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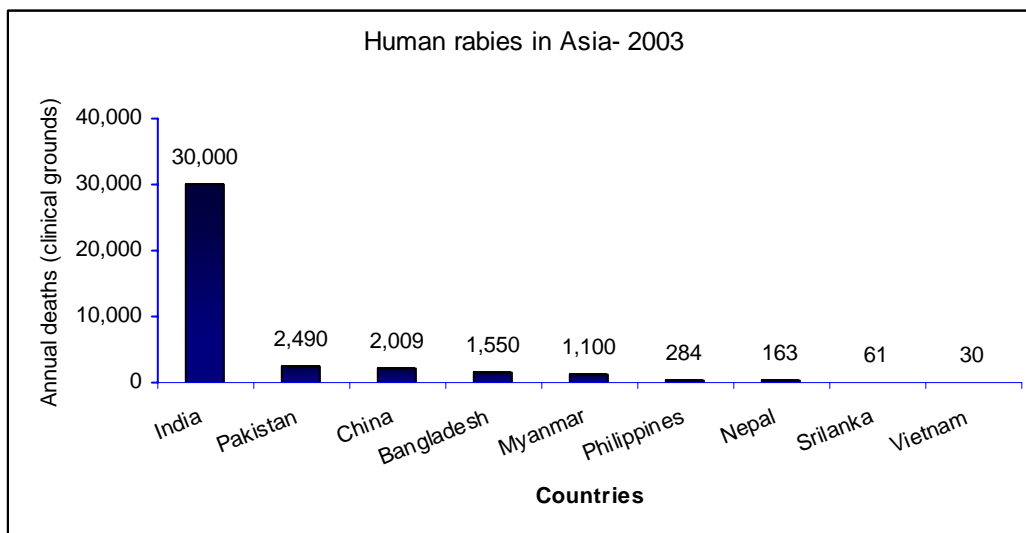
## **Epidemiology and Control of Rabies in Nepal**

### **Abstract**

Rabies is an acute and fatal encephalomyelitis caused by infection with rabies virus or other lyssaviruses of the family *Rhabdoviridae*. Although the disease occurs worldwide, developing countries of Asia and Africa are endemic. A wide range of mammals of the orders Carnivora and Chiroptera serve as the host of rabies virus, but rabid dogs are the most important hazards. Transmission occurs by introduction of virus-laden saliva in mucous membranes and open wounds, usually through animal bite. Virus spread occurs by centripetal movement towards the central nervous system, replication in the neurons followed by centrifugal spread to the salivary glands. Average incubation period varies from 1-3 months. Clinical manifestations mainly involve altered mentation followed by paralytic seizures. The disease can be diagnosed definitively only after death of victim which involves demonstration of viral antigen in the brain-tissues. Current treatment practices include preexposure and postexposure immunization of individuals by using vaccines and immunoglobulins. The disease is 100% fatal after the onset of clinical symptoms. Approximately 55,000 human deaths occur each year worldwide, about 56% of them in Asian countries. Relative to the Western World, rabies has been poorly studied in these countries. Rabies has been endemic in Nepal since time immemorial, but the exact data on magnitude of human and animal rabies is not available as surveillance mechanism for rabies hardly exists. Dog is the principal reservoir, and most common victims are children, and people from low-income group. The use of 'modern rabies vaccines' is low and immunoglobulins negligible. Simple type vaccine made from sheep brain is still in use despite of its adverse reaction and other potential complications. Control program has not been initiated at the national level as yet. Epidemiological surveillance of rabies and study of dog ecology at the local level seems important for designing an effective control program. As the magnitude of rabies burden seems negligible compared to other public health problems, it is highly unlikely in the near future that government of Nepal will initiate a control program. If funding is available and the international organizations like the World Health Organization (WHO) and the World Organization for Animal Health (OIE) are willing collaborate with the public health authorities in Nepal, rabies may be controlled in this country. This essay reviews the current status of rabies in Nepal and suggests priority areas needed to be given consideration for designing and implementation of an effective rabies control program.

