

RABIES REVIEW: IMMUNOPATHOLOGY, CLINICAL ASPECTS AND TREATMENT

CONSALES C. A. (1), BOLZAN V. L. (1)

(1) Pasteur Institute, São Paulo, Brazil.

ABSTRACT: Among the diseases of viral origin, rabies is unique in its distribution and range of victims since it can afflict all warm-blooded animals. The interaction between the virus and the host population has facilitated the survival of the disease. The rabies virus (RV) has not changed in any significant way and has been capable of taking advantage of conditions suited to the continuance of rabies. Infection by RV is invariably lethal in the absence of protective immune response which, however, can contribute to the pathogenesis of rabies. Proinflammatory cytokines might affect, directly or indirectly, the levels of neurotrophins, growth factors, neurotransmitters and neurotoxins in the brain by activating glia, neurons, and vascular and immune cells. Although understanding of the bases for neuronal dysfunction and neuronal death during RV infection has progressed, no fundamental abnormality has been identified so far.

KEY WORDS: rabies, immunopathology, clinical aspects, treatment.

CONFLICTS OF INTEREST: There is no conflict.

CORRESPONDENCE TO:

CLEIDE ASCHENBRENNER CONSALES, Instituto Pasteur, Av. Paulista, 393, 01311-000, São Paulo, SP, Brasil. Phone: +55 11 3288 0088 ext. 108/116.
Email: cleide135@yahoo.com.br.

ETIOLOGY

Lyssavirus is one of the seven genera that form the family Rhabdoviridae, within the order Mononegavirales. It comprises classical rabies virus (RABV; genotype 1), Lagos bat virus (LBV; genotype 2), Mokola virus (MKV; genotype 3), Duvenhage virus (DV; genotype 4), European bat lyssavirus 1 (EBLV-1; genotype 5), European bat lyssavirus 2 (EBLV-2; genotype 6), and Australian bat lyssavirus (ABLV; genotype 7). Recently, four additional viruses, isolated from insectivorous bats, have been proposed as new members of *Lyssavirus* genus: Aravan virus (AV), Khujand virus (KV), Irkut virus (IV), and West Caucasian bat virus (WCBV) (10, 33, 60, 97).

Rabies viruses are bullet-shaped structures of about 75 nm X 200 nm and can be roughly divided into a structural and a functional unit: the viral envelope and the ribonucleocapsid core. Five monocistronic genes relate to five viral proteins: The N gene codes for a nucleoprotein that encapsulates the viral and the unsegmented negative-stranded RNA. The P gene produces a phosphoprotein, which is important not only for transcription and replication but also for interactions with cellular protein components during axoplasmic transport. The M gene codes for a matrix protein. The G gene produces a single transmembrane glycoprotein which is assembled as a trimeric spike. This glycoprotein is responsible for the initial binding during infection of susceptible cells and is the only target for virus-neutralizing antibodies. The L gene encodes a polymerase for RNA synthesis (11, 81).

RABIES TRANSMISSION

The commonest way of rabies transmission is by the bite of an infected mammal (Figure 1) (2).

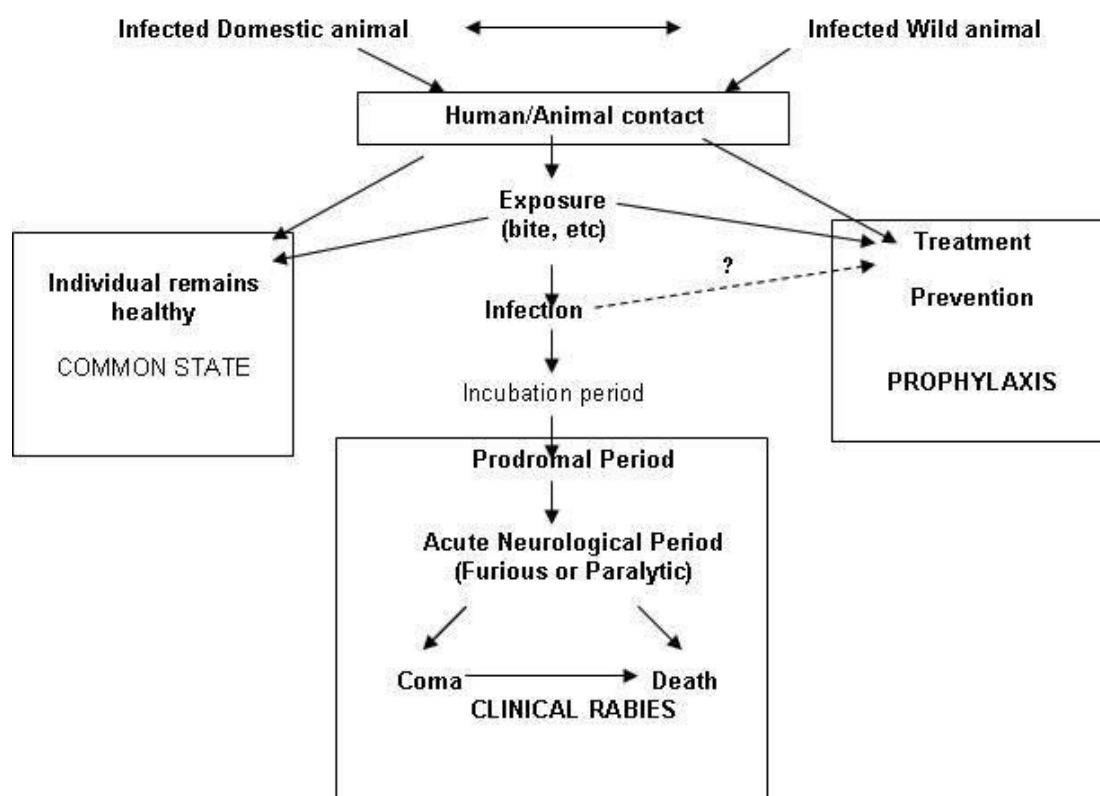


Figure 1: Course of rabies transmission. The first step is the transmission of the disease following exposure. The incubation period varies greatly. It ends when the virus begins to spread from the bite site to the surrounding peripheral nerves. Adapted from Baer *et al.*, (2).

Bites by rabid animals generally inoculate virus-laden saliva through the skin into muscle and subcutaneous tissues (94). Other inoculation routes are rare. Rabies virus entry occurs through wounds or direct contact with mucosal surfaces. The virus cannot cross intact skin. The risk of rabies infection by a bite (5%-80%) is at least 50 times greater than that by a scratch (0.1%-1%) (45).

Mortality after untreated bites by rabid dogs ranges from 38% to 57% and depends on the severity and location of the wound as well as on the presumed virus concentration in the saliva (45, 94).

