Tissues should be kept cold for storage and transport to the laboratory. They should not be placed in formalin, as this precludes their use or reduces their reliability for all the principal diagnostic tests. However, unpreserved and formalin-fixed samples of other tissues should be collected at necropsy to aid differential diagnosis.

Freezing of specimens should be avoided unless chilling is not possible. However, freezing should be considered if long-term storage is necessary. Freezing does not affect the major diagnostic tests, but thawing of large specimens may increase the time to obtain results. Decomposition may affect the reliability of diagnosis, particularly culture methods. However, provided tissue material is present, the state of decomposition should not influence the decision to test, as antigens and viral RNA can successfully be detected in even the most severely decomposed tissues.

It is important that all the appropriate epidemiological information — including precise and accurate identification of species and geographical origin — is acquired and included on the laboratory test request form. If necessary, a taxonomist should be consulted for the correct identification of wildlife.

Transport of specimens

For cost-effectiveness and ease of shipping, it may be necessary to consider removing the head or brain of larger animals in the field, or at a regional veterinary facility, rather than at the diagnostic laboratory. Many diagnostic laboratories do not have the facilities to deal adequately with large carcasses. However, the risk of self-inoculation should be carefully managed. It is preferable that the procedure is undertaken by vaccinated and experienced operators.

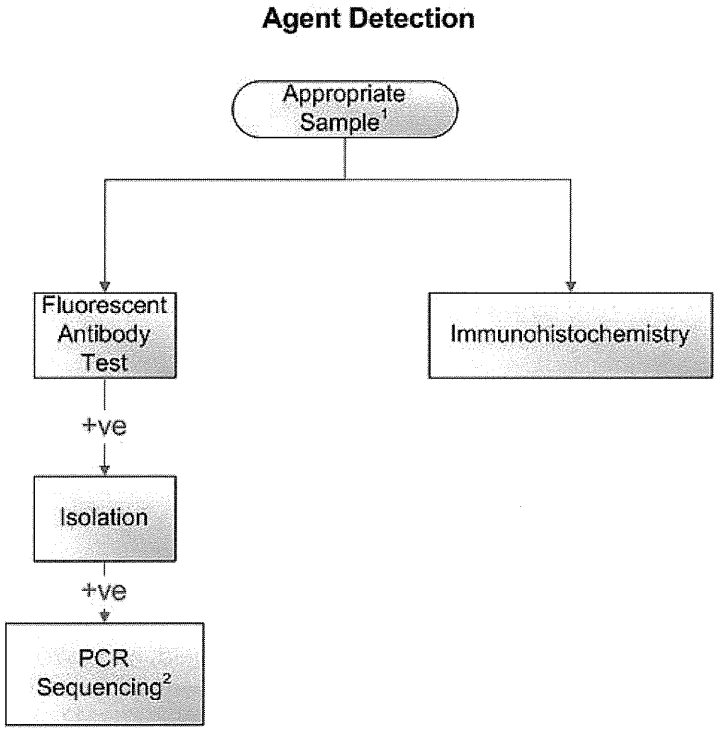
Firstly, the necessary clearance from the CVO of the state or territory of the disease outbreak should be obtained, and the CVO of Victoria should be informed about the transport of the specimens to Geelong. Then specimens should be sent to the state or territory diagnostic laboratory, from where they will be forwarded to AAHL, Geelong, for emergency disease testing.

Laboratory diagnosis

The testing method used by CSIRO-AAHL is shown in Figure 1.1. Further details of tests currently available at AAHL are shown in Table 1.3.

The fluorescent antibody test (FAT) is the initial test of choice for the diagnosis of lyssavirus infections in domestic or wild animals because it is the most rapid and reliable of the tests used. It involves applying a specific fluorescein-labelled antibody — directed against the viral nucleocapsid protein — to a smear of brain tissue. Current FAT reagents react to all lyssaviruses and are not rabies specific. Differentiation of rabies from other lyssaviruses requires characterisation of the viral genome by molecular genetic techniques (eg polymerase chain reaction [PCR] and sequencing).

AAHL Rabies Testing Algorithm



1. Brain, salivary gland, CSF, saliva. Fixed brain or salivary gland for IHC.
2. Selected isolates

Figure 1.1 The current approach to diagnostic testing for rabies used at CSIRO-AAHL

Table 1. 3 Tests currently available at the CSIRO-AAHL for use in the diagnosis and control of rabies

Test	Specimen	Test detects	Time taken to obtain result
Agent detection			
FAT	Fresh brain	Viral antigen	1 day
Immunohistochemistry	Formalin-fixed brain	Viral antigen	2 days
Agent characterisation			
Virus isolation in neuroblastoma cell cultures	Fresh brain	Live virus	5 days
PCR and sequencing	Fresh brain, cultured virus	Viral genome	3–4 days
Virus isolation in mice	Fresh brain	Live virus	28 days
Serology			
ELISA	Serum	Antibodies	1 day
RFFIT (serum neutralisation test)	Serum	Antibodies	3 days
FAVN (serum neutralisation test)	Serum	Antibodies	3 days

ELISA = enzyme-linked immunosorbent assay; FAT = fluorescent antibody test; FAVN = fluorescent antibody virus neutralisation; RFFIT = rapid fluorescent focus inhibition test; PCR = polymerase chain reaction

Source: CSIRO-AAHL, 2010 (refer to CSIRO-AAHL for most up-to-date information)

As at October 2009, appropriate laboratories for confirming results are:

- CSIRO-AAHL[8] (see Table 1.3 for current diagnostic tests);
- Queensland Health Forensic and Scientific Services (QHFSS);[9] and
- Queensland Primary Industries and Fisheries Biosecurity Sciences Laboratory (BSL).[10]

Although other state and public health laboratories may offer some diagnostic tests, the confirmation of diagnostic results must be by one of the laboratories listed above.

