Chapter 26

RABIES VIRUS INFECTION

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Introduction and history

Terrestrial rabies is a neurotropic disease of virtually all mammals. It exists worldwide, except in places such as Australia and New Zealand, and terrestrial rabies has been eradicated from the British isles by quarantine and other measures.

The disease has been recognised since antiquity. The Latin word *rabies* comes from an old Sanskrit word *Rabhas*, which means 'to do violence' (Steele, 1975). In Britain, the earliest mention of the disease was in 1026, but is was not until the late eighteenth and nineteenth centuries that it became frequent. Dogs were recognised as the major source of infection (urban rabies) and the first attempt to deal with it on a national basis was made in 1831 when a Bill was drafted to 'prevent the spreading of canine madness' (Waterhouse, 1971). The Bill did not become law, but local authorities were later given the power to require muzzling, to restrict the movement of dogs and to destroy strays. These methods were only partly successful when left to local authorities, but when the Board of Agriculture assumed overall responsibility and introduced quarantine, among other measures, there was a marked decline in the number of rabies cases each year. The disease appeared to have been eradicated in 1903, was reintroduced in 1918, but was finally eliminated by 1922.

The absence of records of terrestrial rabies in wildlife in Britain, and the success of the straightforward measures by which the disease was eventually eliminated through the control of dogs, suggest that it was never established in the United Kingdom in a permanent and widespread form. Between 1886 and 1903 in the whole of Great Britain, 3056 animals are known to have died of rabies, but of these, the only wild animals affected were a herd of deer, and it is presumed that this outbreak originated from attacks by a stray, rabid dog (Waterhouse, 1971).

Since the final eradication of terrestrial rabies from Great Britain in 1922, although several cases have occurred in animals while in quarantine, only two cases have occurred in dogs that had passed through the specified 6 month quarantine period (Morgan-Jones, 1969; Peace & Hopes, 1970). Fortunately, in both cases, the disease was contained, but as a result of this, quarantine procedures were changed and animals entering quarantine were later required to be vaccinated; only two deaths from rabies have since occurred in quarantine (King *et al.*, 1985; Department of Health Memorandum, 2000). However, more recently, rabies control measures in the UK have changed again with the introduction of the Pet Travel Scheme (PETS). This scheme allows pet dogs and cats from certain countries to enter the UK without quarantine as long as they meet certain conditions (see below).

The causative agent

Rabies virus is a bullet-shaped virus, a member of the Rhabdoviridae. The Rhabdoviridae are enveloped RNA viruses characterised by their shape (Greek *rhabdos*, rod) and by the presence of helical ribonucleocapsids enclosed in a lipid envelope bearing surface projections. The genome is a single molecule of negative sense single-stranded RNA which is non-infectious and is transcribed into five mRNAs, each of which codes for a single protein. The gene order is 3′-N-NS-M-G-L-5′, representing the nucleocapsid protein (N), the non-structural protein (NS), the matrix protein (M), the envelope glycoprotein (G) and a large protein (L), the RNA-dependent RNA polymerase (Tordo *et al.*, 1988; Wunner *et al.*, 1988; Pastoret & Brochier, 1992).

Rabies virus belongs to the genus *Lyssavirus*, named after rabies in Greek (Greek *Lyssa*, madness). For many years rabies virus was thought to be unique. It is now clear that antigenic variation exists within the rabies virus (Schneider, 1982) and the existence of several distinct rabies-related viruses is now recognised (King *et al.*, 1990; Pastoret & Brochier, 1992; Bourhy *et al.*, 1993). These viruses can be distinguished from rabies virus using conventional sera or monoclonal antibodies and sequence-based phylogeny. The exact significance of rabies-related viruses in the epidemiology of classical rabies is not well understood, but some rabies-related viruses may infect cats (Foggin, 1982; King & Crick, 1988). Of special interest are lyssaviruses isolated from insectivorous bats which can be occasionally transmitted to terrestrial mammals (Office International des Epizooties, 1998; McColl *et al.*, 2000).

The biological behaviour of conventional isolates of rabies virus also varies in that there are differences in pathogenicity between strains, depending on their species of origin or on their passage history in the laboratory; the dose and route of exposure also influence the outcome of infection (Charlton, 1988). There is also an intrinsic difference in species susceptibility to rabies virus infection in that the amount of infectious virus needed to induce the disease experimentally differs markedly between species (Sikes, 1962; Parker & Wilsnack, 1966; Smith & Baer, 1988). The level of virus excretion and the percentage of virus excretors within an infected group also vary according to the species and the infecting virus strain (Blancou & Barrat, 1988). In some species a paradox has been observed when a high dose of virus has been used for infecting animals; such animals seem to be more resistant to challenge than animals given a lower infecting dose (Blancou *et al.*, 1983).

