UNDERSTANDING RABIES

By

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From Association for Prevention and Control of Rabies in India

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This book is dedicated to all those who are working together to make Rabies history

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Preface

Rabies is acute viral encephalitis. It is a zoonotic disease (i.e. transmitted by animals). All mammals, but mainly carnivores and bats, are susceptible and can transmit rabies virus. Human exposures are most frequently associated with bites by rabid dogs and transmission of virus from dogs' saliva. Rabies is the only communicable disease of man that is practically 100% fatal even today but easily preventable. Till date only seven survivors have been recorded.

Few countries are free of rabies as a result of their privileged geographical situation & strict application of stringent legislation.

The Association for the Prevention and Control of Rabies in India (APCRI) reported in 2004 that there were 20,565 reported human deaths over the period of one year. True incidence of human rabies could be even 10 times more than those officially reported. Current statistics of animal bites/rabies in animal population are scanty, unreliable and controversial due to poor surveillance/reporting system.

Dog is the commonest source of human rabies in Asia and Africa. It causes over 99% of all global human rabies deaths. Nearly 96% of cases are due to bites from stray, ownerless, dogs. 40-65% of dog bite victims are children <15 years.

Hydrophobia and aerophobia are pathognomonic for rabies and occur in 50% of patients. Attempting to drink or having air blown in the face produces severe laryngeal or diaphragmatic spasms and a sensation of asphyxia. This may be related to a violent response of the airway irritant mechanisms. Even the suggestion of drinking may induce hydrophobic spasm. Hydrophobia is typically unique to human beings.

There is no specific treatment for clinical rabies. Key to survival after exposure to rabies virus is administration of post-exposure prophylaxis (PEP) as soon as possible. Death is virtually inevitable once clinical signs develop. Medical management is supportive and palliative.

Rabies can be prevented by:

- Avoiding contact with unfamiliar animals. Do not handle, feed, or attract wild animals. Place litter in closed garbage cans. Never adopt or bring wild animals into your home. Teach your children to never handle unfamiliar animals, wild or domestic, even if they appear friendly.

- Being a responsible pet owner. Keep rabies vaccinations up-to-date for all cats and dogs. Neuter your pets to help reduce the number of unwanted pets that may not be properly cared for or vaccinated regularly.

Let us work together to make rabies history.

17th March, 2013

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Dr. A.K. Gupta

Epidemiology

Rabies is one of the oldest and most feared zoonotic diseases, and has been a threat to human health for more than 4000 years. Rabies is a neglected and severely under-reported disease. **The WHO estimated the annual number of human rabies deaths to be between 40,000 and 70, 000.** All mammals, but mainly carnivores and bats, are susceptible and can transmit rabies virus. Human exposures are most frequently associated with bites by rabid dogs and transmission of virus from dogs' saliva. The Association for the Prevention and Control of Rabies in India **(APCRI)** estimated in 2004 that **in India**, there were **20,565** reported human **deaths** every year. Human rabies in India accounts to 36 % of global rabies deaths and 56 % of human rabies deaths in Asia. Rabies is the only communicable disease of man that is practically **100% fatal** even today but easily preventable.

About half of the world's population lives in areas in which rabies is enzootic.

Till date only seven survivors have been recorded. These patients survived not due to any specific anti-rabies therapy but following intensive life support and excellent nursing care. These patients survived for variable periods with residual neurological deficits. All the survivors had paralytic form of rabies and majority had history of some anti-rabies vaccination in the past.

There are about 59 countries that do not report rabies.

Few countries are free of rabies as a result of their privileged geographical situation & strict application of stringent legislation.

According to Centers for Disease Control and Prevention, Countries and political units that reported no indigenous cases of rabies are:

REGION	COUNTRIES
Africa	Cape Verde, Libya, Mauritius, Réunion, São Tomé and Príncipe, and Seychelles
Americas	North: Bermuda, St. Pierre and Miquelon Caribbean: Antigua and Barbuda, Aruba, The Bahamas, Barbados, Cayman Islands, Dominica, Guadeloupe, Jamaica, Martinique, Montserrat, Netherlands Antilles, Saint Kitts (Saint Christopher) and Nevis, Saint Lucia, Saint Martin, Saint Vincent and Grenadines, Turks and Caicos, and Virgin Islands (UK and US)

REGION	COUNTRIES
Asia and the Middle East	Hong Kong, Japan, Kuwait, Lebanon, Malaysia (Sabah), Qatar, Singapore, United Arab Emirates
Europe	Austria, Belgium, Cyprus, Czech Republic,[2] Denmark,[2] Finland, Gibraltar, Greece, Iceland, Ireland, Isle of Man, Luxemburg, Netherlands,[2] Norway, Portugal, Spain[2] (except Ceuta and Melilla), Sweden, Switzerland.
Oceania[3]	Cook Islands, Fiji, French Polynesia, Guam, Hawaii, Kiribati, Micronesia, New Caledonia, New Zealand, Northern Mariana Islands, Palau, Papua New Guinea, Samoa, and Vanuatu

[1] Bat rabies may exist in some areas that are reportedly free of rabies in other mammals.
[2] Bat lyssaviruses are known to exist in these areas that are reportedly free of rabies in other mammals.
[3] Most of Pacific Oceania is reportedly "rabies-free."

Rabies statistics in India

- Each year approximately 7 million people undergo post exposure rabies treatment after a dog bite. Besides, there is an estimated **17.4 million animal bites** annually in India. Majority (75%) of animal bite victims belong to poor or low-income group.
- The Association for the Prevention and Control of Rabies in India **(APCRI)** reported in 2004 that there were **20,565** reported human **deaths** over the period of **one year**. True incidence of human rabies could be even 10 times more than those officially reported. Current statistics of animal bites/rabies in animal population are scanty, unreliable and controversial due to poor surveillance/reporting system.
- Nearly **96%** of cases are due to bites from **stray, ownerless, dogs**.
- **40-65% of dog bite victims are children <15 years**. Children are at a significant higher risk of getting bitten by a provoked bite. Children do not recognize the angry or defensive behavior of the dog and continue to play with them which the dog considers as the invasion of territory and may incite an attack.

In India the islands of Andaman and Nicobar and Lakshadweep are free of rabies.

Rabies virus

Rabies is acute viral encephalitis **caused by** a RNA **virus** (Genus: Lyssavirus, Family: Rhabdoviridae). The genus name derives from lyssa, the Greek goddess of frenzy.

Virion morphology and size: Enveloped, bullet shaped 45-100 nm in diameter, 100-430 nm in length.

Nucleic acid: Single-stranded, linear, negative-sense RNA genome, ~11.9 kb in length.

These viruses have a phospholipids envelope with glycoprotein surface spikes. The rabies virus **genome** encodes **five proteins**: the nucleoprotein (N), the matrix protein (M), the glycoprotein (**G-protein** is an antigen, to which protective anti-bodies are induced), the phosphorylated protein (P) and a large polymerase protein (L).

The existence of lyssaviruses that are closely related to rabies virus and that can also cause clinical disease (Duvenhage virus, Lagos bat virus, Mokola virus, Shimoni bat virus, and Ikoma virus) has been known for several decades.

Types of rabies virus

There are mainly **two types** of rabies virus.

1. **Street virus** which is **virulent**, having a long and variable incubation period of about 3 weeks to 3 months. When first isolated from natural human or animal hosts, rabies virus preserves its natural properties and is referred to as street virus. Most of the street virus isolates generally cause a lethal CNS infection.
2. **Fixed virus** which is an attenuated street virus. It is a rabies virus that has been passaged in tissue culture or animals. The term fixed indicates only that the incubation period and virulence has been stabilized. Fixed virus is **least virulent**, and has a **fixed short incubation period** of 5-9 days. It is used as ***seed virus*** for manufacturing rabies vaccines.

Rabies virus gets ***inactivated*** by:

- By heat (1 hour at 50°C)
- On exposure to ultraviolet radiation
- By exposure to 70% ethanol, phenol, formalin, trypsin, β-propiolactone, 1% sodium Hypochlorite, 2% glutaraldehyde
- Detergents

- By lipid solvents.
- At pH below 3 or above 11.
- Rabies virus is susceptible to sunlight and desiccation. It is inactivated rapidly in sunlight and does not survive for long periods out of the host (in dried blood and secretions)
- Ultra-violet and x-rays.

Rabies virus is **resistant to** cold and freeze drying.

Animal reservoirs

Reservoir means species in which transmission of virus is **sustained** with a maintained circle of infection within the species.

Vector means any animal which **transmits** the disease.

Rabies exists in **two forms**:

1. **Urban Rabies**, propagated chiefly by unimmunized dogs.
2. **Sylvatic rabies**, propagated by skunks, foxes, raccoons, mongooses, wolves, bats etc.

Dog is the commonest source of human rabies in Asia and Africa. It causes over 99% of all global human rabies deaths.

In America, north of Mexico and in Europe, only 0.1% to 5% of the total number of animal rabies cases reported annually involve dogs.

In these areas, three factors may account for the low prevalence of disease in dogs:

1. A large proportion of the dog population is restricted in movements (i.e., dogs are kept indoors or in enclosures and are kept on a leash when outside)
2. Dog vaccination is strongly recommended or even compulsory; and
3. It is possible that strains of virus that are adapted to wild species are not well suited for propagation within dog populations.