Laboratory procedures for the diagnosis of rabies fall into three categories: antigen detection assays, nucleic acid detection assays and culture for live virus. Before the development of antigen detection tests in the 1950s, histological techniques, using chemical stains such as Seller's stain, were used for the detection of the characteristic Negri bodies. However, such assays are so insensitive that they are of almost no value for the medical management of contact victims. Biopsy of the brain may also be required.

The first-line diagnostic test in most laboratories is the FAT, which can be performed and produce results within 1–2 hours.

For this test, fresh brain or other nerve tissue is used to make impression smears, which are then fixed and stained by incubating with fluorescein-labelled anti-rabies antibody, and observed under a fluorescence microscope. In experienced hands and with superior reagents, this test is highly reliable and back-up tests rarely change the outcome. These features, combined with its relative low cost and rapid test time, make the FAT a highly robust assay that would be difficult to surpass. However, the FAT cannot be performed reliably on formalin-fixed samples.

In recent years, PCR methods have become the main auxiliary tests for rabies. Real-time PCR tests using TaqMan technology are reliable and rapid, with results available on the same day. They are, however, considerably less sensitive if the samples are fixed in formalin. Subsequent sequencing of PCR products may provide important epidemiological information on the virus.

Culture methods are the oldest diagnostic tests. Cell cultures are performed using mouse neuroblastoma cells, a cell line that is highly sensitive to rabies virus. Mouse inoculation is also a reliable test, although now rarely used as a routine detection test. Apart from their value as diagnostic tests, culture methods are appropriate where virus needs to be amplified for detailed antigenic and genetic characterisation. Their main disadvantage as diagnostic assays is their long performance times – up to 5 days for cell cultures and up to 3–4 weeks to confirm a negative result in mice. Culture for rabies virus also has the disadvantage that it

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[8] CSIRO Australian Animal Health Laboratory, 5 Portarlington Road, Geelong, Victoria 3219 (contact the duty veterinarian)

[9] Biosecurity Services Laboratory (contact the duty veterinarian). This laboratory is colocated with QHFSS at 39 Kessels Road, Coopers Plains, Queensland, 4108.

[10] Health and Food Sciences Precinct, specimen receipt (loading block 12), 39 Kessels Road, Coopers Plains, Queensland, 4108

cannot be performed on formalin-fixed tissues. Culture methods are also less sensitive than the FAT and PCR tests when specimens are decomposed.

Immunohistochemistry is an antigen-detection assay that is performed on sections of tissue. If appropriately selected anti-rabies antibodies are used, this test can be highly sensitive and specific on formalin-fixed tissues. A minimum of 2 days are needed to return a test result.

Most high-proficiency laboratories will attempt to use more than one test to reach a final diagnosis, particularly where human contact is involved. The reliability of a diagnostic test is dependent on several factors. Given optimal equipment and operator performance, the two primary areas that affect test performance are specimen quality, and the quality and design of the reagent probe (antibody or primer). The most common reasons for test failure are examination of a single, rather than a composite, brain sample; diagnostic antibody or primer mismatch, particularly with unusual lyssavirus types; and severe decomposition of the specimen.

Virus typing is important to characterise the probable origins of virus strains. Once the sample has been diagnosed as positive, the virus can be typed by PCR amplification and gene sequencing, or by antigenic reactivity to panels of monoclonal antibodies.

Serology tests are of no value for the diagnosis of rabies, although they have supported diagnosis in several human cases that have exhibited symptoms but have survived the infection. These patients showed high levels of antibodies with no detectable antigen at presentation. Serology tests are useful for confirming vaccine responses in animals and humans. Virus neutralisation tests (rapid fluorescent focus inhibition test [RFFIT] or fluorescent antibody virus neutralisation [FAVN]) have been developed for this purpose. As these tests are performed on cell cultures, the serum samples should be taken carefully and separated from the cellular components of the blood as soon as possible to minimise the toxic effects of cell lysis.

Further characterisation

The virus isolate in an outbreak will be further characterised by gene sequencing. This not only differentiates rabies virus from other lyssaviruses, but can also provide valuable epidemiological information on the possible origin of the virus and its likely maintenance host(s) (ie the biotype), which is of central importance in developing the response strategy.

Weak or negative fluorescent antibody staining may be obtained from brain specimens of human or animal patients that have had clinical signs indicative of rabies. If neurological signs and death occur in mice inoculated with such brain specimens, a divergent lyssavirus should be suspected as the aetiological agent.

Case definition

For the purposes of an emergency disease response, a case of rabies is one that is confirmed by any of the tests listed in Table 1.3 and gene sequence analysis indicating that the agent belongs to a lyssavirus genotype 1 lineage that is known to be a terrestrial mammal or bat biotype (see Table 1.1).

1.4.4 Differential diagnosis

Any other causes of neurological dysfunction should be considered as differential diagnoses for rabies. Change in behaviour is the key clinical sign for wildlife and domesticated animals, and this sign may be missed by the owner or handlers of an affected animal. The disease in all mammals is acute, progressive and fatal. Where this is not the case, rabies can usually be excluded.