Rabies virus is sensitive to lipid solvents and emulsifying agents and thus is quickly inactivated by a number of disinfectants, including formalin, soap and quaternary ammonium compounds (Waterhouse, 1971; Dean, 1975). The virus is easily inactivated by heat and sunlight, but stable at low temperatures. Under normal environmental conditions, therefore, it does not remain infective for long outside the host in contaminated secretions.

Pathogenesis and pathology

Rabies virus is excreted in the saliva of infected animals and is mainly transmitted by biting, or less frequently, by contamination of mucosa or a superficial wound (Afshar, 1979). In the USA, airborne transmission has also been recorded in humans, coyotes and foxes following exposure to caves heavily populated by infected bats (Constantine, 1962; Winkler, 1975). However, several dogs and cats exposed in the same way did not become infected. Experimentally, rabies has been transmitted by the oral route to various laboratory animals, foxes, skunks and cats, and infection of dogs from eating rabid fox carcases has been reported from the Arctic (Soave, 1966; Bell & Moore, 1971; Charlton, 1988). An important sequela to the demonstration of oral infection is the development of oral rabies vaccines for wildlife.

On gaining entry to the body, the virus multiplies locally within myocytes at the site of exposure before moving up the axon of the associated nerve to the central nervous system (CNS) to produce an encephalitis; there is also evidence that in some cases virus may enter peripheral nerves directly, without preliminary replication in non-nervous tissue (Murphy *et al.*, 1973a, b; Baer, 1975a; Charlton, 1988). During the spread of virus through the CNS, virus moves centrifugally into the peripheral nerves, viral antigens appearing in nerve endings in salivary glands, skin, mucosal surfaces, gut and most other organs. Thus saliva may be infective for a few days before clinical signs appear.

As in other species, infection of the salivary glands is important in the spread of the disease: at least 70% of rabid cats have virus in their saliva and are able to transmit infection (Vaughn *et al.*, 1963; Chantal & Blancou, 1985; Blancou & Pastoret, 1990), and this may vary according to the virus strain.

Gross pathological findings are minimal in rabies. The carcass may be emaciated and there may be evidence of self-trauma. Although in dogs there may be foreign bodies in the alimentary tract as a result of pica, this is less common in cats. Histologically, there is diffuse encephalitis with mononuclear cell perivascular cuffing and focal gliosis, constituting an inflammatory response typical of any non-suppurative infection of the brain (Jubb *et al.*, 1985). Spongiform lesions in the brains of rabid animals have also been reported (Charlton, 1988).

There may also be a ganglioneuritis in the paravertebral ganglia and degenerative changes in the salivary gland. Neuronal degeneration in rabies is usually relatively severe compared with some other viral infections of the CNS, and in carnivores in particular it is often quite extensive. In addition, the neurons may contain characteristic intracytoplasmic inclusions containing viral antigen, called Negri bodies. These can

occur in several areas of the brain, but are usually most prominent in the hippocampus; they are pathognomonic for rabies in most species (Atanasiu, 1975). In cats, however, some confusion may arise, in that even in normal cats inclusions that resemble Negri bodies may be found in the cytoplasm of some nerve cells (Szlachta & Habel, 1953).

Although Negri bodies are found in the majority of cases of rabies (McQueen, 1960; Tustin & Smit, 1962) and have in the past been used extensively in diagnosis, their absence does not exclude the disease. In addition, when identification of Negri bodies was used as the predominant method of rabies diagnosis, it was usually considered necessary to keep the animal alive until it died naturally, to increase the sensitivity of the test (Tierkel, 1959). The technique has therefore now been superseded in most countries by more reliable techniques in terms of specificity, sensitivity and speed of diagnosis (see below).

As already mentioned, the susceptibility of animals to rabies virus varies a great deal, depending on the animal species, the virus dose and the virus strain (biotype). For example, foxes are highly susceptible to their own virus, but humans seem to be fairly resistant to this biotype. In general, cats are probably of intermediate susceptibility (Jaeger & Barth, 1979; Soulebot *et al.*, 1981). Cats appear to be more susceptible than dogs to attenuated strains of vaccines (Dean & Guevin, 1963; Vaughn, 1975; Pastoret *et al.*, 1985). Other factors significant in determining an individual's susceptibility to the disease include the age of the animal, and the nature and site of the wound. Thus, a young cat suffering a deep wound in the head region, heavily contaminated with saliva, is more likely to develop the disease, with a shorter incubation period, than an older animal with a superficial wound on the extremities. In mice, resistance to rabies virus infection has been shown to be under genetic control (Lodmell, 1988).

