MEDICAL VIROLOGY

# Rabies virus

## Rhabdoviruses

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## 2. introduction :

The Rhabdoviridae family is comprised of over 150 viruses of vertebrates, invertebrates and plants. The Rhabdoviruses are approximately 70 nm wide and 170 nm long. They consist of a lipid envelope with glycoprotein peplomers surrounding a helically wound nucleocapsid. It is the arrangement of the peplomers and the nucleocapsid that give the virus its distinct bullet- shaped morphology. The viruses contain a single linear molecule of minus sense ssRNA, 11-12 kb in size. The pathogen that humans should be most concerned with is the rabies virus which is capable of infecting all warm blooded animals. Rabies occurs in most geographic regions but has been successfully eradicated in Australia, Japan, Great Britian, Hawaii, and the islands of the Carribean basin. Dog rabies is an important human concern as the virus in the saliva of infected dogs is responsible for majority of the 75,000 cases of human rabies that occur each year worldwide. The prevalence of wildlife rabies in the United States, Europe and Canada has become an increasing concern for humans in these geographic regions.

### 2.1 History of the disease:

It was quickly understood even in ancient history that the rabies virus could be passed on via an animal bite. Rabies is mentioned in several ancient literature works, such as the paper by Aristotle (300BC) that notes rabies as one of the diseases that affects dogs and any animal that the dog bites.

Also in early historical times, the owner of a dog displaying symptoms of rabies such as excessive salivation was required to take precautions to prevent their dog from biting someone.

### 2.2 Introduction of the virus :

Rabies is a very serious viral infection that targets the brain and nervous system. You can catch rabies if you are bitten by an infected animal and haven't been vaccinated.
It's almost always fatal once symptoms develop, but can be prevented if treatment is given promptly after exposure.
In the UK, rabies is not found in the animal population (with the exception of bats) and infections are almost always picked up during travel abroad.

## 2.3 Epidemic:

A very wide distribution


Tens of thousands of people die each year from rabies.

Latin America and the Caribbean have had a substantial reduction in the number of human and animal rabies cases following the implementation of dog rabies control programmes. Official reports of cases of human rabies transmitted by dogs decreased from about 250 in 1990 to fewer than 10 in 2010. The Pan American Health Organization has set a target to eliminate dog-mediated rabies from the Americas by 2015.
Globally, estimates indicate that human mortality (due to endemic canine-mediated rabies) is highest in Asia, with the highest incidence and deaths reported in India. This

is closely followed by Africa, however estimates of burden have always been uncertain due to the absence of reliable data.

More investigation into the scale of the burden of rabies in the Middle East and Central Asia is required, where minimal information is available.

## 3. Classification of the virus :

Rabies is caused by negative strand RNA-viruses belonging to the genus Lyssavirus, family Rhabdoviridae of the order Mononegavirales. According to the International Committee on Taxonomy of Viruses (ICTV) the genus Lyssavirus is delineated into different virus species based on demarcation criteria such as genetic distance and antigenic patterns in reactions with panels of antinucleocapsid monoclonal antibodies. This demarcation is further supported by geographic distribution and host range

### 3.1 Family :

The family Rhabdoviridae consists of more than 100 single-stranded, negative-sense, nonsegmented viruses that infect a wide variety of hosts, including vertebrates, invertebrates, and plants. Common to all members of the family is a distinctive rod- or bullet-shaped morphology. Human pathogens of medical importance are found in the generaLyssavirus and Vesiculovirus.Only rabies virus, medically the most significant member of the genus Lyssavirus, is reviewed in this chapter.

### 3.2 Order :

Rabies virus belongs to the order Mononegavirales, viruses with a nonsegmented, negative-stranded RNA genomes. Within this group, viruses with a distinct "bullet" shape are classified in the Rhabdoviridae family, which includes at least three genera of animal viruses, Lyssavirus, Ephemerovirus, and Vesiculovirus.

