Rabies and other lyssavirus diseases

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The full scale of the global burden of human rabies is unknown, owing to inadequate surveillance of this fatal disease. However, the terror of hydrophobia, a cardinal symptom of rabies encephalitis, is suffered by tens of thousands of people each year. The recent discovery of enzootic European bat lyssavirus infection in the UK is indicative of our expanding awareness of the Lyssavirus genus. The main mammalian vector species vary geographically, so the health problems created by the lyssaviruses and their management differ throughout the world. The methods by which these neurotropic viruses hijack neurophysiological mechanisms while evading immune surveillance is beginning to be unravelled by, for example, studies of molecular motor transport systems. Meanwhile, enormous challenges remain in the control of animal rabies and the provision of accessible, appropriate human prophylaxis worldwide.

For more than three millennia, rabies has been one of the best known and most feared human diseases. Each year, more than ten million people, many of whom are unvaccinated, endure protracted anxiety after exposure to an animal with suspected rabies.1 Although human rabies encephalitis remains untreatable, the infection is preventable. However, the genus Lyssavirus can still cause some surprises. In 1996 and 1998, two women died in Queensland, Australia, from infections with a newly discovered rabies-related virus (Australian bat lyssavirus [ABLV]).2,3 In 2002, a man died in Scotland with a newly discovered rabies-related virus (European bat lyssavirus [EBLV]).4-6 In 2003, a man died in Finland from infection with EBLV type 2b;7 and a Swiss bat zoologist who died in 2004 with a newly discovered rabies-related virus (Australian bat lyssavirus [ABLVR]).8

EBLV in human beings

Bat rabies has been documented in continental Europe for about 50 years, but only four human infections are known. All the patients presented with clinical features of classic rabies: a girl in the Ukraine and another in the Russian Federation,9 one infected by EBLV 1a, the other by an untyped lyssavirus; a Swiss bat zoologist who died in Finland from infection with EBLV type 2b;10 and a Scottish bat conservationist who died of EBLV type 2a infection.11

Importance of EBLV

European insectivorous bats are protected. Epidemiological data depend on the examination of bats submitted for testing. The scale of this surveillance varies greatly. In the Netherlands in the 1980s hundreds of bats were examined, and about 7% were rabid.12 By contrast, in Belgium only 77 bats have been tested in 15 years, and none has been reported rabid (F Costy, personal communication). The reason for the poor surveillance of bat infection may be that the importance of EBLV infection in Europe has been overshadowed by the more obvious danger of fox rabies. Oral vaccination of foxes has controlled this epizootic in western Europe, but no means of vaccinating bats has emerged, and population control of protected species would be inappropriate. The control

Genus lyssavirus

Rabies, a single-stranded RNA virus, was the first of the seven lyssavirus genotypes to be identified. Of the other six rabies-related viruses, all but Lagos bat virus have caused fatal encephalitis in people, clinically indistinguishable from classic rabies, with the exception of Mokola virus (table 1). New lyssaviruses have recently been reported, Aravan from a single bat in Kyrghyzstan and Khujand from a bat in Tajikistan.10

EBLV in the UK

The UK was apparently free of indigenous rabies for a century, but in 1996 a Daubenton’s bat (Myotis daubentoni) infected with EBLV was found at Newhaven on the south coast of England.13 The animal was thought to have been imported from the continent; carriage of bats on the wind or in ships is well documented.13 However, the isolation of EBLV from a juvenile Daubenton’s bat in an established colony near the Lancashire canal in the northwest of England in 200214 and the death of a man in Scotland15 confirmed that the virus was indigenous. All three UK isolates were EBLV type 2a. This virus could have originated in continental Europe and have been enzootic in the British Isles for years. Changes in the prevalence of the virus, in human behaviour, or bat ecology could have created the opportunity for increased human contact.

Search strategy

We searched PubMed, Current Contents including "Agriculture, Biology and Environmental Sciences", "Life Sciences", and "Focus on Veterinary Science", and our own literature accumulated over many years. The restricted number of references and text permitted have constrained the citation of original papers. These should be sought in the reviews supplied.
of EBLV infection is therefore regarded as an intractable problem, incurring only the costs of postexposure treatment, the protracted anxiety of exposed patients, and very rare deaths. The direct immunofluorescent screening test with genotype 1 conjugate has been unreliable for ABLV, genotype 7. Whether the use of a specific EBLV antibody conjugate would increase the sensitivity of the test is not known.