Experimentally in cats the incubation period has been shown to range from 9 to 51 days (median 18 days), while the periods of clinical illness until death ranged from 1 to 8 days (median 5 days) (Vaughn *et al.*, 1963). Virus was first detected in the saliva of these cats, by conventional virological techniques, 1 day before the appearance of clinical signs, and virus shedding usually then continued until the cat died. Under natural conditions, the incubation period is generally similar, although in occasional cases it may be much longer, probably up to 6 months or more. In dogs, for example, cases have occasionally been recorded beyond this time (Waterhouse, 1971).

Clinical signs

The clinical course of rabies is classically described as three, often overlapping, phases: the prodromal period, the excitative phase ('furious' rabies; Figure 26.1) and the paralytic stage ('dumb' rabies; Figure 26.2). In cats, the prodromal phase usually lasts for only 1 day and is manifested by a marked change in behaviour (Vaughn, 1975). Sullen cats may become more alert, restless and friendly, whereas amicable cats may scratch or bite without provocation, or become depressed and withdrawn, hiding in dark places. Aggression is more commonly observed in the cat than in the dog (Tierkel, 1959). During this initial period, there may be slight pyrexia and some dilatation of the pupils, with an impaired corneal reflex.

Gradually, the excitement phase predominates, and it is in this form that rabies is most easily recognised and the animals are at their most hazardous to others. The cat becomes increasingly nervous, irritable and vicious, and may show muscle tremors, flaccidity or

Figure 26.1 'Furious' rabies.

Figure 26.2 'Dumb' rabies.

incoordination. As in humans, there is difficulty in swallowing owing to spasm, and eventual paralysis of the muscles of deglutition. This in turn leads to the accumulation of saliva, and drooling or frothing. As the cat enters the paralytic stage, muscular incoordination and convulsions gradually lead to generalised paralysis, coma and death. The excitement phase may last for up to 7 days, but sometimes it is virtually non-existent and cats progress directly from the prodromal phase to the paralytic stage.

Although in classical rabies there is undelayed progression of the characteristic clinical signs through to death, in some cases infection may lead to a more variable outcome. Thus, in dogs in Ethiopia a carrier state has been reported, which may or may not be associated with a period of clinical signs (Andral & Serie, 1965; Fekadu, 1975; Carey & McLean, 1983; Charlton, 1988). Chronic recrudescent rabies has also been observed experimentally in a cat (Perl *et al.*, 1977). Recovery from CNS infection in both animals and humans has also been reported, albeit rarely (Bell, 1975; Fedaku *et al.*, 1981; Charlton, 1988). A serological survey in South America showed that 1% of the cats included in the study had antirabies neutralising antibodies (Diaz *et al.*, 1975). However, this may have been only as a result of the peripheral infection and its significance is not clear.

Since most animals die from rabies and no serological response can be detected before the onset of clinical signs, most information on protective immune mechanisms in rabies has been obtained from vaccination studies. There is a good correlation between antibody titres obtained after vaccination and protection (Blancou *et al.*, 1986a), and protection against rabies can be obtained through passive transfer of specific antibodies. However, vaccinated animals without detectable neutralising antibodies can still resist challenge, and undoubtedly other immunological mechanisms, particularly cell-mediated immunity, are involved in protection.

Diagnosis and action in suspect cases

In Britain, where rabies is not endemic, most veterinary surgeons are inexperienced in dealing with the disease. Nevertheless, because of the public health risk and the great danger that the disease may become established in the terrestrial wildlife of the country, a diagnosis of rabies should always be considered when certain clinical signs are present. The furious form of rabies, where the cat shows unprovoked aggression, is probably most easily recognisable, but should be distinguished from pseudorabies (Aujeszky's disease; see Chapter 30). However, it is when an incoordinated, paralytic or moribund animal is presented that there may be most difficulty. The differential diagnosis includes toxoplasmosis, CNS infection, neoplasia or trauma, thiamin deficiency, oral and pharyngeal foreign bodies, and poisoning with substances such as lead, organochlorine compounds, benzoic acid and strychnine. The syndrome of spongiform encephalopathy should also be considered (Wyatt *et al.*, 1990, 1991). The severe weakness seen in animals moribund from infectious diseases such as panleucopenia and feline leukaemia virus infection may also on occasion simulate paralytic or terminal rabies.

A detailed account of the action to be taken in Britain in a case of suspected rabies has been given by members of the State Veterinary Service (Anon, 1987; Department of Health Memorandum, 2000). In brief, a suspect case should be detained in isolation on the premises on which it has been examined and the Divisional Veterinary Manager (DVM) of the Department for Environment, Food and Rural Affairs (DEFRA) must be notified immediately. The veterinary surgeon and any other handler should carry out a thorough personal disinfection in soap or detergent and water and any contaminated clothing should be changed. If anyone is bitten or scratched, it is imperative to wash and flush the wound immediately with soap or detergent (not both, as soap inhibits quaternary ammonium

compounds) and water, then water alone, followed by the application of 40–70% alcohol, tincture or aqueous solutions of iodine, or 0.1% quaternary ammonium compounds (e.g. cetrimide BP). No further animals should enter the consulting room until the case is diagnosed as negative or the premises have been satisfactorily cleaned and disinfected under the supervision of the DVM. The names and addresses of any contacts (e.g. those in the waiting room) should be recorded. The Veterinary Officer will inform the public health authorities of any necessary further action.