## 1 General Introduction

Rabies is an acute and progressive zoonotic infection occurring in a number of mammalian species. The disease has the highest case-fatality ratio and is almost 100% fatal following the onset of clinical symptoms <sup>1, 2</sup>. The causative agents, genotype 1 rabies virus, are neurotropic RNA viruses belonging to the family *Rhabdoviridae*, genus *Lyssavirus* <sup>3</sup>. Rabies is one of the oldest diseases known <sup>2</sup> which has killed substantial number of humans and animals for many years. Although all mammals may serve as a source of human exposure, rabid dogs pose the greatest hazard. It has been estimated that approximately 55 000 people die annually due to canine rabies <sup>4</sup>. With the exception of very few countries, rabies occurs worldwide. However over 99% of the human rabies deaths occur in the developing countries of Asia, Africa and Latin America <sup>1, 5</sup>, out of which 50% are children <sup>6</sup>. The Asian countries account for 56% of the global human deaths due to rabies (figure 1)<sup>1</sup>, and India alone reports about 30, 000 human deaths per year, and one million postexposure immunizations <sup>7, 8</sup>. It is generally accepted that these officially reported numbers greatly underestimates the true incidence of rabies <sup>4</sup>. The actual numbers are believed to be quite high as rabies is not a notifiable disease in most of the developing countries, and the figures reported accounts only clinically confirmed cases. It is estimated that the number of deaths due to rabies may be 10 times more than those reported cases <sup>9</sup>.



**Figure 1 Human deaths in Asian countries due to rabies (source WHO, 2005 <sup>7</sup>).**

The disease has been controlled in most of the developed countries through effective control of rabies in reservoir animals, and by administering highly effective treatments for humans. However, in the developing world, rabies continues to remain a huge burden, mainly because of the unaffordability and inaccessibility of post-exposure treatments and higher risk of exposure to rabid dogs. In these countries, it is estimated that, one person dies from rabies each 15 minutes, and more than 300 others are exposed<sup>2</sup>. In economic terms, the annual cost of rabies has been calculated to be US\$ 583.5 million<sup>4</sup>, and that of livestock losses as a result of rabies is estimated to be US\$ 12.3 million<sup>1</sup>.

The control attempts in the developing countries have not been effective mainly because of the poorly designed controlled programmes. An effective control programs should be based on understanding of the epidemiology and natural history of the disease in that particular region. Taking Nepal, a rabies endemic country in the Indian Subcontinent, as an example, this essay reviews the epidemiology, diagnosis, prevention and control of rabies in the less developed countries. As little information is available about rabies in Nepal, most of the information is extrapolated from the neighbouring countries, particularly India.

## 2 The rabies virus

### 2.1 Classification

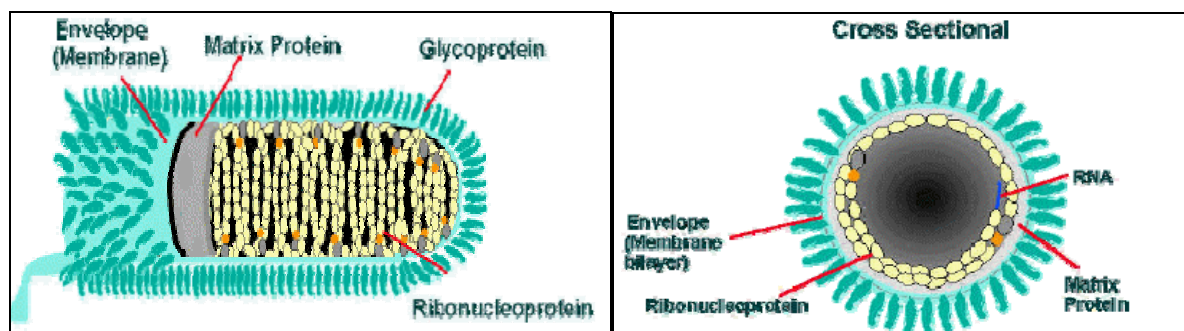
The rabies virus belongs to the order *Mononegavirales*, family *Rhabdoviridae*, and the genus *Lyssavirus*. Based on their nucleotide sequence and percentage of amino acid similarity in the nucleoprotein, the genus *Lyssavirus* is classified into seven different genotypes (Table 1). Further characterization of subtypes within each serotype is possible by the use of use of monoclonal antibodies (MAbs) directed against viral nucleocapsid or glycoprotein antigens, and the sequencing of defined genomic areas<sup>10</sup>. Although all lyssaviruses can cause rabies, the most characterized viruses are classical rabies viruses of serotype 1<sup>11</sup>. The more than 160 viral species in the *Rhabdoviridae* family are morphologically similar and genetically related, and these viruses not only induce severe diseases in humans and animals, but also cause problems in plants<sup>12</sup>.

