

Rabies

Anthony R. Fooks^{1–3}, Florence Cliquet⁴, Stefan Finke⁵, Conrad Freuling⁵, Thiravat Hemachudha^{6,7}, Reeta S. Mani⁸, Thomas Müller⁵, Susan Nadin-Davis⁹, Evelyne Picard-Meyer⁴, Henry Wilde⁶ and Ashley C. Banyard¹

Abstract | Rabies is a life-threatening neglected tropical disease: tens of thousands of cases are reported annually in endemic countries (mainly in Africa and Asia), although the actual numbers are most likely underestimated. Rabies is a zoonotic disease that is caused by infection with viruses of the *Lyssavirus* genus, which are transmitted via the saliva of an infected animal. Dogs are the most important reservoir for rabies viruses, and dog bites account for >99% of human cases. The virus first infects peripheral motor neurons, and symptoms occur after the virus reaches the central nervous system. Once clinical disease develops, it is almost certainly fatal. Primary prevention involves dog vaccination campaigns to reduce the virus reservoir. If exposure occurs, timely post-exposure prophylaxis can prevent the progression to clinical disease and involves appropriate wound care, the administration of rabies immunoglobulin and vaccination. A multifaceted approach for human rabies eradication that involves government support, disease awareness, vaccination of at-risk human populations and, most importantly, dog rabies control is necessary to achieve the WHO goal of reducing the number of cases of dog-mediated human rabies to zero by 2030.

Rabies is a zoonotic disease caused by viruses of the *Lyssavirus* genus (in the family Rhabdoviridae of the order Mononegavirales) that was first described in the 4th century BC¹. Rabies virus (RABV), the prototype virus of the *Lyssavirus* genus (TABLE 1), is by far the most common causative agent of rabies² and is most readily transmitted by the bite of an infected mammal (FIG. 1). Dog-transmitted rabies causes >99% of the human cases reported. Both animal and human rabies are entirely preventable through vaccination, and the first efficacious rabies vaccines for human use were developed in the 19th century. However, in the 21st century, the virus is still enzootic (that is, endemic in animals) in many regions of the world, and human rabies remains one of the most serious and distressing diseases and an important threat to public health³. Indeed, when an individual with rabies develops symptoms, the disease is nearly always fatal⁴. Rabies is often considered a disease of poverty, ignorance and, in some circumstances, misinformation⁵.

RABV enters peripheral nerves at the synapse level at the site of the bite and is transported to neurons in the central nervous system (CNS); the virus then replicates and causes cerebral damage. Rabies can manifest in two classical forms (furious and paralytic) with a range of symptoms, but ultimately leads to coma and death. The priority for reducing the burden of human rabies is controlling dog rabies, especially in free-roaming community dogs^{6–9}. Rabies elimination was achieved in domestic

dogs in North America, Europe and some countries of Latin America as well as in wildlife species in Europe and Canada¹⁰. The subsequent substantial reduction in cases of human rabies in such regions advocates for the ‘One Health’ approach to rabies control (that is, a collaborative and transdisciplinary approach at the local, national and global level that aims to achieve optimal health outcomes by recognizing that the health of people is connected to the health of animals and the environment)¹¹. With this in mind, the WHO, World Organisation for Animal Health (OIE) and Food and Agriculture Organization of the United Nations (FAO) have set the target to eliminate dog-transmitted human rabies in endemic countries by 2030 (REF. 12). This Primer summarizes the epidemiology of RABV; global efforts to diagnose, control and eliminate the transmission of RABV by terrestrial animals; and the mechanisms and pathophysiology of RABV infection. Conundrums regarding treatment options and both existing and potential future prophylactic tools to combat infection of the CNS are discussed, along with initiatives aimed at the global elimination of this deadly zoonotic virus.

Epidemiology *Lyssaviruses and their hosts*

Since the 1970s, methods of viral characterization have revealed the extensive diversity of the *Lyssavirus* genus¹³ (TABLE 1), and the taxonomy of this genus continues to

Correspondence to A.R.F. Animal and Plant Health Agency (APHA), Wildlife Zoonoses and Vector Borne Diseases Research Group, (WHO Collaborating Centre for the Characterisation of Rabies and Rabies-Related Viruses, World Organisation for Animal Health (OIE) Reference Laboratory for Rabies), Weybridge, New Haw, Addlestone, Surrey KT15 3NB, UK. Tony.Fooks@apha.gsi.gov.uk

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Author addresses

¹Animal and Plant Health Agency (APHA), Wildlife Zoonoses and Vector Borne Diseases Research Group, (WHO Collaborating Centre for the Characterisation of Rabies and Rabies-Related Viruses, World Organisation for Animal Health (OIE) Reference Laboratory for Rabies), Weybridge, New Haw, Addlestone, Surrey KT15 3NB, UK.

²Institute of Infection & Global Health, University of Liverpool, Liverpool, UK.

³Institute for Infection and Immunity, St. George's Hospital Medical School, University of London, London, UK.

⁴French Agency for Food, Environmental and Occupational Health & Safety (ANSES)-Nancy Laboratory for Rabies and Wildlife (European Union Reference Laboratory for Rabies, WHO Collaborating Centre for Research and Management in Zoonoses Control, OIE Reference Laboratory for Rabies, European Union Reference Institute for Rabies Serology), Technopôle Agricole et Vétérinaire de Pixérécourt, Malzéville, France.

⁵Institute of Molecular Virology and Cell Biology (WHO Collaborating Centre for Rabies Surveillance and Research, OIE Reference Laboratory for Rabies), Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany.

⁶Department of Medicine (Neurology) and (WHO Collaborating Centre for Research and Training on Viral Zoonoses), Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

⁷Thai Red Cross Emerging Infectious Disease-Health Science Centre, Thai Red Cross Society, Bangkok, Thailand.

⁸Department of Neurovirology (WHO Collaborating Centre for Reference and Research in Rabies), National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India.

⁹Ottawa Laboratory Fallowfield, Canadian Food Inspection Agency (WHO Collaborating Centre for Control, Pathogenesis and Epidemiology of Rabies in Carnivores), Ottawa, Ontario, Canada.

evolve, owing to enhanced surveillance and sequencing technologies. Most *Lyssavirus* spp. are maintained by chiropteran hosts (the only mammals capable of true flight; that is, bats). The reservoir hosts and geographical range of some of these viruses, such as the European bat lyssaviruses (EBLVs) in Europe, are quite well documented, but similar information for many others is lacking, owing to the recovery of only small numbers of isolates¹⁴. By contrast, RABV is maintained in most parts of the world by mesocarnivores (animals whose diet consists of 50–70% meat), including dogs, foxes, raccoon dogs, raccoons, mongooses and skunks³. Exceptions include the maintenance of RABV in multiple bat species (only in the Americas) and the reported role of a herbivore, the kudu, as an RABV reservoir in Namibia¹⁵. For all *Lyssavirus* spp., other mammals, including humans, cattle and equids, represent cases of cross-species transmission (or spillover; that is, the ability of a virus to infect a member of a new host species)³. Although such spillover infections normally result in 'dead-end' infections with no further spread to other hosts, neuroinvasion leading to clinical disease can still occur.

RABV is estimated to be responsible for hundreds of thousands of cases of animal rabies annually, and the application of sequencing methods to this extensive pool of data has enabled the investigation of the epidemiology and evolution of RABV and has provided insights into the detailed phylogeography of disease outbreaks¹⁶. Such studies identify natural landscape features such as mountain ranges and waterways as frequent barriers to epizootic spread, whereas man-made roads and human-mediated movement of animals, often accompanying large-scale human migrations, can facilitate disease dispersion; such information is valuable to control programmes¹⁷.

RABV probably evolved from a bat-associated progenitor¹⁸, and several host-switching events yielded a complex pattern of RABV variants. Worldwide, seven main lineages of RABV are recognized^{13,19}; within each lineage, multiple variants, each associated with a particular mammalian host and geographical range, have emerged. The most widespread lineage is found in several continents (Europe, the Americas, Africa and Asia), and it is maintained by dog populations in many countries and by several wildlife species (for example, foxes, raccoon dogs, skunks and jackals)²⁰. Throughout the Americas, many bat-associated RABV variants persist²¹, including one harboured by the vampire bat, which is an important source of disease for humans and domestic livestock in Latin America.

Disease

Large-scale control programmes have eliminated dog rabies in Europe, the United States and Canada and substantially reduced its prevalence in Latin America, with a concomitant decrease in cases of human rabies²². However, as dog rabies is controlled, the existence of sympatric (occurring in the same area) wildlife rabies reservoirs often becomes more-evident, and sylvatic rabies (affecting wildlife) has been identified in countries that were historically considered free of rabies²³. In Europe, oral vaccination campaigns have demonstrated the feasibility of eliminating rabies from wildlife reservoirs, but the continued circulation of *Lyssavirus* spp. in bat hosts with an aerial lifestyle will challenge future global rabies eradication efforts, owing to difficulties in the development and administration of vaccines to these highly mobile, poorly accessible species³.

Globally, human rabies is considered a neglected zoonosis, especially in resource-limited countries in Africa and Asia where the disease burden falls disproportionately and mortality is substantial²⁴ (FIG. 2). It has been estimated that someone dies from rabies in a rabies-endemic country every 10–20 minutes and that 40–50% of fatalities are in children <15 years of age³. Owing to their small size and frequent interactions with domestic or free-roaming dogs, children are at a higher risk of being bitten, not only on their extremities but also on the head, which leads to shorter incubation times. Thus, in endemic countries, rabies should be considered a neglected paediatric disease²⁵.

The estimated 60,000 human deaths annually worldwide^{3,26} do not capture the true burden of this disease. Predictions suggest that as many as 20,000 lives are lost annually in India alone²⁶. Lack of surveillance²⁷ due to limited health-care services, such as laboratory capabilities, qualified personnel and medical and veterinary infrastructures, results in under-reporting of human cases, which is further compounded by cultural, religious and social taboos²⁸. Furthermore, in humans, rabies can present with symptoms that are common to other clinically indistinguishable conditions (for example, Guillain-Barré syndrome and cerebral malaria)²⁹; consequently, without confirmation of the diagnosis using established laboratory protocols³⁰, rabies might be misdiagnosed. These inaccuracies in

collating empirical data of disease incidence can discourage policymakers from considering rabies as a high-priority disease^{26,31}.

Mechanisms/pathophysiology

Host infection

The main mode of RABV transmission is through exposure of broken skin to the saliva of an infected animal. Exposures that can lead to infection can vary from severe bites, most often by a dog, to superficial skin lesions. These lesions are often described as cryptic infection because the exposure goes unrecognized and unreported³², and most frequently result from contact with bats. Other potential transmission routes include organ transplantation from donors with undiagnosed or misdiagnosed rabies, which caused several cases of rabies in transplant recipients^{33–36}. Infection via the aerosol route has also been rarely reported^{37–39} and probably depends on efficient viral excretion in the saliva and exposed ocular or nasal mucosa^{37,40}.

