In addition, *Exotic Diseases of Animals: A Field Guide for Australian Veterinarians* by WA Geering, AJ Forman and MJ Nunn, Australian Government Publishing Service, Canberra, 1995 (to be updated) is a source for some of the information about the aetiology, diagnosis and epidemiology of the disease.

AUSVETPLAN manuals[5]

Disease strategies

- Individual strategies for each of 35 diseases
- Bee diseases and pests
- Response policy briefs (for diseases not covered by individual manuals)

Operational procedures manuals

- Decontamination
- Destruction of animals
- Disposal
- Livestock welfare and management
- Public relations
- Valuation and compensation

Enterprise manuals

- Artificial breeding centres
- Feedlots
- Meat processing
- Saleyards and transport
- Poultry industry
- Zoos

Management manuals

- Control centres management (Parts 1 and 2)
- Laboratory preparedness

Wild animal response strategy

Summary document

[5] The complete series of AUSVETPLAN documents is available on the internet at: www.animalhealthaustralia.com.au/programs/eadp/ausvetplan/ausvetplan_home.cfm

Contents

Figures

1 Nature of the disease

This AUSVETPLAN manual considers only rabies caused by lyssaviruses that are maintained and transmitted in warm-blooded terrestrial animals and bats. Infection with Australian bat lyssavirus is covered in a specific AUSVETPLAN disease strategy manual.

Rabies is a viral encephalitis of mammals that is almost invariably fatal. It is usually transmitted by bites and has a variable incubation period of days to years. Globally, the disease is of both public health and animal health significance.

Although an endemic lyssavirus is present in bats in Australia and can cause a fatal encephalitis – which is indistinguishable from rabies in humans – Australia is free from the rabies virus. Rabies is a disease that is present in most of the world, and is maintained in various hosts such as dogs and other carnivores, and bats.

1.1 Aetiology

Rabies is caused by infection with viruses of the genus *Lyssavirus,* family *Rhabdoviridae.* Lyssaviruses are genetically and serologically related, and all cause similar diseases in mammals. The genus is classified phylogenetically into seven genotypes, with four proposed new genotypes each from central Asian microchiropterid bat (Table 1.1). Lyssavirus genotypes can be further classified into variants or biotypes, defined by their maintenance-host species. A virus biotype is adapted to a single maintenance-host species, where infection and transmission by members of this species are highly efficient. Other species may also be infected by the virus biotype, but these hosts may be too inefficient as vectors or may not be numerous enough to maintain a cycle.

Example of classification:
Family: *Rhabdoviridae*
Genus: *Lyssavirus*
Genotype: for example, classical rabies = genotype 1

Rabies infection in people causes a fatal encephalitic disease. It is a significant public health issue in those areas of the world where it is present. It is virtually always fatal in humans once symptoms appear, and medical advice should be immediately sought if there is a risk of infection.

Table 1.1 Lyssavirus genotypes: common name, numerical genotype classification, geographic location, maintenance hosts and known spillover hosts

