Although there is debate about the estimated health burden of rabies, the estimates of direct mortality and the DALYs due to rabies are among the highest of the neglected tropical diseases. Poor surveillance, underreporting in many developing countries, frequent misdiagnosis of rabies, and an absence of coordination among all the sectors involved are likely to lead to underestimation of the scale of the disease It is clear, however, that rabies disproportionately affects poor rural communities, and particularly children. Most of the expenditure for post-exposure prophylaxis is borne by those who can least afford it. As a result of growing dog and human populations, the burden of human deaths from rabies and the economic costs will continue to escalate in the absence of concerted efforts and investment for control.

Since the first WHO Expert Consultation on Rabies in 2004, WHO and its network of collaborating centres on rabies, specialized national institutions, members of the WHO Expert Advisory Panel on Rabies, and partners such as the Gates Foundation, the Global Alliance for Rabies Control and the Partnership for Rabies Prevention, have been advocating the feasibility of rabies elimination regionally and globally and promoting research into sustainable cost-effective strategies. Those joint efforts have begun to break the cycle of rabies neglect, and rabies is becoming recognized as a priority for investment.

This Consultation concluded that human dog-transmitted rabies is readily amenable to control, regional elimination in the medium term and even global elimination in the long term. A resolution on major neglected tropical diseases, including rabies, prepared for submission to the World Health Assembly in May 2013 aims at securing Member States' commitment to the control, elimination or eradication of these diseases. Endorsement of the resolution would open the door for exciting advances in rabies prevention and control.

9 789241 209823

982
WHO Expert Consultation on Rabies
WHO Technical Report Series

WHO

WHO Technical Report Series
982

# WHO Expert Consultation on Rabies

Second report

World Health Organization

The World Health Organization was established in 1948 as a specialized agency of the United Nations serving as the directing and coordinating authority for international health matters and public health. One of WHO's constitutional functions is to provide objective and reliable information and advice in the field of human health, a responsibility that it fulfils in part through its extensive programme of publications.

The Organization seeks through its publications to support national health strategies and address the most pressing public health concerns of populations around the world. To respond to the needs of Member States at all levels of development, WHO publishes practical manuals, handbooks and training material for specific categories of health workers; internationally applicable guidelines and standards; reviews and analyses of health policies, programmes and research; and state-of-the-art consensus reports that offer technical advice and recommendations for decision-makers. These books are closely tied to the Organization's priority activities, encompassing disease prevention and control, the development of equitable health systems based on primary health care, and health promotion for individuals and communities. Progress towards better health for all also demands the global dissemination and exchange of information that draws on the knowledge and experience of all WHO's Member countries and the collaboration of world leaders in public health and the biomedical sciences.

To ensure the widest possible availability of authoritative information and guidance on health matters, WHO secures the broad international distribution of its publications and encourages their translation and adaptation. By helping to promote and protect health and prevent and control disease throughout the world, WHO's books contribute to achieving the Organization's principal objective – the attainment by all people of the highest possible level of health.

The *WHO Technical Report Series* makes available the findings of various international groups of experts that provide WHO with the latest scientific and technical advice on a broad range of medical and public health subjects. Members of such expert groups serve without remuneration in their personal capacities rather than as representatives of governments or other bodies; their views do not necessarily reflect the decisions or the stated policy of WHO.

For further information, please contact WHO Press, World Health Organization; 1211 Geneva 27, Switzerland; www.who.int/bookorders; tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int.

## SELECTED WHO PUBLICATIONS OF RELATED INTEREST

**WHO Position Paper on Rabies Vaccines**
*Weekly Epidemiological Record*, 2010, 85: 309-320

**WHO Expert Consultation on Rabies. First report.**
Geneva, World Health Organization, 2005
WHO Technical Report Series, No. 931

**WHO Expert Committee on Rabies. Eighth report.**
Geneva, World Health Organization, 1992
WHO Technical Report Series, No. 824

**Laboratory Techniques in Rabies. Fourth edition.**
Geneva, World Health Organization, 1996

Further information on these and other WHO publications can be obtained from
WHO Press, World Health Organization ▪ 1211 Geneva 27, Switzerland ▪ www.who.int/bookorders
**tel.:** +41 22 791 3264; **fax:** +41 22 791 4857; **e-mail:** bookorders@who.int

WHO Technical Report Series
982

# WHO Expert Consultation on Rabies

Second report

*This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization*

World Health Organization

WHO Library Cataloguing-in-Publication Data

WHO Expert Consultation on Rabies: second report.

(WHO technical report series ; no. 982)

1.Rabies – prevention and control. 2.Rabies – diagnosis. 3.Rabies – epidemiology. 4.Rabies vaccines. 5.Rabies virus. 6.National health programs. I.World Health Organization. II.Series.

ISBN 978 92 4 120982 3 (NLM classification: WC 550)
ISBN 978 92 4 069094 3 (PDF)
ISSN 0512-3054



Design: WHP (Sophie Guetaneh Aguettant)

# Contents

# Introduction

The World Health Organization (WHO) Expert Consultation on Rabies met in Geneva, Switzerland, on 18–20 September 2012. Dr Denis Daumerie, Project Manager, welcomed the participants on behalf of Dr Lorenzo Savioli, Director, Department of Control of Neglected Tropical Diseases, and the Director-General. He pointed out that rabies, like the tropical diseases covered by the Department, affected mainly people whose deaths are not accounted for. The disease continues to occur mainly in poor communities, where measures that could prevent it in humans by controlling dog rabies are not implemented, even though a resolution adopted by the Third World Health Assembly in 1950 already mentioned the need for prevention of rabies in humans and its control in dogs. Advances have been made in the field of rabies, particularly in the production and use of human and animal biologicals, but the disease is still neglected, and no new WHO resolutions on rabies have been proposed to address human rabies transmitted by dogs. Dr Daumerie described the successful collaboration between the Department of Control of Neglected Tropical Diseases and major drug manufacturers for the control and elimination of tropical diseases such as leprosy, lymphatic filariasis and human African trypanosomiasis, and advised the consultation to explore the benefits of such partnerships for rabies prevention and control.

Dr François-Xavier Meslin, Neglected Zoonotic Diseases, recalled that WHO had been denouncing and combating the 'cycle of neglect' with regard to rabies for more than a decade. Since the first WHO Expert Consultation on Rabies, in 2004, WHO and its network of collaborating centres on rabies, specialized national institutions, members of the WHO Expert Advisory Panel on Rabies and partners such as the Bill & Melinda Gates Foundation, the Global Alliance for Rabies Control and the Partnership for Rabies Prevention, have been advocating the feasibility of rabies elimination regionally and globally, and promoting research into strategies. Those joint efforts have begun to break the cycle of rabies neglect, and rabies is becoming recognized as a priority for investment.

Dr Louis Nel was appointed Chairperson and Dr Naseem Salahuddin was appointed Rapporteur of the Consultation. The list of participants is given in *Annex 1*.

The information in this report should be considered the most current data on rabies prevention and control, and supersedes that of the report of the first WHO Expert Consultation on Rabies, published in 2005 (*1*).

# 1. The burden of rabies

Information on disease burden is widely used to set public health priorities, allocate limited resources for disease prevention and control and assess the impacts and cost–effectiveness of interventions (*1*). Standardized metrics, such as the disability-adjusted life year (DALY), have been widely adopted to evaluate the burden of disease at regional and global levels and have become an essential tool for decision-making by policy-makers (*2*). The major burden of rabies is attributable to dog-mediated transmission, and therefore this chapter focuses on dog-mediated rabies and only briefly addresses the burden attributable to other host species (*3*). Estimates of disease burden can be contentious when the underlying data are of poor quality; nevertheless, the resulting information is a useful starting point for more accurate estimates as better data become available.

## 1.1 Methods for estimating the burden of rabies

Several factors contribute to significant underreporting of human deaths from rabies in many parts of the world. Methods have therefore been developed to estimate the mortality attributable to rabies, which account for the quality of reporting in countries with endemic canine rabies. In particular, a predictive approach based on a probability decision-tree has been devised to determine the likelihood of the onset of clinical rabies in humans after a bite by a dog suspected of being rabid. This method, initially used to estimate human deaths from rabies in the United Republic of Tanzania (*4*), has resulted in a revised estimate of the burden of rabies in Africa and Asia (*5*). More recently, the approach has been tailored for estimating mortality due to rabies in specific countries in Asia (e.g. Bhutan (*6*) and Cambodia (*7*)). Empirical studies to both parameterize and validate such estimates include community surveys (*8*), large-scale verbal autopsy surveys (*9*) and active surveillance and contact tracing (*10*).

DALYs incorporate both premature mortality and disability (*2*). The most critical element in calculating DALYs for rabies is premature death (*5*); because of the short duration of the disease, disability accounts for a relatively small part of the burden of rabies. Disability can, however, occur after administration of nerve tissue vaccine, which is still in use in a few countries. These vaccines have severe side-effects lasting from 4 to 7 months, depending on the type of vaccine used, in an estimated 0.3–0.8 cases out of 1000 (*5*).

The economic burden of disease is typically calculated from a combination of direct and indirect costs. For rabies, the direct costs of post-exposure prophylaxis depend on the vaccine, regimen and route of administration as well as the type of rabies immunoglobulin used; the indirect costs include those for visiting a clinic (or accompanying a bite victim to a clinic) and associated income

loss. The scale of such costs is particularly important for rabies, as lack of post-exposure prophylaxis translates directly into human deaths. A further economic component is productivity loss, calculated by weighting the discounted years of life lost by the country's gross domestic product and using a 3% discounting rate. Up to now, productivity losses have not been considered in studies of the burden of rabies. The cost of rabies prevention, control and elimination (including surveillance) in animal reservoirs and losses in the animal production sector should also be taken into account. An additional component of the burden of rabies is its emotional and psychological impact, particularly the trauma and the long periods of uncertainty after a bite by a rabid animal when post-exposure prophylaxis is either unreliable or unavailable.

A working group was convened by the Partners for Rabies Prevention to collate and review the most recent data and use the probability decision-tree approach to assess the global burden of canine rabies. The Institute for Health Metrics and Evaluation, as part of the study, also generated estimates of the global burden of rabies using a 'cause of death ensemble' model (*11*,*12*). The preliminary results of these studies are discussed here; however, both indicated that their estimates are highly uncertain, owing to lack of accurate data. Field data to validate these estimates are therefore needed to address this enduring problem.

## 1.2 Estimated burden of rabies in the world

In the following section, information on the rabies burden in various countries is grouped according to epidemiological similarity and geographical proximity. For each region, results of local studies that have provided the most accurate data are given, as well as less certain regional estimates based on extrapolations.

### 1.2.1 Countries that are free of canine rabies

Canine rabies has been eliminated from western Europe, Canada, the United States of America (USA), Japan, Malaysia and a few Latin American countries; while Australia is free from carnivore rabies, and many Pacific island nations have always been free from rabies and related viruses. In these areas, human deaths from rabies are restricted to people exposed while living or travelling in areas endemic for canine rabies. About two deaths per year due to imported human rabies have been reported in Europe, North America and Japan (*13*,*14*). One third of the imported cases in 1990–2010 originated in South and South-East Asia (predominantly India and the Philippines), another third in Africa, almost 20% in Latin America and the Caribbean and over 10% in eastern Europe and Central Asia. The costs of post-exposure prophylaxis for travellers returning from overseas and for pre-exposure prophylaxis are often substantial. The cost of post-

exposure prophylaxis in otherwise rabies-free areas escalates after incidents of imported rabid animals and is higher in places where illegal entry from endemic countries is common, putting a considerable burden on the health services (*15*). In countries bordering areas endemic for canine rabies, border campaigns and intensified surveillance are required to maintain rabies-free status. Quarantine procedures and legislation are needed in all rabies-free countries.

The costs of prevention in many countries where wildlife rabies or bat rabies viruses circulate must also be taken into account. Millions of dollars have been spent annually to eliminate wildlife rabies by administering oral rabies vaccine, and the cost varies substantially according to the setting and tactics (*16*). For instance, one to eight human rabies deaths occur annually in the USA as a result of wildlife rabies (*17*), and, according to the Centers for Disease Control and Prevention, an estimated US$ 300 million are spent per annum for rabies prevention. Several states are attempting to eliminate raccoon rabies to reduce the demand for post-exposure prophylaxis. Since fox rabies was eliminated from western Europe, the costs for oral vaccination have been substantially reduced (*Table 1*), but other European countries now striving to eliminate fox rabies are incurring high costs. Recent incursions into Italy, although now under control, required substantial financial commitments, and costs may escalate elsewhere, given the threat of emergence in rabies-free countries such as Greece. The cost of setting up a cordon sanitaire along the entire eastern border of the European Union to prevent such incursions is estimated to exceed US$ 6.5 million per year (*21*).

Table 1
**Examples of costs associated with rabies and its elimination from Europe**

| Country (reference) | Period of programme | Costs included in programme | Cost of programme (million US$) |
|---|---|---|---|
| France (*18*) | 1988–1993 | Post-exposure prophylaxis, preventive vaccination of cattle, dogs and cats, oral rabies vaccination | 261 |
| Germany (*19*) | 1983–2008 | Oral rabies vaccination | 122 |
| Estonia (*20*) | 2005–2010 | Oral rabies vaccination and surveillance | 15.5 |

### 1.2.2 Countries in which canine rabies is endemic

#### *Latin America and the Caribbean*

Canine rabies control programmes during the past two decades have had substantial success in this region. Official reports of cases of human rabies transmitted by dogs decreased from about 250 in 1990 to fewer than 10 in 2010, with concomitant declines in dog rabies (*22*). In foci where canine rabies continues to circulate, however, official reports probably underestimate the scale of the problem, particularly in the Plurinational State of Bolivia, Cuba, the Dominican Republic, El Salvador, Guatemala, Haiti, Honduras and parts of Brazil, Mexico and Peru. In these countries, human deaths from rabies are either still occurring or are at risk of occurring. Preliminary estimates with the probabilistic decision-tree model suggest that the number of human deaths due to canine rabies in the Americas is more likely to be of the order of 200 cases per annum, most occurring in Haiti.

Although progress has been made in phasing out nerve tissue vaccines in the Americas, their use is still widespread in Argentina, the Plurinational State of Bolivia, Honduras, Peru and the Bolivarian Republic of Venezuela, and therefore adverse events and the resulting disabilities are still a problem. The annual public health burden of rabies in this region probably exceeds 15 000 DALYs, about 100 of which are probably attributable to adverse events from nerve tissue vaccines; however, appropriate systems for reporting adverse events are required to accurately quantify the number.

The Pan American Health Organization has set a target to eliminate canine rabies from the Americas by 2015. To reach this target, an estimated total budget of more than US$ 20 million per year is required (*23*); however, there is currently an annual budget shortfall of around US$ 4 million (*24*). Almost 75% of this estimated total annual budget is allocated to dog vaccination, and 5–10% is associated with post-exposure prophylaxis. The costs incurred by people seeking post-exposure prophylaxis (including time lost, income loss and side-effects) were not included in these estimates, nor were the costs of bat-related rabies in humans or livestock.

#### *Asia*

More human deaths from rabies occur in Asia than anywhere else in the world, with estimates of human mortality due to endemic canine rabies exceeding 30 000 per annum (95% confidence interval [CI], 8100–61 400) in 2003 (*5*). Since 2003, the epidemiological situation in many parts of the region has changed, with improvements in rabies control and prevention in many areas, particularly in delivery of post-exposure prophylaxis. There have, however, been emergences elsewhere.

Nerve tissue vaccines have been almost completely phased out in the region; only Mongolia, Myanmar and Pakistan still use these vaccines. The DALYs attributable to adverse events from the vaccines are estimated to have decreased from over 40 000 (5) to around 10 000 in 2010. Bangladesh phased out nerve tissue vaccines in late 2011, and plans are under way to discontinue their production and use in both Myanmar and Pakistan. Wider availability of post-exposure prophylaxis might have reduced the death toll in many areas, including India, but its increased use has been costly, as dog rabies control programmes have not been given the same priority, and exposure to the risk of contracting rabies remains and may be increasing. The costs associated with post-exposure prophylaxis are higher in Asia than anywhere else, estimated at around US$ 1.5 billion. Extreme examples include Sri Lanka and Thailand, where the annual direct costs of post-exposure prophylaxis in both countries exceed US$ 10 million (*25*).

Estimates suggest that in 2010 between 15 900 ('cause of death ensemble' model approach) and 34 500 (probability decision-tree approach) human rabies deaths occurred in Asia, excluding Central Asia, with about 1.2 million DALYs lost in the region. Both estimates are uncertain, with overlapping confidence intervals, and field data are required to validate the model results. In excellent examples of such studies, the incidence of human deaths from rabies was estimated to be 1.1–1.8 deaths/100 000 in rural Bangladesh (*8*), 2.5–7.5 deaths/100 0000 in populations at risk in Bhutan (*6*) and 2.8–11.5 deaths/100 000 in Cambodia (*7*).

India is reported to have the highest incidence of rabies globally. A multi-centre study in 2003 showed that 20 565 human deaths occur annually (*26*), and a large-scale verbal autopsy study in 2005 put the figure conservatively at 12 700, without adjustment for atypical cases not captured by this latter method (9). Most cases were reported in rural communities (*9*, *26*) where no large-scale dog vaccination programmes have been conducted and where the incidence of dog rabies presumably remains high. While the availability of post-exposure prophylaxis has improved, it is not clear how much rural communities have benefited; furthermore, most deaths occur among people who do not seek medical care. The number of deaths due to rabies in India therefore remains uncertain.

Estimates of the burden of rabies in China are also uncertain. Surveillance records indicate that the incidence has decreased since 2007, when over 3300 suspect (clinically diagnosed) rabies deaths were recorded officially (*27*). These records may, however, underreport the incidence of the disease (*27*), and field investigations are therefore urgently needed.

Despite the uncertainty of these estimates, rabies is clearly a major problem in Asia, mainly affecting the rural poor. In many countries, official records substantially underestimate the scale of the problem (*6*,*7*,*9*), and re-assessments are therefore encouraged. This is already planned for India.

#### *Africa*

The number of deaths from endemic canine rabies in Africa was estimated in 2003 to be about 23 700 (95% CI, 6900–45 900) (*5*). Estimates of the burden of rabies in Africa have always been uncertain, however, because of the lack of good data. Few large-scale dog vaccination programmes were implemented in the region during the past decade, and the disease continues to circulate largely unregulated. Recent surveys have also indicated extremely limited availability of post-exposure prophylaxis in most of sub-Saharan Africa. In-depth studies show that official reports may underestimate the incidence of rabies by more than 100-fold, because most deaths occur in communities rather than in hospitals (*4*,*10*), and those that occur in hospitals are frequently misdiagnosed as forms of encephalitis (*29*).

The revised 2010 estimate of the rabies burden in Africa by the probability decision-tree approach of about 23 800 deaths (95% CI, 21 000–28 000) and 609 000 DALYs (95% CI, 522 000–707 000) is consistent with the earlier estimate (*5*). In the study of the Institute for Health Metrics and Evaluation, 9500 rabies deaths were estimated to occur in 2010 (*11*), although the number of DALYs was similar (750 000; 95% CI, 169 000–2 733 000). These figures should be interpreted with caution, as there are few data for validation, and they should be the subject of further investigation in the region.

Use of nerve tissue vaccine remains widespread in Ethiopia, contributing about 1000 DALYs per annum. Algeria still produces nerve tissue vaccines, but the situation in some other countries in North Africa and in the horn of Africa is unknown.

#### *Central Asia and the Middle East*

Little information is available on rabies in the Middle East or Central Asia, and the scale of the rabies burden in these regions has not been investigated previously. On the basis of the literature and population data in the probability decision-tree model, initial estimates can be made of 350 deaths (95% CI, 270–450) and 13 100 DALYs (95% CI, 11 100–15 900) in the Middle East and 1900 deaths (95% CI, 1600–2350) and 55 200 DALYs (95% CI, 47 500–66 600) in Central Asia.

### 1.2.3 Vampire bat rabies

In Latin America and the Caribbean, cases due to vampire bat rabies virus are largely underreported. In 1985, it was estimated that the death toll among cattle was of the order of 100 000 per year, at an annual estimated cost of US$ 30 million. Evidence suggests, however, that the incidence of bat rabies has increased, probably resulting in more human cases and livestock losses (*30*).

## 1.3 Global summary

The annual number of human rabies deaths globally is estimated in 2010 to be from 26 400 (95% CI 15 200–45 200) ('cause of death ensemble' model approach) to 61 000 (95% CI 37 000–86 000) (probability decision-tree approach) (*Table 2*). The vast majority of deaths (84%) occur in rural areas. These estimates represent about 1.9 million (95% CI, 1.3–2.6 million) DALYs. About 12 600 DALYs are due to morbidity following adverse events due to nerve tissue vaccine. The estimated annual cost of rabies is US$ 6 billion (95% CI, 4.6–7.3 billion), with almost US$ 2 billion (~40%) due to lost productivity after premature deaths and a further US$ 1.6 billion spent directly on post-exposure prophylaxis.

Although there is considerable debate about the estimated burden of neglected tropical diseases, the estimates of direct mortality due to rabies are among the highest (possibly the highest), and the DALYs due to rabies are also high (*31*).

The cost of life-saving prophylaxis is a major burden both to national economies and to poor families as more data on increasing numbers of post-exposure prophylaxis provided annually are becoming available from countries such as China (e.g. reports of 10 million post-exposure prophylaxis treatments delivered in 2010) and India since 2004, suggesting higher exposure to the risk of contracting rabies even if a large proportion of these are not from rabid animals. The psychological impact of fear and trauma after a suspected rabid dog bite is difficult to translate into a monetary value but was estimated to account for about 32 000 DALYs in Africa and 140 000 DALYs in Asia (*32*). These effects are heightened by uncertainty about the availability, quality and affordability of post-exposure prophylaxis in many countries endemic for canine rabies.

Poor surveillance, underreporting in many developing countries, frequent misdiagnosis of rabies (*29*) and an absence of coordination among all the sectors involved are likely to lead to underestimation of the scale of the disease burden. Both country-specific burden studies and improved surveillance (see section 11) should be encouraged in order to obtain more reliable global estimates of the burden of rabies.

Nonetheless, it is clear that rabies disproportionately affects poor rural communities, and particularly children. Most of the expenditure for post-exposure prophylaxis is borne by those who can least afford it. For example, in India, patients pay nearly half the financial burden of rabies. Previous estimates indicated that a full course of post-exposure prophylaxis represents as much as 3.87% of the gross national income for a person in Asia and 5.80% for a person in Africa (equivalent to 51 days' wages for an average African, and 31 days' wages for an average Asian). A recent field study in the United Republic of Tanzania suggests, however, that these are still considerable underestimates of the true cost for high-risk populations. As a result, many patients do not complete their treatment courses and often use regimens that are not recommended. The

Table 2
**Estimated numbers of deaths from rabies (with 95% confidence intervals) in various areas of the world**

| Year of estimate | Reference /source | Methods | Africa | China | India | Other Asian countries | All Asia | All Asia and Africa | World |
|---|---|---|---|---|---|---|---|---|---|
| 2003 | (*26*) | Multi-centre study (community surveys and hospital records) | | | 20 565 (16 931–24 198) | | | | |
| 2005 | (*9*) | Verbal autopsies | | | 12 700 (10 000–15 000) | | | | |
| 2003 | (*5*) | Probability decision-tree approach | 23 700 (6900–45 900) | 2336 (565–5049) | 19 713 (4192–39 733) | 9489 (2281–19 503) | 30–000 (8100–61 400) | 55 270 (23 910–93 057) | |
| 2010 | (*11*,*12*) | 'Cause-of-death ensemble' model | 9500** | | | | 16 000 | 25 500 | 26 400 (15 181–45 184) |
| 2010 | (*27*) | National surveillance data | | 2213 | | | | | |
| 2010 | PRP | Probability decision-tree approach | 23 800 (21 000–28 000) | 7450 (2000–13 000) | 16 450 (6000–27 000) | 10 550 * (6000–14 000) | 34 500 * (14 000–54 000) | 58 300 (35 000–82 000) | 61 000 (37 000–86 000) |

PRP, Partners for Rabies Prevention

*Excluding Central Asia

**Excluding North Africa

annual cost of livestock losses due to rabies is also substantial: approximately US$ 12.3 million (90% CI, 11–13.7 million) (5), disproportionately affecting the rural poor who depend upon livestock for subsistence.

As a result of growing dog and human populations, the burden of human deaths from rabies and the economic costs will continue to escalate in the absence of concerted efforts and investment for control. Rabies is entirely preventable. As countries strive to reduce the number of human deaths and improve the availability of post-exposure prophylaxis, the costs will rise; however, if dog rabies control and ultimately elimination are achieved by mass dog vaccination, both the demand for post-exposure prophylaxis and the costs should decline. National vaccination programmes will require consistent, sustained commitment but will have widespread health benefits, particularly for the poorest communities in the world.

## 1.4 References

1. Murray CJL et al. *Summary measures of population health: concepts, ethics, measurements, and applications*. Geneva, World Health Organization, 2002.
2. Stein C et al. The global burden of disease assessments—who is responsible? *PLoS Neglected Tropical Diseases*, 2007, 1(3):e161.
3. *Essential rabies maps*. Geneva, World Health Organization (http://www.who.int/rabies/rabies_maps/en/; accessed March 2013).
4. Cleaveland S et al. Estimating human rabies mortality in the United Republic of Tanzania from dog bite injuries. *Bulletin of the World Health Organization*, 2002, 80(4):304–310.
5. Knobel DL et al. Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization*, 2005, 83(5):360–368.
6. Tenzin et al. Dog bites in humans and estimating human rabies mortality in rabies endemic areas of Bhutan. *PLoS Neglected Tropical Diseases*, 2011, 5(11):e1391.
7. Ly S et al. Rabies situation in Cambodia. *PLoS Neglected Tropical Diseases*, 2009, 3(9):e511.
8. Hossain M et al. Human rabies in rural Bangladesh. *Epidemiology and Infection*, 2012, 140(11):1964–1971.
9. Suraweera W et al. Deaths from symptomatically identifiable furious rabies in India: a nationally representative mortality survey. *PLoS Neglected Tropical Diseases*, 2012, 6(10):e1847.

10. Hampson K et al. Rabies exposures, post-exposure prophylaxis and deaths in a region of endemic canine rabies. *PLoS Neglected Tropical Diseases,* 2008, 2(11):e339.

11. Lozano R et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 2012, 380(9859):2095–2128.

12. Murray CJL et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380(9859):2197–2223.

13. Gautret P, Parola P. Rabies vaccination for international travelers. *Vaccine,* 2012, 30(2):126–133.

14. Malerczyk C, DeTora L, Gniel D. Imported human rabies cases in Europe, the United States, and Japan, 1990 to 2010. *Journal of Travel Medicine*, 2011, 18:402–407.

15. Lardon Zl et al. Imported episodic rabies increases patient demand for and physician delivery of antirabies prophylaxis. *PLoS Neglected Tropical Diseases*, 2010, 4(6):e723.

16. Sterner RT et al. Tactics and economics of wildlife oral rabies vaccination, Canada and the United States. *Emerging Infectious Diseases*, 2009, 15(8):1176–1184.

17. Blanton JD et al. Rabies surveillance in the United States during 2010. *Journal of the American Veterinary Medicine Association*, 2011, 239(6):773–783.

18. Aubert MF. Costs and benefits of rabies control in wildlife in France. *Revue Scientifique et Technique* (International Office of Epizootics), 1999, 18(2):533–543.

19. Müller T et al. Elimination of terrestrial rabies in Germany using oral vaccination of foxes. *Berliner und Munchener tierarztliche Wochenschrift*, 2012, 125(5–6):178–190.

20. Cliquet F et al. Eliminating rabies in Estonia. *PLoS Neglected Tropical Diseases*, 2012, 6(2):e1535.

21. Demetriou P, Moynagh J. The European Union strategy for external cooperation with neighbouring countries on rabies control. *Rabies Bulletin Europe*, 2011, 35(1):5–7.

22. *Sistema de Información Epidemiológica*. Washington DC, Pan American Health Organization and World Health Organization. (http://siepi.panaftosa.org.br; accessed March 2013).

23. *Elimination of neglected diseases and other poverty-related infections*. Pan American Health Organization and World Health Organization. 49th Directing Council. 61st session of the Regional Committee. Washington DC, 2009 [resolution CD49.R19]. (http://new.paho.org/hq/dmdocuments/2009/CD49.R19%20(Eng.).pdf; accessed March 2013).

24. *Interagency meeting on planning the prevention and control of neglected zoonotic diseases, Geneva, 5–6 July 2011*. Geneva, World Health Organization, 2011 (WHO/HTM/NTD/NZD/2011; also available at whqlibdoc.who.int/publications/2011/9789241502931_eng.pdf; accessed March 2013).

25. *Strategic framework for elimination of human rabies transmitted by dogs in the South-East Asia Region*. New Delhi, WHO Regional Office for South-East Asia, 2012 (http://www.searo.who.int/topics/rabies/en/; accessed March 2013).

26. Sudarshan MK et al. Assessing the burden of human rabies in India: results of a national multi-center epidemiological survey. *International Journal of Infectious Diseases*, 2007, 11(1):29–35.

27. Yu J et al. The spatial and temporal dynamics of rabies in China. *PLoS Neglected Tropical Diseases*, 2012, 6(5):e1640.

28. Yin C-P. Analysis on factors related to rabies epidemic in China from 2007–2011. *Virologica Sinica*, 2012, 27(2):132–143.

29. Mallewa M et al. Rabies encephalitis in malaria-endemic area, Malawi, Africa. *Emerging Infectious Diseases*, 2007, 13(1):136–139.

30. Streicker DG et al. Ecological and anthropogenic drivers of rabies exposure in vampire bats: implications for transmission and control. *Proceedings of the Royal Society B. Biological Sciences*, 2012, 279(1742):3384–3392.

31. Mathers CD, Ezzati M, Lopez AD. Measuring the burden of neglected tropical diseases: the Global Burden of Disease Framework. *PLoS Neglected Tropical Diseases*, 2007, 1(2):e114.

32. *WHO Expert Consultation on Rabies. First report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 931).

# 2. Classification of lyssaviruses

## 2.1 Distinguishing features of lyssaviruses

Rabies is an acute encephalitis or meningoencephalitis due to a lyssavirus infection. The etiological agents of rabies encephalitis belong to the Mononegavirales order, the Rhabdoviridae family and the *Lyssavirus* genus. Lyssaviruses have a 12-kb nonsegmented RNA genome of negative polarity that encodes five viral proteins (3′ to 5′): a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G) and an RNA-dependent RNA polymerase (or large protein, L). The lyssavirus particle is shaped like a bullet, 100–300 nm long and 75 nm in diameter. It is composed of two structural and functional units: an internal helical nucleocapsid and an external envelope. The nucleocapsid consists of a ribonucleoprotein complex comprising the genomic RNA and tightly bound N protein together with the L and P proteins. The nucleocapsid is active for transcription and replication: the N-RNA template is processed by the L protein, which contains most of the RNA polymerase activities, and its cofactor, the P protein. The lipid envelope is derived from the host cytoplasmic membrane during budding. Knobbed glycoprotein spikes (5–10 nm long and about 3 nm in diameter) consisting of three glycosylated ectodomains, which binds the virions to host cell receptors, protrude through the virion membrane. The M protein forms oligomers that bind to the outside of the nucleocapsid, giving rigidity to the virion structure and providing a binding platform for the viral glycoprotein and the envelope membrane (*1*,*2*).

## 2.2 Criteria for differentiating between lyssaviruses

Until the 1950s, the rabies virus was considered to be unique. Identification of serologically related viruses in Nigeria—Lagos bat virus from a pteropodid bat (*3*) and Mokola virus from a shrew (*4*)—showed that the structure of this virus group was more complex, and the terms 'rabies-related viruses' and 'rabies serogroup' were introduced (*4*). Another serologically related virus, Duvenhage virus, was isolated from a man who died of rabies after a bite of an insectivorous bat in 1970 in South Africa (*5*), representing a fourth serotype.

The viruses regularly isolated from bats in Europe since the 1950s were related serologically to Duvenhage virus and were initially included in the Duvenhage serotype (*6*,*7*). Later, use of monoclonal antibodies made it possible to refine the classification of the 'rabies serogroup' (*8*). European bat lyssaviruses were not only distinguished from the African Duvenhage virus (*9*) but also separated into two distinct serotypes (*10*), temporally termed 'biotypes' (*11*). This differentiation was later supported by gene sequencing and phylogenetic analysis (*12*,*13*). Extensive phylogenetic studies of the diversity of rabies-related

viruses led to the creation of the operational term 'genotype', which has since been used broadly in the scientific literature (*12*). New genotypes were identified, and quantitative criteria for their differentiation were proposed (*12,14–18*).

To accommodate the growing variety of 'rabies-related' viruses, the genus *Lyssavirus* was established under the auspices of the International Committee on the Taxonomy of Viruses. The name of the genus was derived from Greek mythology: Lyssa (Λυσσα) was a goddess or spirit of rage, fury, raging madness and frenzy. The existing 'genotypes' served as a basis for the taxonomy of lyssavirus but were refined to satisfy the official rules of the International Committee, which apply to more complex entities such as viral species.

The demarcation criteria for lyssavirus species include (*19*):

- Genetic distance, with a threshold of 80–82% nucleotide identity for the complete N gene, which provides better quantitative resolution than other genes, or 80–81% nucleotide identity for concatenated coding regions of the N+P+M+G+L genes. In general, all isolates belonging to the same species have higher identity values than the threshold, except the viruses currently included in the Lagos bat virus species. For that reason, some authors have suggested that Lagos bat virus be subdivided into several genotypes (*20,21*). In the absence of other sufficient demarcation characters, however, Lagos bat virus has not been separated into several species, as these representatives segregate into a monophyletic cluster in most phylogenetic reconstructions.
- Topology and consistency of phylogenetic trees obtained with various evolutionary models
- Antigenic patterns in reactions with nucleocapsid monoclonal antibodies (preceded by serological cross-reactivity and definition of lyssavirus serotypes with polyclonal antisera)
- When available, additional characteristics, such as ecological properties, host, geographical range and pathological features.

## 2.3 Present structure of the *Lyssavirus* genus

Currently, the International Committee on the Taxonomy of Viruses recognizes 12 *Lyssavirus* species (*Table 3*). On the basis of genetic distances and serological cross-reactivity, the genus has been subdivided into two phylogroups:

- Phylogroup I contains the species rabies virus, European bat lyssaviruses type 1 and type 2, Duvenhage virus, Australian bat lyssavirus, Aravan virus, Khujand virus and Irkut virus.

- Phylogroup II contains Lagos bat virus, Mokola virus and Shimoni bat virus.

The remaining species of the genus, West Caucasian bat virus, cannot be included in either of these phylogroups and is suggested to be considered a representative of an independent phylogroup III.

