Rabies

An acute, central nervous system infection, characterized by CNS irritation, followed by paralysis and death. Rabies, a member of the Rhabdovirus family, is caused by the virus *Neurotropic lyssavirus*. It is a single-stranded, neurotropic, negative-sense RNA virus which encodes 5 proteins: a glycoprotein, a nucleoprotein, and three others. The mature virus has a bullet shape, a protein coat, and a lipid envelope. The outer surface of the virus is covered with thumblike glycoprotein projections 5-10 nm long and 3 nm in diameter. The virus averages approximately 780 nm in length.

**Rabies virus:**

The virus is found in the salivary glands and CNS of infected, warm-blooded animals (including humans). Worldwide, where approximately 50,000 people die each year from rabies, the highest risk of human infection is from rabid dogs. In the U.S., where domestic dogs are usually vaccinated, only occasional cases of human rabies usually occur, usually from the bites of wild animals.

The word rabies comes from the Sanskrit *rabhas*, which means, "to do violence." Rabies is thought to be the oldest communicable disease of humans.

There are few viruses as successful as rabies virus. It has survived for millennia in most parts of the world. It can replicate in all warm-blooded animals, where the resultant disease is almost invariably fatal. The virus ensures its own survival by causing the afflicted host to find another host for it. It was thought that a single virus type is responsible for all the rabies diseases. It was not until the discovery of rabies-related viruses such as the Lagos bat, Mokola and Duvenhage viruses in the Lyassavirus genus, was this view seriously challenged.
Geographic distribution of (A) human (including 5 formalin-fixed samples not shown in Figure 1) and (B) terrestrial mammal cases identified by rabies virus variant isolated.
Properties

member of the Lyassavirus of the Rhabdoviridae
ssRNA enveloped virus, helical symmetry
infectivity destroyed by lipid solvents
6-7 nm spike projections are present on the envelope
characteristic bullet-shaped appearance
virion 130-240nm * 80nm
-ve stranded RNA codes for 5 proteins; G, M, N, L, S
Exceeding wide range of hosts

Electronmicrograph and schematic diagram of rabies virus particles
(Source: CDC)
Rabies virus have been adapted to growth in a wide variety of primary and continuous cell systems, not only from cells of warm blooded animals but also ones of poikilothermic vertebrate origin. The virus is grown in human diploid cells for the purpose of producing a vaccine. It has also been adapted to growth in avian embryos. Monoclonal and polyclonal studies of rabies isolates from many animal studies worldwide have led to the following classification of the rabies group of Rhabdoviridae, genus Lyssavirus. Lagos bat, Mokola and Duvenhage viruses have been isolated from various animals in a number of African countries. Their natural hosts are unknown and are thought to be bats or rodents. EBL-1 and EBL-2 have been isolated from European bats. Duvenhage and EBL-2 viruses have been associated with human infection that resulted in a rabies-like illness and death.

Serotype 1 prototype rabies virus
Serotype 2 Lagos bat
Serotype 3 Mokola
Serotype 4 Duvenhage
Serotype 5 EBL-1 ((European bat lyssaviruses)
Serotype 6 EBL-2

Epidemiology
Rabies is a zoonosis which is prevalent in wildlife. The main animals involved differs from continent to continent.

Europe fox, bats
Middle East wolf, dog
Asia dog
Africa dog, mongoose, antelope
N America foxes, skunks, raccoons, insectivorous bats
S America dog, vampire bats
Increasingly, bats have been recognized as important reservoirs of rabies in many parts of the world. Cases of rabies in humans have been reported after bites by bats. Rabies virus had been shown to infect all mammals so far tested. Dogs, cats and cattle are particularly susceptible. Skunks, bats, foxes, squirrels, badgers, raccoons and mongooses are the principle wildlife host. Foxes are the main carriers in Europe. Birds have also been shown to be susceptible to infection. Compartmentation occurs with rabies, so that the disease is reported in one major host species in certain geographical areas while it is reported less frequently in the same species in other areas of endemic rabies.