Table 1 Classification of lyssaviruses <sup>1, 10</sup>.

Species	Genotype	Serotype	Abbreviation	Geographical distribution	Potential reservoir
Rabies virus	genotype 1	serotype 1	RABV	Worldwide, except some islands	Carnivores, bats
Lagos bat virus	genotype 2	serotype 2	LBV	Sub-Saharan Africa	Furgivorous bats
Mokola virus	genotype 3	serotype 3	MOKV	Sub-Saharan Africa	Unknown
Duvenhage virus	genotype 4	serotype 4	DUUV	Southern Africa	Insectivorous bats
European bat lyssavirus	genotype 5	Not typed	EBLV1	Europe	Insectivorous bats
European bat lyssavirus	genotype 6	Not typed	EBLV2	Europe	Insectivorous bats
Australian bat lyssavirus	genotype 7	Not typed	ABLV	Australia	Furgivorous/ Insectivorous bats

## 2.2 Structure

The virus particles have a bullet-shaped structure with an average length of 180 nm and a diameter of 75 nm <sup>13</sup>. Each particle contains a helical nucleocapsid surrounded by a lipid bilayer envelope (figure 2). The outer surface is covered with spike-like projections, embedded in the envelope <sup>13</sup>. These glycoprotein trimers are about 10 nm long, and function to recognize viral receptors on susceptible cell membranes <sup>1</sup>.



**Figure 2 Diagram of a typical bullet-shaped lyssavirus showing a stylised view of the genome and viral antigens <sup>2</sup>.** (The cross-sectional diagram demonstrates the concentric layers: envelope membrane bilayer, M protein, and tightly coiled encased genomic RNA).

The genome is a negative-sense non-segmented RNA of 12 kb length which encodes five viral proteins, namely, nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and polymerase (L) <sup>12</sup>. The nucleocapsid is a complex composed of the genomic RNA, proteins N, L, and P, which functions to ensure genome transcription and replication in the cytoplasm <sup>1</sup>. Protein M is necessary for virus budding and bullet-shaped morphology. The order and relative

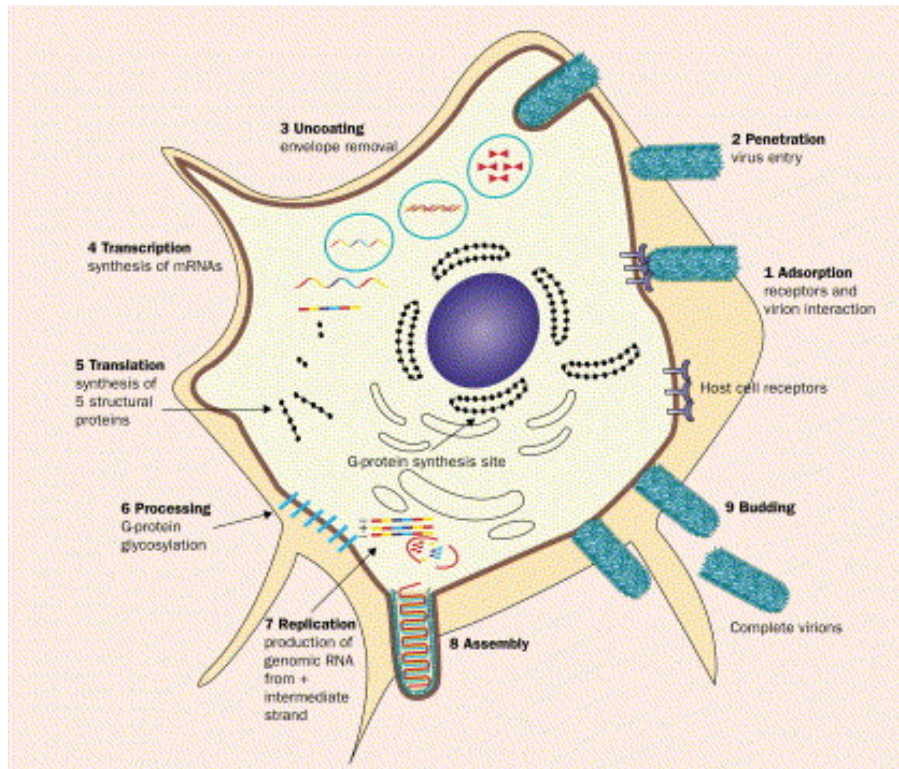
size of the genes in the genome are shown in the figure 3. There is a leader-sequence (LDR) of approximately 50 nucleotides which is followed by N, P, M, G, and L genes<sup>14</sup>.