A further potential extension of the genus, a novel Bokeloh bat lyssavirus, was recently isolated from an insectivorous bat (*Myotis nattereri*) in France and Germany. This virus is related phylogenetically to European bat lyssavirus type 2 and Khujand virus (*17,22*). Another divergent lyssavirus, related phylogenetically to West Caucasian bat virus (therefore potentially a member of the proposed phylogroup III) and tentatively named Ikoma lyssavirus, was detected in an African civet (*Civettictis civetta*) in the United Republic of Tanzania (*18*). Bats are the reservoirs and vectors of lyssaviruses for 12 of the 14 recognized and proposed species, while the reservoirs of Mokola virus and Ikoma lyssavirus remain to be determined.

Lyssaviruses show broad antigenic cross-reactivity at the nucleocapsid level, mainly because of sequence conservation of the N protein. Therefore, similar reagents can be used for diagnosis by immunofluorescence. The ectodomain of the G protein (which carries the main antigenic sites) is more variable, and there is cross-neutralization among lyssaviruses of the same phylogroup (amino acid identity in the ectodomain, >74%) but not between phylogroups (amino acid identity in the ectodomain, <62%). Experimental evidence indicates that the available vaccine strains, which all belong to rabies virus species in phylogroup I, are ineffective against infection with lyssaviruses in phylogroup II and West Caucasian bat virus. A similar lack of protection is likely for Ikoma lyssavirus.

## 2.4 References

1. Graham SC et al. Rhabdovirus matrix protein structures reveal a novel mode of self-association. *PLoS Pathogens*, 2008, 4:e1000251.
2. Ge P et al. Cryo-EM model of the bullet-shaped vesicular stomatitis virus. Science, 2010, 327:689–693.
3. Boulger LR, Porterfield JS. Isolation of a virus from Nigerian fruit bats. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1958, 52:421–424.

Table 3
**Viruses currently included in the genus *Lyssavirus***

| Recognized and proposed species (abbreviation) | Primary host | Geographical range | Comments |
|---|---|---|---|
| Rabies virus (RABV) | *Carnivora* and bats (*Chiroptera*) | Terrestrial mammals worldwide except in Australia, Antarctica and several islands; bats in the New World only | 1 |
| Australian bat lyssavirus (ABLV) | Pteropodid bats (at least four species of *Pteropus* genus) and insectivorous bats (*Saccolaimus albiventris*) | Australia (and perhaps several nearby islands) | 2 |
| European bat lyssavirus, type 1 (EBL1) | Insectivorous bats (predominantly *Eptesicus serotinus*) | Most of Europe, from Spain to the Ukraine | 3 |
| European bat lyssavirus, type 2 (EBL2) | Insectivorous bats (predominantly *Myotis daubentonii* and *M. dasycneme*) | North-western Europe | 4 |
| Khujand virus (KHUV) | Insectivorous bat *Myotis mystacinus* | Central Asia | 5 |
| Aravan virus (ARAV) | Insectivorous bat *Myotis blythi* | Central Asia | 6 |
| Bokeloh bat lyssavirus (BBLV) | Insectivorous bat *Myotis nattereri* | France, Germany | 7 |
| Irkut virus (IRKV) | Insectivorous bat *Murina leucogaster* | Eastern Asia | 8 |
| Duvenhage virus (DUVV) | Insectivorous bats | Sub-Saharan Africa | 9 |
| Lagos bat virus (LBV) | Pteropodid bats of several genera (e.g. *Eidolon helvum, Rousettus aegyptiacus, Epomophorus* spp.) | Sub-Saharan Africa | 10 |
| Mokola virus (MOKV) | Unknown | Sub-Saharan Africa | 11 |
| Shimoni bat virus (SHIBV) | Insectivorous bat *Hipposideros commersoni* | Kenya | 12 |
| West Caucasian bat virus (WCBV) | Insectivorous bats from genus *Miniopterus* | South-eastern Europe | 13 |
| Ikoma lyssavirus (IKOV) | Not known | United Republic of Tanzania | 14 |

1. Responsible for the vast majority of human rabies cases in the world. All currently available human and veterinary vaccine strains originate from this species.
2. Given limited surveillance, the host range among insectivorous bats may be greater. Two human cases have been documented.
3. Given the limited surveillance in eastern Europe and Asia, may be distributed more broadly, along the reservoir species range. Spillover infections in wild and companion animals and a very small number of human cases have been documented.
4. Two human cases have been documented.
5. Known from a single isolate. Given the limited surveillance in eastern Europe and Asia, may be distributed more broadly. No human cases have been documented.
6. Known from two isolates. Given the limited surveillance in eastern Europe and Asia, may be distributed more broadly. No human cases have been documented.
7. Known from a single isolate. Does not have species status and is not listed in current International Committee on the Taxonomy of Viruses documents. No human cases have been documented.
8. Known from two isolates, from a bat and from a human.
9. Known from four isolates, three of which came from humans bitten by bats and one from a bat, presumably of the *Miniopterus* species.
10. Constitutes several lineages with long genetic distances. In the future, may be subdivided into two or three separate species. Spillover infections reported in wild and companion animals. No human cases documented to date.
11. Twice isolated from shrews, once from a rodent. Most other isolates were obtained from companion animals, such as cats, as the result of spillover infection. Two human cases have been reported.
12. Known from a single isolate. Serological surveys suggest that *H. commersoni* is the probable reservoir. No human cases have been documented.
13. Known from a single isolate; however, serological surveys suggest that West Caucasian bat virus (or another serologically related virus) is present in *Miniopterus* bats in Africa (Kenya). No human cases have been documented.
14. Known from a single isolate from an African civet (*Civettictis civetta*). The natural host is unknown. Given the phylogenetic relatedness to the West Caucasian bat virus, the index case in an African civet may have resulted from a spillover infection of bat origin. No human cases have been documented.

4. Shope RE et al. Two African viruses serologically and morphologically related to rabies virus. *Journal of Virology*, 1970, 6:690–692.

5. Meredith CD, Rossouw AP, van Praag Koch H. An unusual case of human rabies thought to be of chiropteran origin. *South African Medical Journal*, 1971, 45:767–769.

6. Schneider LG. Antigenic variants of rabies virus. Comparative Immunology, *Microbiology and Infectious Diseases*, 1982, 5:101–107.

7. Schneider LG, Barnard BJH, Schneider HP. Application of monoclonal antibodies for epidemiological investigations and oral vaccination studies: I. African viruses. In: Kuwert E et al., eds, *Rabies in the tropics*. Berlin, Springer-Verlag, 1985:49–53.

8. Wiktor TJ, Koprowski H. Monoclonal antibodies against rabies virus produced by somatic cell hybridization: detection of antigenic variants. *Proceedings of the National Academy of Sciences of the United States of America*, 1978, 75:3938–3942.

9. Dietzschold B et al. Antigenic diversity of the glycoprotein and nucleocapsid proteins of rabies and rabies-related viruses: implications for epidemiology and control of rabies. *Reviews of Infectious Diseases*, 1988, 10(S4):785–798

10. Bourhy H et al. Antigenic and molecular characterization of bat rabies virus in Europe. *Journal of Clinical Microbiology*, 1992, 30:2419–2426.

11. King A, Davis P, Lawrie A. The rabies viruses of bats. *Veterinary Microbiology*, 1990, 23:165–174.

12. Bourhy H, Kissi B, Tordo N. Molecular diversity of the *Lyssavirus* genus. *Virology,* 1993, 194:70–81.

13. Davis PL et al. Phylogeography, population dynamics, and molecular evolution of European bat lyssaviruses. *Journal of Virology*, 2005, 79:10487–10497.

14. Fraser GC et al. Encephalitis caused by a lyssavirus in fruit bats in Australia. *Emerging Infectious Diseases*, 1996, 2:327–331.

15. Kuzmin IV et al. Bat lyssaviruses (Aravan and Khujand) from Central Asia: phylogenetic relationships according to N, P and G gene sequences. *Virus Research*, 2003, 97:65–79.

16. Kuzmin IV et al. Phylogenetic relationships of Irkut and West Caucasian bat viruses within the *Lyssavirus* genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. *Virus Research*, 2005, 111:28–43.

17. Freuling C et al. Novel lyssavirus in a Natterer's bat (*Myotis nattereri*), Germany. *Emerging Infectious Diseases*, 2011, 17:1519–1522.

18. Marston DA et al. Ikoma lyssavirus: identification of a highly divergent novel lyssavirus in an African civet (*Civettictis civetta*). *Emerging Infectious Diseases*, 2012, 18:664–667.

19. Dietzgen RG et al. Family Rhabdoviridae. In: King AMQ et al., eds. *Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses*. Oxford, Elsevier, 2011:686–714.

20. Delmas O et al. Genomic diversity and evolution of the lyssaviruses. *PLoS One*, 2008, 3:e2057.

21. Markotter W et al. Phylogeny of Lagos bat virus: challenges for lyssavirus taxonomy. *Virus Research*, 2008, 135:10–21.

22. Picard-Meyer E et al. Découverte d'une chauve-souris de Natterer infectée par un lyssavirus Bokeloh en Moselle en 2012. *Bulletin Epidémiologique Santé animale, Alimentation*, 2012, 55:25 (http://www.anses.fr/bulletin-epidemiologique/).

## 3. Pathogenesis

Rabies virus enters the body through wounds or by direct contact with mucosal surfaces. It cannot cross intact skin. Rabies virus replicates in the bitten muscle and gains access to motor endplates and motor axons to reach the central nervous system (*1–5*). Virions are carried in transport vesicles (*6*) and travel to the central nervous system exclusively by fast retrograde transport along motor axons, with no uptake by sensory or sympathetic endings (*1–3,5*). Viruses can also enter motor axons in peripheral nerves directly during a penetrating injury (*1,3,4*). In some bat variants, viral propagation may also occur via sensory nerves due to skin tropism (*3,7,8*). The incubation period varies from 5 days to several years (usually 2–3 months; rarely more than 1 year), depending on the amount of virus in the inoculum, the density of motor endplates at the wound site and the proximity of virus entry to the central nervous system (*3–5*). Muscle-specific micro-RNA may

contribute to this eclipse phase by suppressing viral transcription and replication in the muscle (*9,10*). The estimated speed of virus migration depends on whether it moves by centripetal retrograde axonal transport or centrifugal spread. In centripetal retrograde axonal transport, migration is fast, with speeds of 5–100 mm/day or even faster, because neuronal populations of the same synaptic order located at various distances, e.g. 10 μm to 2 cm, are infected simultaneously (*1,5*). Conversely, centrifugal spread is slow, probably mediated by passive diffusion rather than active transport (*1–3,5*).

The first rapid centripetal phase leads to wide transneuronal transfer within the central nervous system and to infection of dorsal root ganglia via their central connections with the initially infected motor neurons and spinal interneurons (*1–3,5*). The virus then moves centrifugally from the central nervous system via slow anterograde axoplasmic flow in motor axons to the ventral roots and nerves and in peripheral sensory axons of the infected dorsal root ganglia, leading to infection of muscle spindles, skin, hair follicles and other non-nervous tissues, such as salivary glands, heart muscle, lung and abdominal visceral organs via their sensory innervation (*3–5*). By the time of clinical onset, the virus is widely disseminated throughout the central nervous system and probably to extra-neural organs (*11*).

The first specific clinical symptom is neuropathic pain at the site of the bite. This is caused by virus replication in dorsal root ganglia and inflammation induced by cellular immunity (*12*). Human rabies can manifest as furious or paralytic forms, which cannot be correlated with a specific anatomical localization of rabies virus in the central nervous system (*12–14*). The major clinical signs are probably due to different site-specific responses (*14*). Functional neuronal impairment also explains coma. Electrophysiological studies with pathological correlates show that peripheral nerve axonopathy or myelinopathy is responsible for weakness in paralytic rabies (*7,12*). Preferential entry via the motor route explains why subclinical anterior horn cell dysfunction precedes sensory loss in furious rabies and is initially localized at body segments corresponding to the site of the bite, progressively spreading to other locations (*3,5,12*). The same considerations apply to prodromal symptoms and signs in paralysed patients (*3–5*). It is likely that less virus is present in the brain in paralytic rabies (when consciousness is preserved) than in furious rabies. Diffusion tensor imaging in canine paralytic rabies showed that neural tract integrity is compromised at brain-stem level, limiting viral propagation to the forebrain (*5,15,16*). A viral immune evasive strategy with blood–brain barrier integrity prevents eradication of the virus in the central nervous system (*4,16–21*). There is no evidence of immune suppression or accelerated death in rabies-infected patients (*15,16*).

Rabies with atypical clinical and/or neuroimaging features is increasingly recognized (*4,22–26*). Whether this is due to atypical virus variants, a host immune

response or large doses of virus inoculum (as in the case of organ transplantation from rabies-infected donors) is unknown. Without intensive care, death occurs within 2 weeks after the appearance of clinical symptoms (*5,7*).

## 3.1 References


1. Ugolini G. Use of rabies virus as a transneuronal tracer of neuronal connections: implications for the understanding of rabies pathogenesis. *Developments in Biologicals* (Basel), 2008, 131:493–506.
2. Ugolini G. Advances in viral transneuronal tracing. *Journal of Neuroscience Methods*, 2010, 194:2–20.
3. Ugolini G. Rabies virus as a transneuronal tracer of neuronal connections. *Advances in Virus Research*, 2011, 79:165–202.
4. Hemachudha T, Laothamatas J, Rupprecht CE. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. *Lancet Neurology*, 2002, 1(2):101–109.
5. Hemachudha T et al. Human rabies: neuropathogenesis, diagnosis and management. *Lancet Neurology*, 2013, 12(5):498–513.
6. Klingen Y, Conzelmann KK, Finke S. Double-labeled rabies virus: live tracking of enveloped virus transport. *Journal of Virology*, 2008, 82(1):237–245.
7. Hemachudha T et al. Pathophysiology of human paralytic rabies. *Journal of Neurovirology*, 2005, 11(1):93–100.
8. Morimoto K et al. Characterization of a unique variant of bat rabies virus responsible for newly emerging human cases in North America. *Proceedings of the National Academy of Sciences of the United States of America*, 1996, 93(11):5653–5658.
9. Israsena N et al. Inhibition of rabies virus replication by multiple artificial microRNAs. *Antiviral Research*, 2009, 84(1):76–83.
10. Israsena N, Mahavihakanont A, Hemachudha T. Rabies virus infection and microRNAs. *Advances in Virus Research*, 2011, 79:329–344.
11. Hemachudha T et al. Rabies. *Current Neurology and Neuroscience Reports*, 2006, 6(6):460–468.
12. Mitrabhakdi E et al. Difference in neuropathogenetic mechanisms in human furious and paralytic rabies. *Journal of Neurological Science*, 2005, 238(1–2):3–10.

13. Dumrongphol H et al. Alteration of muscarinic acetylcholine receptors in rabies viral-infected dog brains. *Journal of Neurological Science*, 1996, 137(1):1–6.

14. Thanomsridetchai N et al. Comprehensive proteome analysis of hippocampus, brainstem, and spinal cord from paralytic and furious dogs naturally infected with rabies. *Journal of Proteome Research*, 2011, 10(11):4911–4924.

15. Laothamatas J et al. Furious and paralytic rabies of canine origin: neuroimaging with virological and cytokine studies. *Journal of Neurovirology,* 2008, 14(2):119–129.

16. Laothamatas J, Sungkarat W, Hemachudha T. Neuroimaging in rabies. *Advances in Virus Research*, 2011, 79:309–327.

17. Lafon M. Evasive strategies in rabies virus infection. *Advances in Virus Research*, 2011, 79:33–53.

18. Laothamatas J et al. MR imaging in human rabies. *American Journal of Neuroradiology*, 2003, 24(6):1102–1109.

19. Roy A et al. Failure to open the blood–brain barrier and deliver immune effectors to central nervous system tissues leads to the lethal outcome of silver-haired bat rabies virus infection. *Journal of Virology*, 2007, 81(3):1110–1118.

20. Roy A, Hooper DC. Immune evasion by rabies viruses through the maintenance of blood–brain barrier integrity. *Journal of Neurovirology*, 2008, 14(5):401–411.

21. Kasempimolporn S et al. Human immune response to rabies nucleocapsid and glycoprotein antigens. *Clinical and Experimental Immunology*, 1991, 84(2):195–199.

22. Hemachudha T, Phuapradit P. Rabies. *Current Opinions in Neurology*, 1997, 10(3):260–267.

23. Burton EC et al. Rabies encephalomyelitis: clinical, neuroradiological, and pathological findings in 4 transplant recipients. *Archives of Neurology*, 2005, 62(6):873–882.

24. Maier T et al. Management and outcomes after multiple corneal and solid organ transplantations from a donor infected with rabies virus. *Clinical Infectious Diseases*, 2010, 50(8):1112–1119.

25. Shantavasinkul P et al. Failure of rabies postexposure prophylaxis in patients presenting with unusual manifestations. *Clinical Infectious Diseases*, 2010, 50(1):77–79.

26. Human rabies—Minnesota, 2007. *Morbidity and Mortality Weekly Report*, 2008, 57(17):460–462.

# 4. Diagnosis

Rabies is an acute, progressive encephalitis caused by a lyssavirus. Clinical diagnosis of encephalitis can be challenging, and all suspected and probable clinical cases of rabies should be confirmed by laboratory methods when possible. During the past decade, significant progress has been made in laboratory diagnostic methods for clinical case confirmation. Each country should have a national reference laboratory with the capacity for basic rabies diagnosis and case confirmation by suggested modern techniques (*1–7*). Where such expertise is lacking, training and reference diagnostic capability can be obtained from WHO collaborating centres (*8*) (*Annex 8*) and from reference centres of the World Organisation for Animal Health (OIE) for animal rabies (*9*).

## 4.1 Standard case definitions for rabies

All countries should use standard case definitions for rabies supported by laboratory-based surveillance of suspected cases in humans and animals. According to the WHO recommended standards and strategies for surveillance, prevention and control of communicable diseases, a clinical case of rabies is defined as:

*a subject presenting with an acute neurological syndrome (i.e. encephalitis) dominated by forms of hyperactivity (i.e. furious rabies) or paralytic syndromes (i.e. dumb rabies) progressing towards coma and death, usually by cardiac or respiratory failure, typically within 7–10 days after the first sign, if no intensive care is instituted.*

One or more of the following laboratory criteria should be used to confirm a clinical case:

- presence of viral antigens;
- isolation of virus in cell culture or in laboratory animals;
- presence of viral-specific antibodies in the cerebrospinal fluid or the serum of an unvaccinated person; or

- presence of viral nucleic acids detected by molecular methods in samples (e.g. brain biopsy, skin, saliva, concentrated urine) collected post mortem or intra vitam.

Cases of rabies are basically classified as follows:

- **suspected**: a case that is compatible with a clinical case definition
- **probable**: a suspected case plus a reliable history of contact with a suspected rabid animal
- **confirmed**: a suspected or probable case that is laboratory-confirmed. In some situations, a clinical suspicion of encephalitis or a history of animal exposure may be lacking; however, a case would still be considered confirmed by appropriate laboratory diagnostic testing.

A record form for possible exposure to rabies is given in *Annex 2*.

## 4.2 Clinical diagnosis

A presumptive diagnosis of rabies, an acute, progressive encephalomyelitis, with the highest case fatality rate of any infectious disease, is simple in a person presenting with a compatible illness after documented exposure to a laboratory-confirmed rabid animal. Specific clinical signs of hydro- or aerophobia in humans provide a strong suspicion of rabies, if they are well documented. In the absence of a history of exposure or paramount signs, however, the diagnosis of rabies on clinical grounds alone is difficult and often unreliable. For example, some patients can present with a paralytic or Guillain-Barré-like syndrome or other atypical features (*10*). Atypical or non-classical rabies is increasingly recognized and may be responsible for underreporting of cases. Detailed clinical information on patients with atypical rabies, especially cases associated with exposure to bats or other wildlife, has been reported (*11,12*). Human case reports can be found in a variety of peer-reviewed publications, national and international reports and electronic sources, such as the website of the United States Centers for Disease Control and Prevention.

Classical signs of brain involvement include spasms in response to tactile, auditory, visual or olfactory stimuli (e.g. aerophobia and hydrophobia) alternating with periods of lucidity, agitation, confusion and signs of autonomic dysfunction (*10*). Spasms may occur in rabid patients in whom excitation is prominent. Spontaneous inspiratory spasms can occur continuously until death, and their presence may facilitate a clinical diagnosis. Excitation is less evident in paralytic rabies, and phobic spasms may appear in only 50% of such patients. During the early stages of paralytic rabies, notable signs may include myoedema at percussion sites, usually in the region of the chest, deltoid muscle and thigh, piloerection and fasciculations.

Magnetic resonance imaging, performed with adequate precautions for potentially infectious patients, can be helpful (*10,13*). Abnormal, ill-defined, mildly hypersignal T2 images involving the brain-stem, hippocampus, hypothalamus, deep and subcortical white matter and deep and cortical grey matter indicate a diagnosis of rabies, regardless of clinical type. Gadolinium enhancement may appear clearly only in later stages, when patients lapse into a coma. Such patterns can help differentiate rabies from other viral encephalitides, not in terms of location, but in the T2 image appearance and in the pattern of contrast enhancement, when compared to consciousness status. Computerized tomography of the brain is of little diagnostic value.

Rabies should be included in the differential diagnosis of all patients who present with unexplained, acute, progressive viral encephalitis, even in areas where the disease is rare, as it can occur locally in wildlife, such as bats, can be acquired during travel to enzootic areas and because imported cases of human and animal rabies continue to occur (*2,12*). In addition, rabies may be misdiagnosed and death ascribed to another cause (e.g. cerebral malaria), without adequate epidemiological scrutiny and laboratory confirmation (*2,4,14*). As transmission of rabies virus to recipients of solid organ transplants has been described, all potential organ donors who present with a compatible encephalitis should be screened and tested to determine whether they present an infectious risk, by examining suitable ante- or post-mortem specimens by sensitive, specific laboratory methods (*2,4,6*).

## 4.3 Biosafety, sampling and specimen transport for laboratory diagnosis

### 4.3.1 Biosafety

Rabies has the highest case fatality rate of any currently recognized infectious disease. Safety is therefore of paramount importance when working with lyssaviruses. In general, biosafety level 2 safety practices are adequate for routine laboratory activities such as handling animals, necropsy, collection preparation and processing samples (*5–7*). The basic facility design should be adequate, and precautions should include personal protective equipment (e.g. clothing, gloves, eye protection) and vaccination. Certain activities may require a biosafety level 3 classification, such as production of large quantities of concentrated virus, procedures that may generate aerosols (e.g. homogenization of tissue suspensions) and working with newly isolated lyssaviruses for which the effectiveness of current prophylaxis is not known. All national safety guidelines for working with infectious agents should be followed.

### 4.3.2 Sampling for intra-vitam diagnosis in humans

Secretions, biological fluids (e.g. saliva, spinal fluid, tears) and tissues (skin biopsy samples and hair follicles at the nape of the neck) can be used to diagnose rabies during life (*1,2,5,6,15,16*). Three saliva samples taken at intervals of 3–6 h, skin and hair follicules are the most sensitive samples. Ideally, samples should be stored at –20 °C or less. Serum should be collected from blood samples before freezing and stored at –20 °C or less.

### 4.3.3 Sampling for post-mortem diagnosis in humans and animals

Brain tissue is the preferred specimen for post-mortem diagnosis in both humans and other animals (*4,5,7*). If a brain biopsy cannot be performed, such as in field studies, tissue samples can be collected via the trans-orbital or trans-foramen magnum route (*1*). Preservation in glycerine (at +4 °C or –20 °C) or drying smears of brain tissue on filter paper containing proper inactivating chemicals (at +30 °C) allows safe, stable transport of infected material, but safe, effective viral inactivation must be ensured before shipment (*1,17*). Other specimens, such as skin and hair follicles taken at the nape of the neck, are also highly sensitive for post-mortem diagnosis (*5,6,18*).

### 4.3.4 Transport of specimens

Specimens for a diagnosis of rabies should be shipped according to national and international regulations to avoid exposure. Information on the appropriate International Air Transport Association shipment classification can be found on the Association's website (*19*), and packing instructions are given in the WHO recommendations on transport of infectious substances (*20*). Diagnostic specimens should be frozen or refrigerated; if they are shipped at ambient temperature, they should be preserved in 50% glycerine–saline solution. The source of specimens for diagnosis and the storage conditions clearly affect the results of any laboratory procedure. Rabies can be diagnosed in fresh (unfixed) specimens from several different tissue sources, but they are preferably refrigerated or frozen.

If samples are stored in 50% glycerol–saline before testing, they must be washed thoroughly; freezing and long-term storage are not recommended. Unlike the processing of fresh or frozen tissues, acetone fixation is not recommended before direct fluorescent antibody testing of samples stored in glycerol saline. The choice of specimens and handling depend on the test to be performed and the stage of the disease (*1,6*).

Examination of chemically fixed specimens for viral antigens can be both sensitive and specific if appropriate tissues and tests are used (*21*). Formalin fixation of brain tissue is not, however, a suitable method for routine diagnosis, because it delays the test results. If specimens are received in formalin, the

duration of fixation should be approximately 7–14 days before embedding in paraffin. Wet tissue specimens should be transferred from formalin to absolute ethanol for subsequent molecular diagnosis and antigen detection. Typical intracytoplasmic inclusions in fixed brain tissue can be detected in neurons by validated immunohistochemical methods (*22*).

## 4.4 Laboratory techniques for post-mortem diagnosis of rabies

A definitive diagnosis of rabies can be made only with the appropriate laboratory methods. The basic techniques are described in the WHO publication *Laboratory techniques in rabies (5) and the OIE Manual of diagnostic tests and vaccines for terrestrial animals* (*7*). Participation in routine quality management is strongly recommended when using any of the laboratory techniques described (*6*).

### 4.4.1 Viral antigen detection

The direct fluorescent antibody technique is a rapid, sensitive, specific method for diagnosing rabies in animals and humans (*5–7,23,24*) and is the gold standard for rabies diagnosis. The accuracy of the test depends, however, on variables such as the expertise of the examiner, the quality of the anti-rabies conjugate and basic equipment, including the fluorescence microscope. The test is based on microscopic examination of impressions or smears of brain tissue after incubation with anti-rabies polyclonal globulin or broadly cross-reactive monoclonal antibodies conjugated with fluorescein isothiocyanate. The diagnostic conjugate should be of high quality, and the appropriate working dilution for optimal performance and detection of virus-specific antigens must be determined.

Impressions (or smears) of samples from the brain-stem and cerebellum are recommended for high sensitivity of the test (*6*). The hippocampi (Ammon horns) may be included but are not necessary for a definitive diagnosis.

Other methods for the detection of lyssavirus antigens, such as enzyme-linked immunosorbent assays (ELISAs) and direct rapid immunohistochemistry tests, have provided consistently reproducible results in several laboratories (*6,25–28*). Extensive evaluation of direct rapid immunohistochemistry tests has shown that their sensitivity and specificity are at least comparable to those of the direct fluorescent antibody test, the traditional standard in rabies diagnosis. This test allows rapid onsite testing by light microscopy and should facilitate decentralized epidemiological surveys if the reagents become commercially available. The Consultation recommends further development of direct rapid immunohistochemistry tests as an alternative to the direct fluorescent antibody test for improved decentralized laboratory-based surveillance.

Lateral flow tests for rapid detection of rabies virus antigen under field conditions have been developed (*29–31*); however, the procedures for the

commercially available assays have not been standardized or harmonized for proper use and adequate validation according to international standards (*5*).

### 4.4.2 Virus isolation

Virus might have to be isolated to confirm the results of antigen detection tests and for further amplification or characterization of an isolate (*5*). Viruses can be isolated in cell cultures, such as neuroblastoma cells, or by intracranial inoculation into mice. Virus isolation in animals should be replaced by alternative methods, whenever possible.

Murine neuroblastoma cells (e.g. NA C1300) are more susceptible to field isolates of lyssavirus than other cell lines tested (*5,6*). Virus isolation in neuroblastoma cell culture is at least as efficient as animal inoculation, especially for small quantities of virus. Cell culture isolation also reduces the time required for diagnosis, from 10–21 days with the mouse inoculation test to only 1–2 days. If the conditions are not optimal, however, such as decomposed brain, false-negative results may be obtained. When cell culture facilities or molecular methods are not available, animal inoculation can be used. If a rapid answer is required, suckling mice (<3 days old) are preferred to weanling or adult mice, because they are more susceptible than older animals. The observation period may be shortened by fluorescent antibody examination of brains of inoculated mice euthanized 14–21 days (or more) after inoculation or when clinical signs appear.

### 4.4.3 Viral RNA detection

Molecular methods, such as the reverse transcription polymerase chain reaction (RT-PCR) and other amplification techniques, are playing an increasingly important role in many countries but are not recommended currently for routine post-mortem diagnosis of rabies if brain tissue is available, when the direct fluorescent antibody test should be used (*5*). Molecular techniques can be used, however, for epidemiological surveys in laboratories with strict quality control procedures and with experience and expertise in using such techniques; they can also be used for ante-mortem diagnosis in humans. The use of robust positive controls or in-process controls is strongly recommended.

## 4.5 Techniques for intra-vitam diagnosis of rabies in humans

Many laboratory methods can be used to confirm a clinical case of rabies while the patient is still alive (*2,32*). Use of intra-vitam techniques for the diagnosis of rabies in animals is, however, strongly discouraged. The sensitivity of a technique for diagnosing rabies varies widely according to the stage of the

disease, immunological status, intermittent viral excretion and the training of the technical staff. While a positive validated result is indicative of rabies, a negative result does not necessarily rule out the infection. It is not recommended that a brain biopsy sample be taken solely for the diagnosis of rabies, but it can be useful when obtained (*6,10*). A diagnosis of rabies in a patient suspected of having the disease is valuable for multiple reasons, including: specific characterization of the causative agent and of the potential source of infection, especially when a history of exposure to an animal is lacking; identification of other people who may have been exposed to the same animal during the public health investigation; application of appropriate measures for infection control to prevent exposure from contact with the patient; administration of post-exposure prophylaxis to people exposed to the patient's infectious secretions; case closure and grief counselling with family members; consideration of experimental therapeutic options; monitoring of viral loads and patient response if treatment is undertaken; less invasive techniques for documenting the human burden of disease, given the infrequency of autopsies; and indication of another infectious agent if the tests are negative.

### 4.5.1 Viral antigen detection

Viral antigens can be detected with the direct fluorescent antibody test in skin biopsy samples or hair follices from patients with clinical rabies (*33*). The results are independent of the antibody status of the patient, and specimens may be positive during the early phase of the disease. Skin samples are usually taken from the nuchal area of the neck, with hair follicles containing peripheral nerves. Examination of several sections may be required to detect viral antigens around the base of hair follicles. The quality of the samples is of paramount importance, as the absence of follicles decreases the sensitivity of the test. This technique may not be practicable in all settings, because a cryostat is required to prepare frozen sections of skin; it should be replaced by detection of viral RNA (*6,12,18,34*). Fluorescent antibody testing of corneal impressions is rarely reliable in most clinical settings, and it is not recommended as a routine test because of the risk of corneal scarification, particularly in patients with encephalitis and not rabies. Immunochromatographic methods have been developed to detect rabies antigen directly in saliva or in brain tissue from animals (*29–31*) but still require standardization and stringent quality control.

### 4.5.2 Viral antibody detection

Neutralizing antibodies in the serum of unvaccinated patients or in cerebrospinal fluid can be measured with a virus neutralization test, including the rapid fluorescent focus inhibition test and the fluorescent antibody virus neutralization test (*27,35–37*). If virus-neutralizing antibodies are present in serum, they tend

to appear on average 7–8 days after clinical symptoms. Viral antibodies are infrequently found in cerebrospinal fluid, depending in part on the clinical stage of the disease. Antibody titres against rabies glycoprotein measured by ELISA correlate well with those measured by virus neutralization, and ELISA is easier to perform routinely (*27,35*). Rapid detection of antibodies (immunoglobulins G and M) to other viral antigens, (e.g. nucleoprotein) may also be useful, as they may appear before neutralizing antibodies (*12*).

### 4.5.3 Viral RNA detection

Molecular detection methods are highly sensitive for diagnosis (*1–3,5,10,11,14–18,24,32,38–42*), although, like all laboratory methods, they require standardization and stringent quality control. Lyssavirus RNA can be detected and amplified not only from brain tissue but also from other biological fluids and tissue samples (e.g. saliva, cerebrospinal fluid, tears, skin, concentrated urine and hair follicles). Serial samples of, for example, saliva and urine should be tested, as the virus is excreted intermittently.

### 4.5.4 Virus isolation

Virus is preferably isolated from brain or saliva or other biological samples in which it is highly likely to be detected (*1–7*). The success rate depends in part on the immunological status of the patient (more positive results are obtained in those without antibodies), the intermittence of viral excretion and the number of consecutive passages in cell culture. Liquid specimens or swabs should be frozen after collection, the content of the swab having been expelled into the collection medium. Under no circumstances should preservatives be added to the collection medium. Specimens may contain no infectious virus even during the late stage of the disease.

## 4.6 Virus identification with molecular techniques: epidemiological considerations

Thousands of lyssavirus isolates from humans, domestic animals and wildlife have been compared with molecular techniques, leading to basic identification and classification of lyssaviruses and the demonstration that virus isolates from a given geographical area or species have unique genetic sequences. In most cases, these differences can be used to identify the principal animal hosts (e.g. bat, dog, fox) and to infer the source of infection when a definitive history of exposure is lacking (*1–3,5,10–12,14–17,29,32,33,38–42*).

## 4.7 References


1. Barrat J et al. Rabies diagnosis. *Developments in Biologics* (Basel), 2006, 125:71–77.
2. Dacheux L et al. More accurate insight into the incidence of human rabies in developing countries through validated laboratory techniques. *PLoS Neglected Tropical Diseases*, 2010, 4:e765.
3. Dürr S et al. Rabies diagnosis for developing countries. *PLoS Neglected Tropical Diseases*, 2008, 2(3):e206.
4. Fooks AR et al. Emerging technologies for the detection of rabies virus: challenges and hopes in the 21st century. *PLoS Neglected Tropical Diseases,* 2009, 3(9):e530.
5. Meslin FX et al., eds. *Laboratory techniques in rabies, 4th ed*. Geneva, World Health Organization, 1996.
6. Orciari LA, Rupprecht CE. Rabies. In: Versalovic J et al., eds. *Manual of clinical microbiology*, 10th ed. Washington DC, ASM Press, 2011:1470–1478.
7. *Manual of diagnostic tests and vaccines for terrestrial animals,* 6th ed. Paris, World Organisation for Animal Health, 2011 (http://www.oie.int/international-standard-setting/terrestrial-manual).
8. *WHO collaborating centres database and portal*. Geneva, World Health Organization (http://apps.who.int/whocc/).
9. *Reference experts and laboratories*. Paris, World Organisation for Animal Health (http://www.oie.int/our-scientific-expertise/references-laboratories/list-of-laboratories/).
10. Rupprecht CE, Hemachudha T. Rabies. In: Scheld M, Whitley RJ, Marra C, eds. *Infections of the central nervous system*. Philadelphia, Lippincott, Williams & Wilkins, 2004:243–259.
11. Feder HM et al. Rabies: still a uniformly fatal disease? Historical occurrence, epidemiological trends, and paradigm shifts. *Current Infectious Disease Reports*, 2012, 14:408–422.
12. Petersen BW, Rupprecht CE. Human rabies epidemiology and diagnosis. In: Tkachev S, ed. *Non-flavivirus encephalitis*. Rijeka, InTech, 2011.
13. Laothamatas J et al. MR imaging in human rabies. *American Journal of Neuroradiology*, 2003, 24:1102–1109.