Bat virus might be more infectious when inoculated superficially into the epidermis since it replicates more rapidly in non-neuronal cells and at lower temperatures than do dog rabies viruses. Percutaneous infection probably occurs during unnoticed skin contact, which may result in a minute bite. The route of viral entry into epithelial nerves and eventually into the central nervous system (CNS) is unknown (23, 65).

Inhalation of aerosolized RV occurred accidentally in laboratories of vaccine production (102) or in caves inhabited by numerous infected bats (36). Infection through the digestive tract has also been reported (16, 19).

Contact with animal vaccines may be significant when attenuated vaccine is used. In these situations, rabies prophylaxis is necessary. The handling and skinning of infected carcasses can be of risk for workers in refrigeration plants and butchers' shops, and veterinarians.

The contact with infected people could be a potential risk for their relatives and health workers when unprotected direct contact with secretions from a patient containing viable virus occurs (30, 44).

There are many reports of organ transplantation involved in the transmission of rabies. The most frequent cases have been observed in corneal transplantation (53). The most recent case reported was of a German patient and occurred in 2005 (43, 54).

In 2004, the Centers for Disease Control (CDC) in the United States confirmed the first case of rabies transmission through solid organ transplantation by testing autopsy samples after the death of 4 patients who received organ transplants (two kidney receptors, one liver receptor, and one receptor of an arterial segment) from the same donor. Subsequently, it was learned that a bat had bitten the donor (83). Three other cases of rabies-related organ transplantation were reported in Germany in 2005. These three patients received lung, kidney and kidney/pancreas transplants following the donor's death (43).

Before transplantation, the donor (if possible) and his relatives and friends should be questioned as to any history of physical contact with bats or bites by them or other mammals anywhere in the world. Patients with such a history should not be accepted as donors, even if post-exposure prophylaxis was carried out. There are no suitable screening tests to distinguish whether potential donors are infected. It has been recommended that donors, particularly those with neurological symptoms, should be screened for rabies (24).

So far, cases of rabies infection via blood transfusion have not been reported and rabies viraemia has not been demonstrated in animals or men. The virus seems to be strictly intra-neuronal during the incubation phase of the disease. There are no evidences that apparently healthy blood donors can transmit rabies, even if they incubate the infection. The one-year deferral of donation following post-exposure

rabies prophylaxis remains a reasonable precaution (54). In Brazil, the Sanitary Surveillance Center (ANVISA) has decided that the interval between rabies post-exposure treatment and blood donation should be one year and that for rabies pre-exposure vaccination, 4 weeks (12).

The RV migrates along peripheral nerves (via the fast axonal transport system) towards the CNS at about 50-100 mm per day. This movement is strictly retrograde, which indicates infection is via sensory and motor nerves. Invasion of the CNS by the RV glycoprotein may not occur via the sensory nerve pathway (Figure 2) (64).

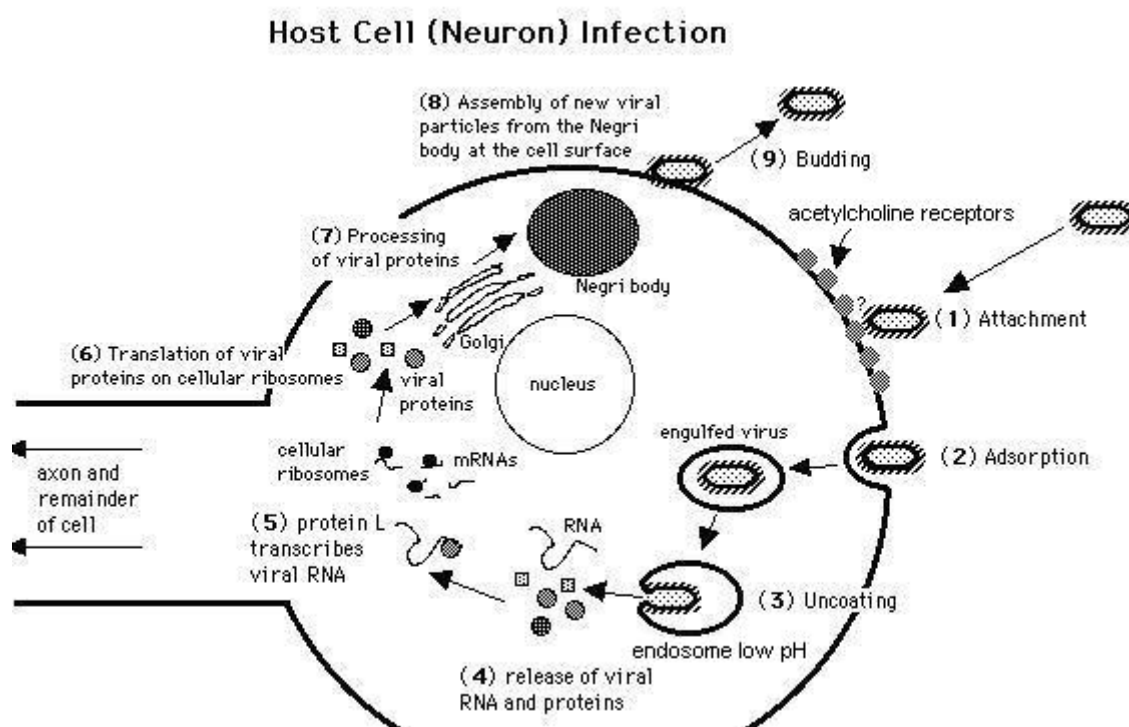


Figure 2: Cycle of viral infection and replication. Adapted from Mazarakis *et al.* (64).

The incubation period or eclipse phase varies from 2 weeks to 6 years (average: 2 to 3 months) according to the amount of viral inoculum and the inoculation site. Bites on the head, face, neck and hands, particularly together with bleeding, offer the highest risk and are generally associated with a shorter incubation period. The RV can stay in the muscle tissue for long periods and in certain circumstances, its long persistence may provide an opportunity for host immune clearance and post-exposure treatment (45, 94).

IMMUNOPATHOLOGY

One of the most surprising aspects of rabies immunopathology is the almost complete lack of an inflammatory response within the CNS characterized by perivascular cuffing with mononuclear cells, local gliosis and neuronophagia. Lesions occur in most areas of the CNS but are frequently more severe in the brainstem. This contrasts with other viral diseases of the CNS, in which inflammation is the major pathological characteristic. These observations suggest that neuronal dysfunction, rather than neuronal death, is probably responsible for the fatal outcome of rabies under normal conditions.

Contrasting with the protective role of immunity, immune mechanisms (even those generally thought to be protective) often have pathological attributes depending on the extent of the infection when immune effectors come into play. This is the case of reactions occurring in the nervous tissue. The anti-RV immune response elicited after exposure to the virus can prevent rabies, indicating that the pathogenicity of a particular RV strain may depend on its capability to spread without inducing a protective immune response. The virus neurotropism is probably a key element in this process, as CNS tissues are naturally sequestered from the immune system. Other aspects of the RV nature clearly have a major impact on the virus capacity to induce a protective antiviral immune response, which has been extensively studied (48, 55,103).