**Figure 3** The rabies virus genome- a single-stranded, negative-sense, non-segmented RNA (from CDC, 2005<sup>14</sup>).

### 2.3 Replication

Under natural conditions, rabies virus infects neurons almost exclusively with the exception of muscle cells in entry site and the acinar cells of the salivary glands at the final stage<sup>15</sup>. The three main aspects of the molecular mechanism of classical rabies virus infection are: neurotropism, neuroinvasiveness, and impairment of neuronal functions<sup>15</sup>. Neuroinvasion is mainly contributed by the G protein, and transsynaptic spread by the RNP.

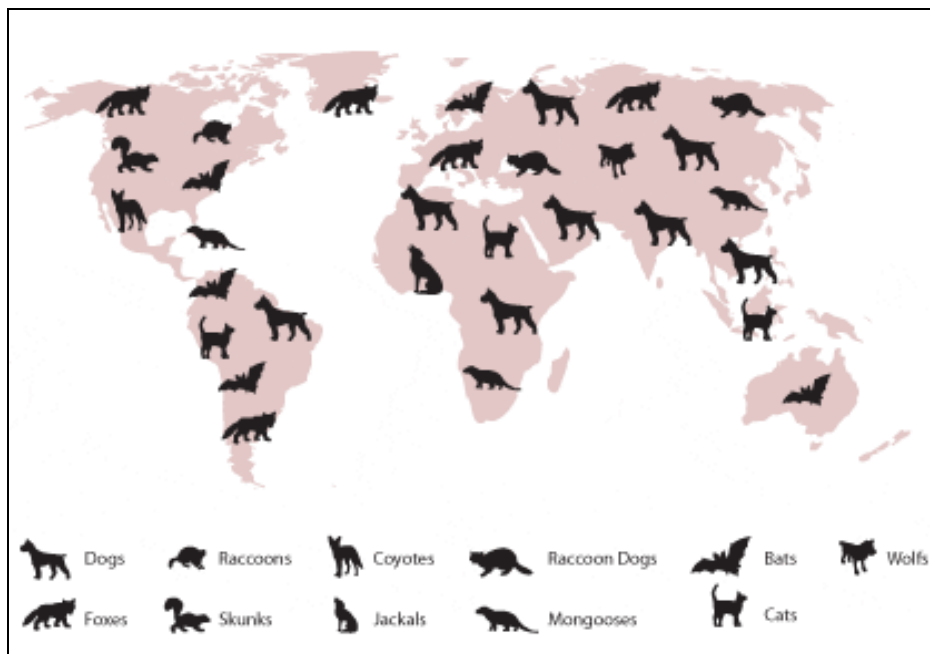


**Figure 4** Conceptual flow of lyssavirus reception, entry, transcription, translation, replication, and exit from a generalized host cell (from Rupprecht, 2002<sup>2</sup>).

The events of replication of *Lyssavirus* can be divided into three steps<sup>2</sup>: attachment and entry to the host cell, transcription and translation, and budding and release of the virion (figure 4). Attachment to the host cell is mediated by the receptor -nicotine acetylcholine<sup>2 15</sup>. The negative-sense RNA acts as template for both transcription and translation<sup>12</sup>. In spite of being neurotropic in nature, they can be propagated in several cell types like the neuroblastoma, Vero cells etc, under laboratory conditions<sup>2</sup>.

## 2.4 Reservoirs and Vectors

Rabies virus has a very wide range of hosts. Almost all the warm-blooded mammals of the animal kingdom are susceptible to rabies virus infection, at least under experimental conditions<sup>16</sup>. Even avian species are susceptible<sup>2,17</sup>. However, the degree of susceptibility greatly varies with species, and only a few species can maintain the infection as reservoirs<sup>18</sup>. The wild carnivores like the foxes, wolves, coyotes and jackals are considered to be highly susceptible, whereas domestic animals and man are considered to be moderately susceptible<sup>19</sup>. Infection in human beings however results in a dead-end<sup>2</sup>. The species of animals serve as major hosts in different parts of the world is shown in figure 5.