14. Mallawa M et al. Rabies encephalitis in a malaria-endemic area of Malawi, Africa. E*merging Infectious Diseases*, 2007, 13:136–139.

15. Madhusudana SN, Sukumaran SM. Antemortem diagnosis and prevention of human rabies. *Annals of Indian Academy of Neurology*, 2008, 11(1):3–12.

16. Wacharapluesadee S, Hemachudha T. Ante- and post-mortem diagnosis of rabies using nucleic acid-amplification tests. *Expert Review of Molecular Diagnosis*, 2010, 10(2):207–218.

17. Picard-Meyer E et al. Use of filter paper (FTA) technology for sampling, recovery and molecular characterisation of rabies viruses. *Journal of Virological Methods*, 2007, 140(1–2):174–182.

18. Dacheux L et al. A reliable diagnosis of human rabies based on analysis of skin biopsy specimens. *Clinical Infectious Diseases*, 2008, 47(11):1410–1417.

19. *Shipping guidelines for hazardous goods*. Montreal, Quebec, International Air Transport Association (www.iata.org/whatwedo/cargo/dangerous_goods/pages/infectious_substances.aspx).

20. *Guidance on the regulations for transport of infectious substances 2007–2008*. Geneva, World Health Organization. (www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2cc.pdf).

21. Coertse J et al. A case study of rabies diagnosis from formalin-fixed brain material. *Journal of the South African Veterinary Association*, 2011, 82(4):250–253.

22. Stein LT et al. Immunohistochemical study of rabies virus within the central nervous system of domestic and wildlife species. *Veterinary Pathology*, 2010, 47(4):630–633.

23. Robardet E et al. International interlaboratory trials on rabies diagnosis: an overview of results and variation in reference diagnosis techniques (fluorescent antibody test, rabies tissue culture infection test, mouse inoculation test) and molecular biology techniques. *Journal of Virological Methods*, 2011, 177:15–25.

24. Rudd RJ et al. A need for standardized rabies-virus diagnostic procedures: effect of cover-glass mountant on the reliability of antigen detection by the fluorescent antibody test. *Virus Research*, 2005, 111(1):83–88.

25. Lembo T et al. Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerging Infectious Diseases*, 2006, 12(2):310–313.

26. Madhusudana SN et al. Evaluation of a direct rapid immunohistochemical test (dRIT) for rapid diagnosis of rabies in animals and humans. *Virologica Sinica*, 2012, 27(5):299–302.

27. Welch RJ et al. An evaluation of two commercially available ELISAs and one in-house reference laboratory ELISA for the determination of human anti-rabies virus antibodies. *Journal of Medical Microbiology*, 2009, 58(6):806–810.

28. Xu G et al. WELYSSA: a simple tool using mouse monoclonal antibodies for the detection of lyssavirus nucleocapsid in rabies suspected specimens. *Developments in Biologics* (Basel), 2008, 131:555–561.

29. Kasempimolporn S et al. Evaluation of a rapid immunochromatographic test strip for detection of rabies virus in dog saliva samples. *Journal of Veterinary Diagnostic Investigation*, 2011, 23(6):1197–1201.

30. Markotter W et al. Evaluation of a rapid immunodiagnostic test kit for detection of African lyssaviruses from brain material. *Onderstepoort Journal of Veterinary Research*, 2009, 76(2):257–262.

31. Servat A et al Evaluation of a rapid immunochromatographic diagnostic test for the detection of rabies from brain material of European mammals. *Biologicals*, 2012, 40(1):61–66.

32. Hemachudha T, Wacharapluesadee S. Ante-mortem diagnosis of human rabies. *Clinical Infectious Diseases*, 2004, 39:1085–1086.

33. Crepin P et al. Intravitam diagnosis of human rabies by PCR using saliva and cerebrospinal fluid. *Journal of Clinical Microbiology*, 1998, 36(4):1117–1121.

34. Macedo CI et al. Diagnosis of human rabies cases by polymerase chain reaction of neck-skin samples. *Brazilian Journal of Infectious Diseases*, 2006, 10(5):341–345.

35. Feyssaguet M et al. Multicenter comparative study of a new ELISA, Platelia Rabies II, for the detection and titration of anti-rabies glycoprotein antibodies and comparison with the rapid fluorescent focus inhibition test (RFFIT) on human samples from vaccinated and non-vaccinated people. *Vaccine*, 2007, 25(12):2244–2251.

36. Nishizono A et al. Evaluation of an improved rapid neutralizing antibody detection test (RAPINA) for qualitative and semiquantitative detection of rabies neutralizing antibody in humans and dogs. *Vaccine*, 2012, 30(26):3891–3896.

37. Wright E et al. A robust lentiviral pseudotype neutralisation assay for in-field serosurveillance of rabies and lyssaviruses in Africa. *Vaccine*, 2009, 27(51):7178–7186.

38. Hughes GJ et al. Evaluation of a TaqMan PCR assay to detect rabies virus RNA: influence of sequence variation and application to quantification of viral loads. *Journal of Clinical Microbiology*, 2004, 42:299–306.

39. Wacharapluesadee S et al. Development of a TaqMan real-time RT-PCR assay for the detection of rabies virus. *Journal of Virological Methods*, 2008, 151:317–320.

40. Wacharapluesadee S et al. Comparative detection of rabies RNA by NASBA, real-time PCR and conventional PCR. *Journal of Virological Methods*, 2011, 175(2):278–282.

41. Wacharapluesadee S et al. Detection of rabies viral RNA by TaqMan real-time RT-PCR using non-neural specimens from dogs infected with rabies virus. *Journal of Virological Methods*, 2012, 184(1–2):109–112.

42. Wakeley PR et al. Development of a real-time, TaqMan reverse transcription-PCR assay for detection and differentiation of lyssavirus genotypes 1, 5, and 6. *Journal of Clinical Microbiology*, 2005, 43:2786–2792.

# 5. Management of patients before and after death

## 5.1 Rabies survivors and treatment protocols

Although rabies is considered a fatal disease, survival has been documented during the past few decades, particularly in cases associated with bat variants (*1*). After successful treatment in 2004 of an adolescent in the USA with the Milwaukee protocol (*2*), attempts were made in the USA and in some countries of South America to treat rabies patients, albeit with little success (*3*).

In considering a possible treatment modality for rabies patients, the following should be kept in mind (*1*).

- Rabies is not invariably fatal in animals, but a very small number of humans have recovered.
- At present, it is not possible to predict which patients are likely to recover.
- All survivors, with or without treatment, had a vigorous, early immune response.
- Studies to identify management protocols, procedures for immunomodulation and new medications, including antiviral drugs, are encouraged.
- Human treatment must be proven to be safe and not further harm the patient.

## 5.2 Clinical management of rabies patients

Patients remain conscious, are often aware of the nature of their illness and are usually extremely agitated, particularly when excitation is predominant. Furthermore, they are often isolated because of the perceived risk of transmission of the virus through contact. Patients with confirmed rabies should receive adequate sedation and care in an appropriate medical facility, preferably in a private room, with suitable emotional and physical support. Repeated intravenous morphine or benzodiazepines is effective in relieving the severe agitation, anxiety and phobic spasms that afflict patients with furious rabies (*1*). Once furious rabies has been diagnosed, invasive procedures should be avoided, and the patient should be cared for in a private, quiet, draft-free area. In view of the inevitability of death in most cases, treatment should focus on comfort, with heavy sedation (barbiturates, morphine) and avoidance of intubation or life-support measures once the diagnosis is certain (*1*).

## 5.3 Transmission via organ transplantation

Rabies virus is present in many tissues in the terminal stages of disease. Caution should be exercised before transplanting organs from people who have died with neurological symptoms and signs, as several cases of rabies due to organ and tissue transplantation have been documented (*4,5*). Testing for common or highly fatal infections should be balanced against the urgency of transplanting a viable organ. Rare diseases will not be identified until the techniques become available. Corneal transplantation, which is common in developing countries, should be performed with caution.

## 5.4 Recommendations for health care personnel and patients' family members

The care of people in whom rabies is diagnosed may create anxiety among medical and nursing staff and in the media and the public. Human rabies does not pose any greater risk to health care staff than most bacterial or viral infections if routine precautions are used, especially during intubation and suctioning.

Post-exposure prophylaxis should be provided for health care personnel considered to be at risk after careful assessment, and they should be reminded of the importance of adhering to barrier nursing, as recommended for all infectious diseases. Hospitals that are likely to receive rabies patients may consider pre-exposure vaccination for health care staff who may be involved in their management.

It may sometimes be necessary to immunize the partners of patients, as close contact and sexual intercourse in the early stages of the disease carry a risk for transmission.

## 5.5 Management of the bodies of patients who have died of rabies

The body of a patient suspected to have died of rabies should be labelled as infectious. The risk for transmission to others is, however, small if normal precautions are taken. Blood does not contain the virus, but it is present in many tissues and fluids, such as those of the central nervous system and salivary glands (*1*). If embalming or autopsy is performed, it should be undertaken carefully, with appropriate precautions and personal protective equipment. Tissues and body fluids should be disposed of in the same manner as for other infectious diseases. The body of the deceased should be buried or cremated, depending on their religious practice.

## 5.6 References

1. Hemachudha T et al. Human rabies: neuropathogenesis, diagnosis and management. *Lancet Neurology*, 2013, 12(5):498–513.
2. Willoughby RE et al. Survival after treatment of rabies with induction of coma. *New England Journal of Medicine,* 2005, 352:2508–2514.
3. Jackson AC. Therapy of human rabies. *Advances in Virus Research*, 2011, 79:365–375.
4. Srinivasan A et al. Transmission of rabies from an organ donor to four transplant recipients. *New England Journal of Medicine*, 2005, 352:1103–1111.

5. Maier T et al. Management and outcomes after multiple corneal and solid organ transplantation from a donor infected with rabies virus. *Clinical Infectious Diseases*, 2010, 50(8):1112–1119.

# 6. Vaccines and rabies immunoglobulin for humans

Since their development more than four decades ago, concentrated, purified cell culture and embryonated egg-based rabies vaccines (jointly referred to as CCEEVs) have proved to be safe and effective in preventing rabies. These vaccines are intended for both pre- and post-exposure prophylaxis and have been administered to millions of people worldwide (*1*). Prompt administration of CCEEVs after exposure combined with proper wound management and simultaneous administration of rabies immunoglobulins is almost invariably effective in preventing rabies, even after high-risk exposure (*1*) (see also section 8).

## 6.1 Vaccine types

### 6.1.1 Cell culture and embryonated egg-based rabies vaccines

CCEEVs contain rabies virus that has been propagated in cell substrates such as human diploid cells, Vero cells, primary chick embryo cells or embryonated duck eggs. Recently developed vaccines based on chick embryo and Vero cells are as safe and effective as human diploid cell vaccines and are less expensive.

After growth in cell culture (or embryonic egg), the viral harvest is concentrated, purified, inactivated and lyophilized. In some CCEEVs, human albumin or processed gelatine is used as a stabilizer. Rabies vaccines are not supplied in multidose vials for intramuscular injection, and those prequalified by WHO do not contain preservatives such as thiomersal. The shelf-life of these vaccines is ≥3 years, provided they are stored at 2–8 °C and protected from sunlight. After reconstitution with sterile diluent, the vaccines should be used immediately or within 6 h if kept at the correct temperature (*1*), as partially used vials of rabies vaccine may become contaminated.

Rabies vaccines for humans should meet WHO recommendations for characterization, production and control, as set out by the WHO Expert Committee on Biological Standardization (*2*). Presently, WHO recommendations apply only to inactivated rabies vaccines produced in cell culture or embryonated eggs.

### 6.1.2 Nerve tissue vaccines

Nerve tissue vaccines induce more severe adverse reactions and are less immunogenic than CCEEVs. Since 1984, WHO has recommended discontinuation

of the production and use of nerve tissue vaccines and their replacement by CCEEVs. Many developing countries have followed this recommendation (see below list of countries and dates at which discontinuation took place) and meet their requirements for rabies biologicals by either importing vaccine, developing or acquiring technology for producing CCEEVs. In a few countries, mainly in Asia and Latin America, populations at high risk for rabies still depend on vaccines derived from animal nerve tissues for post-exposure prophylaxis. Ecuador and Peru in Latin America and Myanmar and Pakistan in Asia are investigating affordable, sustainable alternatives to the use of nerve tissue vaccines.

This Consultation again strongly recommends that production and administration of vaccines based on animal central nervous systems, including suckling mouse brain, be discontinued and replaced by CCEEVs. A four-step strategy to replace nervous tissue vaccine by modern rabies vaccines produced on cell culture or embryonated eggs has been developed (*3*) and is attached as *Annex 3* to this report.

**Region, country and date of discontinuation of nerve tissue vaccines**

**South East Asia (1987–2011)**

- Bangladesh (2011)
- Bhutan (1995)
- India (2004)
- Indonesia (1992)
- Nepal (2006)
- Sri Lanka (1995)
- Thailand (1987)

**Western Pacific Region (1997–2007)**

- Cambodia (2005)
- China (1990)
- Lao People's Democratic Republic (2005)
- Philippines (1997)
- Viet Nam (2007)

**Region of the Americas (2002–2009)**

- Brazil (2002)

- Chile (2003)
- Dominican Republic (2009)
- El Salvador (2009)
- Mexico (1995)
- Nicaragua (2005)
- Paraguay (2006)

## 6.2 WHO prequalification of human rabies vaccines

Vaccines supplied through United Nations agencies such as UNICEF should be prequalified by WHO. This is an established procedure, initiated voluntarily by vaccine manufacturers, for initial and continuous evaluation by WHO of nationally licensed vaccines. After initial prequalification, products are reassessed at regular intervals to ensure continuing quality. A revised procedure for WHO prequalification of vaccines was endorsed by the WHO Expert Committee on Biological Standardization in October 2010 and has been in effect since 1 February 2012 (*4*).

National regulatory authorities can assume responsibility for regulatory control of a vaccine, and a vaccine must be licensed in the country of manufacture as a prerequisite to prequalification. WHO prequalification ensures the quality, safety and efficacy of vaccines and their suitability for use in national immunization programmes in low- and middle-income countries. The vaccine characteristics must be suitable for use in such programmes with regard to e.g. potency, thermostability, presentation, labelling and cold chain volume. The producer must then meet international standards of quality and comply with international standards of good manufacturing practice.

Prequalification involves a review of the production process and quality control procedures, testing the consistency of lots, an audit of the manufacturing facilities by WHO with observers from the responsible national regulatory authority, assurance of continued acceptability and reassessments at regular intervals. Continued compliance is monitored.

In 2012, only three rabies vaccines were prequalified for intramuscular use: purified Vero cell rabies vaccine, purified chick embryo cell vaccine and purified duck embryo vaccine. The list, which is updated when necessary, can be found at http://www.who.int/immunization_standards/vaccine_quality/prequalification_vaccine_list_en/en/.

The WHO Consultation encourages rabies vaccine manufacturers to enter the WHO prequalification process and Member States to purchase WHO prequalified vaccines.

## 6.3 Requirements for human rabies vaccines

### 6.3.1 Potency requirements, tests and standards

The minimal acceptable potency of CCEEVs is 2.5 international units (IU) per intramuscular dose, as determined in the mouse protection potency test (*5*,*6*). Alternative assays based on serum neutralization (*7*), ELISAs (*8*), fewer animals (*9*), peripheral challenge (*10*) and others (*11*) are being explored. The efficacy of these alternative tests should be established in multicentre studies carried by WHO collaborating centres, national regulatory authorities and control laboratories, in collaboration with manufacturers.

The international standard for rabies vaccine is used in standardizing the mouse protection test and in vitro assays for glycoprotein content. In 2008, a candidate vaccine was calibrated against the fifth international standard in a collaborative study and became the sixth international standard for rabies vaccine. When used in mouse protection tests, this standard contains 8 IU per ampoule, i.e. 8 IU/ml, when reconstituted in 1 ml of distilled water. Other units are used in in vitro assays, such as enzyme immunoassays and single radial immunodiffusion tests, to determine the rabies virus glycoprotein antigen content (*12*).

### 6.3.2 Characterization and evaluation of rabies vaccines

More than a dozen species or genotypes of *Lyssavirus* have been described as causative agents of rabies (see section 2). *Lyssavirus* genomes vary considerably, rabies virus being by far the commonest causative virus for human rabies and the only virus used to date in vaccines. Current vaccines may not protect against lyssaviruses other than those in phylogroup I (see section 2). The virus strains used for vaccines must be carefully selected, and the antigenic identity of the virus strains and the identity and purity of the cell lines used for production should be evaluated periodically. Comprehensive genetic characterization by full genome sequencing of vaccine virus strains is recommended.

General principles for nonclinical and clinical evaluation of inactivated rabies vaccines have been published by WHO (*4*). Preclinical testing is a prerequisite for the initiation of clinical trials in humans and includes immunogenicity studies (proof of concept) and safety testing in animals. Clinical development of rabies vaccines should include evaluation of their use for pre- and post-exposure prophylaxis, with various vaccination schedules and routes of administration, the onset, extent and duration of protection, and the requirement for and timing of booster vaccination. Clinical trials should adhere to the principles described in the WHO *Guidelines for good clinical practice* (*13*) and to those for the design, conduct and analysis of vaccine clinical trials, described in the WHO Guidelines for clinical evaluation of vaccines (*4*). All clinical trials should be approved by the relevant national regulatory authority.

## 6.4 Routes of vaccine administration

Current rabies vaccines are produced as individual doses for intramuscular injection. CCEEVs reconstituted with 0.5 or 1 ml of solvent in one intramuscular dose vial with a potency of ≥2.5 IU/dose can be used for both pre- and post-exposure prophylaxis.

The cost of cell culture-based vaccines for intramuscular administration limits, however, their widespread use in many areas where rabies is present. Intradermal administration of these vaccines is an equally safe and immunogenic alternative. Only one or two vials of vaccine are required to complete a full course of post-exposure prophylaxis by the intradermal route, thereby reducing the volume used and the direct cost of vaccine by 60–80% in comparison with standard intramuscular injection (*14–18*). There is no evidence that vaccines administered intradermally must be more potent than those recommended for intramuscular administration (*3,19,20*). Intradermal vaccination results in an equivalent immune response at a lower dose, thus sparing vaccine in pre- and post-exposure prophylaxis. Appropriate training should be given to ensure full intradermal instillation of the vaccine and to avoid accidental subcutaneous injection. An intradermal dose of 0.1 ml per site represents one fifth to one tenth of the intramuscular dose, depending on its volume after reconstitution. Although antibody titres are higher and more sustained after intramuscular injection, both routes induce rapid recall responses upon booster immunization. Intradermal vaccination is not recommended in immunocompromised individuals (*21,22*), as the underlying disease appears to impair transport of antigen-presenting dendritic cells to draining lymph nodes and thereby the magnitude of the antibody response.

Once opened, vials should be stored for no longer than 6 h, resulting in some wastage, particularly in centres where the number of patients injected daily is small. Nevertheless, intradermal administration remains cost-effective for both pre- and post-exposure prophylaxis (*23*).

Only two of the three WHO prequalified vaccines—purified Vero cell rabies vaccine and purified chick embryo cell vaccine—have been shown to be safe and effective when administered intradermally at a dose of 0.1 ml in a WHO-recommended pre- or post-exposure prophylaxis regimen.

Vaccine manufacturers should provide clinical evidence that new products are immunogenic, effective and safe when given intradermally. Administration should adhere to WHO guidance for that route and prior approval by the national health authorities. In particular, the vaccine should have been compared with a vaccine of known immunogenicity, efficacy and safety and should have undergone serological testing with the rapid fluorescent focus inhibition test, and the results should have been published in an international, peer-reviewed journal.

In countries where intradermal administration is an approved route for pre- or post-exposure prophylaxis, manufacturers of vaccines proven to be

safe and effective when given by this route should register their product for intradermal use and state in the product insert that their vaccine can be used intradermally.

## 6.5 Adverse events after active immunization

In general, CCEEVs are safe and well tolerated. Adverse events may occur, however, depending in part on the purity of the inactivated rabies virus, which may vary among lots (*24*). In 35–45% of vaccinated people, minor, transient erythema, pain or swelling occurs at the site of injection, particularly after intradermal administration of a booster. Mild systemic adverse events, such as transient fever, headache, dizziness and gastrointestinal symptoms, have been observed in 5–15% of vaccinated people. Serious adverse events are rare and include Guille-Barre syndrome and allergic reactions (*25*).

## 6.6 Duration of immunity

CCEEVs establish immunological memory that presumably persists for the life of the individual even after titres of neutralizing antibodies decline. Clinical data confirm that vaccinated people respond to booster immunization (*26–28*), even if the initial course of pre- or post-exposure prophylaxis was administered years previously and regardless of the route of priming or booster immunization (intramuscular or intradermal) and the presence or absence of detectable titres of rabies virus-specific antibodies at the time of the booster. In addition, published data indicate that periodic booster doses of vaccine are not required after primary rabies vaccination (*29,30*), except as an additional precaution for people whose occupation puts them at continual or frequent risk of exposure (see section 8.4). Nevertheless, all vaccinated individuals subsequently exposed to rabies, according to the WHO definition of exposure, should receive an abbreviated course of post-exposure prophylaxis, as specified in section 8.

## 6.7 Rabies vaccine and full post-exposure prophylaxis failures

Post-exposure prophylaxis failures, when a patient dies despite having received the correct protocol in a timely manner, are very rare among the estimated 20 million people who receive post-exposure prophylaxis each year. Although such cases are certainly underreported, only a few have been notified, all in developing countries and most involving deviations from the WHO-recommended prophylaxis protocol (*31,32*). Most deviations from the recommended protocol leading to death are: delay in seeking rabies prophylaxis; lack of or improper administration of rabies immunoglobulin (e.g. failure to inject all bite sites); lack of or improper primary wound care, and/or poor-quality rabies vaccine (*33*).

## 6.8 Rabies immunoglobulins

In order to protect people from developing rabies, those who were previously unvaccinated or incompletely vaccinated, in category III of exposure or severely immunocompromised (e.g. AIDS patients or transplant recipients) people with category II exposure should receive both an effective rabies vaccine and rabies immunoglobulin (*34*). Rabies immunoglobulins should preferably be administered into and around the wound site to neutralize the rabies virus still present (see section 8).

Three classes of biological product are available for passive immunization: human rabies immunoglobulin, equine rabies immunoglobulin and highly purified F(ab′)2 fragments produced from equine immunoglobulin (*35*). In this latter preparation the deletion of the Fc fragment might reduce the immunological functions of the antibody preparation, including its immunogenicity and thus the reactogenicity of the product.

The second international standard preparation of human immunoglobulin is held and distributed on request by the WHO International Laboratory for Biological Standards at the National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, United Kingdom (*12*). The current WHO reference serum for standardization contains 30 IU per ampoule.

Rabies immunoglobulin should be given with the first dose of vaccine into and around the wound site. Human immunoglobulin should be given at 20 IU/kg of body weight, while equine immunoglobulin has a shorter half-life in humans, and 40 IU/kg of body weight are required. Equine immunoglobulin is considerably less expensive than the human product, and most of the new equine preparations are potent, highly purified and safe, with few adverse events. Serum sickness can occur 1 week after administration of highly purified equine rabies immunoglobulin in <1–3% of recipients. The risk for anaphylactic reaction is low (1/150 000), and the reaction is generally treatable.

Skin tests are not recommended before administration of equine rabies immunoglobulin, as such tests poorly predict severe adverse events and should not be the basis for not giving equine immunoglobulin if it is needed. Equine immunoglobulin should be administered under conditions that would allow management of an anaphylactic reaction.

Rabies immunoglobulins are in short supply throughout the world. New technology may lead to use of monoclonal antibodies in post-exposure prophylaxis. WHO has recommended the use of monoclonal antibody 'cocktails' containing at least two antibodies against rabies virus, as alternatives for rabies immunoglobulins in post-exposure prophylaxis (*36*). A fully human monoclonal antibody cocktail is being evaluated for clinical safety and efficacy (*37*), and WHO is designing a humanized mouse monoclonal antibody cocktail for use in post-exposure prophylaxis for developing countries (*38*). WHO monoclonal

antibodies have been licensed to a number of development partners for commercialization, and one initiated a phase-I clinical evaluation of a cocktail in 2012. These products are therefore expected to become available in the near future.

## 6.9 References


1. WHO position paper on rabies vaccines. *Weekly Epidemiological Record*, 2010, 85:309–320.
2. *WHO Expert Committee on Biological Standardization. Fifty-sixth report*, Annex 2. Geneva, World Health Organization, 2007 (WHO Technical Report Series, No. 941).
3. *Human and dog rabies prevention and control: report of the WHO/Bill & Melinda Gates Foundation consultation, Annecy, France, 7–9 October 2009*. Geneva, World Health Organization, 2010 (WHO/HTM/NTD/NZD/2010.1) (http://whqlibdoc.who.int/hq/2010/WHO_HTM_NTD_NZD_2010.1_eng.pdf).
4. *WHO Expert Committee on Biological Standardization. Fifty-third report,* Annex 1. Geneva, World Health Organization, 2004 (WHO Technical Report Series, No. 924). (http://www.who.int/immunization_standards/vaccine_quality/pq_revision2010/en/index.html).
5. Seligmann EB Jr. Laboratory techniques in rabies: the NIH test for potency. *Monograph Series*. Geneva, World Health Organization, 1973, 23:279–286
6. Wilber LA, Aubert MFA. The NIH test for potency. In: Meslin FX, Kaplan MM, Koprowski H, eds. *Laboratory techniques in rabies*, 4th ed. Geneva, World Health Organization, 1996:360–368.
7. Kamphuis E et al. Potency testing of inactivated rabies vaccines using a serological method. *Developments in Biologics* (Basel), 2012, 134:23–27.
8. Nimmagadda SV et al. Recombinant diabody-based immunocapture enzyme-linked immunosorbent assay for quantification of rabies virus glycoprotein. *Clinical and Vaccine Immunology*, 2010, 17:1261–1268.
9. de Moura WC et al. Potency evaluation of rabies vaccine for human use: the impact of the reduction in the number of animals per dilution. *Journal of Virological Methods*, 2009, 158:84–92.

10. Wunderli PS et al. The rabies peripheral challenge test: more accurate determination of vaccine potency. *Vaccine*, 2006, 24:7115–7123.

11. Stokes W et al. Report on the international workshop on alternative methods for human and veterinary rabies vaccine testing: state of the science and planning the way forward. *Biologicals*, 2012, 40:369–381.

12. National Institute for Biological Standards and Control. *WHO international standard. Sixth international standard for rabies vaccine* (NIBSC code: 07/162. Instructions for use, Version 1.0, dated 10/11/2008) (http://www.nibsc.ac.uk/products/biological_reference_materials/product_catalogue/detail_page.aspx?catid=07/162).

13. *WHO Expert Committee on the Use of Essential Drugs. Sixth report*, Annex 3. Geneva, World Health Organization, 1995 (WHO Technical Report Series, No. 850).

14. Warrell MJ et al. Economical multiple-site intradermal immunisation with human diploid-cell-strain vaccine is effective for post-exposure rabies prophylaxis. *Lancet*, 1985, i:1059–1062.

15. *WHO Expert Committee on Rabies. Eighth report*. Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 824).

16. Briggs DJ et al. Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine. *Bulletin of the World Health Organization*, 2000, 78:693–698.

17. Quiambao BP et al. Reducing the cost of post-exposure rabies prophylaxis: efficacy of 0.1 ml PCEC rabies vaccine administered intradermally using the Thai Red Cross post-exposure regimen in patients severely exposed to laboratory-confirmed rabid animals. *Vaccine*, 2005, 23:1709–1714.

18. Ambrozaitis A et al. Rabies post-exposure prophylaxis vaccination with purified chick embryo cell vaccine (PCECV) and purified Vero cell rabies vaccine (PVRV) in a four-site intradermal schedule (4-0-2-0-1-1): an immunogenic, cost-effective and practical regimen. *Vaccine*, 2006, 24:4116–4121.

19. Beran J et al. Potency requirements of vaccines administered intradermally using the Thai Red Cross regimen: investigation of the immunogenicity of serially diluted purified chick embryo cell rabies vaccine. *Vaccine*, 2005, 23:3902–3907.

20. Sudarshan MK et al. Assessing the relationship between antigenicity and immunogenicity of human rabies vaccines. Results of a meta-analysis. *Human Vaccines*, 2005, 1:187–190.

21. Kopel E et al. Inadequate antibody response to rabies vaccine in immunocompromised patient. *Emerging Infectious Diseases*, 2012, 18:1493–1495.

22. Tantawichien T et al. Failure of multiple-site intradermal postexposure rabies vaccination in patients with human immunodeficiency virus with low CD4+ T lymphocyte counts. *Clinical and Infectious Diseases*, 2001, 33:E122–E124.

23. Hampson K, Cleaveland S, Briggs D. Evaluation of cost-effective strategies for rabies post-exposure vaccination in low-income countries. *PLoS Neglected Tropical Diseases*, 2011, 5:e982.

24. Finke S et al. Assessment of inactivated human rabies vaccines: biochemical characterization and genetic identification of virus strains. *Vaccine*, 2012, 30:3603–3609.

25. *Grading of scientific evidence. Table III. Safety of cell-culture-based rabies vaccines*. Geneva, World Health Organization, 2010 (http://www.who.int/entity/immunization/rabies_grad_safety.pdf).

26. Suwansrinon K et al. Survival of neutralizing antibody in previously rabies vaccinated subjects: a prospective study showing long lasting immunity. *Vaccine*, 2006, 24:3878–3880.

27. Brown D et al. Intradermal pre-exposure rabies vaccine elicits long lasting immunity. *Vaccine*, 2008, 26:3909–3912.

28. Naraporn N et al. Immune response to rabies booster vaccination in subjects who had postexposure treatment more than 5 years previously. *Journal of Travel Medicine*, 1999, 6:134–136.

29. Strady A et al. Antibody persistence following preexposure regimens of cell-culture rabies vaccines: 10-year follow-up and proposal for a new booster policy. *Journal of Infectious Diseases*, 1998, 177:1290–1295.

30. *The immunological basis for immunization series, module 17: Rabies*. Geneva, World Health Organization, 2011.

31. Wilde H et al. Failure of postexposure treatment of rabies in children. *Clinical and Infectious Diseases*, 1996, 22:228–232.

32. Wilde, H. Failures of post-exposure rabies prophylaxis. *Vaccine*, 2007, 25:7605–7609.

33. Rupprecht CE et al. Evidence for a 4-dose vaccine schedule for human rabies post-exposure prophylaxis in previously non-vaccinated individuals. *Vaccine*, 2009, 27:7141–7148.

34. *Guide for post-exposure prophylaxis*. Geneva, World Health Organization, 2012 (http://www.who.int/rabies/human/postexp/en/).

35. Lang J et al. Evaluation of the safety, immunogenicity, and pharmacokinetic profile of a new, highly purified, heat-treated equine rabies immunoglobulin, administered either alone or in association with a purified, Vero-cell rabies vaccine. *Acta Tropica*, 1998, 70(3):317–333.

36. *Consultation on a rabies monoclonal antibody cocktail for rabies post-exposure treatment. Geneva, 23–24 May 2002*. Geneva, World Health Organization (available at www.who.int/rabies/vaccine/en/mabs_final_report.pdf; accessed March 2013).

37. Bakker AB et al. First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. *Vaccine,* 2008, 26(47):5922–5927.

38. Müller T et al. Development of a mouse monoclonal antibody cocktail for post-exposure rabies prophylaxis in humans. *PLoS Neglected Tropical Diseases*, 2009, 3(11):e542. Erratum in: PLoS Neglected Tropical Diseases, 2009,3(11):10.1371/annotation/df98339d-6bdb-40ed-af83-cc38b249264a.

## 7. Vaccines for animals

Veterinary vaccines have been developed for use against rabies in domestic mammals and wildlife. These vaccines are either inactivated (killed), modified-live or biotechnology-derived products. Whatever the method for vaccine production, the quality of the source material and standards (e.g. virus master seed, specific pathogen-free eggs, cell seed) should be clearly documented, particularly with regard to sterility and safety. Rabies vaccines for animals should be approved by the competent state authorities and comply with national requirements for vaccines. When there are no adequate national regulations for veterinary biologicals (pre- and post-marketing requirements) with regard to potency, sterility, safety and efficacy, reference should be made to the relevant international standards (1–8). Vaccine strains should be genetically characterized, preferably by full genome sequencing.

## 7.1 Vaccine types

Vaccines should be administered by or under the supervision of a competent person, such as a veterinarian, according to the producer's recommendations for e.g. minimum age, route of administration (oral, intramuscular or subcutaneous), duration of immunity and time between doses. Scientific evidence and the absence of contraindications may, however, allow adaptation of the vaccination schedules recommended by the producer, such as parenteral vaccination of animals younger than 3 months during mass programmes, in order to optimize herd immunity (see section 9).

### 7.1.1 Vaccines for domestic animals

***Injectable modified-live virus vaccines***

Modified-live vaccines are produced from a modified egg-adapted strain of virus (e.g. Flury strain) serially passaged in embryonated chicken eggs. They can also be produced from strains adapted to cell culture (e.g. SAD/ERA). These vaccines are no longer considered safe because of their inherent ability to cause rabies, and their use in domestic animals should be discontinued.

***Injectable inactivated vaccines (monovalent or in combination)***

The vaccines most commonly used in domestic animal species are inactivated (killed) injectable vaccines, which are safe and inexpensive. The safety, potency and purity of such vaccines should be assessed by validated methods before use. Inactivated vaccines are produced in cell culture with either primary cells or continuous cell lines infected with an adapted strain of rabies virus. Various methods of inactivation are used, beta propiolactone or ultraviolet light being the most frequent. An adjuvant is recommended; one of the commonest is aluminium hydroxide. Inactivated rabies vaccines are available in either liquid or lyophilized form.

Inactivated rabies vaccines can be used in combination with bacterins (e.g. Leptospira) and other viral antigens (polyvalent), such as canine distemper virus, canine adenovirus type 2 and canine parvovirus. Combined vaccines currently available for cats include various antigens, such as feline panleukopenia virus, feline calicivirus and feline herpesvirus. A combined rabies and foot-and-mouth disease vaccine is available for use in cattle, sheep and goats. An inactivated rabies vaccine combined with bacterin against Potomac fever (caused by Ehrlichia risticii) is available for horses.