The host animals of the rabies virus differ among regions. The main vectors are foxes in Europe and Canada, raccoons, skunks, and fruit-eating and insectivorous bats in the United States, dogs in Asia, mongooses, jackals, and dogs in Africa, and dogs and vampire bats in Latin America.

In India, the animals commonly responsible for **transmission** of rabies are **dogs and cats** (97%) followed by wild animals like mongoose, foxes & jackals (2%) and occasionally by horses, donkeys, monkeys, cows, goats, sheep and pigs.

India has approximately 25 million dogs, with an estimated dog: man ratio of 1:36.2. The dogs fall into 4 broad categories: pets (restricted and supervised); family dogs (partially restricted, wholly dependent); community dogs (unrestricted, partially dependent); and undomesticated stray dogs (unrestricted, independent). Most dogs in India, perhaps 80%, would fall into the last 3 categories. The majority of the stray dog population is found in rural areas.

Animal bites are very common in India. The annual incidence of animal bites is high, 1.7% and it is more in rural areas (1.8%), children (2.6%) and poor/low income group (75%). The main biting animal is dog (91.5%), mostly stray (63%), followed by cat (4.7%). Neither the age nor the breed or sex of the dog is important in transmission of rabies.

In India most rodents, rats, squirrel, rabbits, birds and bats have been found to be **free of rabies**. However, following exposure to mongoose, PEP is recommended.

Rat bite cases do not require rabies vaccination but this is a right opportunity to start pre-exposure vaccination.

Transmission

Modes of exposures: Human exposures to rabies can generally be categorized as bite, open wound, mucous membrane, or other types of exposure:

Bite exposure: Any penetration of the skin of a person by the teeth of a rabid or potentially rabid animal.

Open wound exposure: Introduction of saliva or other potentially infectious material (cerebrospinal fluid, spinal cord, or brain tissue) from a rabid or potentially rabid animal into an open wound (e.g., broken skin that bled within the past 24 hours).

Mucous membrane exposure: Introduction of saliva or other potentially infectious material (cerebrospinal fluid, spinal cord, or brain tissue) from a rabid or potentially rabid animal onto any mucous membrane (eyes, nose, mouth).

Other exposure: Any interaction with a rabid or potentially rabid animal where a bite, open wound, or mucous membrane exposure cannot be definitively ruled out.

Airborne infections, such as inhaling an aerosol of infected animal brain tissue in virus laboratories, or of contaminated air in bat-inhabited caves, have been reported. Bats that are the easiest to approach and capture (unable to fly, etc.) are the most likely to have rabies. **Bat Rabies is not present in India.**

Iatrogenic rabies cases have occurred in patients who received cornea, kidney, liver, or blood vessel graft transplantation from donors who had undiagnosed rabies.

Transmission of rabies by blood transfusion

There has **never** been a reported case of rabies infection via a blood transfusion. Viremia has not been demonstrated, and the virus is intraneuronal during the incubation period. There is **no evidence** to suggest that an apparently healthy blood donor can transmit rabies, even if incubating clinical rabies.

Rabies **cannot** be transmitted to doctors/assistants conducting **postmortem** of a person who died of rabies. As per Centre for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Report (MMWR), no confirmed case of rabies has ever been reported in persons who performed a postmortem examination of people or animals, although contact with decedents with confirmed or suspected rabies can cause anxiety. Even from living patients with rabies, human-to-human transmission has been documented only rarely, (in cases of organ or tissue transplantation).

Both CDC and the World Health Organization (WHO) have stated that the infection risk to health-care personnel from human rabies patients is no greater than from patients with other viral or bacterial infections. In addition, rabies post-exposure prophylaxis (PEP) is available for exposed personnel. Nevertheless, because of the nearly universal fatal outcome from rabies, both CDC and WHO recommend that all personnel working with rabies patients or decedents adhere to recommended precautions. Even the minimal risk for rabies virus transmission at autopsy can be reduced by using careful dissection techniques and appropriate personal protective equipment, including an N95 or higher respirator, full face shield, goggles, gloves, complete body coverage by protective wear, and heavy or chain mail gloves to help prevent cuts or sticks from sharp instruments or bone fragments.

Participation in the autopsy should be limited to persons directly involved in the procedure and collection of specimens. Previous vaccination against rabies is not required for persons performing such autopsies. PEP of autopsy personnel is recommended only if contamination of a wound or mucous membrane with patient saliva or other potentially infectious material (e.g., neural tissue) occurs during the procedure.

Pathogenesis

The first event in rabies is the **inoculation of virus** through the **skin**, usually through a bite that delivers virus-laden saliva. Initial viral replication appears to occur within striated muscle cells at the site of inoculation. Replication may occur in muscle/subcutaneous /nerve tissues. The virus gets attached to nicotinic acetylcholine receptors at the neuromuscular junction and enters the peripheral nerves.The virus then spreads centripetally up the nerve to the **CNS**, probably via peripheral nerve axoplasm, at a rate of 3-10 mm/hour. Once the virus reaches the CNS, it replicates almost exclusively within the grey matter and then passes centrifugally along autonomic nerves to other tissues – the salivary glands, adrenal medulla, kidneys, lungs, liver, skeletal muscles, skin, and heart. Passage of virus into the salivary glands and viral replication in mucinogenic acinar cells facilitates further transmission via infected saliva.

There is **no** viremia in Rabies.

Essentials of rabies diagnosis

Hydrophobia and **aerophobia** are pathognomonic for rabies and occur in 50% of patients. Attempting to drink or having air blown in the face produces severe laryngeal or diaphragmatic spasms and a sensation of asphyxia. This may be related to a violent response of the airway irritant mechanisms. Even the suggestion of drinking may induce hydrophobic spasm. Hydrophobia is typically unique to human beings.

Types of rabies

There are mainly **two types** of rabies:

1. ***Furious*** **(encephalitic)** *type, t*wo-third of rabies patients suffer from typical furious type of rabies. The virus replicates in portions of the brain including the hippocampus, amygdala, anterior thalamic nuclei and limbic cortex. Furious rabies has **three cardinal signs:**

- Fluctuating consciousness, episodes of excitement and hallucinations.
- Phobic spasms – Aerophobia, Hydrophobia and Photophobia.
- Autonomic dysfunctions like increased salivation, excessive sweating, priapism & pupillary abnormalities.

It is typically believed that salivation and vomiting are linked, and contribute to the apparent hydrophobia (fear of water) in patients. These symptoms can last for few days, after which the patient may suffer from the second type of rabies, or may slip into a coma and die. It is when suffering from furious rabies that a person or animal is likely to attack those near them, and spread the disease.

2. ***Dumb*** **(paralytic)** type which is characterized by flaccid muscle weakness, constipation, urinary retention, stupor and coma. **Hydrophobia** is usually ***absent*** in these cases. This is a condition resembling Guillain Barre Syndrome. Dumb Rabies occurs as the result of the virus replicating in the brain's neocortex.

It is much harder for a doctor to diagnose rabies in its "dumb" form than it is in its "furious" form, because the symptoms are less indicative of a specific medical issue.

Both forms are progressive and will lead to death, usually within 7 days in patients with encephalitic rabies and 3 weeks in those with paralytic rabies.

Clinical features of rabies in humans

The **incubation period** of rabies that is the time interval between the exposure to virus and the onset of symptoms, is usually from 03 weeks to 03 months (rarely 04 days to 02 years).

In rabies, a long and variable incubation period is well known. The **recorded longest incubation periods** have been 19 years and 27 years. The later was recently reported from Philippines. During most of the long incubation period of rabies, the virus likely remains close to the site of viral entry. Centripetal spread to the central nervous system and spread within the central nervous system occur by fast axonal transport.

The incubation period varies with the amount of virus transmitted, virus strain, site of inoculation (bites closer to the head have a shorter incubation period), host immunity and nature of the wound.

Children are at an increased risk of a shorter incubation period because of their short stature and bites are often closer to CNS. Multiple bites to the head and neck are associated with very short incubation periods less than 1 month.

People who are immuno-compromised will most likely be more susceptible to rabies. Rabies is usually undetectable during the incubation period, and infections can also be difficult to diagnose when the clinical signs first appear.

Prodromal syndrome (Lasts for 2-10 days)

- Pain or paraesthesiae at the site of the bite is well known as a diagnostically useful prodromal symptom occurring in one-third to two-thirds of cases.
- In Thailand, however, a specific type of paraesthesiae-itching-was the earliest symptom in >40% of cases. Itching occurred at the site of the healed bite wound or involved the whole bitten limb and was sometimes so intense as to provoke frenzied scratching and excoriation of the skin. The explanation for local paraesthesiae may be the multiplication of virus in the dorsal root ganglion of the sensory nerve supplying the area of the bite.
- Pain behind the grafted eye was an early symptom in three of the four patients who developed rabies following corneal transplants.
- Priapism with frequent spontaneous orgasms was the first symptom in one Thai patient.
- Fever, malaise, nausea and vomiting.
- The skin becomes sensitive to changes of temperature, especially air currents.

Neurologic stage (Lasts for 2-7 days)

- Aphasia
- In coordination
- Paresis
- Paralysis
- Mental status changes
- Hyperactivity

Late symptoms

- Hypotension
- Cardiac arrhythmias
- Disseminated intravascular coagulation (DIC)
- Cardiac arrest

Coma (May last for 0-14 days)

Death usually occurs within a few days after appearance of clinical symptoms.

Detection of rabies virus

(a)Diagnosis of rabies in animals

In animals, rabies is diagnosed using the **direct fluorescent antibody (dFA) test**, which looks for the presence of rabies virus antigens in brain tissue.