**Figure 5 Worldwide animal reservoirs of rabies (from Anon, 2004<sup>18</sup>).**

Most of these reservoir species belong to the orders Carnivora and Chiroptera, dogs being the most important reservoir for human rabies. Besides dogs, cats, wolves, foxes, jackals,

mongooses, skunks, racoons, coyotes and bats are the animals that have been source of exposure of rabies virus to man for many years. In addition, there are several species of animals that may serve as vectors of rabies virus. As rabies viruses are capable of crossing the species barriers, 'spillover' into a new species may occur when ecological conditions are favourable<sup>20</sup>. Cats are a good example. They are primarily regarded as incidental hosts since the infection occurs as a result of 'spillover' infections in dogs or wildlife species<sup>21</sup>. Although they do not serve as reservoirs, both domestic and wild cats are efficient vectors of rabies virus transmission<sup>2</sup>.

## **2.5 Persistence and spread of infection**

At present, in most parts of the world, the rabies virus circulates either in the urban or in the sylvatic cycle, for which domestic dogs and wild canids, respectively, are the principal hosts. Before 18<sup>th</sup> century, there was little role of domestic dog in the spread of rabies, and majority of the infection in humans was due to wild animal exposures<sup>17</sup>. Even today, there is significant role of wild animals and bats (sylvatic cycle), in maintaining the infection and transmitting to the urban cycle.

To date, it is not clearly known how the infection persists in dog population<sup>16</sup>. However, it has been demonstrated that higher dog densities ( $>5$  dog/sq<sup>2</sup>) favors the maintenance of infection in some African countries<sup>22</sup>. As high density of the dog population enhances contact between infected and healthy individuals, the rate of transmission of rabies virus increases, resulting in endemic canine rabies<sup>16</sup>. These high numbers of rabid animals, seeking other animals, wildlife or humans to bite, dramatically increase the incidence of rabies in a given area. It may also be possible that urban cycle will maintain the sylvatic cycle when canine rabies becomes endemic.

## **3 The disease**

### **3.1 Transmission and risk factors**

Rabies transmission in humans and animals occur almost invariably by introduction of virus-laden saliva into a bite wound (bite exposure) or an open lesion (non-bite exposure)<sup>2, 23</sup>. For transmission to occur, virus needs be inoculated into the tissues of susceptible host<sup>24</sup>. The virus cannot cross the intact skin surfaces<sup>1</sup>. So, infection occurs almost always by bite or scratch of the rabid animals<sup>17</sup>. After being inoculated, the virus enters into nervous-tissues, either directly or after brief multiplication in non-nervous tissues<sup>24</sup>.

Very rarely, transmission of rabies virus may occur through some apparent routes (non-bite exposure). As lyssaviruses are fairly fragile and do not persist in the environment<sup>2</sup>, indirect transmission rarely occurs. It has been mentioned that rabies virus might be transmitted by ingestion, rectal instillation, inhalation or vertically<sup>3, 23</sup>. Transmission by inhalation of virus containing aerosol does not occur under natural conditions<sup>24</sup>. Also as the viruses are susceptible to gastric juice and intestinal fluids<sup>3</sup>, transmission may occur via oral route. Non-bite transmission (ingestion, inhalation and other mucosal exposure) is occasionally implicated in dog to dog transmission<sup>16</sup>.

Meat or milk from infected animals are not believed to be transmitting the virus, but precaution should be taken. Consumption of milk is not considered a hazard as pasteurization readily inactivates the virus<sup>19</sup>. However, it has been mentioned that transmission of rabies virus through unpasteurized milk is theoretically possible<sup>25</sup>. In spite of this, the presence of virus in milk of lactating animals has not been considered an important transmission hazard, as infected animals quickly cease production or produce grossly abnormal-appearing milk<sup>3</sup>. Rabies did not occur in a child who was being breastfed even when the mother had developed clinical signs, and died later after 2 days<sup>26</sup>.