***Injectable live recombinant vectored vaccine (monovalent or in combination)***

A canarypox virus expressing the rabies virus glycoprotein has been licensed in the USA as a parenteral vaccine for cats. The commercially available vaccine

combines the rabies–canarypox with feline panleukopenia, feline calicivirosis and feline herpesvirus components.

#### *Live replication-competent vaccines for oral use*

The parenteral route is preferred for dog vaccination, although oral vaccination may be appropriate under specific conditions. The oral route should be used as a complement to parenteral mass vaccination campaigns, to improve vaccination coverage of the dog population by targeting individuals that are inaccessible for injectable vaccines (see section 9). The liquid vaccine usually contained in a sachet or blister pack should be incorporated in a bait, the taste, size, texture of which should be adapted to dogs. To maximize its use and to prevent the occurrence of vaccine-related untoward events in humans, WHO has established requirements for the safety and efficacy of candidate oral vaccines and for the design, testing and distribution of dog baits (*9,10*). Only vaccines with the lowest known residual pathogenicity should be used in dogs. To date, one attenuated vaccine (*11*) and one recombinant vaccine (*12*) have met the WHO minimum recommendations (*10*).

Only one oral vaccine for dogs has been licensed so far. Use of oral vaccines in domestic species should be evaluated case by case, on the basis of preliminary knowledge of the structure (owned, ownerless) and accessibility of the dog population to interventions (*13–15*). As oral rabies vaccines are costly and safe distribution tends to be time-consuming, the cost–benefit ratio of administering these vaccines to dogs should be carefully assessed.

## 7.2 Potency requirements for animal rabies vaccines

### 7.2.1 Inactivated animal rabies vaccines

The rabies vaccines used to immunize wild animals are usually live vaccines delivered by the oral route. The liquid vaccine usually contained in a sachet or blister pack should be incorporated in a bait preferably adapted to the target species with regard to taste, size and texture. Inactivated vaccines licensed for domestic animals can also be used for wildlife in trap–vaccinate–release programmes

#### *Modified-live virus vaccines*

All attenuated vaccines currently used are derived from the original ERA/SAD (Street Alabama Dufferin) strain, with various levels of attenuation after passage in cell cultures. Several vaccines are attenuated by serial in-vitro selection on cloned baby hamster kidney cells or by passaging in mice in vivo. One vaccine was developed by using rabies virus glycoprotein monoclonal antibodies to select an attenuated virus carrying two mutations in position 333 (*11,16*). Use of rabies virus strains that can cause rabies in wildlife species is not recommended.

***Live recombinant vaccines***

Several recombinant vaccines have been developed: a recombinant vaccinia virus and, more recently, a human adenovirus vector, both expressing the glycoprotein gene of rabies virus (*17,18*). Recombinant vaccines based on rabies virus with site-directed mutagenesis (reverse genetics) are also available. Some of these constructs have been approved by competent national authorities

### 7.2.2 Animal rabies vaccines for oral vaccination

A batch-release titre is established before marketing release, which represents the lowest titre of vaccine that can protect 100% of the target experimental animals against a virulent rabies challenge. The batch-release titre in the vaccine bait should correspond to at least 10 times the minimum 100% protective dose found during the challenge test (*3,21*).

National control laboratories or government institutions involved in licensing vaccines or evaluating oral rabies vaccination programmes may verify the viral titre of all batches of vaccine bait before and during a campaign (*7,21,22*). Such tests should be conducted in qualified laboratories with documented, validated methods and appropriate standards.

## 7.3 Safety of animal vaccines

### 7.3.1 Vaccines for parenteral use

An effective pharmocovigilance system should be in place to detect vaccine-associated problems during post-marketing authorization (field use). Safety tests should be conducted by intracerebral inoculation of mice or, preferably, if validated, in cell culture (*2,5,7,19*).

### 7.3.2 Vaccines for oral use

The safety of vaccines is assessed in target and non-target species, i.e. relevant wild rodents and other wild and domestic species that live in the area and may consume baits, as well as in non-human primates (*10*). The vaccine should not induce any adverse signs in either target or non-target species.

Some modified-live rabies virus oral vaccines used in the field for wildlife may have residual pathogenicity, depending on the level of attenuation of the viral strain. Therefore, any rabies virus isolated from animals in the area of vaccination should be characterized with monoclonal antibodies or molecular techniques to ensure that no vaccine-induced rabies has occurred.

In the event of accidental human exposure to attenuated rabies virus vaccines, medical attention should be sought and post-exposure prophylaxis considered. The potential risk to animals, humans and the environment of recombinant vaccines, such as those containing live pox or adenovirus vectors,

should be assessed, and methods for mitigation or treatment, particularly in humans, should be identified early in research and development (*10,23*).

When oral vaccines are used to vaccinate dogs, the risk for vaccine virus transmission among target and non-target species (including humans) should be assessed by testing for rabies virus in saliva and faecal samples from dogs up to 7 days after administration. No viable virus should be detectable after vaccination, as it would suggest replication and excretion. Any virus that is recovered should be characterized with molecular techniques or monoclonal antibodies.

## 7.4 Parenteral rabies vaccination

Specific conditions may apply to the use of veterinary vaccines in mass vaccination programmes to control dog rabies. The implementation and monitoring of mass vaccination campaigns for dogs are described in section 9.

To preserve the immunological properties of rabies vaccines, the manufacturers' recommendations for storage should be respected. Particularly, prolonged breaks in the cold chain, exposure to sunlight and temperature fluctuations should be avoided. Opened vials should be used within 2–3 days (inactivated vaccines), provided sterile techniques are used to withdraw vaccine from multidose vials.

If possible, dogs should also be vaccinated against other diseases, dewormed, spayed or neutered, to improve their health. Such a 'visible' effect might prompt people to bring their dogs for booster vaccinations in the future.

A peak in rabies virus neutralizing antibodies is generally reached 4–6 weeks after initial antigenic stimulation. Thereafter, the levels of antibodies decrease rapidly and may be below the threshold of detection as soon as several weeks after vaccination. In dogs vaccinated several times, including those vaccinated twice 12 months apart, antibodies titres are generally higher, irrespective of the date of the serum test (*24*). Depending on the competent national authorities, the duration of efficacy of inactivated vaccines is considered to be 1–3 years.

## 7.5 References

1. *WHO Expert Committee on Rabies. Eighth report*. Geneva, World Health Organization, 1992 (WHO Technical Report Series, No. 824).
2. Meslin FX, Kaplan MM, Koprowski H, eds. *Laboratory techniques in rabies,* 4th ed. Geneva, World Health Organization, 1996.
3. *WHO Expert Consultation on Rabies. First report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 931).

4. *Guidelines on nonclinical evaluation of vaccines,* Annex 1. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 927).

5. Rabies. In: *Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees),* 7th ed. Paris, World Organisation for Animal Health, 2012:263–282.

6. Principles of veterinary vaccine production. In: *Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees),* 7th ed. Paris, World Organisation for Animal Health, 2012:52–63.

7. *Code of Federal Regulations, Title 9, Parts 1–199 (1-1-12 edition).* Washington DC, Government Printing Office, United States Department of Agriculture–Animal and Plant Health Inspection Service, 2012.

8. Brown CM et al. Compendium of animal rabies prevention and control, 2011. *Journal of the American Veterinary Medicine Association*, 2011, 239(5):609–617.

9. Matter HC. *Suggestion for the development of a research project for the field evaluation of several vaccine-bait delivery techniques to vaccinate dogs orally against rabies*. Geneva, World Health Organization, 1993 (WHO/Rab.Res./93.40).

10. *Guidance for research on oral rabies vaccines and field application of oral vaccination of dogs against rabies*. Geneva, World Health Organization, 2007.

11. Cliquet F et al. The safety and efficacy of the oral rabies vaccine SAG2 in Indian stray dogs. *Vaccine*, 2007, 25:3409–3418.

12. Blancou J et al. Innocuité et efficacité d'un vaccine antirabique recombinant vaccine virus rabique administré par voie orale au renard, chien et chat [Safety and efficacy of an antirabies vaccine consisting of recombinant vaccinia-rabies virus administered orally to the fox, dog and cat.] *Annales de Recherche Vétérinaire*, 1989, 20(2):195–204.

13. *Field application of oral rabies vaccines for dogs: report of a WHO consultation organized with the participation of the Office International des Epizooties*, Geneva, Switzerland, 20–22 July 1998. Geneva, World Health Organization, 1998 (WHO/EMC/ZDI/98.15).

14. Matter HC, Fico R. *Accessibility of dogs to oral and parenteral vaccination against rabies in Tunisia and Turkey.* Geneva, World Health Organization, 1992 (WHO/Rabies/93.206).

15. Matter H et al. Field evaluation of two bait delivery systems for the oral immunization of dogs against rabies in Tunisia. *Vaccine*, 1998, 16(7): 657–665.

16. Cliquet F et al. Eliminating rabies in Estonia. *PLoS Neglected Tropical Diseases*, 2012, 6(2):1–17.

17. Rosatte RC et al. Prevalence of tetracycline and rabies virus antibody in raccoons, skunks and red foxes following aerial distribution of V-RG baits to control raccoon rabies in Ontario Canada. *Journal of Wildlife Diseases*, 2008, 44:946–964.

18. Yarosh OK et al. Human adenovirus type 5 vectors expressing rabies glycoprotein. *Vaccine*, 1996, 14:1257–1264.

19. Rabies vaccines (inactivated) for veterinary use. In: *European Pharmacopoeia*. Strasbourg, Council of Europe, European Directorate for the Quality of Medicines and Health Care, 2010:734–736.

20. Servat A et al. In vivo potency tests of rabies vaccines for veterinary use. A 2-year retrospective analysis of data according to the criteria of the European Pharmacopoeia. *Pharmeuropa*, 2008, 20(4):655–664.

21. *The oral vaccination of foxes against rabies. Report of the Scientific Committee on Animal Health and Animal Welfare*. Luxembourg, European Commission, 2002.

22. Rabies vaccines (live, oral) for foxes. In: *European Pharmacopoeia*. Strasbourg, Council of Europe, European Directorate for the Quality of Medicines and Health Care, 2008:736–743.

23. Human vaccinia infection after contact with a raccoon rabies vaccine bait—Pennsylvania, 2009. *Morbidity and Mortality Weekly Report*, 2009, 58:1204.

24. Cliquet F et al. Neutralising antibody titration in 25,000 sera of dogs and cats vaccinated against rabies in France, in the framework of the new regulations that offer an alternative to quarantine. *Revue Scientifique et Technique (International Office of Epizootics)*, 2003, 22(3):857–866.

# 8. Prevention of human rabies

Rabies is almost always fatal. Thus, it is important to prevent it by immunization before and after suspect or proven exposure to the virus. The rabies vaccines and immunoglobulins used for prophylaxis should comply with WHO recommendations for production and control and for immunogenicity and safety for use by both the intramuscular and the intradermal route (see section 6).

## 8.1 General considerations

After suspected or proven exposure to rabies virus, prompt use of CCEEVs with proper wound management and simultaneous administration of rabies immunoglobulin is almost invariably effective in preventing rabies, even after severe exposure. Assessment of potential exposure can be complex and confusing. When in doubt, post-exposure prophylaxis should be initiated, and the attending physician should consult an infectious disease specialist with expert knowledge of rabies.

Pre-exposure prophylaxis for people who are at risk of exposure to lyssaviruses because of their job, residence or travel is strongly recommended.

Vaccines can be administered intramuscularly or intradermally at certain sites. For intramuscular administration, the vaccine should be injected into the deltoid muscle for adults and children aged ≥2 years; for children aged <2 years, the anterolateral thigh is recommended. Rabies vaccine should not be administered in the gluteal area, as induction of an adequate immune response is less reliable. For intradermal administration, the recommended sites include the deltoids, lateral thighs or suprascapular areas (see *Annex 4*). The site is selected on the basis of the level of privacy that can be provided and sociocultural acceptance. Devices are available to facilitate intradermal injection.

## 8.2 Pre-exposure prophylaxis

Pre-exposure prophylaxis is recommended for anyone who is at continual, frequent or increased risk for exposure to the rabies virus, as a result of their residence or occupation, such as laboratory workers dealing with rabies virus and other lyssaviruses, veterinarians and animal handlers. Travellers in high-risk areas should be vaccinated after a risk assessment. Children living in or visiting rabies-affected areas are at particular risk and should be given pre-exposure prophylaxis on an individual basis or in mass campaigns when there are no economic, programmatic or logistical obstacles (see also section 8.8).

As far as possible, the vaccination series listed below must be completed in the stipulated time; however, there is no need to restart the series if the doses are not given on the exact schedule (*1*).

*Intramuscular administration*: One intramuscular dose is given on each of days 0, 7 and 21 or 28. Day 0 is the date of administration of the first dose of vaccine.

*Intradermal administration*: One intradermal injection of 0.1 ml is given on each of days 0, 7 and 21 or 28. To maximize savings, sessions of intradermal pre-exposure prophylaxis should involve enough individuals to use all opened vials within 6 h.

## 8.3 Post-exposure prophylaxis

Post-exposure prophylaxis consists of:

- local treatment of the wound as soon as possible after exposure,
- a course of potent, effective rabies vaccine that meets WHO recommendations and
- administration of rabies immunoglobulin, if indicated.

Factors that should be taken into consideration in deciding to initiate post-exposure prophylaxis include the epidemiological likelihood that the implicated animal was rabid, the severity of exposure (see section 8.3.2), the clinical features of the animal, its vaccination status (particularly for dogs and cats) and its availability for observation and laboratory testing. All exposures determined to represent a risk for rabies require post-exposure prophylaxis.

Prophylaxis should be instituted immediately. If possible, the suspect animal should be identified, quarantined for observation (for healthy dogs and cats) or euthanized for laboratory examination. Prophylaxis should be continued while awaiting laboratory results or during the observation period. If the laboratory tests are positive, an immediate retrospective risk assessment should be conducted to identify all people who may have been exposed, and they should be given post-exposure prophylaxis.

Prophylaxis should be completed if the suspect animal is not available for testing or observation but may be discontinued if the animal is proved by appropriate laboratory examination to be free of rabies. When the domestic dog, cat or ferret at the origin of human exposure is healthy, properly vaccinated (at least two documented vaccinations with a potent vaccine) and easily accessible for observation for 10 days, proper wound management should be ensured and

booster vaccination can be deferred, especially if the patient had received pre-exposure prophylaxis or previous post-exposure prophylaxis in the past 3 months (*2*).

All bite victims and other people with suspect animal contacts presenting at a health care facility should immediately be reported to a veterinary expert to conduct an investigation of the animal and ensure laboratory examination if it is suspected of having rabies or to monitor the animal's state of health if it is under observation.

When animal bites, scratches and other contacts (excluding contacts with bats) occur in an area free of carnivore rabies and where there is adequate rabies surveillance, post-exposure prophylaxis may not be required. The decision should be based on a risk assessment conducted by a medical expert knowledgeable in the local epidemiology of rabies.

In areas where canine and/or wildlife rabies is enzootic, prophylaxis should be instituted immediately after a suspected exposure, unless adequate laboratory surveillance is in place and data from laboratory and field sources indicate that the species involved is not a vector of rabies; for example, bites by rodents, rabbits and hares do not routinely require post-exposure prophylaxis.

The recommendations given here are a general guide; they might be modified in certain situations, such as when a reliable exposure history cannot be obtained (e.g. from infants or mentally challenged people). This is particularly true in areas where rabies is enzootic and follow-up observation of the biting animal and/or laboratory testing are not readily available. A careful risk assessment should ideally be conducted by a qualified medical professional on every patient exposed to an animal suspected of being rabid.

### 8.3.1 Local treatment of wounds

Prompt local treatment of all bite wounds and scratches is an important step in post-exposure prophylaxis. The recommended first-aid procedures include immediate, thorough flushing and washing of the wound with soap and water, detergent, povidone iodine or other substances with virucidal activity. If soap or a virucidal agent is not available, the wound should be thoroughly and extensively washed with water. People who live in areas endemic for rabies should be taught simple local wound treatment and warned not to use procedures that may further contaminate or enlarge the wound.

A bleeding wound at any site indicates potentially severe exposure and must be infiltrated with either human or equine rabies immunoglobulin. Most severe

bite wounds are best treated by daily dressing, followed by secondary suturing when necessary. If suturing after wound cleansing cannot be avoided, the wound should first be infiltrated with human or equine rabies immunoglobulin and suturing delayed for several hours to allow diffusion of the immunoglobulin through the tissues before minimal sutures are applied. Secondary sutures are less likely to become infected and present better cosmetic results if carried out under optimal conditions. An infected bite wound is no contraindication to injection of rabies immunoglobulin (*3*). Bites on the finger or toe tip, ear lobe or nasal area can be safely injected with rabies immunoglobulin, provided excessive pressure is not applied, as this can cause compression syndromes (*4*). Other treatments, such as administration of antibiotics and tetanus prophylaxis, should be applied as appropriate for potentially contaminated wounds.

### 8.3.2 Categories of exposure and post-exposure prophylaxis (*Annex 5*)

In countries or areas enzootic for rabies, exposure to suspected or confirmed rabid (domestic or wild) animals is categorized as follows:

- **category I**: touching or feeding animals, licks on intact skin, contact of intact skin with secretions or excretions of a rabid animal or human. These are not regarded as exposures, and no post-exposure prophylaxis is required.
- **category II**: nibbling of uncovered skin, minor scratches or abrasions without bleeding. Vaccine should be injected as soon as possible.
- **category III**: single or multiple transdermal bites or scratches, licks on broken skin, contamination of mucous membrane with saliva from licks and exposure to bats. Vaccine and rabies immunoglobulin should be administered at distant sites as soon as possible. Immunoglobulin can be administered up to day 7 after injection of the first dose of vaccine.

For categories II and III, thorough local wound treatment (see 8.3.1) is of paramount importance. Post-exposure prophylaxis, including rabies immunoglobulin, should always be administered when category III exposure is recognized, even months or years after contact.

When it is not possible to complete post-exposure prophylaxis with the same cell culture-based or embryonated egg-based vaccine, a rabies cell culture-based vaccine that fulfils WHO requirements should be used. Thus should, however, be an exception.

### 8.3.3 WHO-recommended post-exposure prophylaxis regimens

Day 0 is the date of administration of the first dose of vaccine. It is important to complete the initial three doses within 1 week.

***Intramuscular administration***

The recommended regimen consists of either a five-dose (1-1-1-1-1) or a four-dose schedule (2-0-1-0-1 or 2-1-1):

- The five-dose 'Essen' regimen (1-1-1-1-1) consists of one dose administered on each of days 0, 3, 7, 14 and 28. A reduced, four-dose vaccine schedule (1-1-1-1-0) for healthy people is supported by the peer-reviewed literature, unpublished data, epidemiological reviews and expert opinion. This shortened Essen regimen, consisting of one dose on each of days 0, 3, 7 and 14, may be used as an alternative for healthy, fully immune competent, exposed people provided they receive wound care plus rabies immunoglobulin in category III as well as in category II exposures and a WHO-prequalified rabies vaccine (*5*).
- The four-dose 'Zagreb' regimen (2-0-1-0-1 or 2-1-1) consists of two doses of vaccine injected on day 0 (one into each of the two deltoid or thigh sites) followed by one dose on each of days 7 and 21.

***Intradermal administration***

The updated two-site Thai Red Cross regimen (2–2–2–0–2) consists of injections of 0.1 ml of vaccine at two different intradermal sites on each of days 0, 3, 7 and 28 (*6*). This regimen can be used for people with category II or III exposure in countries in which the intradermal route has been endorsed by the national health authorities.

### 8.3.4 Short post-exposure prophylaxis for previously vaccinated individuals

Exposed or re-exposed patients who can document previous complete pre-exposure prophylaxis or complete post-exposure prophylaxis with rabies CCEEVs should receive:

- one dose of vaccine intramuscularly or intradermally at one site on both days 0 and 3. Rabies immunoglobulin is not indicated in such cases. This regimen can also be given to people vaccinated against rabies who have detectable rabies virus neutralizing antibody.
- As an alternative to this regimen, the patient may be offered a 'one visit four-site' intradermal regimen consisting of four injections of 0.1 ml equally distributed over the left and right deltoids, thigh or suprascapular areas at a single visit (*7,8*).

For people who have received complete pre- or post-exposure prophylaxis within a maximum delay of 3 months before exposure or re-exposure to a bite or other contact, proper wound management should be ensured, and booster vaccination can be safely deferred, if the biting dog or cat is healthy, vaccinated and available for an observation period of 10 days (*2*).

People with category III exposure who have received complete pre- or post-exposure prophylaxis with a vaccine of unproven potency, including nerve tissue vaccines, or an incomplete course of pre- or post-exposure prophylaxis should receive a full post-exposure vaccination course, including rabies immunoglobulin.

## 8.4 Requirements for periodic booster injections

Periodic booster doses of rabies vaccine are not necessary for people living in or travelling to high-risk areas who have received a complete primary series of pre- or post-exposure prophylaxis with rabies CCEEVs. Only people whose occupation puts them at continual or frequent risk of exposure should receive periodic booster injections as an extra precaution in the absence of recognized exposure. If available, monitoring of rabies virus neutralizing antibody in personnel at risk is preferred to routine boosters. For people potentially at high risk for laboratory exposure to high concentrations of live rabies virus, neutralizing antibody titration should be done every 6 months. If the titre falls below 0.5 IU/ml of serum, one booster dose of vaccine should be given intramuscularly or intradermally.

Professionals who are not at continual risk of exposure, such as certain categories of veterinarians and animal health officers, should undergo serological monitoring every 2 years. As vaccine-induced immunological memory persists in most cases for years, a booster is recommended only if the rabies virus neutralizing antibody titre has dropped below 0.5 IU/ml.

## 8.5 Vaccination of immunocompromised individuals

Several studies of patients with HIV/AIDS have shown that those with very low CD4 counts mount a significantly lower or no detectable neutralizing antibody response to rabies virus. In these patients and others in whom the presence of immunological memory is no longer assured, proper, thorough wound treatment and antisepsis accompanied by local infiltration of human or equine rabies immunoglobulin and a complete series of five intramuscular doses of rabies CCEEV is required for category II and III exposures. When feasible, the rabies-virus neutralizing antibody response should be determined 2–4 weeks after vaccination to assess whether an additional dose of vaccine is required. When in doubt, consult an infectious disease specialist with expert knowledge of HIV/AIDS and rabies prevention.

## 8.6 Rabies immunoglobulin for passive immunization

The role of rabies immunoglobulin in passive immunization is to provide neutralizing antibodies at the site of exposure before patients can begin producing their own antibodies physiologically after vaccination. Therefore, rabies immunoglobulin should be administered to all patients presenting with category III exposure.

Rabies immunoglobulin is administered only once, preferably at or as soon as possible after initiation of post-exposure vaccination. It is not indicated beyond the seventh day after the first dose of rabies vaccine, regardless of whether the day 3 and day 7 doses were received, because an active antibody response to the CCEEV has already started, and there may be interference between active and passive immunization. The dose of human rabies immunoglobulin is 20 IU/kg of body weight, while that of equine immunoglobulin and F(ab')2 products is 40 IU/kg of body weight. All the immunoglobulin, or as much as anatomically possible (but avoiding possible compartment syndrome), should be administered carefully into or around the wound site or sites. The remaining product, if any, should be injected intramuscularly at a site distant from the site of vaccine administration.

Use of the same syringe or mixing rabies vaccine and rabies immunoglobulin must be avoided. For severe and multiple wounds, which require more immunoglobulin than the calculated dose, the product may be diluted with sterile normal saline to a volume sufficient for effective, safe infiltration of all wounds (*8*). Post-exposure prophylaxis, including rabies immunoglobulin for category III exposure, should be administered after exposure is recognized, even months or years later.

## 8.7 Contraindications and precautions

As rabies is fatal, there are no contraindications to post-exposure prophylaxis, and it should be given as indicated by the nature of the exposure in a setting in which staff are adequately trained in its administration and in the management of possible adverse reactions. There are no contraindications for post-exposure prophylaxis in infants, pregnant women or immunocompromised individuals, including children with HIV/AIDS.

People taking chloroquine for malaria treatment or prophylaxis may have a reduced response to intradermal rabies vaccination and should receive the vaccine intramuscularly. As with all vaccinations, recipients should be kept under medical supervision for at least 15–20 min after vaccination. A previous severe reaction to any component of a vaccine (except rabies immunoglobulin) is a contraindication to use of the same vaccine for pre- or post-exposure prophylaxis.

## 8.8 Travellers to and residents of rabies-affected countries and areas, and indications for pre-exposure prophylaxis

Travellers to and residents of rabies-affected countries and areas should avoid contact with free-roaming animals, especially dogs and cats, and with wild, free-ranging or captive animals. For people who participate in spelunking, casual exposure to cave air is not a concern, but cavers should be warned not to handle bats. Contact with bats should be followed by post-exposure prophylaxis.

The map in *Figure 1* shows four categories of countries or areas, from those at no risk to those at low, moderate and high risk. The categorization is based on the major animal host/transmitter and lyssavirus species involved, and the availability of reliable, laboratory-based surveillance data on these reservoir species. Access to proper medical care and the availability of CCEEVs and other rabies biological products were also taken into consideration.

- **category 1**, no risk: lyssavirus risk-free countries or areas.
- **category 2**, low risk: countries or areas with either only rabies-related lyssaviruses circulating in bats or rabies virus circulating in bats (non haematophagous) and other wildlife.

In both groups of countries or areas, proper medical care, CCEEVs and other rabies biological products are easily accessible, and reliable laboratory-based surveillance data are available.

- **category 3**, moderate risk: countries or areas where rabies virus circulates in bats (non haematophagous) and other wildlife.
- **category 4**, high risk: countries or areas with sustained dog-to-dog transmission of the rabies virus and/or where vampire bat rabies is reported (*9*).

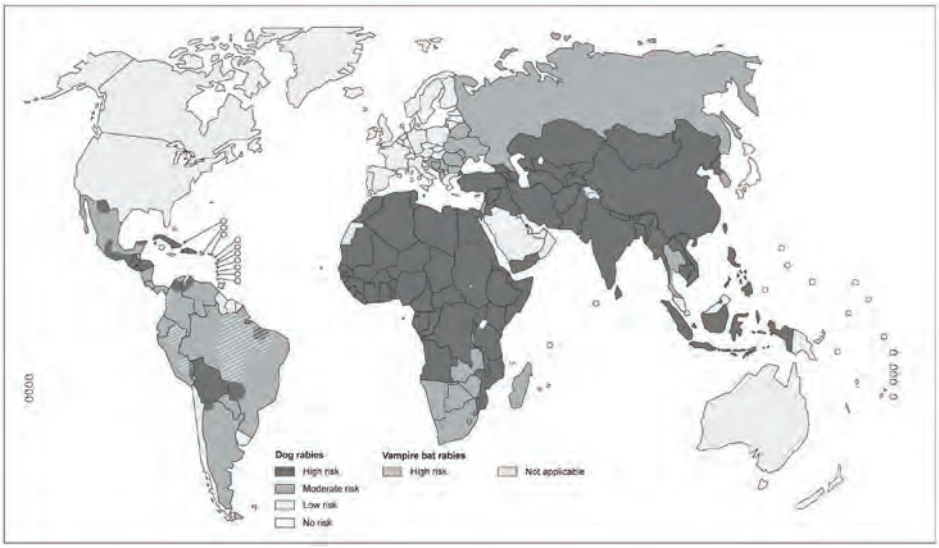
*Advice to travellers and residents according to level of risk:*

*No risk*: No need for pre-exposure prophylaxis.

*Low risk and moderate risk*: People involved in any activities that might bring them into direct contact with non haematophagous bats and other wild animals, especially carnivores (for example, wildlife professionals, researchers, veterinarians and adventure travellers visiting areas where bats and other wildlife are commonly found) should receive pre-exposure prophylaxis.

*High risk*: People travelling to rural areas or involved in activities such as running, bicycling, camping or hiking should receive pre-exposure prophylaxis. Prophylaxis is also recommended for people with significant occupational risks, such as veterinarians, and residents of areas with a significant risk for exposure to domestic animals, particularly dogs and cats as well as wildlife including vampire

Figure 1
**Four categories of countries or areas, from those at no risk to those at low, moderate and high risk**




bats. Children should be preventively immunized as they are at higher risk.

When potentially exposed in a low, moderate or high risk country or area people who have received pre-exposure prophylaxis should receive booster vaccination (see section 8.3.4) and people who have not been previously vaccinated should consult a physician and if indicated receive post-exposure prophylaxis within the shortest possible delay (see section 8.3.3).

Suggested certificates of pre- and post-exposure vaccination against rabies are shown in *Annex 6*.

## 8.9 References

1. *Recommendations for routine immunization. Summary tables*. Geneva, World Health Organization, 2012 (www.who.int/immunization/policy/immunization_tables/en; accessed March 2013).
2. Sudarshan MK, Ravish HS, Ashwath Narayana DH. Time interval for booster vaccination following re-exposure to rabies in previously vaccinated persons. *Asian Biomedicine*, 2011, 5(5):589–593.
3. Wilde H et al. Is injection of contaminated animal bite wounds with rabies immune globulin a safe practice? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1992, 86:86–88.

4. Suwansrinon K et al. Is injecting a finger with rabies immunoglobulin dangerous? *American Journal of Tropical Medicine and Hygiene*, 2006, 75:363–364.

5. Rupprecht CE et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies, recommendations of the Advisory Committee on Immunization Practices. *Morbidity and Mortality Weekly Report*, 2010, 59(RR02):1–9.

6. Madhusudana SN et al. Comparison of safety and immunogenicity of purified chick embryo cell rabies vaccine (PCECV) and purified Vero cell rabies vaccine (PVRV) using the Thai Red Cross intradermal regimen at a dose of 0.1 ml. *Human Vaccines,* 2006, 2(5):200–204.

7. Rabies vaccines: WHO position paper. *Weekly Epidemiological Record*, 2010, 85:309–320.

8. *Human and dog rabies prevention and control, report of the WHO/Bill & Melinda Gates Foundation Consultation, 2009, Annecy, France*. Geneva, World Health Organization, 2010 (WHO/HTM/NTD/NZD 2010.1).

9. Schneider MC. et al Rabies transmitted by Vampire bats to humans: an emerging zoonosis in Latin America? *Pan American Journal of Public Health*, 2009, 25(3):260–269.

## 9. National programmes for dog rabies control

Canine rabies can be eliminated, as demonstrated in North America, western Europe, Japan and many areas of South America and parts of Asia. It is, however, still widespread, occurring in over 80 countries and territories, predominantly in the developing world. In more than 99% of all cases of human rabies, the virus is transmitted via dogs; half the global human population lives in canine rabies-endemic areas and is considered at risk for contracting rabies. Controlling and eventually eliminating the disease in dogs would have major benefits for human health by prevention at source of most human deaths from this cause.

Animal vaccines that provide a considerable duration of immunity are commercially available, and mass parenteral vaccination programmes are the mainstay of canine rabies control. In recent years, rabies control and elimination programmes through mass vaccination of dogs have resulted in marked reductions or elimination of human rabies cases (*1–4*). In a few instances, rabies vaccination coupled with sterilization of dogs has resulted in local elimination of cases (5) or has been predicted to lead to elimination of human rabies (*6*). The contribution of sterilization, over and above vaccination alone, to the control of dog rabies has not been fully evaluated.

Vaccination programmes should take into account the local ecology of the dog population, including the degree of ownership (owned and confined, owned and roaming, community-owned or ownerless). This knowledge is essential to ensure that the method of vaccination delivery maximizes access to dogs and in order to provide culturally appropriate education. The success of vaccination campaigns in Latin America was due to the central coordinating role of the public health sector and the involvement of communities in rabies control. In addition, as rabies control programmes must involve multiple agencies and sectors, including animal and public health, they require a 'one health' approach, with effective interministerial cooperation.

While mass dog vaccination has repeatedly been shown to be effective for controlling canine rabies, there is no evidence that removal of dogs has a significant impact on the dog population density or the spread of rabies. Mass culling of dogs should not be an element of a rabies control strategy: it is ineffective and can be counterproductive to vaccination programmes.

Euthanasia of a dog suspected of being rabid reduces human health risks and prevents further animal suffering. A list of the classical clinical signs of rabies in dogs is given in section 11. When the diagnosis is unclear, the dog can be quarantined and observed; however, if the signs progress, euthanasia should be performed (*7*).

## 9.1 Canine mass parenteral vaccination campaigns

To achieve control and eventual elimination of rabies, programmes must ensure recurrent (usually annual) campaigns and achieve a vaccination coverage of at least 70% (*8,9*). This coverage should be sufficient to maintain the required level of herd immunity in the vaccinated population in spite of dog population turnover (births, deaths, emigration, immigration) in the period between campaigns (*8,10*).

Latin America is an example of a region in which several countries have successfully controlled rabies. Since their formal pledge in 1983 to eliminate human deaths from rabies transmitted by dogs, the countries of the region have had a decrease of over 90% in rabies in dogs and hence a similar decrease in human deaths (*9,12*). This has been achieved predominately by mass vaccination of over 45 million dogs annually, with concurrent appropriate treatment of people potentially at risk for rabies (pre- and post-exposure prophylaxis) and epidemiological surveillance.

Other recent examples are KwaZulu-Natal (South Africa), the Visayas (the Philippines) and Bali (Indonesia). The KwaZulu-Natal province of South Africa had been plagued by dog rabies for several decades. During 1983–2007, 79% of laboratory-confirmed human cases in South Africa occurred in this

province, with a human population estimated at 10.6 million. The dog rabies elimination project illustrates the effectiveness of collaboration between the provincial government, donors (e.g. the Bill & Melinda Gates Foundation) and WHO. More than 1.5 million dogs have been vaccinated since the beginning of the project in 2009. In 2012, more than 630 000 dogs were vaccinated, the highest number immunized in a year by the provincial veterinary services. The occurrence of animal rabies has been halved in 3 years, with an initial decrease in human cases (*11*): for the first time in 20 years, KwaZulu-Natal reported in 2010–2011 a continuous 12-month period without a single human case (*12*). Despite many challenges, the project is now being extended across southern Africa, with renewed support and momentum.

The regional programme for rabies elimination in the Visayas is part of the national rabies programme jointly implemented by the departments of agriculture, health and education and chaired by the agriculture department's Bureau of Animal Industry on the basis of the National Rabies Act 9482. The 'rabies-free Visayas' project is being carried out in collaboration with partners such as WHO, the Bill & Melinda Gates Foundation, the Global Alliance for Rabies Control (GARC) and the Optimus Foundation. The project involves vaccination of more than 3 million dogs over 5 years, and campaigns have been conducted in the Western Visayas, parts of Central Visayas including the island of Bohol (*13*) and the Eastern Visayas. Intensive information and education campaigns are conducted to strengthen community support and volunteer engagement in order to increase dog vaccination and responsible pet ownership and improve clinical management of human rabies and surveillance and diagnostic capability. The number of human deaths from rabies in the Visayas has decreased significantly, from 48 cases in 2008 to 13 in 2012, a 70% reduction (*12*).