A diagnosis of rabies can be made after detection of rabies virus from any part of the affected brain, but in order to rule out rabies, the test must include tissue from at least two locations in the brain, preferably the brain stem and cerebellum.

(b)Diagnosis of rabies in humans

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.

Ante Mortem testing

All samples should be considered as potentially infectious. Test tubes and other sample containers must be securely sealed (tape around the cap will insure that the containers do not open during transit).

All four samples listed below are required to provide an ante mortem rule out of rabies.

1. Saliva

Using a sterile eyedropper pipette, collect saliva and place it in a small sterile container which can be sealed securely. No preservatives or additional material should be added. Laboratory tests to be performed include detection of rabies RNA (by reverse transcription and polymerase chain reaction, RT/PCR, of extracted nucleic acids) and isolation of infectious virus in cell culture. *Tracheal aspirates and sputum are not suitable for rabies tests.*

2. Neck biopsy

A section of skin 5 to 6 mm in diameter should be taken from the posterior region of the neck at the hairline. The biopsy specimen should contain a minimum of 10 hair follicles and be of sufficient depth to include the cutaneous nerves at the base of the follicle. Place the specimen on a piece of sterile gauze moistened with sterile water and place in a sealed container. Do not add preservatives or additional fluids. Laboratory tests to be performed include RT/PCR and immunofluorescent staining for viral antigen in frozen sections of the biopsy.

3. Serum and cerebral spinal fluid (CSF)

At least 0.5 ml each of serum and CSF should be collected; no preservatives should be added. Do not send whole blood. If no vaccine or rabies immune serum has been given, **the presence of antibody to rabies virus in the serum is diagnostic** and tests of CSF are unnecessary. **Antibody to rabies virus in the CSF, regardless of the immunization history, suggests a rabies virus infection**. Laboratory tests for antibody include indirect immunofluorescence and virus neutralization.

4. Brain biopsy

The rarity of rabies and the lack of an effective treatment make the collection of a brain biopsy for ante mortem testing unwarranted; however, biopsy samples negative for herpes encephalitis should be tested for evidence of rabies infection. The biopsy is placed in a sterile sealed container; do not add preservatives or additional fluids. Laboratory tests to be performed include RT/PCR and immunofluorescent staining for viral antigen in touch impressions.

Postmortem testing

In certain cases, human samples may need to be tested for rabies postmortem. Fresh tissue samples from the central nervous system (brain) should be submitted. Postmortem diagnosis of rabies is **made by immunofluorescent staining** of viral antigen in touch impressions of brain tissue. Portions of the medulla (brain stem), the cerebellum, and the hippocampus should be frozen and sent on dry ice to a laboratory. Preservation of tissues by fixation in formalin is not recommended if rabies diagnosis is desired.

About various available tests

1. Direct fluorescent antibody test

The dFA test is based on the observation that animals infected by rabies virus have rabies virus proteins (antigen) present in their tissues. Because rabies is present in nervous tissue (and not blood like many other viruses), the ideal tissue to test for rabies antigen is brain. The most important part of a dFA test is fluorescently-labeled anti-rabies antibody. When labeled antibody is incubated with rabies-suspect brain tissue, it will bind to rabies antigen. Unbound antibody can be washed away and areas where antigen is present can be visualized as fluorescent-apple-green areas using a fluorescence microscope. If rabies virus is absent there will be no staining.

2. Rapid Fluorescent Focus Inhibition Test (RFFIT)

The test measures the ability of antibodies that may be present in a sample to neutralize and block rabies virus from infecting the cells used in the test. These antibodies are called rabies virus neutralizing antibodies (RVNA). Immunofluorescent staining of infected cells is used as an indicator of rabies virus replication. The RFFIT takes ~20 hours and is both sensitive and specific.

While rabies virus antibodies can be measured through the application of an Enzyme-Linked Immunosorbant Assay (ELISA), these tests measure binding antibodies to viral antigens rather than functional neutralizing antibodies measured in the RFFIT. In some cases, ELISA tests can provide false positive results if they detect antibodies that bind to rabies virus, but are unable to neutralize the virus. The **RFFIT is the current gold standard serological assay** recommended by both the Advisory Committee on Immunization Practices (ACIP) and the World Health Organization (WHO). Other serological tests, such as an ELISA, are more appropriate for research, and are not recommended for samples requiring clinical decision making by clinicians based upon current ACIP and WHO recommendations.

3. Histological examination

General histopathology

Histological examination of biopsy or autopsy tissues is occasionally useful in diagnosing unsuspected cases of rabies that have not been tested by routine methods. When brain tissue from rabies virus-infected animals are stained with a histological stain, such as hematoxylin and eosin, evidence of encephalomyelitis may be recognized by a trained microscopist. This method is nonspecific and not considered diagnostic for rabies.

Before current diagnostic methods were available, rabies diagnosis was made using this method and the clinical case history. Histopathology evidence of rabies encephalomyelitis (inflammation) in brain tissue and meninges includes the following:

1. Mononuclear infiltration
2. Perivascular cuffing of lymphocytes or polymorphonuclear cells
3. Lymphocytic foci
4. Babes nodules consisting of glial cells
5. Negri bodies

Negri bodies

Negri bodies are round or oval inclusions within the cytoplasm of nerve cells of animals infected with rabies. Negri bodies may vary in size from 0.25 to 27 µm. They are **found most frequently in** the pyramidal cells of Ammon's horn, and the Purkinje cells of the cerebellum. They are also found in the cells of the medulla and various other ganglia. Negri bodies can also be found in the neurons of the salivary glands, tongue, or other organs. Staining with Mann's, giemsa, or Sellers stains can permit differentiation of rabies inclusions from other intracellular inclusions. With these stains, Negri bodies appear magenta in color and have small (0.2 µm to 0.5 µm), dark-blue interior basophilic granules.

The presence of Negri bodies is variable. Histological staining for Negri bodies is neither as sensitive nor as specific as other tests. Some experimentally-infected cases of rabies display Negri bodies in brain tissue; others do not. Histological examination of tissues from clinically rabid animals show Negri bodies in about 50% of the samples; in contrast, the dFA test shows rabies antigen in nearly 100% of the samples. In other cases, non-rabid tissues have shown inclusions indistinguishable from Negri bodies. Because of these problems, **the presence of Negri bodies should not be considered diagnostic for rabies.**

4. Immunohistochemistry (IHC)

IHC methods for rabies detection provide sensitive and specific means to detect rabies in formalin-fixed tissues. These methods are more sensitive than histological staining methods, such as H&E and Sellers stains. Like the dFA test, these procedures use specific antibodies to detect rabies virus inclusions. The techniques use enzyme-labeling systems that increase sensitivity. In addition, monoclonal antibodies may be used to detect rabies virus variants.

5. Electron microscopy

The ultra structure of viruses can be examined by electron microscopy. Using this method, the structural components of viruses and their inclusions can be observed in detail.

6. Amplification methods

Samples containing small amounts of rabies virus may be difficult to confirm as rabies-positive by routine methods. Virus isolation in cell cultures increases the virus concentration because the virus replicates in cell cultures. Mouse neuroblastoma cells (MNA) and baby hamster kidney (BHK) cells provide an excellent environment for amplification of rabies virus without the use of animals.

Another method for amplifying the nucleic acid portion of rabies virus uses biochemical methods. With this procedure, rabies virus RNA can be enzymatically amplified as DNA copies. Rabies RNA can be copied into a DNA molecule using reverse transcriptase (RT). The DNA copy of rabies can then be amplified using polymerase chain reaction (PCR). This technique can confirm dFA results and can detect rabies virus in saliva and skin biopsy samples.

Accuracy of the tests

Because of high sensitivity and specificity of the direct fluorescent antibody (DFA) test, in comparison to virus isolation methods, the **dFA test is the "gold standard" diagnostic method for rabies.**

During clinical disease, millions of viral particles may be found intermittently in the saliva. In theory, only a single rabies particle or virion is required to result in a productive infection.

The mass of a single virion has been estimated to be approximately 221 thousand kilodaltons. A small proportion of this amount includes viral RNA which accounts for less than 2% of the mass of a single virion.

Any rabies test proposed on living animals would need to be extremely sensitive to detect very minute quantities of protein or nucleic acid. In addition, several repeat tests would be needed over time to ensure that rabies virus excretion is not missed since viral shedding in the saliva is inconsistent.

Differential diagnosis of rabies

- Encephalitis
- Guillain-Barre Syndrome
- Herpes Simplex
- Herpes Simplex Encephalitis
- Poliomyelitis
- Tetanus

During differential diagnosis, the most important viruses to rule out are **Herpes Simplex virus type 1and Varicella-zoster virus**.

Management of animal bites

Post Exposure Prophylaxis (PEP)

As there is **no specific** treatment for clinical rabies, key to survival after exposure to rabies virus is administration of post-exposure prophylaxis (PEP) as soon as possible. **Death** is virtually **inevitable** once clinical signs develop. Medical management is supportive and palliative.

Improper treatment to animal bite victims may lead to rabies death and litigation under **Consumer Protection Act (COPRA).** According to Consumer Protection Act, the animal bite should be considered as **"medical urgency"** and treated with due care.

PEP includes

1. Local treatment of wound(s)
2. Categorization of animal bite wound(s)
3. Anti Rabies Vaccination (ARV)
4. Administration of Rabies Immunoglobulin (RIG)
5. **In addition**, Tetanus prophylaxis, analgesics & systemic antibiotics may be given.