The following conditions must be considered in the differential diagnosis:

- viral encephalitides
  - canine distemper and infectious canine hepatitis
  - Aujeszky's disease
  - Borna disease
  - eastern, western and Venezuelan equine encephalomyelitis viruses
  - West Nile virus, Japanese encephalitis virus and other flaviviruses
  - various insect-borne reoviruses
  - Australian bat lyssavirus;[11]
- bacterial and mycotic diseases of the CNS, including listeriosis and cryptococcosis;
- poisonings, including by '1080' (sodium fluoroacetate), heavy metals (eg lead), chlorinated hydrocarbon and organophosphate pesticides, urea, and nitrogen trichloride;
- protozoal infections, including babesiosis and toxoplasmosis;
- foreign bodies in the oropharynx or oesophagus, and other traumatic injuries; and
- acute psychoses in dogs and cats
- bovine spongiform encephalopathy.

1.4.5 Treatment of infected animals

The treatment of infected animals is ineffective.

1.5 Resistance and immunity

1.5.1 Innate and passive immunity

There is no evidence of naturally occurring innate immunity to rabies virus. Transient passive immunity occurs in offspring of vaccinated animals.

[11] www.animalhealthaustralia.com.au/aahc/index.cfm?3C0BD05B-9FCB-F40F-5367-0F5F6F2ED92C

1.5.2 Active immunity

Active immunity can be induced by vaccination. In humans and some animals, this can be used to prevent clinical disease. Active immunity is ineffective in resolving clinical disease, and there is no carrier state.

Antibody has been detected in clinically normal, nonimmunised animals and is thought to be associated with transient infection without the development of clinical disease. There is no evidence that these animals pose a risk of transmission.

1.5.3 Vaccination

Parenteral vaccination programs are widely implemented overseas for rabies control. Oral administration of vaccine is generally used in the control or reduction of wildlife rabies. Modified oral live virus vaccines and live recombinant vaccines have been very effective in inducing adequate immunity in many species, including foxes, and for significantly reducing the incidence of rabies in wildlife. All vaccines currently used for oral vaccination programs are either modified live virus vaccines or live recombinant vaccines.

Domestic animals

Nobivac Rabies (Intervet) is the only registered parenteral rabies vaccine in Australia. Currently, it can only be used for vaccinating animals for export, so in the event of a rabies outbreak, the vaccine would have to be approved for domestic use. This would be done through the Australian Pesticides and Veterinary Medicines Authority (APVMA) and the Australian Government Department of Agriculture, Fisheries and Forestry.

The Nobivac Rabies vaccine is inactivated, and has been assessed as being safe and efficacious. It is therefore unlikely to induce disease in the recipient animal.

Some animals in Australia may have been vaccinated overseas before importation. Maternal antibodies persist until 10–12 weeks of age (Precausta et al 1982); therefore, offspring from vaccinated dams must be at least 3 months old before receiving their first vaccination. Vaccinations and boosters should be delivered in accordance with the manufacturer's recommendations. Revaccination will be considered in the face of an outbreak regardless of an animal's rabies vaccination history.

WHO recommends that, for mass canine vaccination campaigns, at least 80% of target populations need to be vaccinated to control rabies (WHO 2007).

Post-vaccination serology

The OIE accepts a minimal rabies serum neutralising antibody titre of 0.5 international units (IU)/mL as an indication that the animal has responded to vaccination. Following vaccination of dogs and cats, serological testing is important to determine if adequate seroconversion has occurred. Measurement of virus-neutralising antibodies is used to indirectly assess vaccine efficacy.

Where exposure of a domestic animal to a rabid animal is unlikely but unknown, postexposure treatment may be considered by the Consultative Committee on Emergency Animal Diseases (CCEAD) on a case-by-case basis.

Further detailed information on immunisation of domestic animals can be found in the *Compendium of Animal Rabies Prevention and Control* (National Association of State Public Health Veterinarians 2008).

Vaccination of livestock is not essential for eradication, but may be desirable to prevent sporadic cases in these animals and the subsequent risk to humans. Pleasure horses, valuable stud animals and any other animal that comes into frequent human contact during the incursion should be considered for vaccination.

Humans

Safe and efficacious vaccines are available for human use, both for pre- and post-exposure prophylaxis. Information can be found in the *Australian Immunisation Handbook*, 9th edition (NHMRC 2008). Section 3.2.2 has further information on who should be vaccinated and when.

Wildlife

Vaccination

Overseas, oral vaccination is an important tool to control the spread of rabies in wildlife populations – ongoing programs are implemented annually, particularly in Europe and North America (Blanton et al 2007). Programs involve distributing baits containing orally immunogenic vaccines throughout the landscape, targeting wildlife. The programs aim to establish population immunity, and thus prevent spread of rabies or eliminate species-specific variants (Sterner et al 2009). Oral vaccines are also effective in immunising domestic dogs under experimental conditions, and experimental trials have demonstrated their potential in field situations.

Oral vaccination is made possible by the ability of vaccine strains to elicit an immune response through the oral/pharyngeal route by local infection of mucous membranes (Wandeler 1991). For this reason, oral rabies vaccines consist of live viruses. Inactivated antigens are not effective.