**Pathogenesis**

The commonest mode of transmission in man is by the bite of a rabid animal or the contamination of scratch wounds by virus-infected saliva. However, other routes have been implicated in the past, such as through mucous membranes of the mouth, conjunctiva, anus and genitalia. Infection by aerosol transmission had been demonstrated in experimental animals and has been implicated in human infection in rabies-infected bat caverns and in several laboratory accidents. Man to man transmission by transplantation of infected corneas were reported in 5 instances. Rabies is an acute infection of the CNS which is almost invariably fatal. The virus is similar to VSV of cattle. Following inoculation, the virus replicates in the striated or connective tissue at the site of inoculation and enters the peripheral nerves through the neuromuscular junction. It then spreads to the CNS in the endoneurium of the Schwann cells. Terminally, there is widespread CNS involvement but few neurons infected with the virus show structural abnormalities. The nature of the profound disorder is still not understood.
**Life cycle**

After receptor binding, rabies virus enters its host cells through the endosomal transport pathway. Inside the endosome, the low pH value induces the membrane fusion process, thus enabling the viral genome to reach the cytosol. Both processes, receptor binding and membrane fusion, are catalyzed by the glycoprotein G which plays a critical role in pathogenesis (mutant virus without G proteins cannot propagate).

The next step after entry is the transcription of the viral genome by the P-L polymerase (P is an essential cofactor for the L polymerase) in order to make new viral protein. The viral polymerase can only recognize ribonucleoprotein and cannot use free RNA as template. Transcription is regulated by cis-acting sequences on the virus genome and by protein M which is not only essential for virus budding but also regulates the fraction of mRNA production to replication. Later in infection, the activity of the polymerase switches to replication in order to produce full-length positive-strand RNA copies. These complementary RNAs are used as templates to make new negative-strand RNA genomes. They are packaged together with protein N to form ribonucleoprotein which then can form new viruses.
Clinical Features

The incubation period is highly variable, ranging from 7 days to several years. It depends on several factors such as:

1. Dose of inoculum
2. The severity of the wound
3. The length of the neural path from the wound to the brain e.g. wounds on the face have a shorter incubation period than wounds in the leg.
The illness begins with a non-specific prodrome period, comprising of fever, malaise, anorexia, N+V, sore throat, myalgia and headache. The patient may exhibit irritability and abnormal sensations around the wound. The prodrome is followed by one of two basic clinical patterns: the more common "furious" form characterized by hyperexcitability, spasms and hydrophobia; or "dumb" rabies featuring an ascending paralysis. Survival tends to be longer for patients with "dumb" rabies than those with "furious" rabies.

Complications involving the Cardiovascular System, CNS, and the respiratory systems eventually develop and contribute to death. Cardiac dysrhythmias of all types occur and respiratory disturbances occur in all cases. Raised intracranial pressure contributes to the decreased level of consciousness and to focal convulsions. Other CNS complications include disturbances of thermoregulation, diabetes insipidus, autonomic dysfunction and convulsions. The differential diagnosis of rabies includes tetanus, poliomyelitis, Guillain-Barre syndrome, viral encephalitis and poisonings and drugs.
Figure 1. The infectious path of rabies virus

1. Raccoon is bitten by a rabid animal.

2. Rabies virus enters the raccoon through infected saliva.

3. Rabies virus spreads through the nerves to the spinal cord and brain.

4. The virus incubates in raccoon’s body for approximately 3-12 weeks. The raccoon has no signs of illness during this time.

5. When it reaches the brain, the virus multiplies rapidly, passes to the salivary glands, and the raccoon begins to show signs of disease.

6. The infected animal usually dies within 7 days of becoming sick.
Laboratory Diagnosis

The diagnosis of animal and human rabies can be made by 4 methods: (1) histopathology (2) virus cultivation (3) Serology (4) virus antigen detection. Although each of the first 3 methods have distinct advantages, none provide a rapid definitive diagnosis.

1. **Histopathology** - Negri bodies are pathognomonic of rabies. However, Negri bodies are only present in 71% of cases.

2. **Virus cultivation** - The most definitive means of diagnosis is by virus cultivation from infected tissue. Tissue culture lines, such as WI-38, BHK-21, or CER. Since rabiesvirus induce minimal CPE, IF is routinely used to detect the presence of rabiesvirus Ag in the tissue culture. The more commonly used method for virus isolation is by the inoculation of saliva, salivary gland tissue and brain tissue.
intracerebrally into infant mice. The mice should develop paralysis and death within 28 days. Upon death, the brains are examined for the presence of the virus by immunofluorescence.

3. **Serology** - circulating antibodies appear slowly in the course of infection but they are usually present by the time of onset of clinical symptoms. The most commonly used serological tests were the mouse infection neutralization test (MNT) or the rapid fluorescent focus inhibition test (RFFIT). These tests have now been largely superseded by EIAs. Serology had been reported to be the most useful method for the diagnosis of rabies.