In vitro, lyssaviruses can infect most cell types, although the underlying molecular interactions are not completely understood. In the host, lyssaviruses generally infect peripheral nerves (sometimes also muscle cells) at the motor endplate of neuromuscular junctions or at other innervated tissues (FIG. 3). After receptor-mediated

entry, the virus is contained in endosomal transport vesicles^{41,42} and retrogradely transported along axons via microtubules^{43–47}. In the neuronal soma, the ribonucleoproteins (RNPs), which are complexes formed by the viral RNA, nucleoproteins and phosphoproteins, are released into the cytoplasm from the vesicles⁴⁸ and primary transcription occurs, leading to the production of viral proteins. With the accumulation of viral proteins, cytoplasmic inclusion bodies are formed, which are sites of viral RNA synthesis (secondary transcription)⁴⁹. From replication sites in inclusion bodies, newly generated RNPs are transported to postsynaptic membranes, where the new virions are assembled and transsynaptically transmitted⁵⁰ to next-order neurons in a viral glycoprotein-dependent manner⁵¹. This process includes budding in the synaptic cleft and subsequent receptor-mediated entry at presynaptic membranes (FIG. 3). The virus eventually reaches the brain; what brain area is infected is determined by what motor neurons innervate the site of entry. From the brain, the virus spreads to the salivary glands and is intermittently excreted in the saliva, ready to be transmitted to another host. Local symptoms start once the virus, after disseminating in the CNS, centrifugally spreads and reaches the dorsal root ganglia corresponding to the nerves at the site of entry; in the dorsal root ganglia, viral replication causes inflammation,

Table 1 | The Lyssavirus genus

Species	Virus	Countries of virus isolation*	Most common reservoir based on virus detection
Phylogroup I			
Rabies lyssavirus	Rabies virus (RABV) [†]	Global	All mammals
Aravan lyssavirus	Aravan virus (ARAV)	Kyrgyzstan	Bats
Australian bat lyssavirus	Australian bat lyssavirus (ABLV) [†]	Australia	Bats
Bokeloh bat lyssavirus	Bokeloh bat lyssavirus (BBLV)	France and Germany	Bats
Duvenhage lyssavirus	Duvenhage virus (DUVV) [†]	Kenya and South Africa	Bats
European bat 1 lyssavirus	European bat lyssavirus 1 (EBLV-1) [†]	Belgium, Denmark, France, Germany, the Netherlands, Poland, Russia, Slovakia, Spain and Ukraine	Bats
European bat 2 lyssavirus	European bat lyssavirus 2 (EBLV-2) [†]	Denmark, Finland, France, Germany, the Netherlands, Norway, Switzerland and the United Kingdom	Bats
Gannoruwa bat lyssavirus	Gannoruwa bat lyssavirus (GBLV)	Sri Lanka	Bats
Irkut lyssavirus	Irkut virus (IRKV) [†]	China and Russia	Bats
Khujand lyssavirus	Khujand virus (KHUV)	Tajikistan	Bats
Phylogroup II			
Lagos bat lyssavirus	Lagos bat virus (LBV)	Central African Republic, Ethiopia, France [§] , Ghana, Nigeria, Senegal, South Africa and Zimbabwe	Bats
Mokola lyssavirus	Mokola virus (MOKV) [†]	Cameroon, Central African Republic, Ethiopia, Nigeria, South Africa and Zimbabwe	Rodents and domestic animals
Shimoni bat lyssavirus	Shimoni bat virus (SHIBV)	Kenya	Bats
Phylogroup III			
Ikoma lyssavirus	Ikoma lyssavirus (IKOV)	Tanzania	African civet
Lleida bat lyssavirus	Lleida bat lyssavirus (LLEBV)	Spain	Bats
West Caucasian bat lyssavirus	West Caucasian bat virus (WCBV)	Russia	Bats

Based on the International Committee on Taxonomy of Viruses classification¹⁹⁴. *Countries where the virus was isolated. [†]Associated with human fatality. [§]It is unknown whether the infected animals arrived in France from Egypt or Togo. ^{||}Could be single member of new phylogroup.

which triggers pain, paraesthesia (a tingling sensation) and/or pruritus (itch)⁵². The virus could also reach peripheral nerves; inflammation in these nerves (caused by viral replication) could result in weakness in paralytic rabies. The mechanisms underlying the symptoms that manifest during the acute neurological phase are not completely understood.

Receptors and neuroinvasion

The nicotinic acetylcholine receptor (nAChR), neuronal cell adhesion molecule (NCAM) and tumour necrosis factor receptor superfamily member 16 (TNFRSF16; also known as p75NTR) have been proposed to be

involved, to a greater or lesser degree, in RABV cell entry^{53–55} and, therefore, are considered as RABV receptors (FIG. 3). However, there is conflicting evidence on the role of p75NTR⁵⁶, and other potential receptors have also been suggested⁵⁷. p75NTR co-internalization and subsequent retrograde axonal co-transport with RABV have been directly shown by live-cell imaging in sensory dorsal root ganglion neurons⁴². Nevertheless, how RABV enters neurons at peripheral inoculation sites has yet to be completely defined. Within neuromuscular junctions, the presence of nAChR at, or close to, the postsynaptic membranes of muscle cells might support the infection of muscle cells before neuronal infection. Virus amplification (due to replication) in muscle cells and the subsequent budding of progeny virions in the synaptic cleft could increase the efficiency of neuroinvasion through receptors at presynaptic axonal membranes. However, nAChR could also act as an attachment receptor that could concentrate extracellular virus in the synaptic cleft for presentation to receptors such as NCAM and p75NTR on the presynaptic membranes of motor neurons⁵⁷ (FIG. 3). Finally, RABV could directly bind to neuronal receptors and infect the neuron independent of nAChR binding or muscle cell infection. In fact, whether one or more of these receptor-mediated pathways occur and whether infection of non-neuronal cells at peripheral sites contributes to the efficiency of neuroinvasion and disease development remains controversial. In addition to neuromuscular junctions, sensory neurons could also serve as entry routes, as RABV has been detected in sensory nerves⁵⁸. Both retrograde and anterograde axonal transport have been observed in cultivated dorsal root ganglion neurons^{44,59} (Supplementary information S1 (video)), and *in vivo* anterograde trans-synaptic transfer in sensory neuronal circuits has been demonstrated⁶⁰.

Immune response and host manipulation

RABV virulence resides in its ability to escape the host's immune response while retaining the viability of infected neurons. The concept of the CNS as an immune-privileged cellular environment has been challenged by the discovery that peripheral immune cells can cross the intact blood–brain barrier and that CNS neurons and glia can regulate macrophage and lymphocyte responses. Thus, it must be assumed that RABV replication in the CNS occurs in a fully immunocompetent cellular environment. However, in experimental models, attenuated and pathogenic RABV strains induce different inflammatory markers and other host parameters^{61,62}, and virus clearance from the brain, which correlates with the level of B cell infiltration and antibody production in the CNS^{63,64}, is reduced in models infected with pathogenic RABV strains. These findings support the hypothesis that adaptive immune responses are impaired in natural RABV infections.

Enhancing the permeability of the blood–brain barrier to the infiltration of immune cells is considered necessary for successful virus clearance, and enhanced permeability increased the clearance of attenuated RABV from the CNS in experimental models⁶⁵.

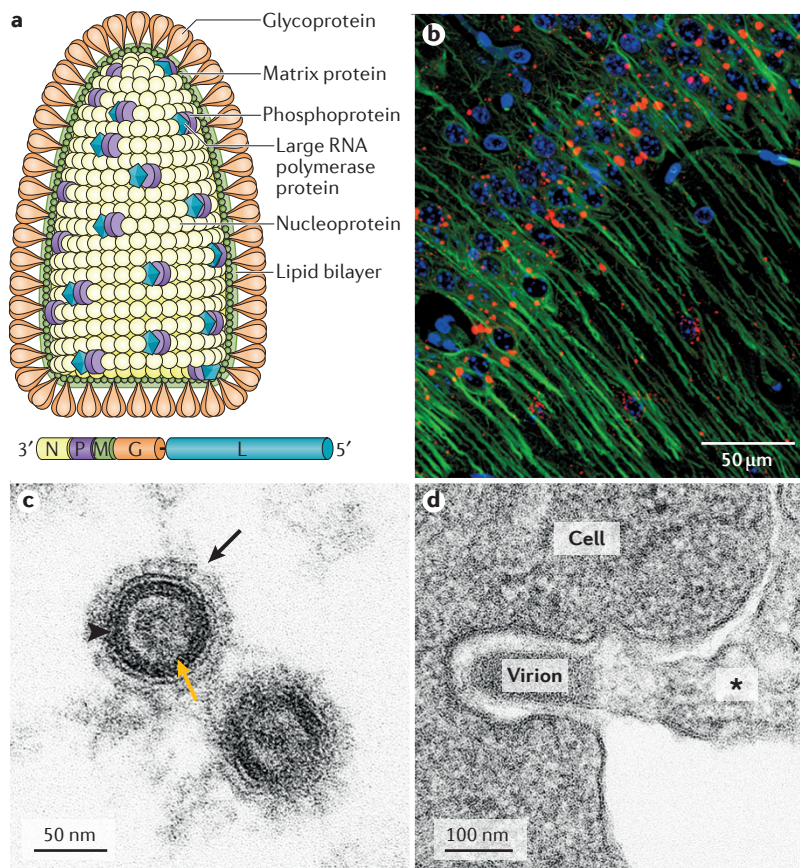


Figure 1 | Structure of the rabies virus. a | The rabies virus (RABV) virion is bullet-shaped, with a plasma membrane covered in homotrimers of type 1 transmembrane glycoprotein with peplomers (glycoprotein spikes). The ~12 kb negative-sense RNA genome of lyssaviruses encodes five viral proteins: the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and large RNA polymerase protein (L). Within the virion, the genomic RNA is tightly encapsidated with N and P to form a ribonucleoprotein (RNP), which is closely associated with a viral RNA polymerase complex consisting of P and L. The RNP forms a helix that is encapsidated with M, which in turn connects the RNP and the lipid bilayer membrane envelope. **b** | Hippocampal neurons of a laboratory mouse infected with street RABV (that is, obtained from a naturally infected dog) that were isolated during the clinical phase of the disease. Despite clear neuronal infection (shown by the presence of N, labelled in red), the structural integrity of the neurons seems to be preserved. Green labelling denotes the neuronal marker tubulin $\beta 3$ chain; blue labelling indicates cell nuclei. **c** | A cross section of the virion demonstrates the distinct G peplomers (arrow), plasma membrane (arrowhead) and RNP core (yellow arrow). **d** | Longitudinal view of the virion, highlighting the bullet shape. Here a virion is being engulfed by a cell, presumably through endocytic mechanisms. *Indicates the cell from which the virion is budding. Parts **c** and **d** are reproduced with permission from Kati Franzke.