Name	Genotype designation	Locality	Maintenance hosts	Spillover hosts reported
Rabies virus	1	Worldwide (with exceptions) Bat biotypes are confined to the American continents — insectivorous bats mainly in North America, haematophagous bats in South and Central America, and the Caribbean.	Multiple American insectivorous bats: highest frequency in *Eptesicus fuscus*, *Lasionycteris noctivagens*, *Lasiuris* spp., *Myotis* spp., *Pipistrellus* spp., *Tadarida brasiliensis* Haematophagous (vampire) bats: *Desmodus* spp. Carnivores	Insectivorous bat strains: humans, foxes, skunks Vampire bat rabies: mainly cattle, horses, humans Carnivore rabies: several spillover hosts reported, including cats, dogs, humans, cattle, horses and wildlife
Lagos bat virus	2	Sub-Saharan Africa One case from France in a fruit bat imported from West Africa (1999)	Fruit bats: *Eidolon helvum*, *Micropterus pusillus*, *Epomophorus wahlbergi* Single isolate from insectivorous bat: *Nycteris gambiensis*	Cats, dogs, *Atilax paludinosus* (water mongoose)
Mokola virus	3	Sub-Saharan Africa	Not known. Has been isolated from shrews (*Crocidura* spp.)	Cats, dogs, humans, shrews
Duvenhage virus	4	Southern and eastern Africa	Insectivorous bats: *Nycteris thebaica*, possibly *Miniopterus schreibersi*	Humans
European bat lyssavirus 1	5	Europe (continental)	Insectivorous bats, particularly *Eptesicus serotinus*	Sheep, stone martens (*Martes foina*), cats, humans
European bat lyssavirus 2	6	Europe (continental, United Kingdom)	Insectivorous bats, particularly *Myotis daubentonii*, *Myotis dasycneme*	Humans
Australian bat lyssavirus	7	Australia	Flying foxes (*Pteropus* spp.) Insectivorous bat: *Saccolaimus flaviventris*	Humans
Aravan, Khujand, Irkut, West Caucasian bat virus (WCBV)	Undesignated Proposed new genotypes	Central Asia	Single isolates from insectivorous bats: *Myotis blythi* (Aravan virus), *Myotis mystacinus* (Khujand virus), *Murina leucogaster* (Irkut virus), *Miniopterus schreibersi* (WCBV)	None recorded

1.2 Susceptible host species

Lyssaviruses infect warm-blooded animals. However, rabies virus (genotype 1) can be further classified into variants or biotypes that have adapted to a specific host species and are referred to as the host biotype. The term biotype, defined by its maintenance-host species, will be used throughout this document for purposes of control and management.

The rabies virus life cycle involves maintenance-host species and spillover-host species:

- *Maintenance host.* The species that principally sustains the virus life cycle is highly susceptible to its biotype, but less susceptible to other biotypes. Successful control of rabies in the maintenance host will lead to eradication of the virus cycle in the ecological community.
- *Spillover host.* Infected hosts that belong to species that do not normally maintain the virus biotype in question. These hosts are not maintenance hosts and have no epidemiological significance in sustaining rabies epidemics. Spillover hosts are often, but not always, dead-end hosts. They may transmit infection to other hosts, although such events are relatively uncommon. Spillover hosts include humans and other primates, horses, cattle, sheep, pigs and some wild species.

The virus dose required to cause infection is higher for nonmaintenance species than for the maintenance host. The probability of establishing infection is also lower, the clinical and pathological course of the disease is less consistent, and virus shedding, and therefore transmission, is less effective.

The maintenance hosts of rabies virus are usually members of the orders Carnivora and Chiroptera. Domestic dogs are a major maintenance host in much of the world, as they were in Europe and North America before the early decades of the 20th century. Dogs still cause the majority of human rabies in the world today. In some areas of the world where dog rabies has been controlled, wildlife species have become more important for maintenance of the disease. This includes the red fox and raccoon dog in Europe; striped skunks, raccoons, red and grey foxes, and coyotes in North America; side-striped and black-backed jackals, various mongoose species (particularly the yellow mongooses) and bat-eared foxes in southern Africa; and the Arctic fox in the northern polar areas. Table 1.2 gives examples of terrestrial maintenance-host species and their localities.

Other hosts include many species of American bats. The disease appeared in the Americas during the 20th century following ecological changes that allowed major increases in bat density.

Although most, if not all, warm-blooded animals are susceptible to infection with rabies virus, rabies is not regarded as a disease of avian species. In most cases where the infection has been observed in birds, it was experimentally induced (Gough and Jorgenson 1976; Scott 1993). Birds therefore do not play a significant part in the maintenance or spread of rabies virus. The susceptibility of Australian native animals is unknown.