As rabies is 100% fatal, there are no contraindications to post exposure prophylaxis.

Local treatment of wound(s)

By mere washing of wounds and application of antiseptics, the risk of rabies reduces by about 50%.

The maximum benefit of the wound washing is obtained when fresh wound is cleaned immediately. It is important to remove saliva containing rabies virus at the site of bite by physical or chemical means. This can be done by prompt and gentle thorough washing with ordinary soap or detergent and flushing the wound with running tap water for at least 15 minutes. If soap or antiviral agent is not available, the wound should be thoroughly washed with water.

Washing of the wound must be done as long as the wound is raw; irrespective of the time elapsed since the exposure. Care must be taken not to disturb the scab, if formed.

After thorough washing and drying the wound, any one of the available chemical agents should be applied viz Povidone iodine (Betadine), Alcohol, Chloroxylenol (Dettol), Chlorhexidine Gluconate and Cetrimide solution (Savlon - in appropriate recommended dilution), etc.

Discourage local wound applicants like turmeric, neem, red chili, lime, plant juices, coffee powder etc.

Theoretically, the richly innervated areas like head, neck, face, hands and genitals are the most dangerous sites of bite in man. But practically, it is often the wounds on legs, which are ignored/neglected, that cause rabies.

Avoid

1. Avoid **direct touching** of wounds with bare hands. Considering the importance of this step, the anti-rabies clinics should have proper wound washing facilities.
2. **Cauterizing** the wound is not advisable as it leaves a very bad scar and also does not confer any additional advantage over washing the wound with water and soap. It amounts to malpractice and the doctor can be sued for compensation under COPRA.
3. **Avoid suturing** of the bite wound as a rule since it may risk inoculation of the virus deeply into the wound. However, if the wound has to be sutured, it should be done as late as possible from several hours to 3 days after infiltration of RIGs. If RIGs is not available, as a last resort, the wound must be flushed with povidone iodine before suturing. The suture should be loose and not interfere with free bleeding and drainage. Human and animal bite wounds are best closed by **secondary sutures** after one week and after proper cleansing and daily wound care. Primary surgical intervention must be avoided if possible.

4. We should **never try to deepen** the bite wound. Deepening of wound for cleaning depends on area of injury, extent of injury and the aim should be to preserve as much tissue as possible and to excise dead tissue only.
5. **Do not bandage** the wound as far as possible and if unavoidable, apply non-adherent, absorbent dressings (paraffin gauze or Melolin) to absorb the discharge from the wound.

Animal bites and Infections

15 to 20 percent of dog bite wounds become infected. Puncture wounds and hand wounds are more likely to become infected than scratches.

Most infected dog bite wounds yield polymicrobial organisms. *Pasteurella multocida* and *Staphylococcus aureus* are the **most common** aerobic organisms

Systemic antibiotic treatment for animal bites

Using antibiotics may be helpful, particularly in high-risk wounds.

Antimicrobials effective in the empiric treatment of patients with animal bite and human bite wounds		
	Animal bite	**Human bite**
Amoxicillin-clavulanate (PO)	+	+
Ampicillin-sulbactam (IV)	+	+
Moxifloxacin (PO, IV)	+	+
Gatifloxacin (PO, IV)	+	+
Doxycycline (PO, IV)	+	+

Medical Conditions Associated with a High Risk of Infection After a Dog Bite

1. Chronic disease
2. Chronic edema of the extremity
3. Diabetes mellitus
4. Immunosuppression
5. Liver dysfunction
6. Previous mastectomy
7. Prosthetic valve or joint
8. Splenectomy
9. Systemic lupus erythematosus

Categorization of animal bite wound(s)

WHO classified exposures into three categories.

Category (Type of contact with a suspect or confirmed rabid domestic or wild animal, or animal unavailable for observation)	Recommended treatment.
	None, if reliable case history is available
I. Touching or feeding of animals, Licks on intact skin	Administer vaccine immediately. Stop treatment if animal remains healthy throughout an observation period of 10 days or if animal is killed humanely and found to be negative for rabies by appropriate laboratory techniques.
II. Nibbling of uncovered skin, Minor scratches or abrasions without bleeding,	
III. Single or multiple transdermal bites or scratches, Contamination of mucous membrane with saliva (i.e. licks), Licks on broken skin	Administer rabies immunoglobulin and vaccine immediately. Stop treatment if animal remains healthy throughout an observation period' of 10 days or if animal is killed humanely and found to be negative for rabies by appropriate laboratory techniques.

When in doubt of degree of exposure to rabies risk, it is safer to over treat than under treat.

Biting animal

It is very important to elicit information about biting animal.

Low risk *category* includes healthy pets and regularly vaccinated dogs/cats.

Moderate risk *category* includes healthy pets but vaccination status doubtful or not done.

High risk *category* includes rabid, sick, died, stray dogs/cats, or wild animals.

Observation of biting animal

Carrier state of rabies in dogs/cats is not yet conclusively proven and established. Hence, **both WHO and Govt. of India** recommend observation of

animal for **10 days** during post-exposure treatment. **In Europe** which is almost rabies free, observation period is **15 days**.

The treatment should be started immediately after the bite. The treatment may be modified if animal involved **(dog or cat)** remains healthy throughout the observation period of 10 days by converting post-exposure prophylaxis to pre-exposure vaccination by skipping the vaccine dose on day 14 and administering it on day 28 while using Essen Schedule. The observation period is **valid for dogs and cats only**. The natural history of rabies in mammals other than dogs or cats is not fully understood and therefore the 10-day observation period may not be applicable. The rationale for observation is that if the animal is incubating rabies, they will show signs of disease in the next 3-5 days and die subsequently in another 3-5 days.

Whether a dog bite was provoked rather than unprovoked should not be considered a guarantee that the animal is not rabid as it can be difficult to understand what an attacking dog considers provocation for an attack.

Anti Rabies Vaccination (ARV)

First Rabies Vaccine: Pasteur treatment

All human cases of rabies were fatal until a vaccine was developed in 1885 by Louis Pasteur and Émile Roux.

- Joseph Meister, a 9 year old boy was severely bitten by a rabid dog.
- On July 6th, 1885, Louis Pasteur administered the first of 14 injections of infected rabbit spinal cord suspension; inactivated by desiccation.
- The immunization was successful and Joseph survived.

Historical development of rabies vaccines

1885: Pasteur vaccine, Louis Pasteur developed the first rabies vaccine

1911: Semple vaccine developed by Sir David Semple

- Inactivated virus using formalin and phenol
- Infected adult rabbit, sheep, or goat brain tissue

1955: Fuenzalida vaccine, Drs Fuenzalida and Palacios

- Suckling mouse brain (SMB) vaccine
- Decreased reactogenicity with improved immunogenicity 10-15 doses required

1956: Duck embryo vaccine, first effective attempt at cell culture vaccine

- Duck embryo vaccine (DEV)
- Poorly immunogenic, high rate of allergic reaction. Stopped in 1980

1970s: Modern cell culture vaccines

- Highly immunogenic with good safety profile

Modern Anti-rabies vaccines being used now are **Tissue Culture Vaccines (TCV)** inactivated by **beta-propiolactone** (BPL). Beta-propiolactone is carcinogenic but it loses its carcinogenicity because of its hydrolysis during the process of inactivation of rabies virus.

As a general rule, live attenuated viral vaccines induce better immunity. But, **WHO has forbidden the use of live attenuated rabies vaccine in humans** because live attenuated vaccines always carry risk of reverting to virulent forms. If such a vaccine is given to human beings, it may cause fatal disease.

Ideal requirements of a rabies vaccine

- **Pure:** Highly purified and well-tolerated primary cell culture vaccine with excellent safety profile
- **Powerful:** Highly potent, provides early and long-lasting protective antibiotic titers
- **Protection:** Highly efficacious and trusted for use in all WHO recommended IM and ID regimens.

Various types of modern anti-rabies vaccines

These are:

1. Purified vero cell rabies vaccine (PVRV) - Verorab, Xprab, Abhayrab, Indirab and Verovax-R.
2. Purified chick embryo cell vaccine (PCECV) - Rabipur, Vaxirab-N
3. Human diploid cell vaccine (HDCV)- Rabivax
4. Purified duck embryo vaccine (PDEV) - Vaxirab

Each IM dose is available in a single dose vial only.

Response to rabies vaccination is a unique mix of specificities to rabies virus antigens. Antibodies develop out of intrinsic host responses and in response to extrinsic factors such as the amount of antigen given, type of antigen, and route of exposure or vaccination. There may be substantial variation in the neutralizing activity and quantity of rabies virus neutralizing antibodies (RVNA)

produced. Therefore, the strain of virus used in antirabies vaccine plays a role in eliciting the required response.

Compared to other continuous cell lines based rabies vaccines, PCEC vaccine with PM strain has several advantages, these includes:

- This method provides vaccine with high yield, greater potency and immunogenicity which makes the vaccine comparatively cost effective and unique.
- It is more readily scalable to large scale commercial vaccine production.

Of the currently available TCVs such as HDCV, PCECV, PVRV, and PDEV, **all are equally good** and approved by WHO. All are **interchangeable** following non availability of one brand or due to allergy to one of the CCVs or PDEV. All are considered **protective** throughout the world against **different strains** of rabies viruses in different parts of the world.

Each vaccine has its **own merit**.