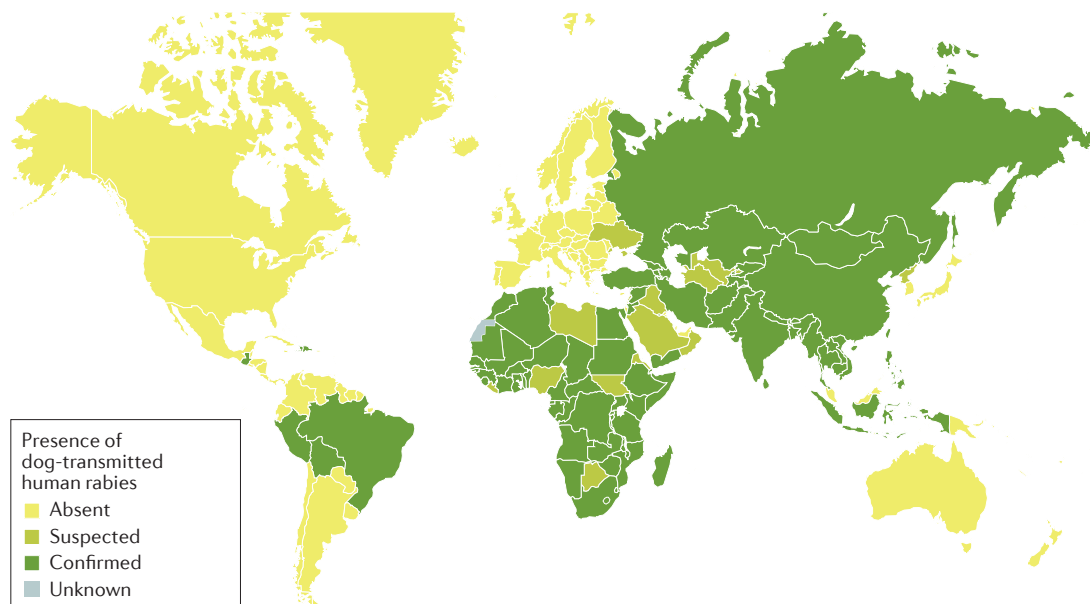


Figure 2 | **The global distribution of dog-transmitted human rabies.** Dog-transmitted human rabies caused by the rabies virus (RABV) is responsible for most human fatalities. The map does not reflect very low numbers of cases of human rabies resulting from exposure to RABV via wildlife reservoirs in areas where dog rabies has been eliminated (for example, North America). Figure reprinted from World Health Organization. Rabies: epidemiology and burden of disease. <http://www.who.int/rabies/epidemiology/en/> (date accessed: 17/10/2017) (REF. 174).

Consistent with the findings in the canine model that infected animals develop limited immune responses⁶⁶, wild-type RABV infection does not increase the permeability of the blood–brain barrier⁶⁷. The permeability of the blood–brain barrier remains unaltered in dogs and patients in both furious and paralytic rabies⁶⁸ during early-stage clinical disease, as demonstrated by MRI (in humans and dogs)^{52,69} and diffusion tensor imaging (in dogs). However, the permeability of the blood–brain barrier is considered one of the main reasons for the rare survival of patients with clinical rabies and might be a potential target for future treatments⁷⁰. Nevertheless, whether the diminished immune response in the brain of patients infected by wild-type lyssaviruses is the result of a reduced permeability of the blood–brain barrier or the virus-dependent inhibition of immune cell infiltration to the CNS remains to be investigated. In any case, virus-neutralizing antibodies (VNAs), which might be detected in the acute neurological phase of naturally acquired RABV infection in humans, are considered to be unable to prevent a fatal outcome of the infection.

Immune evasion. Numerous mechanisms have been proposed to explain how RABV can evade or counteract the host's defence strategies^{71,72}, and RABV replication in the CNS seems to be characterized by limited pathogen recognition and immune reactions (FIG. 4). Type I interferon is the main mediator of antiviral innate immune responses, but RABVs seem to be poor inducers of type I interferon⁶², and such dampened type I interferon initial responses at the virus peripheral entry sites might only partially eliminate replication.

Several mechanisms could enable RABV to escape or manipulate adaptive immune surveillance in the nervous system. Upregulation of *FASLG* expression⁷³ and subsequent activation of Fas ligand could induce apoptosis of antigen-activated T cells and contribute to the termination of the immune response. The expression of HLA class I histocompatibility antigen, α chain G (HLA-G), a nonclassical, immunosuppressive HLA^{74,75}, and upregulation of the expression of programmed cell death 1 ligand 1 (PDL1; also known as B7H1) could inhibit T cell proliferation and apoptosis^{73,74,76}.

Neuronal survival. Although the clinical manifestations of RABV infection can be severe, both macroscopic and histopathological changes are often quite mild. RABV replication is self-limited by suboptimal transcription signals and large RNA polymerase protein expression to avert cytopathogenic effects⁷⁷, thereby supporting the maintenance of vital CNS functions and intraneuronal transport machineries. The viral load in the brain is greater in dogs that develop furious rabies than in dogs with paralytic rabies, and in dogs with paralytic rabies the viral intracellular spread is reduced^{69,78}. In paralytic dog rabies, axonal integrity is also disrupted at the level of the brainstem, impairing the viral spread to each hemisphere^{78,79}. Similar data for human infection are lacking.

Viral infections cause cellular stress responses that modulate the host's gene expression by affecting the regulation of mRNA translation, localization and degradation while promoting viral transcription, replication and translation. One stress response induced

by RABV infection is the assembly of both viral and host mRNA complexes into dynamic cytoplasmic structures known as stress granules⁸⁰. Although an antiviral role for stress granules has been suggested⁸¹, it is not clear

whether the formation of stress granules is directly stimulated by RABV as a means to control the number of viral transcripts and limit cytopathogenic effects and consequently cellular damage⁸⁰.

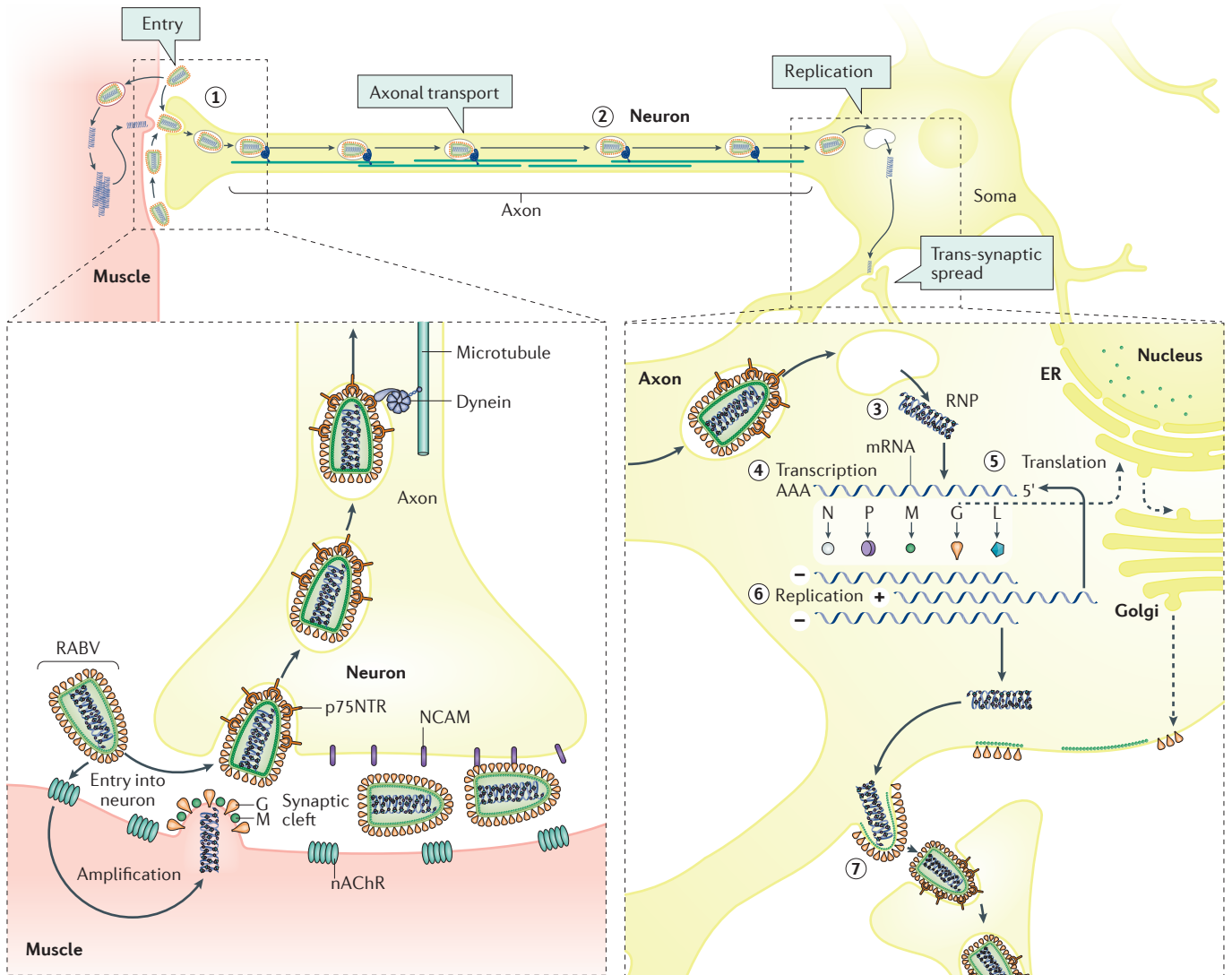


Figure 3 | Spread of rabies virus within the nervous system. At the motor endplate, after receptor-mediated endocytosis at the presynaptic membrane (step 1), the virion travels by retrograde axonal transport towards the neuron soma (step 2), probably in a dynein-dependent manner, as suggested by transport directionality⁴³, measured transport velocities⁵⁹ and co-transport with the dynein-motor transported tumour necrosis factor receptor superfamily member 16 (p75NTR)⁴². In the soma, the ribonucleoprotein (RNP) is released from the endocytic vesicle (step 3), and primary transcription produces 5'-capped and polyadenylated (AAA) viral mRNAs, a process driven by the virion-associated large RNA polymerase protein (L) and the phosphoprotein (P) (step 4). Viral proteins are translated on free ribosomes in the cytoplasm with the exception of the G protein, which is translated through the endoplasmic reticulum (ER) Golgi network (step 5). In later phases, the matrix protein (M) shifts the activity of the RNA polymerase complex (enzymatic subunit L and cofactor P) from transcriptase to replicase^{175,176} and replication of full-length RNA genomes (step 6) occurs. The negative-sense RNA genome is first transcribed in full-length, positive-sense RNA strands, which are subsequently transcribed into full-length, negative-sense RNA strands. Both replication products, full-length positive-sense and negative-sense RNA genomes, start to be

assembled with the nucleoprotein (N) to form new RNPs while the replication process is ongoing and can either be used as templates for further RNA synthesis (full-length genome replication and mRNA transcription) or encapsidated into progeny virions. Assembly and budding of virions is essentially mediated by M¹⁷⁷, but how M is recruited to budding sites is unknown. M is located in the cytoplasm, inclusion bodies (not shown) and the nucleus and accumulates at cellular membranes¹⁷⁸. Oligomerization of M at membranes could increase the binding affinity of M for the lipid bilayer and could play a major part in membrane curvature, envelopment of RNPs and virus egress (step 7). A late domain (a protein interaction motif that has a role in the budding process) in M increases the efficiency of the virus egress¹⁷⁹. The glycoprotein (G) on the surface of virions is required for receptor binding and subsequent pH-dependent membrane fusion of the endocytic vesicle¹⁸⁰. G is transported to budding sites through the secretory pathway, which includes translation at the rough ER and transport through the Golgi apparatus. During surface transport, G is glycosylated¹⁸¹ and forms homotrimers that are incorporated into budding virions¹⁸². nAChR, nicotinic acetylcholine receptor; NCAM, neural cell adhesion molecule; RABV, rabies virus. Figure adapted with permission from REF. 182, Macmillan Publishers Limited.

