests based on antigen detection or virus isolation are referenced tests by WHO (World Health Organisation, 2005) and World Organisation for Animal Health (OIE) (Cliquet and Barrat, 2008) and are currently used by most laboratories. Besides those techniques, tools have been developed to enable lyssavirus typing, either by the use of monoclonal antibodies or by the sequencing of amplified products (for review see Fooks et al., 2009).

Unfortunately, sample size, collection procedures and specimen type are only well regulated in MSs for ORV programmes. Rabies surveillance is variable in MSs according to the status of countries regarding rabies, e.g. in rabies-free countries and in rabies-endemic countries in which ORV programmes have not yet been established.

Involvement of international organizations and of the EC has existed for a long time in European countries involved in rabies control. The WHO and OIE regularly publish recommendations dealing with surveillance, control and prevention of rabies in animals and in humans (WHO, 2005; Cliquet and Barrat, 2008). Several techniques and technical specifications are also recommended through EC
rabies experts groups (EC - SCAHAW, 2002) and the European Directorate for the Quality of Medicines and Healthcare\(^4\) (available monographs on rabies vaccines for animal and human use and on different techniques). Different tools for the reporting of rabies incidence also exist but have different objectives.

The objective of this project was to reconsider the existing system in place in Europe for the monitoring and reporting of animal rabies. Information on diagnostic methods currently available and used in National Reference Laboratories (NRLs) for rabies are compiled and discussed among the consortium of experts, and focus particularly on new tools not yet recommended by WHO and OIE. The sampling strategy (particularly sample size of wild animals to be analysed and collection procedure) and the current existing reporting system of rabies in terrestrial non-flying animals and in bats are reviewed and analysed and possible gaps are identified.

The assessment of the objective of the development of a harmonised scheme for the monitoring and the reporting of rabies was studied in this report in several milestones. The first milestone was to review the current disease situation in MSs through existing report schemes and the current national level of monitoring and reporting. The following milestone assessed animal species to identify those relevant to public health. Analytical diagnostic methods were then discussed in order to harmonise the tests used to be undertaken on animals received for diagnosis. One milestone targets bat rabies surveillance to provide recommendations on bat rabies epidemiology based on passive and active surveillance. The last milestones were to identify several recommendations for rabies harmonised monitoring and reporting in the EU.

\(^4\) The EDQM (Council of Europe) is a key European organisation involved in harmonisation and co-ordination of standardisation, regulation and quality control of medicines, blood transfusion, organ transplantation, pharmaceuticals and pharmaceutical care.
OBJECTIVES

Objective 1. Identifying the current disease situation in Member States and the current national level of monitoring and reporting

1.1 Rationale

This section is intended to provide an overview on the current disease situation in Europe. Also, existing systems of rabies reporting in MSs will be assessed to explain their respective objectives and how information from different countries is collected and analysed. This section will be the basis for Objectives 6 and 7, consisting of propositions for a harmonised monitoring and reporting scheme.

1.2 Approach

The epidemiological situation of rabies in Europe is analysed by evaluating current publications and information provided by the RBE (Rabies information system of the WHO Collaborating Centre for Rabies Surveillance and Research of FLI, Wusterhausen, Germany, http://www.who-rabies-bulletin.org) and the World Animal Health Information Database (WAHID) Interface (World Organisation for Animal Health (OIE), http://www.oie.int/wahis/public.php?page=home). In a second step, these existing systems of data collection and reporting systems are evaluated. Further disease reporting systems are also assessed, such as the world rabies survey (WRS), and reports to EU institutions and agencies (e.g. EC, EFSA).

1.3 Results

Current disease situation in MSs.

1.3.1 Classical rabies

In large parts of Europe, rabies was endemic before the 1980s with around 16,000 to 25,000 annual animal and human cases (compilation from WHO RBE data). The incidence of annual animal and human rabies cases in Europe decreased dramatically since the late 1980s largely as a result of the ORV of wildlife reservoirs with a concomitant decrease of annual animal cases, below 10,000 for the first time in 1983 (compilation from WHO RBE data). Please see Appendix D.

To date, 17 MSs are officially rabies-free according to OIE (see Table 1); of these, 9 countries have a long history of freedom from the disease, and eight have become rabies-free thanks to ORV programmes.

In February 2008, France lost its rabies-free status for two years due to secondary cases after the illegal importation of a rabid dog (Allibert et al., 2008). Italy was re-infected at the end of 2008 by fox-mediated rabies from rabies-endemic regions on the Balkan (de Benedictis et al., 2008). Those two countries became free of rabies in 2000 and in 1997, respectively, following the use of ORV against wildlife rabies. The above described two recent examples of re-infection of rabies clearly demonstrate the need for rabies-free countries to maintain rabies expertise and effective disease surveillance both for domestic animals and for wildlife.

Rabies cases in animals are reported in 10 MSs. The disease situation is improving with only a few reported cases in those countries that successfully implement ORV programmes. Several countries are bordering rabies-endemic regions and have to maintain a cordon sanitaire (protecting buffer area) along the border. Among MSs, the country with the highest number of reported rabies cases is Romania. In Bulgaria, the disease seems to be restricted to the western and northern regions of the country.
In the EU, human rabies cases are very rare. In the EU, human rabies cases are very rare. Between 2000 and 2009, 13 human imported cases of rabies and five indigenous cases were recorded in the EU (WHO RBE).

**Table 1:** Overview on the rabies situation in animals in 2008 in MSs, Switzerland, and Norway

<table>
<thead>
<tr>
<th>Country</th>
<th>OIE status (a)</th>
<th>Last case (b)</th>
<th>ORV in course</th>
<th>ORV used in the past</th>
<th>Presence of bat rabies</th>
<th>Rabies cases</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wildlife</td>
<td>Domestic</td>
<td>Bats</td>
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<tr>
<td>Austria</td>
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<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Belgium</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>10</td>
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<tr>
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<td>-</td>
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<td>Finland</td>
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<td>Free</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Italy</td>
<td>Disease present</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Latvia</td>
<td>Disease present</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>90</td>
<td>20</td>
<td>-</td>
<td>110</td>
</tr>
<tr>
<td>Lithuania</td>
<td>Disease present</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>47</td>
<td>22</td>
<td>-</td>
<td>69</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>Free</td>
<td>1999</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malta</td>
<td>Free</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Free</td>
<td>1989</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Norway</td>
<td>Free</td>
<td>1814</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Poland</td>
<td>Disease present</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>21</td>
<td>5</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>Portugal</td>
<td>Free</td>
<td>1960</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Romania</td>
<td>Disease present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>906</td>
<td>183</td>
<td>-</td>
<td>1089</td>
</tr>
<tr>
<td>Slovakia</td>
<td>Disease present</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Slovenia</td>
<td>Disease present</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>53</td>
<td>2</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Spain</td>
<td>Free</td>
<td>1979</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>2 (d)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Sweden</td>
<td>Free</td>
<td>1886</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Free</td>
<td>1996</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Free</td>
<td>1922</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>1(e)</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Source: OIE and WHO RBE.

(a) Based on self declaration of countries to the OIE.
(b) Indigenous only, imported cases as well as bat cases excluded.
(c) Two indigenous dogs infected by an illegally imported rabid dog.
(d) Cases in North Africa.
(e) Imported case of dog rabies to a quarantine facility. This did not affect the rabies-free status of the United Kingdom.
1.3.2 Bat rabies

Two specific lyssavirus genotypes, European bat lyssavirus type 1 (EBLV-1) and EBLV type 2 (EBLV-2) have been isolated from bats in the EU. Recently, West Caucasian Bat Lyssavirus (WCBV), a new member of the lyssavirus genus has been detected in the European part of the Caucasus (Kuzmin et al., 2005). Bat rabies is recorded in Europe since 1954. The majority of rabid bats were diagnosed in Denmark, followed by the Netherlands, Germany and Poland. Bat rabies was also reported from France, Spain, Switzerland, the United Kingdom (Great Britain), the Czech Republic, Slovakia, Hungary, Ukraine and Russia (WHO RBE; Van der Poel et al., 2005)). Based on current knowledge of the disease and the limitations in bat rabies surveillance, it can be assumed that bat rabies is present throughout the EU. Though bat rabies is widespread in Europe, it is very rarely transmitted to terrestrial mammals. EBLV-1 has been isolated from several Danish sheep in 1998 and 2002 (Rønsholt et al., 2002), from a German stone marten in 2001 (Muller et al., 2004) and from a cat in France in 2007 (Dacheux et al., 2009).

Between 1977 and 2006, EBLVs caused four human casualties (Fooks et al., 2003a; Botvinkin et al., 2005), of which two occurred in MSs, one in Finland and the other in Scotland, United Kingdom (Fooks et al., 2003b; Lumio et al., 1986).

One imported human rabies case in the Netherlands was caused by the Duvenhage virus (DUVV), an African bat lyssavirus (van Thiel et al., 2008).

1.3.3 Current national level monitoring and reporting

1.3.3.1 Reporting to European Commission and EFSA

In accordance with the Directive on animal health problems affecting intra-Community trade in bovine animals and swine\(^5\), MSs are obliged to report each year to the EC by 31 May, details of the occurrence of diseases of bovine animals and swine (i.e. rabies cases in these species) listed in Annex E (I) to the Directive and of any other diseases covered by the additional guarantees provided for by EU legislation in its territory.

On the basis of this Article, Commission Decision of 10 December 2003\(^6\) laying down criteria for information to be provided laid down the format of rabies reporting which corresponds to the format of the quarterly reports sent by the MSs to the WHO Collaborating Centre for Rabies Surveillance and Research. MSs may also provide the information in the format of the annual report on the occurrence of rabies as published in the WHO RBE. This report concerns only rabies cases in bovine animals and swine, however MSs also provide data on cases in other species on a voluntary basis.

Information on zoonosis and zoonotic agents is collected by MSs and transmitted on an annual basis in reports to the EC who forwards them to EFSA in accordance with Directive 2003/99/EC\(^7\) of 17 November 2003 on the monitoring of zoonoses and zoonotic agents. However, the information is in practise directly reported by MSs through the EFSA web reporting application. A reporting manual (EFSA, 2008) provides guidance and advice for the reporting of all agents. EFSA provides precise guideline information in the Manual on Reporting on Zoonoses to assist MSs in preparing the annual report of epidemiological data: relevant animal species to be tested and reported, relevant agents species of lyssavirus to be tested and reported, description of the monitoring and control system, reporting on the status as free, diagnostic methods typically used and a table to complete (Appendix C the EFSA guideline chapter dedicated to rabies). Reported data are then analysed and published in the

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CSR (see EFSA 2009, chapters 3-6). For 2007, 22 MSs reported data on rabies to EFSA. Table 2 provides information (EFSA, 2009) on the proportion of rabies cases from countries providing continuous data from foxes.

Table 2: Proportion of positive rabies samples from countries providing continuous data to EFSA from foxes 2004-2007

<table>
<thead>
<tr>
<th>Countries with a monitoring programme</th>
<th>2007</th>
<th>2006</th>
<th>2005</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>Total</td>
<td>% pos</td>
<td>Total</td>
<td>% pos</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Austria (a)</td>
<td>8,190</td>
<td>0</td>
<td>7,215</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>4,424</td>
<td>0</td>
<td>7,066</td>
<td>0</td>
</tr>
<tr>
<td>Estonia</td>
<td>83</td>
<td>0</td>
<td>111</td>
<td>34.2</td>
</tr>
<tr>
<td>Finland</td>
<td>261</td>
<td>0</td>
<td>230</td>
<td>0</td>
</tr>
<tr>
<td>Latvia</td>
<td>5,124</td>
<td>1.9</td>
<td>336</td>
<td>55.7</td>
</tr>
<tr>
<td>Lithuania</td>
<td>824</td>
<td>83.4</td>
<td>778</td>
<td>68.5</td>
</tr>
<tr>
<td>Poland</td>
<td>16,044</td>
<td>0.3</td>
<td>21,908</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Countries with annual data, but no information on monitoring provided</th>
<th>2007</th>
<th>2006</th>
<th>2005</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>Total</td>
<td>% pos</td>
<td>Total</td>
<td>% pos</td>
</tr>
<tr>
<td>-----------------------------------------------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Belgium</td>
<td>141</td>
<td>0</td>
<td>94</td>
<td>0</td>
</tr>
<tr>
<td>France</td>
<td>220</td>
<td>0</td>
<td>336</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>14,845</td>
<td>0.1</td>
<td>13,763</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Hungary</td>
<td>4,496</td>
<td>0.1</td>
<td>3,601</td>
<td>0.1</td>
</tr>
<tr>
<td>Italy</td>
<td>2,143</td>
<td>0</td>
<td>2,303</td>
<td>0</td>
</tr>
<tr>
<td>Portugal</td>
<td>53</td>
<td>0</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>Slovakia</td>
<td>3,747</td>
<td>0</td>
<td>3,630</td>
<td>0.1</td>
</tr>
<tr>
<td>Switzerland</td>
<td>41</td>
<td>0</td>
<td>52</td>
<td>0</td>
</tr>
</tbody>
</table>

Source: EFSA (2009)

(a) In Austria in 2006, one fox tested positive with the vaccination strain not with the wild strain.