Table 1. 2 Examples of maintenance-host species for rabies virus (genotype 1) biotypes

Maintenance host	Locality
Family Canidae	
Domestic dog (*Canis lupus*)	Africa, Asia, and Central and South America
Arctic fox (*Alopes lagopus*)	Arctic regions
Raccoon dog (*Nyctereutes procyonoides*)	Eastern Baltic states
Red fox (*Vulpes vulpes*)	Europe,[a] Canada (Ontario), USA (northeast)
Bat-eared fox (*Otocyon megalotis*)	South Africa (Cape)
Black-backed jackal (*Canis mesomelas*)	Southern Africa
Side-striped jackal (*Canis adustus*)	Zimbabwe
Other Carnivora	
Striped skunk (*Mephitis mephitis*)	USA, Canada
Raccoon (*Procyon lotor*)	USA, Canada
Indian mongoose (*Herpestes auropunctatus*)	Caribbean
Yellow mongoose (*Cynictis penicillata*)	South Africa

USA = United States of America
a Rabies has been eradicated in western Europe.

1.3 World distribution and occurrence in Australia

Rabies virus occurs throughout most of the world except in Australia, New Zealand, Papua New Guinea, Japan, Great Britain and Ireland, and many small island nations. In some areas of the world (eg parts of western Europe), effective management of rabies in animals has reduced the frequency of occurrence of rabies in humans, and led to its eradication. However, the number and size of rabies-free countries, territories or areas is small compared with those of rabies-affected areas. Recently, there has been an outbreak of rabies in Indonesian islands such as Bali and Flores; previously, these islands had been considered rabies free.

Human rabies is found wherever animal rabies is found. Globally, human deaths from rabies are estimated to be between 55 000 and 100 000 per year. About 95% of these deaths occur in Asia and Africa. World Health Organization (WHO) data on human and animal rabies are available from the WHO website.[6] The most up-to-date information on the global rabies situation is given in the World Animal Health Information Database (WAHID) Interface.[7]

In Australia, there was one probable occurrence of transmission of rabies. This occurred in Tasmania in 1867 and involved several dogs, a pig and a child bitten by one of the dogs. In two more recent cases (1987 and 1990), individual children who contracted the infection in endemic countries developed the clinical disease in Australia after a protracted incubation.

[6] http://apps.who.int/globalatlas/default.asp
[7] www.oie.int/wahid-prod/public.php?page=disease_outbreak_map

1.4 Diagnostic criteria

1.4.1 Clinical signs

Animals

It is important to recognise the variability of the clinical syndrome. The clinical signs of rabies can, in many instances, be subtle and even unremarkable.

Clinical signs of the disease are attributable to the neurological effects of the infection. Clinical signs may change as the disease progresses and may be intermittent, alternating between different states during the course of the disease. They fall into six main categories:

- *Excitation*. This includes unprovoked aggression, overreaction to external or perceived stimuli, aimless wandering, restlessness, self-inflicted trauma (eg scratching, biting).
- *Paralysis*. This can affect any of the motor systems, causing ataxia; knuckling of distal limbs; paresis; the inability to swallow, close the jaws and lips, or retract the tongue; and facial asymmetry, including drooping ears and eyelids.
- *Loss of normal social and behavioural responses*. Wild animals will often lose their natural fear of humans. These animals may wander into urban areas and into buildings, and can be attacked and killed by dogs. Frequently, this happens at unusual times; for example, nocturnal animals may appear during the day. In companion animals, owners may report a 'personality change'.
- *Unusual vocalisation*. Many rabid animals will vocalise relentlessly. In dogs, the tone is altered, due to muscular incoordination of the larynx, causing a characteristic low-pitched, hoarse howling.
- *Pica*. Rabid animals, particularly carnivores, will chew, and often swallow, anything in their environment, including soil, plant material and bedding.
- *Coma*. This is seen in the terminal stage of the disease.

A rabid animal's signs can often rapidly change; for example, a dog may change, without provocation, from resting quietly to running frenziedly. Cats may attack suddenly and without warning. In addition to the above, animals may suffer from signs secondary to the direct neurological changes, including dehydration, red eyes, salivation, poor condition, unkempt pelage and signs of trauma. However, cases have been reported where death has occurred with few, if any, premonitory signs.

In much of the traditional literature, the clinical signs of rabies are classified into furious and dumb syndromes. While this may have been a useful classification, particularly for dog rabies, the clinician must take care not to let this bias prevent them from noticing other manifestations of the disease.