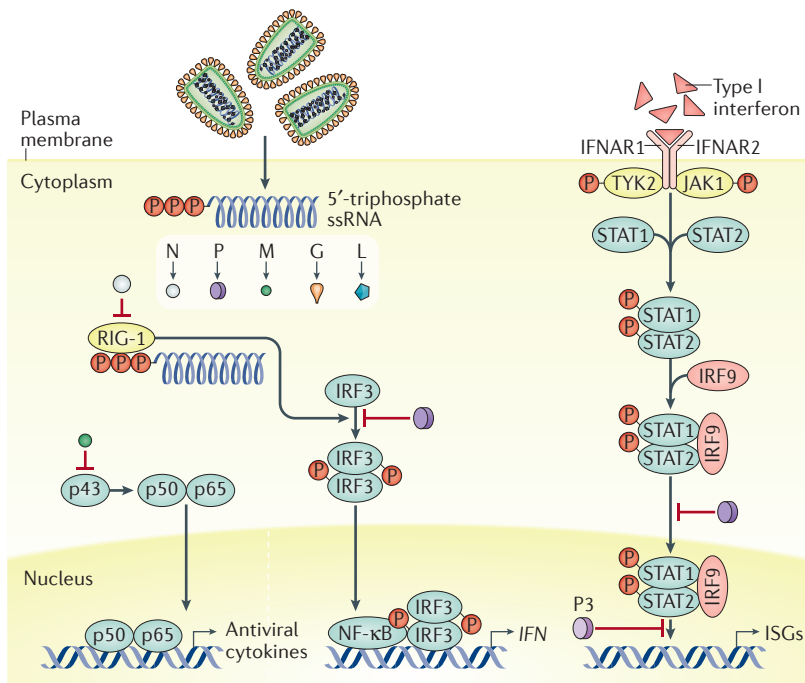


Figure 4 | Mechanisms of immune evasion of the rabies virus. Viral infection triggers numerous signalling cascades, including the induction of the genes encoding type I interferons and chemoattractive and inflammatory responses, resulting in an antiviral environment and efficient innate immune responses^{182,183}. However, the rabies virus (RABV) has developed several different mechanisms to counteract these innate signalling cascades^{184–188}. Expression of antiviral cytokines is activated by the transcription factor nuclear factor- κ B (NF- κ B). NF- κ B is a homodimeric or heterodimeric complex of various proteins, of which the heterodimeric complex composed of transcription factor p65 (p65) and NF- κ B p50 subunit (p50) seems to be the most abundant. A variant of p65, p43, which stabilizes p50-containing dimers and promotes their activation, is inhibited by RABV matrix protein (M)^{189,190}. The innate immune system recognizes intracellular RABV replication through internal receptors such as probable ATP-dependent RNA helicase DDX58 (also known as retinoic acid-inducible gene 1 protein (RIG-1))^{186,191,192}, which promotes type I interferon regulator factor 3 (IRF3) phosphorylation and *IFN* transcription. RABV nucleoprotein (N) interferes with RIG-1 activation¹⁸⁶, whereas RABV phosphoprotein (P) inhibits *IFN* induction by blocking IRF3 phosphorylation. The binding of interferon to its receptors triggers the dimerization, phosphorylation, association with interferon regulatory factor 9 (IRF9) and nuclear import of signal transducer and activator of transcription 1 (STAT1) and STAT2 heterodimers, which promote the transcription of interferon stimulated genes (ISGs). The nuclear import of STAT1-STAT2 is prevented by P¹⁹³, and, within the nucleus, an N-terminal truncated phosphoprotein form of P (P3) interferes with STAT1-STAT2-dependent transcription¹⁹³. P also inhibits type II IFN- γ -dependent signalling through STAT1 homodimers (not shown)¹⁹³. IFNAR, IFN- α and IFN- β receptor; JAK1, tyrosine-protein kinase JAK1; ssRNA, single-stranded RNA; TYK2, nonreceptor tyrosine-protein kinase TYK2. Figure adapted with permission from REF. 182, Macmillan Publishers Limited.

Furthermore, RABV could also directly modulate neuronal cell death, as it has been hypothesized that a PDZ-binding domain in the cytoplasmic tail of glycoprotein could be involved in the regulation of neuronal survival by binding to microtubule-associated serine/threonine protein kinase 2 (MAST2)⁸². The complex formed by MAST2 and phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity phosphatase and tensin homologue (PTEN) can prevent or reduce neurotrophin-dependent apoptosis inhibition. Thus, the binding of glycoprotein to MAST2 could impair the pro-apoptotic function of the MAST2-PTEN

complex. In addition, a high affinity of glycoprotein to the PDZ-binding domain of tyrosine-protein phosphatase non-receptor type 4 (PTPN4) correlated with virulence attenuation^{83,84}. Accordingly, a model has been proposed in which the binding of viral proteins to the PDZ-binding domains of host proteins could represent a decisive virulence marker⁸². This model is supported by the following observations: the strong binding of glycoprotein from an attenuated RABV to the PDZ-binding domain of PTPN4 increased cell death; pathogenicity and apoptosis induction by RABV inversely correlate⁸⁵; and, in histopathological findings, neuronal apoptosis does not have an important role in human rabies-associated encephalitis⁸⁶. Evidence of an autophagic response has been observed in wild-type RABV infection and this response might be influenced by matrix protein expression^{87,88}. Indications of an autophagic response include minimal pathology with minimal or no inflammation⁸⁹; signs (in immunohistochemistry tests) of mitochondrial outer membrane permeabilization (but not of apoptosis) in post-mortem brain examination⁹⁰; and preserved brain and spinal cord function despite abundant viral particles, as in both furious and paralytic rabies coma occurs only at the pre-terminal phase of the disease, and in paralytic rabies electrophysiological and pathological measurements of peripheral nerves show either axonopathy or myelinopathy⁹¹. Apoptosis seems to be more-prevalent in infections with attenuated or fixed viruses (a fixed virus is a cultured strain in which the incubation period and virulence have been stabilized)⁹².

Diagnosis, screening and prevention

Clinical disease

Clinical descriptions have reported many manifestations of rabies⁹³. It has been demonstrated that symptoms do not correlate with the site of viral replication in the brain^{69,89}. Two classical forms of rabies are generally recognized: furious (also called encephalitic) and paralytic. The factors that determine the development of either form remain ill-defined^{69,94}, but each form may be characterized by specific symptoms, although case definition can typically be established with certainty only when the disease reaches the acute neurological phase (FIG. 5). Incubation periods can vary considerably, although most patients develop symptoms 20–90 days after exposure³. The cause of this variation is probably multifactorial and can include the site of virus entry and the viral load, the species and strain of the infecting virus and the immunological competence of the host. Initial symptoms are triggered by the viral replication in the dorsal root ganglia and include pain, paraesthesia and/or pruritus⁵². After a short prodromal phase, the patient enters the acute neurological phase of the disease, which is characterized by clinical manifestations. Furious rabies typically presents during this phase with intermittent agitation, hypersalivation and hydrophobia (a flight response to fluids) (FIG. 5). By contrast, the paralytic form presents with muscle weakness and, eventually, paralysis and generally a longer acute neurological phase than furious rabies⁹⁴. Both forms of rabies lead to coma and death.

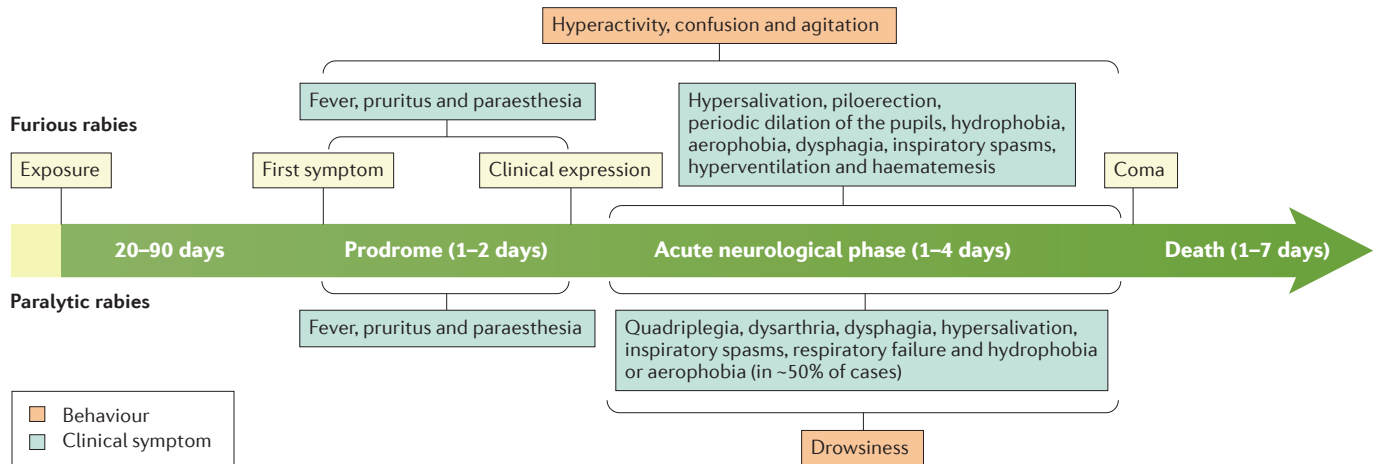


Figure 5 | **The spectrum of clinical rabies.** The clinical presentation of rabies can vary substantially between patients, which makes diagnosis on the basis of observed symptoms problematic. In individuals with furious rabies, hyperactivity manifests during the prodromal phase and can alternate with confusion or agitation once the patient enters the acute neurological phase. In patients with paralytic rabies, during the prodromal phase the patient is fully alert, but confusion or drowsiness can intermittently occur during the acute neurological phase, although not as prominently as seen in the furious form of the disease. The course of clinical disease and some clinical manifestations can be observed in both paralytic and furious rabies; thus, it is generally not possible to differentiate between the two forms until the acute neurological phase. The average time from the onset of symptoms to death is 7.8 days in furious rabies and 11 days in paralytic rabies⁹⁴.