Cryptic bat rabies in the USA
In the Americas, all rabies viruses, including bat strains, are of genotype 1. Of 35 cases of indigenous human rabies in the USA reported between 1958 and 2000, 32 were caused by insectivorous-bat strains of rabies virus. 26 patients had no history of a bat bite, although 12 had had physical contact with a bat. 28 patients were infected by a virus strain associated with the solitary tree-roosting silver-haired bat (Lasionycteris noctivagans) or the eastern pipistrelle bat (Pipistrellus subflavus). Since 1990, 27 people have died of bat variant lyssavirus encephalitis, but only two of them reported a bat bite. Rabies may be underdiagnosed in the USA.

Doubt has been cast on the assumption that the two human cases of bat rabies virus infection in Texas in the 1950s were due to inhalation of virus in caves densely populated by Mexican free-tailed bats (Tadarida brasiliensis mexicana). Although infection by the olfactory route has been shown experimentally in caves and in human beings (two laboratory accidents involved the inhalation of modified virus during vaccine preparation), no other patient who died of bat rabies had been in bat-infested caves. Percutaneous infection is thought more likely to have occurred by unnoticed skin contact perhaps resulting in a minute bite. The virus associated with the silver-haired bat might be more infectious when inoculated superficially into the epidermis, since it replicates more readily in non-neuronal cells and at lower temperatures than dog rabies viruses. The route of viral entry into epithelial nerves and eventually the central nervous system is unknown.

Is rabies a chronic or latent infection in animals?
Animal recovery from rabies
Natural rabies infection in all species generally causes an acute fatal illness, but rabies antibody has been detected in apparently healthy vector species including mongooses, skunks, raccoons, foxes, hyenas, jackals, fruit bats, vampire bats, insectivorous bats, and domestic dogs in Ethiopia. Transmission of rabies by asymptomatic animals is an intriguing possibility. In India a dog that had no detectable antibody was found to excrete rabies virus intermittently in its saliva over 30 months, but the validity of this unusual finding has been questioned. However, virus was isolated repeatedly from 0·5% of asymptomatic, naturally infected Ethiopian dogs, and street rabies virus was isolated once from saliva of 0·3% of healthy dogs in Nigeria. Seropositive vampire bats might have recovered from infection and they were thought to be asymptomatic rabies carriers, but the evidence was incomplete. Attempts to induce chronic infection experimentally have failed. Apparently healthy animals might be infectious during their prodromal illness.

Some wild-caught bats have antibody to rabies-related
**Table 1: Lyssavirus genus of the rhabdovirus family**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Virus</th>
<th>Source</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rabies virus</td>
<td>Dog, fox, raccoon, bat, and others</td>
<td>Widespread</td>
</tr>
<tr>
<td>2</td>
<td>Lagos bat virus*</td>
<td>Bats, cats; has not been detected in humans</td>
<td>Africa (rare)</td>
</tr>
<tr>
<td>3</td>
<td>Mokola*</td>
<td>Shrews, cats</td>
<td>Africa (rare)</td>
</tr>
<tr>
<td>4</td>
<td>Duvenhage</td>
<td>Insectivorous bat</td>
<td>Nigeria,‡ Danmark, Germany, Poland, Hungary, Russian Federation, France, Netherlands;‡ France, Spain, Netherlands;‡ UK, Germany;‡ Ukraine‡, Switzerland (and Swiss man who died in Finland)</td>
</tr>
<tr>
<td>5</td>
<td>EBLV type 1*</td>
<td>Insectivorous bat</td>
<td>Australia, ‡Philippines§</td>
</tr>
<tr>
<td></td>
<td>Type 1a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 1b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>EBLV type 2†</td>
<td>Insectivorous bat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 2a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Australian bat lyssavirus</td>
<td>Frugivorous bat (or flying fox); insectivorous bat</td>
<td>Australia, ‡Philippines§</td>
</tr>
</tbody>
</table>

| § | The genus has been divided into two phylogroups. Phylogroup II is Mokola and Lagos bat viruses. All other genotypes are in phylogroup I, and they cause fatal rabies-like encephalitis in human beings. Phylogroup II viruses are less pathogenic, but Mokola virus has probably caused three known human infections, including one fatal encephalitis without typical features of rabies.† The Netherlands is unusual in having three types of EBLV, including type 2a isolates from pond bats, Myotis dasycneme, which are not found in the UK. ‡Single isolate. §Serological evidence of its presence in the Philippines.