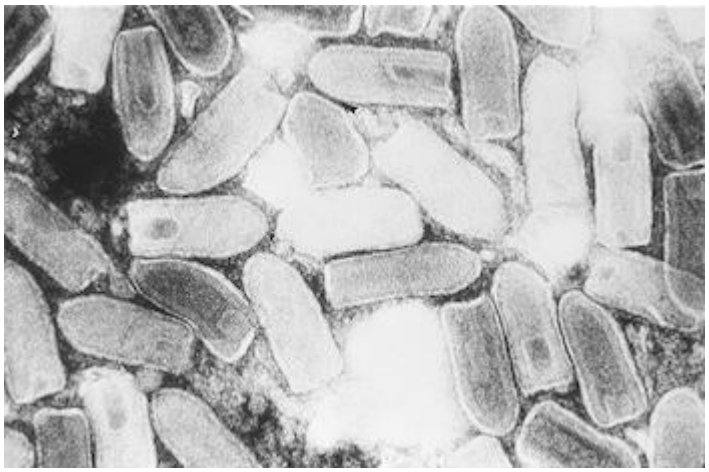
### 3.3 Genus :

The genus Lyssavirus includes rabies virus, Lagos bat, Mokola virus, Duvenhage virus, European bat virus 1 & 2 and Australian bat virus.

## 4. structure and genome :

### 4.1 Shape:

The  virus is rod and bullet-shaped as can be seen in the electron micrograph image below.



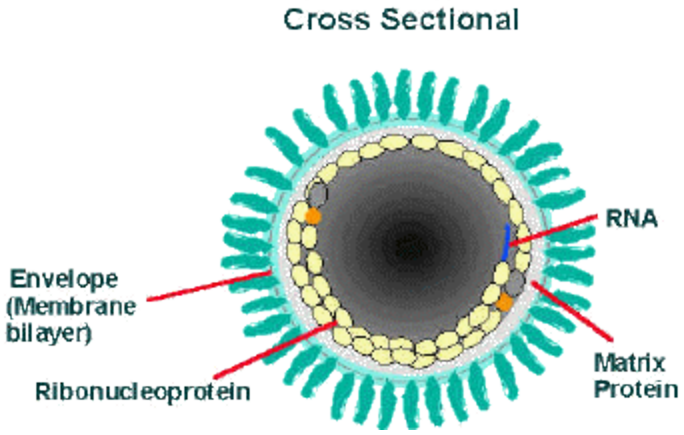
Electron Micrograph of Rabies viruse

### 4.2 Size:

Rhabdoviruses are approximately 180 nm long and 75nm  wide.

### 4.3 Enveloped or not :

Enveloped RNA virus .




### 4.4 Nucleic acid:

Rabies virus is an enveloped rhabdovirus with a single, non-segmented negative-strand RNA.

## 5. Proteins (Virulence Factors):

### 5.1. Structural proteins and their function:

The rabies genome encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L). All rhabdoviruses have two major structural components: a helical ribonucleoprotein core (RNP) and a surrounding envelope. In the RNP, genomic RNA is tightly encased by the nucleoprotein. Two other viral proteins, the phospoprotein and the large protein (L-protein or polymerase) are associated with the RNP. The glycoprotein forms approximately 400 trimeric spikes which are tightly arranged on the surface of the virus. The M protein is associated both with the envelope and the RNP and may be the central protein of rhabdovirus assembly.

### 5.2. Non- Structural proteins:

The non-structural protein (NS; nucleocapsid-associated protein) of the nucleocapsid , the NS protein (groups NS I and NS II).

## 6. Transmission:

All species of mammals are susceptible to rabies virus infection, but only a few species are important as reservoirs for the disease. In the United States, distinct strains of rabies virus have been identified in raccoons, skunks, foxes, and coyotes. Several species of insectivorous bats are also reservoirs for strains of the rabies virus.

Transmission of rabies virus usually begins when infected saliva of a host is passed to an uninfected animal. The most common mode of rabies virus transmission is through the bite and virus-containing saliva of an infected host. Though transmission has been rarely documented via other routes such as contamination of mucous membranes (i.e., eyes, nose, mouth), aerosol transmission, and corneal and organ transplantations.

## 7. Penetration and the Target Organ:

The portal of entry (the alimentary and respiratory tracts), where the infection often causes no significant symptoms or signs of illness.