Dogs

There is a prodromal stage, which lasts 2–3 days, but is often missed by the dog's owner. During this stage, there is a sudden change in temperament. Dogs that are normally friendly towards people may suddenly become snappy and uncertain, and shy dogs may become affectionate. There may be a rise in temperature, dilation of the pupils and hyperaesthesia at the wound site.

A rabid dog will typically become unusually restless, seldom lying or sitting in one place for more than a short time. If confined, it will move around ceaselessly. At certain periods, the dog may seem possessed of abnormal strength and insensitivity to pain. Bars of cages, furniture and other objects are frequently attacked to the point where the animal's teeth are reduced to stumps and the mouth lacerated. If the dog is not under restraint, this excitable energy is manifested by furious, aimless running (sometimes for long distances), and by snapping at animate or inanimate objects in its path. Alternatively, the dog may remain quiet and lethargic; it may hide behind cover and bite only when provoked.

In many cases of rabies, the animal's pupils are dilated, there is loss of the corneal reflex, and there is sometimes a squint. The animal assumes a watchful, puzzled or apprehensive look, and may snap at imaginary objects. There is a change in phonation, often with a characteristic low-pitched, hoarse howling. Their appetite for usual foods decreases, and animals start to eat stones, sticks, earth and other objects. There may be muscle tremors and paralysis of the hindquarters, the jaw ('dropped jaw') and the tongue, which hangs flaccidly from the mouth. There may be drooling from the mouth. Often, the rabid dog is unable to eat or to lap water, although it may repeatedly try to do so. In contrast to human rabies, hydrophobia is a rare sign in dogs and other animals. Within 1–4 days of the onset of symptoms, there is rapidly progressing ataxia. Death supervenes within a few days, usually from paralysis of respiratory muscles.

Cats

The clinical signs in cats are generally similar to those in dogs, but unprovoked aggression is a more common presenting sign. Rabid cats often retreat into hiding, from where they spring to attack humans or other animals ferociously when approached. Their pupils are dilated. They may mew continuously and the vocalisation becomes hoarse. As the disease progresses into the paralytic phase, the animal shows marked incoordination, followed by posterior paralysis. The muscles of the head become paralysed, and the animal soon lapses into a coma and dies.

Horses

Clinical signs of rabies in horses are highly variable and can be easily confused with other diseases affecting the nervous system, such as cervical vertebral malformation or other viral encephalitides.

Periods of marked excitation and aggressiveness alternate with periods of relative calm. In periods of excitation, affected animals become restless, stare, paw, move their ears, draw their upper lips back and forth continually, and salivate excessively. There may be intense sexual excitement. Animals frequently grind their teeth, whinny as if in great pain, and show signs of acute colic, which may present as oesophageal obstruction. They may lash out with great fury at any perceived threat or restraint (donkeys will often attack and bite other animals and people). They often bite or rub at the site of exposure, causing self-mutilation. As

paralysis develops, they fall repeatedly, finally remaining down with their legs thrashing. In some cases, the excitatory phase is absent and there is dysphagia, aimless wandering or staggering, and a rapidly developing paralysis. Equine rabies progresses rapidly, with most affected animals becoming depressed, recumbent and comatose before dying within 5 days of the onset of clinical signs.

Cattle

In cattle, there is initial depression and cessation of milk production. Paralysis of throat muscles with grinding of teeth and excess salivation is common, and may lead to a false diagnosis of oesophageal obstruction. Cattle may bellow frequently in a low-pitched voice. There is increased sexual excitement. Some animals develop one or more furious stages, and may attack other animals or objects; they charge and butt, but seldom bite. Other animals show little excitement.

As paralysis develops, cattle knuckle over at the fetlocks, stumble and fall frequently. Finally, they are unable to rise, lapse into a coma and die.

Sheep

In sheep, a period of excitement occurs, during which affected sheep move restlessly, salivate and grind their teeth. They also show twitching of the lips and oscillation of the tongue, pulling of wool and aggressive butting of other sheep or objects. Rams exhibit sexual excitement. Sheep may either be silent or emit frequent hoarse bleats. The excitation stage is followed by depression, increasing weakness, paralysis and recumbency. Sheep generally die within 72 hours of the onset of clinical signs.