Oral vaccination in foxes was used to control rabies in western Europe in the 1970s. As a result, the prevalence of fox rabies rapidly decreased, and France and Switzerland were declared free from rabies in terrestrial animals by 2000, although bats in these countries still cary lyssaviruses. The oral rabies vaccination programs in Europe mainly used attenuated rabies virus vaccines, which were highly effective in immunising fox populations. However, they caused a small number of vaccine-induced rabies cases and were not very effective in other species (Blanton et al 2007). During the 1980s, a vaccinia–rabies glycoprotein (V-RG) recombinant virus vaccine was developed. V-RG was extensively used in the United States of America to control rabies in various maintenance hosts (Blanton et al 2007). However, the efficacy of V-RG vaccine in some species, including skunks, is limited (Blanton et al 2007). Although safety in animals is generally good, vaccinia recombinant vaccines have occasionally caused local and disseminated vaccinia infections in humans, and for this reason recombinant vaccines using other, less pathogenic viruses are being developed.

The Ministry of Natural Resources in Ontario, Canada, has managed a rabies control program that has been very successful in eliminating fox rabies from many areas with the use of vaccine baits.[12]

Vaccination will markedly reduce the frequency of rabies in maintenance and spillover hosts. In maintenance hosts, immunisation coverage of more than 50% of the population will result in a significant decline. Immunisation coverage of more than 70% should lead to the eventual eradication of the disease. For dogs, a total population vaccination level of 80% is desirable (WHO 2007). Injectable vaccines have played a major role in eradicating dog biotypes in Europe, North America and Japan, and reducing their frequency in many other regions. Oral vaccines, placed into baits, have reduced or eradicated wildlife rabies over large areas of Europe and North America.

Although rabies vaccination campaigns using oral vaccines have been successful in European and North American wildlife populations, oral vaccination of domestic dog populations has not progressed beyond field experimental stages. Dog rabies is predominantly a problem of resource-poor countries. Although oral vaccination may allow the effective immunisation of a proportion of dogs that cannot easily be caught, these campaigns require significant resources. In addition, the use of live vaccines in companion animals is not well supported. This is due to the small risk of the pet acquiring vaccination-induced rabies, which could potentially be transmitted to humans — an impediment to its ready adoption.

Detailed guidelines for oral vaccination programs are available at the WHO website (WHO 2007).

Overseas protocols for oral vaccination of foxes would be expected to be applicable in the Australian situation. Experimental field protocols that have demonstrated the potential for oral vaccination of dogs may be applicable in some circumstances in Australia. However, the safety and efficacy of bait administration in native Australian species has not been assessed, and WHO recommends that a risk assessment be undertaken before the release of vaccine bait into the environment.

Trap–vaccinate–release

Trap–vaccinate–release (TVR) involves capturing live wildlife with cage traps and vaccinating by intramuscular injection. It has been used for rabies management in urban skunks and raccoons in North America. This method could be used to conserve endangered species. It could also be used to manage rabies in wildlife that live in areas inhabited by people where population-reduction methods and oral-baiting methods are unsuitable or unacceptable to the public, or where satisfactory baits have not been developed. TVR may be preferrable to depopulation in some species, as the latter causes a population sink into which infected or susceptible animals migrate (see 'Population reduction', below).

[12] www.mnr.gov.on.ca/en/Business/Rabies

Population reduction

Widespread, sustained population reduction of animal reservoirs (eg by shooting, gassing, poisoning) to eliminate rabies is not justified for epidemiological, ecologic, economic and ethical reasons (Rupprecht et al 1995).

Maintenance hosts of rabies have high potential rates of population replacement. Even in the face of heavily sustained population removal, they are able to replace population losses at a sufficient rate to maintain rabies virus cycles. Paradoxically, unless it is very heavy, host depopulation can be counterproductive. By removing mature, socially stable animals, depopulation leads to dispersal and a younger demographic structure. This results in higher host-contact rates and greater potential to spread disease.

Nevertheless, some degree of population management is necessary, particularly with companion animal species. Stray and nuisance dogs and cats should be impounded and, if necessary, destroyed. Such policy is important at all times, but particularly during a rabies epidemic.

1.6 Epidemiology

In dogs and other maintenance hosts, outbreaks of rabies occur in local, explosive epidemics of particular biotypes that last for several months. After the main epidemic in a particular area (involving a discrete group of animals) has passed, the outbreak will tail off before becoming locally extinct. However, it may take several years before extinction and occasional cases of disease may still occur during this time. The population then becomes susceptible to reinfection. If sufficiently isolated, local host populations may remain free from rabies for long periods before the next epidemic is initiated.

Maintenance hosts are able to establish dense populations and have high potential-replacement rates. In intense epidemics, the disease itself becomes the major factor controlling host population size. A significant proportion of the maintenance host population is affected and the population density falls, often catastrophically. The transmission ratio falls below the threshold of sustainability and the disease dies down, allowing the host density to recover to its pre-epidemic level. Variation in the relationships and contact between different local populations will increase the chance that there is always at least one infected local population to sustain the disease over the long term.

In cattle, sheep and other herbivores, spillover cases of rabies coincide with rabies in a maintenance host. There are often several nearly simultaneous cases in cattle and sheep herds, resulting from multiple attacks by a rabid animal. However, it is possible that limited transmission between animals in a herd may occur through grooming and drinking from the same water trough. Cattle appear to be highly sensitive to rabies, as its incidence is high in cattle where the disease is present in a maintenance host. This may be due partially to their innate curiosity, which would draw them towards rabid animals.