Viral glycoprotein is the target for most RV-neutralizing antibodies and has been a strong inducer of apoptosis in infected cells, which is evidently an immunogenic process in rabies (28, 79). If an immune response to rabies develops either inappropriately or after the infection has spread sufficiently, an extensive immune-mediated damage of CNS tissues could be expected (49).

Researches using mice showed that when the infection route were the extremities such as the mouse footpad, peripheral nerve damage was more likely to occur leading to paralytic rabies with some prospects of survival; differently from lethal encephalitic rabies following intracerebral infection by the same strain (84).

The possibility that immunity contributes to the pathogenesis of rabies is also supported by what has been termed the "early death" phenomenon, in which inadequately immunized mice may die more rapidly of rabies than unvaccinated controls. Insights into the contribution of the immune response to the accelerated death of RV-infected animals result from classic experiments in which

immunocompromised mice survived RV infection for longer periods than normal animals. However, immunosuppression can increase the overall mortality. Clinical signs of rabies and death were accelerated in immunosuppressed mice when immune serum was administered or the immune response to rabies re-appeared, suggesting that an antiviral antibody can contribute to rabies immunopathogenesis (78, 82).

Pathological aspects of the immune reactivity in the CNS are sufficient to explain the contribution of immune responses to rabies pathogenesis. However, there is another potential contribution that has not been examined in great detail: elements of either the adaptive or the innate immune response may directly or indirectly stimulate the virus replication and dissemination. Evidences that cells of the immune system are infected (90) suggest that such cells might transport the virus from poorly to highly innervated areas such as the lymph nodes, facilitating the RV spread to the CNS. This could explain how the RV can enter the nervous system when introduced via organ transplants, as has recently occurred (15).

Cells of the immune and central nervous systems may share similar functions: secretion of immunoregulatory cytokines, response to cytokines, and antigen presentation. These properties allow physical contact between the two systems, i.e. microglia and/or astrocytes presenting antigens to T cells, and communication with soluble factors such as cytokines. There is a complex circuit of interactions mediated by cytokines, especially during lymphoid/mononuclear cell infiltration into the CNS. Secretion of IFN- γ by infiltrating activated T cells could initially induce astrocytes and microglia to express class I and class II major histocompatibility complex (MHC) antigens, and to prime these class I and class II MHC antigens for subsequent cytokine production. The activation of astrocytes and microglia may contribute to the initiation and/or propagation of intracerebral immune and inflammatory responses. A number of mediators present in the CNS, such as prostaglandin, cytokines IFN- α , IFN- β and IFN- γ , and endogenous neuropeptides like norepinephrine and vasointestinal peptides, can suppress these responses by inhibiting both class I and class II MHC expression and cytokine production by glial cells. The induction and ultimate downregulation of the immune response and cytokine production within the CNS is dependent on: a dynamic interaction between a variety of peripheral immune and CNS cells; the activation status of these cells; the presence of cytokines with pleiotropic effects (IFN- γ , IL-1, IL-6, IFN- α , and others); the concentration and

location of these cytokines in the CNS; and the temporal sequence in which a particular cell responds to the cytokines (6).

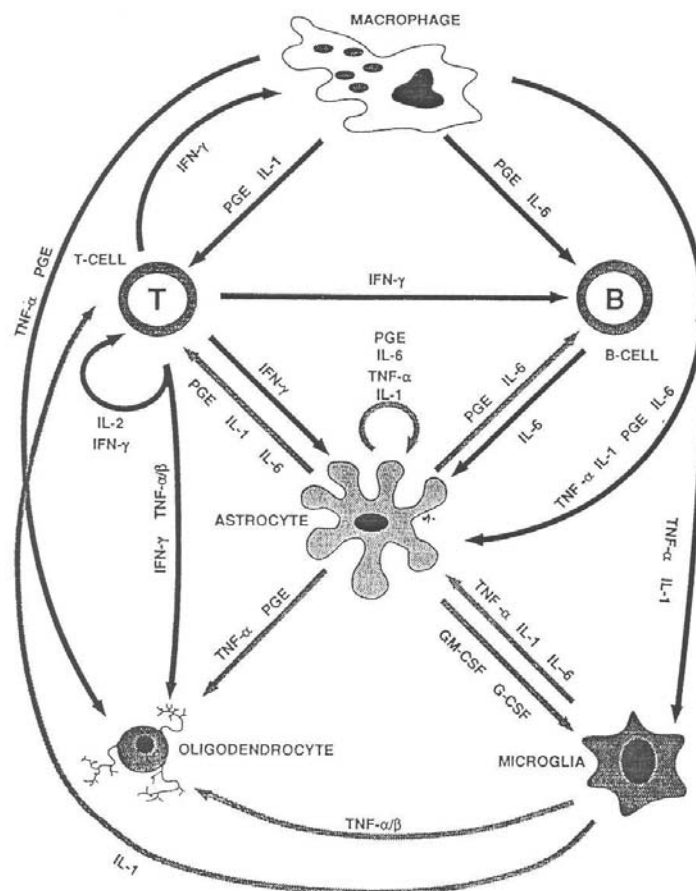


Figure 3: Potential interactions between cells of the immune system (T and B cells, and macrophages) and those of the central nervous system (astrocytes, microglia and oligodendrocytes). Solid arrow: cytokine effects mediated by immune system cells. Stippled arrows: cytokine effects mediated by nervous system cells. Adapted from Benveniste (6).

The cause of functional alterations in RV-infected neurons is not clear yet. Experimental studies have investigated possible abnormalities in the neurotransmission involving acetylcholine (25, 50, 92). Defective neurotransmission involving neurotransmitters other than acetylcholine could be important in the pathogenesis of rabies, and both serotonin and gamma-aminobutyric acid (GABA) have been studied (14).

Neurotransmission essentially involves four steps, namely: synthesis, storage, release and interaction with postsynaptic receptors, and intracellular events (71). Measurement of one, some, or all of the aspects of neurotransmission has been used as an indicator of neuronal activity (35). Koprowski *et al.* have hypothesized