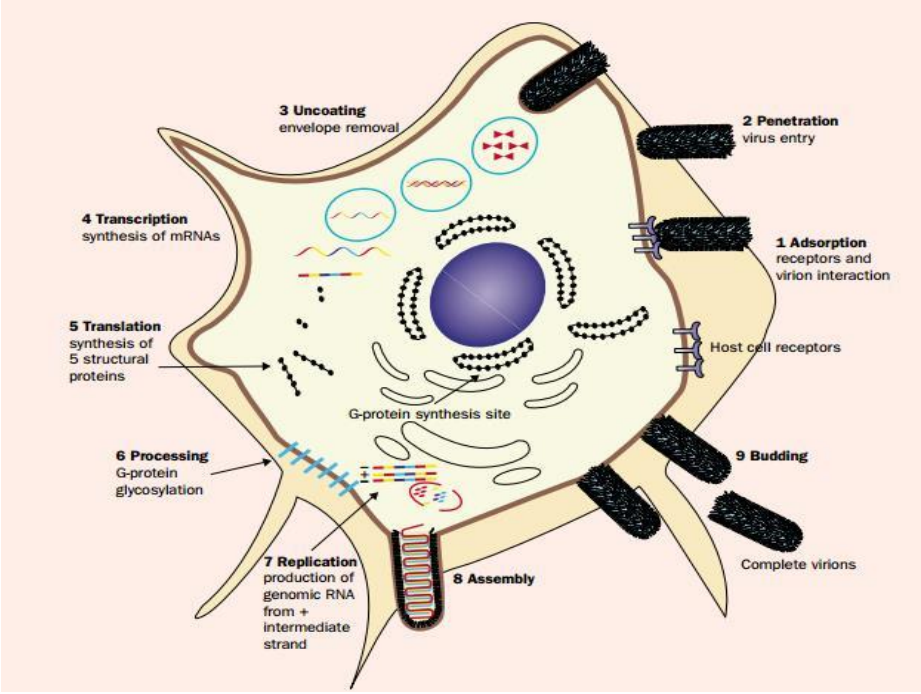
Virus replication in the target organ resembles replication at other body sites except that the target organ in systemic infections is usually reached late during the stepwise progression of virus through the body, and clinical disease originates there. At each step of virus progression through the body, the local recovery mechanisms (local body defenses, including interferon, local inflammation, and local immunity) are activated. Thus, when the target organ is infected, the previously infected sites may have reached various stages of recovery. illustrates this staging of infection and recovery in different tissues during a spreading surface infection. Circulating interferon and immune responses probably account for the termination of viremia, but these responses may be

too late to prevent seeding of virus into the target organ and into sites of shedding. Nevertheless, these systemic defenses can diffuse in various degrees into target organs and thereby help retard virus replication and disease.

Depending on the balance between virus and host defenses, virus multiplication in the target organ may be sufficient to produce dysfunction manifested by disease or death. Additional constitutional disease such as fever and malaise may result from diffusion of toxic products of virus replication and cell necrosis, as well as from release of lymphokines and other inflammatory mediators. Release of leukotriene C4 during respiratory infection may cause bronchospasm. Viral antigens also may participate in immune reactions, leading to disease manifestations. In addition, impairment of leukocytes and immunosuppression by some viruses may cause secondary bacterial infection.

## 8. Replication Cycle (the main site):

The fusion of the rabies virus envelope to the host cell membrane (adsorption) initiates the infection process. The interaction of the G protein and specific cell surface receptors may be involved. After adsorption, the virus penetrates the host cell and enters the cytoplasm by pinocytosis (via clathrin-coated pits). The virions aggregate in the large endosomes (cytoplasmic vesicles). The viral membranes fuse to the endosomal membranes, causing the release of viral RNP into the cytoplasm (uncoating). Because lyssaviruses have a linear single-negative-stranded ribonucleic acid (RNA) genome, messenger RNAs (mRNAs) must be transcribed to permit virus replication.