**Figure 2: Rabies virion**

Rabies virions measure 180×75 nm. The genome, a single non-segmented strand of negative-sense RNA of 11.9 kb, a nucleoprotein, a phosphoprotein, and an RNA-dependent RNA polymerase form the helical coil of the ribonucleoprotein complex core. A layer of matrix protein covers this cylindrical structure. The lipoprotein envelope is a host-derived lipid bilayer studded with rabies glycoprotein bearing trimeric spikes.
Rabies virus can infect a great variety of neuronal and non-neuronal cells in vitro. Non-specific viral attachment to several types of cell-surface receptors including carbohydrates, phospholipids, and sialylated gangliosides has been demonstrated. Specific binding occurs at neuromuscular junctions, where virus colocalises with the nicotinic acetylcholine receptor. Binding at this postsynaptic site is competitive with cholinergic ligands, including the snake venom neurotoxin α-bungarotoxin, which shows sequence homology with the envelope glycoprotein of rabies virus. Concentration of virus at this site increases its chances of entering the axon terminal across the synaptic cleft.

Rabies virus attaches specifically to two other receptors on neuronal cell membranes: the neural cell adhesion molecule and the p75 neurotrophin receptor (p75NTR). Two neurotransmitter receptors in the central nervous system, for N-methyl-D-aspartate subtype R1 and GABA, have been suggested as possible receptors for rabies virus.

Rabies genotype 1 and EBLV type 2 (genotype 6) bind avidly to p75NTR, but EBLV type 1 (genotype 5) and the other lyssaviruses do not. Rabies virus binds to mammalian but not to avian p75NTR, which is consistent with the lack of rabies pathogenicity in birds. Mazarakis and colleagues have suggested that binding to this receptor might not only enable entry into a cell but also facilitate axonal transport.

Rabies virus enters cells by adsorptive endocytosis into endosomes. Soon after infection, virus may be associated with synaptic vesicles, since it colocalises with synapsin I, or with early acidic endosomes, in which case viral glycoprotein could fuse with the endosomal membrane releasing the core ribonucleoprotein complex (figure 2) into the cytosol. The rabdovirus glycoprotein has a unique reversible fusion inactive state at low pH, so it can resist fusion and could remain intact in vesicles.

Transport of virus to the brain
Rabies virus migrates along peripheral nerves towards the central nervous system at about 50–100 mm per day via the fast axonal transport system. Because this movement is strictly retrograde, it is used experimentally to track neural pathways. Infection is thought to be via sensory as well as motor nerves, because antigen was detected in sensory nerve endings and dorsal root ganglia soon after peripheral inoculation in several studies. However, Mazarakis and colleagues have shown that rabies glycoprotein invasion of the central nervous system apparently does not occur via this sensory nerve pathway (figure 3). Using a rabies-glycoprotein-pseudotyped lentivirus vector, which is effectively transported in a single neuron but cannot cross synapses, they showed that injection of muscle with the lentivector resulted in transgene expression in the ventral horn of the spinal cord. Injection of skin resulted in transgene expression in dorsal root ganglia but not further into the dorsal horn of the spinal cord. Injection into the dorsal horn of the spinal cord, however, produced transgene expression in the relevant dorsal root ganglia but not in the skin (Mazarakis ND, personal communication). This finding shows that rabies-glycoprotein-pseudotyped lentivirus is retrogradely transported in neurons, and thus it can pass to the spinal cord through the motor nerves, but not via this pathway in peripheral sensory nerves. If any lentivirus entered sympathetic motor nerves supplying the skin, it might reach the sympathetic ganglia, but not the spinal cord in this experiment. Further studies showed the abolition of this retrograde transport by substitution of the arginine-333 residue on the glycoprotein.

Two groups of researchers found independently that, like some other virus proteins, rabies phosphoprotein can interact with the light-chain LC8, a highly conserved cytoplasmic component of dynein cargo-binding complex and myosin V, and an inhibitor of neuronal nitric oxide synthase. LC8 also colocalises with viral ribonucleoprotein in vitro. The myosin V actin-based motor complex drives cytoplasmic vesicular transport in the endoplasmic reticulum, so LC8 binding to rabies proteins indicates its possible involvement in viral pathogenesis early in the cycle of neuronal infection. The association of rabies phosphoprotein with the LC8 component of dynein would enable axonal retrograde transport of the viral ribonucleoprotein complex, as predicted by Murphy. However, retrograde transport of virus can still occur if the phosphoprotein binding site is deleted, hence binding of viral proteins to the LC8 molecule is not essential for pathogenesis.