1. Purified Vero cell Rabies Vaccine (PVRV): Verorab, Xprab, Abhayrab, Indirab.

Vero cell line is a continuous cell line initiated in 1962 by Yasumura (Japan) from kidneys of an African green monkey.

Verorab was developed in 1984.It is a freeze-dried product composed of PM/WI 38 1503-3M strain. The rabies virus is produced on Vero cell lines, inactivated with beta propiolactone and purified so that the potency of one dose of vaccine is superior to 2.5 IU.

2. Purified chick embryo cell vaccine (PCECV): Vaxirab-N, Rabipur

a. VAXIRAB-N

- Vaxirab N is manufactured in Zydus Vaccine Plant which has state-of-the-art manufacturing facility.
- Vaxirab N is World's 1st Purified Chick Embryo Cell Culture Rabies Vaccine (PCEC) with *Pitman-Moore (PM) Advantage.*
- Pitman Moore Strain is the most Stable & Safe Strain for biologicals.
- Vaxirab N contains
  - Intact Inactivated Virus
  - High G & N Protein
- Vaxirab N has double Stabilizer.
- Approved by DCGI both for Pre exposure and Post exposure prophylaxis.
- Approved both for Intramuscular (I.M.) or Intradermal (I.D.) route of Administration.

b. **Rabipur**

Rabipur is a purified, potent and efficacious PCECV rabies vaccine. In the US, PCECV has received FDA approval and is marked under the brand name of RabAvert.

It is manufactured from the Flury LEP rabies virus strain grown in a culture of chick embryo fibroblast cells, inactivated with beta-propiolactone, stabilized with a gelatin product and purified by zonal centrifugation.

PCECV vaccine is generally contraindicated in persons allergic to egg proteins.

3. Human diploid cell vaccine (HDCV) – Rabivax

HDCV is of homologous (human) origin, considered by many as the "purest" antigen. It is prepared with Pitman-Moore strain of rabies virus. HDCV vaccine was developed by propagating the PM-1503-3M strain of the rabies virus on diploid cells of human origin. In 1964, Wiktor et al used the WI38 Line, later replaced by the MRC 5 line. After achieving growth of the virus, it is purified, inactivated, stabilized and lyophilized. Each dose is reconstituted in a volume of 1 ml. Large-scale production is impossible due to low yield of virus, duration and difficulties of human diploid cell culture, the need for a heavy viral antigen concentration and the complicated and lengthy controls that make production difficult.

Window period

It is the **time taken** by Anti-Rabies vaccine **to produce protective levels of antibodies** in the patient. The window period is of 7-14 days.

Vaccine potency and storage

WHO recommends that the **vaccine potency** should be at least 2.5 IU per dose. The potency is the capacity of the vaccine to induce immune response. Vaccine must be **stored at** $+2^0$ C to $+ 8^0$ C.

Caution

- The modern anti-rabies vaccines should **not be diluted** with tetanus toxoid or any other diluents other than that provided with the vaccine.
- If the vaccine was accidentally kept in freezer, it should **not** be used.
- **Full course** of rabies vaccine must be given even if sero-protective levels are obtained after 2-3 injections. This is to cover a longer incubation period of rabies as it can be more than 3 weeks and also to increase the **cellular**

immunity and interferon production that also play a role in the mechanism of protection.

- A person receiving/completed antirabies immunization **can donate blood.** However, the recipient does not benefit from the transfer of rabies-neutralizing antibodies due to hemodilution.

Vaccination in special medical conditions

- Rabies vaccine can be given to a child with **chicken pox or measles** and it is effective. If possible administration of measles vaccine should be postponed by a fortnight after the completion of antirabies immunization.
- Rabies vaccine can be given to a patient with **jaundice.**
- Several studies of patients with **HIV/AIDS** have reported that those with low CD4 (<200 counts) will mount a significantly lower or no detectable neutralizing antibody response to rabies. In such patients and those in whom the presence of immunological memory is no longer assured as a result of other causes, proper and thorough wound management and antisepsis accompanied by local infiltration of rabies Immunoglobulins followed by anti-rabies vaccination are of utmost importance. Even immuno compromised patients with category II exposures should receive rabies immunoglobulin in addition to a full post-exposure vaccination. Preferably, if the facilities are available, antirabies antibody estimation should be done 10 days after the completion of course of vaccination.

Bite by a vaccinated dog

Although unvaccinated animals are more likely to transmit rabies, vaccinated animals can also do so if the vaccination of the biting animal was ineffective for any reason. A history of rabies vaccination in an animal is not always a guarantee that the biting animal is not rabid. Animal vaccine failures may occur because of improper administration or poor quality of the vaccine, poor health status of the animal, and the fact that one vaccine dose does not always provide long-lasting protection against infection in dogs. **PEP is required for a person bitten by a vaccinated dog.** Vaccinating the pet dog is primarily to protect it against contracting rabies following bites by stray rabid dogs/animals.

It should be noted that:

1. No veterinary vaccine offers 100% protection against rabies.
2. Rabies is enzootic in our stray dog/animal population.
3. The facility of measuring protective rabies antibody titer is available only at few centers in India.

4. The immunization record of dog may not be always available.
5. Booster dose may not have been given to dog.
6. 6% of dogs found rabid have a reliable pre-exposure rabies vaccine history
7. 62% of dogs found rabid are less than 1 year old and
8. 40% of dogs' vaccinated only one time lose most of their immunity 4-6 months later.

So in view of above facts and practical difficulties, rabies being 100% fatal, we should start rabies PEP vaccination even if **a person is bitten by a vaccinated dog**.

PEP vaccination schedule (WHO ESSEN-IM)

The schedule is one injection on days 0, 03, 07, 14 and 28. (Day 0 means day of first dose of vaccine and not the day of bite).

Vaccine should be injected deep into deltoid muscle (in Adults) or antero-lateral aspect of thigh (in Children).

Note that

- **First three doses** of modern rabies vaccine must be **very timely** and for the fourth and fifth, one or two days of variation is permissible.
- These vaccines ***must not*** be administered in **gluteal** region as the gluteal fat may retard vaccine absorption resulting in delayed and lower seroconversion.
- All modern rabies vaccines have a **uniform dosage for all age** groups.
- **If due to some reasons** only initial three injections on days 0, 3 and 7 were given, three inj. may provide protective antibody titers for about/up to 3 months.
- **In case**, day 0 and 3 inj. were given and inj. due on day 7 was postponed because the dog was kept under observation but the dog dies between 8 and 15 days, three doses of vaccine must be given as close to the original dates of the schedule and **all five inj. must be completed by day 28.**
- There is **no single dose** vaccine or a vaccine that gives lifelong immunity.
- Any **major surgery** can be conducted along with antirabies treatment.

***Day 0 dose should be doubled in*:**

1. Patients who seek treatment after a delay of 48 hours or even months after having been bitten should be dealt in the same manner as if the exposure occurred recently.
2. Patients with very high risk exposures or extensive bites,

3. Immunodeficient patients or on immunosuppressive drugs, such as steroids, antimalarials, anticancer drugs.
4. Severely malnourished patients,
5. Patients with underlying chronic disease like liver cirrhosis and in
6. Patients where RIG is indicated but unavailable.

Double dose of vaccine is not a substitute for RIG.

Reasons for Rabies Post-Exposure Prophylaxis Failures

1. Inappropriate local wound treatment
2. Poor quality of rabies vaccine
3. No RIG administered
4. Faulty handing / mistakes (improper dilution, spills, etc)
5. Improper administration of vaccine
6. Delayed initiation of PEP
7. Vaccination schedule not complete

PEP compliance is low in India

- Use of rabies vaccination in exposed humans is low= 40%
- Use of RIG negligible= 2.1%
- Human rabies victims did not receive any ARV = 79%
- Compliance to the full course is low = 40.5%

Intradermal vaccination (IDRV Rabies) in India

The production of Semple Vaccine, which was a sheep brain derived **Nerve Tissue Vaccine (NTV)** has **stopped totally** in **India** after 31st December, 2004. The last dose of NTV was used in September, 2005. The NTV was mainly used for providing free treatment to animal bite victims attending Anti-Rabies Clinics (ARCs) in govt. hospitals and health centers. After the complete disappearance of NTV, there was a great shortage of Anti-Rabies Vaccines (ARV) in the ARCs in govt. hospitals and health centers.

WHO approved IDRV in 1992. It is considered as an ethical and **cost-effective** replacement of NTV. **In India**, Intra-dermal Rabies Vaccination (IDRV) started on 19th May, 2006 in Uttar Pradesh followed by few other states.

The ID route has been permitted to be used in **selected anti-rabies clinics (ARCs)** having an adequate number of patients (at least 5/day) seeking post-exposure prophylaxis against rabies every day to make IDRV viable and cost-effective.

Mode of action

It is deposition of approved modern rabies vaccine (or antigen) in the layers of dermis of skin by which the immuno-receptive Langerhan cells present within the dermis are stimulated. Subsequently the antigen is carried by antigen presenting cells via the lymphatic drainage to the regional lymph nodes and later to the reticulo-endothelial system eliciting a prompt and highly protective antibody response. Immunity is believed to depend mainly upon the CD 4 + T-cell dependent neutralizing antibody response to the G protein. In addition, cell-mediated immunity has long been reported as an important part of the defense against rabies. Cells presenting the fragments of G protein are the targets of cytotoxic T- cells and the N protein induced T helper cells. The immune response induced by IDRV is adequate and protective against rabies.