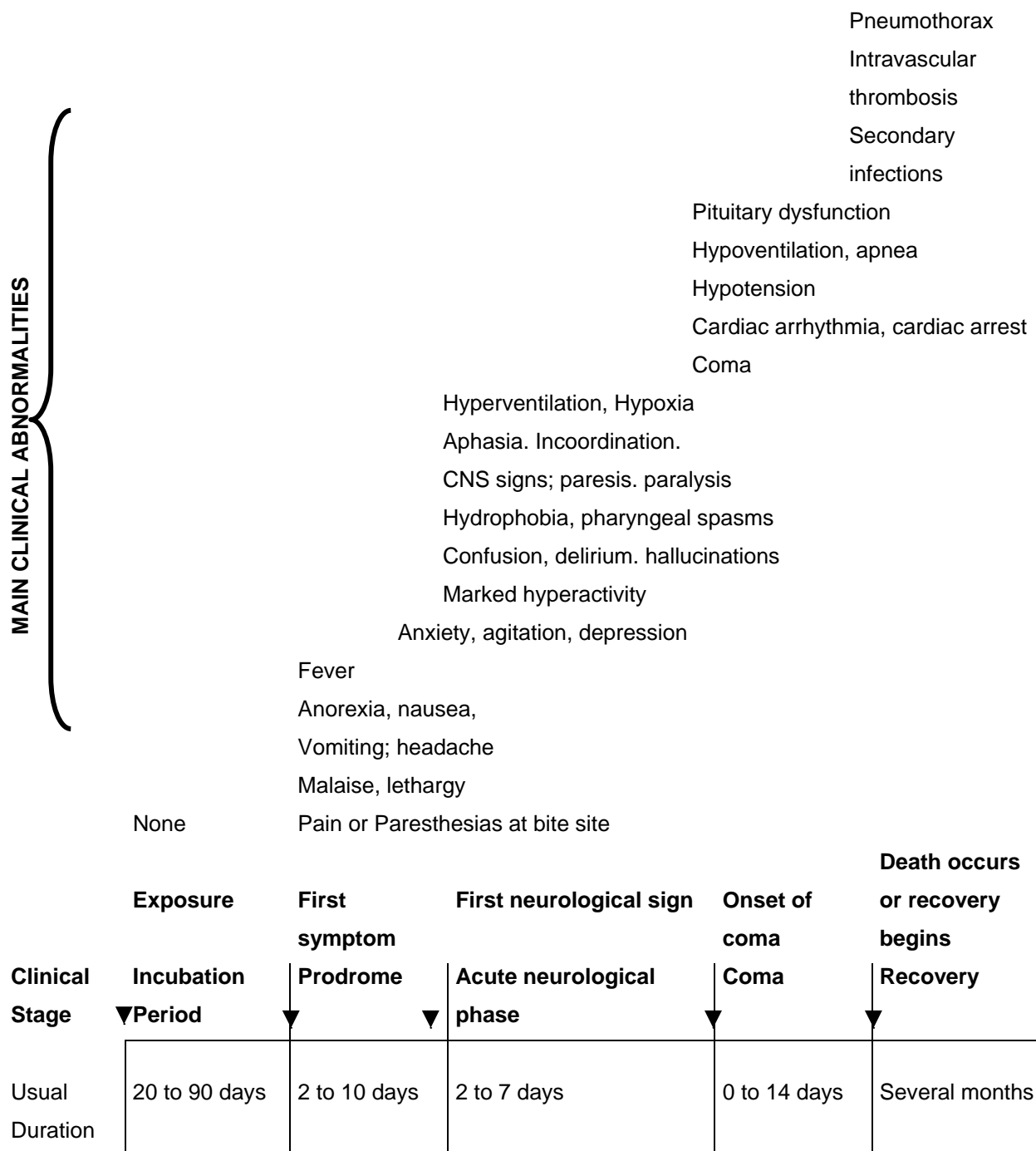
that the nitric oxide neurotoxicity may mediate neuronal dysfunction in rabies and induction of inducible nitric oxide synthase (iNOS) mRNA in mice experimentally infected with street RV (59, 93). Neurotropic viruses may cause cell death through either apoptosis or necrosis. Apoptosis depends on the synthesis of macromolecules and requires energy whereas necrosis is associated with energy failure (29, 40). Apoptosis is induced via both virus-dependent and cell-dependent mechanisms. In RV infection, complex mechanisms may be involved in the death or survival of neurons both *in vitro* and *in vivo* using different viral strains and inoculation routes. Both *in vitro* and *in vivo* observations demonstrated that apoptosis might be a protective rather than a pathogenic mechanism in RV infections because few pathogenic viruses induced more apoptosis than a higher number of pathogenic viruses both *in vitro* and *in vivo* using peripheral inoculation routes. Thus, preservation of the neurons and limitation of such network by inhibiting apoptosis and limiting inflammation and destruction of T cells that invade the CNS in response to the infection is crucial for the RV neuroinvasion and transmission to another animal (3, 79).

CLINICAL MANIFESTATIONS

Human rabies may manifest in encephalitic (furious) or paralytic (dumb) forms. The brainstem is involved in both clinical forms, although there are no clinical signs of dysfunction in this portion of the brain. Differences in the tropism at the inoculation site or in the CNS, in the dissemination route, or in the triggering of the immune cascade in the brainstem may account for clinical variations (45).

Classical signs of brain involvement include spasms in response to tactile, auditory, visual or olfactory stimuli (aerophobia and hydrophobia) alternating with periods of lucidity, agitation, confusion and signs of autonomic dysfunction. Spasms occur in almost all rabid patients in whom excitation is prominent. However, spontaneous inspiratory spasms usually occur continuously until death. Excitation is less evident in paralytic rabies, and phobic spasms appear in only 50% of these patients (97). Clinical features may be divided into 5 stages: incubation period, prodrome, acute neurological phase, coma, and death (Table 1).

Table 1: Natural history of rabies in humans. Hypothetical composite case. Not all clinical abnormalities are necessarily present in every case. Adapted from Fishbein and Robinson (32).



Non-classical symptoms can be observed in patients with bat-related rabies and consist of neuropathic pain, radicular pain, objective sensory or motor deficits, and choreiform movements of the bitten limb during the prodromal phase. Both focal brainstem signs and myoclonus are common. Localized signs like hemiparesis or hemisensory loss, ataxia, Hener's vertigo syndrome, convulsive and non-convulsive

seizures, and hallucinations are frequent. Myoclonus and hemichorea, agitation at night and calm during the day, repeated spontaneous ejaculation, paraphasia, facial and bulbar weakness with preserved arm strength, or bilateral weakness may occur. Patients do not present phobic spasms or autonomic hyperactivity. During coma, inspiratory spasms increase; in the paralytic form, weakness is intense. Viral involvement at the sinus or atrioventricular node, myocarditis as well as changes in the cardiac rhythm and function may happen. Coma precedes circulatory insufficiency, a prime cause of death. Hematemesis occurs in 30%-60% of patients approximately 6 hours before death (46). Renal dysfunction is secondary to dehydration (22).

RABIES DIAGNOSIS

Laboratory diagnosis of rabies in humans and animals is essential for timely post-exposure prophylaxis (45, 47). Rabies diagnosis may be carried out either *in vivo* or postmortem (Table 2) (94).

Table 2: Diagnosis of human rabies.