If the animal dies or is killed, the head and neck should be removed by a DEFRA veterinary officer and transported fresh and intact to an appropriate diagnostic laboratory (in the UK, the Veterinary Laboratories Agency, Weybridge). It should be packed in a sealed container, held at a low temperature, but must not be frozen.

A combination of several laboratory techniques is generally used to diagnose rabies.

- Fluorescent antibody test on brain smears to demonstrate viral antigen: results are available within 2–3 hours and the test has a high degree of accuracy, correlating well with mouse inoculation.
- Histological examination of brain material, usually from the hippocampus, for the specific Negri body inclusions: results are available within 2 days, but are only 40–50% accurate, especially if the animal is killed (Kissling, 1975). Formalin-fixed brain tissue may also be examined by the fluorescent antibody test, if the tissues are trypsin treated (Webster & Casey, 1988). Immunochemical techniques have also been developed.
- Mouse inoculation: mice are inoculated intracerebrally with a suspension of brain tissue, and observed for up to 28 days for mortality. If sufficient mice are inoculated the observation time may be reduced by killing mice at intervals and examining their brains by the fluorescent antibody test (Koprowski, 1973; Webster & Casey, 1988).
- Cell culture isolation: it has been found that some cell cultures [e.g. BHK-21 cells and a murine neuroblastoma (NA) cell line] appear to be at least as sensitive as mouse inoculation for isolation of virus, and this has largely replaced the mouse inoculation test in several laboratories (Crick & King, 1988; Webster & Casey, 1988).
- Panels of monoclonal antibodies may be used in fluorescent antibody tests on brain smears or infected cell cultures, to determine the origin of the rabies virus, e.g. vaccine or field virus (Esh *et al.*, 1982; Bellinger *et al.*, 1983; Whetstone *et al.*, 1984), or rabies or rabies-related virus (Webster & Casey, 1988), or for epidemiological typing, e.g. skunk versus fox rabies (Smith & Baer, 1988).
- Detection of rabies virus nucleic acid after gene amplification is also increasingly being used (Bourhy & Sureau, 1990); followed by sequencing, it allows precise typing of the strain.

Epidemiology

Although virtually every mammal is susceptible to rabies, the natural disease occurs predominantly in carnivores. Thus, in Europe, the red fox is the most important species affected, whereas in Asia it is the domestic dog (Blancou, 1988). In the USA and Canada, rabies is enzootic in several species of animals, such as skunks, foxes and racoons (Smith & Baer, 1988). Monoclonal antibody studies and sequencing studies have shown that although each strain of rabies virus has the potential to infect many species, in different geographical areas usually only one or two vectors predominate.

In many parts of the world, including Europe and the Americas, bats also harbour rabies. Bat rabies seems to be endemic in the UK (McColl *et al.*, 2000). However, bat rabies exists largely as an independent cycle and is not generally linked to terrestrial rabies, although spill-over can and does occur (Smith & Baer, 1988). In contrast, in Latin America, vampire bats are a frequent source of infection for cattle, although the dog is the major vector for humans. In Latin America, therefore, sylvatic rabies (i.e. in wildlife) and urban rabies (i.e. in dogs) coexist. Sylvatic and urban rabies also coexist in parts of Eastern Europe, Africa and Asia, but here a number of wildlife species, including wolves, jackals and mongooses, also act as reservoir hosts; these animals fill the same ecological niche as foxes in other countries (Winkler, 1975; Pastoret *et al.*, 1988b).

Fox rabies in Europe

In continental Europe, epizootics of fox rabies have occurred several times in the past, but the disease then appeared to terminate without specific control measures around the end of the nineteenth century (Winkler, 1975; Blancou, 1988). In 1939,

however, a new, stable, epizootic began, with numerous cases of rabies being reported in foxes and badgers in Poland, and since then it has become an increasing and important problem (Pastoret *et al.*, 1989).

The present European epizootic of rabies has spread some 1400 km westward from Poland since 1939. For several years the front of the epizootic advanced by 20–60 km/year (Toma & Andral, 1977; Macdonald, 1988). This epizootic is sylvatic: the reservoir of infection is in wildlife. While it involves all susceptible species, both wild and domestic, the red fox (*Vulpes vulpes*) is involved in more than 75% of cases. The red fox is both the vector of the disease and its reservoir. It plays a key role in the maintenance of the disease, but usually it does not transmit it directly to humans. People are mainly at risk from affected domestic animals such as cattle and cats, although human deaths in Europe from rabies are now very rare (Blancou, 1988).