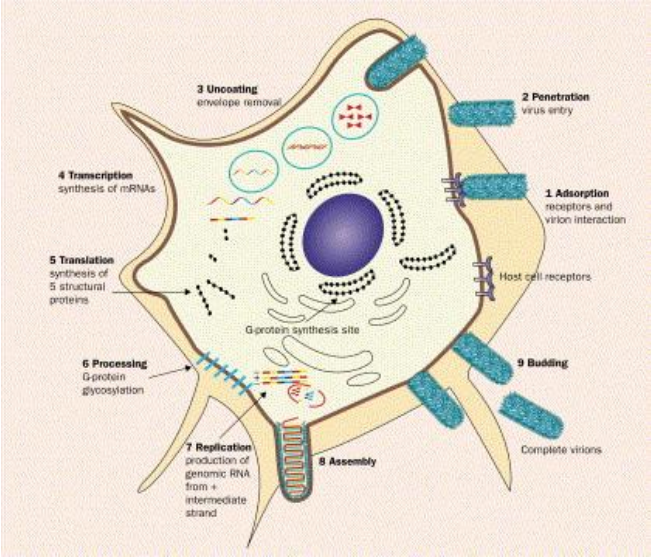

1: Adsorption (receptors and virion interation). 2: Penetration (virus entry). 3: Uncoating (envelope removal). 4. Transcription (synthesis of mRNAs). 5. Translation (Synthesis of structural proteins). 6. Processing (G-protein gycosylation). 7. Replication (production of genomic RNA from intermediate strand. 8. Assembly. 9: Budding (complete virions).

A viral-encoded polymerase (L gene) transcribes the genomic strand of rabies RNA into leader RNA and five capped and polyadenylated mRNAs, which are translated into proteins. Translation, which involves the synthesis of the N, P, M, G and L proteins, occurs on free ribosomes in the cytoplasm. Although G protein synthesis is initiated on free ribosomes, completion of synthesis and glycosylation (processing of the glycoprotein), occurs in the endoplamsic reticulum (ER) and Golgi apparatus. The intracellular ratio of leader RNA to N protein regulates the switch from transcription to replication. When this switch is activated, replication of the viral genome begins. The first step in viral replication is synthesis of full-length copies (postive strands) of the viral genome. When the switch to replication occurs, RNA transcription becomes "non-stop" and stop codons are ignored. The viral polymerase enters a single site on the 3’ end of the genome, and proceeds to synthesize full-length copies of the genome. These positive

strands of rabies RNA serve as templates for synthesis of full-length negative strands of the viral genome.

## 9. Assembly and Egression:

During the assembly process, the N-P-L complex encapsulates negative-stranded genomic RNA to form the RNP core, and the M protein forms a capsule, or matrix, around the RNP. The RNP-M complex migrates to an area of the plasma membrane containing glycoprotein inserts, and the M-protein initiates coiling. The M-RNP complex binds with the glycoprotein, and the completed virus buds from the plasma membrane. Within the central nervous system (CNS), there is preferential viral budding from plasma membranes. Conversely, virus in the salivary glands buds primarily from the cell membrane into the acinar lumen. Viral budding into the salivary gland and virus-induced aggressive biting-behavior in the host animal maximize chances of viral infection of a new host.




## 10. Symptoms:

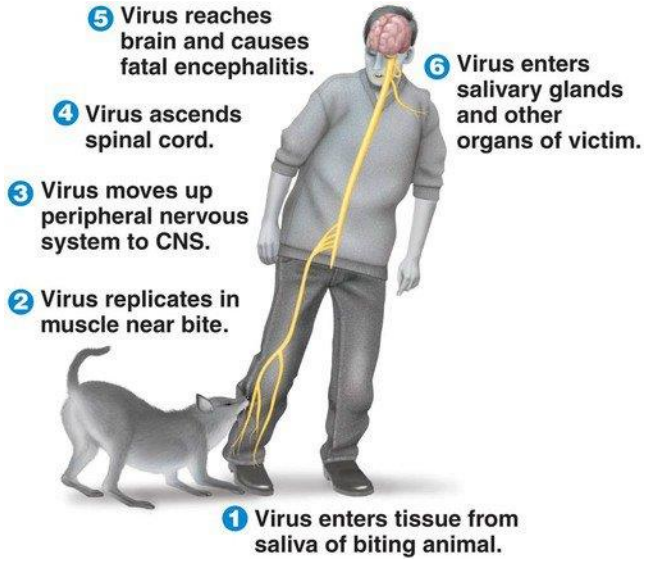
The period between the bite and the onset of symptoms is called the incubation period. It usually takes four to 12 weeks for a person to develop rabies symptoms once they're infected. However, incubation periods can also range from a few days to six years.

The initial onset of rabies begins with flu-like symptoms, including:

- fever
- muscle weakness
- tingling

You may also feel burning at the bite site.

As the virus continues to attack the central nervous system, there are two different types of the disease that can develop.