SPECIMEN	TEST	DETECTION
ante mortem		
skin punch biopsy; repeat until a diagnosis is obtained	FAT test on frozen section RT-PCR	antigen detection viral RNA virus isolation
saliva, tears, CSF; repeat until a diagnosis is obtained	Tissue culture Suckling mouse inoculation RT-PCR	virus isolation viral RNA antibody detection
serum	Unvaccinated; test immediately*	antibody detection
CSF	Vaccinated; save and compare a few days later test immediately with serum*	antibody detection antibody detection
postmortem		
brain needle necropsy** of two or more samples (brainstem and cerebellum)	FAT test of impressions smear RT-PCR suckling mouse inoculation	Antigen detection Viral RNA Viral isolation
retrospective diagnosis	enzyme methods	antigen detection in formalin-fixed tissue

FAT: Fluorescent antibody test

RT-PCR: Reverse transcription polymerase chain reaction

CSF: Cerebrospinal fluid

* In unvaccinated patients, rabies antibody generally appears in the second week of the disease. Rabies specific IgM has been detected in the serum and in some cases in the CSF at low concentration, but no earlier than IgG. High concentrations of antibody in the CSF have been considered diagnostic despite vaccination.

** Needle necropsy: with a Vim-Silverman's or other long biopsy needle.

Source: Warrell and Warrell, 2004 (94).

Serological tests may help but RV antibodies have been detected in only 20% of unvaccinated rabies patients tested 1-26 days after the onset of the disease. Antibodies appear in the cerebrospinal fluid later (47, 48).

Skin biopsy samples are usually taken from the nuchal area containing hair follicles with peripheral nerves. Examination of at least 20 sections is recommended to detect rabies nucleocapsid inclusions around the hair follicles. Sensitivity is 82% when the test is carried out within 4 days after the onset of the disease and 60% when it is performed between 5 and 8 days after infection (47).

Corneal imprint using fluorescent antibody technique can be carried out for detection of RV antigen. Results depend on the quality of the material sent to the laboratory. Sensitivity is around 42% and specificity, 100% (58).

Brain biopsy is not practicable, nor is it recommended for the diagnosis of rabies, but it could be of high sensitivity (47). False negative results may occur when biopsy of the frontal and temporal regions is carried out on the first day of the disease.

Laboratory tests of secretions and biological fluids such as saliva, spinal fluid, tears, and tissues may be used to diagnose rabies. The specimens should be stored at -20°C or below. A positive result is indicative of rabies but a negative result does not rule out the possibility of infection (97). Such specimens can be used to detect the RV using intracranial inoculation into mice or neuroblast cell cultures, and to detect the virus RNA using reverse transcription polymerase chain reaction. Serial samples should be tested since not all of them are positive due to the intermittent shedding of the virus (21, 45).

Molecular detection using polymerase chain reaction (PCR) and nucleic acid sequence-based amplification techniques shows the highest level of sensitivity but can produce false positive or false negative results and should only be used in combination with other conventional techniques. Virus identification using molecular techniques is of epidemiological importance. Studies on molecular virology have permitted the classification of lyssavirus in genotypes and demonstrated the virus isolation from a particular geographic area (9, 91, 96). Indirect immunofluorescence uses a panel of antinucleocapsid monoclonal antibodies for antigenic typing, which is provided by the Centers for Disease Control and Prevention (CDC), Atlanta, USA (97).

POSTMORTEM DIAGNOSIS

Brain tissue specimens are the preferred sampling for postmortem diagnosis in both humans and animals. In cases where brain tissue is not viable, other tissues may be of diagnostic value; also, other routes such as transorbital or trans-foramen magnum should be used to obtain brain tissue. To preserve tissues refrigeration, glycerin can be used. Dried smear of brain tissue can be kept on filter papers when safe transportation of the infected material is possible (97).

The amount of antigens has varied among different regions of the brain (7). The thalamus, pons and medulla can be considered the most reliable parts of the brain, as they were positive in all the specimens tested. The cerebellum, hippocampus and other parts of brain were negative in 4.5%, 4.9%, and 3.9%-11.1%, respectively, out of positive brains. The thalamus was positive in all specimens and had the most frequent prevalence (97.8%) of abundant antigens.

Antigen detection may be performed using the fluorescent antibody test (FAT), a rapid and sensitive method to diagnose rabies infection in animals and humans. This is the gold standard for rabies diagnosis. However, FAT can lead to a false negative result when bat or horse specimens are involved (61). Virus isolation may be necessary to confirm the results of antigen detection tests and for further characterization of the isolate using intracranial inoculation into mice or neuroblast cells. Molecular detection using PCR and other amplification techniques are not currently recommended for routine postmortem diagnosis of rabies (96).

DIFFERENTIAL DIAGNOSES

Differential diagnosis of rabies includes: encephalitis caused by arboviruses such as Japanese, Eastern equine and West Nile viruses, enteroviruses, Nepah virus, and Herpes virus; acute hepatic porphyria with neuropsychiatric disturbances and signs of autonomic dysfunction; substance abuse like alcohol withdrawal or delirium tremens; acute serotonin syndrome due to administration of serotonin uptake inhibitors; tetanus; Guillain-Barré syndrome; transverse myelitis; neuroparalytic accidents, when the patient is from a country where nerve-type vaccines are used; psychiatric disturbances; cerebrovascular accidents; epilepsy; poisoning by atropine-like compounds; poliomyelitis; and intracranial mass (31, 96, 97).

MANAGEMENT OF RABIES IN HUMANS

Efforts to prevent fatal outcome have failed, and no spontaneous recovery has been observed (96). Rabies in humans progresses to death five to seven days after the onset of symptoms. Medical management may prolong survival by 133 days (26). Treatment seems to be incapable of changing the evolution to death.

Treatment is basically to comfort the patient and his/her family. Rabies experts have recommended that the approach to rabies encephalitis management be only palliative. The following characteristics and resources could be considered favorable for an aggressive therapy: administration of any rabies vaccine before the clinical onset of rabies; manifestation of the disease at a very early stage, with minimal neurological symptoms or signs; previously good health and absence of chronic diseases; relatives who accept both the high probability of an unsuccessful outcome and the possibility of neurological disability in a rabies survivor; and access to appropriate resources and facilities (38).

Therapy should combine antiexcitatory and antiviral drugs as well as intense care while natural native immune response combats the viral infection (52); rabies vaccine at multiple sites by intradermal route (the intramuscular route takes at least one week to produce immunity and the intradermal route accelerates the immune response); rabies immunoglobulin (human or equine rabies immunoglobulin can lead to viral clearance); monoclonal antibodies (RV-neutralizing antibodies by intravenous and intrathecal route); ribavirin and amantadine (*in vitro* antiviral activity) (87); interferon-alpha (administered by intravenous and intrathecal route through the Ommaya reservoir); ketamine (an anesthetic agent that has anti-rabies activity by interacting with N-methyl-D-aspartate receptor antagonist) (62, 77); benzodiazepines and barbiturates (GABA-receptor agonists and induced therapeutic coma reduce the brain excitatory metabolism and autonomic reactivity). Corticosteroids should not be used (in mouse models, the use of corticosteroids increased the mortality rate and shortened the incubation period) (27). Severe edema associated with risk of brain herniation is rare in rabies patients, although this is a possible complication in intrathecal therapy using human rabies immunoglobulin. Therefore, administration of corticosteroids is not recommended for rabies therapy, except for the treatment of adrenocortical insufficiency. Corticosteroids may reduce the passage of therapeutic agents through the blood brain barrier (4).