Figure 4 shows two methods by which rabies proteins might be transported. In theory, if on entry to a neuron the virus in an acidic endosome fused with the vesicle membrane, the liberated naked nucleocapsid (including the phosphoprotein) could be attached to dynein via LC8; if the virus remained intact, the envelope glycoprotein could be the ligand for vesicular transport through the p75NTR as suggested by Mazarakis and colleagues. Free nucleocapsids and whole virions in vesicles within axons have been shown in electron micrographs by Gosztolyi. This process is found with herpes simplex virus, the components of which are transported separately along microtubules, as either nucleocapsids or glycoprotein within vesicles. Whether either mechanism is used by rabies virus remains to be proved.

Spread of virus within the CNS
Viral replication is intraneuronal, but the mechanism of interneuronal spread is unknown. The fact that budding of virus is very rarely seen at synapses by electron microscopy suggests that infectious naked nucleocapsids are transferred across synapses. However, interneuronal infection is dependent on the presence of viral...
the presence of virus.38,62,74 Results show no clear serotonin, opioid, GABA, and muscarinic acetylcholine progression, no consistent pattern has yet emerged. A range of abnormalities as the human encephalitis infection indicates neuronal dysfunction.62 Although MRI shows a variety of abnormalities during animal data of uncertain relevance to human disease.49,87,88 Local paraesthesia at the site of the bite is the cause of paralysis. 77,86 Species associated with the interferon response, host-cell protein synthesis, synaptic vesicle function, and neuron growth and spread, even in some uninfected or non-neuronal cells. 71 One hypothesis on the cause of death is therefore that short-circuiting of normal neural pathways results from the formation of new interneuronal connections.74 Another hypothesis is that disruption of neuronal metabolism ends in the exhaustion of metabolic pools.79

**Viral virulence and the immunology of recovery**

Rabies virus virulence is influenced by its glycoprotein envelope. Factors associated with increased virulence experimentally are the presence of the surface amino acid residue arginine-333,41 the very low external expression of viral glycoprotein on infected cells, and the absence of apoptosis until a terminal stage.77,81 Recovery from predictably fatal rabies encephalitis has been achieved only in rats treated with one monoclonal antibody.41 Animal experiments show that early induction of neutralising antibody is essential for recovery, associated with the inhibition of intercellular viral spread, reduction of viral gene expression, and the early induction of inflammation.82,83 Dietzschold and colleagues suggest that viral glycoprotein on the cell surface acts as a signal-transducing receptor. The external trigger of antibody binding to the protein could initiate changes probably ending in apoptosis. This idea might explain why administration of antibody at a late stage results in acceleration of the disease.74 The important consequence in human infection is that recovery is inevitably accompanied by neuronal loss.77 Although a cell-mediated immune response and interferons (predominantly interferon γ) contribute to recovery from attenuated virus infection, they are not essential. CD8-positive T cells might have a minor role.43,44 Expression of MHC class I mRNA is slightly upregulated in the central nervous system in rabies infection but this change is unrelated to the outcome. MHC class II mRNA is greatly upregulated in avirulent virus infection, but there is very little expression in lethal disease, indicating the importance of a T-helper-cell response.45 In mice, the immune response, not the direct effect of the virus, is the cause of paralysis.74,80 Species variation in response to rabies makes interpretation of animal data of uncertain relevance to human disease.

**Clinical issues**

In its classic furious form with hydrophobia or aerophobia, human rabies encephalitis is unmistakable. However, clinical descriptions over the past two centuries have shown the protean manifestations of this disease.95,97,98 Local paraesthesia at the site of the bite (most commonly itching) is the only reasonably suggestive prodromal symptom. Paralytic forms of rabies are rare presentations with subtle symptoms or with psychiatric disturbances are especially likely to be misdiagnosed. In a patient with an acute neuropsychiatric illness, a history of travel to a rabies-endemic area during the previous
months or even years, and a history of a bite by a domestic or wild mammal, especially a carnivore or bat, will raise the possibility of rabies. However, rabies has developed in people in America who have had such trivial contacts with bats that they passed unnoticed. So far, no distinctive clinical features have been associated with infections by any of the rabies-related viruses. Furious, paralytic, and atypical manifestations have been reported in these patients.2,14,88