Diagnostic evaluation

The specific clinical symptoms of hydrophobia or aerophobia (fear of drafts or fresh air) provide a strong indication of RABV infection. However, only the results of laboratory tests can provide a definitive diagnosis of rabies³⁰, as other differential diagnoses are possible (FIG. 6). In fact, the absence of pathognomonic symptoms means that fluctuating consciousness, phobic or inspiratory spasms and autonomic stimulation can indicate infection with RABV. Some presentations can commonly occur in paralytic rabies or other atypical forms, caused by infection with dog or bat variants of RABV⁹⁰. Although early diagnosis is important, the lack of tools to enable survival once the patient develops clinical disease means that, regardless of the timeliness of the diagnosis, the prognosis is extremely poor and will almost certainly result in death if RABV infection is confirmed²⁹.

Ante-mortem diagnostic testing. In endemic settings, ante-mortem diagnostic tests are not routinely conducted, as facilities often lack the necessary resources. This situation is unfortunate, as ante-mortem confirmation of infection enables appropriate patient care and alerts caregivers to potential risks of RABV transmission. The most useful samples for ante-mortem diagnosis are obtained from nuchal (nape) skin biopsies and saliva (TABLE 2). Live virus has been occasionally isolated from human saliva^{93,95}; in dog models, high viral titres can be intermittently detected in secretions (saliva) up to 10 days before the onset of clinical disease⁹⁶. Skin specimens can be tested for the presence of virus antigen (that is, the nucleoprotein) in neurons in close proximity to hair follicles, and both skin and saliva samples can be tested for the presence of viral RNA by reverse transcription PCR (RT-PCR)⁹⁷. Indeed,

a number of RT-PCR-based and quantitative RT-PCR (RT-qPCR)-based assays have been proved rapid, highly sensitive and specific and are increasingly used for ante-mortem RABV testing⁹⁸⁻¹⁰¹. Other biological samples that could be used for RT-PCR testing include urine and extracted hair follicles¹⁰²; as detecting the virus, especially in the early stages of disease, is challenging, increasing the number of tested samples can improve the probability of identifying the virus, if present. Ante-mortem testing of corneal impressions is of limited value and is no longer recommended³⁰, and other diagnostic methods such as viral culture are not sufficiently sensitive or timely for diagnostic application and require tissue culture facilities that are often unavailable. Although saliva can be obtained quite easily, because of the intermittent secretion of the virus into this fluid the diagnostic findings should be interpreted with caution, and multiple samples collected daily might be required to confirm virus presence^{30,97,102}. However, it must be stressed that laboratory tests can only confirm infection: a negative ante-mortem test result does not necessarily rule out infection.

Humoral immune responses to RABV usually arise in the serum or the cerebral spinal fluid (CSF) at late stages of infection¹⁰³. Nevertheless, although it is not directly considered a diagnostic test, the detection of virus-specific antibodies against viral antigens in the serum of unvaccinated individuals, or in the CSF of both vaccinated and unvaccinated individuals, can be of diagnostic value. VNAs in the CSF were detected in 20 (22.4%) of 89 clinically suspected cases of rabies in India¹⁰⁴. By contrast, antibodies in the CSF were not detected in 31 Thai patients with rabies and 43 unvaccinated patients who were infected with dog RABV variants from Cambodia, Madagascar and Senegal⁵². Individuals who develop VNAs in the serum

or CSF early during the disease course are considered potentially favourable candidates for aggressive management¹⁰⁵. Of note, most documented survivors of rabies were diagnosed solely by the presence of VNAs in the CSF or serum, and no viral RNA or antigens were detected in biological samples (TABLE 3).

Post-mortem diagnostic testing. Brain tissue is the specimen of choice for post-mortem diagnosis²⁹. Historically, the presence of Negri bodies (intracellular accumulations of RABV particles), first described in 1903, was considered indicative of rabies, but this test has now been replaced by more-sensitive and specific methods^{98,106}. However, the detection of Negri bodies upon autopsy can warrant further testing for RABV infection. The fluorescent antibody test (FAT) to detect virus antigen in brain impressions is the diagnostic test recommended by both the WHO and the OIE, and it yields reliable results in fresh specimens in <4 hours¹⁰⁷ (TABLE 2). The sensitivity of the FAT depends on the quality of the specimen and the degree of autolysis of the brain tissue. However, the FAT requires expensive equipment; thus, it is often unavailable in resource-limited settings. To overcome this limitation, a direct immunohistochemical test (dRIT) that uses a mixture of biotinylated antibodies and light microscopy has been generated; dRIT is cost-effective and can rapidly detect virus antigen in fresh brain material¹⁰⁸ (TABLE 2). Extensive validation of dRIT, through evaluation against the gold-standard FAT and comparison

with other molecular-based methods, has been completed, and this procedure, or variations thereof¹⁰⁹, is expected to increase the availability of antigen detection methods to endemic regions and could enable reliable primary diagnosis in resource-limited areas²⁷. Further antigen detection tests, including lateral flow devices (LFDs), have been developed (TABLE 2) but lack validation. Depending on the sensitivity and specificity of routine diagnostic tests (such as the FAT and RT-PCR), a confirmation of results could be required by a validated secondary method, such as the rabies tissue culture isolation test (RTCIT) for virus isolation or the mouse inoculation test (MIT), in which brain samples are inoculated intracranially in laboratory mice, in areas where tissue culture facilities for RTCIT are unavailable¹⁰⁷ (TABLE 2).

Molecular diagnostic assays that can detect the RNA of many lyssaviruses are available^{110,111} (TABLE 2) and will replace the cumbersome existing secondary tests for rabies diagnosis in animals. Compared with the FAT, molecular methods are less prone to subjective interpretation and have the added benefit that they can identify the virus species, a useful tool in cases with undefined history of potential exposure to RABV. Molecular assays are now recognized by the OIE; however, these tests need to be performed in well-controlled environments to avoid false-positive results and, as a consequence, the standardization of the assays and use of appropriate controls are required before these tools can be used for routine post-mortem diagnosis of rabies in animals in resource-limited settings³⁰. Most post-mortem diagnoses are currently based on the previously recommended techniques (the FAT, RTCIT and MIT): adoption of new reliable and validated tools (dRIT and RT-PCR) will improve diagnostic options for rabies in endemic areas where empirical data are lacking and the burden of the disease is disproportionately high.

Prevention

Primary prevention is the best defence against rabies. It includes: elimination of animal rabies through dog vaccination campaigns; promoting awareness of the disease and available prevention; and encouraging responsible dog ownership and vaccination, particularly in endemic areas, where existing cultural practices might need to be adapted to integrate these interventions²⁷. Where possible, rabies pre-exposure prophylaxis (PrEP) via immunization is recommended for travellers to endemic regions, although such advice might not always be followed¹¹². However, in the absence of infrastructure to support such initiatives, secondary prevention attempts to prevent the onset of clinical disease through post-exposure prophylaxis (PEP). Assessing the category of exposure as defined by the WHO (BOX 1) determines the course of action required. The combination of PrEP and PEP can successfully prevent infection in virtually 100% of cases and, regardless of whether or not PrEP has been administered, appropriate wound care and application of PEP are almost invariably effective in preventing deaths³⁰.

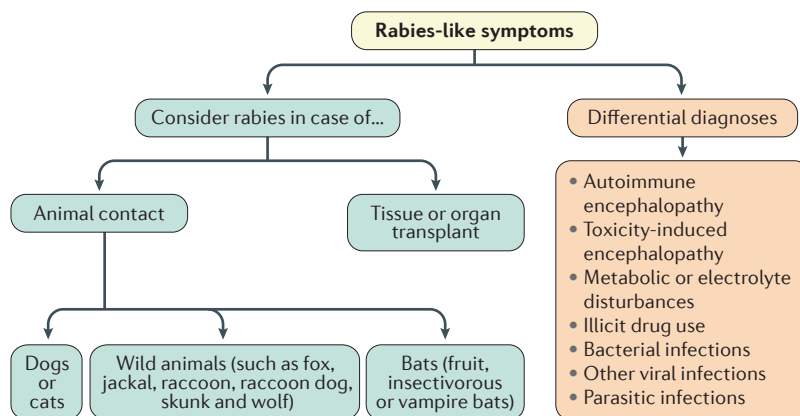


Figure 6 | Algorithm for the evaluation of differential diagnoses. The diagnosis of rabies cannot be made solely on the basis of clinical presentation, as several other infectious or noninfectious aetiologies are possible. The geographical context can provide valuable information during the evaluation of a patient presenting with rabies-like symptoms. It is also important to establish whether the patient had contact with potentially infected animals and, if this is the case, what kind. Rarely, rabies can occur after interaction with less commonly reported animal hosts, such as rodents. The circumstance of the bite can provide information on the category of exposure that occurred. Several autoimmune encephalitis are thought to be quite common in rabies-endemic areas and, therefore, should be considered alongside other potential aetiologies, such as bacterial infections (for example, *Mycobacterium tuberculosis*, *Clostridium tetani* or *Rickettsia* spp.), other viral infections (for example, herpes simplex or enteroviruses) and Guillain-Barré syndrome. Parasitic infections (for example, *Plasmodium* spp.) are another possibility, as rabies could be misdiagnosed as cerebral malaria in the absence of laboratory evaluation. Importantly, the exclusion of other rabies mimics and confirmation of diagnosis can cause delay in management planning.

Table 2 | Diagnostic tests

Test	Target	Turnaround time	Advantages	Disadvantages
Ante-mortem				
Conventional RT-PCR* [§]	Viral nucleic acid	3–4 hours	<ul style="list-style-type: none"> • Rapid • High sensitivity and specificity • One-tube systems reduce contamination risks • Amplicons can be used for genetic characterization 	<ul style="list-style-type: none"> • Requires specialist equipment • Potential for contamination
Real-time RT-PCR* [§]	Viral nucleic acid	2–3 hours	<ul style="list-style-type: none"> • Rapid • High sensitivity and specificity • One-tube systems reduce contamination risks 	<ul style="list-style-type: none"> • Requires specialist equipment • Potential for contamination • Amplicon not useful for genetic characterization
Fluorescent antibody virus neutralization (FAVN) and rapid fluorescent focus inhibition test (RFFIT)	Virus-neutralizing antibodies	5–8 days	<ul style="list-style-type: none"> • Useful for assessment after vaccination 	<ul style="list-style-type: none"> • Long turnaround time • Incompatible with ante-mortem confirmation of diagnosis, as antibodies are usually detected after the onset of clinical signs • Requires specialist containment and operator expertise
Post-mortem				
Fluorescent antibody test (FAT)* [‡]	Virus antigen	2–3 hours	<ul style="list-style-type: none"> • Rapid • High sensitivity and specificity 	<ul style="list-style-type: none"> • Expensive conjugate • Requires an ultraviolet light microscope • Can be difficult to interpret results
Direct immunohistochemical test (dRIT)* [‡]	Virus antigen	2–3 hours	<ul style="list-style-type: none"> • Rapid • Requires only a light microscope 	<ul style="list-style-type: none"> • Can be difficult to interpret results
Lateral flow devices (LFDs) [‡]	Virus antigen or antibody	30–60 minutes	<ul style="list-style-type: none"> • Simple point-of-care test • Safety risk associated with sample preparation in the field 	<ul style="list-style-type: none"> • Requires validation • Batch-to-batch variation reported
Rabies tissue culture isolation test (RTCIT)*	Live virus	4–6 days	<ul style="list-style-type: none"> • Enables propagation of virus for characterization 	<ul style="list-style-type: none"> • Requires specialist facilities and operator expertise • Long turnaround time
Mouse inoculation test (MIT)*	Live virus	Up to 28 days	<ul style="list-style-type: none"> • Enables propagation of virus for characterization 	<ul style="list-style-type: none"> • Requires specialist facilities and operator expertise • Very long turnaround time • Ethical issues owing to the use of <i>in vivo</i> models

RT-PCR, reverse transcription PCR. *Awaiting validation by the World Organisation for Animal Health (OIE). [‡]Low relative cost per test. [§]Can also be used post-mortem. ^{||}High relative cost per test.