Ecological factors are critical in determining the epidemiology in the different host species. Dog biotypes are dominant in human societies, both urban and rural, that do not place restricted movement on the dog population. Conversely, in societies that confine dogs to yards and homes, dog rabies is likely to be reduced. The most effective wildlife maintenance hosts establish dense populations, have high

potential replacement rates, and have adapted well to urbanisation and habitat pressure.

1.6.1 Incubation period

The incubation period in animals, including humans, is highly variable. It is generally 3–8 weeks, but can vary from 2 days to 6 months or even longer — up to 6.5 years in humans (Bek et al 1992). Incubation periods in excess of one year have been documented in animals, but rarely so. The OIE *Terrestrial Animal Health Code* gives a maximum incubation period, for regulatory purposes, of 6 months.

Several factors influence the duration of the incubation period, including the virus strain, the virus dose, the distance of the bite site from the central nervous system and the richness of the sensory innervation at the site of virus entry into the body. The last two of these factors are most important. For example, the incubation period following a bite on the face or muzzle could be expected to be much shorter than that after a bite on the trunk or limbs.

1.6.2 Persistence of agent

General properties and the environment

Rhabdoviruses are bullet-shaped and contain single-stranded, unsegmented RNA, which is complementary to messenger RNA and is enclosed in a nucleocapsid protein complex. Rabies viruses are relatively large and contain lipids; hence they are susceptible to a wide range of disinfectants, including warm soapy water, iodine preparations and detergents (see Section 3.2.3, 'First aid').

Rabies virus is comparatively fragile and does not survive for long periods outside the host. The virus is inactivated by heat, and is susceptible to ultraviolet (UV) light, lipid solvents (soapy water, ether, chloroform, acetone), 45–75% ethanol, quaternary ammonium compounds (eg 0.2% cetrimide) and 5–7% iodine preparations. However, the rate of inactivation of rabies virus by physical and chemical conditions is greatly modified by the stabilising effects of polypeptides and other compounds (Kaplan et al 1966, Michalski et al 1976, Matouch et al 1987, Scott Williams Consulting Pty Ltd 2003).

Some authors have suggested that refrigerated virus or virus preparation at pH 5–10 may remain stable for extended periods. Others have thought that the virus may be inactivated by direct sunlight and UV light, and that the virus is labile to proteolytic enzymes, but no supporting data are provided (Fernandes et al 1963, Swanepoel 1994). Survival of rabies virus in the saliva of dead carcasses is unknown, but continued infectivity for a period postmortem cannot be dismissed, particularly in temperate conditions.

Although aerosol contamination in bat caves is well recognised, there are no studies proving that other routes of environmental contamination play any significant role in transmission of rabies (Gibbons 2002).

Live animals

The virus is shed in saliva from about the time of onset of clinical signs. Virus shedding 1–5 days and up to 13 days before clinical signs appear has been reported. Rabies virus has not been identified in other bodily secretions more than 2 weeks before confirmed infection (Fekadu 1988, Greene and Rupprecht 2006).

It is generally accepted that there is no carrier or latent state for rabies.

1.6.3 Modes of transmission

Live animals

Rabies virus is transmitted by contamination of a fresh wound with virus laden saliva. This is usually from the bite of a rabid animal, but can also result from licking abraded skin or mucous membranes. The virus cannot penetrate intact skin.

Respiratory and oral transmission can also occur, but is considered uncommon. In exceptional circumstances, transmission from mother to suckling young has been reported. For practical purposes, these routes can be ignored in framing control strategies.

Transmission risk in laboratory situations includes splashing onto mucous membranes and aerosol exposure (Gibbons 2002).

Animal carcasses

There is neural spread of virus from the brain to various organs and tissues during the clinical phase of the disease. Therefore, the entire carcass is regarded as potentially contaminated with rabies virus.

Animal products and byproducts

Any products or byproducts from a rabid animal should be regarded as potentially infectious and not permitted into the food chain.

Equipment and personnel

Equipment and personnel are not recognised as significant in the transmission of rabies virus.

Vectors

Transmission of rabies virus by arthropod vectors is not known to occur.

Semen and embryos

No evidence exists for transmission in semen or embryos.

Other modes of transmission

Transmission of rabies by the transplant of the cornea and other organs has occurred in humans (Gibbons 2002).

In several species of mammals, including dogs, cattle, bats and laboratory rodents, rabies has been reported to have been transmitted across the placenta from mother to fetus. This is considered an infrequent mode of transmission.

In two separate incidents, aerosols created during laboratory procedures infected two staff members. One person had been using a blender to homogenise rabid goat brains (Winkler et al 1973), and the other had been spraying live rabies virus in a pharmaceutical manufacturing machine (Tillotson et al 1977).

1.6.4 Factors influencing transmission

The population density of susceptible (ie nonimmunised) maintenance-host species is important for transmission. Epidemics often spread on a slow-moving front; for example, 30–60 km per year in fox rabies in Europe (Toma and Andral 1977). However, this is influenced by migration and seasonal dispersal patterns of the host species. Dog rabies can be rapidly spread to new areas by dogs with furious rabies that have running fits (where they may travel distances of more than 30 km) or by pets moved to new areas by their owners.