### Diagnosis of human rabies encephalitis

The laboratory diagnosis of rabies is rarely attempted in less developed countries, but confirmation of infection during life will guide management of the patient, relatives, and staff; prevent unnecessary investigations; and allow characterisation of the virus. Routine tests might show plasma neutrophil leucocytosis. Mild pleiocytosis is seen in only 60% of patients in the first week.89 The diagnosis can be made by early identification of antigen or viral RNA or by virus isolation, and in unvaccinated people, antibody detection (table 2).49,89–94

### Survivors of rabies

Since 1970, there have been reports of five patients said to have survived rabies encephalitis (table 3).21,22,94–97 All these patients had received some rabies vaccine before the onset of symptoms, but none had had rabies immune globulin (RIG). Neither rabies virus nor viral antigen was detected, but samples were taken when neutralising antibody was present. All the diagnoses were based on high antibody concentrations in the cerebrospinal fluid. In the future, the diagnosis of rabies in such cases might be confirmed by RNA detection by RT-PCR. The diagnosis remains in doubt, however, in the patients who were given vaccines of nervous-tissue origin, because postvaccinal encephalitis can produce similar signs and symptoms.96,97 The term “limited survival” is more appropriate than “recovery” in the three patients given tissue-culture vaccines, since all had profound residual neurological deficits. Severe impairment of nervous function was irreversible despite control of the infection, presumably by the immune response.
Management
The mortality from rabies is 100% in unvaccinated patients. Despite many attempts at intensive-care treatment over the past 30 years, no vaccinated patient has recovered without severe sequelae. Life can be prolonged, but many complications arise. Heavy sedation and analgesia should be given to relieve the agonising symptoms. Ketamine is an appropriate anaesthetic, although the concentrations reached in the central nervous system are probably insufficient for an antiviral effect as discussed above. Immunosuppressive and antiviral drugs have not proved useful.

Until a new specific therapy is available, palliative care is recommended. Patients and their relatives should be advised that although intensive-care therapy may prolong life, there can be no expectation of survival in unvaccinated patients. Previously immunised patients will have severe permanent neurological disabilities.

Prevention
Since rabies is untreatable, prevention of infection is paramount. The most efficient way to control human rabies is to eliminate infection in animal vectors. Domestic-dog strains of rabies virus account for more than 90% of human disease worldwide. Rabies in stray dogs can be reduced by parenteral vaccination, fertility control, and clearing rubbish to reduce the food supply. Vaccination of wildlife vectors with oral live attenuated rabies virus or vaccinia-recombinant vaccines has virtually eliminated fox rabies in western Europe, and vaccinia-recombinant vaccines have been used against coyote, raccoon, and fox rabies in North America. Other similar vaccines are being developed. A single human infection by vaccinia-recombinant animal rabies vaccine has been reported in a pregnant woman with a chronic skin condition, epidermolytic hyperkeratosis. Despite much effort, DNA vaccination against rabies has not proved practicable.

So far there is no means of controlling rabies in some inaccessible vector species, such as insectivorous bats, despite their potential for infecting people. Avoidance of contact with bats, pre-exposure vaccination, and prompt postexposure treatment for people who may have been exposed are the only means of preventing human infection.

Human prophylaxis
Rabies vaccines
Two rabies vaccines are now licensed for use in the UK and USA: human-diploid-cell vaccine (HDCV; Imovax Rabies, Aventis Pasteur, Lyon, France) and purified chick-embryo-cell vaccine (PCECV; Rabipur, RabAvert, Chiron Behring). Both are sold in single-dose 1 mL vials. Elsewhere, purified vero-cell vaccine (PVRV; Verorab, Aventis Pasteur) is widely available in a single-dose 0.5 mL vial. Rabies vaccine adsorbed (BioPort, Lansing, MI, USA) is also licensed in the USA.

Pre-exposure treatment
The most successful form of rabies prevention is pre-exposure vaccination. No rabies deaths have been reported in anyone who has had pre-exposure treatment followed by a booster dose after exposure. For people given pre-exposure vaccine, postexposure treatment is simplified—only two doses of vaccine (days 0 and 3) and no RIG. Prophylaxis is recommended for people at occupational risk and for travellers to areas where dog rabies is endemic, mainly in Asia and Africa.