## 11. Diagnosis and cytopathic effect :

Several tests are necessary to diagnose rabies ante-mortem (before death) in humans; no single test is sufficient. Tests are performed on samples of saliva, serum, spinal fluid, and skin biopsies of hair follicles at the nape of the neck. Saliva can be tested by virus isolation or reverse transcription followed by polymerase chain reaction (RT-PCR). Serum and spinal fluid are tested for antibodies to rabies virus. Skin biopsy specimens are examined for rabies antigen in the cutaneous nerves at the base of hair follicles.

Both fixed and street rabies virus when cultivated in McCoy cells caused cytopathic changes 24 to 72 h after infection, depending on the multiplicity of infection. The cytopathic effect (CPE) was easily recognizable and resembles that induced by other members of the Rhabdovirus group, such as vesicular stomatitis virus, in several cell cultures. Higher titers of the Pasteur strain (PV) of fixed rabies virus were found in supernatants of McCoy cells when compared to those in VERO cells. The virus titer increased with the number of passages attaining a high titer after three passages. Rabies antigens were detected by direct immunofluorescence labeling in most McCoy cells of the infected culture, and specific antibodies neutralized the virus growth and CPE. There was also inhibition by treatment of the cells with human interferon (HuIFN) -alpha or -gamma, but not by murine interferon (MuIFN) -alpha, -beta or -gamma. Rabies-infected McCoy cell cultures may provide a useful assay system, based on the induction of CPE, the high virus production and the sensitivity to IFN.

## 12. Control the virus and Prevention:

Vaccination of susceptible animal species, particularly dogs and cats, will control this zoonotic disease.

Rabies in humans is 100% preventable through prompt appropriate medical care. Yet, more than 55,000 people, mostly in Africa and Asia, die from rabies every year - a rate of one person every ten minutes.

The most important global source of rabies in humans is from uncontrolled rabies in dogs. Children are often at greatest risk from rabies. They are more likely to be bitten by dogs, and are also more likely to be severely exposed through multiple bites in high-risk sites on the body. Severe exposures make it more difficult to prevent rabies unless access to good medical care is immediately available.

This major source of rabies in humans can be eliminated through ensuring adequate animal vaccination and control, educating those at risk, and enhancing access of those bitten to appropriate medical care.

In 2006, a group of researchers and professionals formed a global Alliance for Rabies Control. They created and began inviting partners to join the World Rabies Day initiative.

The goal of this outreach is to mobilize awareness and resources in support of human rabies prevention and animal rabies control. The inaugural campaign on September 8, 2007 saw participation of nearly 400,000 individuals from at least 74 countries! This overwhelming response was an important step forward for rabies prevention and control and further illustrates the widespread recognition of the need for action to control this easily preventable disease.

What can you do?

- Vaccinate your pet
- Maintain control of your pets to reduce their exposure to wildlife
- Spay or neuter to decrease the number of stray animals
- Report any stray or ill animals to animal control

## 13. Treatment:

### 13.1 Vaccines:

A regimen of four 1-mL doses of Human diploid cell culture rabies vaccine HDCV or purified chick embryo cell culture rabies vaccine PCEC vaccines should be administered intramuscularly to previously unvaccinated persons.

### 13.2 Medication:

The first dose of the four-dose course should be administered as soon as possible after exposure. Additional doses should be administered on days 3, 7, and 14 after the first vaccination. For adults, the vaccination should always be administered intramuscularly in the deltoid area (arm). For children, the anterolateral aspect of the thigh is also acceptable. The gluteal area should never be used for rabies vaccine injections because

observations suggest administration in this area results in lower neutralizing antibody titers.

## 14. Host Immune Defense:

The host animal species, viral variant, inoculum concentration, body location and severity of exposure, and host immune status have been associated with overt susceptibility to infection and with different incubation periods. The association of virus-neutralizing antibody, principally IgG, and protective immunity is well known. Production of cytokine, such as interferon, induced during rabies virus infection or vaccination, has been reported to abort the disease if it occurs shortly after viral infection. In one clinical trial, however, all subjects died despite experimental treatment with high doses of alpha interferon.

Recently it has been demonstrated that animals immunized with purified RNP complexes or recombinant nucleoprotein vaccines resisted lethal challenge with rabies virus, although the role of N protein in protection, illness, or recovery is unclear.