1.3.3.2 Reporting to OIE

According to the Terrestrial Animal Health Code, Article 1.1.3. (see Appendix 1) OIE rabies code, the OIE delegate shall send an “Immediate notification” followed by weekly reports on the (re-)occurrence of a listed disease such as rabies to the OIE. Furthermore, a six-monthly report on the absence or presence, and evolution of diseases listed by the OIE is required. From 1996 to 2004, data on listed diseases is available in the Handistatus II (http://www.oie.int/hs2/sit_mald_freq_pl.asp?c_cont=4&c_mald=26). Here, only information on the current status of the disease is provided. Data from 2005 onwards are shown on the WAHID interface. Rabies cases can be summarised monthly and annually and also at country level or by lower administrative units, depending on the reporting country. Six-monthly reports describe the listed disease situations in each country. However, it is not clear whether only “terrestrial rabies” cases caused by RABV are subject to notification or any confirmed rabies case in animals. Information on control measures is also provided.

The “Immediate notification” reporting to the OIE is performed by the national authorities of different countries and is particularly useful as an alert system for exceptional events (disease reintroduction for example).
Appendix B represents an example of immediate notification (summary of all notification reports) by authorities in Italy during the reoccurrence of rabies in October 2008. The document is a summary with all links to previous related reports.

1.3.3.3 Reporting to WHO (Rabnet)

Since 1959, data on human and animal rabies from countries which are members of the WHO are collected through the WRS questionnaire. The data concern also post-exposure prophylaxis (number of persons vaccinated). Since the late 1990s, the questionnaire is accessible electronically (Rabnet, WHO, http://www.who.int/rabies/rabnet/en/).

The Rabnet website contains rabies data, ready-made maps and rabies related documents. Rabies data can also be linked to several country-specific indicators (population, education and health services). It appears that this system of data collection is presently poorly used by rabies-infected countries.

1.3.3.4 Reporting to WHO Rabies Bulletin Europe

With the WHO RBE, a European rabies reporting system was established in 1977 and is maintained by the WHO Collaborating Centre for Rabies Surveillance and Research in Tübingen (now Wusterhausen, Germany). Veterinary and human health authorities from 41 different European countries (MSs and non-MSs) submit data on officially confirmed rabies cases per species regularly to the RBE. Human cases are also reported to the RBE. Furthermore, additional epidemiological data such as the number of animals tested negative and details of ORV campaigns are provided by public health and veterinary competent authorities and are transmitted to the RBE. All data is summarised at a certain regional level depending on the administrative unit (Nomenclature des Unités Territoriales Statistiques - NUTS) and then are aggregated per country. The NUTS level of reporting differs from country to country. The most common and requested reporting area is NUTS 3, i.e. small regions. These NUTS 3 regions may differ in size depending upon the overall size of the country. All data reported to the RBE is transferred into a database. For technical reasons cumulative data for each NUTS region are stored. For displaying the data in online available maps (http://rbe1.fli.bund.de/Queries/Maps.aspx), Geographic Information System (GIS) data for all European countries with the respective NUTS level need to be available.
Table 3: Summary of the reporting of surveillance data for MSs, Switzerland and Norway

<table>
<thead>
<tr>
<th>Name</th>
<th>Code</th>
<th>Notification</th>
<th>Level (NUTS)*</th>
<th>Species</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>AUT</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>BEL</td>
<td>quarterly/annually</td>
<td>3</td>
<td>yes</td>
<td>printed only</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>BGR</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Czech Republic</td>
<td>CZH</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>DNK</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>EST</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>FIN</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>FRA</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>DEU</td>
<td>quarterly</td>
<td>4</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>GRC</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td>HUN</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>IRA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>ITA</td>
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<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
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<td>LVA</td>
<td>quarterly</td>
<td>3</td>
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<td></td>
</tr>
<tr>
<td>Lithuania</td>
<td>LTU</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Luxembourg</td>
<td>LUX</td>
<td>quarterly</td>
<td>1</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Malta</td>
<td>MLT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>NED</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>POL</td>
<td>quarterly</td>
<td>4</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Portugal</td>
<td>PRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Romania</td>
<td>ROU</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slovakia</td>
<td>SVK</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Slovenia</td>
<td>SVN</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>ESP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>SWE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United Kingdom</td>
<td>UNK</td>
<td>quarterly</td>
<td>3</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>NOR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>CHE</td>
<td>quarterly</td>
<td>4</td>
<td>yes</td>
<td></td>
</tr>
</tbody>
</table>

Source: WHO RBE.

To increase the value of reported rabies data, the number of animals tested negative is also requested beginning in 2002. As shown in Table 3, most MSs report surveillance data quarterly in the same way as positive cases are reported. The number of animals tested depend on the rabies status of the country, the implementation of ORV programmes and the country’s individual approach in rabies surveillance. Thus far, the submitted surveillance data are collected and aggregated at country level and published annually in the RBE. From the issue III/2009 onwards surveillance data can also be displayed in maps.

The RBE is issued on a quarterly basis in printed format, and a free version is also available electronically via internet. This bulletin provides epidemiological data and information on the disease and its control.
1.4 Objective 1 conclusions

All MSs report rabies data to international organisations and to the EC and EFSA. Based on the data from the different reporting systems, the situation of classical rabies in wildlife in the EU can be summarised as follows:

- several countries have been free of rabies for a long time: Cyprus, Denmark, Greece, Ireland, Malta, Portugal, Spain, Sweden, the United Kingdom;
- other countries have not reported rabies cases for several years, and are rabies-free as a result of ORV programmes: Austria, Belgium, the Czech Republic, Finland, France, Germany, Luxembourg, the Netherlands; and
- other countries report endemic areas with cases and are (or will be soon) engaged in ORV programmes: Bulgaria, Estonia, Hungary, Italy, Latvia, Lithuania, Poland, Romania, Slovakia, Slovenia.

The situation regarding rabies may change rapidly:

- reinfection from an infected area (e.g. Italy was reinfected in 2008 by fox-mediated rabies from rabies-endemic regions in the Balkans), particularly for those rabies-free countries bordering infected ones; and
- illegal importation of rabid pets into a rabies-free country (a recent example in 2008 in France with two dog cases from Gambia and Morocco, and the United Kingdom with one dog case from Sri Lanka). Most of these cases concern non-vaccinated puppies or young dogs. These imported cases of canine rabies may be (rarely) at the origin of transmission to indigenous pets. None of these imported cases started the establishment of a canine rabies cycle in the infected areas. The most likely explanation is the rapid detection of such cases prior to further widespread transmission.

Different reporting schemes are in place and provide information on rabies, each system having a specific objective. The main differences in the reporting systems are set out below.

- The OIE WAHID system was implemented for the notification of exceptional disease events, such as re-occurrence and disease outbreaks. Compulsory notification is generally submitted and published electronically rapidly after the event. When analysing rabies information which is presently available on the website, it is clear that only some countries are reporting data regularly. This is particularly true for the notification alert system.
- Quarterly data submission to the WHO RBE is on a voluntary basis. Data are reliable as the interface is regularly updated and the compliance for reporting is high. This database is the most commonly used among rabies epidemiologists. The RBE is published quarterly and focuses on the epidemiology of rabies in Europe both in animals and humans. The web interface is simple to use and allows an analysis of rabies epidemiology with respect to time, locality and species.
- As rabies is a notifiable disease, it has to be monitored and data and information have to be reported by MSs according to their respective epidemiological situation. Therefore, MSs have to undertake rabies surveillance and report on rabies. The EFSA CSR on Zoonoses (EFSA, 2009) constitutes a synthesis and analysis of rabies cases in the EU for the respective year. Data can present minor discrepancies compared to those of the RBE, probably because different MS representatives are involved in this task.

In conclusion, it appears that several reporting data systems for rabies already exist in Europe, with different objectives and edition periodicity. The quality of data primarily depends upon the willingness and participation of the respective countries to submit valid data. In order to ensure good data quality and consistency and to reduce the load of reporting duties of MSs by avoiding duplicative efforts, which may undermine the willingness to report, submission of rabies data to the different databases
should be harmonised as much as possible. When reporting to the EC\textsuperscript{8} the reports are in the same format as those sent to the WHO RBE. Thus, it would be desirable to use the same reports as a foundation for the report to EFSA. Additional information could be added, such as molecular characterisations to identify possible spill-over infections from bats, imported cases or cases from vaccine virus strains. Also, details of ORV campaigns as reported to other European institutions could be added.

Objective 2. Identifying possible infected animal species and specifying which should be monitored

2.1 Rationale

Identifying the most relevant species and new cases can help to identify the emergence and introduction of new virus variants into the animal population and provide a better understanding of epidemiology of rabies in each MS.

Since the late 1930s, the main reservoir and vector of classical rabies (RABV, genotype 1) in Europe has been the red fox (Vulpes vulpes). Though it has not yet been proven, there is an indication that the raccoon dog (Nyctereutes procyonoides) could act as another reservoir, as it is the second most reported species to be infected in central and Baltic Europe. Cases of rabies in wildlife mammals other than foxes and raccoon dogs have also been reported. In endemic European countries, domestic animals are generally infected by wild animals. However, cases of rabies in domestic species may also be the result of importation of an animal incubating rabies.

The reporting of bat rabies cases needs to be improved as bat species may be reported in existing reporting schemes under the category “wildlife” instead of the category “bat species” (Objective 5).

2.2 Approach

A compilation of data was undertaken with animal species in which rabies has been previously reported in existing reporting systems (RBE, EFSA, WHO) and published literature.

2.3 Results

Suspect animals are monitored for rabies in most European countries.

The following paragraphs record the lists of animal species in which the RABV has been reported in EFSA CSR on zoonoses and in the RBE.

The RBE reports on a quarterly basis all mammal species that have been found positive for rabies. Wildlife species most frequently infected with rabies are the red fox and the raccoon dog (Nyctereutes procyonoides) accounting for 90-94% of rabies cases in the EU between 1994 and 2004. Rabies is also detected in a wide range of other wildlife and domestic species, usually in spatial and temporal association with fox rabies. Other species reported infected in wildlife are martens, other mustelids, roe deer, wolves, badgers, raccoons, other carnivores, wild boar, red deer, fallow deer, bats and other wildlife. Apart from foxes and raccoon dogs, rabies cases were reported in jackals (Canis latrans) and in raccoons (Procyon lotor) in the EU. It is possible that the latter species are capable of establishing an independent transmission cycle.

Domestic animals (mainly dogs, cats and cattle accounting for 89-95% of rabies cases in domestic animals during the last decade) are considered spill-over infections since rabies ceased to persist in those areas where fox-mediated rabies was successfully eliminated. To identify those species that are mostly affected, the subsection “domestic animals” is divided into the following species: cats, dogs, cattle, goat/sheep, equine, pigs, stray dogs and other domestic animals. No information is available for the presence of dog-mediated rabies within the EU.

The rabies chapter of the EFSA Manual on Reporting of Zoonoses (EFSA, 2008) indicates to MSs the following relevant animal species to be regularly tested and reported on an annual basis: all domestic species, including pets (cats and dogs) and farm animals (sheep, goats and bovine animals) and also stray dogs and stray cats. For wildlife species, foxes, raccoon dogs, wolves, badgers and bats are recommended. Appendix C reports the prevalence table provided by EFSA to each MS to complete...
with all data obtained annually on each species. Compared to the RBE data, all minor infected species are included in a category “other”. In the RBE, these species which are dead-end or incidental rare species are summarised in “other domestic”, “other wildlife”, “other carnivores” and “other mustelids”.

Illegally imported pet or wildlife animals pose a threat to the reintroduction of rabies into rabies-free areas. Such imported animal further diagnosed positive for rabies should be clearly identified as “imported dog” and not “dog” during the reporting process.

The list of tested species of RBE is regarded to be sufficient and could be generalised for the reporting. Whenever feasible, additional data should be reported and specified in the reporting form:

- when rabies is diagnosed for an imported animal, it should be reported as such with the mention “imported dog” and not just “dog”;
- results of virus characterisation in imported cases, i.e. phylogenetic information should be submitted since it will allow further epidemiological investigations;
- for bat rabies surveillance, animals that were tested (negative and positive) should be speciated and such data should be submitted (see Objective 5); and
- the genotype responsible for each bat rabies case should be determined to distinguish between possible EBLV-1 and EBLV-2.