Although rabies has been considered 100 per cent fatal, there are well-documented reports of animal survivals and six cases of human survival. Such patients received immunoprophylaxis before the onset of symptoms (39, 42). All these cases were of the paralytic type and diagnosed using indirect evidence of high titers of rabies specific antibodies in the cerebral spinal fluid; however, neither the virus nor the viral antigen was demonstrated (Table 3) (94).

Table 3: Human recovery from rabies encephalitis.

PATIENT AND REFERENCE	EXPOSURE	TREATMENT	INCUBATION PERIOD AND COMPLICATIONS	DIAGNOSIS	OUTCOME
9-year-old boy, Ohio, USA, 1970 (42)	Thumb bite by rabid big brown bat	Duck-embryo vaccine without rabies immunoglobulin on the following day	20 days. Encephalitis, paralysis, coma, cardiac arrhythmia	High concentration of antibodies in the CSF and serum	Intensive care, complete recovery in 6 months
45-year-old woman, Argentina, 1972 (76)	Bite by clinically rabid dog which died 4 days later	Suckling-mouse brain rabies vaccine 10 days later	21 days. Cerebellum dysfunction, quadriplegia, varying levels of consciousness, cardiac arrhythmia	High concentration of antibodies in the CSF and serum	Recovered but relapsed twice after vaccine boosters, slow resolution over 1 year
32-year-old man, New York, USA, 1977 (17)	Inhaled aerosol of fixed rabies virus, SAD strain, in laboratory	Only pre-exposure duck-embryo cell vaccine	21 days. Encephalitis, spastic hemiparesis, impaired consciousness	High concentration of antibodies in the CSF and serum	Gradual improvement, personality disorder, dementia
9-year-old boy, Mexico, 1992 (1)	Head bite by rabid dog	Vero-cell vaccine without rabies immunoglobulin on the following day	19 days. Encephalitis, convulsions, deep coma, quadriplegia	High concentration of antibodies in the CSF and serum	Slight improvement, reaction to painful stimuli, blindness and deafness, death after 34 months
6-year-old girl, India, 2000 (63)	Face and hand bites by stray dog which died 4 days later	No wound cleaning, chick-embryo rabies vaccine without rabies immunoglobulin on the same day	16 days. Hallucinations, coma, hydrophobia, focal seizures	High concentration of antibodies in the CSF and serum	3 months of coma, slow improvement Spasticity, tremors and involuntary movements after 18 months

CSF: Cerebrospinal fluid

Source: Warrell and Warrell, 2004 (94).

The first case of human survival after rabies, without administration of rabies vaccine or immunoglobulin, was reported in 2004. A fifteen-year-old girl developed clinical rabies one month after she had been bitten by a bat (101). The possibility that the survival was due to an unusual but more moderate or attenuated variant of the virus, or to a rare host polymorphism, cannot be ruled out (51).

RABIES PROPHYLAXIS

After exposure to the RV, the most effective treatment should be used to combat the disease. Therapy must begin as soon as possible. Prophylaxis consists of the wound care and vaccination associated or not with rabies immunoglobulin (96).

During the management of a case of exposure to the RV, it is important to examine the wound and the immune situation of the patient (considering previous rabies) as well as to evaluate the animal involved in the accident (including a laboratory diagnosis of rabies) and the transmission area (13, 20).

The wound must be washed with soap and water and treated with an antiseptic solution that has antiviral properties, such as povidone-iodine or ethanol. The risk of rabies infection is higher in anatomical regions with a great concentration of nerve endings; when wounds are deep and present bleeding; and if lesions are extensive and numerous (96).

Antibiotic solutions are useless and may increase tissue irritation. Systemic antibiotic therapy will not prevent infection after bite wounds. It is only recommended for high-risk wounds such as deep punctures, i.e. those repaired surgically, and bites in the hands. Patients with a medical history of chronic disease such as diabetes, vascular disorders, prosthetic heart valves or presenting high risk of infection can be treated with antibiotics. Prophylactic antibiotics should be administered for 3 to 5 days. For cellulites, the treatment must continue from 10 to 14 days (80, 86).

According to the tetanus immunization status of the patient, tetanus prophylaxis may be necessary, and it may be simultaneous with rabies prophylaxis, including vaccination and anti-tetanus immunoglobulin.

RABIES VACCINE

There are many rabies vaccines already in use or undergoing evaluation around the world, and compared with HDCV (human diploid cell rabies vaccine, a gold standard

of cell culture vaccine), they are less expensive to produce and more purified, showing greater effectiveness and weaker adverse reactions (32, 97).

An anti-rabies vaccine of cell culture for rabies prophylaxis has been used in São Paulo State since 2000 and throughout Brazil since 2003. A rabies vaccine produced from purified Vero cells (VEROCELL from Pasteur Merrioux, France) has also been used (20).

The Vero cell vaccine is produced in kidney cells from African green monkey using the Pitman-Moore virus strain inactivated with betapropiolactone (69). Each vaccine dose consists of 0.5 ml, and its potency is higher than 2.5 UI/ml per dose. It must be kept under refrigeration at 2°C-8°C.

Doses are the same for children, old people and immunosuppressed patients. There is no contraindication for the use of such vaccine; it can be administered to children, pregnant women, and people who are ill or taking any kind of medicine (73).

Side effects of the rabies vaccine are frequently related to local reactions, fever, malaise, nausea, vomiting and headaches. Deaths due to the use of cell culture vaccine have never been reported.

Reports of neurological reactions associated with cell culture vaccine are rare in medical literature; they occur in approximately 1/500.000 patients. Only six cases (prior to 1996) of neurological reactions such as weakness or paresthesias and permanent deficit of the deltoid muscle have been recorded. One patient developed a disease similar to multiple sclerosis. In the USA, the incidence of allergic reactions such as urticaria and anaphylaxis due to diploid cell rabies vaccine was 0.11% (11 cases out of 10,000 patients) (70).

Approximately 6% of people receiving booster vaccination may experience an immune complication such as a reaction characterized by urticaria, pruritus and malaise. Once initiated, post-exposure rabies prophylaxis should not be interrupted because of possible local or mild systemic reactions to the rabies vaccine (18, 95).