The percentage of rabid foxes is almost certainly underestimated, for several reasons (Braunschweig, 1982). For example, the occurrence of the disease in cubs in the den is always neglected but may be relatively frequent (Thiriart *et al.*, 1985). The fox seems to be the only species to play a role in the maintenance of the present epizootic; it has been shown that where rabies has been eliminated from the fox population in certain areas of Europe, the disease then disappears from all other species, except for bats (Wandeler, 1988).

The prevailing hypothesis is that the virus originated in dogs and became adapted, by successive passages, for foxes (Winkler, 1975). This virus is classified as lyssavirus serotype 1. The virus is highly pathogenic for the fox: a dose of 0.3 mouse intracerebral LD50 is sufficient to kill one in two foxes, whereas domestic carnivores require a dose 100 000 times greater to produce the same effect (Blancou, 1985). When the disease front penetrates a new area the foxes within the area suffer an epizootic of rabies. When the fox population is heavily decimated, the incidence of the disease decreases and remains low for a 'silent' period of some 2–3 years. After the initial epizootic, secondary enzootic outbreaks recur, often at intervals of 3–5 years (Aguilar-Setién *et al.*, 1985; Macdonald & Voigt, 1985).

Superimposed upon the annual variation in the incidence of reported cases of vulpine rabies is a seasonal pattern. Cases tend to peak in late winter and reach a trough in mid-summer. The late winter/early spring peak of rabies seems to be linked to the mating season of foxes. In recent years the advance of the rabies epizootic seemed to have stopped, as exemplified by the situation in France (Blancou *et al.*, 1988a), before its elimination by fox vaccination. France is now rabies free, having met the World Health Organisation (WHO) criteria of rabies freedom, that is, not having a case of rabies in humans or terrestrial mammals acquired indigenously for 2 years. The last case of terrestrial rabies in France was in December 1998 (Rabies Bulletin Europe, 2000).

In Europe, the fox is the animal that is most susceptible to rabies. The proportion of rabid foxes that excrete the virus is very high: 93–100% of rabid foxes harbour the virus in the salivary glands and excrete it in large quantities in saliva. Furthermore, virus may be excreted for 5 days or more before the onset of the disease (Blancou *et al.*, 1979a, b; Aubert *et al.*, 1991). The incubation period of the disease in foxes depends mainly on the dose of virus with which the animal has been infected and varies from 10 to 41 days. The clinical disease lasts for 3–5 days. The symptoms are variable (George *et al.*, 1980). The furious form is relatively rare; instead, most animals become apathetic and develop paralysis (Steck & Wandeler, 1980).

Rabies virus is transmitted by biting. Since the dumb or apathetic form of the disease predominates in the fox, rabid foxes usually do not wander far from their original territory (Artois & Aubert, 1985). This may explain why the front of a fox rabies epizootic progresses slowly.

In continental Europe, rabies in domestic animals tends to follow the pattern of disease seen in wildlife (Blancou & Barrat, 1988). Thus, when the incidence of rabies increases in foxes, it also increases in domestic species such as cattle, sheep, cats and dogs. Human exposure occurs through contact with infected domestic species. Cats become infected relatively often, possibly because they tend to have regular contact with wildlife and vaccines are used less frequently in this species (Wachendörfer, 1962; Diesch *et al.*, 1982). There is no evidence, however, that rabies persists among cats in areas where canine (urban) and wildlife (sylvatic) rabies have been eliminated.

In other areas of the world where urban rabies occurs, stray dogs are common and these animals are one of the reservoirs of infection. Human exposure tends to reflect contact between people and animals, and the control of the disease in the dog population by vaccination leads to a marked drop in human exposure rates (Larghi *et al.*, 1988).

Prevention and control

Introduction

There are two aspects to be considered with respect to rabies vaccination: preventive vaccination and postexposure treatment. The first human vaccination by Louis Pasteur in 1885 was for postexposure treatment. Nowadays, human postexposure treatment requires several vaccinations, together with an injection of specific immunoglobulin if the exposure was severe. Veterinarians and others exposed to an increased risk of infection should be protected by preventive vaccination. In animals only preventive vaccination is generally carried out. In some countries, postexposure vaccination of domestic animals may be allowed, but only if the animal has previously been vaccinated.

Whether current vaccines based on serotype 1 (classical rabies virus) confer adequate protection against the rabies-related viruses is still not clear; some studies have demonstrated adequate protection whereas others have not, and it has been suggested there may be a case for producing polyvalent vaccines in some parts of the world (King & Crick, 1988).