The standard pre-exposure regimen is three doses of a tissue-culture vaccine intramuscularly (deltoid) on days 0, 7, and 28 (or day 21). A booster dose after 1 year increases and prolongs the antibody response. The frequency of vaccine booster doses varies according to the risk of exposure to rabies, and treatment can be avoided if adequate neutralising antibody is shown. In the USA, antibody testing or booster vaccination is recommended every 6 months for people at high risk (eg, laboratory staff handling the virus) and every 2 years for those at frequent risk (eg, some wildlife officers). At the other extreme, no booster doses are deemed necessary for travellers and others at low risk. The secondary immune response to emergency booster doses of rabies vaccine is predictably prompt, and the rapidity is likely to be the important component of postexposure treatment, rather than the actual antibody concentration achieved. Evidence of immunity persists for 5–10 years, although in one study, the antibody concentration declined in 3.5% of vaccinees but responded to boosting, albeit at a lower concentration than the rest. This finding accords with Kuwert and colleagues’ identification of “poor responders” with lower later antibody induction. Repeated vaccine treatment can be avoided if the presence of neutralising antibody is proven.

An economical pre-exposure regimen, recommended by WHO and approved and previously used in the USA, is to give 0.1 mL vaccine intradermally on the same 3 days as for intramuscular vaccination. The pharmaceutical companies cannot now sanction this approach because only 1 mL ampoules are produced, which do not comply with regulations for multidose vials.

Postexposure treatment
Rabies virus in an animal’s saliva can infect mucous membranes or tissues through broken skin. Intact skin is protective, but minute lesions caused by bats may result in infection. Other routes of infection are rare. Rabies mortality after untreated bites by proven rabid dogs was 35–57% in India. The risk of infection is increased in severe exposure: if bites are on the head, neck, or hands, or are multiple or deep. Modern postexposure treatment is highly successful; failures of optimum treatment are exceptional. However, in many cases complete treatment starting on the day of the bite is not given. Wound care with passive and active rabies immunisation are essential especially after severe exposure. Postexposure treatment is assumed to neutralise or inactivate virus while it is still in the wounds, before it gains access to the nervous system where it is protected from immune attack. Therefore, treatment after exposure to rabies virus is very urgent, even if the patient was bitten months before. The decision to give postexposure treatment depends on the assessment of risk of infection in each patient, influenced by the circumstances of the exposure, the mammal concerned, and results of tests.

Experiments have shown that thorough washing of rabies-infected wounds with soap and water can increase survival by 50%. However, this cheap, available treatment is omitted in most cases. Virucidal agents based on iodine or alcohol are advised, and antibacterial treatment should be considered.

The standard vaccine regimen is five doses intramuscularly into the deltoid on days 0, 3, 7, 14, and 28. Injections can be given into the anterolateral thigh for children, but should not be given into the buttock. Non-specific side-effects have been attributed to tissue-culture rabies vaccines, but they are very rarely associated with neurological features.

Passive immunisation with human RIG lowers mortality after severe exposure, but many years of
clinical experience suggest that it is not as important for milder rabies exposure (eg, single bites on limbs) as for more severe exposures. A single dose is infiltrated locally around the bite wound as soon as possible after the incident.106 RIG is expensive and increasingly scarce, so other products are under investigation including a mixture of specific monoclonal antibodies.117

The success of postexposure treatment depends on the efficient, immediate, accurate delivery of all three components of recommended treatment; the competence of the host immune response, and the susceptibility of the infecting virus to the immunity induced by the vaccine. Although delay and errors in implementing treatment can in theory be overcome, immunosuppression by drugs or chronic diseases such as cirrhosis could prove fatal. The antibody response to rabies vaccine is greatly impaired or absent in HIV-infected patients who have low counts of CD4-positive lymphocytes.116 Use of a double dose of vaccine has been suggested in these circumstances, but in the absence of good data, use of the most immunogenic vaccine regimen (with multiple-site intradermal injections on the first day) might be the most appropriate approach.117