4. **Rapid virus antigen detection** - in recent years, virus antigen detection by IF had become widely used. The potentially infected tissue is incubated with fluorescein-labeled antibody. The cells are examined by fluorescent microscopy for the presence of fluorescent intracytoplasmic inclusions. The specimens which are usually used are corneal impressions (obtained by gently abrading the cornea with a microscopic slide) or neck skin biopsy (the cells examined are the sensory nerves). In an American series, IF of corneal impressions or neck skin impressions was diagnostic only in 50% of cases early in the course of the clinical illness.

![Negri body in body of neuron and positive IF test for rabies antigen](Source: CDC)

**Susceptibility to rabies infection**

H<sub>III</sub> and L<sub>III</sub> mice were equally susceptible to the experimental rabies infection, showing 39 and 45% of mortality, respectively, and mean survival time of 12
days (Table 1). Nevertheless, as shown in Figure 1, L\textsubscript{III} mice were more permissible to viral replication in the central nervous system than H\textsubscript{III}. Titers as high as $10^{3.5}$ and $10^{3.3}$ LD\textsubscript{50}/0.03ml were found in brains of L\textsubscript{III} mice on days 5 and 6 of infection, respectively, in comparison to $10^{1.1}$ and $10^{2.3}$ LD\textsubscript{50}/0.03ml found in brains of H\textsubscript{III} mice on the same days. The H\textsubscript{III}-L\textsubscript{III} mice interline differences in virus replication were 1000 and 80 folds on days 5 and 6, respectively.

<table>
<thead>
<tr>
<th>Mice</th>
<th>Mortality/Total</th>
<th>Mean survival time [x±s] (days)</th>
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<tbody>
<tr>
<td>H\textsubscript{III}</td>
<td>39/46</td>
<td>12.0±7.0</td>
</tr>
<tr>
<td>L\textsubscript{III}</td>
<td>45/49</td>
<td>12.0±5.8</td>
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Table 1: Mortality of H\textsubscript{III} and L\textsubscript{III} genetically selected mice after PV rabies virus strain infection. Mice were intramuscularly infected with PV rabies virus (titer of $10^{4.6}$ LD\textsubscript{50}/0.03ml) and observed for 30 days.
Isotype profiles

The isotype detected in sera of mice after different times of infection showed that H\textsubscript{III} mice were capable of regularly producing a higher amount of IgG2a when compared to L\textsubscript{III} mice (Figure 2). Interline differences of at least 25 folds were observed throughout the infection period. H\textsubscript{III} mice were also capable of producing IgE in high amounts from the beginning to the end of the infection and L\textsubscript{III} mice showed at the initial phase a quite low amount of IgE, which rose during a late period of the infection. Thus, interline differences for IgE syntheses were highly significant at the beginning of the infection only.
Figure 2. Kinetics of IgG2a (a) and IgE (b) synthesis in H\textsubscript{III} and L\textsubscript{III} responder mice during PV rabies virus strain infection. Animals were intramuscularly infected with 0.1 ml of PV rabies virus (titer of $10^{4.9}$ LD\textsubscript{50}/0.03ml); sera were collected on days 3, 6, 12, 22 and 30 and the isotype profile of antibodies were determined by ELISA.
Management and Prevention

Once rabies is established, there is nothing much that could be done except intensive supportive care. To date, only 2 persons with proven rabies have survived, and 3 persons with probable rabies. However, one survivor was left with severe neurological sequelae and all 3 who recovered were vaccinated beforehand. Numerous antiviral agents have been tried with no success.

1. Postexposure prophylaxis

In cases of animal bites, dogs and cats in a rabies endemic area should be held for 10 days for observation. If signs develop, they should be killed and their tissue examined in the laboratory. Wild animals are not observed but if captured, the animal should be killed and examined. The essential components of postexposure prophylaxis are the local treatment of wounds and active and passive immunization.

a. **Wound treatment** - surgical debridement should be carried out. The wound should not be sutured up. Experimentally, the incidence of rabies in animals can be reduced by local treatment alone.

b. **Passive immunization** - human rabies immunoglobulin around the area of the wound; to be supplemented with an i.m. dose to confer short term protection. There is convincing evidence that combined treatment with rabies immunoglobulin and active immunization is much more effective than active immunization alone. Equine rabies immunoglobulin (ERIG) is available in many countries and is considerably cheaper than HRIG.

c. **Active immunization** - the human diploid cell vaccine is the best preparation available. The vaccine is usually administered into the deltoid region, and 5 doses are usually given.