In countries using ORVs, rabies vaccine-induced cases should be clearly mentioned (assessed by strain typing using monoclonal antibodies or sequencing) as recommended by OIE and WHO.
Objective 3. Identifying the most suitable diagnostic methods

3.1 Rationale

Rabies diagnosis based upon clinical presentation or gross pathognomonic lesion is unreliable, because signs of the disease are not characteristic and may vary greatly from one animal to another, therefore, confirmation of infection can only be achieved by laboratory techniques. Evidence of a RABV infection can be demonstrated through the detection of the infectious virion (rabies tissue-culture infection test (RTCIT), mouse inoculation test (MIT)), its antigens (Fluorescent antibody test (FAT), Enzyme-linked immunosorbent assay (ELISA)) or Ribonucleic Acid (RNA) (reverse transcription polymerase chain reaction (RT-PCR)).

Referenced techniques for post-mortem diagnosis of rabies in animals and humans are detailed in the WHO Laboratory Techniques in Rabies (Meslin et al., 1996) and in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Cliquet and Barrat, 2008). These methods are also recommended for use in the Manual on Reporting on Zoonoses (EFSA, 2008).

Three principal routine methods of laboratory diagnosis of rabies are recommended both by WHO and OIE, i.e. FAT for antigen detection, the RTCIT and MIT for virus isolation. Both international organisations recommend that in vivo test (MIT) should be replaced by in vitro methods (RTCIT).

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

However, based on results obtained in recent proficiency tests undertaken by the Rabies Community Reference Laboratory (CRL) ANSES-Nancy, it seems that there is a need to standardise and harmonise further the diagnostic techniques used for routine rabies diagnosis in the EU. Also, the sensitivity of most of the molecular methods (RT-PCR) established in individual European laboratories turned out to be high with genotype 1 strains but may be reduced with other genotypes, e.g. EBLVs 1 and 2, representing genotypes 5 and 6 of the lyssavirus genus, respectively.

This section is intended to specify the recommended diagnosis methods for rabies diagnosis in animals.

3.2 Approach

Standard literature on rabies diagnostics published by WHO and OIE (WHO, 2005; Cliquet and Barrat, 2008; Rupprecht et al., 2008) as well as the EFSA CSR (EFSA, 2008) were reviewed.

An inquiry involving all NRLs has been performed in 2008 by the Rabies CRL to collect information on techniques routinely used in MSs for rabies diagnosis. In 2009, a proficiency testing for rabies diagnosis (FAT, RTCIT, MIT and PCR) was organised and a technical questionnaire was sent to NRLs. A short synthesis of results is reviewed focusing on technical performances of currently used tests.
3.3 Results

3.3.1 Techniques available and performance characteristics

3.3.1.1 Antigen detection

FAT is a rapid and sensitive method for diagnosing rabies infection in animals and humans, allowing specific and highly sensitive detection of the rabies antigen. FAT is the gold standard test for rabies diagnosis. The technique is based on impressions or smears made from brain samples, tissue fixation, mostly in cold acetone, and staining with fluorescein isothiocyanate-labelled polyclonal or monoclonal anti-rabies antibodies (Kissling, 1975; Dean et al., 1996; OIE: Cliquet and Barrat, 2008).

In general, the sensitivity and specificity of the FAT is very high but may be influenced by the quality of the specimen, conjugate, equipment and the skills and experience of the people involved in rabies diagnosis. The sensitivity of the FAT could be reduced in case of autolysis and putrification of the samples. Under certain circumstances failure to identify the presence of RABV in brain samples in a single test does not always confirm absence of infection. Therefore, in the case of FAT-negative results with human exposure or FAT-inconclusive results such tests should be confirmed using other recommended techniques (WHO, 2005; OIE: Cliquet and Barrat, 2008).

3.3.1.2 Virus isolation

Virus isolation can be performed on cells or upon intracranial inoculation of mice using RTCIT and the MIT. Both tests aim at detecting complete and fully infective RABV particles.

- The MIT was one of the first diagnostic tests for rabies, and is a sensitive and robust technique. Laboratory mice are inoculated intracerebrally with supernatant of a brain suspension and observed for up to 30 days after inoculation. Death during the first 48 hours after inoculation must be considered as non-specific; all dead animals must be dissected and brain samples tested for rabies by FAT to confirm diagnosis (Koprowski, 1996).

- The RTCIT has been shown to be as sensitive and specific as the MIT but is less time consuming and avoids the use of live animals for rabies diagnosis. Therefore, the RTCIT has already replaced the MIT in many countries. This test implies the isolation of RABV in a cell culture monolayer, e.g. mouse neuroblastoma cells, by subsequent visualisation by FAT. Murine neuroblastoma cells are more susceptible to field isolates of RABV than other cell lines tested such as cattle brain cells, chicken embryo fibroblasts, Vero cells, baby hamster kidney cells 21 (BHK-21). The method is described in detail by Webster et al. (1996), and in the OIE Manual on Diagnostic Tests and Vaccines, rabies chapter (Cliquet and Barrat, 2008).

From an ethical point of view, MIT should be replaced by RTCIT (WHO, 2005; Cliquet and Barrat, 2008).

3.3.1.3 Detection of viral genome

More recently, RNA detection by RT-PCR followed by restriction fragment length polymorphism (RFLP), PCR-ELISA, hybridisation in situ and realtime PCR have been proposed as rapid and sensitive alternative techniques (for review see Fooks et al., 2009). Many laboratories adopted RT-PCR for laboratory diagnosis and have been developing tools to type the RABV strain either with monoclonal antibodies or by amplified sequencing of RT-PCR products.

The amplification procedure consists of the reverse transcription of the target RNA into complementary DNA (cDNA) followed by the amplification of the cDNA by PCR (Kissi et al., 1995). The RT-PCR is widely used for rabies diagnosis, different parts of the genome can be targeted, but in
most cases, the N gene is used (Sacramento et al., 1991; Kamolvarin et al., 1993; Heaton et al., 1997; Picard-Meyer et al., 2004a; Trimarchi and Nadin-Davis, 2007).

The use of RT-PCR (and other amplification) is not currently recommended for routine post-mortem diagnosis of rabies (WHO, 2005), but allows the rapid diagnosis of rabies and typing if subsequent sequencing is undertaken as well as molecular epidemiological studies.

Due to their high sensitivity, molecular methods can be applied as confirmatory or alternative tests on poor quality brain tissue samples (autolysis, decomposition). Also, RT-PCR could also be considered a confirmatory test for FAT-negative samples with human exposure and as a result can be obtained in less time than RTCIT and MIT. Also RT-PCR is a prerequisite to group lyssaviruses into genotypes which is important for bat rabies and possible spill-over cases. It is of utmost importance that molecular methods should only be used in well experienced laboratories with strict quality assurance because molecular methods are highly sensitive. Hence, there is a risk of producing false-positive results due to cross-contamination.

To avoid false-positive and false-negative results validation and quality control of molecular methods should be implemented according to OIE guideline (Belak and Thoren, 2008).

Other tests have been established in many laboratories and are used as (confirmatory) back-up tests for rabies, e.g. MIT, RTCIT and immunochemical tests for antigen detection such as ELISA and rapid rabies enzyme immunodiagnosis (RREID). However, the reliability of the latter has been questioned.

### 3.3.2 Current diagnosis techniques used in MSs and performances

All NRLs for rabies in MSs have established FAT and virus isolation tests (mainly RTCIT). Also, the majority of these laboratories have the equipment and facilities for typing lyssaviruses either with monoclonal antibodies or by sequencing the amplified products of RT-PCR.

Considering proficiency testing for rabies diagnosis organised in 2009, the panel included eight coded samples, all positive for rabies, and three controls (two positive and one negative) were sent to each participating laboratory. The samples were field rabies strains belonging to different species and different genotypes.

Most laboratories produced satisfactory results (data not published). Results revealed that FAT was the diagnosis technique harbouring the lowest rate of discordant results, i.e. false-positive or false-negative results (1.78%), followed by the RT-PCR diagnosis (2.6% for qualitative diagnosis and 4.7% for genotyping diagnosis), then RTCIT (5.8% of discordant results) and MIT (8.9% of discordant results). False-positive results were identified using RT-PCR and FAT diagnosis (8.8% and 2.4% respectively) while false-negative results were found using FAT, RTCIT and MIT (7.7% and 11.9% respectively). Results on this proficiency test indicated a high level of sensitivity of RT-PCR through MSs and a high level of specificity of RTCIT and MIT. The low rate of discordant results obtained by FAT strengthens the status of this technique as the gold standard method. Focusing on the FAT proficiency test, false negative results were only identified for EBLV strains (genotypes 5 and 6). However, RT-PCR was the only technique able to guarantee 100% of efficiency to detect positive cases. As false-negative results cannot be tolerated when human exposition occurs, since they can cause interruption of post-exposure immunoprophilaxis leading to fatal cases of human rabies, an active effort of the international standardisation of RT-PCR procedures is recommended, so that this technique can be included in the standard guides for screening together with antigen detection in all cases of human exposition within the EU.

This highlights that special care must be taken for the diagnosis of rabies in bats and that technical staff must be highly trained to discriminate fluorescence obtained with EBLV from fluorescence obtained with the classical genotype 1 virus. Moreover, it should be noted that depending upon the
fluorescent conjugate used, the sensitivity of most of these methods is high with genotype 1 strains but may be reduced with other genotypes.

The analysis of the associated technical questionnaires revealed many variations in the diagnosis procedures used by MSs. These examples of variations could lead to a lack of efficiency of the diagnosis and emphasise the need for MS procedural harmonisation.

3.4 Objective 3 conclusions

The only way to undertake a reliable diagnosis of rabies is by the use of standardised reference tests. Although FAT, RTCIT and MIT have been standardised internationally and are widely used on a routine basis, efficacy, specificity and reliability may vary slightly if adapted to local laboratory conditions.

It is crucial for routine rabies diagnosis to follow a diagnostic hierarchy:

- to use FAT as the gold standard
  - FAT-positive result: presence of lyssavirus infection proven
  - FAT-negative result: absence of lyssavirus infection proven
  - FAT-inconclusive result: further confirmation using recommended techniques
  - FAT-negative result (in a context of human exposure): further confirmation using recommended techniques
- autolysed or putrified samples: use FAT and other recommended techniques. RTCIT and RT-PCR are considered confirmatory tests.

It is of utmost importance to use strictly validated techniques referenced by WHO and OIE. Laboratories should work in accordance with quality assurance schemes following the requirements of ISO/IEC 17025 (2005+Ap1:2007). Participation in inter-laboratory proficiency testing as organised and conducted by the CRL for rabies as a form of external quality control should be obligatory. RT-PCR techniques should be standardised internationally, at least within the EU, to be recommended for screening together with antigen detection in all cases with human exposition within the EU.
Objective 4. Define sample size and collection procedure, specimen types and sampling techniques

4.1 Rationale

This section is dedicated to sampling considerations for mammals other than bats (for bat surveillance, see Objective 5).

Most MSs report surveillance data in the same way as positive cases are reported. However, the numbers of animals tested differ considerably as they might depend on the rabies status of the country, the implementation of ORV campaigns and the country’s individual approach to rabies surveillance (Table 4). Also, the animal species targeted differ. These differences make it difficult for EU and national competent authorities to evaluate the real rabies situation in a country because low and high numbers of animals tested would seem to represent poor versus good surveillance. In contrast, data may not even be available or only be reported spontaneously and not regularly, so it is unclear whether adequate rabies surveillance is still implemented in these countries. Usually, disease surveillance is based on laboratory investigations on samples taken from susceptible animal species. The type and number of samples should be sufficient to allow the detection of the disease.

A defined sample size seems a prerequisite for the surveillance of many diseases, since it provides information on the validity of the status gained and gives guidance to the respective authorities on the number of animals to be sampled. Even though rabies is the oldest known zoonotic disease, there are no standard recommendations on the sample size for rabies surveillance. Here, we suggest introducing situation-based surveillance.

Furthermore, it is often not clear what animals need to be sampled in the field, and how to package and transport/ship samples to guarantee proper rabies diagnosis. In the context of international transport regulations, infectious substances are defined as substances that are known or are reasonably expected to contain pathogens. Pathogens are defined as microorganisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents, such as prions, which can cause disease in humans or animals. Therefore, it appears important to provide brief practical information to MSs for the transport of specimens to be tested as diagnosis is crucial for decisions related to human post-exposure prophylaxis and for the elaboration of measures of control in animals.