Australia has widespread and abundant populations of wildlife and feral animals that are known to be maintenance hosts of rabies in other countries. Carnivore species in Australia that may be potential hosts are the European red fox, the feral cat, the feral dog and the dingo. It is difficult to predict with certainty which wildlife species would be involved in an outbreak in Australia. Although threshold densities needed to maintain rabies vary widely, even within the same species (eg the red fox in Canada and Europe), it is known that Australia has densities of the European red fox that greatly exceed the densities of rabies-infected populations within endemic countries.

1.7 Manner and risk of introduction to Australia

The highest risk for a rabies virus dog biotype to enter Australia is by the illegal entry of an infected animal (eg through smuggling or itinerant yachts). The possibility of a fox biotype entering Australia via a smuggled fox is remote. Other routes of entry – such as an infected dog with a nondog biotype being undiagnosed and entering Australia illegally or through quarantine, followed by transmission back to the maintenance host – are unlikely.

There is negligible risk that human cases of rabies will spread to animals or other people.

2 Principles of control and eradication

2.1 Critical factors assessed in formulating response strategy

2.1.1 Features of the disease

- Rabies is almost invariably fatal in both humans and animals.
- Rabies has a broad host range.
  - There are species-specific biotypes of rabies virus.
  - Hosts that are expected to maintain existing biotypes are present in Australia (eg dogs, red foxes, dingoes).
  - Rabies virus can spill over to other host species (eg humans, livestock, cats).
  - Any animal that has rabies can potentially transmit it to other animals and people.
  - Marsupial susceptibility is unknown.
- Most commonly, clinical disease will be associated with behavioural change (eg friendly dogs become aggressive, wildlife lose fear of people).
  - Clinical signs can be variable and unremarkable.
  - Clinical signs are not diagnostic; laboratory testing is required.
- In an outbreak, rapid laboratory diagnosis is needed and adequate laboratory surge capacity is necessary.
  - Definitive diagnosis requires laboratory examination of the brain, and biotype determination requires DNA sequencing. Biotype knowledge is crucial to control and management. Live animal testing does not exclude a diagnosis of rabies. Diagnosis will usually take a day from the time of receipt at the appropriate diagnostic laboratory. Early cases may go unnoticed in an outbreak.
- Rabies virus is fragile and does not persist in the environment.
- Transmission is most often by transfer of saliva, usually through biting or scratching. Parenteral or mucosal membrane exposure (eg though the eyes, oral mucosa, bites, scratches) is required.

2.1.2 Vaccination

- Vaccination is an effective technique for controlling rabies.
- Availability of vaccine for humans and animals is essential.
  - Safe and effective registered parenteral vaccines for humans and animals are available in Australia.
  - Oral vaccination programs in some species have effectively eradicated or controlled rabies in wildlife overseas.

  - Overseas information suggests that more than 70% of the population needs to be vaccinated to ensure population protection in wildlife.
  - The use of oral vaccine in wild animal management has been considered as an option in Australia; however, safety and efficacy would need to be evaluated, and an emergency-use permit would need to be obtained.
  - The possibility of reversion to virulence of an oral vaccinal strain in Australian animals would also need to be evaluated.
- All persons involved in the operational management of rabies (eg veterinarians, field officers and their staff who may handle animals) should be vaccinated in accordance with the *Australian Immunisation Handbook*, 9th edition (NHMRC 2008). This may delay the involvement of some personnel in a response for days or weeks.
- Rabies-specific occupational health and safety issues must be considered in field operations.

2.1.3 Features of the populations

- Wildlife control expertise is available in Australia.
- Wildlife population reduction programs are not considered effective.
- There would be significant human and social impacts if rabies became established. Public outreach, communications and liaison are paramount.

2.2 Options for control and eradication

In this description of options for the control and eradication of rabies, the following terms are used:

- *Infected animal.* A live animal that develops clinical signs consistent with the disease and is known to have an epidemiological link (eg in a known infected area or area of epidemiological interest).
- *Confirmed case.* A laboratory-confirmed rabies-positive animal.
- *Susceptible animal.* Mammals are susceptible; members of Carnivora and Chiroptera are recognised as significant reservoirs. In Australia, dogs, cats, horses, cattle, sheep, pigs and foxes are important susceptible species. Wildlife and feral species may also be important.
- *Suspect animal.* An animal not known to have been exposed to a disease agent but showing clinical signs requiring differential diagnosis (ie with no epidemiological link to the disease).
- *Dangerous contact animal.* A susceptible animal that has been designated as being exposed to rabies following tracing and epidemiological investigation, and considered highly likely to be infected.
- *Trace animal.* An animal not showing clinical signs, but with an epidemiological link to the disease.

Initially, action should be directed towards preventing human exposure as far as is practicable and taking every possible precaution to reduce the risk to those involved in the handling of infected animals.

Control measures could involve any or all of the following:

- recognising rabies cases in animals as early as possible;
- defining the geographic area of the outbreak;
- seizing, and quarantining or destroying infected animals;
- tracing, seizing, and quarantining or destroying dangerous contact animals;
- controlling zoning and movement over animals, including prohibiting gatherings, sporting and recreational activities involving animals (eg an embargo on hunting dogs, and mustering that uses working dogs);
- muzzling all domestic dogs when in public to minimise the risk of transmission;
- vaccinating key populations (eg guide dogs, police dogs) early in the response;
- alerting all veterinary practices, state and territory health departments, wildlife carers, the RSPCA, animal shelters, local government animal control organisations, feral animal control organisations and other relevant stakeholder groups;
- controlling stray animals; and seizing, and detaining or destroying animals not properly controlled or vaccinated;
- vaccinating individual animals and using oral vaccinations (eg through baiting) for large populations;
- identifying vaccinated animals;
- detecting and managing the disease in wildlife;
- mounting publicity campaigns;
- reporting human exposure to possibly infected animals; and
- identifying and assessing trace animals.