ID injection technique

Using aseptic technique, reconstitute the vial of freeze-dried vaccine with the diluents supplied by the manufacturer. With 1 ml syringe draw 0.2 ml (up to 20 units if a 100 units syringe is used or up to 8 units if a 40 units syringe is used) of vaccine needed for one patient (i.e. 0.1 ml per ID site X 2 sites) and expel the air bubbles carefully from the syringe thereby removing any dead space in the syringe.

Using the technique of BCG inoculation, stretch the surface of the skin and insert the tip of the needle with bevel upwards, almost parallel to the skin surface and slowly inject half the volume of vaccine in the syringe (i.e. 0.1ml; either 10 or 4 units) into the uppermost dermal layer of skin, over the deltoid area, preferably an inch above the insertion of deltoid muscle. If the needle is correctly placed inside the dermis, considerable resistance is felt while injecting the vaccine. A raised papule should begin to appear immediately, causing a **peau d' orange (orange peel) appearance**. Inject the remaining half the volume of vaccine (i.e. 0.1ml; either 10 or 4 units) on the opposite deltoid area.

IDRV vaccines

The following vaccines have been approved by DCGI for use by intra-dermal route.

1. Purified Chick Embryo Cell-Culture Vaccine (PCEC) - Rabipur & Vaxirab-N.
2. PVRV (Purified verocell rabies vaccine) – Verorab-vial of 0.5 ml,
3. PVRV – Abhayrab – vial of 0.5 ml., Human Biological Institute
4. PVRV – Indirab, vial of 0.5 ml/1.0 ml. Bharath Biotech, Hyderabad.

As far as possible, the same vaccine should be used throughout a course of IDRV. However, in exigencies, the permitted vaccines are interchangeable.

Caution

1. The ID injections must be administered by staff trained in this technique.
2. The Vaccine vials must be stored at $+2^0$ C to $+ 8^0$ C after reconstitution and
3. The total content should be used as soon as possible, but at least within 8 hours.
4. The 0.1 ml. ID administration of cell-culture vaccine should create a wheal of at least 5 mm diameter with **"peau de orange"** appearance.
5. WHO recommends that in cases where the wheal over the skin is not formed then the patient should receive another dose of vaccine at a site nearby. If the IDRV fails in any one of the sites after two attempts, then the vaccine must be given by IM route and the remaining doses of the schedule given by IM route only.

Note:

- There are no dietary restrictions during IDRV. However, alcohol may be avoided as it may affect the immune response.
- Routine sera testing for rabies antibodies to know IDRV efficacy is not required.
- Pregnancy and lactation are not contraindications for IDRV.
- There is no need to alter the dose or schedule of any concomitant medication during IDRV.

Following **re-exposure** the bitten person needs only two doses of 0.1 ml. of ID dose at one site only, on day 0 and day 3. RIGs are not needed. Proper wound treatment is very important.

Side effects

Cell culture vaccines have proved remarkably safe and free of significant adverse events. However, mild symptoms of pain, erythema, irritation or swelling at the intradermal injection sites occur in 3% to 92% of patients. The most frequent symptom is local irritation in 7% to 64% of vaccines. Generalized symptoms reported by 3% to 14% of recipients include headache, fever and influenza- like illness. Transient macula papular and urticarial rashes are occasionally seen. All these adverse effects are mild, transient and self limiting and rarely call for the use of anti-histamines (tablet or syrup Avil) and analgesics.

Dose and schedule of IDRV

The IM dose of Verorab (PVRV) and Abhayrab (PVRV) is 0.5mL; that of Vaxirab N, Rabipur (PCEC) and PVRV (Coonoor) is 1mL. Still the ID dosage of all vaccines is **uniformly 0.1ml.**

For **PEP**, the **modified "TRC ID" schedule** (2-2-2-0-2) is the only schedule approved by the DCGI at present. ID vaccine is given on days 0, 03, 07, and 28.

For **pre-exposure** vaccination, 0.1 ml of ID-approved vaccine is to be given ID over one deltoid on days 0, 7 and 21 or 28 days.

Eight-site intradermal regimen ("8–0–4–0–1–1" regimen)

One dose of 0.1 ml is administered intradermally at eight different sites (Either upper arms, lateral thighs, supra-scapular region, and lower quadrant of abdomen) on day 0. On day 7, four 0.1 ml injections are administered intradermally into each upper arm (deltoid region) and each lateral thigh. Following these injections, one additional 0.1 ml dose is administered on days 28 and 90. This regimen lowers the cost of vaccine administered by intramuscular regimens and generally produces a higher antibody response than the other recommended schedules by day 14. It does not result in a significantly earlier antibody response and in order to ensure optimal treatment, a passive immune product must be administered to patients presenting with severe exposures. **However, this regimen is not approved for use in India by DCGI**.

It is recommended by many internationally reputed experts that the phenomenon of **mixing of IM and ID schedules** is not to be practiced and **must be avoided** as far as possible.

Sites for IDRV

IDRV can be given in deltoid region, supra-scapular, anterior abdominal wall and the upper part of thigh.

Contraindications to IDRV

1. Patient on chloroquine.
2. Patient on long term steroid usage.
3. Immunocompromised patient or on any immunosuppressant therapy.

In such cases, the rabies vaccine should be given IM.

Potency of IDRV

Only three countries are practicing IDRV in regular patients attending regular anti-rabies clinics (ARCs) for more than ten years. These countries are Thailand, Philippines and Sri Lanka.

In Thailand and Sri Lanka, the potency requirement is 0.7 IU/ID dose and in Philippines it is 0.5 IU/ID dose.

The ESSEN (IM) schedule is a better schedule, with a large margin of safety, easy to administer, better compliance for both the patient and the physician and always the route of choice in cases where the immune response to vaccination is doubtful.

Few suggestions by APCRI *for more effective implementation of IDRV*

a. The accurate dosage of vaccine can be done if separate insulin syringes are used for each site of administration.
b. For children below 5 years of age, it is advisable to adopt IM vaccination.

Criterion for protection (Seroconversion) and its importance

The effectiveness of modern tissue rabies vaccines is measured by their ability to protect persons exposed to rabies and to induce antibodies to rabies virus.

The definition of a minimally acceptable antibody titer varies between laboratories and is influenced by the type of test conducted. The WHO specifies a rabies virus neutralizing antibody (RVNA) titer of 0.5 IU/ml as adequate for protection.

The **facility for this test** is available at NCDC Delhi, CRI Kasauli, Pasteur institute Coonnor, NIV Pune and NIMHANS Bangalore.

Serological assays to measure rabies neutralizing antibodies

- **Two** recognized tests by WHO:
  - **RFFIT** (rapid fluorescent foci inhibition test), **'gold standard'.** RFFIT test is **the only internationally approved** procedure for measuring rabies neutralizing antibodies.
  - **FAVN** (fluorescent antibody virus neutralizing test). Neutralizing tests can only be done in labs approved to work with live rabies virus and which have the practiced expertise to do these tests.

ELISA is not an appropriate test system, as it does not measure neutralizing antibodies.

Points that should be considered as to whether a person should receive a **booster dose** of rabies vaccine when their antibody level falls below 0.5 IU/mL are:

- Anticipated risk of exposure (i.e., routinely handling sick animals or rabies reservoir species in enzootic areas)
- Length of time until the next antibody measurement
- Previous rabies antibody levels and the probability of decay to low or undetectable levels in the intervening period
- Individual health status (consider immuno-compromising conditions or a history of poor vaccine response)
- Timely access to vaccine and administration should a potential exposure occur

Rabies Immunoglobulin (RIG)

RIG is a life saving drug in all category 3 exposures and in a few category 2 exposures. **Use of RIG is as low as 2.1%** in our country. ***Failure to advice RIGs attracts litigation/compensation under Consumer Protection Act for deficient/faulty medical service.***

Following situations need RIG:

- All Category III exposures.
- Even Category II exposures in immuno-compromised/ immunosuppressed individuals.
- Bites by all wild animals viz. by mongoose, jackal, fox etc.

Importance of RIG

The rationale behind the use of RIG is above all; to rapidly neutralize the virus locally inside of the wound(s) before it enters the peripheral nerve endings. RIGs are specific rabies-virus-neutralizing antibodies (or their immune-active fragments) that immediately bind to the rabies virus on contact. Once the viruses are coated with antibodies, they cannot adsorb onto and enter the nerve endings. This results in a further reduction (and in some cases complete obliteration) of the inoculated virus, even in deeper tissues where washing and cleansing may not have reached. RIG provides local protection during the time gap until the appearance of vaccine-induced anti-bodies in protective concentration 7 to 10 days later, thereby protecting the patient during the critical first week after infection.

RIGs are **never** to be used **alone** to treat animal bite victims.

RIG should **not be given after day 07** if a modern rabies vaccination has already been started without RIG.

Types of RIGs

There are two types of RIGs:

1. **Human RIG** (HRIG): Available with a potency of 150 IU/ml.
2. **Equine RIG** (ERIG): Available with a potency of 300 IU/ml.

Dosage of RIGs

Dosage for administration is decided on the basis of body weight.

For HRIGs: Dosage is 20 IU per kg body weight subject to a maximum of 1500 IU. HRIG has a longer half-life (about 21 days).

For ERIGs: Dosage is 40 IU per kg body weight subject to a maximum of 3000 IU.