Pigs

Affected pigs tend to stand trembling in a darkened corner, but may dash out and bite if provoked. They may rub or gnaw at the bite site. There is abnormal deep grunting. Depraved appetite is common. There may be alternating periods of intense activity and recumbency. Sows may kill their offspring. There is increasing dullness, incoordination and paralysis.

Foxes

In foxes, the normal fear of people and other animals is lost. The normal social etiquette between foxes, particularly with regard to territoriality, is not observed by the rabid fox, leading to conflict. Anorexia, agitation and a characteristic abnormal cry occur. A rabid fox may charge at and bite passing people, animals and even vehicles. As the disease progresses, the fox becomes more confused and uncoordinated. With the onset of paralysis, it falls and may be unable to rise. It may attempt to drag itself before finally lapsing into a coma and dying.

Other wildlife species

In other wildlife species, the clinical signs are variable. The most important common feature is loss of normal shyness and fear of people and other animals. This makes such animals particularly dangerous to children, who wrongly interpret this behaviour as indicating friendliness.

Humans

The clinical manifestations of rabies in humans are well described (Heymann 2008), and the disease is almost invariably fatal.

1.4.2 Pathology

Gross lesions

Usually, no remarkable gross pathological signs are evident; when present, they are secondary to the neurological effects. Carcasses are often dehydrated and in poor physical condition, and may have physical signs of recent trauma; for example, broken teeth. In carnivores, signs of pica, such as soil and plant material in the mouth and stomach, may be present.

Microscopic lesions (histopathology)

Microscopically, the most significant lesions are in the central nervous system, and cranial and spinal ganglia. There is usually perivascular cuffing, focal and diffuse gliosis, neuronal degeneration, and intracytoplasmic inclusion bodies (or Negri bodies) in the neurones. Negri bodies vary in size with the host – they are large in dogs and cattle. Negri bodies are found most commonly in the neurones of the hippocampus or in the Purkinje cells of the cerebellum in cattle. They are found less frequently in the glial cells, in ganglion cells of the salivary glands and adrenal medulla, and in the retina.

Pathogenesis

Rabies virus is transmitted through saliva by the bite of a rabid animal. After the inoculation of virus into a wound, virus replicates in local tissues. Within hours to days after a bite, there is invasion of peripheral nerve endings, followed by centripetal movement of virions along axons to the central nervous system (CNS). Once the CNS is invaded by virus, clinical signs become apparent and the disease course is irreversible. CNS infection patterns and therefore clinical signs vary, but often include behavioural changes that lead to biting other animals. From the CNS, virus invades peripheral nerves, leading to virus infection of many peripheral tissues, including salivary glands. Virus infection of salivary acinar cells leads to shedding of large numbers of virions into saliva. Shedding is coincident with the behavioural changes that lead to biting of other animals.

No signs – clinical, pathological or immunological – are apparent before CNS invasion, a period referred to as the incubation period. The disease, once it appears, is acute and progressive, leading almost invariably to death of the host if the animal is not destroyed beforehand. The overwhelming neural infection is unusual in that it is slow to provoke an inflammatory response. Once the inflammatory response appears, it contributes to irreversible neurological damage. This pattern of viral invasion followed by inflammatory response causes the typical progression of excitatory to paralytic disease.

A significant proportion of bites by rabid animals do not result in transmission and development of disease. This is usually due to a low dose of virus in the bite inoculum, which does not lead to detectable seroconversion. Alternatively, infection may be initiated at the site of inoculation, but is cleared before establishment in the CNS. This is known as 'abortive infection' and does not result in clinical signs of disease, but may result in seroconversion.

Although there are some reports of dogs surviving rabies or developing chronic infection in western Africa, Ethiopia and India (Veeraraghavan et al 1971, Fekadu

1972, Aghomo et al 1989, Fekadu 1991), these findings have not be reproduced in recent years. It is generally accepted that there is no carrier or latent state for rabies.