Note: Population control (through culling or stamping out) has never been an effective technique in the management of rabies.

The policy to be implemented is described in Section 3.

3 Policy and rationale

For the purposes of an emergency disease response, a case of rabies is one that is confirmed by any of the tests listed in Table 1.3 and gene sequence analysis indicating that the agent belongs to a lyssavirus genotype 1 lineage that is known to be a terrestrial mammal or bat biotype (see Table 1.1).

3.1 Introduction

Summary of policy

Rabies is a notifiable disease in all states and territories of Australia, and is listed by the World Organisation for Animal Health (OIE). The detection of rabies in terrestrial (including bat) hosts in Australia would have significant public health and social impacts, particularly if the disease became widespread, or established in stray or wild animal populations. There may also be ecological and conservation concerns.

Rabies is a Category 1 disease under the government–industry Emergency Animal Disease Response Agreement (EADRA) for cost-sharing arrangements. Category 1 diseases are those for which response costs will be borne 100% by governments.

The default policy is to quickly eradicate rabies to prevent spread to domestic and wild animals, and humans through a combination of strategies including:

- ***quarantine and movement controls*** **on susceptible animals in declared areas to minimise the spread of infection;**
- ***destruction of infected animals*** **to remove the most dangerous source of viruses;**
- ***quarantine, vaccination or destruction*** **of exposed animals;**
- ***movement control, vaccination or quarantine*** **of *suspect animals*** **until their rabies status has been clarified;**
- ***vaccination*** **of domesticated carnivores (eg dogs, cats, ferrets), other selected species and targeted animal groups in declared areas to protect animals against infection and reduce exposure of humans;**
- ***monitoring*** **of wild animals and, if disease establishes in those populations, consideration of implementing a vaccination program;**
- ***tracing and surveillance*** **to determine the source and extent of infection, and to provide proof of freedom from the disease;**
- ***linkage and coordination*** **of public health and environmental authorities so that they are co-responders; and**
- ***a public awareness campaign*** **to facilitate public cooperation from animal owners and the community, including other government and nongovernment authorities.**

Successful implementation of the policy will depend on community cooperation and compliance with all control and eradication measures. Advice about immunisation of humans would be provided by public health authorities.

Population reduction of susceptible species is not appropriate.

The chief veterinary officer (CVO) in the state or territory in which the outbreak occurs is responsible for developing an Emergency Animal Disease (EAD) Response Plan for the particular outbreak.

The Consultative Committee on Emergency Animal Diseases (CCEAD), convened for the incident, assesses the response plan drawn up by the affected jurisdiction's CVO for technical soundness and consistency with AUSVETPLAN, and endorses or seeks modifications to it. Overall operational management of the incident rests with the CVO of the affected jurisdiction, with oversight by the CCEAD. Because rabies is a zoonosis, it is essential for human health authorities to be involved in planning and implementing the response.

The National EAD Management Group (NMG), also convened for the specific incident, decides on whether cost sharing will be invoked (following advice from the CCEAD), and manages the national policy and resourcing needs.

For further details, refer to the **Summary Document**.

CVOs of the affected states and territories will implement disease control measures as agreed in the EAD Response Plan and in accordance with relevant legislation. They will make ongoing decisions on follow-up disease-control measures in consultation with the CCEAD and the NMG, based on epidemiological information about the outbreak.

For information on the responsibilities of the state or territory disease control headquarters and local disease control centres, see the **Control Centres Management Manual**.

3.2 Occupational health and safety

3.2.1 Key points

- Every person involved in a rabies eradication program who may come in contact with an infected animal should be immunised against rabies. Procedures for use of vaccine, including storage, dosage and administration, are set out in the *Australian Immunisation Handbook*, 9th edition (the Handbook) (NHMRC 2008). However, as scientific and medical knowledge of rabies and its prevention may change between revisions of the Handbook, advice should be sought from the relevant state health department on the current recommendations.
- Contact between humans and potentially infected animals should be minimised.
- Personnel who have contact with potentially infected animals should always wear appropriate personal protective equipment (PPE).

- If a potential exposure to rabies occurs, first-aid procedures should be undertaken immediately, as detailed in Section 3.2.3. Medical advice should always be sought without delay, irrespective of vaccination status, as postexposure prophylaxis may be needed.

3.2.2 Vaccination

General

Safe and effective vaccines for protecting humans against rabies are available. There are two protocols for rabies vaccination: pre-exposure vaccination and postexposure vaccination. Vaccination recommendations for staff involved in a rabies eradication program may differ from person to person, depending on the potential for exposure to live virus.

In particular, vaccination status of veterinary staff in the declared areas should be determined. Unprotected veterinary staff should be advised of risks and should be advised to seek medical advice regarding vaccination options. Unprotected veterinary staff should not handle suspect animals.

Antibody titres in vaccinated staff are likely to wane with time. It is important that an adequate titre be maintained in staff exposed to potentially rabid animals and in laboratory staff working with live virus. Regular antibody titre measurements or a vaccine booster dose may be required. The specific regimens differ depending on the nature of the potential exposure; these are outlined in the Handbook (NHMRC 2008).