Rabies was introduced to Bali in 2008 and spread rapidly throughout the island, causing 141 human deaths by the end of 2012. Initial attempts to contain the spread of the disease involved indiscriminate mass culling of dogs. Since the introduction of mass canine vaccination as the main strategy from late 2010, the numbers of human and animal cases of rabies have dropped dramatically: the number of human cases decreased by 72% between 2010 and 2011 and by 90% between 2010 and 2012. Two mass vaccination campaigns have been completed, and a third campaign is nearing completion. The feasibility of an island-wide vaccination campaign was demonstrated by a local nongovernmental organization, the Balinese Animal Welfare Association, with funding from the World Society for the Protection of Animals. The Government of Indonesia with technical assistance from the FAO assumes responsibility for the second and subsequent campaigns. The reasons for the success of the programme have been: a clear operational goal of vaccinating 70% of dogs in each locality on the island during each campaign; daily reporting of vaccination and post-vaccination

survey results, by SMS and on paper; daily, weekly and monthly Government coordination meetings during vaccination campaigns; and campaign-specific standard operating procedures, with in-service training of field staff (*14,15*).

## 9.2 Strategic planning and management of vaccination campaigns

Vaccination campaigns must be strategically planned, well managed and adequately resourced and funded. The 'rabies blueprint' prepared by the Partners for Rabies Prevention provides guidance on planning and implementing dog vaccination campaigns (*16*).

### 9.2.1 Studies of dog ecology

To plan a vaccination campaign, the dog population must be estimated and dog-keeping practices ascertained in order to calculate the resources required and the appropriate methods for accessing dogs for vaccination (*17*). The dog population can be estimated from the human:dog ratio, but these ratios vary widely by community. Low levels of reported dog ownership and variable ownership patterns in urban areas make it difficult to estimate urban dog populations accurately. Other methods for estimating dog populations include questionnaire surveys, which provide information on owned dogs only, and capture–mark–recapture approaches, which cover the free-roaming population (*18*). Details of these methods are available from the International Companion Animal Management Coalition (*19*) and the Partnership for Rabies Prevention (*20*). Such surveys are often usefully combined with post-vaccination surveys to evaluate vaccination coverage, and population estimates can be revised for future campaigns. Information from dog registries can be useful, but, as these do not include unregistered or ownerless dogs, use only of this source will lead to underestimates of the total dog population.

### 9.2.2 Vaccination and immunization coverage

Low or patchy vaccination coverage of the target population is directly correlated with the persistence of rabies and hence jeopardizes the prospects of elimination over an entire region, even is coverage elsewhere is high. Vaccination may be more effective if carried out comprehensively in a small contiguous area than in many separate areas. Models of rabies transmission are helpful in identifying the best strategy for such situations (*21*).

Reactive vaccination is not recommended unless increased surveillance shows that the incidence has been reduced to low levels in a few remaining foci. Reactive strategies take longer to control rabies and are less likely to lead to successful control than systematic vaccination in an entire area.

The required immunization coverage can be achieved by well-designed educational campaigns, intersectoral and interdisciplinary cooperation, community participation, local commitment to planning and execution, the availability of high-quality vaccine, media support, and effective general coordination and supervision of activities by the appropriate authorities.

## 9.3 Implementing and monitoring dog vaccination campaigns

### 9.3.1 Target animals and vaccination methods

During mass campaigns, all dogs should be vaccinated, regardless of age, weight or state of health. Although the aim should be to vaccinate as many dogs as feasible, herd immunity is achieved by vaccinating at least 70% of the population. As cats are important vectors of rabies to humans, cats should also be vaccinated when presented at vaccination campaigns.

A common reason for low coverage is the misperception that puppies should not be vaccinated (*22–24*). In many countries endemic for canine rabies, young dogs comprise a large proportion of the population, and owners and vaccination teams must be made aware that puppies, including newborns, should also be vaccinated to ensure adequate population coverage.

Three basic approaches have been used, either alone or in combination, for accessing dogs for vaccination campaigns: house-to-house visits, fixed vaccination posts in well-recognized sites within a community, and temporary vaccination posts set up by mobile teams. Such posts are usually sufficiently attended only when they are at less than 500 m or about a 10-min walk (*25*). The choice of approach depends on the community and should be made at local level. A combination of approaches may be required.

### 9.3.2 Timing of campaigns

Rabies vaccination campaigns are generally conducted annually, but more frequent campaigns may be conducted in areas where population turnover is high.

Intensive vaccination campaigns lasting from 1 day to 1 month have been effective in rabies control, most notably in Latin America. Campaigns must, however, reach at least 70% of the dog population, and coverage should not be compromised in pursuit of speed.

Campaigns might be organized on weekends or during school holidays to improve turnout, as children often bring their dogs for vaccination.

### 9.3.3 Monitoring vaccination campaigns

Registration and permanent identification of vaccinated dogs is recommended; however, effective methods of identification are not widely available, and further research is required. Lack of resources or capacity to permanently identify dogs should not obviate implementation of a vaccination campaign. The use of coloured tags or plastic collars as temporary marking has proven to be useful in identifying vaccinated dogs (*25*) and motivates owners to take their pets for vaccination. Identification of vaccinated dogs is necessary in order to evaluate the vaccination coverage rate and to differentiate unvaccinated dogs for follow-up vaccination. For example, in Bali, red collars or red spray paint (for puppies that were still growing) were used to mark every dog that was vaccinated. A survey was then conducted within 3 days of the campaign to assess the numbers of marked and unmarked dogs; where coverage was calculated to be less than 70%, a revaccination campaign was organized to access unvaccinated dogs.

Routine serological monitoring in the context of mass dog vaccination campaigns is not recommended if:

- a reputable vaccine has been used (defined as a vaccine that has been demonstrated to confer protection for 2 years or more after a single injection against a virulent challenge, killing at least 80% of controls);
- vaccination teams have been trained and have used proper injection technique, dog handling and vaccine vial management; and
- the cold chain has been maintained throughout.

If repeated annual vaccination campaigns that reach the targeted coverage are not resulting in a decrease in the number of animal rabies cases, one or more of the above elements may not have not been complied with. Well-designed serological and other studies (e.g. vaccine potency, cold chain monitoring) may then be warranted.

### 9.3.4 Cost–effectiveness of dog vaccination

Several theoretical studies have indicated that dog vaccination in combination with post-exposure prophylaxis is more cost–effective for preventing human deaths from rabies than post-exposure prophylaxis alone (*26,27*). This conclusion remains uncertain, however, as the costs of different campaigns vary widely, and the operational costs can be substantially higher than those used in modelling studies, such as US$ 1.73–5.50 in rural United Republic of Tanzania (*20,24*). Furthermore, the demand for post-exposure prophylaxis does not invariably decrease with a decrease in the incidence of dog rabies; this relation requires further investigation.

Little research has been done on the effect of requiring owners to contribute to registration or campaign costs, especially for vaccine, or on the options for differential contributions based on owners' capacity to pay, and further evaluation of this approach would be beneficial. When dog owners are unwilling or unable to pay and this jeopardizes a critical level of immunization coverage, the intervention (i.e. registration, marking, vaccination, certificate delivery) must be provided free of charge and the costs balanced against the public health benefits.

### 9.3.5 Vaccines to be used

As vaccines are susceptible to extremes of temperature, including freezing, care should be taken to ensure that the cold chain is maintained within an acceptable temperature range. Long-acting vaccines with a minimum duration of immunity of 2 years should be used in annual campaigns to revaccinate all dogs. Revaccination has no adverse effects, and annual campaigns provide a simple, effective message. Turning people and their dogs away could confuse this message, while the direct costs of revaccination are marginal in comparison with campaign costs.

All members of a vaccination team who handle dogs should receive pre-exposure prophylaxis before the campaign. Adequate post-exposure prophylaxis should be available for people exposed during the campaign.

## 9.4 Increasing access to dogs for vaccination

When the usual approaches for accessing dogs for parenteral vaccination are deemed insufficient, other measures can be used. Increased community engagement and mobilization can improve the turn-out for vaccination campaigns, their cost–effectiveness and sustainability, and the surveillance and management of rabies cases.

When a proportion of the dog population cannot be handled by their owners or when no single owner claims responsibility for vaccination, expert dog handlers can be used to catch and restrain dogs humanely for vaccination. Various methods are available for dog catching. Expert dog handlers require suitable training to ensure they can catch dogs efficiently, reliably and humanely; inexpert handling can injure both catcher and dog and may make future catching for vaccination more difficult.

Oral vaccination of dogs may improve coverage in situations in which dogs cannot be restrained or caught. Further field studies are required to evaluate the cost–effectiveness of oral vaccination for achieving target coverage in different settings with different delivery strategies *(28)*.

## 9.5 Supplementary measure: humane dog population management

Humane management of dog populations is achieved mainly by responsible dog ownership and provision of sterilization services and basic dog health care (*29*). The objective of dog population management in the context of canine rabies control is to improve and maintain vaccination coverage and reduce risky dog behaviour. As there is no evidence that rabies transmission depends on the density of dog populations, reducing the population size through humane means may not be the most important factor, although it may have other benefits (e.g. with regard to dog welfare or nuisance behaviour). Dog population management may therefore be beneficial in canine rabies control. Work on the impact of humane dog population management programmes on rabies (and other associated benefits) has been relatively limited (*5,6,29*) and further evaluation of this approach would be beneficial.

Humane dog population management is an effective strategy for reducing dog population turnover and creating a healthy, sustainable population. As the status and composition of dog populations varies from country to country, no one intervention will work in all situations. Authorities should work with people who know the local dog population in order to understand ownership, demographics and the attitude of the local community towards dogs. This information can form the basis for a tailored package of humane dog population management tools for long-term, sustainable management (*16,19,29*).

India has an unusually high proportion of ownerless dogs. Dog population management has been used for canine rabies control in animal birth control programmes, in which free-roaming dogs are caught, sterilized and vaccinated before being released. Several locations have reported reductions in the number of human deaths from rabies during such programmes (*5,30,31*). The contribution of sterilization, over and above vaccination alone, to the control of dog rabies has, however, not been fully evaluated.

## 9.6 Main components of a dog rabies control programme

The Consultation recommended that the following components be included in a dog rabies control programme:

- Establish national focal points and national rabies elimination committees to prepare, implement and monitor long-term plans for management of people at risk with targeted pre- and post-exposure prophylaxis regimens, mass vaccination of dogs and humane management of dog populations.

- Strengthen surveillance and diagnostic facilities to include rapid diagnostic measures.
- Ensure sustainable community, district, national and regional rabies control programmes.
- Develop effective cross-border collaboration for rabies control and elimination.
- Promote through campaigns and child education programmes increased awareness in the general public of the benefits of responsible dog ownership, basic care of suspected rabid bites and avoiding animal exposure
- Foster cooperation among all relevant sectors, including veterinary services, public health, wildlife management and ecologists, to develop evidence-based approaches to human and animal rabies elimination.
- Support integration of rabies control activities at all levels of the health services, aligning them with other public health programmes, such as those for bacterial (e.g. tuberculosis), parasitic (e.g. neurocysticercosis, cystic echinococcosis) and vector-borne diseases (e.g. human African trypanosomiasis, leishmaniasis). Synergies among programmes improve the logistics of use of human, material and financial resources.
- Seek funding from bilateral and multilateral agencies and other donors in the framework of technical cooperation or humanitarian aid.
- Strengthen coordination and collaboration among international organizations, such as WHO, the Food and Agriculture Organization of the United Nations (FAO), OIE with their specialized networks of collaborating centres and reference laboratories and nongovernmental global and regional organizations (such as GARC, the World Veterinary Association, the Commonwealth Veterinary Association, the World Society for the Protection of Animals, and other international animal welfare organizations and coalitions).
- Stimulate cooperation with the pharmaceutical industry and institutions for the provision of vaccines, both human and veterinary, and technical cooperation to ensure proper vaccine storage, delivery and administration.

## 9.7 Operational research for dog rabies control

Operational research on dog rabies control is conducted during interventions (e.g. vaccination, population control), taking advantage of the fact that animals are handled and can be inspected and marked. In operational research, efforts should be made to adhere to proper protocols, and ensure rigorous statistical standards and unbiased sampling. When possible, controls should be included.

The main areas in which further operational research is needed are as follows.

- Questionnaire surveys on owned dogs and to elicit opinions about ownerless dogs should be conducted to determine dog population size (per person, per household, per surface area), demography, dynamics, and distribution before and after interventions (*32,33*). Vaccination and other veterinary interventions can provide the opportunity to apply a visual mark temporarily or permanently, such as a collar, ear notch, ear tag or tattoo for recapture studies. Better methods are needed for marking dogs rapidly and cost–effectively and for subsequent mark–recapture analyses, which include, for example, information on short-term movements of dogs (home range for 1 day to 1 week). Methods are needed to integrate questionnaire surveys and wildlife census methods to better determine the numbers of truly ownerless dogs that may not be readily accessible for vaccination.
- Questionnaire surveys can also be used to collect information on awareness about rabies, social attitudes to dogs and methods of dog population control as part of an education programme.
- Direct observation and questionnaire surveys should be used to collect data on the extent of supervision, which must be clearly defined. This information can be used to estimate the accessibility of dogs for veterinary interventions, which depend on the extent of supervision, culture, habitat and ecology (climate, meteorology).
- Methods for marking dogs that are sterilized or vaccinated temporarily (e.g. collars, stains, microchips) or permanently (e.g. tattoos, ear tags) should be explored.
- Better methods are needed for recording the absolute numbers and proportions of the dog population in different classes (e.g. age, sex,

treatment) with hand-held devices, positioning devices or computer software and for managing the results for rapid use to assess the incidence of disease in relation to vaccination coverage.

- Means could be explored for classifying and recording dog condition, diseases and parasites (e.g. during veterinary interventions, field surveys, household visits) to determine the health of the dog population. This would allow assessment of the effects of diseases on dog population dynamics and management of dog health.
- Applied research is required on the economics of dog vaccination, the sustainability of programmes, willingness to pay, cost–effectiveness and cost–benefit analyses in different cultural, ecological and economic contexts (*13,26,27,34,35*), including large-scale and regional models. The research should include socioeconomic barriers to programme implementation, translation of research into policy and practice and potential integration of dog rabies control into programmes for other dog-borne zoonoses, such as echinococcosis and leishmaniasis.
- The relevant information and methods of disseminating it should be assessed. The impact of education campaigns can be judged by analysing questionnaire surveys conducted before and after a campaign. Other means that can be used to evaluate education include changes in the numbers of dog bites, hospital visits and free-roaming dogs.
- As dog population management moves towards surgical or nonsurgical sterilization or contraception, questions remain on the impact of fertility control on dog population size and rabies control, including effects on population dynamics, social behaviour and disease transmission (*5,36*). Research should conducted to assess whether fertility control reduces the contact rate, home range and aggressiveness (particularly of males) and the disease transmission rate.
- Recently developed nonsurgical sterilants and contraceptives, such as immunocontraceptives and intratesticular sterilants, should be tested when dogs can be closely monitored to determine their humaneness, the longevity of the effect at population level and the feasibility of using and delivering these drugs (*37*).
- The cost, feasibility and sustainability of combining surgical or nonsurgical sterilization with rabies vaccination should be assessed. In parallel, cost–benefit analyses should be carried out to compare dif-

ferent dog population management options and to determine whether and how fertility control could be used as an adjunct to optimize rabies elimination programmes in some contexts.

## 9.8 References


1. Lembo T et al. Renewed global partnerships and redesigned roadmaps for rabies control. *Veterinary Medicine International*, 2011 (doi:10.4061/2011/923149).
2. Lembo T et al. Zoonoses prevention, control, and elimination in dogs. In: Macpherson CNL, Meslin F-X, Wandeler AI, eds. *Dogs, zoonoses and public health*, 2nd ed. Wallingford, Oxon., CAB International, 2013:205–258.
3. Nel L, Le Roux K, Atlas R. Meeting the rabies control challenge in South Africa. *Microbe*, 2009, 4(2):61–65.
4. Wandeler AI et al. Dogs and rabies. In: Macpherson CNL, Meslin F-X, Wandeler AI, eds. *Dogs, zoonoses and public health*, 2nd ed. Wallingford, Oxon., CAB International, 2013:43–66.
5. Reece JF, Chawla SK. Control of rabies in Jaipur, India, by the sterilisation and vaccination of neighbourhood dogs. *Veterinary Record*, 2006, 159:379–383.
6. Totton SC et al. Stray dog population demographics in Jodhpur, India following a population control/rabies vaccination program. *Preventive Veterinary Medicine*, 2010, 97:51–77.
7. *Terrestrial animal health code.* Chapter 7.7. Stray dog control. Paris, World Organisation for Animal Health, 2011 (http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.7.htm; accessed 29 November 2012).
8. Coleman PG, Dye C. Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine*, 1996, 14:185–186.
9. Cleaveland S et al. Dog rabies vaccination campaign in rural Africa: impact on the incidence of dog rabies and human dog-bite injuries. *Vaccine,* 2003, 21:1965–1973.
10. Tamayo H et al. Case report (4) Americas. Elimination of human rabies transmitted by dogs in Latin America and the Caribbean: achievements. In: *OIE Global Conference on Rabies Control, Republic of Korea, 7–9 September 2011* (http://www.oie.int/eng/A_RABIES/presentations.htm; accessed 29 November 2012).

11. *Report of the 4th meeting of the international coordination group of the Gates Foundation/WHO project for human and dog rabies elimination in low-income countries, 2–4 October 2012, Cebu, Philippines*. Geneva, World Health Organization, 2013 (http://www.who.int/rabies/bmgf_who_project/en).

12. *Report of the 3rd meeting of the international coordination group of the Gates Foundation/WHO project for human and dog rabies elimination in low-income countries, 19–21 October 2011, PieterMaritzburg, KwaZulu-Natal, South Africa*. Geneva, World Health Organization, 2011 (http://www.who.int/rabies/bmgf_who_project/en/).

13. Lapiz SMD et al. Implementation of an intersectoral programme to eliminate human and canine rabies. The Bohol Rabies Prevention and Elimination Project. *PLoS Neglected Tropical Diseases*, 2012, 6(12):e1891.

14. Suseno PS et al. Dog vaccination and campaign management for effective rabies control: the Bali experience. In: *International Conference on Emerging Infectious Diseases, 11–14 March 2012, Atlanta, Georgia.* Atlanta, Georgia, United States Centers for Disease Control and Prevention, 2012.

15. Suseno PP et al. Integrated bite case management for rabies in Bali: putting one health into action. In: *International Conference on Emerging Infectious Diseases, 11–14 March 2012, Atlanta, Georgia.* Atlanta, Georgia, Centers for Disease Control and Prevention, 2012.

16. Lembo T et al. The blueprint for rabies prevention and control: a novel operational toolkit for rabies elimination. *PLoS Neglected Tropical Diseases*, 2012, 6(2):e1388.

17. *Report of a WHO consultation on dog ecology studies related to rabies control*. Geneva, World Health Organization, 1988 (WHO/Rab. Res./88.25).

18. Hiby LR et al. A mark–resight survey method to estimate the roaming dog population in three cities in Rajasthan, India. *BMC Veterinary Research*, 2011, 7:46.

19. *Humane dog population management guidance*. International Companion Animal Management Coalition, 2008 (http://www.wsava.org/PDF/2008/Misc/AWC_ICAM_Coalition.pdf).

20. *Blueprint for rabies prevention and control* [canine rabies blueprint]. Partners for Rabies Prevention (www.rabiesblueprint.com; accessed March 2013).

21. Townsend SE et al. Surveillance guidelines for disease elimination: a case study of canine rabies. *Comparative Immunology, Microbiology and Infectious Diseases*, 2012 (http://dx.doi.org/10.1016/j.cimid.2012.10.008).

22. Suzuki K et al. Rabies vaccination coverage and profiles of the owned-dog population in Santa Cruz de la Sierra: Bolivia. *Zoonoses and Public Health*, 2008, 55(4):177–183.

23. Flores-Ibarra M, Estrella-Valenzuela G. Canine ecology and socioeconomic factors associated with dogs unvaccinated against rabies in a Mexican city across the US–Mexico border. *Preventive Veterinary Medicine*, 2004, 62(2):79–87.

24. Kaare M et al. Rabies control in rural Africa: evaluating strategies for effective domestic dog vaccination. *Vaccine*, 2009, 27:152–160.

25. Kappeler A, Wandeler A. *Dog population studies related to a vaccination campaign against rabies in Lalitpur City, Nepal. Report to WHO*. Geneva, 1989 (whqlibdoc.who.int/Kappeler_Wandeler_Nepal_Report_1989_eng; accessed 18 February 2012).

26. Boegel K, Meslin FX. Economics of human and canine rabies elimination: guidelines for programme orientation. *Bulletin of the World Health Organization*, 1990, 68:281–291.

27. Zinsstag J et al. Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, 106:14996–15001.

28. *Oral vaccination of dogs against rabies: guidance for research on oral rabies vaccines and field application of oral vaccination of dogs against rabies*. Geneva, World Health Organization, 2007 (http://www.who.int/rabies/vaccines/veterinary_ vaccines/en/index.html; accessed May 2012).

29. Hiby E. Dog population management. In: Macpherson CNL, Meslin F-X, Wandeler AI, eds. *Dogs, zoonoses and public health*, 2nd ed. Wallingford, Oxon., CAB International, 2013:177–204.

30. Chinny Krishna S. Control of rabies—Has the ABC programme been a success in India? *Indian Journal of Environmental Education*, 2003, 2:5–8.

31. Tenzin, Ward MP. Review of rabies epidemiology and control in South, South East and East Asia: past, present and prospects for elimination. *Zoonoses and Public Health*, 2012 (doi:10.1111/j.1863-2378.2012.01489.x).

32. Jackman J, Rowan AN. Free-roaming dogs in developing countries: the benefits of capture, neuter, and return programs. In: Salem DJ, Rowan AN, eds. *The state of the animals IV*. Washington DC, Humane Society Press, 2007:55–64.

33. Lembo T et al. The feasibility of canine rabies elimination in Africa: dispelling doubts with data. *PLoS Neglected Tropical Diseases*, 2010, 4:e626

34. Knobel DL et al. Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization*, 2005, 83:360–368.

35. Kayali U et al. Cost-description of a pilot parenteral vaccination campaign against rabies in dogs in N'Djaména, Chad. *Tropical Medicine and International Health*, 2006, 11:1058–1065.

36. Carroll MJ et al. The use of immunocontraception to improve rabies eradication in urban dog populations. *Wildlife Research*, 2010, 37:1–12.

37. Massei G. Fertility control in dogs. In: Macpherson CNL, Meslin F-X, Wandeler AI, eds. *Dogs, zoonoses and public health*, 2nd ed. Wallingford, Oxon., CAB International, 2013:205–258.

# 10. Prevention and control of rabies in wild animals

In the past, rabies was seen predominantly in domestic dogs, although there were occasional reports indicating the involvement of wildlife. Strict implementation of dog mass vaccination and other measures resulted in the virtual disappearance of dog-mediated rabies in Europe and North America during the 1940s, but the disease unexpectedly re-emerged in wildlife. With progress in molecular approaches to the identification and phylogeny of virus variants, understanding of lyssavirus epidemiology has improved significantly. Rabies is a viral zoonosis associated with many species of Carnivora and Chiroptera, which are the primary hosts of the rabies virus; only Chiroptera species are the primary hosts of almost all other lyssaviruses (see section 2).

## 10.1 Epidemiology and ecology of rabies in carnivore species

### 10.1.1 Africa

The cosmopolitan lineage of canine rabies virus is believed to have spread across the African continent during the European colonization. Domestic dogs remain the major primary hosts of rabies virus in Africa (*1*). Although sporadic cases of rabies in wildlife have been documented across the African continent, convincing

evidence for the circulation of rabies in populations of wild carnivores has been found only in southern Africa, where wild canids, such as jackals (*Canis adustus* and *C. mesomelas*) and bat-eared foxes (*Otocyon megalotis*) are assumed to be primary hosts of rabies virus (*2,3*). Additionally, members of the Herpestidae family (e.g. mongooses) appear to be responsible for transmission of a distinct variant of rabies virus in southern Africa (*4*). Infection with a canid rabies virus has been shown to be the cause of significant mortality among kudus (Tragelaphus strepsiceros) in Namibia, and direct oral transmission of infective saliva from kudu to kudu is suspected (*5,6*).

Spillover rabies virus from dogs is threatening endangered wild African canids such as the Ethiopian wolf (*C. simensis*) and the African wild dog (*Lycaon pictus*) (*7–10*).

### 10.1.2 Middle East and Asia

While dog rabies predominates in central and tropical Asia, rabies is maintained by wild canids in the forest–steppe and steppe zones of continental Asia, primarily by the red fox (*Vulpes vulpes*) and in the Russian far east by the raccoon dog (*Nyctereutes procyonoides*) (*11,12*). In southern China, the ferret badger (*Melogale moschata*) has been associated with human rabies for several years and is considered to be a primary host in this region (*13*).

Although occasional cases of rabies have been reported in wild carnivores in a number of countries in the Middle East and central, South and South-East Asia, it is unclear whether wildlife rabies is independent of the dog rabies transmission cycle in these regions. Fox rabies is present in Israel, the West Bank and Gaza Strip and has emerged in Turkey, where most cases of cattle rabies result from contacts with rabid foxes (*14*). Furthermore, certain countries in the Middle East region are reporting increasing numbers of cases of wildlife rabies, including the Islamic Republic of Iran, Oman, Saudi Arabia and Yemen. Red foxes and golden jackals (*C. aureus*) are usually implicated in those regions (*15–17*).

### 10.1.3 Europe

Wildlife rabies emerged in Europe after dog rabies was eliminated, the new primary host being the red fox (*V. vulpes*). Coming from the east, fox rabies spread inexorably across the continent within a few decades. By the mid-1980s, large parts of central and western Europe were affected. The westward expansion came to a halt in areas such as France and northern Italy, where foxes were treated with oral rabies vaccine (*17*).

Infected foxes are responsible for maintaining rabies virus within the fox population and also for transmission to other wildlife species and domestic animals. In affected areas, rabies is detected in a wide variety of species at different

frequencies. The animals most likely to come into contact with rabid foxes, such as roe deer, cattle and other domestic ruminants, represent most of the victims. There are indications that the raccoon dog (*Nyctereutes procyonoides*) may act as another primary wildlife host, as it is the second most frequently reported infected species in central and Baltic Europe (*18*).

Presently, fox-mediated rabies is still prevalent in eastern and south-eastern Europe, while large parts of western and central Europe have been freed from fox rabies by implementation of national and regional oral rabies vaccination programmes (*19*). Some southern and insular Mediterranean countries were never affected by the fox rabies epizootic, although a case was reported in northern Greece in October 2012 (*20*). Other countries have never had fox rabies, e.g. Sweden and the United Kingdom (*17*).

### 10.1.4 North America

With successful elimination of canine rabies in Canada and the USA in the middle of the 20th century and substantial progress in prevention and control of canine rabies in Mexico, wildlife rabies began to emerge in North America, as in Europe. In contrast to other parts of the world, wildlife rabies in temperate North America involves many primary host cycles, often with overlapping geographical ranges, making animal rabies control a major challenge. The commonest primary hosts are red foxes (*V. vulpes*) in parts of Alaska and Canada and raccoons (*Procyon lotor*) in the east. While the North American fox rabies epizootic extended its range in Canada, a different rabies virus variant emerged in raccoons in Florida (USA) and spread to neighbouring states. The spread was accelerated by translocation of rabid raccoons into the mid-Atlantic area in the 1970s, and the outbreak extended south and north as far as Quebec. Whereas the epizootic of fox rabies in southeastern Canada was eventually eliminated towards the end of the twentieth century, largely as a result of oral rabies vaccination, raccoon rabies still poses a serious problem in the region (*21–23*). The Arctic fox (*Alopex lagopus*) is a primary host in the polar regions of the continent, and the striped skunk (*Mephitis mephitis*) is a major host throughout the central plains and in California (*22,23*). In addition, grey foxes (*Urocyon cinereoargenteus*) are involved, particularly in southwest USA, and several species of skunk (*Spilogale* spp.) are recognized as primary hosts in Mexico. Each wild species maintains one predominant host-adapted rabies virus variant but can also harbour distinct spillover variants of rabies virus from other primary host species. Spillover to other wild and domestic animals is frequent in all areas. To date, oral rabies vaccine has played a major role in the prevention and control of rabies in red foxes and raccoons and in the elimination of rabies in coyotes and grey foxes in Texas.

### 10.1.5 South America

Rabies has been documented in wild carnivores in several areas, and phylogenetic studies of the genomes of rabies virus isolates from a variety of species indicate the presence of several distinct wildlife primary hosts, including the marmoset (*Callithrix* spp.) and the crab-eating fox (*Cerdocyon* spp.). Surveillance of wildlife for rabies is, however, generally inadequate to allow major epidemiological inferences. Information on the presence of rabies can be obtained from the Pan American Health Organization (http://new.paho.org/rabies).

### 10.1.6 Caribbean islands

The small Indian mongoose (*Herpestes auropunctatus*), which was introduced from South Asia to many Caribbean islands in the second half of the 19th century for rodent control, is a primary rabies host in parts of the Caribbean. For example, mongoose rabies is currently reported in Cuba, the Dominican Republic, Grenada and Puerto Rico. Other Caribbean islands are considered free of rabies among domestic and wild carnivores.

### 10.1.7 Eurasian and American arctic and subarctic regions

Arctic foxes (*Alopex lagopus*), domestic dogs and red foxes participate in the propagation of arctic rabies or 'polar madness', although the epidemiology is not well understood in these thinly populated areas with incomplete surveillance. Interestingly, arctic-like rabies virus lineages are also found in central and South-East Asia.

## 10.2 Epidemiology and ecology of rabies in bats

Lyssaviruses have been detected in bats throughout the world, although different species are present in different regions (24; see also Table 1 in section 2). Bats have been identified as vectors for all *Lyssavirus* species except Mokola virus and Ikoma lyssavirus (see section 2), for which the true primary host is yet to be found. This observation strongly suggests that bats are true primary hosts for lyssaviruses.

Bats have several traits (e.g. small size, long life, low intrinsic population growth rates and a variety of well-defined ecological niches) that are different from those of carnivore rabies hosts. Consequently, the properties of the lyssaviruses adapted to bats must be different from those that cause rabies in carnivores. The factors involved in maintenance of lyssaviruses in bats are insufficiently explored.

### 10.2.1 Lyssaviruses in Africa, Australia and Eurasia

At least four lyssavirus species are known to circulate in populations of insectivorous and frugivorous African bats (see Table 2, section 2). Lagos bat

virus, a lyssavirus predominantly associated with various large African fruit bat species (Megachiroptera) was originally isolated from *Eidolon helvum* in Nigeria in 1956 and later from other bat species in the Central African Republic, Senegal and South Africa. An epizootic that resulted in significant mortality among Epomophorus bats was observed in Natal, South Africa, where the virus is still occasionally isolated. Lagos bat virus has also occasionally been isolated from the insectivorous Gambian slit-faced bat (*Nycteris gambiensis*). No human cases have been confirmed to date, perhaps due to insufficient surveillance and virus characterization. Spillover of Lagos bat virus to other mammals has been reported infrequently (*1,25*).

Duvenhage virus was first isolated in 1970 from a person in Transvaal, South Africa, who died of rabies encephalitis after being bitten by an insectivorous bat reported to be associated with *Miniopterus* spp. Two further cases of rabies due to Duvenhage virus in humans have been reported, one in South Africa and the other in the Netherlands, the latter infection having been contracted in Kenya (*1*). In 2009, a bat-associated lyssavirus called Shimoni bat virus was isolated from the insectivorous Commerson leaf-nosed bat (*Hipposideros commersoni*) in Kenya. With Mokola virus and Lagos bat virus, it belongs to phylogroup II (26) (see section 2.3).

In 1996, Australian bat lyssavirus was isolated from fruit-eating bats (flying foxes, *Pteropus alecto*) on the eastern coast of Australia, a country considered to be 'rabies-free' since 1867. Two human deaths due to rabies caused by Australian bat lyssavirus were confirmed in 1996 and 1998. Australian bat lyssavirus has been isolated from all four species of frugivorous megabat (genus *Pteropus*, family Pteropodidae) in Australia and from an insectivorous bat species, the yellow-bellied sheath-tailed bat (*Saccolaimus flaviventris*) (*27,28*).

In Europe, sporadic cases of rabies have been diagnosed in bats in the past 60 years. Most cases are in serotine bats (*Eptesicus serotinus*), the viruses being identified as European bat lyssavirus type 1, while those from *Myotis* bats (*M. dasycneme* and *M. daubentonii*) are characterized as European bat lyssavirus type 2 (*29,30*). Cases of bat rabies appear to be less frequent in Europe than in the New World; however, the level of surveillance in Europe is still very heterogeneous, despite international recommendations. In total, four autochthonous human rabies cases transmitted by bats have been confirmed in Europe: two in the Russian Federation (1977 and 1985), one in Finland (1985) and one in Scotland (2002) (*24*). In 2002, a common bent-wing bat (*Miniopterus schreibersii*) was captured in the Russian Federation near the Georgian border and subsequently tested positive for lyssavirus infection. The virus, named West Caucasian bat virus, was a genetically divergent bat-derived member of the Lyssavirus genus, representing a member of phylogroup III, with no serological cross-reactivity to other lyssaviruses (*31*).

Bokeloh bat lyssavirus, isolated from a Natterer bat (*Myotis nattereri*) in Germany in 2010 and France in 2012, has been shown to differ from all previously known lyssaviruses occurring in Europe but to be antigenically and genetically close to European bat lyssavirus type 2 and Khujand virus (*32,33*). In 2012, an Ikoma lyssavirus-like virus was detected in *Miniopterus schreibersi* on the Iberian Peninsula (*34*).

In central Asia, three bat-associated lyssaviruses have been isolated. In 1991, an apparently healthy lesser mouse-eared bat (*Myotis blythi*) captured in the Aravan district, Kyrgyzstan, tested positive for rabies by the mouse inoculation test. Ten years later, near the town of Khujand, Tajikistan, a whiskered bat (*Myotis mystacinus*) also tested positive. Subsequent characterization of the isolated viruses revealed two new lyssavirus species: Aravan virus and Khujand virus (*35*). In 2002, a virus from *Murina* spp., commonly known as tube-nosed bats, was classified as a lyssavirus and named Irkut virus after a village in Irkutsk Province. One human case of rabies reported in Far East Russia in 2007 was due to infection with a virus similar to the original Irkut virus (*31*). Little is known about the epidemiology of bat lyssaviruses that have been isolated only once.