Laboratory diagnosis techniques for rabies are classically applied to brain tissue. The results obtained depend on different factors, e.g. methods/techniques used and choice of appropriate parts of brain tissue to be analysed. Although there is extensive literature on the subject, there is often a lack of awareness concerning this matter. A short description of the most appropriate brain tissues to be analysed is provided in the following section.
## Table 4: Rabies surveillance data (animals tested negative) as reported to the World Health Organisation Rabies Bulletin Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Bats</td>
<td>Wildlife</td>
</tr>
<tr>
<td>Austria</td>
<td>8,239</td>
<td>2</td>
<td>8,063</td>
</tr>
<tr>
<td>Belgium</td>
<td>488</td>
<td>21</td>
<td>170</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>158</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>7,927</td>
<td>12</td>
<td>7,318</td>
</tr>
<tr>
<td>Cyprus</td>
<td>no data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>49</td>
<td>39</td>
<td>7</td>
</tr>
<tr>
<td>Estonia</td>
<td>475</td>
<td>1</td>
<td>228</td>
</tr>
<tr>
<td>Finland</td>
<td>554</td>
<td>1</td>
<td>533</td>
</tr>
<tr>
<td>France</td>
<td>1,774</td>
<td>200</td>
<td>375</td>
</tr>
<tr>
<td>Germany</td>
<td>16,252</td>
<td>61</td>
<td>15,391</td>
</tr>
<tr>
<td>Greece</td>
<td>no data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td>3,982</td>
<td>8</td>
<td>3,523</td>
</tr>
<tr>
<td>Ireland</td>
<td>no data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>3,622</td>
<td>2</td>
<td>3,064</td>
</tr>
<tr>
<td>Latvia</td>
<td>572</td>
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<td>271</td>
</tr>
<tr>
<td>Lithuania</td>
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<td>1,280</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>28</td>
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<td>22</td>
</tr>
<tr>
<td>Malta</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>121</td>
<td>113</td>
<td>3</td>
</tr>
<tr>
<td>Poland</td>
<td>26,800</td>
<td>118</td>
<td>24,544</td>
</tr>
<tr>
<td>Portugal</td>
<td>58</td>
<td>-</td>
<td>42</td>
</tr>
<tr>
<td>Romania</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Slovak Republic</td>
<td>4,241</td>
<td>14</td>
<td>3,721</td>
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<tr>
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<tr>
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<td></td>
</tr>
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<td>Sweden</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Switzerland+Liechtenstein</td>
<td>99</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>922</td>
<td>867</td>
<td>10</td>
</tr>
</tbody>
</table>

### 4.2 Approach

A review was carried out on international standard literature from the WHO, OIE, International Air Transport Association (IATA, 2009) and the “Accord européen relatif au transport international des marchandises Dangereuses par Route”\(^9\) (ADR, 2009) as well as specific national legislation related to recommendations of sample sizes for rabies surveillance, transport and shipment of infectious substances, and selection of appropriate brain tissues.

\(^9\) European Agreement concerning the International Carriage of Dangerous Goods by Road
4.3 Results

4.3.1 Surveillance sample size (passive and active) and sample collection procedure

Sampling for surveillance is often divided into two approaches, either testing on suspicion, or using a systematic sampling plan (passive versus active surveillance).

Rabies passive surveillance is based on laboratory investigations of dead animals for rabies diagnosis using reference techniques (see Objective 3). A passive surveillance system should be in place in all countries, whatever their rabies status (free or infected). Suspect animals (domestic or wildlife) likely to be submitted for rabies diagnosis are listed in Objective 2.

There are no current sample size recommendations for passive surveillance in rabies-endemic countries.

Considering the (passive) surveillance in rabies-free countries/regions, the WHO report that “an adequate passive surveillance system should be in operation in rabies-free countries and a minimum number of homogeneously distributed samples from suspect cases belonging to the major susceptible domestic and wild animal species present in the country should be tested on a regular basis. National public health and veterinary authorities, in collaboration with relevant international entities, should define the appropriate number of samples to be tested from the different susceptible wild and domestic animal. For domestic animals, in particular dogs and cats, the number of samples to be tested should be between 0.01% and 0.02% of the estimated population” (WHO, 2005). In both cases it is suggested to give priority to the testing of those animals suspected of being rabid, and those found dead such as road kills. The OIE only recommends that an effective system should be in place, but no specific sample size recommendations are given.

Recommendations for rabies surveillance are only given for countries having implemented ORV campaigns (mainly foxes and raccoon dogs), where it is addressed under the term “rabies monitoring”. The objective of monitoring in this proposed scheme is to sample animals in vaccination areas to evaluate the efficacy of ORV campaigns in terms of bait consumption (bait-uptake), herd immunity of the target population against rabies as well as incidence of rabies. Therefore, animals that are specifically sampled and killed for this purpose correspond to the healthy subpopulation targeted by oral vaccines, i.e. susceptible or protected/treated foxes and raccoon dogs. Those animals are considered not suspect for rabies but healthy animals. The following laboratory investigations for those animals are recommended:

- rabies diagnosis using reference tests;
- determination of the level of rabies neutralising antibodies in blood samples to evaluate the immunity of the animal population; and
- analysis of the occurrence of a biomarker: baits contain a biomarker (generally tetracycline) that provides a life-long marking of bones and teeth.

In the latest technical EU report (EC - SCAHAW, 2002) of the WHO expert committee on Rabies (WHO, 2005) the recommended sample size for monitoring the efficacy of ORV programmes (biomarker detection, serological testing and rabies incidence) was reduced to a minimum of four target animals per 100 km² annually, from the 8/100 km² recommended in 1992. The EU report (EC - SCAHAW, 2002) recommends testing at least eight foxes/100 km²/year for rabies in vaccination areas.

Unfortunately, in this specific case based on standard definitions, the terms monitoring and surveillance are not appropriately used here and lead to confusion and misinterpretation. According to OIE (2009), surveillance is defined as a continuous investigation of a given population to detect the occurrence of a disease to be controlled, which can include testing of parts of the population. In
contrast, monitoring is defined as an ongoing programme aimed at the detection of changes of disease prevalence in a given population and its environment.

4.3.1.1 Reconsideration of recommendations on rabies surveillance

Rabies pathogenesis in terrestrial mammals is distinctive, as infected animals will eventually die from the disease. The testing of healthy animals is likely to lead to negative results of no value as the presence of the virus can only be confirmed in the late stage of the disease and no “carrier state” or asymptomatic sub-clinical infection exists. Taking this concept into consideration, disease surveillance for important diseases in wildlife that show clinically visible alterations has recently been reconsidered (Thulke et al., 2009). The purpose was to evaluate common sampling schemes to derive one single improved "situation-based" strategy for wildlife diseases that cause mortality or morbidity events, including rabies. The aim was to relate surveillance more closely to actual epidemic situations.

The evaluation was motivated by (i) missing or vague recommendations on surveillance for countries with endemic wildlife-mediated rabies, (ii) the need for infected MSs to specify their national rabies surveillance programmes in wildlife with respect to demonstrating the efficacy of current ORV programmes according to existing EU regulations; and (iii) the urgent need for a surveillance scheme for rabies in foxes after the countrywide elimination of this disease in western and central Europe.

The following is a digest of the paper mentioned above highlighting the rationale, main findings and conclusions of the study, focusing on rabies as an example:

In guidelines published by the World Health Organization (WHO), the World Organization for Animal Health (OIE), and national documents, surveillance programmes for wildlife diseases including rabies are often adapted from livestock surveillance approaches. The applied schemes are therefore based on continuing tests of quotas of the total population to demonstrate the absence of disease. In particular, these schemes involve the continuation of testing protocols after elimination (or even before arrival) of the disease of concern. Sample design for wildlife disease surveillance, however, is more complex than in livestock due to the limited knowledge on census, and limited access to the population at risk. Consequently, statistical confidence might also be limited unless the sample size is increased. Considering the huge economic burden caused by continuous testing of presumably healthy animals, reconsideration of the aims, sample sources and sampling designs of common investigation efforts is required.

A situation-based scheme has been proposed showing that adapting surveillance to the actual epidemic situation provides a straightforward and cost-efficient solution for an overall surveillance (Thulke et al., 2009). The study provided evidence that a sample size cannot be defined for proving the absence or the presence of rabies in wildlife regardless of the reservoir species. Instead, the scheme recommended the use of indicator animals, the number of which cannot be predetermined.

4.3.2 Rabies surveillance and rabies monitoring

The following paragraphs to consider will detail the different animals to consider for rabies surveillance and rabies monitoring and answered whether rabies surveillance should more effectively target indicator or hunted animals.

4.3.2.1 Rabies surveillance: based on indicators animals

All MSs should undertake rabies surveillance (infected or rabies-free countries). For rabies-free countries, demonstrating that the animal population is free from rabies infection may be difficult to prove as these countries rarely encounter suspected cases.
Indicator animals (IAs) are individuals suspected of having the disease. This includes animals to which humans might have been exposed (biting, scratching or licking on broken skin), animals showing clinical signs or abnormal behaviour suggestive of rabies, animals found dead and road-kills (in rabies-endemic countries only). Animals imported and showing clinical signs suggestive of rabies are included in this surveillance. It should be noted that in the absence of suspect clinical signs or information suggesting illegal import, rabies-free countries should not precipitate a rabies investigation in those domestic animals at the origin of a human contamination.

For diseases that cause mortality or morbidity events the sample source will, by definition, be representative of the infected population, focusing the sampling effort in area and time towards the outbreak.

The rationale for focusing sampling on IAs is based on three facts: (i) the chances of finding a positive animal is higher in suspect animals; (ii) the numbers of samples submitted to diagnostic investigation would be dramatically lowered, with consequently significantly less laboratory effort and relative costs, and (iii) the increased number of IA sample units from affected regions (i.e. where newly introduced rabies causes fatalities) will increase the number of laboratory investigations exactly when needed. With these characteristics the routine sampling of IAs fits the needs of rabies surveillance in both rabies-endemic and rabies-free countries. From the retrospective analysis of historic rabies surveillance data sets, it was found that the sampling of IAs is always more effective for detecting virus-positive animals than sampling hunted animals regardless whether a national ORV programme was implemented or not (Thulke et al., 2009).

The current concept for disease surveillance of epidemics in wildlife is repeated proof of absence via falsifying presence, e.g. investigation of sample sizes large enough to reject prevalence levels of equal or larger than 5% with 95% certainty (Cannon and Roe, 1982). While intended for areas where the disease is suspected to occur (EC - SCAHAW, 2002) the approach is less helpful in areas where disease is absent and prevalence is zero. Further assurance of a lower prevalence threshold might increase the required sample size to an economically impractical amount of tests, and still not solve the conceptual problem. In areas where the disease is absent, numbers of IAs not killed by rabies might be naturally limited and hence a predetermined number of samples from IAs cannot be guaranteed.

After the detection of a rabies case in a region the epidemic situation changes to ‘presence of disease’. In this situation continued IA sampling aims at the detection of diseased animals still present to assist decisions on the termination of control measures: as long as cases are detected, control measures have to be continued. As the time since the last rabies case lengthens, suspicion will eventually arise as to whether the disease is still present. Direct proof of disease absence by statistical testing alone is problematic. Consequently, disease management guidelines incorporate plausibility arguments based on the epidemic character of the disease (most often some time period operating disease surveillance without detecting a case). Under the proposed situation-based surveillance scheme, if the testing of IAs does not provide evidence of disease over an epidemiologically reasonable time period (disease-specific; two years according to OIE for rabies) absence can be proved by stopping control but continuing routine disease surveillance in IA. After a finite time the protective effect of control (e.g. immune animals) will cease in the host population (OIE, 2009). Hence, persistent but unseen disease would recover and finally be shown through IA disease surveillance.

The only issue that must be managed in IA sampling is willingness to deliver IAs. The proposed scheme will be heavily dependent upon the detection and submission of IAs for testing. Individual countries should therefore assess the risk of rabies incursion or spread and ensure that there is sufficient vigilance for IA, and that submission is not biased. For example more road-kills and animals in contact with humans may be submitted from urban than rural areas. Disease awareness is known to decrease when a country is free from a disease (WHO, 2005) and the proposal of fixed-size sampling aims to counter that decreasing awareness. Extensive public awareness of the preventive role of IA
sampling needs to be raised.

### 4.3.2.2 Rabies monitoring: based on hunted animals

All infected countries involved in ORV programmes (and also free countries vaccinating at borders) should organise rabies monitoring. An apparently pragmatic approach is to supplement the routine sampling of IAs with hunted animal samples in order to meet sample size requirements. Hunted animals (HAs) are sampled from regular hunting activities or specific sampling hunts, e.g. structured or non-random selection (OIE, 2009).

This sample source is assumed to be representative of the healthy population (i.e. susceptible or protected/treated) on large spatial and temporal scales and therefore will consist of individuals who are not suspected of having the disease. Although the extra effort of investigation of HA submissions will not limit the functionality of the disease surveillance, there will be a good chance that most HA samples (i.e. samples from healthy animals) are investigated without providing useful information because tested animals are not sampled among a representative population of suspect animals.

Hence, if sample size is augmented with HAs, the majority of tested samples will come from HAs which (i) do not represent the targeted subpopulation of disease surveillance, (ii) provide limited evidence to give a timely alert a disease outbreak, and (iii) require a continual huge diagnostic effort from regions where detection cannot occur. Therefore, the sample size specification might bias diagnostic efforts on HAs when it is not desirable (i.e. if IA incurrence is naturally limited).