Availability of different RIGs in India

HRIG Brands

1. Berirab-P- manufactured by CSL Behring GmbH, Germany and marketed by Bharat Serum & Vaccines Ltd.
2. Kamrab - manufactured by Kamada Ltd., Israel and marketed by Synergy.

ERIG brands

1. Anti Rabies Serum - manufactured by CRI – Kasauli.
2. Equirab - manufactured and marketed by Bharat Serum & Vaccines Ltd.
3. Abhayrig - manufactured by VINS Bioproducts and marketed by Human Biologicals Ltd.
4. Vinirig - manufactured and marketed by VINS Bioproducts.

When to administer RIGs

RIG is more effective if infiltrated immediately or within 24 hours of animal bite along with first dose of vaccine. If vaccine alone was started, then RIG can be given up to 7 days after starting first dose of vaccine. *Ideally*, rabies vaccine should precede RIG. However, in exceptional situations, approximately within 1 hour after administering RIG, the vaccine must be given.

Precautions to be taken while administering RIGs

1. Patient should not be on an empty stomach.
2. RIGs vial taken out from the refrigerator should be kept outside for a few minutes to warm it to room/body temperature.
3. While infiltrating RIGs into the bite wound, care must be taken to avoid injecting into blood vessels and nerves.
4. While injecting into finger tips, care must be taken to avoid compartment syndrome.
5. All emergency drugs and facilities for managing any adverse reactions must be available.
6. For ERIG, keep the patient under observation for at least one hour after ERIG administration and then send home.

Skin sensitivity testing & interpretation

Majority of reactions to ERIG result from complement activation and **are not IgE mediated** and will not be predicted by skin testing.

The recent **WHO recommendation** states that there are no scientific grounds for performing a skin test prior to the administration of ERIG, because testing does not predict reactions and ERIG should be given whatever the result of the test.

However skin test is **mandatory** to avoid any possible litigation under consumer protection Act (COPRA) in India.

Inject 0.1 ml ERIG diluted 1:10 in physiological saline intra-dermally into the flexor surface of the forearm to raise a bleb of about 3-4 mm diameter. Inject an equal amount of normal saline as a negative control on the flexor surface of the other forearm. After 15 minutes an increase in diameter to > 10 mm of indurations surrounded by flare is taken as positive skin test, provided the reaction on the saline test was negative. An increase or abrupt fall in blood pressure, syncope, hurried breathing, palpitations and any other systemic manifestations should be taken as positive test.

A negative skin test must never reassure the physician that no anaphylactic reaction will occur. Those administering ERIG should always be ready to treat early anaphylactic reactions with adrenalin. The dose is 0.5 ml of 0.1 percent solution (1 in 1000, 1mg/ml) for adults and 0.01 ml/kg body weight for children, injected subcutaneously or IM.

Most ERIGs that are manufactured presently are highly purified and the occurrence of adverse events has been significantly reduced. Unlike the original unpurified rabies antisera which resulted in adverse reactions in as many as 40% of recipients, the adverse-reaction rate of patients receiving highly purified ERIGs has been reduced to <1–2%. However adverse event like anaphylaxis cannot be completely ruled out.

Adverse reactions to HRIGs

In rare cases the following adverse reactions may occur:

- Allergic reactions including fall in blood pressure, dyspnoea, cutaneous reactions, in isolated cases reaching as far as anaphylactic shock, even when the patient has shown no hypersensitivity to previous administration of Immunoglobulins.
- Generalized reactions such as chills, fever, headache, malaise, nausea, vomiting, arthralgia and moderate back pain
- Cardiovascular reactions particularly if HRIG is inadvertently injected intravascularly.

Local reactions:

At the injection site local pain, tenderness or swelling can be observed in rare cases.

Adverse reactions to ERIGs

- There may be transient tenderness at the injection site.
- Brief rise in body temperature.
- Skin reactions are extremely rare.
- RIG must never be given intravenously since this could produce symptoms of shock, especially in patients with antibody deficiency syndromes.
- Serum sickness occurs in 1% to 6% of patients usually 7 to 10 days after injection of ERIG, but it has not been reported after treatment with HRIG.

ERIG should preferably be given in a hospital setting.

Adverse reactions to ERIGs are managed as follows:

Anti-sera of equine origin may cause anaphylactic shock.

Management if anaphylactic reaction occurs:

1. Adrenaline: The dose is 0.5 ml of 0.1 percent solution (1 in 1000, 1mg/ml) for adults and 0.01ml/kg body weight for children, injected intramuscularly (IM).
2. Inj Hydrocortisone: 100 mg stat and 6 hourly I/V.
3. Inj Chlorphinaramine I/V.
4. Inj Ranitidine I/V.

If patient is sensitive to ERIG, HRIG should be used. Patient who had prior exposure of anti-sera (e.g.-Anti-tetanus serum, anti-diphtheria serum) should receive subcutaneous dose of Inj adrenaline (the requirement will be half dose of that required for treatment for anaphylaxis).

Mode of administration of full dose of RIGs

It is important to infiltrate all wounds with RIGs. Intra-muscular (IM) administration of RIGs is of very little value. As much of the calculated dose of RIG, as is anatomically feasible, should be infiltrated into & around all the wounds. In the event that some volume of RIGs is left over after all wounds have been infiltrated, it should be administered by deep IM at a site distant from the vaccine injection site.

If the calculated dose of RIG is insufficient (by volume) to infiltrate all wounds, sterile saline can be used (up to equal volume) to dilute it to permit thorough infiltration.

If a wound has healed or healing (scab is formed) the total dose of RIGs should be given IM gluteal only.

Note: RIG should **never** be administered in the same syringe or at the same anatomical site as vaccine administration.

RIGs in re-exposure cases

Managing re-exposure following post-exposure treatment with TCV: If re-exposed, persons who have previously received full post-exposure prophylaxis (Either by IM or ID route) with a potent cell-culture vaccine should now be given only two booster doses, intramuscularly (0.5ml/1ml)/intra-dermally (0.1 ml at 1 site) on days 0 and 3. Proper wound toilet should be done. Treatment with RIG is not necessary.

Managing exposure following pre-exposure prophylaxis with TCV: If after recommended pre-exposure prophylaxis, a vaccinated person is exposed to

rabies, a proper wound toileting should be done and two IM/ID (0.1 ml at 1 site) doses of Cell Culture Vaccine are given on days 0 and 3. Treatment with RIG is not necessary.

Managing re-exposure following post-exposure treatment with NTV: Persons who have previously received full post-exposure treatment with NTV should be treated as fresh case and may be given treatment as per merits of the case.

Approach to a patient requiring RIGs, when none is available

In circumstances where no immunoglobulin are available greater emphasis should be given to proper wound toileting followed by Essen Schedule of Cell Culture Vaccine with double dose on day 0 at 2 different sites intramuscularly (0 day – 2 doses on left and right deltoid, 3, 7, 14 and 28 days). It is emphasized that doubling the first dose of CCV is not a replacement to RIG. A full course of vaccine should follow thorough wound cleansing and passive immunization.

Important considerations

- RIGs can be infiltrated even to already **sutured wounds** without disturbing the sutures.
- RIGs can be safely injected into already **infected** animal bite wounds following proper wound cleansing and administration of appropriate antibiotics.
- **Unboiled milk** of a rabid animal may contain rabies virus. There is a theoretical risk of humans contracting rabies after having consumed unboiled milk of a rabid animal. If a person has consumed milk of a rabid animal, counseling should be done. If counseling is not effective, then PrEP by IM or ID route or as a last resort a course of PEP (only vaccine) should be given. If the milk is boiled or heated then only counseling or at the most a course of PrEP should be given only due to compulsions in medical practice in Indian setting.
- **Kissing** a rabies patient may transmit disease because there may be contact with rabies patient's saliva. Full post-exposure immunization must be given either by Intramuscular (IM) or Intradermal (ID) route. If there are ulcers in the mouth of the exposed person, then RIGs must be given by IM route.
- Rabies virus is present in the **semen** and to some extent in **vaginal secretions**. Priapism, increased sexual libido and indulgence are seen both in male and female rabies cases. Hence, in the exposed person, a full course of rabies post-exposure vaccination either by Intramuscular (IM) or Intradermal (ID) route should be given. If there is any doubt of category III

exposures, that is, abrasion on penis or in vagina, then even RIG must also be given by IM route.

- If a person has handled or eaten the **raw meat** of a rabid animal, he should receive full course of rabies vaccine. If the person has eaten raw meat of a rabid animal and has oral ulcers/lesions, he may be given RIGs in thigh IM on day 0 along with first dose of vaccine.
- If the rabid animal`s saliva falls into the **eyes**, the eyes should be washed with water/saline and then RIGs can be instilled as eye drops, after dilution (1:1) with sterile normal saline along with full course of anti-rabies vaccination.
- Antibodies from vaccination do not cross an intact **blood-brain barrier**.
- A person does not acquire **immunity against natural rabies** infection, as it occurs in other viral infections because there is no viremia in rabies and the virus is not accessible to the normal immune mechanism of the body. The antibody production starts only after travelling efferently from CNS via mostly autonomic nerves to different target organs. But by that time, the neuronal cells of patient`s brain stem are affected.
- In case the **mother** develops rabies, the foetus is safe because rabies virus does not cross **placental barrier**. Still the new born should be given full course of rabies PEP vaccination.
- If a vaccinated **pet dog dies of sudden unexplained death**, then all those who came in contact with the saliva of the animal, directly or through its **fomites**, should be given full PEP vaccination.
- It is advisable to use **human vaccines for human** and use the veterinary vaccines for animals.
- **If unvaccinated** or partially vaccinated **pet dog is bitten** by a suspected rabid stray dog, then the pet dog should ideally be put to sleep (Euthanasia). Otherwise, full PEP vaccination of pet dog with cell culture vaccine and simultaneous observation of dog for 2 to 6 months is recommended. Pre-exposure vaccination of all household members is necessary.