2. Preexposure prophylaxis

Persons who are regularly at high risk of exposure, such as vets, laboratory workers, animal handlers and wildlife officers should be considered for preexposure prophylaxis by active immunization with the cell culture vaccine. Immunization normally consists of 3 doses of vaccine. Antibody can be demonstrated in the sera of virtually 100% of those vaccinated if the diploid cell culture vaccine is used. Booster doses should be offered to persons at continuing risk every one to three years. Local treatment of wounds should always be carried out in exposed persons who have been vaccinated previously. The WHO expert
committee considers that local infiltration with antiserum is optional and systemic passive immunization contraindicated.

3. Rabies Vaccines

Several types of live attenuated vaccines are available for use in animals, but they are considered to be unsuitable for humans. The vaccines which are available for humans are present are inactivated whole virus vaccines.

a. **Nervous tissue preparation** - this consisted of a 5% suspension of infected animal nervous tissue which had been inactivated (eg. the Semple vaccine was derived from phenol-inactivated infected rabbit brain). These preparations are now out of date as they were associated with the rare complication of demyelinating allergic encephalitis. This appears to be related to myelin basic protein in the vaccine. This complication was shown to occur in 4.6 case for 1000 persons vaccinated by the Semple vaccine. The case-fatality proportion was 3.13%. The Semple vaccine is still used in some developing countries. A suckling mouse brain vaccine is used in some Central and S.American countries.

b. **Duck Embryo Vaccine** - this vaccine strain is grown in embryonated duck eggs and is inactivated with B-propiolactone. This vaccine has a lower risk of allergic encephalitis. However, it is considerably less immunogenic and does have minor side effects. Almost all vaccinees experience local reactions, 33% have constitutional symptoms such as fever, malaise, myalgia, and generalized lymphadenopathy.

c. **Human Diploid Cell Vaccine (HDCV)** - HDCV was introduced in 1978. It is a grown on WI-38 (U.S.) or MRC-5 (Europe) cells. The vaccine is highly effective, in several studies, antibodies have been demonstrated in 100% of all recipients. Serious adverse reactions to HDCV are extremely rare. However, the vaccine is very expensive ($100 for 6 doses), as human cell cultures are more difficult to handle than other animal cell culture systems. 5 or 6 doses of the vaccine is normally i.m. However, several studies suggest than smaller intradermal doses of HDCV may be as effective and thus it may be considered for use in poor developing countries.

Another inactivated vaccine is widely used in China. It is derived from virus grown in primary hamster kidney cells and cost less than the other diploid cell culture vaccines. The vaccine had been shown to be as
effective as HDCV. Efforts are being made to use other inexpensive cell culture systems such as VERO cells.

4. Failure of prophylaxis

HDCV has been used to treat many thousands of people exposed to possible rabid animals in the past 12 years and its efficacy has been proven. At least 16 people treated with HDCV after exposure have died of rabies. All of the patients had major exposures and in the majority, the incubation period was short, 21 days or less. Treatment was frequently not started promptly within 24 hours and only half received combined serum and vaccine. At least one person has died despite optimum treatment.

Rabies Control

Urban - canine rabies accounts for more than 99% of all human rabies cases and over 90% of all human post-exposure treatments worldwide. In the past, the Scandinavian countries were able to rid themselves of rabies by sanitary control alone, which included stray dog control. Other countries, such as the UK, have used these techniques allied with quarantine and/or vaccination to eradicate and then maintain freedom from the disease. Currently, the importation of mammals into the UK is controlled by the Rabies order. It applies to a wide range of mammals but livestock including horses, which are covered by separate regulations. The animals must be vaccinated on arrival. Effective animal vaccines are available.

Wildlife - canine rabies can be controlled because in general, dogs live in close association with man and are therefore within physical reach. An attempt was made to vaccinate foxes in an attempt to create an immune barrier at the entrance to the Rhone valley in 1978. The live attenuated virus was contained in small plastic blister packages fixed under the skin of chicken heads used as a bait, and 4050 of these were distributed over an area of 335km². With continued field trials, Switzerland has been freed of rabies. Other field trials are being set up.
Reference

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