This perspective is supported by a recent observation made by the subgroup rabies of the EU task force indicating that “the sample sizes for rabies surveillance as recommended by both WHO (2005) and the SCAHAW of the EU (EC – SCAHAW, 2002) may be difficult to achieve. Rigorous attempts to achieve this target may simply result in the shooting and testing of a large number of healthy animals. The priority categories for rabies surveillance should be “indicator” animals, e.g. animals showing clinical signs of rabies, suspect animals, road kill, animals found dead, and animals involved in human and animal exposure, throughout the country” (EC, 2008).

Therefore, HAs should only be used for the monitoring of the efficacy of ORV programmes (bait consumption, herd immunity); those animals that are targeted by oral vaccines, namely foxes and raccoon dogs. A sufficient number should be gained from all vaccinated areas trying to follow WHO recommendations (four animals per 100 km² annually). The blood sample and bones or teeth of animals should be analysed for serological and biomarker examination, respectively.

### 4.3.2.3 Rabies surveillance conclusions

Considering the situation-based surveillance approach, care is required when interpreting data as presented in Table 4.

Also, in order to avoid future misinterpretation the terms “surveillance” and “monitoring” should be used appropriately. For rabies the term “surveillance” should be used for the continuous investigation of susceptible populations (wildlife and domestic animals) to detect the occurrence of rabies to be controlled. The term “monitoring” when referred to rabies, should only be used for the follow-up of ORV programmes, e.g. bait consumption and herd immunity.

Tables 5 and 6 summarise updated recommendations of sampling for rabies surveillance and monitoring depending on rabies epidemiological situation and laboratory investigations to be undertaken.
Competent veterinary authorities must be made aware that, rather than by defining minimum sample size, effective notification of the rabies outbreaks can be baselined by continuing alertness for, and sampling of indicator animals in order to maintain an effective system of disease surveillance (OIE, 2009).

As all mammals are principally susceptible to rabies, all clinically suspect cases, regardless of the species involved, need to be monitored in order to estimate the spread of the disease and to estimate effectively the risk to human health. Special attention should be paid to those species that could possibly act as reservoirs. In rabies-free countries disease vigilance need to be maintained with special emphasis on (illegally) imported pet or wildlife animals.

**Table 5:** Rabies surveillance and monitoring of Oral Rabies Vaccination

<table>
<thead>
<tr>
<th>Samples</th>
<th>Rabies-free countries</th>
<th>Rabies-free countries bordering infected countries</th>
<th>Rabies-infected countries involved in ORV</th>
<th>Rabies-infected countries not involved in ORV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indicator animals (all domestic and wild species)</td>
<td>Animals sampled throughout the country</td>
<td>Animals sampled throughout the country Enhanced surveillance along the borders</td>
<td>Animals sampled throughout the country</td>
<td>Animals sampled throughout the country</td>
</tr>
<tr>
<td>Hunted animals</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 6:** Laboratory investigations on field samples

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Indicator animals (IAs)</th>
<th>Hunting animals (HAs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabies diagnosis</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>Serology</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Biomarker determination</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* If hunted animals are still analysed for rabies diagnosis data reporting cannot be mixed with those related to indicator animals.

4.3.3 **Transport of samples**

According to IATA (2009) and ADR (2009), infectious substances are classified into two categories of infectious substances, designated as A and B:

- A “Category A” material is an infectious substance that is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A infectious substances are assigned to identification number UN 2814 for substances that cause disease in humans or both in humans and animals, or UN 2900 for substances that cause disease only in animals. For an updated list of indicative samples of Category A substances, please see the latest edition of IATA or ADR.

- A “Category B” infectious substance is one that does not meet the criteria for inclusion in Category A. A Category B infectious substance does not cause permanent disability or life-threatening or fatal disease to humans or animals when exposure to it occurs. The proper shipping name for a Category B infectious substance, “Biological specimen, Category B,” is assigned to identification number “UN 3373”.

Based on the definition described above for Category A and B infectious substances and on the definition of cultures, patient specimens and diagnostic specimens, infectious RABV and specimens for rabies should be classified as follows:
• All infectious RABVs (cell cultures only, positive brain tissue) should be assigned to identification number UN2814. The proper shipping name for UN 2814 is “INFECTIOUS SUBSTANCE, AFFECTING HUMANS”.
• Specimens for rabies diagnosis (brain material, etc.) should be assigned UN3373. The proper shipping name of UN 3373 is “BIOLOGICAL SUBSTANCE, CATEGORY B”.

4.3.4 Specific specimen tissues to be investigated and sampling techniques for rabies diagnosis

4.3.4.1 Biosafety considerations

All persons involved in rabies diagnosis should receive rabies pre-exposure immunisation, their immunological status should be checked regularly with serologic assay of antibody titer and booster injections should be given as necessary. Biosafety level 2 safety practices are adequate for routine laboratory activities such as diagnosis and animal handling (WHO, 2005), RABV is categorised as a Biosafety level 2 pathogen in diagnostic settings in the USA (Trimarchi and Nadin-Davis, 2007) and in many other rabies-endemic countries. It should be noted that in certain countries, depending upon national safety rules, Biosafety level 3 level can be recommended. In certain research and vaccine production settings, and for diagnostic samples with the additional suspicion of infection with a Biosafety level 3 agent, it may be elevated to Biosafety level 3 status. Level 3 of biological safety is recommended by WHO (2005) for the production of large quantities of concentrated viruses, conducting procedures that may generate aerosols and when working with lyssaviruses for which the effectiveness of a current prophylaxis is not known.

All procedures that could generate aerosols, such as the grinding of tissues for performing cell inoculation tests, should be undertaken in a biological safety cabinet. During necropsy, barrier protection is required for the safe removal of brain tissue from animals submitted for rabies testing and should include the following personal protective equipment: gloves, waterproof apron, boots, surgical masks, protective sleeves and a face shield.

4.3.4.2 Transport of specimens

As described in section 4.3.2, specimens for rabies diagnosis should be shipped, with triple packaging, according to national and international regulations to avoid exposure hazards. The transit time of specimens should be as short as possible, preferably within 48 hours. The specimen should be preserved by refrigeration or freezing during transport to the laboratory, refrigeration will preserve a sample for at least 48 hours; freezing of the sample for transit may introduce additional testing delays.

4.3.4.3 Source of specimens for diagnosis and storage conditions

The carcass submitted for rabies diagnosis should be refrigerated immediately following the death of the animal to retard decomposition and brain autolysis. Samples submitted to the laboratory may be a complete carcass, an intact head, an intact brain or dissected brain tissues. The intact head or an intact brain of the animal, constituting the ideal specimen for rabies testing, should be submitted for analysis. In the same manner, the entire body of a bat should be submitted. For large livestock, such as cattle and horses, and due to the fact that the shipping of the complete carcass or the entire head can pose a specific problem (size, weight, cost), veterinary laboratories or a veterinarian clinician can remove the entire head and/or perform the brain dissection following decapitation. Dissected brain tissues must include a complete cross-section of the brain stem and either cerebellum or hippocampus.
4.3.4.4 Sampling for postmortem diagnosis

The accuracy of rabies diagnosis is dependent on the quality of the sample, virus antigen distribution and areas of the brain tested. Because the animal species, site of exposure, variant of RABV and time and cause of death can all affect the terminal distribution of RABV in the brain of an infected animal, multiple areas of two or three regions of the brain should be tested to achieve reliable results (Trimarchi and Nadin-Davis, 2007). The hippocampus is reported to be FAT-negative in 3% to 5% of rabid animals; cerebellum and other parts of the cerebrum may then be negative in up to 11.1% of the cases (Bingham and van der Merwe, 2002). Full brain stem cross-sections, such as the medulla and pons, shown as the most valuable sample for the demonstration of RABV infection, should be routinely included in the rabies diagnosis, as well as the cerebellum and the hippocampus.

For very small animals such as bats, combined areas, including parts of the cerebellum and mid-brain as well as both cerebral hemispheres, should be examined.
Objective 5. Enhancement of bat rabies surveillance in the European Union

5.1 Rationale

Bat rabies is thought to occur throughout Europe. Indigenous bats are protected and sometimes endangered, which prevents a strategic surveillance approach such as that used for classical rabies. As a result, the numbers of animals tested differ considerably. Also, in many MSs there is no rabies surveillance programme in bats (Objective 4, Table 4). In countries where rabies surveillance in bats is established, the programme is often a re-active (passive) surveillance. In a few countries active (pro-active) surveillance programmes have also been initiated, in which bats are captured for sampling to be investigated for rabies by often targeting specific species and specific regions of the country only. Therefore, an evaluation of the real situation and the associated animal and public health risk is difficult. For effective public health protection, enhancement of bat rabies surveillance in Europe is needed.

5.2 Approach

Current re-active and pro-active EBLV harmonised surveillance protocols were presented by the WP5 MedVetedNet working group (Med-Vet-Net 2005) and adopted by EUROBATS (5th Session of the Meeting of Parties, Ljubljana, Slovenia, 4-6 September 2006). Other relevant literature include: recommendations of the First International Conference “Rabies in Europe”, Kiev, Ukraine, 2005 (Dodet B et al., 2006).

5.3 Results

Three known bat lyssaviruses can potentially occur in Europe: EBLV-1 and EBLV-2 in western and central Europe and West Caucasian bat virus in eastern parts of Europe.

A national bat rabies surveillance network is desirable in all European countries in close collaboration with bat specialists including international bat agencies. Bats are protected species; hence sampling for surveillance for bat lyssavirus infections has to comply either with regulations of Council Directive 92/43/EEC of the European Union on the conservation of natural habitats and of wild fauna and flora, with the Agreement on the Conservation of Bats in Europe, EUROBATS, 1991 or national legislation. The following protocols for passive and active surveillance are based on the recommendations of the First International Conference “Rabies in Europe”, Kiev, Ukraine, 2005.

5.3.1 Passive surveillance

Passive surveillance (re-active surveillance) is based on the testing of sick, rabies suspect (showing clinical signs or abnormal behaviour) or dead bats of all bat species, for lyssavirus infections. Also, bats involved in contact incidents, e.g. biting or scratching, or animals caught by pets should be included. Further sources of frozen or formalin preserved bat samples can be archives of zoological institutions or private bat collections. All dead bats (regardless of species) should be submitted to the NRLs 1 Rabies for lyssavirus testing.

5.3.2 Active surveillance

Active surveillance (pro-active surveillance) is based on the monitoring of free-living indigenous bat populations for lyssavirus infections. The focus of research can either be on the screening of all abundant bat species or on the surveillance of high risk bat species in a particular area. Sampling (generally blood and saliva) has to be done without damaging bat populations: killing bats for active surveillance is illegal and unacceptable. Capturing of bats should be conducted in close collaboration with bat conservationists previously vaccinated against rabies. Bats can best be captured when leaving their shelters using mist-nets, harp traps, hand-nets, etc., according to the particular species and...
roosting sites. Sampling of micro-biological material should be undertaken on an annual basis, preferably in the same month in order to get comparable data. Repeated sampling in the same year should be discouraged because it could cause excessive disturbance to bat colonies.

For both passive and active surveillance the following data should be collected: (i) ring identification number, (ii) species, (iii) gender and reproductive state, (iv) age (estimated by the degree of ossification in fingers’ metacarpals and phalanxes, together with tooth wear levels), (v) weight and forearm length (active surveillance), (vi) collector (name, address, telephone number, email), (vii) accurate location, (viii) details of exposure (contact, biting, scratching, part of the body), (ix) information on abnormal behaviour, etc. and (x) diagnostic test results (FAT, virus isolation tests, PCR, serology and others if applicable). A uniform sample submission form is recommended for data collection.

5.3.3 Recommendations on bat rabies surveillance

A fact often disregarded by veterinary authorities is that the word “bats” is a general term for a number of different species, which can differ considerably in their distribution, abundance and apparent likelihood of carrying lyssavirus.

According to EUROBATS (www.eurobats.org) more than 45 different bat species occur in Europe. Therefore, in contrast to terrestrial rabies, the establishment of an adequate surveillance for bat rabies is much more complicated as from an epidemiological point of view, all bat species occurring in any country, would need to be included to the same extent. For the reasons explained above and because of conservation issues, however, as for terrestrial rabies defining a sample size for harmonised bat rabies surveillance is unsuitable.