## 15. Genetics (Gene Mutation):

In an attempt to compare intrinsic and extrinsic genetic diversity of the lyssavirus genotypes, 69 rabies virus isolates from various part of the world were partially sequenced and compared to 13 representative isolates of the 6 lyssavirus genotypes. The analysis of their phylogenetic relationships, performed on the complete nucleoprotein (N) coding gene (1350 bases), established that the rabies virus isolates all belonged to genotype 1 and that at least 11 phylogenetic lineages could be identified in accordance with their geographical localization and species of origin. These lineages diverged mostly by the accumulation of synonymous mutations. Stabilizing selection, possibly related to host specificity, limits amino acid sequence and antigenic drift. Analyses were also performed either on the highly variable 400-base region coding for the amino terminus of the N protein or on the 93-base noncoding region corresponding to the 3′ end of the N mRNA, the intergenic N-phosphoprotein (M1) region, and the 5′ end of the M1 mRNA.

These shorter nucleotide sequences were shown to provide phylogenetic data suitable for the completion of large epidemiological studies, but with less robustness. This latter noncoding sequence, despite a 3.1 times higher mutation rate than its adjacent coding N gene, followed a parallel evolutionary pattern.

## 16. Recent discoveries:

### 1-Transmission of Rabies Virus from an Organ Donor to Four Transplant Recipients

Rabies is an acute encephalitis caused by viruses in the genus Lyssavirus, family Rhabdoviridae, that is nearly uniformly fatal in unvaccinated hosts. Although the virus is present in animal reservoirs, infection in humans is rare in the United States, with only two cases reported in 2003 and no more than six cases reported in any year in the past decade.The primary mode of transmission is through the bite of an infected animal, most commonly a bat in the United States.Although transmission of rabies virus from corneal transplants has previously been described,to our knowledge, no cases ascribed to organ or vascular-tissue transplants have been reported.

### 2-The immune response to rabies virus infection and vaccination.

Infection with rabies virus causes encephalitis in humans that has a case fatality rate of almost 100%. This inability to resolve infection is surprising since both pre-exposure vaccination and, if given promptly, post-exposure vaccination is highly effective at preventing encephalitic disease. The principal immunological correlate of protection produced by vaccination is neutralizing antibody. T-helper cells contribute to the development of immunity whereas cytotoxic T cells do not appear to play a role in protection and may actually be detrimental to the host. One reason for a failure to protect in humans may be the poor immunological response the virus provokes, despite the period between exposure to virus and the development of disease being measured in months. Few individuals have measurable neutralizing antibody on presentation with disease, although in many cases this develops as symptoms become more severe.

Furthermore, when antibody is detected in serum it rarely appears in cerebrospinal fluid suggesting limited penetration into the CNS, the site where it is most needed. The role of the modest mononuclear cell infiltrate into the brain parenchyma is unclear. Some studies suggest the virus can suppress cell-mediated immunity early during the infection although there is little mechanistic evidence to support this beyond suppression of intracellular interferon production by the viral phosphoprotein. In contrast, levels of antibody in the CNS correlate to the peak virus production within the CNS. Here we review the current understanding of immune responses to rabies infection and vaccination against this disease. This article identifies a need to understand how rabies antigens are initially presented and how this can influence the subsequent development of antibody responses. This could help identify ways in which the response to prophylactic vaccination can be enhanced and how the natural immune response to infection can be boosted to combat neuroinvasion.



## 3-Laboratory diagnostics in dog-mediated rabies-an overview of performance and a proposed strategy for various settings.

Dog-mediated rabies diagnosis in humans and animals has greatly benefited from technical advances in the laboratory setting. Approaches to diagnosis now include detection of rabies virus (RABV), of RABV RNA or of RABV antigens. These assays are an important tool in the current effort for the global elimination of dog-mediated rabies. We review assays available for use in laboratories and their strong or weaker points, which vary with the types of sample analyzed. Depending on the setting, however, the public health objectives and use of RABV diagnosis in the field will also vary. In non-endemic settings, detection of all introduced or emergent animal or human cases justifies exhaustive testing. In dog RABV-endemic settings such as rural areas of developing countries where most cases occur, availability or access to testing may be severely constrained. Therefore, we discuss issues and propose a strategy to prioritize testing while access to rabies testing in the resource-poor, highly endemic setting is improved.

As the epidemiological situation of rabies in a country evolves, the strategy should shift from that of an endemic setting to one more suited when rabies incidence decrease due to the implementation of efficient control measures and when nearing the target of dog-mediated rabieselimination.



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