Pre-exposure vaccination (PrEV)

Pre-exposure (Prebite) vaccination **means** immunization before the bite.

It should be given to:

- Veterinarians and staff
- Rabies laboratory personnel
- Personnel working in rabies vaccine manufacturing plants

- Wildlife rehabilitees and animal control workers
- Military personnel and armed forces
- Adventure travelers to canine rabies endemic countries
- Children in canine rabies endemic high risk areas

Pre-exposure vaccination schedule

The regimen is three IM injections on days 0, 7 & 28.

Pre-exposure vaccination **simplifies** post-exposure vaccination because after bite, those who have received full Pre-exposure vaccination, only **two** doses of vaccine at days 0 & 3 are required. **RIGs are not required** (WHO 2007).

Although no teratogenicity is reported with modern rabies vaccines, pre-exposure vaccination should be **avoided in pregnant** woman**.**

Rabies in animals

The **symptoms of rabies** in dogs/cats are:

Behaviour change – Friendly dog becomes aggressive and vice versa,

Voice change – Distinctive howl or bark,

Choking – Seem to have a bone or other object stuck in throat,

Snapping – Snap at objects that are not there.

As recently as 2004, a new symptom of rabies has been observed in foxes. Probably at the beginning of the prodromal stage, foxes, which are extremely cautious by nature, seem to lose this instinct. Foxes will come into settlements, approach people, and generally behave as if tame.

There are **few variations** in signs of rabies between different species of animals. **Cattle** with furious rabies attack and pursue man and other animals. A common clinical sign is a characteristic **abnormal bellowing. Head butting** is a characteristic sign in case of rabies in **sheep** and **goats.**

The **signs of Rabies** in dogs/cats are:

Motor Paralysis is most consistent sign in all animals. Paralysis develops and is usually an ascending. **First signs** are weakness or uncoordinated hind limbs. As paralysis becomes more extensive, locomotors dysfunction becomes more pronounced.

Development of **paraplegia** without a history of compatible trauma injury is highly suspicious of rabies.

Animals **do not** exhibit **Hydrophobia.**

Control of rabies in dogs

The classic methods are

- Vaccination of dogs and
- Control of dog population

Animal vaccination remains the method of choice to control and eradicate rabies.

Primary vaccination schedule for the dogs and cats, consists of initial two doses of vaccine, that is, one dose given at 3 months of age and the second given 1 month later. This is followed by a **booster** dose of vaccine every year.

Post Exposure Prophylaxis (PEP) vaccination is not very successful in dogs.

The cost of a post-bite treatment in humans is about *twenty to one hundred times* more costly than the vaccination of a dog.

Animal birth control: Under optimal conditions a given population of dogs would nearly triple every year. In the policy of animal birth control, also referred to as the ABC Programme, stray dogs are impounded, surgically sterilized and released back into the area from where they were picked up. The success of this program hinges on the sterilization of 70% of the strays in a given geographic area within six months, before the next reproductive cycle begins; otherwise the entire effort is negated. This target is difficult to achieve, given the large number of strays and the limited resources. Hence the success of the animal birth control program in controlling the stray dog population is a subject of dispute and doubt.

Myths about Rabies in India

Following myths about rabies are very much prevalent in India:-

- People apply turmeric, herbal extracts and sometimes ghee over the wound area. Chilies, hydrogen- peroxide and cow dung are some other wrong practices followed mainly in the rural parts of India.
- In rural areas, people also resort to witchcraft and religious practices.
- Washing of wound(s) can cause hydrophobia.

- Dietary changes can cure, that is, shift from vegetarianism to non-vegetarianism or vice versa; stopping consumption of white things etc.
- A single dose vaccine will prevent rabies.
- Vaccines are more effective if taken on empty stomach.
- One should not take bath; eat meat and eggs during vaccination.
- Gems and stones have magical properties against rabies.

Seasonal variation in dog bite cases

Maximum dog bites were observed in the autumn months. It is observed that there is an increase during warm-weather months (May through August) and a corresponding decrease during colder months (November through March).

How can rabies be prevented?

- Have your **pets vaccinated** against rabies. Any pets which come in contact with wild animals are at risk. If your cat or dog has been bitten by a wild animal or has bites or scratches of unknown origin, consult the veterinarian immediately.
- If your cat or **dog is sick**, seek the advice of your veterinarian.
- Protect your pets from stray or wild animals.
- Report stray animals to your local health department.
- **Do not feed** or handle **wild animals** especially those that appear aggressive or sick.
- Never keep a wild animal as a pet.

Methods of Euthanasia (for animals)

1. Intravenous anesthetic

Pets are almost always euthanized by intravenous injection, typically a very high dose of a barbiturate such as pentobarbital. Unconsciousness, respiratory then cardiac arrest follows rapidly, usually within 30 seconds.

2. **Stray** animals are sometimes put to sleep by animal shelters that put unclaimed and unadopted dogs and cats in a sealed chamber and pump the air out. The animal dies of **anoxia**.

3. Inhalant anesthetic

Gas anesthetics such as isoflurane and sevoflurane can be used for euthanasia in very small animals. The animals are placed in sealed chambers where high levels of anesthetic gas are introduced.

4. Cervical dislocation

Cervical dislocation, or snapping of the neck, is a simple and common method of killing small animals such as rabbits.

5. Intraperitoneal injection

When intravenous injection is not possible, euthanasia drugs such as pentobarbital can be injected directly into a body cavity. Intraperitoneal injection is fully acceptable (although it may take up to 15 minutes in dogs and cats)

6. Shooting

This can be an appropriate means of euthanasia for large animals (e.g., horses, cattle)

The **usual method** of euthanasia **for dogs** is by IV injection of either concentrated Mag. Sulphate solution or euthatol which is concentrated pentabarbitine.

World Rabies Day: It is on 28th September.

Conclusion

- Rabies is the only communicable disease of man that is practically 100% fatal even today but easily preventable.
- The WHO estimated the annual number of human rabies deaths to be between 40,000 and 70, 000.
- Most human deaths follow a bite from an infected dog.
- Wound cleansing and immunizations, done as soon as possible after suspect contact with an animal and following WHO recommendations, can prevent the onset of rabies in virtually 100% of exposures.
- Once the signs and symptoms of rabies start to appear, there is no treatment and the disease is almost always fatal.
- Globally, the most cost-effective strategy for preventing rabies in people is by eliminating rabies in dogs through animal vaccinations.
- 40-65% of dog bite victims are children <15 years. Children often play with animals and are less likely to report bites or scratches.
- In areas known for rabies, professionals with frequent exposure to animals (e.g. veterinarians), or who spend a lot of time outdoors (e.g. wildlife specialists or researchers), particularly in rural areas, should be vaccinated preventively.

Abbreviations and Acronyms

ABC	Animal Birth Control Programme
AEV	Avian Embryo Vaccines
APCRI	Association for Prevention and Control of Rabies in India
ARCs	Anti-Rabies Clinics
ARS	Antirabies Serum
ARV	Antirabies Vaccine
ATS	Antitetanus Serum
AWBI	Animal Welfare Board of India
BBB	Blood-Brain Barrier
BPL	Beta Propio Lactone
CCV	Cell Culture Vaccine
CDC	Centers for Disease Control and Prevention United States
COPRA	Consumer Protection Act
CRI	Central Research Institute, Kasauli, H.P.
DCGI	Drug Controller Govt. of India
DFA	Direct Fluorescent Antibody Testing
EPI	Expanded Programme of Immunization
ERIG	Equine Rabies Immunoglobulin
FAT	Fluorescent Antibody Test
HDCV	Human Diploid Cell Vaccine
HRIG	Human Rabies Immunoglobulin
ID	Intradermal
IDRV	Intradermal Rabies Vaccination
IM	Intramuscular
IU	International Units
MNT	Mouse Neutralization Test
NCDC	National Institute of Communicable Diseases, Delhi

NIMHANS	National Institute of Mental Health and Neurosciences, Bangalore
NIV	National Institute of Virology, Pune
NTV	Nerve Tissue Vaccine
PCECV	Purified Chick Embryo Cell Vaccine
PDEV	Purified Duck Embryo Vaccine
PEP	Post Exposure Prophylaxis
PrEV	Pre Exposure Vaccination
PVRV	Purified Vero Cell Rabies Vaccine
RFFIT	Rapid Fluorescent Focus Inhibition Test
RIG	Rabies Immunoglobulin
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
RVNA	Rabies Virus Neutralizing Antibody
TCV	Tissue Culture Vaccines
TRC ID	Thai Red Cross Intradermal Schedule
WHO	World Health Organization, Geneva, Switzerland
WRD	World Rabies Day

WEBSITES ON RABIES

www.who.int/rabies

www.cdc.gov.in

www.apcri.org

www.rabiesinasia.org

www.kimscommunitymedicine.org

www.rabiescontrol.net

www.worldrabiesday.org

www.drakgupta.in

Although utmost care is taken to provide updated information through this book, ultimately the decision to treat the patient lies solely with the physician.