EBLV-1 and EBLV-2 have been shown to have a specific association with the Serotine bat (Eptesicus serotinus and Eptesicus isabellinus) (Vazquez-Moron et al., 2008; Picard-Meyer et al., 2004b) and the species of Myotis bats (Myotis daubentonii and Myotis dascyneme), respectively (Whitby et al., 2000; Johnson et al., 2003; Harris et al., 2006), whereas rabies cases due to EBLVs in other bat species have only occasionally been reported (Müller et al., 2007). West Caucasian bat virus (WCBV), a recently ratified member of the lyssavirus genus with distinct genetic and biological properties, was isolated from a Schreiber’s long-fingered bat (Miniopterus schreibersii) on the European side of the Caucasus mountain range (Kuzmin et al., 2005). Given the wide distribution of this bat species, it is likely that WCBV also occurs in other parts of Eurasia and Africa, but only serological evidence of WCBV in Africa has been detected (Kuzmin et al., 2008). Therefore, focusing surveillance on those bat species supposed to be the likely reservoir should be given priority.

As passive surveillance is mostly based on bats collected by the public or involved in human contact, it is biased to the bat species more linked to urban areas and human shelters. Collected animals are usually dead, ill or show abnormal behaviour, which are the most prone to interact with humans and, therefore, the most relevant for public health. Indeed, the chance to detect an infected animal is much higher than among healthy bats. However, this approach is very limited for estimating the prevalence and the distribution of the lyssaviruses in a territory or to establish the epidemiology and the patterns of viral circulation. In addition, the presence of lyssaviruses such as WCBV that are linked to non-urban bat species could even remain undetected with this approach. A prerequisite for successful passive surveillance is an efficient network of bat handlers, conservationists and bat biologists on the one hand and regional veterinary laboratories and/or the NRLs for rabies on the other. Depending on the networks existing at national level, countries may often see no possibility of establishing efficient passive surveillance systems and therefore, prefer to focus primarily on active (pro-active) surveillance with great expectations. From a scientific point of view, however, active surveillance has several limitations regarding successful detection of EBLV-infections, that need to be taken into account even if a suggested main reservoir bat species is targeted because (i) in all probability EBLV infections do not homogenously occur among bat colonies, (ii) intermittent viral shedding means, that...
in a bat colony infected with EBLV, animals may not necessarily shed virus at the time point of sampling and (iii) there must be a sufficient viral load in oral swabs to guarantee detection with the diagnostic test used. While the presence of the EBLV-1 in oral swabs from healthy bats has been reported for serotine bats, the EBLV-2 RNA has not been detected in the United Kingdom after extensive testing. Therefore, the efficiency of viral RNA testing in oral swabs cannot be assumed for models different to EBLV-1 in healthy serotine bats. Furthermore, the sensitivity of techniques for diagnosis of EBLVs varies greatly according to the virus and bat species, stage of the disease, antibody status, intermittent nature of viral shedding and the training of technical staff. While a positive result is indicative of rabies, a negative result does not necessarily rule out infection. Also, detection of viral RNA using conventional or realtime PCRs in free-living animals would not result in the confirmation of a “rabies case” or “rabies outbreak” in the sense of national regulations as the presence of rabies has to be confirmed by the detection of viral antigens and/or viruses using prescribed methods. The same applies to the detection of EBLV or WCBV-specific antibodies in sera collected from free-living bats.

Despite both approaches providing complementary information, bat rabies-passive (re-active) surveillance should be given priority, as it is focused on the most immediate concern for public health and the chances of finding EBLV-positive bats are much higher. Active surveillance should be considered only after a passive pro-active surveillance has been established as a valuable scientific tool for analysing the prevalence, dynamics and epidemiology of lyssavirus infections in bat host reservoirs, as well as searching for previously undetected lyssaviruses.
Objective 6. Propose harmonised monitoring and reporting scheme

6.1 Rationale

The reliability of rabies surveillance systems depends on adequate investigations of target populations and on the management of information. Rabies data should be collected, processed, analysed and disseminated rapidly. The great majority of European countries submit data to their competent central authority and also to the international European database. Although the reporting of rabies and surveillance data in animals in MSs is effective, it could still be improved in different ways taking previous conclusions of EFSA’S AHAW panel opinions of 2006 and 2007 into account. Furthermore, Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents as well as EFSA’s BIOHAZ and AHAW panels have stressed the need for (i) improving rabies surveillance and reporting using a common strategy for data collection, and (ii) setting uniform standards to allow the proper comparison of epidemiological data between MSs.

6.2 Approach

Previous objectives served to provide guidelines for the harmonisation of rabies surveillance and reporting.

6.3 Results

6.3.1 Animals to be sampled and reported (bats excluded)

Based on the latest scientific knowledge for rabies a situation-based surveillance is sufficient under any epidemiological situation. No minimum sample size is required for maintaining “an effective system of disease surveillance” (Thulke et al., 2009). Veterinary authorities must be aware, or made aware, that the effective notification of rabies outbreaks can be baselined by continuing vigilance of suspect animals.

6.3.1.1 Rabies surveillance in MSs

A rabies case corresponds to an animal positive for rabies using laboratory diagnosis reference techniques on dead animals. Suspect animals (domestic or wild) that may be submitted to rabies diagnosis are listed in Objective 2.

An adequate surveillance system should be in place in all countries, whatever the rabies status (free or infected), for collecting and diagnosing suspect animals. Surveillance should be evenly distributed in time and space, i.e. suspect animals should be collected at any time in all areas of the country. Results of rabies diagnoses, e.g. positive and negative, should be reported.

Rabies surveillance should target animals suspected of having contracted the disease, designated IAs. This includes animals showing clinical signs or abnormal behaviour suggestive of rabies, animals found dead, animals to which humans might have been exposed, and road-kills (in rabies-endemic countries only). Animals being imported from non-MSs in which rabies is endemic and showing clinical symptoms suggestive of rabies, have to be included in the surveillance.

In rabies-free countries bordering infected countries, national and local veterinary services should be aware of the rabies epidemiological situation at the border for the possible adaptation of surveillance in bordering areas, in order to avoid re-infection from infected areas, and report data.
6.3.1.2 In rabies-infected countries using oral vaccination programmes

ORV of wildlife is the method of choice in rabies control. To be efficient, this method requires a long-term strategy. Therefore, next to adequate rabies surveillance, as described above, thorough monitoring of vaccination efficacy is important for the assessment and adjustment of vaccination campaigns.

This monitoring is based on investigating hunted animals that are sampled only for evaluating the efficacy of ORV programmes, e.g. those animals targeted by the oral vaccines, i.e. susceptible or protected/treated foxes and raccoon dogs. A sufficient number of animals should be investigated from all vaccinated areas trying to follow WHO recommendations (four animals per 100 km² annually). The blood sample and teeth or bones of animals should be analysed for serology and biomarker examination, respectively, and data (positive and negative results) reported.

6.3.2 Bat sampling in all MSs

Bats found sick, suspected rabid bats (showing clinical signs or abnormal behaviour), dead bats of all indigenous bat species as well as bats involved in contact incidents, e.g. biting or scratching, or animals caught by pets should be tested for rabies. This requires the involvement of an efficient network of bat handlers, conservationists and bat biologists as well as and regional veterinary laboratories and/or the NRL for rabies. Considering the protected status of European bats, national bat rabies surveillance networks should be encouraged in all European countries in close collaboration with bat specialists.

Whenever feasible, sampling should be undertaken throughout the country.

6.3.3 Harmonised reporting scheme

Several data reporting systems for rabies already exist in Europe (see Objective 1) with different objectives and periodicities of reporting.

The quality of data primarily depends upon the willingness and participation of respective countries to submit valid data. In order to ensure good data quality and consistency and to reduce the load of reporting duties by avoiding duplicative efforts which undermine the willingness to report, the submission of rabies data to the different databases should be streamlined. The WHO RBE is a European database that currently gathers rabies information from voluntary European countries and that has been issuing a quarterly bulletin since 1977. Regular submission of data by Veterinary Authorities of MS is efficient making this database highly reliable. MS report may be sent to the EC in the same format as those they sent to the WHO RBE. It is therefore recommended that the same reports should also be used as a foundation for the report for EFSA.

It is then recommended to use the RBE as the basis for the reporting scheme of animal rabies in MSs.

However additional information could be reported to both EFSA and RBE to improve the existing data collection system, such as:

• **Imported cases of rabies**: if rabies is diagnosed in an imported animal, reporting should include the notification “imported” as the designation of the species alone, i.e. “dog” would be misleading. Furthermore, results of virus characterisation, i.e. information on sequence and phylogenetic analysis should be submitted since it will allow further epidemiological investigations.

• **Details on the vaccination programmes**: since 2006 the RBE has provided maps showing vaccination areas for countries in which ORV of wildlife has been implemented. Additionally countries should be encouraged to report results of the monitoring of ORV campaigns (bait-
uptake, seroconversion) in target species from vaccination areas on an annual basis: name of the oral RABV vaccine used, number of animals tested for each species, number of positive. Furthermore, any animal rabies vaccine-induced case should be clearly reported and mentioned as such (assessed by strain characterisation using monoclonal antibodies, restriction enzyme analysis of RT-PCR products or sequencing) as recommended by OIE and WHO.

- **Surveillance data**: the surveillance data related to the number of tested suspect cases should be systematically reported, including positive and negative results. Rabies-free countries should also report the number of tested suspect animals. The report should include information on the geographical location of sampled animals.

- **Bat rabies surveillance**: in order to harmonise the data collection through MSs, a standard form for bat rabies surveillance could be elaborated. Animals that are tested (negative and positive) should be identified into species and data submitted. The causative lyssavirus genotype (EBLV-1 or EBLV-2) should be identified.

The following procedure could be proposed for data reporting:

- whenever technically feasible, improvement of the existing data collection system of RBE by integration on a quarterly basis information described above;
- MSs are invited by EFSA to use the RBE shape when submitting their data to EFSA; and
- for EFSA Zoonoses reports such data could then be used by EFSA for the CSR reports to undertake epidemiological analyses of the disease in the EU.

Those issues should be considered and agreed upon between the EC, MSs, EFSA and WHO (FLI).

In order to ensure rapid and recent MS information regarding the epidemiological situation of rabies in other countries, particularly in the case of re-emergences or outbreaks, it is crucial that veterinary authorities report cases on a regular basis in the OIE database interface.
Objective 7. Proposal of information for analysis by the European Commission and EFSA for the detection of trends

Surveillance is an essential component for long-term trend analysis in disease development. Detailed information is needed to assess the disease situation within a country to investigate disease trend and to compare data between MSs.

7.1 Global analysis in MSs

An overall evaluation of rabies surveillance in each MS is assessed using the total number of positive animals and the total number tested. However, the calculation of a prevalence percentage cannot be carried out as the sampling may not be uniform in all parts of the country considered.

In order to analyse the epidemiological situation of rabies within the EU, maps recording spatial distribution of rabies cases within each country and for the reporting period may also be used: positive cases are individually recorded as well as negative cases (NUTS 3). In such maps, clear distinctions should be made for:

- cases of vulpine origin;
- cases recorded in imported animals;
- cases recorded in bats; and
- cases of vaccinal origin.

A record of the total number of rabies-infected countries and rabies-free countries should be established; among infected countries, a distinction should be made between countries implementing regular ORV campaigns and countries not involved in such programmes.

For countries using ORV programmes, maps providing the location of areas vaccinated during spring and autumn campaigns provide an overview of rabies control efficacy in infected countries.

7.2 Monitoring of trends over time

The determination of trends depends on the rabies status of countries. Suggested analysis are recommended for rabies-free and infected countries.

7.3 Rabies-free countries

The awareness of veterinary services to detect possible cases is assessed by the number of annually tested (and reported) samples from suspect cases (wild and domestic animals) and the detection of possible imported cases.

Details on ORV programmes, if any: precise location of vaccinated areas, name of the oral vaccine used. The number of tested samples (from each animal species) for serology and biomarker determination should be determined and percentages of positive responses for serology and biomarker determination according to the species should be considered.

7.4 Rabies-infected countries

Incidence of rabies in wild and domestic animals in each MS assessed by rabies surveillance should be analysed according to control measures implemented in infected areas.

The reporting of regional distribution (NUTS 3) of positive as well as negative cases in each country may be an indicator of the efficiency of surveillance.
Details on ORV programmes, if used, should be indicated in the reports setting out the precise location of the vaccinated area. Furthermore, other important information such as the name of oral vaccine baits and their density per km²; see above. The analysis of rabies incidence in all species (except in bats) in those countries involved in ORV programmes can also be analysed in those areas vaccinated regularly for several years to evaluate the efficiency of the vaccination.

The analysis of rabies incidence data in parallel to those of ORV programmes (areas vaccinated, percentage of positive responses for serology and biomarker determination) may be useful to identify gaps in rabies surveillance and/or monitoring.

7.5 Bat rabies trend over time

Data obtained from passive and surveillance programmes should be analysed, focusing on passive surveillance.

At EU level, the analysis of species reported as positive for rabies is a useful indicator to know more about bat rabies epidemiology in Europe and bat species that are infected.