### 10.2.2 Rabies in insectivorous bats in the Americas

To date, all bat lyssaviruses in the Americas have been categorized as rabies virus. Many genetically and antigenically distinct variants circulate in bat species, several occurring within a single species, and the geographical distribution of variants overlaps. There is, however, an inverse correlation between cross-species transmission and phylogenetic distance among insectivorous bat species (36,37). Spillover to other animals is observed frequently. Although the incidence of human rabies is low in temperate North America, nearly 50% of cases are caused by bat-associated rabies virus (38). The silver-haired bat (*Lasionycteris noctivagans*) and the eastern tri-coloured bat (*Parasrellus subflavus*) play key roles in transmitting bat rabies to humans.

### 10.2.3 Vampire bat rabies

Vampire bat rabies is a major public health problem in the subtropical and tropical areas of the Americas, from Mexico to Argentina. A rabies virus variant related to the other American bat viruses is maintained in haematophagous bats, mainly by *Desmodus rotundus* (*37*) and is transmitted frequently to domestic animals and humans. Vampire bat-transmitted bovine paralytic rabies has a significant economic effect on the livestock industry. Currently, most cases of human rabies in Amazonia are caused by haematophagous bats (*39*).

## 10.3 Rabies in rodents

Testing of tens of thousands of wild and synanthropic rodents in areas endemic for rabies across the world has revealed only exceptional instances of dead-

end spillover of rabies virus infection, indicating that these animals are neither primary hosts nor play a role in the epidemiology and transmission of the disease.

## 10.4 Wildlife species of special concern

Rabies has emerged as a threat to conservation after outbreaks in highly endangered populations of Ethiopian wolves (*C. simensis*) in the Bale Mountains National Park, in African wild dogs (*Lycaon pictus*) in eastern and southern Africa and in the Blanford fox (*V. cana*) in Israel. Ethiopian wolves and African wild dogs are among the world's most highly endangered carnivore species, and transmission of rabies virus from more abundant primary hosts (such as domestic dogs) is considered a threat for extinction of several populations.

Rabies has been recorded in wolves (*C. lupus*) everywhere in the northern hemisphere where rabies occurs in wildlife, and they are therefore often believed to play a major role in transmission. Although wolves are susceptible and readily succumb to the disease, they cannot sustain circulation of rabies virus independently of other wildlife, as wolf population densities and dynamics do not support epizootics, and the highly territorial nature of wolves prevents ready spread of the disease from one pack to another. Once a pack member is infected, the disease can decimate the pack because of its highly social nature, with regular contact among the animals. The genetic make-up of rabies virus isolated from wolves is identical to those found in more abundant carnivore primary hosts in their vicinity (either domestic dog or wild species). Although wolves are more a victim of the disease rather than a true primary host, they can transmit rabies virus to other naive, susceptible hosts. Rabies in wolves is often experienced as a dramatic event, particularly if people are involved. Because they migrate over long distances, wolves that are incubating rabies virus are believed to be able to reintroduce wildlife rabies into freed areas.

## 10.5 Elimination of rabies in wild carnivores

### 10.5.1 Reduction of animal populations

Rabies virus transmission within wild carnivore populations that are capable of sustaining an infection cycle is considered to be density-dependent. Conventional methods of rabies control with drastic decimation of wild carnivore populations have failed to eliminate rabies (*17,40*). The resilience of Carnivora to elimination, their high reproductive potential and the capacity of the environment to provide food, water and shelter often make population control efforts futile. Consideration of humane, economical and ecological aspects will prevent inefficient large-scale culling campaigns.

### 10.5.2 Immunization

Mass vaccination of the principal wildlife hosts is a more effective control method than culling. This method emerged independently in Europe and North America (*22,40*). Since the late 1970s, the oral rabies vaccination strategy originally developed for foxes has been used to eliminate fox rabies in large parts of western and central Europe, Canada and the USA. Its success was due to research on and development of tools including effective, safe vaccines, machine-made baits that are attractive to a variety of species, automated, computer-supported aerial bait distribution, adequate vaccination strategies and strong political commitment (*22,41*).

An oral rabies vaccination strategy that works for one carnivore primary host species will not necessarily work for others. Adapted oral vaccine strategies have been used quite successfully not only for red foxes but also for other primary wildlife hosts, including coyotes, grey foxes and raccoon dogs, although they require optimization for raccoons (*23*). Different strategies are needed for other primary wildlife hosts.

As oral rabies vaccination programmes are designed to eliminate rabies from a defined area or to prevent spread of the disease by creating an immunological barrier (containment, *cordon sanitaire*), they should result in sufficient herd immunity to reduce transmission (i.e. the effective reproductive rate of the disease falls below 1) in the target primary wild host. The level of herd immunity required varies with the transmission dynamics of the disease in particular target species and populations and with local conditions.

Vaccines used in the field must fulfil the requirements of national or international regulatory authorities for biological products, i.e. efficacy, safety and stability, and be licensed or registered (see section 7). Baits must be designed for each target wild animal to ensure that the vaccine is released onto a susceptible target tissue (oropharyngeal mucosa or tonsils) to elicit an immune response. The bait casing must fulfil three functions: to carry the attractant for the target species, to contain a biomarker (usually tetracycline) of bait uptake by the target population, and to protect the vaccine blister, capsule or sachet from ultraviolet light to ensure the stability of the virus titre. The requirements for bait casings are laid down in relevant standards (*42–46*). The bait must be thermostable to guarantee its palatability, and it should be tested before marketing authorization at different temperatures (*44*). As the majority of rabies vaccine baits are consumed within 7 days of distribution in the field, the bait casing should protect the vaccine sachet or blister for this time under local weather conditions. Warnings should be printed on the blister or bait matrix.

Bait uptake and herd immunity in the target population depend on, e.g., vaccine efficacy and stability, the bait casing and attractiveness, the baiting method, the spatial distribution of baits, timing of oral rabies vaccination

campaigns and the abundance of bait competitors. The mode of bait distribution should guarantee that most of the target species has access. Oral rabies vaccine campaigns are usually conducted twice a year, in spring and in autumn in Europe and once a year in North America, with bait delivered mainly from fixed-wing aircrafts or helicopters (*23,44*). Manual distribution should complement aerial distribution or may be the only way to distribute bait in densely populated areas.

### 10.5.3 Planning, implementing and evaluating oral rabies vaccination programmes

Oral rabies vaccine has become the essential tool for preventing geographical spread, control and elimination of rabies when the primary host is wildlife. As oral rabies virus vaccines and baits developed for one primary host species may not work for others, vaccine efficacy, bait design and attractiveness should be evaluated for each new target species. Evaluation of oral vaccination programmes should include a cost–benefit analysis for public health. The basic requirements for planning, implementing and evaluating large-scale vaccination campaigns or field trials have been published (*43,44*) and were revised recently (*45*).

Epidemiological data based on reliable surveillance and laboratory studies of rabies cases in target and non-target species (wild and domestic) must be available before an oral rabies vaccination programme or field trial is initiated.

*Planning*

Strong political commitment is a prerequisite for an oral rabies vaccination programme, as the legal framework, planning, organization and evaluation are vital to its success. A national rabies committee should be constituted that includes all stakeholders. An effective programme is based on a comprehensive plan, outlining the justification (benefits), the objectives, roles (which agencies should be involved), responsibilities (who is responsible for what) and chains of command as well as infrastructure (laboratory requirements and equipment, cold chain), estimated costs (budgetary requirements) and funding. The plan must also include information on the areas to be covered in consecutive years, taking into account the patterns of movement of wildlife populations, the geographical characteristics of the area, the rabies situation in neighbouring countries, details of the vaccination strategy (timing, mode of bait distribution, bait density, flight line distance), safety considerations, surveillance and monitoring of campaigns. The size of the target population should be estimated, with baseline levels of the biomarker (if applicable) in the target species before vaccination.

As a general rule, an oral rabies vaccination programme should consist of two phases: an attack phase (elimination) and a maintenance phase. A long-term, large-scale approach is the most effective, and there must be a guarantee that the programme can be sustained in the long term. The plan should be distributed to

competent authorities well in advance for consideration and evaluation. Upon request, WHO can provide the necessary expertise.

***Implementation***

Delivery of oral rabies vaccine requires infrastructure and logistics that ensure the integrity of the bait and the vaccine (maintenance of cold chain) and that allow distribution of adequate numbers of baits (airports, aircrafts, other personnel) to cover large areas evenly.

Initial meetings should be organized by the national rabies committee for all stakeholders, including hunters, trappers, wildlife service staff, forest officers, physicians, veterinarians and local authorities, to discuss the programme in detail and to agree on the responsibilities of each stakeholder.

Responsible authorities and personnel should be trained in rabies surveillance, database management, data analysis and interpretation to monitor the progress of the intervention; reporting and dissemination of information to the competent authorities; the vaccine bait, the target species and the human component, and sampling of specimens under appropriate conditions.

Trained personnel and laboratory facilities should be available to carry out the recommended standard tests for routine diagnosis of rabies (see section 4) and for monitoring (biomarker detection, serology, virus titration, characterization of rabies virus isolates) the campaign in a quality assurance system.

The awareness of hunters, trappers, the general public and medical and veterinary practitioners about the campaign should be raised, so that they can take appropriate measures in case of accidental exposure to the vaccine. A medical or veterinary advisory group should be established.

Assignment of specialists is strongly encouraged to investigate the prevailing and changing epidemiological situation in both humans and animals and to evaluate the campaign and report regularly to the responsible authorities.

National meetings should be held regularly with all stakeholders to discuss the progress of the campaign and any adaptations required for future campaigns.

***Evaluation***

Surveillance and monitoring of oral rabies vaccination campaigns are essential for evaluating their success. They require a sustained, constant, intensive approach.

Adequate surveillance is important, as the incidence of rabies is the index of the impact of a programme. A risk-based sampling scheme should be used, focused on so-called 'indicator animals' that are ill, suspected of being rabid, show abnormal behaviour, found dead or involved in human exposure. Although the exact number of animals required cannot be predetermined, it should be sufficient to demonstrate an acceptable statistical degree of certainty (*18*). Surveillance

should generally be conducted before, during and after administration of vaccine, not only in the vaccination areas but also in neighbouring areas, particularly those free of rabies, to detect spread of the epizootic or re-infection as early as possible to allow a swift response and countermeasures (*45*). Rabies viruses isolated from animals in the vaccination areas should be characterized. The Consultation stressed the importance of reinforced surveillance in vaccination areas and beyond and requested governments to consider and adopt the above guidelines.

Monitoring the efficacy of an oral rabies vaccination programme (bait uptake, seroconversion) requires adequate sampling of hunted or trapped animals of the target species. The suggested sampling size is four target animals per 100 $km^2$ and year (*18*); however, experience has shown that this sample size can be difficult to achieve, depending on the topographical features of the vaccination area, infrastructure and logistics. If this number of samples cannot be taken, a reference area in which the sample size could be reached may be selected.

Basic or denominator data, e.g. species, date of finding and submission, location (Gauss–Krueger coordinates or lowest national unit of territories), age, sex, results of laboratory investigations (fluorescent antibody or tissue culture infection test, virus characterization, biomarker detection, serology) should be collected for all animals found for stratification and proper epidemiological (temporal and spatial) analysis.

To eliminate rabies in wildlife, 'progressive control pathways' and procedures for international certification of rabies-free status should be established.

***International cooperation***

International cooperation and coordination in planning, implementing and evaluating oral rabies vaccination programmes at all levels is necessary for success and cost–effectiveness. Preliminary contact should be made with neighbouring countries when the policy is decided, and these contacts should be maintained until elimination of the disease. Regular multilateral meetings with representatives of the public health and veterinary authorities of neighbouring regions and countries ensure coordination of activities along common borders and transparency. The involvement of WHO collaborating centres and of other international organizations is recommended. The results of vaccination programmes should be presented at international conferences, as a presence on the international stage can help pressure national governments to remain heavily committed to rabies elimination.

***Other options***

Besides oral vaccination, strategic trapping of wild carnivores and releasing them after parenteral vaccination (trap–vaccinate–release) has been used with

apparent success in some areas of North America, primarily for skunks and raccoons (*46,47*).

## 10.6 Bat rabies control

The goal of eliminating the disease in bats is challenged by the plethora of lyssavirus species and the substantial role of Chiroptera in global ecology, such as in seed dispersal, pollination and arthropod predation. Elimination of bat rabies is therefore not possible at the present time. The public health risk associated with bat rabies (except that transmitted by vampire bats) is lower than those associated with carnivores rabies, although the consequences of infection are also severe. Therefore, any method that indiscriminately destroys bats should be avoided, especially as bats are protected in most countries.

Education of the public is the key to preventing bat-transmitted human rabies. It should include basic information on avoiding potentially infectious contact with bats, seeking proper medical attention after exposure and preventing bats from establishing colonies in sensitive buildings (e.g. hospitals and schools).

Vampire bat-transmitted paralytic rabies of cattle can be controlled by vaccinating cattle. The approaches to controlling vampire bat-transmitted rabies by culling the primary host species with an anticoagulant, by direct application on the backs of captured bats or by intramuscular injection of cattle, is questionable and obsolete. Strict application of post-exposure prophylaxis is recommended in cases of human exposure to vampire bats. Preventive immunization of populations living in highly enzootic areas with limited access to anti-rabies biologicals should be considered.

## 10.7 Other public health measures

The general public should be better informed about avoiding direct contact with wildlife in general and with abnormally behaving and sick animals in particular. Any person bitten by a wild or domestic animal, particularly in areas where wildlife rabies is endemic, should seek medical attention (see section 7). Translocation of wildlife for any purpose except conservation should be banned or strongly discouraged.

## 10.8 References

1. Weyer J et al. Epidemiology of human rabies in South Africa, 1983–2007. *Virus Research*, 2011, 155(1):283–290.
2. Sabeta CT et al. Molecular epidemiology of rabies in bat-eared foxes (*Otocyon megalotis*) in South Africa. *Virus Research*, 2007, 129(1):1–10.

3. Zulu GC et al. Molecular epidemiology of rabies: focus on domestic dogs (*Canis familiaris*) and black-backed jackals (*Canis mesomelas*) from northern South Africa. *Virus Research*, 2009, 140(1–2):71–78.

4. Van Zyl N et al. Evolutionary history of African mongoose rabies. *Virus Research*, 2010, 150(1–2):93–102.

5. Scott T et al. Rabies in kudu (*Tragelaphus strepsiceros*). *Berliner und Munchener tierarztliche Wochenschrift*, 2012, 125(5–6):236–241.

6. Mansfield K et al. A molecular epidemiological study of rabies epizootics in kudu (*Tragelaphus strepsiceros*) in Namibia. *BMC Veterinary Research*, 2006, 2:2.

7. Haydon DT et al. Low-coverage vaccination strategies for the conservation of endangered species. *Nature*, 2006, 443:692–695.

8. Johnson N et al. A new outbreak of rabies in rare Ethiopian wolves (*Canis simensis*). *Archives of Virology*, 2010, 155(7):1175–1177.

9. Hofmeyr M et al. Rabies in African wild dogs (*Lycaon pictus*) in the Madikwe Game Reserve, South Africa. *Veterinary Record*, 2000, 146(2):50–52.

10. Woodroffe R et al. Contact with domestic dogs increases pathogen exposure in endangered African wild dogs (*Lycaon pictus*). *PLoS One*, 2012, 7(1):e30099.

11. Gruzdev KN. The rabies situation in Central Asia. *Developments in Biologics* (Basel), 2008, 131:37–42.

12. Shao XQ et al. Genetic evidence for domestic raccoon dog rabies caused by Arctic-like rabies virus in Inner Mongolia, China. *Epidemiology and Infection*, 2011, 139(4):629–635.

13. Liu Y et al. Ferret badger rabies origin and its revisited importance as potential source of rabies transmission in Southeast China. *BMC Infectious Diseases*, 2010, 10:234.

14. Vos A et al. Rabies in foxes, Aegean region, Turkey. *Emerging Infectious Diseases*, 2009, 15(10):1620–1622.

15. Seimenis A. The rabies situation in the Middle East. *Developments in Biologics* (*Basel*), 2008, 131:43–53.

16. World Health Organization Mediterranean Zoonoses Control Programme and World Organisation for Animal Health. *Inter-country expert workshop on protecting humans from domestic and wildlife rabies*

*in the Middle East, 23–25 June 2008, Amman, Jordan*. Paris, 2008 (www.oie.int/doc/ged/D6490.pdf; accessed 3 December 2012).

17. King AA et al., eds. *Historical perspectives of rabies in Europe and the Mediterranean Basin*. Paris, World Organisation for Animal Health, 2004.
18. Cliquet F et al. *Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union*. Brussels, European Food Safety Agency, 2010 (http://www.efsa.europa.eu/en/scdocs/scdoc/67e.htm).
19. Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit. *WHO rabies bulletin for Europe*. Greifswald-Insel Riems (www.who-rabies-bulletin.org).
20. World Organisation for Animal Health. *Rabies, Greece*. Paris, 2012. (http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/; accessed 23 October 2012).
21. MacInnes CD et al. Elimination of rabies from red foxes in eastern Ontario. *Journal of Wildlife Diseases*, 2001, 37(1):119–132.
22. Rupprecht CE et al. (2008) Can rabies be eradicated? *Developments in Biologics* (*Basel*), 2008, 131:95–121.
23. Slate D et al. Oral rabies vaccination in north America: opportunities, complexities, and challenges. *PLoS Neglected Tropical Diseases*, 2009, 3(12):e549.
24. Banyard AC et al. Bats and lyssaviruses. *Advances in Virus Research*, 2011, 79:239–289.
25. Markotter W et al. Epidemiology and pathogenicity of African bat lyssaviruses. *Developments in Biologics* (*Basel*), 2008, 131:317–325.
26. Kuzmin IV et al. Shimoni bat virus, a new representative of the *Lyssavirus* genus. *Virus Research*, 2010, 149:197–210.
27. Gould AR. et al. Characterisation of a novel lyssavirus isolated from *Pteropid* bats in Australia. *Virus Research*, 1998, 54:165–187.
28. Gould AR et al. Characterisation of an Australian bat lyssavirus variant isolated from an insectivorous bat. *Virus Research*, 2002, 89:1–28.
29. Schatz J et al. Current state of bat rabies surveillance in Europe. *Zoonoses and Public Health*, 2012 (doi: 10.1111/zph.12002).
30. McElhinney LM et al. Molecular epidemiology of bat lyssaviruses in Europe. *Zoonoses and Public Health*, 2012 (doi: 10.1111/zph.12003).

31. Kuzmin IV et al. Phylogenetic relationships of Irkut and West Caucasian bat viruses within the *Lyssavirus* genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. *Virus Research*, 2005, 111:28–43.

32. Freuling CM et al. Novel lyssavirus in Natterer's bat, Germany. *Emerging Infectious Diseases*, 201, 17(8):1519–1522.

33. Picard-Meyer E et al. Short item: Isolation of the novel BBLV *Lyssavirus* in Natterer's bat in France. *Bulletin Epidémiologique—Santé animale, alimentation, 2012*. (http://www.anses.fr/bulletin-epidemiologique/).

34. Aréchiga N et al. Novel lyssavirus from a *Miniopterus schreibersii* bat in Spain. In: *Twenty-third Rabies in the Americas Conference, São Paulo, Brazil, 14–18 October 2012* (abstract CO.04 at http://acontecimento.com.br/rita2012/rita_2012_abstract.pdf; accessed March 2013).

35. Kuzmin IV et al. Bat lyssaviruses (Aravan and Khujand) from Central Asia: phylogenetic relationships according to N, P and G gene sequences. *Virus Research*, 2003, 97:65–79.

36. Streicker DG et al. Host phylogeny constrains cross-species emergence and establishment of rabies virus in bats. *Science*, 2010, 329:676–679.

37. Streicker DG et al. Ecological and anthropogenic drivers of rabies exposure in vampire bats: implications for transmission and control. *Proceedings of the Royal Society B. Biological Sciences*, 2012, 279:3384–3392.

38. De Serres G et al. Bat rabies in the United States and Canada from 1950 through 2007: human cases with and without bat contact. *Clinical Infectious Diseases*, 2008, 46(9):1329–1337.

39. Schneider MC et al. Rabies transmitted by vampire bats to humans: an emerging zoonotic disease in Latin America? *Revista Panamericana de Salud Pública*, 2009, 25(3):260–269.

40. *WHO Expert Consultation on Rabies. First report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 931).

41. Müller T et al. Rabies elimination in Europe—a success story. In: *Compendium of the OIE Global Conference on Rabies Control, Seoul, Korea, 7–9 September 2012*.

42. *Manual of diagnostic tests and vaccines for terrestrial animals*. Chapter 2.1.13. Rabies. Paris, World Organisation for Animal Health, 2012 (http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/; accessed 4 December 2012).

43. *Report of a WHO seminar on wildlife rabies control, Geneva, Switzerland, 2–5 July 1990*. Geneva, World Health Organization, 1990 (WHO/CDS/VPH/90.93).

44. *Report of the WHO/APHIS consultation on baits and baiting delivery systems for oral immunization of wildlife against rabies*. Geneva, World Health Organization, 1990 (WHO/Rab. Res./90.36).

45. *Blueprint for rabies prevention and control* [fox rabies blueprint]. Partners for Rabies Prevention (www.rabiesblueprint.com; accessed March 2013).

46. Rosatte RC et al. Trap–vaccinate–release and oral vaccination for rabies control in urban skunks, raccoons and foxes. *Journal of Wildlife Diseases*, 1992, 28(4):562–571.

47. Slavinski S et al. Trap–vaccinate–release program to control raccoon rabies, New York, USA. *Emerging Infectious Diseases*, 2012, 18(7):1170–1172.

## 11. Rabies surveillance

Surveillance is the systematic, continuous collection, analysis and interpretation of data and their dissemination to appropriate people in order that action be taken (*1*). Its aim is to demonstrate the absence of disease or to identify its presence or distribution in order to allow timely dissemination of information for integrated action among different sectors (*2*). Surveillance is distinct from monitoring, which is defined by the OIE as intermittent performance and analysis of routine measurements and observations to detect changes in the environment or health status of a population. Monitoring in rabies control may include assessment of vaccination coverage through household surveys, observation of marks applied to dogs during mass parenteral vaccination (see section 7) and bait uptake in oral vaccination campaigns for wildlife. Further details on the surveillance of rabies in wild animals and monitoring of oral vaccination programmes are given in section 8.

For rabies, surveillance therefore involves measuring the incidence of the disease in both humans and animals. Measures of incidence are essential in rabies control and prevention to ensure appropriate management of cases and outbreaks, to monitor trends in order to evaluate the effectiveness of interventions and to estimate the burden of disease. Rabies surveillance also includes sharing data through appropriate channels, such as the World Animal Health Information System and Database (WAHIS and WAHID), the Global Early Warning System (GLEWS), the Empress embedded data system, the *Rabies Bulletin Europe* and official regional databases. Rabies should be a notifiable disease in national health and veterinary services. Timely responses to surveillance activities and results

will motivate field and hospital staff to continue reporting cases (see standard case definitions in 4.1 and clinical diagnosis in humans in 4.2). The response should include, at a minimum, prompt acknowledgement of reports, feedback on the results of diagnostic tests and advice on management of cases and outbreaks. Communication with medical and veterinary staff in the field ensures appropriate management and follow-up of cases and improves case detection rates.

To be effective, rabies surveillance must be based on diagnostic confirmation of human and animal suspected and probable cases. It is recommended that countries that lack or have inadequate diagnostic facilities improve their capacity through OIE laboratory twinning projects and links with WHO collaborating centres.

The involvement of private and public veterinarians, animal health workers, game wardens and other such professionals is essential, as they are the most likely professionals to see a clinically rabid dog. They should be aware of the clinical signs in a suspected case, the method of sample collection and the process for reporting. Lack of infrastructure and resources for collecting and submitting samples is often a greater impediment to rabies surveillance than lack of diagnostic facilities (*3*).

Animal rabies surveillance should be based on risk and therefore focused on investigation and diagnosis of suspected cases. Rabies may be suspected when animals show clinical signs of rabies, unprovoked bites have been reported and animals are morbid or found dead. The clinical signs of rabies in animals vary widely. The classical signs include abnormal behaviour, altered vocalization, pica, hypersexuality, drooling saliva, aimless wandering, 'fly-snapping', 'bone-in-the-throat' syndrome, aggression, incoordination, paralysis and convulsions. In rabies-endemic areas, loss of inhibition and abnormal behaviour in wild animals (such as activity of nocturnal animals during the day) should raise suspicion of rabies. In a dead animal, soiling of the mouth can indicate abnormal biting behaviour. Hyperaesthesia is not a feature of rabies in animals.

Surveillance should be maintained even in countries that have successfully eliminated canine rabies. The recent emergence of canine rabies in several rabies-free islands in Indonesia (*4,5*) and costly outbreaks in Europe of rabies transmitted by illegally imported pets and companion animals from rabies-endemic areas (*6*) show the importance of such surveillance.

Routine characterization of virus isolates from cases and outbreaks is encouraged in order to identify animal host origins, sources of infection and geographical origin (*7,8*), particularly in view of increased international travel and animal movement.

Measurement of rabies-specific antibodies is not recommended for routine rabies surveillance. In addition to laboratory-confirmed cases, the numbers of suspected and probable animal cases, animal bites and people seeking and receiving post-exposure prophylaxis should be recorded and reported. This

information should be shared with the medical and veterinary sectors to facilitate management of animal bites, outbreak investigation and implementation of control measures.

## References


1. *Making surveillance work* [modules 1–4]. Geneva, World Health Organization Department of Vaccines and Biologicals, 2001 (V&B/00.08 to 00.11).
2. *Terrestrial animal health code* [Chapter 1. Animal disease diagnosis, surveillance and notification, section 1.4. Surveillance]. Paris, World Organisation for Animal Health, 2012 http://www.oie.int/international-standard-setting/terrestrial-code/access-online/; accessed 26 November 2012).
3. Halliday J et al. Bringing together emerging and endemic zoonoses surveillance: shared challenges and a common solution. *Philosophical Transactions of the Royal Society of London B*, 2012, 367:2872–2880.
4. Windiyaningsih C et al. The rabies epidemic on Flores Island, Indonesia (1998–2003). *Journal of the Medical Association of Thailand*, 2004, 87(11):1389–1393.
5. Susilawathi NM et al. Epidemiological and clinical features of human rabies cases in Bali 2008–2010. *BMC Infectious Diseases*, 2012, 12:81.
6. Lardon Z et al. Imported episodic rabies increases patient demand for and physician delivery of antirabies prophylaxis. *PLoS Neglected Tropical Diseases*, 2010, 4(6):e723.
7. Bourhy H et al. The origin and phylogeography of dog rabies virus. *Journal of General Virology*, 2008, 89:2673–2681.
8. Talbi C et al. Phylodynamics and human-mediated dispersal of a zoonotic virus. *PLoS Pathogens*, 2010, 6(10):e1001166.


# 12. Rabies-free countries or areas

To assist public health authorities in assessing the risk for contracting rabies after contact with animals, this Consultation defined three types of risk-free countries or areas: dog-rabies free, wildlife (excluding bats) rabies-free and *Lyssavirus*-free. These definitions differ from the current OIE definition of rabies-free countries for the purpose of animal movement (*1*).

The following requirements apply to all three definitions:

- Rabies in all animal species and humans is notifiable, and a continuous, effective surveillance system is in operation.
- The system has or has ready access to one rabies laboratory in which WHO (*2*) or OIE-recommended techniques (*3*) for rabies diagnosis are used.
- An adequate number of samples from suspected cases in the main susceptible domestic and wild animal species in the country are tested. The level of statistical significance used to define sample size should be set by the suitable national authority.
- National authorities should ensure that samples are collected throughout the country.
- An effective import policy, i.e. measures to prevent the importation of rabies, especially those in section 13, is in place.

**A country or area that is free of risk for dog rabies is defined as one in which:**

- No case of indigenously acquired infection due to a dog rabies virus has been confirmed in humans, dogs or cats or any other animal species at any time during the previous 2 years.
- Any autochthonous positive case must be shown by molecular characterization to be a spillover from wildlife. If an imported case in carnivores is confirmed, the status of the country or area shall not be affected if molecular characterization confirms the nonindigenous source of the virus and epidemiological tracing backwards and forwards reveals no evidence of secondary dog infections.

**A country or area that is free of risk for wild carnivore rabies is defined as one in which:**

- No case of indigenously acquired infection due to a wild carnivore virus has been confirmed in humans or any domestic or wild species, at any time during the previous 2 years.
- Any autochthonous positive case must be shown to be a spillover from bats or dogs.

- If an imported case is confirmed, the status of the country or area shall not be affected if molecular characterization confirms the nonindigenous source of the virus and epidemiological tracing backwards and forwards reveals no evidence of secondary infections in any wild or domestic carnivores.
- Serological evidence of infection in some wild animals (e.g. mongoose) should be considered an indicator of the presence of rabies.

**A country or area that is free of risk for *Lyssavirus* rabies is defined as one in which:**

- No case of indigenously acquired infection with any *Lyssavirus* has been confirmed in humans or any domestic or wild species, including bats, at any time during the previous 2 years.
- If an imported case is confirmed, the status of the country or area shall not be affected if molecular characterization confirms the nonindigenous source of the virus and epidemiological tracing backwards and forwards reveals no evidence of secondary infections in any species.
- Serological evidence of infection in bats should be considered an indicator of the presence of rabies.

In countries or areas in any of the above categories, additional measures may be in place, such as vaccination of dogs and other pets. Reporting of several cases over time at the borders of a previously defined *Lyssavirus* risk-free country or area should be sufficient for the national authorities to suspect that rabies is likely to have been acquired indigenously rather than to have been imported.

In deciding whether to use human pre- or post-exposure prophylaxis, reference should be made to section 8.

## References

1. *Terrestrial animal health* code [vol. 2, chapter 8.10: Rabies]. Paris, World Organisation for Animal Health, 2011 (http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.8.10.htm; accessed 21 September 2012).
2. *Laboratory techniques in rabies*, 4th ed. Geneva, World Health Organization, 1996.
3. *Manual of diagnostic tests and vaccines for terrestrial animals* [vol. 1, chapter 2.1.12]. Paris, World Organisation for Animal Health, 2011 (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.13_RABIES.pdf; accessed 21 September 2012).

# 13. International movement of animals

Regulations for importing domestic, captive wild and wild mammals from rabies-free countries or from countries considered infected with rabies should comply with OIE standards (*1*), including presentation of a valid international veterinary certificate (*2*).

International standards depend on the rabies status of the country of origin and the animal species involved.

## 13.1 International transport of dogs, cats and ferrets from rabies-infected countries or areas

National importing authorities should require an international veterinary certificate attesting that the animal was not showing signs of rabies at time of shipment, was permanently identified, vaccinated or revaccinated and subjected to a positive serological test prior to shipment. OIE international standards should be followed.

A model of international rabies vaccination certificate is set out as *Annex 7*.

## 13.2 International transport of livestock and animals for zoos, research, shows and other activities from rabies-infected countries or areas

These animals should comply with OIE standards (*3*), which include a veterinary certificate for domestic animals, laboratory rodents/lagomorphs and wildlife, permanent identification and optional vaccination for domestic ruminants, equids, camelids and suids and a statement that the animals showed no sign of rabies on the day of shipment and particularly for laboratory and wild animals that they were kept in quarantine, or other relevant isolation, for 6 months before shipment with no rabies case detected in the isolation establishment for at least 12 months prior to shipment.

Countries that are free from rabies may either prohibit the importation of certain species of mammals, in particular Carnivora and Chiroptera, or permit their entry only under license, subject to quarantine in premises and under conditions approved by the government veterinary service. Entry may be permitted for limited periods or for life. In view of the increase in the number of reported rabies cases in wild animals acquired as pets, national authorities should control the trade in such animals. Keeping such animals as pets should be discouraged.

### 13.3 Special exemption of guide dogs for people with disabilities and of other service dogs

Certified guide dogs for people with disabilities and other service dogs (e.g. military and search dogs) in rabies-free countries should be permitted to accompany their owners into rabies-infected countries if the dogs are vaccinated with a cell-culture vaccine that fulfils WHO and OIE standards and are shown to have an adequate virus-neutralizing antibody titre by one of the methods recommended by the OIE (*3*) and WHO (*4*).

These dogs must be identifiable by means of a microchip. Provided that the owners confirm that they were kept confined, on a leash or under permanent visual supervision while abroad in a rabies-infected country, the dogs should be allowed to remain outside the country for a maximum of 6 months with no requirement for re-entry other than reconfirmation of the antibody titre.

### 13.4 References

1. *Terrestrial animal health code* [vol. 2, chapter 8.10: Rabies]. Paris, World Organisation for Animal Health, 2011.
2. *Terrestrial animal health code* [vol. 1, chapter 5.11: Model international veterinary certificate for dogs and cats originating from rabies infected countries]. Paris, World Organisation for Animal Health, 2012 (http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.5.11.htm; accessed 21 September 2012).
3. *Manual of diagnostic tests and vaccines for terrestrial animals* [vol. 1, chapter 2.1.12]. Paris, World Organisation for Animal Health, 2011 (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.13_RABIES.pdf; accessed 21 September 2012).
4. *WHO Expert Consultation on Rabies. First report.* Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 931).

## 14. Global and regional activities on rabies

Many activities on rabies have taken place at international, regional and national levels since publication of the first report of the WHO Expert Consultation on Rabies (*1*). A growing number of partners (intergovernmental and nongovernmental organizations, public and private institutions and foundations) are contributing to the prevention, control and elimination of human and animal rabies at global, regional and national levels, such as FAO, OIE, the Association

of South-East Asian Nations (ASEAN), the South Asian Association for Regional Cooperation, GARC, the Commonwealth Veterinary Association, Humane Society International, the Rabies in Asia Foundation, Vets Beyond Borders, the World Society for the Protection of Animals, and the Bill & Melinda Gates Foundation. These partners have prepared global standards and policies, helped in resources mobilization, provided regional coordination or directly supported national programmes. The list does not pretend to be exhaustive.

## 14.1 WHO global and regional activities

### 14.1.1 WHO headquarters

The WHO World Survey of Rabies, created in 1990, was subsequently enhanced by a computerized data management system to process data collected online at country level, known as 'Rabnet'. The system was improved from 2000 onwards with the addition of new features, such as the production of interactive maps at global and country levels and customized charts, graphs and maps. The database was designed to analyse global trends in the disease as well as regional and national changes. The system was, however, closed down in 2010, as too few individual reports were entered annually into the system by designated national rabies focal points to make analysis of the data meaningful. WHO, the WHO regional offices and collaborating centres on rabies and GARC are studying alternative ways of collecting data and producing annual reports on human and animal rabies. Information on rabies in humans, domestic animals and wildlife should be shared across sectors.

The concept of 'neglected zoonotic diseases' emerged at a meeting held at WHO headquarters in September 2005 (*2*) and was reinforced at international conferences held in 2007 (*3*) and 2010 (*4*). The term 'neglected' for this group of diseases indicates that they are insufficiently addressed by governments and the international community, and that they are best defined by the people and communities they affect most: poor people living in remote rural areas or urban slums of the developing world. The term is now well accepted internationally. Rabies has unfortunately all the features of a neglected zoonotic disease. It is, however, the disease most amenable to control, as the tools are available. It is the first zoonosis on the list of neglected diseases targeted for regional and eventually global elimination. An interagency meeting proposed investment in a 'priority neglected zoonotic diseases portfolio', comprising regional elimination of human–dog transmitted rabies in Latin America and Asia (5). A first costing indicated that about US$ 10 million per annum in external funding for the next 5 years will be required to achieve the expected outcomes by 2016.