1.4.3 Laboratory tests

Rabies may be suspected in animals that display neurological signs, including behavioural changes and paralysis, followed by death within 10 days. The diagnosis must be confirmed by laboratory tests. A positive result in any species must be notified immediately to the chief veterinary officer (CVO) of the state or territory concerned, who will immediately notify their public health department equivalent.

Specimens should initially be sent to the state or territory veterinary laboratory (or other appropriate laboratory), from where they may be forwarded to the CSIRO Australian Animal Health Laboratory (CSIRO-AAHL) for testing or confirmation of positive or suspicious test results.

Specific laboratory diagnostic tests are necessary to confirm rabies infection as neither clinical signs, nor gross or histological pathology are pathognomonic. The tests currently available for rabies diagnosis are discussed in the section 'Laboratory diagnosis', below.

Further information on testing is available on the website of the Australian Government Department of Health and Ageing (www.health.gov.au).

Specimens required

Because rabies is almost invariably fatal without vaccination, operators should take adequate precautions to prevent accidental exposure or self-inoculation when collecting specimens. If a potential exposure to rabies occurs, first-aid procedures should be undertaken immediately. Medical advice should always be sought without delay, irrespective of vaccination status, as postexposure prophylaxis may be needed. Please also refer to Section 3.2 for more information on occupational health and safety aspects.

Before shipping specimens, submitters should contact the receiving laboratory to discuss arrangements for sampling, transport and sample reception.

For all species, whole animals, severed heads or unpreserved brains should be chilled and forwarded on ice to the testing laboratory. The brain is the most important specimen for laboratory confirmation of rabies. Distribution of virus in the brain is usually diffuse, but may be localised in some cases. Of the structures of the brain, the brain stem is the most consistently reliable area for detection of infectious virus or viral antigen. Other regions of the brain, including the hippocampus, are negative in up to 5% of rabid animals. For this reason, it is important to take a composite brain sample to include several different parts of the brain in the diagnostic test. If the brain is not present, other suitable tissues include spinal cord, the trigeminal ganglion, peripheral nerves (taken from points close to the CNS) and salivary glands.

Unless the operator is vaccinated and experienced, the head or brain should not be removed before submission because of the risk of self-inoculation.

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.

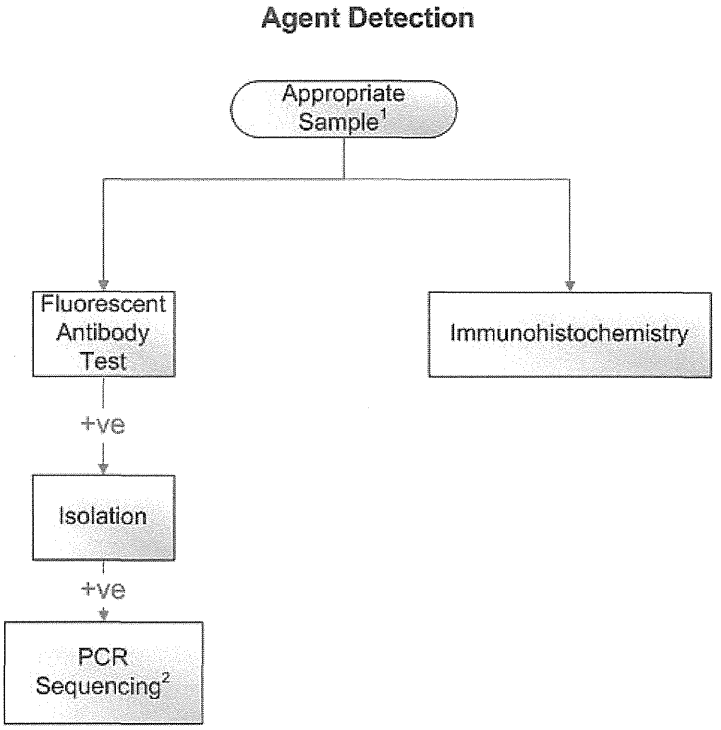
Firstly, the necessary clearance from the CVO of the state or territory of the disease outbreak should be obtained, and the CVO of Victoria should be informed about the transport of the specimens to Geelong. Then specimens should be sent to the state or territory diagnostic laboratory, from where they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