There is no evidence of a lack of efficacy of rabies vaccine against genotype 1 rabies strains, but experiments on their protective effect against EBLV have given varying results, especially with antigens of different viral origins.118 The Pitman-Moore strain is used in HDCV and PVRV manufacture, and the Flury-LEP strain for PCECV, but some studies have used another strain, Pasteur virus. Although some human serology and animal experiments have shown poor results with viruses of vaccines for human use against EBLV type 1 virus,119–122 HDCV afforded protection against challenge with EBLV type 1 and partial protection against EBLV type 2b in mice.123 Furthermore, human vaccinees had neutralising antibody to EBLV type 1,124 and there is some evidence of EBLV-specific cell-mediated immunity.125

Although commercially produced RIG did not protect mice against challenge with EBLV type 1,125 it has neutralised this virus.126 In another study, RIG did not cross-neutralise EBLV type 1, but it did neutralise EBLV type 2.127 Antibody to an experimental DNA rabies glycoprotein antigen of the Pasteur virus strain cross-neutralised fairly well with EBLV type 2a, but poorly with type 1.128 All three studies of the efficacy of vaccines against both EBLV types 1 and 2 showed different results for the two subtypes.129,130,131 Rabies vaccines should be tested against appropriate wild-type lyssaviruses.132

Meanwhile, hundreds of people bitten by bats, some proven EBLV positive, have received postexposure vaccination. None has developed EBLV encephalitis. Although the protection from current rabies vaccines and RIG is likely to be less efficient against EBLV than against genotype 1 rabies infection, there is currently no other treatment. This uncertainty increases the importance of pre-exposure vaccination and the urgency of postexposure treatment in anyone exposed to rabies-related viruses.

**Rabies prophylaxis in less developed countries**

The recommendations for prophylaxis outlined above are applicable worldwide, but implementation is impossible where medications are unaffordable or unobtainable and health facilities remote. In South Africa in 2001, 26% of rabies-vaccine treatment facilities had no vaccine in stock, and 83% had no RIG.133 In another study, HDCV afforded protection against challenge with EBLV type 1, but it did neutralise EBLV type 2.127

Antibody to an experimental DNA rabies glycoprotein antigen of the Pasteur virus strain cross-neutralised fairly well with EBLV type 2a, but poorly with type 1.128 All three studies of the efficacy of vaccines against both EBLV types 1 and 2 showed different results for the two subtypes.129,130,131 Rabies vaccines should be tested against appropriate wild-type lyssaviruses.132

The two-site intradermal postexposure regimen has use the same amount of vaccine, but a comparative study showed that the eight-site method consistently induced significantly higher concentrations of neutralising antibody from day 7 onwards than the two-site regimen, which is important when RIG is not available.137

A further reduction in the cost of the two-site regimen has been proposed by administration of 0·1 mL instead of the standard 0·2 mL PCECV per intradermal site.138 The new regimen was tested in two non-randomised trials in 50139 and 120140 confirmed rabies-exposed patients with transdermal (category III) lesions; some of the former and all the latter also received RIG. This method would be predicted to give better protection than the current Semple vaccine, but in comparison with the standard intradermal regimens, the margin of safety is likely to be lower, especially after severe exposure.

Human RIG is prohibitively expensive, but equine RIG may be available in less developed countries. However, less than 2% of all vaccine-treated patients also receive RIG.141 This low proportion and the high prevalence of HIV and AIDS in many areas combine to reduce the efficacy of postexposure treatment in some patients.
We thank N D Mazarakis for his data and helpful criticism.

MJW has received travel grants from Aventis. Neither this source of data to direct implementation of potentially highly the disease. A current WHO initiative in Asia may yield extent of the dog rabies epizootic in Asia and Africa.

but methods for radical amelioration exist, and should be supply of RIG.

WHO. New monoclonal-antibody products already being culture regimens. This change is strongly endorsed by culture vaccines could be used more effectively in less research. Meanwhile, the excellent human rabies tissue-
effective treatment depend on the results of such future
detail. Any innovations in methods of prophylaxis and
and through the brain. Further knowledge of the influence
RNA, but no virus, is detectable.

Assessment of the risk of human exposure requires better
more detail of the distribution of EBLV infection.

Lyssaviruses cause unrecognised disease and suffering,
but methods for radical amelioration exist, and should be implemented.

Conflict of interest statement
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References


41 Pawan JL. Rabies in the vampire bat of Trinidad with special
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Iwata M, Uno T, Mhapary S, Ohishi K. Rabies virus infection prevents the modulation by alpha(2)- adrenoceptors, but not muscarinic receptors, of Ca(2+)-channels in NG108-15 cells. Eur J Pharmacol 2000; 404: 79–86.