Rabies is discussed at length in the first and second WHO reports on neglected tropical diseases (*6,7*) and is included in the shorter list of targeted

diseases for regional elimination in the executive summary of the 'roadmap for implementation' published in 2012 (*8*). Rabies is also one of the main viral zoonoses in the technical report of the Disease Reference Group on Zoonoses of the Special Programme for Research and Training in Tropical Diseases (TDR) published by WHO in 2012 (*9,10*).

Recommendations and a map are available and updated regularly on the risk for contracting rabies (see section 6.8) in the WHO publication *International travel and health*, to inform international travellers about the necessity for pre-exposure prophylaxis, depending on their destination (*11*).

Since the last Expert Consultation in 2004 and in accordance with its mandate to provide guidance to Member States on rabies prophylaxis, WHO has issued a position paper on rabies vaccines in a series of regularly updated position papers on vaccines and vaccine combinations against diseases of international public health importance (*12*). This position paper, issued in 2010, was based on the outcome of a WHO consultation on rabies prevention and control in humans and animals held in Annecy, France, in 2009 (*13*). The position paper, which replaced one issued in 2002, was reviewed and endorsed by WHO's Strategic Advisory Group of Experts on vaccines and immunization (*12*). Position papers are designed for use mainly by national public health officials and immunization programme managers and are of interest to international funding agencies, the vaccine manufacturing industry, the medical community, the scientific media and the public.

Since 2002, WHO has maintained a website that provides information on rabies in humans and animals, human and animal vaccines and pre- and post-exposure prophylaxis. It also contains selected WHO reports and peer-reviewed articles. Since 2009, the site has provided information on progress made in implementation of the 5-year (2009–2013) pilot project for human and dog rabies elimination in selected developing countries (KwaZulu-Natal in South Africa, southwestern United Republic of Tanzania and the Visayas in the Philippines) funded by the Bill & Melinda Gates Foundation and managed by WHO (*14,15*).

### 14.1.2 WHO regional offices

***Asia***

The WHO Regional Office for South-East Asia has been proactive in preparing standards and guidelines, issuing recommendations and providing technical support to Member States for the prevention and control of human and animal rabies in the region. It advocates use of cost–effective intradermal vaccination to improve the availability and affordability of modern rabies vaccines, and phasing out of the production and use of nerve tissue vaccine. The production and use of this vaccine has been abandoned since 2005 in Bangladesh, Cambodia, India, the Lao People's Democratic Republic, Nepal and Viet Nam. The WHO Collaborating

Centre for Rabies Diagnostics in Bangalore, India, has introduced direct rapid immunohistochemistry tests into the region in collaboration with the WHO Collaborating Centre for Reference and Research on Rabies at the Centers for Disease Control and Prevention in Atlanta, Georgia, USA, and regional hands-on training in rabies diagnosis was organized in Bangalore in 2010 to train laboratory professionals in the use of these tests.

To consolidate achievements in the control of zoonoses, particularly rabies, in Member States, the regional office has organized meetings (*16,17*) and prepared a regional strategy for elimination of human rabies transmitted by dogs. The aim is to eliminate human rabies by progressive control of dog rabies and human prophylaxis in rabies-endemic countries and to maintain the status of rabies-free areas in the region by 2020 (*17*).

***Latin America***

The programme for elimination of humans rabies transmitted by dogs is led by the Veterinary Public Health unit of the Pan American Health Organization/WHO Regional Office for the Americas in Rio de Janeiro, Brazil. The objectives of the plan for eliminating rabies from the principal cities of Latin America, initiated in 1983, were extended in 1992 to elimination of dog-transmitted rabies in small conglomerates and rural areas. Since 1983, the occurrence of dog-transmitted rabies has diminished steadily, with a reduction of approximately 90% in human and canine cases.

A series of inter-American meetings on health and agriculture at ministerial level is organized by the Veterinary Public Health unit to discuss intersectoral policies and include the regional rabies elimination programme. Every 2 years, the unit also convenes a meeting of the directors of national rabies programmes, at which the epidemiological situation and strategies for prevention of rabies are discussed and updated. The conclusions and recommendations are submitted to ministers of health and agriculture during the interministerial meetings for their consideration and endorsement. The eleventh meeting of the directors of national programmes, held in Brasilia in 2006, recommended elimination of human rabies transmitted by dogs from the hemisphere by 2012, and the fifteenth interministerial meeting, held in Rio de Janeiro in 2008, committed the ministers of health and of agriculture to this goal (*18*). In 2009, the forty-ninth Directing Council of the Pan American Health Organization in Resolution CD49.R9 proposed 2015 as the target date for regional elimination of all neglected diseases and other poverty-related infections, including rabies (*19*).

The Regional Information System for Epidemiologic Surveillance of Rabies in the Americas (http://siepi.panaftosa.org.br/) produces reports on human and animal rabies based on official data entered into the system by health and agriculture ministries in Member States. Data from 1970 onwards are available for on-line consultation.

### 14.1.3 WHO network of collaborating centres on rabies

A network of collaborating centres on rabies was established almost at the inception of WHO to support WHO activities at country, intercountry, regional, interregional and global levels. The collaborating centres also participate in strengthening the institutional capacity of Member States in terms of information, services, research and training for rabies-related activities such as diagnosis, surveillance, research and monitoring and evaluation of projects and programme for elimination of rabies in humans and animals.

The centres are officially designated by WHO on the basis of a jointly agreed plan of work, usually for 4 years, renewable after annual evaluation of their performance by WHO. The plan of work depends on the expertise or specificity of the centre but usually covers:

- collection, collation and dissemination of information on rabies;
- standardization of rabies diagnostic reagents, prophylactic and therapeutic substances, as well as methods and procedures for their application;
- design and application of appropriate techniques;
- provision of reference substances and other services;
- participation in collaborative research under the Organization's leadership;
- training, including research training; and
- coordination of activities carried out by several institutions.

There are 12 designated WHO collaborating centres, most for reference and research on rabies. Five are in Asia, four in Europe and three in the USA (see Annex 8). The WHO Collaborating Centre for Rabies Surveillance and Research hosted by the Friedrich-Loeffler-Institute in Germany produces the WHO *Rabies Bulletin Europe* (see section 14.2.2).

## 14.2 Examples of activities by partners

Several global and regional initiatives for rabies control and eventual elimination began rapidly and continued to flourish in the past decade. Examples are described below.

### 14.2.1 Global activities

***Food and Agriculture Organization of the United Nations (FAO)***

FAO contributes to rabies control by raising awareness and providing policy advice and technical support for animal rabies control in a number of African and

Asian countries. It supports animal health clubs in schools in Sierra Leone, and contributes to partnerships and alliances for preventing and controlling rabies, such as the Partnership for Rabies Prevention and GARC. FAO has organized global stakeholder consultations on dog population management for rabies control with the World Society for the Protection of Animals and, with OIE and WHO, is exploring a 'progressive control pathway' to rabies elimination focused on elimination of dog-transmitted human rabies (*20*).

***World Organisation for Animal Health (OIE)***

OIE is an intergovernmental organization that issues science-based standards, guidelines and recommendations for the control of infectious diseases in animals, including those that are transmissible to humans, such as rabies. Internationally agreed diagnostic laboratory methods and requirements for the production and control of animal rabies vaccines and other biological products are published in the OIE *Manual of diagnostic tests and vaccines for terrestrial animals* (*21*). The OIE *Terrestrial animal health code* (*22*) lists measures adopted internationally for the control of rabies. Through its network of reference laboratories (http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories) and collaborating centres (http://www.oie.int/en/our-scientific-expertise/collaborating-centres/list-of-centres), the OIE provides policy advice, strategy design and technical assistance for the diagnosis, control and elimination of rabies in animals. In 2011, the OIE organized, with WHO and FAO, a global conference on rabies entitled 'Towards sustainable prevention at the source' in the Republic of Korea, which raised the awareness of responsible parties and decision-makers on the importance of tackling rabies at its animal source and re-emphasized the role of national veterinary services in preventing and controlling the disease (*23*).

***The Global Alliance for Rabies Control (GARC), Partners for Rabies Prevention and World Rabies Day***

GARC is the only registered charity working specifically on reducing the global burden of rabies. It has two branches: the Global Alliance for Rabies Control in the USA and the Alliance for Rabies Control, established in Scotland. GARC's mission is to eliminate human deaths from rabies and to relieve the burden of rabies in animals, especially dogs (http://www.rabiescontrol.net/).

GARC works with governments and communities in Africa and Asia to plan and conduct intersectoral (or 'one health'), sustainable rabies control programmes, funded through partnerships between governments, international foundations, private donations and animal welfare organizations. GARC has established a repository for educational material that is available on the World Rabies Day website for individuals and organizations that require accurate material to improve awareness in their regions.

GARC was instrumental in establishing and is a member of the Partners for Rabies Prevention (*24*). This informal group comprises the main international agencies involved in rabies: WHO, FAO, OIE, WHO rabies collaborating centres, research scientists, representatives of the Bill & Melinda Gates Foundation, the UBS Optimus Foundation and representatives of industry. The Partners for Rabies Prevention have published a blueprint for rabies prevention and control (*25*).

World Rabies Day was initiated by GARC in 2006. It now involves all partners in human and animal health at international, national, state and local levels, veterinary, medical and other professional and student organizations, and corporate and non-profit partners. Its goal is to raise awareness and mobilize resources for human rabies prevention and animal rabies control. The inaugural campaign in September 2007 was attended by nearly 400 000 people in 74 countries. This response was an important step for rabies prevention and control and shows that the need for action to control this easily preventable disease is widely recognized. World Rabies Day events have been held in 150 countries, with education for 182 million people and vaccination of 7.7 million dogs.

### 14.2.2 Regional activities

*Africa*

The Southern and Eastern African Rabies Group was founded in 1992 for the control of dog rabies. Official meetings are held about every 2 years, for presentation of data on rabies in standardized country reports, which are subsequently published on an open access website (http://www.searg.info). The country reports describe rabies in humans, domestic animals and wildlife and outline requirements for vaccine purchase or production and vaccination strategies. These meetings help to improve diagnosis, surveillance and awareness and highlight the lack of knowledge of the true burden of rabies in Africa. The tenth meeting was held in Maputo, Mozambique, in 2011, and the next will be held in Dar es Salaam, United Republic of Tanzania, in 2013.

The African Rabies Expert Bureau (http://www.afroreb.info/) is an informal network of rabies experts in French-speaking countries of Africa. It was established in 2008. Members meet regularly to review the rabies situation in their countries, share experience and discuss any problems encountered and potential solutions. Reports of their meetings are published in international journals. The Bureau provides a platform for French-speaking rabies experts to exchange information and to link with other networks of rabies experts.

***Asia***

FAO has provided technical support for animal rabies control in a number of Asian countries, particularly Indonesia. OIE advises national veterinary services in the Asia Pacific region on dog rabies control and dog population management and provides dog rabies vaccine to certain counties within a project supported by the European Union. The aim of this 4-year project (2009–2013), conducted by FAO, OIE and WHO, is to strengthen the capacity of countries and of the two main regional organizations, ASEAN and the South Asian Association for Regional Cooperation, in order to enhance regional cooperation on diseases of animal origin, including rabies.

The Global Framework for the Progressive Control of Transboundary Animal Diseases in Asia and the Pacific has identified rabies as a priority at the human–animal interface and called for increased political commitment at national and regional levels.

Member States of ASEAN and the South Asian Association for Regional Cooperation have also identified rabies as a priority public health problem, and governments have expressed concern and commitment for the elimination of human rabies. The ASEAN countries adopted a call for action to prevent and control rabies, with the goal of elimination by 2020 (*26*). The Rabies in Asia Foundation, at a conference in 2009, resolved to take seven steps to achieve human and dog rabies elimination by 2020 and requested the WHO regional committees of the South-East Asia and Western Pacific regions to meet the demands of Member States for technical assistance and technology transfer and to launch regional initiatives for dog rabies control and elimination in Asia in collaboration with regional organizations.

ASEAN, FAO, OIE and WHO organized a rabies workshop in Chiang Mai, Thailand, in January 2012, which was attended by officials responsible for animal and human health from 12 Asian countries. Country progress was described, and the group decided to make a unified effort to eliminate rabies in the region, with a plan for control and eradication (*27*).

The Asian Rabies Expert Bureau is an informal network of rabies experts established in 2004. Its members meet regularly to review the situation in their countries, share experience and discuss any problems encountered and their solutions. Meeting reports are available on their site (http://www.areb.info).

***Latin America***

An international conference on rabies in the Americas (http://www.rabiesintheamericas.org/) is organized annually to review and discuss rabies research and control in the region. The meeting has an international committee consisting of representatives of Brazil, Canada, Mexico and the USA. The twenty-third meeting was held in Brazil in October 2012.

***Middle and Near East***

The Middle East and Eastern Europe Rabies Expert Bureau (http://www.meereb.info) is an informal network of rabies experts established in 2010. Members meet regularly to review the situation in their countries, share experience and discuss any problems and their potential solutions. Reports of their meetings are published in international journals (*28*).

***Europe***

A rabies reporting system, the WHO *Rabies Bulletin Europe*, was created in 1977 by WHO and the Friedrich-Loeffler Institute in Germany; it is hosted by the WHO Collaborating Centre for Rabies Surveillance and Research at the Institute. The system is continuously updated, and all data reported are automatically transferred to a database, summarized by administrative unit and aggregated per country. More than 40 European countries report officially confirmed rabies cases in both wild and domestic animal species and in humans on a quarterly basis. The *Rabies Bulletin Europe* is printed quarterly, and a free version is available electronically from www.who-rabies-bulletin.org/. The website also allows dynamic database queries. Since 1990, maps of rabies cases have been displayed online, and since 2009 surveillance data are also mapped. The *Bulletin* provides valuable information for both the general public and the scientific community.

European Union member states exchange information on rabies regularly at meetings of the Standing Committee of Food Chain and Animal Health. In 2003, the European Union established a subgroup on rabies within a task force for monitoring animal disease eradication to assess co-financed oral rabies vaccination campaigns in member states and neighbouring non-member countries. The subgroup comprises private and governmental rabies experts, who visit member states at the request of the European Commission. Its conclusions and recommendations to improve oral rabies vaccination programmes are submitted to the European Commission and the respective member state for consideration. Its reports are publicly available (http://ec.europa.eu/food/animal/diseases/eradication/taskforce_en.htm).

The Food and Veterinary Office of the European Commission conducts on-the-spot inspections of the execution of co-financed rabies elimination programmes at all levels in member states. Its reports can be obtained from the website (http://ec.europa.eu/food/fvo/inspectprog/policy_papers/index_en.htm).

Since 2008, the European Union Reference Laboratory for Rabies in Nancy, France, has organized annual meetings of European national rabies laboratories in order to harmonize and standardize diagnostic techniques. The European Union also supports partner countries through Technical Assistance and Information Exchange, an instrument managed by the Directorate-General

for Enlargement of the European Commission. Information on recent missions and workshops on rabies is available online (http://ec.europa.eu/enlargement/taiex/dyn/taiex-events/index_en.jsp).

Independent multilateral meetings are organized with representatives of public health and veterinary authorities of neighbouring countries that have oral rabies vaccination programmes. The European Centre for Disease Prevention and Control, which was established to strengthen the capacity of the European Union to prevent and control infectious diseases, organized a consultation in January 2009 with WHO participation to review the epidemiological situation of rabies in Europe, to identify approaches to administering post-exposure prophylaxis and to find solutions to the shortage of rabies biologicals, including the possibility of establishing a virtual stockpile (*29*).

Human rabies cases and the epidemiology of rabies are presented and discussed in Eurosurveillance, a peer-reviewed scientific journal for articles on the epidemiology, surveillance, prevention and control of communicable diseases that are relevant to Europe. It is published by the European Centre for Disease Prevention and Control (http://www.eurosurveillance.org/).

## 14.3 References

1. *WHO Expert Consultation on Rabies. First report*. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 931).
2. *The control of neglected zoonotic diseases: a route to poverty alleviation. Report of a joint WHO/DFID-APHP meeting with the participation of FAO & OIE*. Geneva, World Health Organization, 2006 (WHO/SDE/FOS/2006.1).
3. *Integrated control of neglected zoonotic disease in Africa: applying the 'one health' concept. Report of a joint WHO/EU/ILRI/DBL/FAO/OIE/AU meeting*. Geneva, World Health Organization, 2008 (WHO/HTM/NTD/NZD/2008.1).
4. *The control of neglected zoonotic diseases (NZDs): community-based interventions for prevention and control. Report of the third conference organized by WHO/ICONZ/DFID-RIU/SoS/EU/TDR/FAO with the participation of ILRI and OIE*. Geneva, World Health Organization, 2011 (WHO/HTM/NTD/NZD/2011.1).
5. *Interagency (FAO,OIE,WHO) meeting on planning NZDs prevention and control*. Geneva, World Health Organization, 2011 (WHO/HTM/NTD/NZD/2011.3).

6. *Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases*. Geneva, World Health Organization, 2010 (WHO/HTM/NTD/2010.1).

7. *Sustaining the drive to overcome the global impact of neglected tropical diseases: second report on neglected tropical diseases*. Geneva, World Health Organization, 2013 (WHO/HTM/NTD/2013.1).

8. *Accelerating work to overcome the global impact of neglected tropical diseases: a roadmap for implementation*. Geneva, World Health Organization, 2012 (WHO/HTM/NTD/2012.1).

9. Molyneux D et al. Zoonoses and marginalised infectious diseases of poverty: Where do we stand? *Parasites and Vectors*, 2011, 4(106):1–19.

10. *Research priorities for zoonoses and marginalized infections*. Geneva, World Health Organization, 2012 (WHO Technical Report Series, No. 971).

11. *International travel and health*. Geneva, World Health Organization, 2012 (www.who.int/ith).

12. Rabies vaccines: WHO position paper. *Weekly Epidemiological Record*, 2010, 32(85):309–320.

13. *Human and dog rabies prevention and control: report of the WHO/Bill & Melinda Gates Foundation consultation, Annecy, France, 7–9 October 2009*. Geneva, World Health Organization, 2010 (WHO/HTM/NTD/NZD/2010.1) (http://whqlibdoc.who.int/hq/2010/WHO_HTM_NTD_NZD_2010.1_eng.pdf).

14. *Report of the 4th meeting of the international coordination group of the Bill & Melinda Gates Foundation/WHO project for human and dog rabies elimination in low-income countries, 2–4 October 2012, Cebu, Philippines*. Geneva, World Health Organization, 2013 (http://www.who.int/rabies/bmgf_who_project/en).

15. *Report of the 3rd meeting of the international coordination group of the Bill & Melinda Gates Foundation/WHO project for human and dog rabies elimination in low-income countries, 19–21 October 2011, PieterMaritzburg, KwaZulu-Natal, South Africa*. Geneva, World Health Organization, 2011 (http://www.who.int/rabies/bmgf_who_project/en/).

16. *Regional meeting on zoonotic diseases. Report of the meeting, Jakarta, Indonesia, 6–8 November 2007*. New Delhi, WHO Regional Office for South-East Asia, 2008 (SEA-CD-174).

17. *Report of the informal consultation to finalize a regional strategy framework for the elimination of human rabies transmitted by dogs in the South-East Asia Region, June 2011, Bangkok, Thailand*. New Delhi, WHO Regional Office for South-East Asia, 2012.

18. *15th inter-American meeting at ministerial level, on health and agriculture, Rio de Janeiro, Brazil, 11–12 June 2008*. Washington DC, WHO Regional Office for the Americas/Pan American Health Organization, 2008.

19. *Elimination of neglected diseases and other poverty-related infections*. Pan American Health Organization and World Health Organization. 49th Directing Council. 61st session of the Regional Committee. Washington DC, 2009 [resolution CD49.R19]. (http://new.paho.org/hq/dmdocuments/2009/CD49.R19%20(Eng.).pdf; accessed March 2013).

20. *Rabies: a looming threat*. Rome, Food and Agriculture Organization of the United Nations Animal Production and Health Division, 2010 (http://www.fao.org/ag/againfo/home/en/news_archive/AGA_in_action/2010_rabies.html).

21. *Manual of diagnostic tests and vaccines for terrestrial animals*, 6th ed. Paris, World Organisation for Animal Health, 2011.

22. *Terrestrial animal health code*. Paris, World Organisation for Animal Health, 2011 (http://www.oie.int/index.php?id=169&L=0&.htm; accessed 29 November 2012).

23. *Global conference on rabies control. Towards sustainable prevention at the source, Incheon-Seoul, Republic of Korea, 7–9 September 2011* [recommendations]. Paris, World Organisation for Animal Health, 2011 (http://www.oie.int/fileadmin/Home/eng/Conferences_Events/docs/pdf/recommendations/A_Recommendation_Global%20Rabies%20Conference%20Seoul_final.pdf).

24. Lembo T et al. Renewed global partnerships and redesigned roadmaps for rabies prevention and control. *Veterinary Medicine International*, 2011 (ID 923149, doi:10.4061/2011/923149).

25. Lembo T et al. The blueprint for rabies prevention and control: a novel operational toolkit for rabies elimination. *PLoS Neglected Tropical Diseases,* 2012, 6(2):e1388.

26. *Call for action: towards the elimination of rabies in the ASEAN Member States and the Plus Three Countries*. Jakarta, Association of Southeast Asian Nations, 2010 (http://www.aseanplus3-eid.info/ Rabies_Call_for_Action; accessed 10 December 2012).

27. *Report of the ASEAN/FAO/OIE/WHO rabies workshop, January 2012, Chiang Mai, Thailand*. Jakarta, Association of Southeast Asian Nations, 2012.

28. *Report of the second meeting of the Middle East and Eastern Europe Rabies Expert Bureau (MEEREB), Paris, France, June 5–8, 2012*. Lyon, 2012 (http://www.meereb.info/meetings-concrete-actions; accessed March 2013).

29. *Meeting report: expert consultation on rabies post-exposure prophylaxis, Stockholm, 15 January 2009*. Stockholm, European Centre for Disease Prevention and Control, 2009 (http://www.ecdc.europa.eu/en/publications/Publications/0906_MER_Expert_Consultation_on_Rabies_Post-exposure_Prophylaxis.pdf; accessed March 2013).

# 15. Research

## 15.1 Diagnostics

Although new techniques and protocols have been proposed for the diagnosis of rabies, especially in humans, over the past 10 years, the number of laboratory-confirmed human rabies cases reported is limited and represents an underestimate of the real impact of this neglected zoonotic disease, particularly in Africa and Asia. Better tests for rapid, economical diagnosis, with no loss of sensitivity or specificity, would therefore be welcome (*1–3*). For molecular methods, more universal primers, real-time RT-PCR and nested PCR assays, focus on viral genes other than N and G and improved sequencing protocols are needed, especially for developing countries where the diversity of lyssaviruses is poorly recognized.

International standards are lacking for determining the sensitivity of these techniques, making comparisons difficult. Such standards should be prepared for use in local laboratories in rabies-endemic countries in order to ensure proper evaluation of the new molecular techniques (*3*). Proficiency testing should be organized at international level to ensure reliable data on diagnoses and the incidence of rabies.

Lateral flow and other tests should be devised for rapid detection of rabies viral antigens in the field, with adequate validation according to international standards.

## 15.2 Epidemiology

The lack of accurate data on disease burden, which are required for setting regional and national priorities for research and control, results in a vicious circle of indifference and neglect (*4*). Better decentralized surveillance methods and more sensitive and specific laboratory techniques are therefore needed, including:

- diagnostic approaches based on validated protocols and specimens and evaluated under field conditions; and
- epidemiological models to better estimate the incidence of rabies. Recent research showed that the incidence of rabies in some countries was as much as 15 times higher than that in official reports (*5,6*). Further work on the design and local implementation of these models is encouraged as well as more accurate field data for incorporation into the models (*7*).

These methods and techniques should be used to generate:

- data on disease incidence, a critical input to epidemiological models of rabies and currently the main limitation to accurate estimates of disease burden;
- information on the epidemiology and population dynamics of rabies in natural mammalian host populations (*8,9,10*);
- information on the ecological patterns, frequency and extent of movement of infected animals in order to predict the spread of rabies; and
- extensive genomic and evolutionary analyses to establish the diversity of lyssavirus species and variants in order to identify the determinants of rabies spread. Integration of phylogeographical data with data on viral genetics is a powerful means for characterizing, predicting and ultimately preventing and controlling the spatial spread of rabies.

Recent observations suggest that bats are important lyssavirus reservoirs, and the virus variants associated with Chiroptera may occasionally spill over to other mammals, with potential adaptation and establishment (*11*). Evidence of direct exposure to bats is sometimes lacking in human rabies infections, and research is required on the epidemiology of bat lyssaviruses (*11*) and potential pathogenic mechanisms in such spillover infections. There have been no recent comprehensive studies of relevant hosts and viruses or alternative routes and unusual settings.

## 15.3 Molecular, genetic and epidemiological characterization of new viral isolates

Isolation of new viruses is being reported more and more frequently throughout the world (see section 2). Scientists who identify new lyssaviruses are encouraged to characterize the isolates promptly and to compare them with previously described species. It is particularly important to determine the epidemiology of new species (range of hosts, geographical distribution and significance

for domestic animals and humans) and to verify whether commercial rabies biologicals, such as vaccines and antibodies, protect against them.

## 15.4 Biological medical products

Currently, the recommended prophylaxis for people severely exposed to lyssaviruses is combined administration of rabies vaccine and immunoglobulins. Both products remain expensive for a significant portion of the target human population. Therefore, ways and means should be sought to decrease their cost and to find new therapeutic approaches. Moreover, although nearly all veterinary products are for pre-exposure use, there may be circumstances in which they would be useful for post-exposure prophylaxis of a naive animal. Validated protocols by product and species should be drawn up.

Several new approaches have been proposed. Reverse genetics involves use of negative-stranded RNA viruses as cloning and expression vectors, and newer, safer, more effective recombinant viruses, based for example on adenoviruses, and DNA and plant-based vaccines continue to receive attention (*12,13*). All genetically engineered rabies vaccines must comply with national and international biosafety guidelines.

If new lyssaviruses continue to be identified, especially in bats, vaccines with a broader protection spectrum will be needed. Production of multivalent vaccines by classical methods in cell culture or by molecular techniques (recombinant virus expressing chimeric G protein, insertion of various epitopes into the lyssavirus G protein) should be investigated. Activation of innate immune responses by novel vaccine carriers and adjuvants and their protection when used for post-exposure prophylaxis should be studied further.

Rabies immunoglobulins are a critical element of human rabies post-exposure prophylaxis, particularly after severe or multiple bites on the face by rabid carnivores. More research, development and assessment are needed of suitable immunoglobulins or alternatives, such as human monoclonal antibodies, in rabies prophylaxis (*14*) (see also section 6.8) to ensure wider access to passive immunization at a reduced cost.

In addition to standard laboratory potency tests for rabies immunoglobulin and other products to determine the concentration of virus neutralizing antibodies per unit volume, some measure of expected efficacy is desirable. Reproducible animal models should be found for assessing the effectiveness of various immunoglobulins and other products (monoclonal antibody cocktail) for in situ virus neutralization after infection. The in vivo half-lives of antibody preparations in relevant target tissues should be established for new preparations. The levels of antibody required for passive immunization and their duration should be determined, particularly for those based on human monoclonal antibodies.

The current mouse protection test for vaccine potency is fraught with difficulties, and more appropriate methods are needed to assess the antigenic content and its correlation with protection (see sections 6.3.1 and 7.2).

Appropriate animal models should be found for studying the pathogenesis and intensive care of human rabies patients (see section 5). No commercial antiviral therapy is available. Investigation of therapeutic approaches based on blocking interactions among viral proteins, targeting the viral replication complex, may lead to the development of new small molecules. Current research on short interfering RNA (*15*) should be extended.

A holistic approach should entail rapid intra-vitam diagnostics, intensive patient care, vaccination, administration of immunoglobulins, cytokines and antiviral therapy, as appropriate, and should be based on realistic animal models and inferences from successfully treated human cases.

Current translational and operational research on inactivated vaccines for veterinary use and new modes of delivery should continue to provide better, easier means for controlling rabies in the animal reservoir in tropical and resource-poor areas. Focused research and development is required to produce live replication-competent vaccines for oral and other routes, which are more effective in wildlife primary hosts such as raccoons, mongooses and skunks and safe for non-target species, including humans.

## 15.5 Human rabies prophylaxis

Shorter post-exposure prophylaxis regimens are being evaluated, such as a shortened Essen intramuscular regimen for immunocompromised patients and four-site intradermal regimens with 0.1 ml per site in association with rabies immunoglobulin (*16–18*). If they are found to be suitable, they will reduce the expense of travelling to clinics to receive multiple doses of rabies vaccine over extended periods and will probably improve compliance (*19*). Industry support of such regimens would be welcomed.

WHO encourages the incorporation of rabies vaccination into infant and child immunization programmes in areas where canine rabies is a major public health problem and there are no economic, logistical or programmatic obstacles.

Further studies are needed to find alternative routes of vaccine delivery, appropriate inexpensive devices and prefilled syringes to facilitate pre- and post-exposure prophylaxis for rabies (*20,21*).

## 15.6 Pathobiology

Lyssaviruses naturally infect neurons, resulting in dysfunction and death (*22,23*). Further studies are required to elucidate the molecular basis of the pathobiology of rabies virus in neurons and other tissues. Insights from pathobiological studies can be used in designing additional approaches for the therapy of rabies (*24,25*).

Comprehensive understanding of the pathobiology of lyssaviruses is lacking. Many studies have addressed the nature of the relations between lyssaviruses and their hosts, but the roles of different viral proteins and how they affect the host cellular machinery remain largely a mystery (*26,27*). Retrospective and prospective comparisons of the pathogenic processes in lyssaviruses found in nature to which humans are differentially susceptible could help to resolve the mystery.

Further research is needed on the factors that determine the ability of lyssaviruses to cross species barriers, from wild animal reservoirs to domestic animals and to humans, and their ability to spread in new animal host species. The role of innate immune responses in controlling host switching should also be investigated.

Relevant cell lines are needed to better appreciate the immunobiology of the Chiroptera and their pathogens.

## 15.7 Host ecology

Different species of mammals harbour different virus variants, and host identification is often difficult (e.g. among bat species). Means are needed for identifying different host species. The priorities for research include host identification, distribution and behaviour (e.g. in relation to disease transmission) and population dynamics in relation to disease persistence.

Research should be conducted on innovative attractive baits and on improving vaccine bait delivery to species such as mongooses, skunks, raccoons and dogs.

Development of cost–effective, large-scale oral rabies vaccination strategies in vast areas where rabies is endemic should be continued, taking into account the ecology of the primary host, bait density and the timing and frequency of campaigns.

Novel immunocontraceptive products could improve the management of animal populations in mass vaccination strategies (*28*).

## 15.8 References

1. Durr S et al. Rabies diagnosis for developing countries. *PLoS Neglected Tropical Diseases*, 2008, 2:e206.
2. Lembo T et al. Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerging Infectious Diseases*, 2006, 12:310–313.
3. Dacheux L et al. More accurate insight into the incidence of human rabies in developing countries through validated laboratory techniques. *PLoS Neglected Tropical Diseases*, 2010, 4(11):e765.

4. Knobel DL et al. Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization*, 2005, 83:360–368.

5. Cleaveland S et al. Estimating human rabies mortality in the United Republic of Tanzania from dog bite injuries. *Bulletin of the World Health Organization*, 2002, 80:304–310.

6. Ly S et al. Rabies situation in Cambodia. *PLoS Neglected Tropical Diseases*, 2009, 3:e511.

7. Hampson K et al. Transmission dynamics and prospects for the elimination of canine rabies. *PLoS Biology*, 2009, 7:e53.

8. Zinsstag J et al. Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, 106:14996.

9. Bourhy H et al. The origin and phylogeography of dog rabies virus. *Journal of General Virology*, 2008, 89:2673–2681.

10. Kuzmin IV et al. Molecular inferences suggest multiple host shifts of rabies from bats to mesocarnivores in Arizona during 2001–2009. *PLoS Pathogens*, 2012, 8(6):e1002786.

11. Streicker DG et al. Rates of viral evolution are linked to host geography in bat rabies. *PLoS Pathogens*, 2012, 8(5):e1002720.

12. Bahloul C et al. Field trials of a very potent rabies DNA vaccine which induced long lasting virus neutralizing antibodies and protection in dogs in experimental conditions. *Vaccine*, 2006, 24:1063–1072.

13. Wu X et al. Development of combined vaccines for rabies and immunocontraception. *Vaccine*, 2009, 27:7202–7209.

14. Bakker AB et al. First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. *Vaccine*, 2008, 26:5922–5927.

15. Israsena N, Mahavihakanont A, Hemachudha T. Rabies virus infection and microRNAs. *Advances in Virus Research*, 2011, 79:329–344.

16. Sudarshan MK et al. Evaluation of a one week intradermal regimen for rabies post-exposure prophylaxis: results of a randomized open label, active controlled trial in healthy adult volunteers in India. *Human Vaccines and Immunotherapeutics*, 2012, 8(8):1–5.

17. Prapimporn S et al. Postexposure rabies prophylaxis completed in 1 week: preliminary study. *Clinical Infectious Diseases*, 2010, 50(1):56–60.

18. Warrell M et al. A simplified 4-site economical intradermal post-exposure rabies vaccine regimen: a randomised controlled comparison with standard methods. *PLoS Neglected Tropical Diseases*, 2008, 2:e224.

19. Hampson K, Cleaveland S, Briggs D. Evaluation of cost–effective strategies for rabies post-exposure vaccination in low-income countries. *PLoS Neglected Tropical Diseases*, 2011, 5:e982.

20. Laurent PE et al. Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults. *Vaccine*, *2010, 28(36):5850–5856.*

21. Program for Appropriate Technology in Health (PATH). *Intradermal delivery of vaccines*. Seattle, Washington, 2010–2013 (http://sites.path.org/deliverytech/id/).

22. Schnell MJ et al. The cell biology of rabies virus: using stealth to reach the brain. *Nature Reviews Microbiology*, 2010, 8:51–61.

23. Rieder M, Conzelmann KK. Interferon in rabies virus infection. *Advances in Virus Research*, 2011, 79:91–114.

24. Jackson AC. Update on rabies diagnosis and treatment. *Current Infectious Disease Reports*, 2009, 11:296–301.

25. Jackson AC. Therapy of rabies encephalitis. *Biomedica*, 2009, 29:169–176.

26. Thanomsridetchai N et al. Comprehensive proteome analysis of hippocampus, brainstem, and spinal cord from paralytic and furious dogs naturally infected with rabies. *Journal of Proteome Research*, 2011, 10(11):4911–4924.