Rabies vaccines. Several inactivated preparations of RABV are available as vaccines to immunize humans and domestic animals. For wildlife vaccination, both live-attenuated and subunit vaccines are available. Human rabies vaccines are administered intramuscularly or intradermally, and the same vaccines that are used for PrEP can also be given as part of PEP. The goal of vaccination is to induce VNAs against glycoprotein that provide protection against RABV infection¹¹³, although the precise location and mechanisms by which VNAs inhibit viral replication are unknown. The availability of inactivated, lyophilized, cell culture-based vaccines with increased antigenicity has led to a reduction in the number of doses needed to elicit a sufficient VNA response and, hence, a reduction in the number of clinic visits required, which in turn has improved patient participation, in both PrEP and PEP¹¹³. The WHO has issued recommendations for inactivated rabies vaccines for human use¹¹⁴ and pre-qualified three vaccines (TABLE 4). All licenced vaccines have good safety records, and severe adverse events after vaccination are very rare. However, clinicians should be aware that, as with virtually all vaccines, minor adverse effects, such as mild fever and localized reactions to inoculations, are common.

Although the use of refrigerated reconstituted vaccines has not been sanctioned by any major organization or vaccine manufacturer, studies have demonstrated that it is safe, and such vaccine preparations can retain immunogenicity for weeks to months, as long as uninterrupted local electrical supply can support storage^{115,116}. Vaccines are now locally manufactured in India, Thailand and China, and the WHO is developing methods to ensure the uniformity, safety and efficacy of these new products, in line with existing recommendations^{114–119}. Research has focused on the development of new vaccines that could reduce the number of clinic visits required through over-expression of the virus glycoprotein. Live-attenuated RABV variants in which glycoprotein expression is two-fold to threefold the physiological levels have proven to be effective as PrEP in murine and canine models^{120–122}. An attenuated virus vaccine with three copies of the gene encoding glycoprotein induced RABV VNAs in the CSF of mice following intrathecal administration¹²³ and could be of value as a human therapeutic.

Primary prevention: pre-exposure immunization. The near-100% case fatality rate and high case incidence of RABV infection in children <15 years of age have led

to the proposal that PrEP should be considered as part of a recommended paediatric vaccination strategy in high-risk regions^{25,30,124}. PrEP regimens currently consist of the administration of 4 vaccine doses, on days 0, 7, 14, and 21 or 28 (REF. 30). Where PrEP is implemented, the assessment of serological positivity is important to ensure adequate protection and longevity of the antibody response. Furthermore, the administration of

rabies vaccines as part of the Expanded Programme on Immunization initiative for children would increase the probability of preventing the development of clinical disease in exposed individuals in the absence of rabies immunoglobulin (RIG) treatment¹²⁵. However, in endemic areas economic difficulties limit the availability of vaccines; thus, widespread PrEP coverage is unattainable and, as a result, PEP is the primary barrier

Table 3 | Human rabies survivors reported worldwide

Country and year of disease onset	Sex and age (years) of the patient and disease outcome	Mode and site of exposure	PEP type and doses; type of RIG	Incubation period	Antibodies in CSF and serum (highest titres or concentrations recorded)	Type of antibody and method of testing	Viral material detected (sample tested)	Refs
India, 2014	Male, 6, severe sequelae**	Dog bite; neck and back	PCECV, 4; ERIG	~22 days	CSF (8,192); serum (>200,000)	RVNA; RFFIT	None (saliva, nuchal skin and CSF)	195
India, 2014	Male, 13, severe sequelae	Dog bite; right hand	PCECV, 3; none	~2 weeks	CSF (>64,000); serum (>64,000)	RVNA; RFFIT	Antigen (nuchal skin)	196, 197
South Africa, 2012	Male, 4, severe sequelae	Dog bite on left ankle and scratch on forehead	Type NA, 3; none	~3–7 weeks (multiple exposures to dogs)	CSF (>13,975 IU/ml); serum (>13,975 IU/ml)	RVNA; RFFIT	None (saliva, nuchal skin and CSF)	198
USA, 2011	Female, 8, complete recovery [§]	Scratches from free-roaming, unvaccinated cats (probable source); site NA	None; none	Unknown	CSF (8); serum (160)	IgM antibody; IFA	None (saliva and nuchal skin)	199
India, 2011	Male, 17, severe sequelae	Dog bite; left calf	PCECV, 4; HRIG	~2 weeks	CSF (>8,000); serum (>16,000)	RVNA; RFFIT	None (corneal smear and nuchal skin)	146
India, 2010	Male, 4, severe sequelae [¶]	Dog bite; face	PCECV, 4; HRIG	25 days	CSF (512); serum (16,384)	RVNA; RFFIT	Antigen (nuchal skin) and RNA (nuchal skin and CSF)	200
Turkey, 2008	Male, 17, complete recovery [#]	Dog bites; left forearm and right shoulder	VCV, 1; none	~3 weeks	CSF (NA); serum (3,788)	RVNA; RFFIT	Antigen (corneal smear) and RNA (CSF and saliva)	201
Brazil, 2008	Male, 15, moderate sequelae	Vampire bat bite; site NA	Type NA, 4; none	~29 days	CSF (>100 IU/ml); serum (>100 IU/ml)	RVNA; RFFIT	RNA (nuchal skin hair follicles)	202
USA, 2004	Female, 15, mild sequelae***	Bat bite; left index finger	None; none	1 month	CSF (1,300); serum (1,604)	RVNA; RFFIT	None (saliva and nuchal skin)	148
India, 2000	Female, 6, severe sequelae	Dog bites; face and hands	PCECV, 3; none	20 days	CSF (312,000); serum (182,000)	RVNA; MNT	None (CSF, nuchal skin and corneal smear)	203
Mexico, 1992	Male, 9, severe sequelae	Dog bite; face	VCV, 3 and HDCV, 1; none	18 days	CSF (78,125); serum (134,800)	RVNA; RFFIT	None (saliva, nuchal skin and corneal smear)	204
USA, 1977	Male, 32, severe sequelae ^{§§}	Laboratory exposure to virus (aerosol)	None ^{¶¶} ; none	~2 weeks (probable)	CSF (16,225); serum (175,000)	RVNA; RFFIT	None (nuchal skin and corneal smear)	205, 206
Argentina, 1972	Female, 45, nearly complete recovery	Dog bite; left arm	SMBV, 14 and 2 boosters; none	21 days	CSF (160,000); serum (640,000)	RVNA; MNT	None (saliva, nuchal skin and corneal smear)	207
USA, 1970	Male, 6, complete recovery ^{¶¶¶}	Bat bite; left thumb	DEV, 14; none	20 days	CSF (3,200); serum (63,000)	RVNA; MNT	None (saliva, CSF, brain biopsy, corneal smear)	208

Only published and adequately documented cases are included. CSF, cerebral spinal fluid; DEV, duck embryo vaccine; ERIG, equine rabies immunoglobulin; HDCV, human diploid cell vaccine; HRIG, human rabies immunoglobulin; IFA, indirect immunofluorescence assay; IgM, immunoglobulin M; MNT, mouse neutralization test; NA, not available; PCECV, purified chick embryo cell vaccine; PEP, post-exposure prophylaxis; RFFIT, rapid fluorescent focus inhibition test; RIG, rabies immunoglobulin; RVNA, rabies virus-neutralizing antibodies; SMBV, suckling mouse brain vaccine; VCV, purified vero cell vaccine. *Sequelae generally include neurological problems and can vary depending on cases. Examples include convulsions, poor swallowing reflex and paralysis. [†]Deviations in PEP protocol used. [‡]Rabies-neutralizing antibodies not detected in CSF or serum. Diagnosis based on virus-specific antibodies in CSF and serum detected by IFA. [§]Delay in initiating PEP. [¶]Nuchal skin biopsy sample positive for viral RNA even 4 years after onset of illness. ^{¶¶}ERIG administered after onset of symptoms. ^{¶¶¶}The US case definition for human rabies is "a clinically compatible case that is laboratory confirmed by testing at a state or federal public health laboratory" (<https://wwwn.cdc.gov/nndss/conditions/rabies-human/case-definition/2011/>). The patient met these criteria, and at the time of publication, the state of California has not withdrawn this case notification. ^{§§}The first reported rabies survivor who had never received prior vaccination. ^{§§§}The first reported case of rabies in a pre-immunized individual. ^{¶¶¶¶}Virus isolated from bat. ^{¶¶¶¶}Pre-exposure immunization with DEV.

to preventing deaths³⁰. Population groups who are at increased risk of exposure to lyssaviruses, including veterinarians, scientists and bat workers, should also receive PrEP^{100,126}. Travellers to endemic areas might be unaware of the need for vaccination, and even if they are, misperception of vaccine-associated risks might prevent them from seeking PrEP¹¹².

Secondary prevention: wound care. Appropriate wound care (in combination with PEP regimens) can save lives; in the absence of wound care, the probability of successful PEP decreases¹²⁷. Wounds should be irrigated with soapy water and scrubbed to remove foreign bodies, and the use of iodine-based antiseptics is recommended. Unless absolutely necessary, suturing the wounds should be avoided or delayed, certainly until after the administration of RIG, as neuronal trauma could allow direct viral access to nerve endings. The local treatment of the wound as well as PEP should be sought immediately after exposure.

Secondary prevention: post-exposure prophylaxis. If exposure is suspected, the WHO guidelines for risk assessment should be followed³⁰ (BOX 1) and medical advice should be sought to evaluate the need for PEP. PEP should be administered as soon as possible after a potential exposure to increase the probability of preventing clinical disease; of note, rare cases of very long incubation times in humans suggest that PEP could be of value even if the administration is delayed and should, therefore, always be provided in suspected cases if concerns are raised. Although the development of clinical disease is almost 100% preventable with prompt PEP, failures do occur, most often because of deviations in recommended PEP protocols¹⁰¹. Such deviations include delay in seeking PEP, improper wound care, lack of or incorrect RIG administration and inadequate vaccine doses. Very rarely, failures can result from the use of substandard black-market products¹²⁸. Cases of patients who develop fatal rabies despite receiving adequate PEP

(true PEP failures) are rare¹²⁹ and can be attributed to short incubation periods that result from multiple bites, especially on highly innervated areas such as the face, neck or hands.