Conventional vaccines for human use

Only inactivated vaccines have been licensed for human use. Inactivated vaccines prepared in the nervous systems of adult animals have been used for protection against rabies. However, their use has led to problems because of postvaccinal nervous system reactions. Such reactions are due to the myelin content of nervous tissues; foreign myelin may be responsible for the induction of hypersensitivity reactions in the recipient, leading to paralysis. Because myelination is delayed in neonatal mice, the use of suckling mouse brain reduces this risk, but reactions have still occurred, and thus such vaccines are best avoided.

Since rabies virus can nowadays be grown in cell culture, current vaccines in developed countries are derived from cell culture and are perfectly safe. They can be used for preventive vaccination or for postexposure treatment in conjunction with specific immunoglobulin therapy. In some parts of the world, however, cost precludes the use of such improved vaccines. New cell culture vaccines (Vero cells) or new vaccination procedures (intradermal) may reduce the cost. Vectored vaccines (canarypox) have also been developed for both human and veterinary use (Pastoret *et al.*, 1993).

Vaccines for domestic animals

Attenuated virus vaccines have been widely used in the past for immunisation of domestic animals. However, all of them still had some residual pathogenicity for some species, and cases of vaccine-induced rabies occasionally occurred, often in cats (Dean & Guevin, 1963; Esh *et al.*, 1982; Bellinger *et al.*, 1983; Whetstone *et al.*, 1984; Pastoret *et al.*, 1985). The use of monoclonal antibodies and sequencing studies has made it much easier to distinguish such cases from infection with wild-type virus. Humans exposed to attenuated vaccine strain for veterinary use have been treated in the same way as after wild-type virus exposure.

In the past few years, safe and potent inactivated vaccines have been developed for veterinary use, and these have now essentially superseded injectable attenuated vaccines. However, live oral vaccines are used in foxes, and in some parts of the world where street rabies occurs they are also used in free-roaming dogs. Rabies virus strains have been adapted to cell cultures so that large amounts of virus can be produced without the hazards associated with vaccines prepared in nervous tissue, and newer adjuvants have also increased the immunogenicity of modern inactivated vaccines. A recombinant vaccinia–rabies glycoprotein vaccine has been developed, which has been shown to be both safe and efficacious for the cat (Kieny *et al.*, 1984; Blancou *et al.*, 1989), and also a recombinant canarypox–rabies vaccine (Taylor *et al.*,1994).

Control measures for rabies in Britain

The presence of rabies in continental Europe posed a threat to Britain (Wright, 1977) and for a long time control measures for rabies in Britain relied exclusively on a rigorously enforced 6 month quarantine period for all mammals, except for farm stock and some other herbivores which are subject to other controls (Anon, 1976). Animals entering quarantine are required to be vaccinated with an authorised vaccine. All quarantine kennels are under veterinary supervision. Details of quarantine procedures, including the code of practice for the welfare of dogs and cats in quarantine, are available from DEFRA (Box 26.1).

However, as an alternative to quarantine, following publication of the Kennedy Report (Kennedy, 1998), the UK Government has now introduced PETS (http://www.defra.gov.uk/animalh/quarantine). This was initiated as a pilot scheme in February 2000, allowing pet cats and dogs to enter the UK without quarantine from certain European countries under certain

Box 26.1 Sources of information for the Pet Travel Scheme.

Post:	Pet Travel Scheme Department for Environment, Food and Rural Affairs, Area 201, 1a Page Street London SW1P 4PQ UK
Tel.:	+44 (0)870 241 1710
Fax:	+44 (0)207 904 6834
E-mail:	pets.helpline@defra.gsi.gov.uk
Website:	http://www.defra.gov.uk/animalh/quarantine

Box 26.2 European countries eligible for the Pet Travel Scheme (PETS) (http://www.defra.gov.uk/animalh/quarantine)

Andorra	Gibraltar	Netherlands
Austria	Greece	Norway
Belgium	Iceland	Portugal
Cyprus	Italy	San Marino
Denmark	Liechtenstein	Spain
Finland	Luxembourg	Sweden
France	Malta	Switzerland
Germany	Monaco	Vatican

France excludes French Guyana and St Pierre and Miquelon.
Norway excludes Svalbard.
Portugal includes the Azores and Madeira.
Spain includes the Canary Islands, but excludes Ceuta and Melilla.

Under PETS, pets from the Channel Islands, Isle of Man and the Republic of Ireland can, having travelled to any qualifying country, return to the UK as long as they are accompanied by proper official certification.
Jersey, Guernsey, the Isle of Man and the Republic of Ireland have each produced their own official PETS certificate.

Box 26.3 Long-haul countries eligible for the Pet Travel Scheme (PETS) (http://www.defra.gov.uk/animalh/quarantine).