In Brazil, Butantan Institute has developed a new rabies vaccine from Vero cells adhered to microcarriers, cultured in a bioreactor in serum-free medium and infected with PV/Vero-Paris rabies virus strain. The virus suspension is purified through chromatography and rendered inactivate using betapropiolactone. Clinical tests (phases 1 and 2) carried out by Pasteur Institute, São Paulo, have shown that the vaccine presents high safety, tolerability and immunogenic response (unpublished

data). Neither fetal bovine serum nor albumin (human or bovine) was used in the preparation of this vaccine (34).

Intramuscular administration of rabies vaccine is the gold standard recommended by experts of The World Health Organization (WHO). The subcutaneous route leads to a significant decrease in sera conversion rates and to a more rapid reduction in antibody response (72). The intradermal use of rabies vaccines is considered by WHO as an acceptable alternative regimen as it requires less amount of vaccine to produce a comparable degree of rabies protection (73, 74). Intradermal administration has become a standard practice in a number of countries throughout the world (95). The use of intradermal regimen is acceptable in Brazil only for pre-exposure treatment (13, 20).

The intradermal route should not be used for all patients. The vaccine should be administered by intramuscular route to patients who are immunocompromised or who are taking steroids or chloroquine. Furthermore, the use of chloroquine must be delayed for at least one month after vaccination if the intradermal route was used. Post-immunization antibody titers should be determined to ensure that an acceptable level was achieved (20, 96, 97).

HETEROLOGOUS RABIES IMMUNOGLOBULIN

Rabies serum is a concentrated and purified solution of anti-rabies antibodies prepared from equines immunized with anti-rabies vaccine. In the past, anti-rabies sera were associated with numerous side effects, serum sickness, and anaphylactic reactions in about 40% of cases (56). Modern products, however, show low level of reaction. They are prepared through a specific purification process like enzymatic digestion, ammonia sulfate precipitation and thermocoagulation to remove any excess of protein (13, 20, 99, 100).

The serum must be kept under refrigeration at 2°C-8°C, without freezing. The recommended dose is 40 IU per kilogram of body weight. The most frequent presentation is 200 IU/ml in 10ml ampoules. Rabies serum must be injected preferably into or around the wound; the volume will depend on the anatomical region affected. It is recommended that all lesions be infiltrated with the serum (96, 97).

Anaphylactic reactions are rare with rabies serum. They have been reported in less than 1/40,000 cases. Reactions to heterologous rabies serum can be immediate or occur later. The most important immediate reaction is the anaphylactic shock, a rare

condition that can occur within 2 hours after the serum administration. Later reactions may occur from 2 to 15 days after administration and may manifest as serum sickness or Arthus reaction. Their incidence varies from 1% to 6.2% (13, 20). Less than 10% of the patients who received heterologous serum presented reaction at Pasteur Institute. The majority of cases of serum sickness occurred on day 7 in women around 40 years old (8).

The skin test is controversial. In medical literature, it has been considered of little value for prediction of reactions (88). It is not recommended by rabies experts of the Brazilian Ministry of Health because of the high number of false negative results. A detailed history of the patient is necessary to assess the risk of severe side effects that may follow anti-rabies serum administration. For patients at high risk of reaction, the use of human anti-rabies immunoglobulin (20 IU/kg of body weight), available at the Special Immunobiological Reference Centers of the Immunization Programs of the Brazilian States, should be considered (13, 20).

PRE-EXPOSURE RABIES VACCINATION

Rabies prophylaxis can be recommended for people who foresee exposure to risky situations during their regular professional activities or travels to epidemic regions. Table 4 shows the criteria for pre-exposure rabies vaccination (13, 18, 20).

Table 4: Pre-exposure rabies vaccination.

RISK CATEGORY	NATURE OF RISK	TYPICAL POPULATION	PRE-EXPOSURE REGIMEN
Continuous	-virus continuously present, often in high concentrations -specific exposures likely to be unrecognized -bite, non-bite or aerosol exposure	-rabies research laboratory workers, rabies biologics production workers	-primary course of vaccine (3 doses) -serological test every 6 months -booster vaccination* if antibody titer is below acceptable levels (< 0.5 UI/ml)
Frequent	- exposure usually episodic with known source, but exposure might also be unrecognized -bite, non-bite or aerosol exposure possible	-rabies diagnostic laboratory workers -spelunkers -veterinarians and staff -animal control and wildlife workers in rabies epizootic areas	-primary course of vaccine (3 doses) -serological test every year -booster vaccination if antibody titer is below acceptable levels (< 0.5 UI/ml)
Infrequent (greater than general population)	-exposure nearly always episodic with known source -bite or non-bite exposure	-veterinarians -animal control and wildlife workers in areas with low rabies incidence -veterinary students	-primary course of vaccine (3 doses) -serological test every year -booster vaccination if antibody titer is below acceptable levels (< 0.5 UI/ml)
		-travelers visiting areas where rabies is enzootic and the immediate appropriate medical care including biologics is limited	no serological test or booster vaccination is recommended
Rare (general population)	- exposure always episodic with known source -bite or non-bite exposure	-general population, including individuals in rabies epizootic areas	-no pre-exposure immunization is necessary

* Pre-exposure booster immunization consists of one dose of rabies culture cell vaccine (in Brazil, Vero cell rabies vaccine) injected into the deltoid area by intramuscular (0.5 ml) or intradermal (0.1 ml) route.

Sources: Costa *et al.*, 1999 (20) and Brasil, Ministério da Saúde, 2004 (13).

POST-EXPOSURE RABIES TREATMENT

The recommended schedule is one dose of the vaccine injected into the patient's deltoid area on days 0, 3, 7, 14, and 28. For children aged two years old or younger, the vaccine may be injected into the thigh muscles. The habits of the dog or cat involved in the accident must be evaluated. Treatment of humans may be unnecessary if the animals are considered of low risk for rabies transmission (e.g. animals living inside the houses, without any contact with other animals including bats). In accidents involving bats, it is always necessary to administer anti-rabies

serum regardless of the lesion severity. A patient previously treated for rabies should be considered only for booster doses of the vaccine (Table 5) (13, 20).

Table 5: Post-exposure rabies treatment.