Rabies immunoglobulin and vaccination. PEP can include both the administration of RIG and vaccination, although individuals who received adequate PrEP should receive a booster vaccination, but not RIG. The application of RIG directly to the wound aims to neutralize any live virus in the immediate wound area and prevent the spread of the virus in the time that it takes to develop sufficient immunity in response to vaccination (up to 14 days¹⁰³). Human RIG (HRIG) or, if not available, chromatography-purified, pepsin-digested equine RIG (ERIG) can be used. The WHO recommends a weight-based total dose calculation: 20 IU/kg for HRIG and 40 IU/kg for ERIG. If possible, all of the calculated dose of RIG should be infiltrated into wounds, and any residual RIG should be injected intramuscularly at a distant site³⁰. However, owing to high manufacturing costs and difficulties in large-scale production, RIG is often scarce in endemic countries²⁶. Although ERIG is safe, easier to produce than HRIG, effective and increasingly available in rabies-endemic countries, the development of new alternatives is warranted¹³⁰. Studies have assessed the application of RIG to wounds alone, omitting the injection of the usually large residual volume of RIG to distant sites, in an effort to save RIG supplies for future patients¹³¹. This approach could enable resource-limited, rabies-endemic countries to provide PEP to all patients with category III exposure¹³¹. Nevertheless, a lack of knowledge regarding the potential tools available for PEP means that, even where RIG is available, it might not be used.

In addition to RIG administration, vaccination is advised at a distant site. For previously vaccinated patients, an intradermal or intramuscular booster regimen has been recommended by the WHO^{30,132} (TABLE 4). For unvaccinated individuals, several vaccine regimens are approved for PEP; as all vaccines are considered equally effective, the choice of the regimen is determined by the available vaccine and the local medical centre experience³⁰ (TABLE 4). Immunocompromised patients and those with low CD4⁺ T cell counts might respond poorly or not at all to vaccination¹³³. Such patients require intense PEP, including wound care with RIG infiltration and vaccination, to elicit an adequate immune response. Patients receiving haemodialysis could also have impaired immunological responsiveness to vaccines, although rabies vaccination is safe and effective if applied before the onset of symptoms¹³⁴. Vaccination for PEP can be time consuming and costly, owing to a need for multiple separate clinic visits. Studies have attempted to shorten PEP vaccination regimens to 1 week¹³⁵ to make PEP more-affordable and accessible¹³⁶. Although the use of intradermal rabies vaccination schedules is expanding worldwide^{137,138}, the WHO requirement to use reconstituted vaccine within 6 hours limits this practice to large clinics, which encounter several patients who need PEP each day.

Box 1 | Exposure risk (WHO categories) and appropriate treatment

Category I exposure: touches or licks on intact skin

Contact with intact skin is not considered exposure; thus, post-exposure prophylaxis is not required. However, exposure assessment based on only the information provided by the individual involved might not be sufficient if the person is a minor, as contact with mucous membranes cannot be excluded. In such cases, it is indicated to test for the presence of broken skin with the application of an alcohol swab over the scratches (pain indicates broken skin). If the individual reports pain, the exposure risk is category II.

Category II exposure: nibbling over uncovered skin, minor scratches with broken skin

Rabies vaccination is required, but rabies immunoglobulin (RIG) is not. The exposed area should be vigorously cleansed and irrigated with detergent, alcohol or iodine.

Category III exposure: single or multiple transdermal bites or scratches or contamination by saliva from rabid animals on broken skin or mucous membranes

The exposed area should be vigorously cleansed and irrigated with detergent, alcohol or iodine. Human or equine RIG should be administered and infiltrated as much as possible into the wounds; if the calculated RIG dose is inadequate to infiltrate all wounds, RIG should be diluted with saline to infiltrate all wounds.

Table 4 | Rabies vaccine regimens for post-exposure prophylaxis

Regimen	Route of administration	Days of visits	Regimen details
Pre-immunized individuals			
Booster	i.d. or i.m.	0 and 3	One dose each day
Unvaccinated individuals			
Essen	i.m.	0, 3, 7, 14 and 28	One dose each day
Modified Essen	i.m.	0, 3, 7 and 14	One dose each day
Zagreb (2-1-1)	i.m.	0, 7 and 21	Two doses administered at different sites on day 0; one dose on days 7 and 21
Thai Red Cross Intradermal (TRC-ID)	i.d.	0, 3, 7 and 28	Two doses at different sites on each visit

i.d., intradermal; i.m., intramuscular. Data from REF. 30.

Management

The management of furious and paralytic rabies does not substantially differ. Treatment approaches are mainly symptomatic and might differ on a case-by-case basis owing to the variety of rabies manifestations. Furthermore, the availability of therapies varies greatly across countries. Once the clinical signs and symptoms of rabies are evident, the prognosis is uniformly dismal, generally culminating in death within 5–11 days⁵², although survival can be prolonged by 1–3 months in some patients receiving critical care¹⁰⁴.

Exposure assessment

In patients who present with encephalitis with undefined origin, the initial assessment should involve a thorough evaluation that should focus on looking for minor bites that might have been missed and the examination of existing wounds. Anamnesis (medical history of the patient) should include any complicating factors, such as underlying illnesses or (allergic) reactions to previous vaccinations or drugs. If exposure to RABV is suspected and domestic animals are implicated, the animal should be observed for clinical signs for 10 days³⁰ and PEP should be initiated. Further treatment can be stopped if the suspect animal remains healthy after quarantine. If the animal shows clinical disease and is available for testing, rabies can be excluded only by a negative FAT result that is confirmed by another reference test (RTCIT or MIT), as recommended by the OIE. In areas where terrestrial rabies has been eliminated, bat rabies constitutes a small but not negligible human health threat and, therefore, this possibility should be considered^{139,140}. If possible, a veterinary examination of the implicated animal should be arranged with necropsy when necessary.

Management of overt rabies. The hospitalization of patients with rabies-like clinical manifestations is required to confirm the diagnosis and prevent contamination or exposure of others to virus-containing biological fluids (FIG. 7). Regardless of whether the diagnosis of rabies has been confirmed, these patients should receive intensive care, if available, as recovery is possible (albeit very rarely); the duration of treatment should be

determined on a case-by-case basis. However, in most cases palliative care is the only option, as there is, as yet, no therapy of proven efficacy for human rabies³⁰, and the decision to provide aggressive intensive care is fraught with several medical, ethical, legal, social and economic challenges for physicians as well as caregivers of patients with rabies^{141,142}. The data on *in vitro* efficacy of antiviral drugs have rarely translated to *in vivo* successes in experimental models and, therefore, these research findings have not translated to clinical settings^{143,144}. Intravenous sedatives (for example, diazepam, midazolam or barbiturates) and analgesics (for example, morphine or ketamine) might be required to relieve agitation, phobic spasms, anxiety and other neurological symptoms in patients with furious rabies^{30,141}. Antipsychotics (for example, haloperidol) can be beneficial; however, patients with rabies tend to require higher and more numerous doses than generally prescribed, which could increase the risk of developing neuroleptic malignant syndrome. In furious rabies, all of these symptomatic or palliative treatments aim to alleviate limbic manifestations caused by functional selective impairment via neurotransmitter dysregulation⁵². New approaches (such as experimental drugs or protocols) might be considered to treat a critically ill patient if the potential benefits outweigh the risks.

Palliative care is imperative even if critical care is not feasible because of medical, logistical or financial reasons. Unfortunately, few studies have addressed palliation in rabies^{141,145}, and standard national guidelines for palliative care remain absent in endemic regions. Patients continue to be isolated, often physically restrained, for fear of aggression and transmission of infection to others.

Surviving rabies. Fewer than 20 adequately documented survivors of rabies have been reported worldwide¹⁰⁵ (TABLE 3). Most reports are dated after 2000, which could reflect an increased awareness of the disease and greater access to better critical care facilities in rabies-endemic countries¹⁴². Although almost all described patients had poor functional outcomes that required lengthy rehabilitation and care, with long-term emotional and financial repercussions for family members, complete or good functional recovery is possible. This possibility should compel physicians to consider and manage rabies as a curable disease, which is not usually possible in under-resourced rabies-endemic countries¹⁴⁶. Incremental triage of potential candidates for an intensive therapeutic approach should be implemented, and favourable factors that could indicate a potential candidate include young age and immunocompetence, prior vaccination, early appearance of VNAs in the CSF and serum, rabies due to a bat RABV variant and mild neurological illness at initiation of therapy^{144,147}.

The difficulty in treating clinical rabies is clear. One therapeutic modality that was developed in the United States that seemed to show early promise of success was the ‘Milwaukee protocol’ (REF. 148), which enabled the survival of an unvaccinated 15-year-old girl who was infected with a US bat RABV variant. The Milwaukee protocol focused on supportive critical care

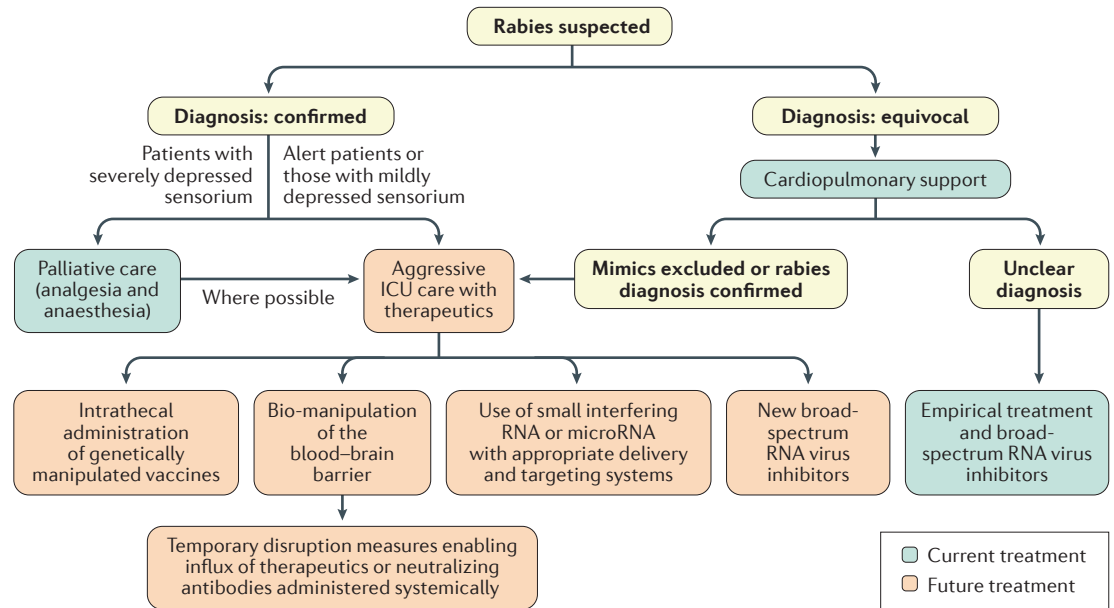


Figure 7 | Management of clinical rabies. The treatment of patients with suspected or laboratory-confirmed rabies at present is purely symptomatic; sedation therapy should alleviate neurological symptoms but avoid compromising cardiopulmonary function. Future therapies should aim to eliminate the virus completely and be applied to alert patients whose nervous system functions are still not corrupted. However, before such therapies can be included in intensive care regimens, it is necessary to ensure that they do not pose any harmful complications. The proposed therapeutics shown remain to be proven effective in experimental animal models. ICU, intensive care unit.

with the aim to evade fatal dysautonomia (dysfunction of the autonomic nervous system) during the first week of hospitalization and to presumably slow the normal metabolic processes, thereby enabling naturally developed immune responses to confer a sterilizing immunity until RABV was cleared from the brain. This goal was achieved by induction of a therapeutic coma (by administering GABA receptor agonists and NMDA (*N*-methyl-*D*-aspartic acid) receptor antagonists) and concurrent administration of antiviral drugs. The original protocol has since undergone several modifications, and induction of coma is no longer recommended. However, despite an initial success, the protocol remains controversial, owing to the associated risks and uncertain benefit¹⁴⁹.