Regional distribution of positive and negative cases and geographical analysis of the different genotypes will allow a better understanding of the different strains of bat rabies circulating in Europe.
REFERENCES


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Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical rabies</td>
<td>This is caused by rabies virus (RABV, genotype 1) the prototype lyssavirus species. It has been known for centuries and is found in many countries in the world, e.g. RABV is responsible for fox-mediated rabies in Europe and for dog rabies around the world.</td>
</tr>
<tr>
<td>Eurobats</td>
<td>This is a United Nations convention on the Conservation of Populations of European Bats within 30 European countries aiming to protect all 45 species of bats identified in Europe (legislation, education, conservation measures and international cooperation).</td>
</tr>
<tr>
<td>Indicator animal</td>
<td>An animal suspected of having rabies (see definition of “suspect animal”).</td>
</tr>
<tr>
<td>Hunted animal</td>
<td>An animal suspected or not of having rabies sampled from regular hunting activities or specific sampling hunts or sampled alive. These animals are healthy and are used for the monitoring of ORV programmes. This definition applies for countries involved in ORV programmes.</td>
</tr>
<tr>
<td>Monitoring of rabies vaccination (also abbreviated to “rabies monitoring”)</td>
<td>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 having implemented ORV programmes.</td>
</tr>
<tr>
<td>Rabies surveillance</td>
<td>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 the whole country. Such a system needs to be established irrespective of the rabies status, i.e. concerns infected and rabies-free countries.</td>
</tr>
<tr>
<td>RabNet</td>
<td>Rabies network website run by WHO (<a href="http://www.who.int/rabies/rabnet">www.who.int/rabies/rabnet</a>). The Rabnet website contains rabies data compiled from the WRS questionnaire, ready-made maps and rabies related documents. Rabies data can also be linked to several country-specific indicators (population, education and health services).</td>
</tr>
<tr>
<td>Suspect animal</td>
<td>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.</td>
</tr>
<tr>
<td>Sylvatic rabies</td>
<td>This term is synonymously used for wildlife-mediated terrestrial rabies. In Europe it refers to classical rabies with the red fox acting as a reservoir.</td>
</tr>
<tr>
<td>Terrestrial rabies</td>
<td>This represents rabies endemic in species of terrestrial mammals, bats being excluded. Non-terrestrial rabies corresponds to bat rabies.</td>
</tr>
<tr>
<td>WAHID</td>
<td>World Animal Health Information Database (WAHID) Interface (World Organisation for Animal Health (OIE), <a href="http://www.oie.int/wahis/public.php?page=home">http://www.oie.int/wahis/public.php?page=home</a>. Data from 2005 onwards are shown on the WAHID interface. Rabies cases can be summarised monthly and annually and also at country level or by lower administrative units, depending on the reporting country. Six-monthly reports describe the listed disease situations in each country. However, it is not clear whether only “terrestrial rabies” cases caused by RABV are subject to notification or any confirmed rabies case in animals. Information on control measures is also provided.</td>
</tr>
</tbody>
</table>
APPENDICES

A. OIE Rabies Code ............................................................................................................................49

B. World Organisation for Animal Health Information database: example of an immediate notification ..........................................................................................................................52

C. EFSA rabies guidelines .................................................................................................................56

D. WHO Rabies Bulletin Europe – reported animal and human rabies cases in Europe in 2008 .......59
A. OIE RABIES CODE

CHAPTER 8.10.

RABIES

Article 8.10.1.

General provisions

For the purposes of the Terrestrial Code, the incubation period for rabies shall be 6 months, and the infective period in domestic carnivores starts 15 days before the onset of the first clinical signs and ends when the animal dies.

Standards for diagnostic tests and vaccines are described in the Terrestrial Manual.

Article 8.10.2.

Rabies free country

For the purposes of international trade, a country may be considered free from rabies when:
1. the disease is notifiable;
2. an effective system of disease surveillance is in operation;
3. all regulatory measures for the prevention and control of rabies have been implemented including effective importation procedures;
4. no case of indigenously acquired rabies infection has been confirmed in man or any animal species during the past 2 years; however, this status would not be affected by the isolation of Bat Lyssavirus;
5. no imported case in carnivores has been confirmed outside a quarantine station for the past 6 months.

Article 8.10.3.

Recommendations for importation from rabies free countries for domestic mammals, and wild mammals reared under confined conditions

Veterinary Authorities should require the presentation of an international veterinary certificate attesting that the animals:
1. showed no clinical sign of rabies on the day of shipment;
2. were kept since birth or for the 6 months prior to shipment in a rabies free country or were imported in conformity with the regulations stipulated in Articles 8.10.5., 8.10.6. or 8.10.7.
A (contd.): OIE RABIES CODE

<table>
<thead>
<tr>
<th>Article 8.10.4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommendations for importation from rabies free countries for wild mammals not reared under confined conditions</td>
</tr>
<tr>
<td>Veterinary Authorities should require the presentation of an international veterinary certificate attesting that the animals:</td>
</tr>
<tr>
<td>1. showed no clinical sign of rabies on the day of shipment;</td>
</tr>
<tr>
<td>2. have been captured in a rabies free country, at a sufficient distance from any infected country. The distance should be defined according to the species exported and the reservoir species in the infected country.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Article 8.10.5.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommendations for importation from countries considered infected with rabies for dogs and cats</td>
</tr>
<tr>
<td>Veterinary Authorities should require the presentation of an international veterinary certificate attesting that the animals:</td>
</tr>
<tr>
<td>1. showed no clinical sign of rabies within 48 hours of shipment;</td>
</tr>
<tr>
<td>AND EITHER</td>
</tr>
<tr>
<td>2. were identified by a permanent mark (such as a microchip) and their identification number shall be stated in the certificate; and</td>
</tr>
<tr>
<td>3. were vaccinated against rabies:</td>
</tr>
<tr>
<td>a) not less than 6 months and not more than one year prior to shipment in the case of a primary vaccination, which should have been carried out when the animals were at least 3 months old;</td>
</tr>
<tr>
<td>b) not more than one year prior to shipment in the case of a booster vaccination;</td>
</tr>
<tr>
<td>c) with an inactivated virus vaccine or with a recombinant vaccine expressing the rabies virus glycoprotein; and</td>
</tr>
<tr>
<td>4. were subjected not less than 3 months and not more than 24 months prior to shipment to an antibody test as prescribed in the Terrestrial Manual with a positive result equivalent to at least 0.5 IU/ml;</td>
</tr>
<tr>
<td>OR</td>
</tr>
<tr>
<td>5. have not been vaccinated against rabies or do not meet all the conditions set out in points 2, 3 and 4 above; in such cases, the importing country may require the placing of the animals in a quarantine station located on its territory, in conformity with the conditions stipulated in its animal health legislation.</td>
</tr>
</tbody>
</table>
A (contd.): OIE RABIES CODE

<table>
<thead>
<tr>
<th>Article 8.10.6.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommendations for importation from countries considered infected with rabies for domestic ruminants, equines and pigs</td>
</tr>
<tr>
<td>Veterinary Authorities should require the presentation of an international veterinary certificate attesting that the animals:</td>
</tr>
<tr>
<td>1. showed no clinical sign of rabies on the day of shipment;</td>
</tr>
<tr>
<td>2. were kept for the 6 months prior to shipment in an establishment where separation from wild and feral animals was maintained and where no case of rabies was reported for at least 12 months prior to shipment.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Article 8.10.7.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommendations for importation from countries considered infected with rabies for domestic ruminants, equines and pigs</td>
</tr>
<tr>
<td>Veterinary Authorities should require the presentation of an international veterinary certificate attesting that the animals:</td>
</tr>
<tr>
<td>1. showed no clinical sign of rabies on the day of shipment;</td>
</tr>
<tr>
<td>2. were kept for the 6 months prior to shipment in an establishment where separation from wild and feral animals was maintained and where no case of rabies was reported for at least 12 months prior to shipment.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Article 8.10.8.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommendations for importation from countries considered infected with rabies for domestic ruminants, equines and pigs</td>
</tr>
<tr>
<td>Veterinary Authorities should require the presentation of an international veterinary certificate attesting that the animals:</td>
</tr>
<tr>
<td>1. showed no clinical sign of rabies on the day of shipment;</td>
</tr>
<tr>
<td>2. were kept for the 6 months prior to shipment in an establishment where separation from wild and feral animals was maintained and where no case of rabies was reported for at least 12 months prior to shipment.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Article 8.10.9.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommendations for importation from countries considered infected with rabies for domestic ruminants, equines and pigs</td>
</tr>
<tr>
<td>Veterinary Authorities should require the presentation of an international veterinary certificate attesting that the animals:</td>
</tr>
<tr>
<td>1. showed no clinical sign of rabies on the day of shipment;</td>
</tr>
<tr>
<td>2. were kept in a quarantine station for the 6 months prior to shipment.</td>
</tr>
</tbody>
</table>

[Note: For non-human primates, reference should be made to Chapter 6.12.]
### B. **World Organisation for Animal Health Information Database: Example of an Immediate Notification**

![OIE Rabies, Italy](image)

**Summary**

<table>
<thead>
<tr>
<th>Report type</th>
<th>Follow-up report No. 33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start date</td>
<td>10/10/2008</td>
</tr>
<tr>
<td>Date of first confirmation of the event</td>
<td>17/10/2008</td>
</tr>
<tr>
<td>Report date</td>
<td>24/10/2010</td>
</tr>
<tr>
<td>Date submitted to OIE</td>
<td>25/10/2010</td>
</tr>
<tr>
<td>Reason for notification</td>
<td>Recurrence of a listed disease</td>
</tr>
<tr>
<td>Date of previous occurrence</td>
<td>1995</td>
</tr>
<tr>
<td>Manifestation of disease</td>
<td>Clinical disease</td>
</tr>
<tr>
<td>Causal agent</td>
<td>Rabies virus</td>
</tr>
<tr>
<td>Serotype</td>
<td>RABV</td>
</tr>
<tr>
<td>Nature of diagnosis</td>
<td>Suscipient, Laboratory (advanced), Necropsy</td>
</tr>
<tr>
<td>Event pertains to area defined zone within the country</td>
<td></td>
</tr>
</tbody>
</table>

**Related reports**

- Immediate notification (21/10/2008)
- Follow-up report No. 1 (29/10/2008)
- Follow-up report No. 2 (03/12/2008)
- Follow-up report No. 3 (09/01/2009)
- Follow-up report No. 4 (15/01/2009)
- Follow-up report No. 5 (23/01/2009)
- Follow-up report No. 6 (05/02/2009)
- Follow-up report No. 7 (31/03/2009)
- Follow-up report No. 8 (07/04/2009)
- Follow-up report No. 9 (08/05/2009)
- Follow-up report No. 10 (15/05/2009)
- Follow-up report No. 11 (30/06/2009)
- Follow-up report No. 12 (02/07/2009)
- Follow-up report No. 13 (18/08/2009)
- Follow-up report No. 14 (17/08/2009)
- Follow-up report No. 15 (27/08/2009)
- Follow-up report No. 16 (02/09/2009)
- Follow-up report No. 17 (23/09/2009)
- Follow-up report No. 18 (23/09/2009)
- Follow-up report No. 19 (30/09/2009)
- Follow-up report No. 20 (04/10/2009)
- Follow-up report No. 21 (14/10/2009)
- Follow-up report No. 22 (19/10/2009)
- Follow-up report No. 23 (24/10/2009)
- Follow-up report No. 24 (09/11/2009)
- Follow-up report No. 25 (23/11/2009)
- Follow-up report No. 26 (30/11/2009)
- Follow-up report No. 27 (07/12/2009)
- Follow-up report No. 28 (11/12/2009)
- Follow-up report No. 29 (18/12/2009)
- Follow-up report No. 30 (31/12/2009)
- Follow-up report No. 31 (14/01/2010)
- Follow-up report No. 32 (21/01/2010)
- Follow-up report No. 33 (24/01/2010)
- Follow-up report No. 34 (02/02/2010)
- Follow-up report No. 35 (10/02/2010)
### B (contd.) WORLD ORGANISATION FOR ANIMAL HEALTH INFORMATION DATABASE: EXAMPLE OF AN IMMEDIATE NOTIFICATION