Animal conditions Kind of exposure	DOG OR CAT (at the moment of the accident)		
	Without rabies signs or symptoms	With clinical suspicion of rabies	With rabies; disappeared or dead wild animals; domestic animals of economic value
Indirect contact Contact with objects contaminated by suspected rabid animals.	Wash the site with soap and water. Treatment is not recommended.	Wash the site with soap and water. Treatment is not recommended.	Wash the site with soap and water. Treatment is not recommended.
Slight accident Bites or scratches with teeth or nails causing slight wounds, small extension and number of lesions, on the trunk and members (except hands and feet), and licking.	Wash the site with soap and water. Observe the animal for 10 days post-exposure. If animal remains healthy during observation period close the case without treatment. If the animal disappears, dies or becomes rabid, start treatment immediately with 5 doses of rabies vaccine on days 0,3,7,14,28.	Wash the site with soap and water. Start rabies vaccine (on days 0, 3 and 7**). Observe the animal for 10 days post-exposure. If the animal remains healthy during the observation period, stop vaccine. If the animal disappears, dies or becomes rabid, complete the treatment with 5 doses of vaccine.	Wash the site with soap and water. Start the treatment with 5 doses of rabies vaccine on days 0, 3, 7, 14, 28.
Serious accident	Wash the site with soap and water. Observe the animal for 10 days post-exposure. Start treatment* with 3 doses of vaccine on days 0, 3, and 7. If the animal remains healthy during the observation period, stop treatment. If the animal disappears, dies or becomes rabid, continue treatment to complete 5 doses of rabies vaccine and use heterologous anti-rabies serum.	Wash the site with soap and water. Observe the animal for 10 days post-exposure. Start treatment† with 3 doses of vaccine on days 0, 3, and 7. If the animal remains healthy during the observation period, stop treatment. If the animal disappears, dies or becomes rabid, continue treatment to complete 5 doses of rabies vaccine and use heterologous anti-rabies serum.	Wash the site with soap and water. Start treatment with heterologous anti-rabies serum and 5 doses of rabies vaccine on days 0, 3, 7, 14, 28.

There are some specific differences between the Brazilian National Rabies Recommendations (13, 20) and those for São Paulo State, they are:

* Start treatment with 2 doses of the vaccine, one on each of days 0 and 3.

**Start treatment with 2 doses of the vaccine, one on each of days 0 and 3.

† Start treatment with heterologous anti-rabies serum and vaccine. If the animal is healthy on the 10th day after the accident, treatment may be suspended.

Source: Costa *et al*, 1999 (20).

RE-EXPOSURE RABIES TREATMENT

A previously immunized person must receive two booster vaccine doses on each of days 1 and 3. If special clinical conditions are present, such as immunosuppression, antimalarial treatment and unsupervised chronic disease, the level of neutralizing antibodies should be assessed 10 days after the last booster vaccine doses (20, 97). The recommended schedule for a person previously vaccinated against rabies is complex and the following factors should be considered: number of vaccines previously received; lack of time between the previous treatment and the recent accident; clinical condition of the patient; and kind of vaccine previously used. A normal person under complete treatment should not receive rabies immunoglobulin in re-exposure treatment, because it might interfere with the immune response to the booster vaccine doses (32). The São Paulo technical report on human rabies prophylaxis recommended that the schedule for re-exposure be as shown in Table 6 (20).

Table 6: Re-exposure rabies treatment.

TIME AFTER PREVIOUS VACCINATION	SCHEDULE	RECOMMENDED VACCINATION (WITH CULTURE CELL RABIES VACCINE)
Less than 15 days	Complete	Vaccine doses are not recommended.
	Incomplete	Indicate vaccine doses needed to complete the schedule
Between 15 and 90 days	Complete	Vaccine doses are not recommended
	Incomplete - 1 or 2 doses	Indicate 4 vaccines doses on day 0, 3, 7, 28.
	Incomplete - 3 or 4 doses	Indicate 2 doses on days 0 and 3.
After 90 days	Complete	Indicate 2 vaccines doses on days 0 and 3
	Incomplete	Indicate complete schedule

Source: Costa *et al.*, 1999 (20).

In case of accidents, patients who have already received pre-exposure prophylaxis can receive the booster vaccine (Table 7) (13, 20).

Table 7: Post-exposure rabies treatment of pre-exposure vaccinated patients.

Serological situation (levels of rabies-neutralizing antibodies)	Recommended schedule of rabies vaccine*
With serological dosage; levels > 0.5 IU/ml	Treatment with 2 doses of rabies vaccine on days 1 and 3
Without dosage of neutralizing antibodies; or level of antibodies < 0.5 IU/ml	Treatment is recommended as for a previously incompletely treated person

* The level of rabies-neutralizing antibodies should be assessed 10-14 days after the previous dose of the vaccine. If it is above 0.5 IU/ml, more doses of booster rabies vaccine and a new assessment of antibodies are recommended.

Source: Costa *et al.*, 1999 (20).

THE ANIMAL'S CONDITION

The characteristics of the animal involved in the accident must be evaluated. If a cat or a dog is involved, their health condition before and at the moment of the accident, their provenance (rabies controlled area), and the possibility of laboratory diagnosis must be taken into consideration. In case the animal dies during the observation period, a sample of its nerve tissue should be sent to a reference laboratory. The observation period of 10 days is restricted to dogs and cats. However, the incubation period of the disease ranges from days to months (frequently 60 days) and these animals can eliminate virus through salivation only a few days before the end of the incubation period, normally 2 or 5 days before the onset of symptoms, which may persist until death (89). Wild animals such as any bat species, monkeys, foxes and skunks are considered potential transmitters of rabies. They are not kept under observation because their rabies pathogenesis is unknown (92). When other domestic animals such as cows, pigs, horses, and sheep are involved, human treatment is suggested according to the characteristics of the wound. If the animal dies, it is recommended that a sample of its nerve tissue be sent to the laboratory. Treatment is not considered necessary for accidents caused by rodents or lagomorphs from urban areas or breeding places (13, 20).

FUTURE PROSPECTS

In the middle of the twentieth century, cell culture was adapted for the growth of viruses. The idea of complete inactivation for the development of vaccines arose in the nineteenth century, not long after Pasteur's original insight (98). The advent of genetic engineering has influenced the development of vaccines, providing more opportunities for the production of inactivated antigens and the attenuation of different viruses through direct mutation. Several new strategies including the use of

vectors, plasmid DNA and lipopeptide vaccines (5) are capable of inducing CD4⁺ and CD8⁺ cellular responses. In addition, the paucity of vaccine adjuvants, which until recently were essentially limited to aluminum salts that stimulate T helper type 2 (Th2) response, is at last being corrected by the production of new oil-in-water emulsions, liposomes, tool-like receptor agonists, cytokines, and other substances that direct the immune system to a T helper type 1 (Th1) response (68, 75).

Another tendency is the stimulation of innate as well as adaptive immune responses, which can be triggered by appropriate adjuvants such as CpG oligonucleotides (57). Proteomics will probably advance to allow *in vitro* production of proteins presenting configurations that are more natural. Lipopeptides, a form of peptide vaccines, are currently under intensive investigation because they can generate comprehensive immune responses, without the use of adjuvants. This observation has been confirmed recently in human volunteers who experienced no significant adverse local or systemic manifestations after injection of HIV lipopeptides without adjuvant. One approach is to develop oral vaccines from plants made transgenic for the production of vaccine antigens (67, 85, 104).

A very important future aspect is the diversification of immunization routes. Many devices have been developed to introduce antigens through the skin. These include patches containing adjuvants applied to lightly abraded skin, and microneedles piercing the stratum corneum. Once past the superficial layer, the antigen comes into contact with dendritic antigen-presenting cells, which migrate to lymph nodes and initiate the immune response (37, 41, 66).

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