Quality of life

Globally, dog-mediated rabies is estimated to cause >3.7 million (95% CI 1.6–10.4 million) disability-adjusted life years (DALYs), resulting, for example, from adverse events of vaccination with nerve tissue vaccines (which are derived from animal brain tissue, for example, sheep, goat or mouse) or mortality due to rabies²⁶. The annual global economic burden of dog-mediated rabies is US\$8.6 billion (95% CI \$2.9–21.5 billion); lost productivity due to premature deaths is a major component (55%), whereas direct costs of PEP (depending on the biologics used and their regimens) and indirect costs of seeking PEP (such as travel, accommodation, multiple clinic visits, lost income and so on) account for 20% and 15% of the economic burden, respectively²⁶. Although difficult to quantify because of the lack of validated methods, anxiety associated with life-threatening

bites from rabid animals can potentially contribute substantially (>10%) to the estimated DALYs²⁶, and is often augmented by uncertainty about the availability and affordability of PEP in many dog rabies-endemic countries³⁰. Livestock losses due to dog-mediated rabies account for a small proportion of the global economic burden (6%); however, they disproportionately affect communities that rely on livestock for subsistence and livelihoods²⁶.

Outlook

In the 21st century, it is anticipated that human rabies will be confined to history: the goal is to eliminate the disease by preventing the transmission of the virus between terrestrial animals (principally dogs) and humans. For this vision to be realized, a combination of improved diagnostic tools and veterinary and medical infrastructures — to enable access to more-inexpensive and efficacious biologics (vaccines, new antivirals and HRIG) — as well as increased education and awareness of rabies in endemic countries is urgently required²⁷.

Diagnosis

One of the first steps towards human rabies elimination is to ensure that all regions have the capability to accurately diagnose the infection using a network of dedicated laboratories with validated diagnostic tools. The development of molecular diagnostic techniques and next-generation sequencing has revolutionized high-throughput diagnostic testing and genetic characterization of microorganisms. However, these techniques are not available in all diagnostic laboratories and remain largely a research tool for pathogen discovery rather

than pathogen diagnosis per se²⁷. The future challenge is to combine molecular assays that can genetically characterize the RABV variant with point-of-care testing⁹⁸. The 'laboratory in a box' concept, which consists of a toolbox of basic laboratory equipment that can be used for preliminary field diagnosis, will enable the rapid assessment of cases of suspected RABV infection and, as a result, to provide initial care early¹⁵⁰, while awaiting confirmation of diagnosis from reference laboratories²⁷. Studies are underway to assess such new technologies.

The WHO and OIE have clarified that both human and animal rabies should be considered notifiable diseases. However, in areas without the appropriate laboratory infrastructures, the timeliness of case reports is often inadequate. Thus, national governments should consider it a high priority to follow a step-wise approach towards dog rabies elimination¹⁵¹. Such approaches link achievable goals on a structured pathway to eliminate the disease¹⁵². The FAO in collaboration with the OIE and the WHO has proposed a Progressive Control Pathway towards rabies elimination, in which the final stage is maintaining both humans and animals free of rabies.

Vector control and disease awareness

The elimination of RABV from dogs (the principal reservoir) and, as a result, the prevention of human disease rely on pragmatic actions that can be adopted where the veterinary structures are adequate to support a sustained approach¹⁵³. The costs required to eliminate dog-mediated rabies in all endemic countries have been estimated at \$6.3 billion, whereas current spending is only \$2.4 billion⁷. Most cases of human rabies would be prevented if dog populations in endemic regions were controlled (principally by immunization but also by reducing their numbers by sterilization or culling of sick animals) and dogs were licensed and vaccinated. Numerous studies have demonstrated that 70% vaccine coverage is required to stop rabies transmission in free-roaming dog communities^{154–156}, and responsible dog ownership and vaccination are pivotal in preventing human rabies¹¹. Targeted campaigns for vaccination of domestic dogs that were supported by adequate supplies and awareness campaigns have demonstrated that dog vaccination can be achieved to a level that prevents virus circulation. However, to be effective, vaccinations require follow-up visits for booster vaccinations; dog owners' compliance with this requirement is scarce and, therefore, these campaigns need to be supported by a global emphasis on eliminating rabies. Oral vaccination of free-roaming dog populations has been proposed as a complementary measure to parenteral vaccination, but assessment of vaccine coverage using oral formulations is challenging¹⁵⁷. The lessons learned from the oral vaccination of wildlife species have demonstrated difficulties in achieving adequate seroconversion with this formulation and, therefore, oral vaccination for dog populations might also be challenging¹⁵⁸. Additionally, the development of vaccines against *Lyssavirus* spp. that are more divergent from RABV has been proposed but is unlikely to gain support unless the burden of these viruses to human or animal populations is demonstrated^{126,153}.

Although the actual burden of human rabies remains difficult to quantify, it is evident that rabies can be managed at a community level by increasing disease awareness in addition to the control of dog populations. Increasing disease awareness in children, and especially what actions to take if an exposure is suspected, could contribute to reducing deaths and could be achieved through the adoption of a few straightforward messages as part of an educational curriculum^{147,159}. Nevertheless, cultural approaches to dog ownership and the multifactorial challenges associated with dog licensing and vaccination mean that any elimination campaign will require long-term investment⁷. The number of animal bites is often high in regions where rabies is endemic, and every bite should be considered a potential exposure¹⁶⁰. It is estimated that PEP is given to >15 million individuals each year to prevent the establishment of disease¹⁶¹. However, owing to lack of awareness, the simplest actions required as part of PEP, including washing the wound and avoiding suturing it, are rarely implemented. Moreover, the biologics required for PEP present challenges, and alternative products are needed. Vaccines require multiple inoculations and are often unavailable because of the lack of a sustained supply across endemic regions. The use of a paediatric rabies vaccine as part of an Expanded Programme on Immunization initiative would increase the probability of exposed children not to develop rabies if they do not receive PEP¹²⁵. Optimization of current vaccines to reduce production costs and the number of doses required and to develop non-injectable formulations could enable the inclusion of rabies vaccines into existing childhood immunization schemes. New approaches to overcome the scarcity of RIG, the high production costs and the problems associated with blood products include the development of murine monoclonal antibody cocktails¹⁶², camelid nanobodies (single-domain antibodies)¹⁶³ and the generation of antibodies *in planta*¹⁶⁴. However, all of these research efforts require substantial economic investments to reach commercial viability.

Management of clinical disease

The outlook for treating clinical rabies remains poor. Despite promising *in vitro* proof-of-principle data, none of the antivirals tested have demonstrated sufficient *in vivo* efficacy in experimental models to warrant further investigation. The development of antivirals for rabies remains an urgent priority, but it is a major challenge because of numerous viral factors (including replication in the CNS and multiple species) and host factors, such as various stages of presentation, exposure history and immune response. An effective antiviral that could be used therapeutically in symptomatic patients with rabies would have to cross the blood–brain barrier, interfere with RABV replication (without affecting the life cycle of the host's cells) and inhibit detrimental host responses to RABV infection. Strategies already attempted include mechanisms to cause temporary disruption of the blood–brain barrier, such as ultrasound and microbubbles, which could facilitate the entry of therapeutics to the optimal site for their action¹⁶⁵.

Small interfering RNA (siRNA) or multiple artificial microRNAs (miRNAs) targeting multiple RABV genome sequences have been shown to suppress viral replication *in vitro*¹⁶⁶. Broad-spectrum antiviral molecules such as favipiravir (6-fluoro-3-hydroxy-2-pyrazinocarboxamide) have demonstrated an *in vitro* effect, and favipiravir was also effective as a PrEP in mice^{167,168}. Appropriate drug delivery and targeting systems that have been developed for neurodegenerative diseases could be applied to rabies therapeutic tools. Transvascular delivery of therapeutic siRNA molecules to neuronal cells using short peptides derived from the RABV glycoprotein has been reported¹⁶⁹. Other delivery methods that have been tested include stable nucleic acid lipid particles or nano-engineered particles with siRNAs or miRNAs¹⁷⁰. If such therapeutics are demonstrated to be beneficial *in vivo*, then their administration to patients with confirmed or even suspected rabies with alert or mildly depressed sensorium could be an option. Certainly, it is only with a better understanding of the host factors and pathogenetic mechanisms of RABV that a safe, effective antiviral therapeutic protocol can be developed in the future.

As part of the Millennium Development Goals to reduce poverty and preventable childhood deaths from infectious diseases, especially in resource-limited regions of the world, dog-mediated rabies has been targeted for global eradication by 2030 within all endemic regions¹². Rabies in wildlife will also need to be controlled to prevent repeated host-switching of the virus from wildlife reservoirs into domestic dogs^{16,171}. Successful elimination of dog rabies from some regions across Latin America has demonstrated once again how targeted initiatives can be successful in clearing the virus^{3,172}. However, the number of rabies cases across endemic regions and the knowledge gained from targeted initiatives suggest that the 2030 eradication target will require additional financial support to be achieved^{7,173}, and the acknowledgement of rabies as a neglected tropical disease by the WHO will encourage investments¹⁷². Despite all of the challenges, the eradication goal should promote global initiatives to encourage pharmaceutical companies, international organizations, governments, charities and non-governmental bodies to work together to achieve human rabies elimination worldwide.

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Competing interests

T.M., C.F. and S.F. have a research cooperation and project (2014–2019) with a German vaccine company on oral vaccination of wildlife (for example, mechanisms of oral vaccination and immunity and development of a novel oral rabies virus vaccine). All other authors declare no competing interests.

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