PETS was extended on 31 January 2001 to include certain long-haul (i.e. non-European) countries and territories. These are:

Antigua and Barbuda	Martinique
Ascension Island	Mauritius
Australia	Mayotte
Barbados	Montserrat
Bermuda	New Caledonia
Cayman Islands	New Zealand
Falkland Islands	Réunion
Fiji	Singapore
French Polynesia	St Helena
Guadaloupe	St Kitts & Nevis
Hawaii	St Vincent
Jamaica	Vanuatu
Japan	Wallis and Futuna

There is a slightly different procedure for bringing pets to the UK from these countries or territories.

Other countries being considered which may be included in the future are:

Cape Verde Islands	St Lucia
Cook Islands	Taiwan
Seychelles	

conditions, and was extended to Cyprus, Malta and certain long-haul countries and territories in January 2001 (Boxes 26.2 and 26.3). The scheme only operates on certain sea, air or mail routes to England. A full, up-to-date list of countries eligible for the scheme, and details of approved routes and transport companies, are available from DEFRA (see Box 26.1).

Although all cats and dogs imported into the UK that do not meet the requirements of PETS must spend 6 months in quarantine, under certain conditions an animal may become eligible for early release from quarantine from the date that it can be shown to comply with the rules of the scheme.

To be eligible to enter the UK under PETS, animals must be fitted with a microchip, vaccinated against rabies and test seropositive following the vaccination. There is a slightly different procedure for France. A list of laboratories currently recognised for serological analysis after vaccination within the European Union is given in Box 26.4. The optimum time for a blood sample to be taken for blood testing is 30 days after the last vaccine injection. A small proportion of vaccinated animals may not show the 0.5 IU antibody titre on blood testing required by PETS, and these animals have to be vaccinated and blood tested again.

Once the animal has been microchipped, vaccinated and blood tested, a government-authorised veterinarian will need to issue an official PETS certificate to the owner to verify that these procedures have been carried out. However, a 6-month interval is required between the date of the blood sample with a successful test result and the day of travel. Before pets are allowed to enter or re-enter the UK under the scheme they must also be treated 24–48 h before re-entry against the fox tapeworm (*Echinococcus multilocularis*) and ticks to

Box 26.4 Laboratories within the European Union recognised for serological analysis after rabies vaccination.

Austria	Federal Institute for the Control of Viral Infection in Animals Department for Equine, Pets and Vaccine Control – Virology Unit Robert Kochgasse 17 2340 Mödling Tel.: +43 2236 46640 902
Belgium	Pasteur Institute of Brussels Department of Rabies Rue Engeland 642 BE-1180 Brussels Tel.: +32 2 373 31 55
Denmark	Danish Veterinary Institute for Virus Research Lindholm DK-4771 Kalvehave Tel.: +45 55 86 02 00
Finland	National Veterinary and Food Research Institute PL 368 (Heimeentie 57) FI-231 Helsinki Tel.: +358 9 393 101
France	Agence française de sécurité sanitaire des aliments de Nancy Domaine de Pixérécourt BP 9 FR-54220 Malzéville Tel.: +333 83 29 89 50
Germany	Institut für Virologie Frankfurter Strasse 107 D-35392 Giessen Tel.: +49 641 99 38350 Eurovir Hygiene Institut Biotechnologiepark D-14943 Luckenwalde Tel.: +49 3371 681 269
Greece	Ministry of Agriculture Centre of Athens Virus Department Neapoleos Street 25 GR-15310 Ag. Paraskevi Athens Tel.: +30 1 6010903
Italy	Instituto Zooprofilattico Sperimentale delle Venezie Via Romea 14/A I-35020 Legnaro (PD) Tel.: +39 049 8084261
Spain	Laboratorio de Sanidad y Produccion Animal del Estado Direccion General de Sanidad de la Produccion Agaria Camino del Jau, S/N ES-18320 Santa Fé (Granada)
Sweden	National Veterinary Institute Commission of Diagnostics Section of Diagnostics Department of Virology PO Box 585 SE-751 23 Uppsala Tel.: +46 1867 4000
Switzerland	Institute of Veterinary Virology Schweizerische Tollwutzentrale Langgass-Strasse 122 CH-3012 Bern Tel.: +41 31 631 23 78
UK	Veterinary Laboratories Agency (Weybridge) New Haw, Addlestone Surrey KT15 3NB Tel.: +44 01 932 357 345 BioBest Pentlands Science Park Bush Loan Penicuik Midlothian EH26 0PZ Tel.: +44 0131 445 6101

prevent potentially serious zoonotic diseases entering the UK. The animal must be accompanied by an official PETS certificate issued by the veterinary surgeon who carried out this treatment.

Details of the scheme for pet owners and procedures for veterinary surgeons (including microchipping, vaccination, blood testing, treatment against ticks and a tapeworm, and certification) are available from DEFRA (see Box 26.1). Further practical details of pet travel outside the UK are also given. It should also be noted that some countries require their own export health certificate or other documentation.