Before engaging in at-risk activities, recently vaccinated people should ensure that they have a protective titre.

Postexposure vaccination

Postexposure prophylaxis (PEP) is used when a person may have been exposed to rabies virus, usually following an animal bite or scratch. PEP varies according to whether or not the person is vaccinated against rabies. For unvaccinated people, PEP includes rabies immunoglobulin. Further information is available in the Handbook (NHMRC 2008).

As rabies may have a long incubation period, medical advice about PEP should be sought regardless of the time that has elapsed since the exposure.

3.2.3 First aid and medical assessment

It is essential that, whenever a potential exposure to rabies virus occurs — via a bite, scratch, or splash onto mucous membranes or aerosol exposure in the laboratory — first aid is commenced as soon as possible to remove the virus from exposed tissue. Medical advice must be sought immediately on the appropriate course of action, irrespective of whether the person has been previously vaccinated against rabies.

Proper cleansing of any wounds, abrasions and splashes is an important first-aid measure in preventing rabies of people. If a person is bitten or scratched, or mucous membranes (eg eyes, nose, mouth or existing wounds) are splashed with any bodily fluids from the animal, the affected area should be immediately and

thoroughly washed with soap and water for approximately 15 minutes. Scrubbing should be avoided, as this may cause abrasions that could facilitate entry of the virus into the wound. A virucidal antiseptic, such as povidone-iodine, iodine tincture, aqueous iodine solution or alcohol (eg ethanol), may be applied to wounds after washing.

3.2.4 Handling of animals

General approach

Suspected rabid animals should be approached and handled only when necessary and only by appropriately trained personnel. Potentially rabid animals should be approached with extreme caution. If it can be done without risk to the operator, every effort should be made to capture and safely confine the animal. If the animal cannot be safely captured or confined, and therefore constitutes a risk to people or other animals, it should be immediately destroyed.

A high level of hygiene and safety measures for personnel are required in the handling of infected and suspect animals. All field and laboratory staff should be trained in the correct use of PPE and in the decontamination of reusable equipment. Contamination with aerosols and saliva is highly possible; therefore, all personnel who are associated with the program, and are handling animals and animal parts must take all necessary precautions. This includes the use of gloves, masks and eye protection.

The medical reasons for rabies pre-exposure and postexposure vaccination should be explained to all staff. They should also be fully conversant with the correct first-aid and medical procedures to be employed after a potential human exposure.

Capture and handling of animals

Nets or dog-catching poles with stout rope or wire loops may be used for small animals, and ropes or other restraints for large animals. Containers, cages or pens must be very strongly constructed and well secured. If a suspect animal is first presented at a veterinary clinic, it should be hospitalised away from other animals. Confined suspect animals should be under veterinary care.

Immediate postexposure first aid of dogs should occur in accordance with first-aid guidelines for people (see Section 3.2.3). When handling an exposed animal, handlers should take due care to minimise any risks to themselves.

Destruction of animals

Animals should be destroyed safely and promptly. When selecting destruction methods, preserving opportunities for sampling for disease should be considered. Destroyed animals, and their excretions and secretions, should be handled with care and while wearing appropriate PPE to avoid potential exposure to live virus through abraded skin or mucous membranes (eg eyes and mouth).

Postmortem examinations and specimen collection

Postmortem examinations and specimen collection in rabies cases pose particular hazards to staff. See Section 1.4.3 for further details.

3.3 Strategy for control and eradication

The default policy is to eradicate the pathogen in animals and to prevent spread of infection to humans.

The control and eradication of an outbreak of rabies will require the collaborative efforts of animal and human health services. Wildlife and other relevant authorities should also be involved in the response.

3.3.1 Stamping out

Destruction of the infected animals (eg maintenance hosts) and dangerous contact animals is necessary because infected animals are the only source of spread. However, the cornerstone of effective response to rabies is vaccination, which will be implemented early in an eradication program.

Although the destruction of some animals may be necessary during rabies control programs, care must be taken in any policy that involves widespread destruction of animals. Experience has shown this to be ineffective, costly and unpopular.

Culling of wild or feral animals may be counterproductive. New animals move into depopulated territories, with concurrent behaviours such as increased fighting and territory protection that can lead to an increased rate of infection. However, population management strategies (eg fertility control, environmental modification) to reduce the carrying capacity may be effective in principle.

3.3.2 Quarantine and movement controls

Infected premises

The infected premises (IP) will be immediately declared and quarantine requirements imposed. The area around the IP will be declared as a restricted area (RA), and movement controls will apply. Infected animals will be destroyed.

Restricted area

The movement of susceptible animals into and within the RA will be controlled under a permit system (see Section 4.2).

A trace animal in an RA will be confined so that there is no contact with other susceptible animals. During confinement, it will be vaccinated and observed until it either demonstrates infection or a titre of more than 0.5 international units (IU)/mL postvaccination.

An animal that is known to have been exposed to rabies and that is considered highly likely to be infected (ie a dangerous contact animal) will not be vaccinated, but will be destroyed.

On a case-by-case basis and only where security can be assured, the CVO may decide, in consultation with the diagnostic team, that a suspect animal (an animal not known to have been exposed to rabies, but showing clinical signs requiring differential diagnosis) may not be immediately destroyed. In such cases, a conservative approach would be taken unless available information indicates otherwise. For example, the animal may be held in quarantine, such as on an