<table>
<thead>
<tr>
<th>New outbreaks</th>
<th>LOCALITA’ TIERE, PADAVENA, BELLUNO, VENETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outbreak 1 (96 RABIES)</td>
<td>17/01/2010</td>
</tr>
<tr>
<td>Date of start of the outbreak</td>
<td>Continuing (or date resolved not provided)</td>
</tr>
<tr>
<td>Outbreak status</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Epidemiological unit</td>
<td>Wild species</td>
</tr>
<tr>
<td>Affected animals</td>
<td>Species</td>
</tr>
<tr>
<td>Affected Population</td>
<td>The positivity regards a fox culled.</td>
</tr>
<tr>
<td>Outbreak 2 (97 RABIES)</td>
<td>12/01/2010</td>
</tr>
<tr>
<td>Date of start of the outbreak</td>
<td>Continuing (or date resolved not provided)</td>
</tr>
<tr>
<td>Outbreak status</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Epidemiological unit</td>
<td>Wild species</td>
</tr>
<tr>
<td>Affected animals</td>
<td>Species</td>
</tr>
<tr>
<td>Affected Population</td>
<td>The positivity regards a fox culled.</td>
</tr>
<tr>
<td>Outbreak 3 (98 RABIES)</td>
<td>18/01/2010</td>
</tr>
<tr>
<td>Date of start of the outbreak</td>
<td>Continuing (or date resolved not provided)</td>
</tr>
<tr>
<td>Outbreak status</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Epidemiological unit</td>
<td>Wild species</td>
</tr>
<tr>
<td>Affected animals</td>
<td>Species</td>
</tr>
<tr>
<td>Affected Population</td>
<td>The positivity regards a fox culled.</td>
</tr>
<tr>
<td>Outbreak 4 (101 RABIES)</td>
<td>18/01/2010</td>
</tr>
<tr>
<td>Date of start of the outbreak</td>
<td>Continuing (or date resolved not provided)</td>
</tr>
<tr>
<td>Outbreak status</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Epidemiological unit</td>
<td>Wild species</td>
</tr>
<tr>
<td>Affected animals</td>
<td>Species</td>
</tr>
<tr>
<td>Affected Population</td>
<td>The positivity regards a fox culled.</td>
</tr>
<tr>
<td>Outbreak 5 (100 RABIES)</td>
<td>17/01/2010</td>
</tr>
<tr>
<td>Date of start of the outbreak</td>
<td>Continuing (or date resolved not provided)</td>
</tr>
<tr>
<td>Outbreak status</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Epidemiological unit</td>
<td>Wild species</td>
</tr>
<tr>
<td>Affected animals</td>
<td>Species</td>
</tr>
<tr>
<td>Affected Population</td>
<td>The positivity regards a fox culled.</td>
</tr>
<tr>
<td>Outbreak 6 (101 RABIES)</td>
<td>14/01/2010</td>
</tr>
<tr>
<td>Date of start of the outbreak</td>
<td>Continuing (or date resolved not provided)</td>
</tr>
<tr>
<td>Outbreak status</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Epidemiological unit</td>
<td>Wild species</td>
</tr>
<tr>
<td>Affected animals</td>
<td>Species</td>
</tr>
</tbody>
</table>

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### B (contd.) WORLD ORGANISATION FOR ANIMAL HEALTH INFORMATION DATABASE: EXAMPLE OF AN IMMEDIATE NOTIFICATION

<table>
<thead>
<tr>
<th>Outbreak 7 (96 RABIES)</th>
<th>LOCALITA' LAGO, LORENZO DI CADORE, BELUNO, VENETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of start of the outbreak</td>
<td>18/01/2010</td>
</tr>
<tr>
<td>Outbreak status</td>
<td>Continuing (or date resolved not provided)</td>
</tr>
<tr>
<td>Epidemiological unit</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Affected animals</td>
<td>Species</td>
</tr>
<tr>
<td>Wild species</td>
<td>1</td>
</tr>
<tr>
<td>Affected Population</td>
<td>The positivity regards a badger culled.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outbreak 8 (99 RABIES)</th>
<th>LOCALITA' VIA OLIVO, VALLE DI CADORE, BELUNO, VENETO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of start of the outbreak</td>
<td>18/01/2010</td>
</tr>
<tr>
<td>Outbreak status</td>
<td>Continuing (or date resolved not provided)</td>
</tr>
<tr>
<td>Epidemiological unit</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Affected animals</td>
<td>Species</td>
</tr>
<tr>
<td>Wild species</td>
<td>1</td>
</tr>
<tr>
<td>Affected Population</td>
<td>The positivity regards a fox found dead.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Summary of outbreaks</th>
<th>Total outbreaks: 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total animals affected</td>
<td>Species</td>
</tr>
<tr>
<td>Wild species</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outbreak statistics</th>
<th>Species</th>
<th>Apparent morbidity rate</th>
<th>Apparent mortality rate</th>
<th>Apparent case fatality rate</th>
<th>Proportion susceptible animals lost*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild species</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

* Removed from the susceptible population through death, destruction and/or slaughter
** Not calculated because of missing information

### Epidemiology

- **Source of the outbreak(s) or origin of infection**
  - Unknown or inconclusive
  - Contact with wild species

### Control measures

- **Measures applied**
  - Control of wildlife reservoirs
  - Vaccination in response to the outbreak(s)
  - Administrative division
    - Friuli-Venezia Giulia
    - Wld species
    - 122000
    - Bats for fines - modified live vaccine Rabigen SA62 - Virbac

- **Measures to be applied**
  - No treatment of affected animals
  - No other measures

### Diagnostic test results

- **Laboratory name and type**
  - Experimental Zooprophylactic Institute (IZS), Venezia - Padova, National Reference Laboratory (National laboratory)

<table>
<thead>
<tr>
<th>Tests and results</th>
<th>Species</th>
<th>Test</th>
<th>Test date</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wld species</td>
<td>direct immunofluorescence (DIF) test</td>
<td>19/01/2010</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Wld species</td>
<td>direct immunofluorescence (DIF) test</td>
<td>21/01/2010</td>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>

### Future Reporting

- The event is continuing. Weekly follow-up reports will be submitted.
B (contd.) WORLD ORGANISATION FOR ANIMAL HEALTH INFORMATION DATABASE: EXAMPLE OF AN IMMEDIATE NOTIFICATION
C. EFSA RABIES GUIDELINES

5.12. Rabies in animals

Relevant animal and agent species to be tested and reported

All domestic animal species, including pets and farm animals and wildlife animals, especially dogs and cats, including stray dogs and stray cats. Domestic farm animals typically to be reported on are species kept in free range production systems, such as sheep, goats or bovine animals. From wildlife species are foxes, raccoon dogs, wolves, badgers. Bats that are known to harbour bat type Lyssavirus.

Relevant agent species of Lyssavirus to be tested and reported

Information on the Lyssavirus species is of particular interest. Whenever possible, the differentiation between European Bat Lyssavirus (unspecified, EBL1 or EBL2) and the classical rabies virus (genotype 1) should be made.

Description of the monitoring and control system

It is recommended to report national control strategy and vaccination programmes.

Reporting on the status as free

A country may be recognised “free from rabies” by OIE or by WHO, according to their specific criteria. There are no officially free regions or MSs according to EU legislation.

A country may be considered free from rabies in accordance with the OIE Terrestrial Animal Health Code conditions, when:

- the disease is notifiable;
- an effective system of disease surveillance is in operation;
- all regulatory measures for the prevention and control of rabies have been implemented, including effective importation procedures;
- no case of indigenously acquired rabies infection has been confirmed in man or in any animal species during the past 2 years (however, this status will not be affected by the isolation of the European Bat Lyssavirus - EBL 1 or EBL 2);
- no imported cases in carnivores have been confirmed outside a quarantine station for the past 6 months.

Note that for WHO, detection of the European Bat Lyssavirus (EBL 1 or EBL 2) will prevent countries from being considered free from rabies.

Diagnostic methods typically used

Agent identification is preferably done using the Fluorescent Antibody Test (FAT). For a large number of samples the immunoenzyme technique can provide rapid results; however, at present, such test is not commercially available. As a single negative test on fresh material does not rule out the possibility of infection, inoculation tests (performed on neuroblastoma cells or upon intracranial inoculation of mice) should be carried out simultaneously.

The identification of the agent can be supplemented in specialised laboratories by identifying any variant virus strains through the use of monoclonal antibodies, specific nucleic acid probes, or Polymerase Chain Reaction followed by DNA sequencing of genomic areas. Typing of rabies virus isolates should be performed for any isolated cases of rabies and in case attenuated oral rabies vaccines are used.
C. (contd.) EFSA RABIES GUIDELINES

Analyses of the results
In the analyses of results, it is preferable to address:

- Number of confirmed rabies cases in animals and the sources of infection. The number of investigated animals should be recorded as well as species tested;
- The results and effectiveness of the vaccination programmes in domestic and wildlife animals;
- A clear distinction between sylvatic and bat rabies cases when describing rabies in wildlife;
- Lyssavirus type and subtypes, and distinction of virus isolates from terrestrial animal species (classical rabies virus) from those circulating in European bats (European Bat Lyssavirus, EBL 1 or EBL 2).

Reporting the results in tables
For reporting of data, use table named "Rabies in animals".

Specific guidelines for entering data in the prevalence tables:

- **Sampling stage** – where the samples have been collected (i.e. at farm) and the sample type (i.e. animal sample/ blood) should be reported;
- **Sampling context** – in this column information on the context of the sampling (i.e. monitoring), who collected the samples (i.e. competent authority) and the sample strategy (i.e. suspect sampling) should be inserted;
- **Sampling details** – free text to be used for further information on samples;
- **Sampling Unit** – in rabies, this is typically the "Animal".
- **Total units positive for Lyssavirus (rabies)** - in this column, the total number of animals found positive for rabies should be inserted. The information about occurrence of European Bat Lyssavirus should be provided as a comment or footnote.
- **Classical rabies virus (genotype 1)** – in this column the number of animals found positive for classical rabies virus is reported.
- **European bat Lyssavirus, unspecified** – in this column the number of animals positive for European bat virus are reported. If the bat virus type is known (EBL 1 or EBL 2), these specific columns can be added from the picklist.
- **Unspecified Lyssavirus** – this column is used to indicate the number of sampling units where the subspecies of the virus is unknown.

It is highly recommended to report whether the virus found was the classical rabies virus or the European bat Lyssavirus.
### C. EFSA RABIES GUIDELINES (contd.)

#### Table Rabies in animals

<table>
<thead>
<tr>
<th>Source of information</th>
<th>Sampling unit</th>
<th>Units tested</th>
<th>Total units positive for Lyssaviruses (rabies)</th>
<th>Unspecified Lyssavirus</th>
<th>Classical rabies virus (genotype 1)</th>
<th>European Bat Lyssavirus - unspecified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badgers - wild</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bats - wild</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cats - stray cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle (bovine animals)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deer - wild - fallow deer</td>
<td></td>
<td></td>
<td></td>
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<td>Deer - wild - red deer</td>
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<td>Deer - wild - roe deer</td>
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<td>Foxes - wild</td>
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<td>Marten - wild</td>
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<td>Raccoon dogs - wild</td>
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<td>Raccoons - wild</td>
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<td>Solpeds, domestic</td>
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<td>Wolves - wild</td>
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D. WHO RABIES BULLETIN EUROPE – REPORTED ANIMAL AND HUMAN RABIES CASES IN EUROPE IN 2008

- Red dots: animal rabies (RABV)
- Green dots: bat rabies
- Stars: imported cases
- Blue squares: human cases

(Source: www.who-rabies-bulletin.org)
ABBREVIATIONS

ADR  Accord européen relatif au transport international des marchandises Dangereuses par Route
ANSES  Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, France
AHAW  Animal Health and Welfare
BHK-21  Baby Hamster Kidney cells 21
cDNA  complementary DNA
CRL  Community Reference Laboratory
CSR  Community Summary Report
DNA  DeoxyriboNucleic Acid
DUVV  Duvenhage Virus
EBLVs  European Bat Lyssaviruses
EBLV-1  European Bat Lyssavirus 1
EBLV-2  European Bat Lyssavirus 2
EC  European Commission
ECDC  European Centre for Disease Prevention and Control
EFSA  European Food Safety Authority
ELISA  Enzyme-linked immunosorbent assay
EU  European Union
FAT  Fluorescent antibody test
FLI  Friedrich-Loeffler-Institut, Germany
GIS  Geographic Information System
HA  Hunting animal
IA  Indicator animal
IATA  International Air Transport Association
ICTV  International Committee on Taxonomy of Viruses
MIT  Mouse inoculation test
MS  Member State
NRL  National Reference Laboratory
NUTS  Nomenclature des Unités Territoriales Statistiques
OIE  World organisation for animal health
ORV  Oral rabies vaccination
PCR  Polymerase chain reaction
RABV  rabies virus (classical rabies)
RBE  Rabies Bulletin Europe
RFLP  Restriction fragment length polymorphism
RNA  Ribo Nucleic Acid
RREID  Rapid Rabies Enzyme Immunodiagnosis
RTCIT  Rabies tissue-culture infection test
RT-PCR  Reverse transcription polymerase chain reaction
SCAHAW  Scientific Committee on Animal Health and Animal Welfare
VLA  Veterinary Laboratories Agency, UK
WAHID  World Animal Health Information Database
WCBV  West Caucasian Bat Lyssavirus
WHO  World Health Organisation
WRS  World Rabies Survey