Control measures in countries where rabies is endemic

In countries where rabies is endemic, apart from measures aimed at wildlife control, the incidence of the disease in cats and dogs may be reduced by the elimination of stray animals, by licensing, by restriction of movement and by vaccination. Several vaccines are available for use in dogs and cats (e.g. for the USA, see vaccines listed under the Compendium of Animal Rabies, Prevention and Control, 2001). Since the use of any live vaccine carries an inherent risk, the newer, potent inactivated vaccines have now essentially

superseded the use of live injectable vaccines, although live oral vaccines are used in free-roaming dogs in some countries where street rabies occurs.

In cats and dogs, primary vaccination is generally not recommended before 3 months of age, followed by a booster 1 year later; thereafter, 1–3 year boosters are recommended, depending on the vaccine. At least 1 month should be allowed for the development of immunity. It should be remembered, however, that although available vaccines confer reasonable protection, fully vaccinated cats may in some cases still develop rabies.

Veterinarians and others exposed to an increased risk of infection should also be protected by prophylactic vaccination.

Control of rabies in wildlife

In Western Europe, prophylactic measures taken in the past (Taylor, 1976; Bögel *et al.*, 1981), such as the destruction of foxes, did not prevent the spread of the epizootic, for although such measures may be temporarily effective, the fox population rapidly recovers and rabies then recurs. However in recent years, fox rabies has nearly been eliminated in most of continental Western Europe, by oral vaccination of foxes (Blancou *et al.*, 1988a; Brochier *et al.*, 2000).

Thus, during recent years, most of the research on the control of fox rabies has concentrated on the development of methods of vaccination of the fox by the oral route (Mayr *et al.*, 1972; Baer, 1975b; Steck *et al.*, 1982a, b). After an initial trial, this method has now been used extensively in most European countries (Kappeler *et al.*, 1988; Schneider & Cox, 1988; Brochier *et al.*, 1988c, 2000) under the control of the WHO (Blancou *et al.*, 1988b).

One of the vaccines used (SADB19 attenuated strain of rabies virus) consists of 1.8 ml of virus suspension in a hermetically sealed capsule contained in a bait (Schneider & Cox, 1988). Initially chicken head baits were used, but baits now consist of a machine-made mixture of fat, bone and fish meal. When a fox chews the bait, the vaccine capsule bursts and the virus suspension spreads over the oral mucosa. Each bait contains 150 mg of tetracycline, which is used as a marker of bait uptake.

Attempts to control rabies by vaccinating wild carnivores have been very successful, and many areas of Europe are now free from rabies as a result of this (Wandeler, 1988; Brochier *et al.*, 2000). However, the use of attenuated rabies virus remains controversial as far as safety and stability are concerned (Leblois & Flamand, 1988), since these vaccine strains are pathogenic for some non-target species, such as rodents. There is no evidence, though, that they can become established in the small mammals in the wild (Wandeler, 1988).

To improve both the safety and the stability of the vaccine, a recombinant vaccinia virus (VVTGgRAB 187 XP-26D3 strain) expressing the immunising glycoprotein of rabies virus has been developed (Kieny *et al.*, 1984; Wiktor *et al.*, 1984). The ability of this VVTGgRAB strain to protect foxes against rabies has been demonstrated (Blancou *et al.*, 1986b; Brochier *et al.*, 1988a).

The safety of this recombinant virus for non-target species has been tested in many laboratory animals, domestic animals and wild animals. In addition, horizontal transmission of the recombinant virus did not occur in foxes, badgers, wild boars, cattle, dogs and ferrets (Brochier *et al.*, 1988b, 1989b). The site as well as the degree of multiplication of the recombinant virus in the fox was also studied. The virus multiplied for a short time only in the same organs as the vector vaccinia strain without any modification of its tissue specificity (Thomas *et al.*, 1990). Under experimental conditions, vaccination of rabid foxes during the incubation period with the recombinant virus did not produce asymptomatic carriers of the virus (Brochier *et al.*, 1989a).

An initial, restricted trial was carried out with this recombinant vaccine in foxes in October 1987 in Belgium (Pastoret *et al.*, 1988a). Larger trials were then carried out in 1988, 1989 and 1990, and the vaccine used in all rabies-infected areas of Belgium (Newmark, 1988; Brochier *et al.*, 1990, 1991) was leading to the elimination of wildlife rabies (Brochier *et al.*, 2001). The recombinant vaccinia–rabies glycoprotein vaccine has also been shown to be efficacious and safe for the oral immunisation of the main vectors in North America (Rupprecht *et al.*, 1986; Tolson *et al.*, 1987). Because of its high thermostability it should also be tested for the control of wildlife rabies in Africa.

The use of this recombinant vaccine has allowed the elimination of fox rabies in Belgium, France and the Grand Duchy of Luxembourg (Brochier *et al.*, 2000).

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