27. Reinke SN et al. Metagenomic and metabolomic characterization of rabies encephalitis: new insights into the treatment of an ancient disease. *Journal of Infectious Diseases*, 2012 (doi: 10.1093/infdis/jis479).

28. Carroll MJ et al. The use of immunocontraception to improve rabies eradication in urban dog populations. *Wildlife Research*, 2010, 37:1–12.

## Concluding remarks

The meeting was closed by Dr Hiroki Nakatani, Assistant Director-General of the WHO cluster for HIV/AIDS, Tuberculosis, Malaria and Neglected Tropical Diseases, and Dr F.X. Meslin. Dr Nakatani thanked the participants, observers and representatives of other international governmental and nongovernmental organizations on behalf of the Director-General of WHO, Dr Margaret Chan, for their work and support for a neglected zoonosis with a substantial public health and economic impact. In particular, he thanked the heads of the WHO collaborating centres and members of the WHO Expert Advisory Panel on Rabies. Dr Nakatani stressed the need for strong, effective, cross-sectoral collaboration on the human–animal interface for rabies control, and welcomed the participation of FAO and OIE in the Consultation. He noted with appreciation the interest of private manufacturers of human and animal rabies vaccines.

Dr Nakatani described the significant health and economic burden represented by rabies, incurring use of 70 million doses of human rabies vaccines in an estimated 20 million people, mostly in developing countries; the societal cost of rabies worldwide is estimated to be in excess of US$ 6 billion including US$ 1.6 billion spent on post-exposure prophylaxis. Those figures will continue to escalate as the demand for safe cell-culture human vaccines increases. Dr Nakatani confirmed the conclusion of the Consultation that human dog-transmitted rabies is readily amenable to control, regional elimination in the medium term and even global elimination in the long term. He closed the Consultation, commenting that a resolution on major neglected tropical diseases, including rabies, was being prepared for submission to the World Health Assembly in May 2013 in the expectation of securing Member States' commitment to the control, elimination or eradication of these diseases. Endorsement of the resolution would open the door for exciting advances in rabies prevention and control.

## Acknowledgements

The Expert Consultation and the WHO Secretariat acknowledge the special contributions to drafting the background documents made by Dr K. Hampson, Dr I. Kuzmin, Dr T. Hemachuda, Dr C. Rupprecht, Professor S. Madhusudana, Dr D. Briggs, Dr H. Ertl, Dr H. Wilde, Dr F. Cliquet, Dr M.K. Sudarshan, Dr B. Quiambao, Dr A. Rahman, Dr E. Russell, Dr G. Massei, Dr A. Wandeler, Dr T. Müller, Professor S. Cleaveland, Dr M. Vigilato and Dr H. Bourhy.

The financial contribution of the Bill & Melinda Gates Foundation is gratefully acknowledged.

# Annexes

## Annex 1. List of participants

### Heads of WHO collaborating centres

Dr Hervé Bourhy, WHO Collaborating Centre for Reference and Research on Rabies, Institut Pasteur, Paris, France

Dr Florence Cliquet, WHO Collaborating Centre on Research and Management on Zoonoses Control, AFSSA-LERPAS, Laboratoire d'études sur la rage et la pathologie, des animaux sauvages, Malzéville, France

Dr Bernhard Dietzschold, WHO Collaborating Centre for Neurovirology, Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, USA

Dr Hildegund Ertl, WHO Collaborating Centre for Reference and Research on Rabies, Immunology Program Leader, The Wistar Institute, Philadelphia,USA

Dr Anthony Fooks, WHO Collaborating Centre for the Characterization of Rabies and Rabies-related Viruses, Animal Health and Veterinary Laboratries Agency, Weybridge, England

Dr Thiravat Hemachudha, WHO Collaborating Centre for Research and Training on Viral Zoonoses, Member of the WHO Expert Advisory Panel on Rabies, Professor of Neurology, Neurology Division, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand

Dr Rattan Lal Ichhpujani, WHO Collaborating Centre for Rabies Epidemiology, Member of the WHO Expert Advisory Panel on Rabies, Additional Director, Microbiology Department, Centre for AIDS and Related Diseases, National Centre for Disease Control, Delhi, India

Professor S.N. Madhusudana, WHO Collaborating Centre for Reference and Research in Rabies, Member of the WHO Expert Advisory Panel on Rabies, Department of Neurovirology, National Institute of Mental Health and Neurosciences, Bangalore, India

Dr Thomas Müller, Head, WHO Collaborating Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut, Federal Research Institute for Animal Health, Greifswald-Insel Reims, Germany

## Members of the WHO Expert Advisory Panel on Rabies

Dr Ahmad Fayaz, Former Head, Rabies Laboratory, Pasteur Institute, Tehran, Islamic Republic of Iran

Professor Louis Hendrik Nel, Professor of Virology, Department of Microbiology and Plant Pathology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hillcrest, South Africa, President of the South Eastern Africa Rabies Group (SEARG) (*Chair*)

Dr Beatriz P. Quiambao, Chief, Clinical Research Division, Research Institute for Tropical Medicine, Philippines. President of the Rabies Asia Foundation (RIA)

Dr Charles E. Rupprecht, Former Chief, Rabies Section, Centers for Disease Control and Prevention, Atlanta, USA

Dr Naseem Salahuddin, Indus Hospital, Korangi, Karachi, Pakistan (*Rapporteur*)

Professor Dr Mysore K. Sudarshan, Dean, Principal and Professor of Community Medicine, Kempegowda Institute of Medical Sciences, Bangalore, India. President of the Rabies Asia Foundation (RIA)

Dr Alexander Wandeler, Former Head, rabies laboratory, Canadian Food Inspection Service, Scientist Emeritus, Carp, Canada

## Other experts

Professor Sarah Cleaveland, Consultant, University of Glasgow, Glasgow, Scotland

Dr Raffy Deray, National Program Manager, National Rabies Prevention and Control Program, National Center for Disease Prevention and Control, Department of Health, Manila, Philippines

Dr Katie Hampson, Research Scientist, Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Scotland

Mr Kevin Le Roux, Rabies Project Management, Veterinary Services, Pietermaritzburg, KwaZulu-Natal, South Africa

Dr Giovanna Massei, Ecologist, Food and Environment Research Agency, York, England

Dr Mathew Maziku, National Project Coordinator, Rabies, WHO Country Office, Dar es Salaam, United Republic of Tanzania

Dr Maria P. Rebollo, Scientific Manager, Monitoring and Evaluation, Liverpool School of Tropical Medicine, Centre for Neglected Tropical Diseases, Liverpool, England

Dr Graham Smith, Senior Researcher, Food and Environment Research Agency, York, England

Dr Mathurin Cyrille Tejiokem, Epidemiologist, Epidemiology and Public Health Laboratory, Centre Pasteur du Cameroun, Yaoundé, Cameroon

Dr Henry Wilde, Professor of Medicine, Senior Consultant, WHO Collaborating Centre for Zoonoses and Rabies, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

**Representatives of other organizations**

*Intergovernmental organizations*

**World Organisation for Animal Health (OIE)**

Dr Marta Martinez, Veterinary Epidemiologist

Dr Dietrich Rassow, Chargé de mission, WHO

*Nongovernmental organizations*

**Association for the Prevention and Control of Rabies in India**

Dr A. Rahman, President, Commonwealth Veterinary Association for Prevention and Control of Rabies in India, Department of Community Medicine, Bangalore, India

**Dodet Science**

Dr Betty Dodet, Caluire et Cuire, France

**Global Alliance for Rabies Control**

Dr Kim Doyle, Trustee of the Alliance for Rabies Control, Global Alliance for Rabies Control c/o Balfour and Manson, Edinburgh, Scotland

Ms Maylin Meincke, Development Studies, University of Helsinki, Helsinki, Finland

Dr Elizabeth Miranda, Asian Coordinator, Global Alliance for Rabies Control, Laguna, Philippines

**Marwar Trust**

Mr Federico Spinola, Founder, Partnership for Animals, Geneva, Switzerland

**PATH**

Dr Darin Zehrung, Technical Officer, Portfolio Leader Vaccine Delivery Technologies, Seattle, USA

**World Society for the Protection of Animals**

Dr Elly Hiby, Scientific Advisor, World Society for the Protection of Animals, Cambridge, England

Dr Esmée Russell, Campaign Manager, World Society for the Protection of Animals, London, England

## Observers

Dr Michaël Attlan, Director, Traveler Endemic and Emerging Vaccines Franchise, Sanofi-Pasteur, Lyon, France

Dr Jac Bergman, Global Marketing Director, Small Animal Vaccines, Global Companion Animal Business Unit, Boxmeer, the Netherlands

Dr Rachel Chikwamba, Technical Lead, Rabies Initiatives, Pretoria 0001, South Africa

Dr Pradip Desai, Director, Span Diagnostics Ltd, Surat, India

Dr Alexandra Giesen, Novartis Vaccines and Diagnostics, Global Medical Affairs, Munich, Germany

Dr Reinhard Glueck, CSO, Zydus Cadila Healthcare, Zydus Research Centre, Gujarat, India

Dr Françoise Guinet-Morlot, Project Director New Vaccines, Sanofi Pasteur, Marcy l'Etoile, France

Dr Gaurav Gupta, Head, Viral Vaccines, Zydus Cadila, Zydus Research Centre, Gujarat, India

Dr K. Jager, Intervet, Boxmeer, the Netherlands

Dr Philipe Mahl, Rabies Programme Manager, Virbac, Carros, France

Dr Joanne Maki, Veterinary Public Health, Global Public Health Director, Athens, Georgia, USA

Dr Claudius Malerczyk, Head, Medical Affairs, Middle East and Africa, Novartis Vaccines and Diagnostics, Marburg, Germany

Dr Stephanus Francois Marais, Commercialisation Manager, CSIR Biosciences, Pretoria, South Africa

Dr Wilfred Marissen, Programme Director, Crucell Holland B.V., Leiden, the Netherlands

Dr Anvar Rasuli, Medical Product Leader, Global Medical Affairs, Sanofi Pasteur, 2 Avenue Pont Pasteur, Lyon, France

Dr Micha Roumiantzeff, Fondation M. Merieux, 1 rue Dangon, 69004 Lyon, France

Dr Carolin Schumacher, Director, Corporate Public Affairs, Merial, Lyon, France

Dr Daniela Todorova-Balvay, R&D Manager, Span Diagnostics SARL, Compiegne, France

Dr Adriaan Vos, Head, Vaccine Development Technologies, IDT Biologika GmbH, Dessau-Rosslau, Germany

## WHO secretariat

### WHO headquarters, Geneva, Switzerland

Dr Hiroki Nakatani, Assistant Director-General, HIV/AIDS, Tuberculosis, Malaria and Neglected Tropical Diseases

Dr Lorenzo Savioli, Director, Department of Control of Neglected Tropical Diseases

Dr François Meslin, Team Leader, Neglected Zoonotic Diseases, Department of Control of Neglected Tropical Diseases (*Organizer and convener*)

Dr Bernadette Abela-Ridder, Foodborne Disease and Epidemiology, Food Safety, Zoonoses and Foodborne Diseases

Dr Simone Magnino, Foodborne Disease and Epidemiology, Food Safety, Zoonoses and Foodborne Diseases

Dr Arve Willingham, Special Programme for Research and Training in Tropical Diseases (TDR)

Dr Martin Friede, Innovation, Innovation, Evidence and Research

Ms Erin Sparrow, Technology Transfer Initiative, Innovation, Information, Evidence and Research

Dr Philippe Duclos, Immunization, Vaccines and Biologicals

Dr Ivana Knezevic, Quality, Safety and Standards, Immunization, Vaccines and Biologicals

Dr Jinho Shin, Quality, Safety and Standards, Immunization, Vaccines and Biologicals

Dr David Wood, Coordinator, Quality, Safety and Standards, Immunization, Vaccines and Biologicals

Dr Ana Padilla, Quality Assurance and Safety: Medicines

Mrs Beatrice Wamutitu, Secretary, Neglected Zoonotic Diseases, Department of Control of Neglected Tropical Diseases

### WHO Regional Office for Africa

Dr Landry Bidé, Medical Officer, Neglected Tropical Diseases, Brazzaville, Congo

### WHO Regional Office for the Americas/Pan American Health Organization

Dr Alfonso Clavijo, Veterinary Public Health, Panamerican Centre for Foot-and-Mouth Disease, São Bento, Duque de Caxias, Rio de Janeiro, Brazil

Dr Marco Vigilato, Veterinary Public Health, Panamerican Centre for Foot-and-Mouth Disease, São Bento, 25045-002 Duque de Caxias, Rio de Janeiro, Brazil

### WHO Regional Office for South-East Asia

Dr Gyanendra Gongal, Scientist, Veterinary Public Health, Disease Surveillance and Epidemiology, New Delhi, India

### Invited but unable to attend

Dr Katinka de Balogh, Senior Officer, Veterinary Public Health, Food and Agriculture Organization of the United Nations, Rome, Italy

Dr Philip Binu, Business Development, Zydus Research Centre, Gujarat, India

Dr Deborah Briggs, Executive Director, Global Alliance for Rabies Control, c/o Balfour and Manson, Edinburgh, Scotland

Dr Yu Hongjie, Director, Division of Infectious Disease Control, Centre for Disease Control, Beijing, China

Dr Ivan V. Kuzmin, Rabies Program, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Dr Tiziana Lembo, Institute of Comparative Epidemiology, Faculty of Veterinary Medicine, University of Glasgow, Glasgow, Scotland

Dr Anastasia Pantelias, Bill & Melinda Gates Foundation, Seattle, Washingthon, USA

Dr A. Rowan, Humane Society International, Washington, District of Columbia, USA

Dr Louis Taylor, PRP Coordinator, Global Alliance for Rabies Control, Manhattan, Kansas, USA

## Annex 2. Record form for cases of possible exposure to rabies

Case no.: Date: Time:

**Patient**

Name: Age: Sex:

Address:

Telephone (home and mobile):

Telephone (work):

GP and Tel:

**Details of exposure**

Country and town:

Date of exposure:

Date of travel:

Nature of exposure: bite/lick/saliva/scratch/other (to specify)

Site of exposure:

Was the skin broken? Yes/No

Did the wound/s bleed? Yes/No

Number of wounds:

Depth of bite/s: superficial/deep

Category of exposure:

**Details of animal**

Type of animal/species:

Wild/domestic:

Provoked/unprovoked (give details):

Is the animal's owner/home known? Yes/No:

Were efforts made to trace the animal? Yes/No:

When was the animal last seen alive?

Animal's vaccination status, if known:

**Previous rabies vaccination history of the patient**

Did s/he have a 3-dose intramuscular/intradermal pre-exposure rabies vaccination? Yes/No

Details:
Was anti-rabies post-exposure prophylaxis given previously? Yes/No
Which rabies vaccine was given?
Details (day/date etc.):
Was rabies immunoglobulin given? Yes/No
Locally/systemically: Other information
Contact on-call virologist/physician for advice with above information
If unavailable, contact:

**Recommended treatment**

- 1. Wound washing using water/ soap/ antiviral agent.
- 2. Rabies vaccination:
- Modified course of treatment for those with previous pre-exposure prophylaxis: days 0 and 3
- Standard course for unvaccinated:
  - Intramuscular – days 0, 3, 7, 14, 28 or 0, 3, 7, 14
  - Intramuscular – days 0 (2 doses), 7, 21
  - Intradermal – days 0 (2 sites), 3 (2 sites), 7 (2 sites), 28 (2 sites)
- 3. Rabies immunoglobulin: human rabies immunoglobulin, 20 IU/kg body weight; equine rabies immunoglobulin, 40 IU/kg body weight

Injection site:
Patient weight (kg): Volume recommended (IU and ml):
Post-exposure course arranged? Yes/No
General practitioner informed via letter/e-mail/phone/SMS text? Yes/No
Name, telephone and signature of completing physician

## Annex 3. Four steps for replacing nervous tissue vaccine by modern rabies vaccines produced on cell culture or embryonated eggs

Countries that are still producing or using neural tissue-based vaccines should follow this proposed four-step strategy to replace nerve tissue vaccines by modern vaccines.

**Step 1**: Relevant national authorities, usually under the leadership of national health authorities, must make the final decision to change from nerve tissue vaccines to modern vaccines. After reviewing the safety, immunogenicity and efficacy of modern vaccines, the authorities should evaluate the local conditions, and assess the feasibility and cost of replacing nerve tissue vaccine. Consideration should be given to the use of the cost-saving intradermal regimens for rabies pre- and post-exposure prophylaxis.

**Step 2:** National guidelines should be formulated that give clear instructions on modern vaccines for pre- and post-exposure prophylaxis, including indications for their use and routes of administration; similarly, guidance should be given for rabies immunoglobulin and other products. The guidelines should be drawn up by technically competent experts on the basis of the recommendations in reports of the WHO Expert Advisory Group on Rabies, other WHO advisory groups, up-to-date scientific literature, the experience of international and national experts and observations. They should be disseminated to all centres that provide pre- and post-exposure prophylaxis. The guidelines must be based on clear policies concerning, e.g. vaccine subsidy (if any) and handling leftover vaccine, and should be regularly updated.

**Step 3:** Rabies centres should receive a constant supply of safe, effective, WHO-recommended rabies vaccines and immunoglobulin from a central office. Once the decision is made to stop nerve tissue vaccine production and use, the procurement of modern vaccines should start, to avoid any gap in provision of treatment once the nerve tissue vaccine supplies run out. Coordination with regulatory bodies for registration of new rabies biologicals and for post-marketing surveillance of new rabies vaccines and rabies immunoglobulin is also important.

**Step 4**: A network of specialized bite centres should be set up, in which the staff are trained in giving pre- and post-exposure prophylaxis and managing adverse reactions; adequate quantities of rabies biologicals at these centres must be ensured. A referral system should be established to maximize the benefit of the intradermal regimen and to reduce the amount of leftover vaccine. A quality assurance system should be instituted, with standards that are followed by all centres. Provincial and municipal governments should be involved in establishing new centres, ensuring a sustainable supply of rabies vaccines, immunoglobulin and other supplies and guaranteeing reporting, investigation of human rabies cases and monitoring of the rabies programme.

## Annex 4. Technique for intradermal administration of rabies vaccine and precautions to be taken

Intradermal administration can be used in all countries in which the intradermal route has regulatory approval for pre- or post-exposure prophylaxis for rabies.

The vaccines administered must be licensed for administration by this route and recommended by WHO (see section 5.1).

Intradermal administration should not be used for immunocompromised individuals or individuals receiving chloroquine-based antimalarial treatment or long-term corticosteroid or other immunosuppressive therapy.

As the volume of an intradermal vaccine dose is smaller than an intramuscular dose, the intradermal route is especially suitable for treating many patients at the same centre within a short time, i.e. within the recommended period of 6–8 h after reconstitution of the vaccine. As the currently available vaccines do not contain preservatives, they must be refrigerated after reconstitution and must be discarded after 6–8 h.

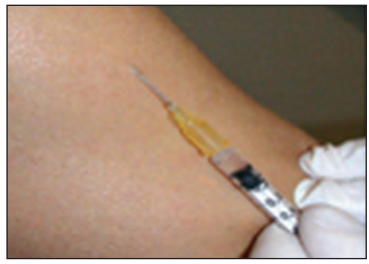
The intradermal route is more cost–effective than the standard intramuscular route and is therefore appropriate when vaccine and money are in short supply and in centres where exposed patients are treated.

### Preliminary steps

Before administering rabies vaccine intradermally:

- All staff must be adequately trained in the intradermal injection technique.
- If the vaccine is given as part of post-exposure prophylaxis, the additional steps should be followed; i.e. the wound must be washed and, if applicable, the appropriate dose of rabies immunoglobulin administered.
- An appropriate 1.0-ml syringe (insulin or tuberculin syringe) and a short, fine hypodermic needle should be used. More costs are saved if a fixed-needle syringe is used, as the void volume is reduced.
- The intradermal schedule should be selected. WHO recommends the 2-2-2-0-2 updated Thai Red Cross schedule (see section 8.3.3).

**Step 1**

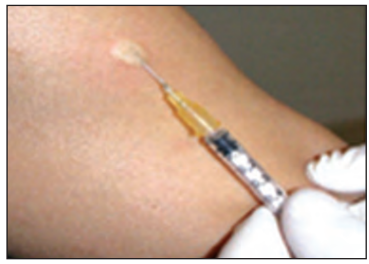


Aseptically reconstitute the vaccine immediately before administration with the appropriate volume of diluent provided by the manufacturer. Do not use a different diluent. Do not use a different amount of diluent.

Draw enough vaccine into the syringe to inject a single patient, using appropriate sterile precautions. Carefully remove any air bubbles.

Disinfect the injection site with antiseptic, then stretch the surface of the skin and insert the tip of the needle (bevelled edge facing upwards) into the upper layer of the skin (dermis), ensuring that the needle and syringe are almost parallel to the skin surface.

**Step 2**

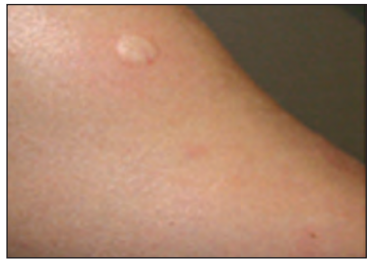


Begin injecting the vaccine. If the needle is in the correct position, there is considerable resistance.

A raised papule, which looks like orange peel, will appear immediately, measuring 6–8 mm in diameter.

If the vaccine is injected easily, or if the papule does not appear, it has been given subcutaneously, i.e. too deeply. In such cases, the correct injection should be repeated.

**Step 3**



Once all doses of 0.1 ml of vaccine have been injected into the same patient, discard the needle and the syringe.

Reconstituted vaccine can be used for more than one patient; however, a sterile syringe and needle must be used to draw up vaccine for each patient.

The reconstituted vaccine must be stored in a refrigerator at 2–8 °C and used within 6–8 h.

# Annex 5. Recommended post-exposure prophylaxis according to type of exposure

| Category of exposure | Type of exposure to a domestic or wild[a] animal suspected or confirmed to be rabid, or animal unavailable for testing | Recommended post-exposure prophylaxis |
|---|---|---|
| I | Touching or feeding animals<br>Licks on intact skin<br>Contact of intact skin with secretions or excretions of a rabid animal or human case | None, if reliable case history is available |
| I I | Nibbling of uncovered skin<br>Minor scratches or abrasions without bleeding | Administer vaccine immediately[b]<br>Stop treatment if animal remains healthy throughout an observation period of 10 days[c] or is proven to be negative for rabies by a reliable laboratory using appropriate diagnostic techniques. |
| I I I | Single or multiple transdermal bites[d] or scratches, licks on broken skin<br>Contamination of mucous membrane with saliva (i.e. licks)<br>Exposure to bats[e] | Administer rabies vaccine immediately, and rabies immunoglobulin, preferably as soon as possible after initiation of post-exposure prophylaxis. Rabies immunoglobulin can be injected up to 7 days after first vaccine dose administration.<br>Stop treatment if animal remains healthy throughout an observation period of 10 days or is proven to be negative for rabies by a reliable laboratory using appropriate diagnostic techniques. |

[a] Exposure to rodents, rabbits or hares does not routinely require rabies post-exposure prophylaxis.

[b] If an apparently healthy dog or cat in or from a low-risk area is placed under observation, treatment may be delayed.

[c] This observation period applies only to dogs and cats. Except for threatened or endangered species, other domestic and wild animals suspected of being rabid should be euthanized and their tissues examined for the presence of rabies antigen by appropriate laboratory techniques.

[d] Bites especially on the head, neck, face, hands and genitals are category III exposures because of the rich innervation of these areas.

[e] Post-exposure prophylaxis should be considered when contact between a human and a bat has occurred, unless the exposed person can rule out a bite or scratch or exposure of a mucous membrane.

## Annex 6. Suggested rabies vaccination certificates for humans

The vaccination certificates below are provided as models. The certificates should be kept carefully by the vaccinated person with his or her personal health documents. Blank certificates should be supplied by the manufacturer of the vaccines.

### Certificate of pre-exposure vaccination against rabies

Name_____

Date of birth/Age (years)_____ Sex_____Occupation_____

Address_____

_____

Tel. no. _____

Signature _____

### Primary vaccination

| Date of vaccination | Day 0 | Day 7 | Day 21 or 28 |
|---|---|---|---|
| Vaccination centre/Place | | | |
| Type/Name of vaccine | | | |
| Manufacturer (batch no.)/Expiry date | | | |
| Dose (ml) | | | |
| Route of administration (intramuscular or intradermal) | | | |
| Site of vaccination | | | |
| Adverse event, if any | | | |
| Rabies virus neutralizing antibody titre, if done/Method | | | |
| Signature of physician | | | |

### Booster doses for people at high risk of exposure

| **Date of booster vaccination** | | | |
|---|---|---|---|
| Vaccination centre/Place | | | |
| Type/Name of vaccine | | | |
| Manufacturer (batch no.)/Expiry date | | | |
| Dose (ml) | | | |
| Route of administration (intramuscular or intradermal) | | | |
| Site of vaccination | | | |
| Adverse event, if any | | | |
| Rabies virus neutralizing antibody titre, if done/Method | | | |
| Signature of physician | | | |

### Certificate of post-exposure vaccination against rabies

Name_____

Date of birth/Age (years)_____Sex_____ Occupation _____

Address _____

_____

Tel. no. _____

Date of exposure_____WHO category of exposure_____Biting animal_____ Healthy/Sick _____Animal vaccination status_____Rabies virus neutralizing antibody titre/Method_____

Observations after 10 days (when relevant) _____

_____

1. Wound washed with water/soap/antiviral agent_____
2. Rabies immunoglobulin:

   Date of treatment_____ Clinic/hospital name _____

   Place_____

   Name/Type of rabies immunoglobulin (human/equine) _____

   _____

Manufacturer (batch no./Expiry date)_____

Weight of patient_____kg. Dose (IU)_____Total volume (ml)_____

Rabies immunoglobulin infiltrated into and around wound / intramuscular (ml) _____

Remaining immunoglobulin injected at site away from site of vaccine injection intramuscularly (ml)_____

3. Rabies vaccine:

   Vaccine regimen:

   Five-dose Essen (1-1-1-1-1) or four-dose Essen (1-1-1-1-0)

   Zagreb (2-1-1)

   Updated Thai Red Cross two-site intradermal (2-2-2-0-2)

   Other

| **Date of vaccination** | **Day 0** | **Day 3** | **Day 7** | **Day 14** | **Day 21** | **Day 28** |
|---|---|---|---|---|---|---|
| Vaccination centre/ Place | | | | | | |
| Type/Name of vaccine | | | | | | |
| Manufacturer (batch no.)/Expiry date | | | | | | |
| Dose (ml) | | | | | | |
| Route of administration (intramuscular or intradermal) | | | | | | |
| Site of vaccination | | | | | | |
| Adverse event, if any | | | | | | |
| Rabies virus neutralizing antibody titre, if done/Method | | | | | | |
| Signature of physician | | | | | | |

**General remarks (if any)**

## Annex 7. International rabies vaccination certificate for dogs, cats and ferrets

The vaccination certificate below is provided as a model. It is based on the OIE certificate.[1] Some countries may require additional information.

**Certificat international de vaccination antirabique pour chiens, chats et furets/ International rabies vaccination certificate for dogs, cats and ferrets**

I. Propriétaire/Owner

Nom et adresse/Name and address _____

_____

_____

_____

II. Signalement/Description

Espèce/Species _____

Age ou date de naissance (si possible)/Age or date of birth (when known)

_____

Sexe/Sex _____

Race/Breed _____

Robe/Coat colour _____

Type de pelage et marques/signes particuliers/Coat type and marking/distinguishing marks _____

Numéro de micro chip/Microchip no._____

Type de lecteur du micro chip/Microchip scanner type _____

Emplacement du micro chip/Location of microchip_____

Numéro et emplacement du tatouage (si présent)/Location and tattoo number (if applicable) _____

III. Vaccinations antirabiques/Rabies vaccinations

Le soussigné certifie avoir vacciné contre la rage l'animal décrit à la page 1, comme il est indiqué ci-après. Au moment de la vaccination, l'animal a été reconnu en bonne santé.

The undersigned declares herewith that she or he has vaccinated the animal described on page 1 against rabies, as shown below. The animal was found to be healthy on the day of vaccination.

[1] *Terrestrial animal health code* [vol. 2, chapter 8.10: Rabies]. Paris, World Organisation for Animal Health, 2011 (http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.8.10.htm; accessed 21 September 2012).

| (1) Date de vaccination/Vaccination date | (2) Nom du vaccin/ Name of vaccine | (3) Nom du fabricant/ Name of manufacturer | (4) Numéro de lot/ Batch no. | (5) Date d'expiration/ Expiry date | (6) signature et cachet du vétérinaire officiel/ Signature and stamp of official veterinary surgeon | (7) Valable jusqu'au/ Valid until |
|---|---|---|---|---|---|---|
| | | | | | | |

IV. Tests sérologiques antirabiques/Rabies serological tests
Déclaration du vétérinaire/Veterinary declaration

Je soussigné(e) certifie avoir pris connaissance des résultats officiels du test sérologique pratiqué sur l'animal décrit ci-dessus à la date du (jj/mm/aa)_____, conduit par un laboratoire agréé confirmant que le titre d'anticorps neutralisants anti-rage était supérieur ou égal à 0.5 UI/ml.

Période de validité:

Nom, date, et cachet du vétérinaire officiel

I have seen an official record of the result of a serological test for the animal, carried out on a sample taken on (dd/mm/yy)_____ and tested in an approved laboratory, which states that the rabies-neutralizing antibody titre was equal to or greater than 0.5 IU/ml.

Period of validity:

Name, date and signature of the authorized veterinarian:

Tests supplémentaires/Further tests:

| Date | Résultat/ Result | Laboratoire agréé/ Approved laboratory | Signature et cachet du vétérinaire/ Signature and stamp of veterinary surgeon |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

V. Autres vaccinations/Other vaccinations

| Date | Vaccin utilisé/ Type of vaccine | Numéro de lot/ Batch no. | Signature et cachet du vétérinaire/ Signature and stamp of veterinary surgeon |
|---|---|---|---|
| | | | |
| | | | |
| | | | |

VI. Informations complémentaires/Additional information

Pays d'origine/Country of origin_____
_____

Pays dans lesquels l'animal a séjourné, selon les déclarations du propriétaire (indiquer les dates)/Countries visited by the animal as declared by the owner (give dates)_____
_____
_____

**Notes:**

Le présent certificat ne dispense pas de l'application des autres dispositions en vigueur pour l'entrée dans chaque pays. Prière de lire la section VII.

This certificate may not be sufficient to meet all the requirements of the countries of destination. Please read Section VII.

Autorisation d'imprimer délivrée par (indiquer l'autorité nationale compétente): Printing authorized by (indicate the national responsible authority):

Pour être valable, le présent certificat doit porter un numéro perforé à chaque page. To be valid, this certificate must bear a number perforated on each page.

VII. Passage de frontière/Frontier crossing

Le propriétaire de l'animal doit, avant de se rendre à l'étranger avec celui-ci, s'assurer des conditions sanitaires imposées par les autorités du pays de destination, le présent certificat ne dispensant pas de l'application des autres dispositions en vigueur dans certains pays.

The owner of the animal must, before going abroad with it, make sure of the veterinary requirements laid down by the authorities of the country of destination, as this certificate may not be sufficient to meet all the requirements of the country of destination.

Le présent certificat est valable à partir du trentième jour et jusqu'à la fin du douzième mois après la date de la première vaccination ; dans le cas d'une revaccination au cours de la période de validité, pendant les douze mois qui suivent la date de revaccination.

This certificate is valid from the 30th day until the end of the 12th month after the date of the first vaccination; in the case of revaccination within the validity period, for 12 months from the date of revaccination.

Le présent certificat doit être imprimé et complété en Français et en Anglais, et si nécessaire, dans la langue du pays d'origine.

This certificate must be printed and completed in French and English and, if necessary, the language of the country of origin.

## Annex 8. WHO collaborating centres on rabies, neurovirology, viral zoonoses and zoonoses control

WHO Collaborating Centre for Reference and Research on Rabies, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris, France
Head, Dr Hervé Bourhy; e-mail: herve.bourhy@pasteur.fr

WHO Collaborating Centre on Research and Management on Zoonoses Control, AFSSA-LERPAS, Laboratoire d'études sur la rage et la pathologie, des animaux sauvages, Domaine de Pixérécourt, BP 9, 54220 Malzéville, France
Head, Dr Florence Cliquet; e-mail: florence.cliquet@anses.fr

WHO Collaborating Centre for the Characterization of Rabies and Rabies-related Viruses, Animal Health and Veterinary Laboratories Agency, Weybridge, Surrey KT15 3NB, United Kingdom
Head, Dr Anthony Fooks; e-mail: t.fooks@ahvla.gsi.gov.uk

WHO Collaborating Centre for Rabies Surveillance and Research, Friedrich-Loeffler Institut, Federal Research Institute for Animal Health, Sudufer 10, 17493 Greifswald-Insel Reims, Germany
Head, Dr Thomas Müller; e-mail: thomas.mueller@fli.bund.de

WHO Collaborating Centre for Control, Pathogenesis and Epidemiology of Rabies in Carnivores, 106 Pineridge Road, Carp, ON, Canada
Head, Dr Christine Fehlner-Gardiner; e-mail: Christine.Fehlner-Gardiner@inspection.gc.ca

WHO Collaborating Centre for Neurovirology, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19105, USA
Head, Dr Bernhard Dietzschold; e-mail: bernhard.dietzschold@jefferson.edu

WHO Collaborating Centre for Reference and Research on Rabies, Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104, USA
Head, Dr Hildegund Ertl; e-mail: ertl@wistar.upenn.edu

WHO Collaborating Centre for Reference and Research on Rabies, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA
Head ad interim, Inger Damon, Chief, Poxvirus and Rabies Branch, CDC

WHO Collaborating Centre for Rabies Epidemiology, Centre for AIDS and Related Diseases, National Centre for Disease Control, 22-Sham Nath, Delhi 110054, India
Head, Dr Rattan Lal Ichhpujani; e-mail: ichhpujani@hotmail.com

WHO Collaborating Centre for Reference and Research in Rabies, Department of Neurovirology, National Institute of Mental Health and Neurosciences, PO Box 2900, 560029 Bangalore, India
Head, Professor S.N. Madhusudana; e-mail: mshampur@hotmail.com

WHO Collaborating Centre for Research and Training on Viral Zoonoses, Chulalongkorn University Hospital, Rama 4 Road, Bangkok 10330, Thailand
Head, Dr Thiravat Hemachudha; e-mail: fmedthm@gmail.com

WHO Collaborating Centre for Research on Rabies Pathogenesis and Prevention, Queen Saovabha Memorial Institute, Thai Red Cross Society, 1871 Rama IV Road, 10330 Bangkok, Thailand
Head, Professor Visith Sitprija; e-mail: sitprija@yahoo.com; and Dr Pakmanee Narumol; e-mail: npakmanee@yahoo.com