The Centers for Disease Control and Prevention of the United States (CDC) has made the following recommendations regarding consumption of meat and milk from rabid animals<sup>27</sup>:

- 1) *If the animal is slaughtered within 7 days of being bitten, its tissues may be eaten without risk of infection, provided that liberal portions of the exposed area are discarded. .... any animal known to have been exposed to rabies within 8 months be rejected for slaughter.*
- 2) *Neither tissues nor milk from a rabid animal should be used for human or animal consumption.*
- 3)..... *drinking pasteurized milk or eating cooked meat does not constitute a rabies exposure.*

Human to human transmission does not occur. There are, however, some reports of humans infected following transplant of the organs –liver, kidney, cornea, and arterial stents- from individuals who were later diagnosed to be rabies infected<sup>28-31</sup>. There is small risk of transmission of rabies virus from humans died of rabies as the virus is not detected in blood<sup>1</sup>. However, persons handling the deadbodies should take precaution as the virus may be present in CNS fluid, saliva, tears and urine<sup>1, 32</sup>, or even in solid organ like liver and kidney<sup>29</sup>.

Rabies viruses are quite fragile and do not persist in environment. They are rapidly killed under sunlight and can't survive long outside hosts<sup>2</sup>. Therefore, fomites like bodies of water and inanimate objects, do not have a role in rabies transmission<sup>2</sup>. Under natural conditions, the viruses present on open surfaces of host animals are inactivated within few days in summer but

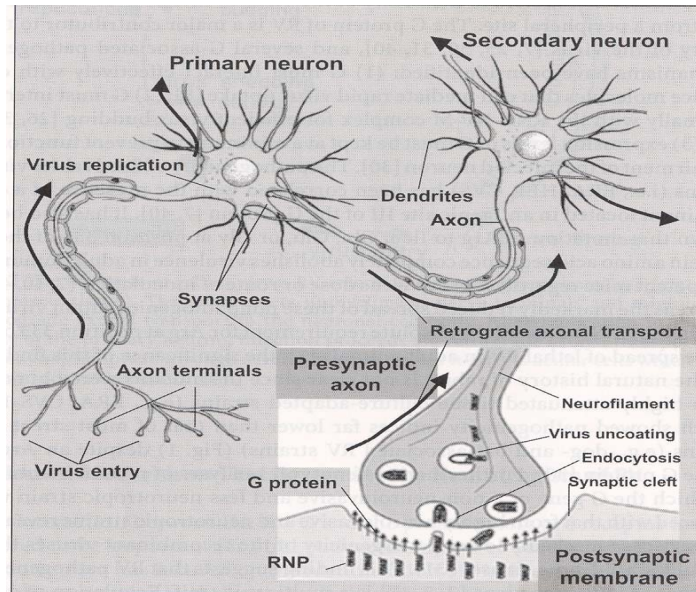
in cool weather conditions, they may persist for weeks<sup>16</sup>. The viruses are readily inactivated by heating at 56°C, exposing to UV light, treating with organic solvents and detergents (1% sodium hypochlorite, 2% glutaraldehyde, 70% ethanol, formaldehyde etc), and by exposing to extreme pH<sup>19</sup>.

### 3.2 Pathogenesis

The average incubation period of rabies in humans is 20-90 days<sup>18</sup>, but may vary from very short (7 days) to very long periods (6 years)<sup>1, 3, 18</sup>. The duration of incubation depends on the following factors<sup>1, 3, 18</sup>:

- Site of exposure: Exposures involving highly innervated areas of the face, neck, hands, and especially the fingertips lead more frequently to infection and to shorter incubation periods.
- Age and immunity status of the host: The incubation period is shorter in children and immunocompromised people.
- The genotype of rabies virus: Street virus strains are more lethal<sup>15</sup>.
- The viral load that has infiltrated the wound site(s)

The virus is neurotropic and neuroinvasiveness is the major defining characteristic of a classic rabies virus infection<sup>15</sup>. Following inoculation of virus-laden saliva into wound or mucosal surfaces, the virus either replicates on the site or directly invades the neurons (figure 6). Then via the peripheral nerves, the virus migrates by retrograde axoplasmic flow, at the rate of about 15–100 mm per day, to the central nervous system (CNS)<sup>1</sup>. Following multiplication in the cell body of the primary neuron, infection continues by retrograde axonal transport and the transsynaptic spread through several neurons before it infects salivary gland cells which release the virus into the oral cavity<sup>15</sup>. The shedding of rabies virus in saliva generally coincides with, or slightly precedes, the onset of clinical disease<sup>1, 3</sup>. Then neuronal dysfunction in rabies is caused by drastic inhibition of synthesis of proteins necessary for maintaining the neuronal functions<sup>15</sup>. Death occurs within 1–5 days of the development of neurological signs, usually due to cardiac arrest and respiratory failure<sup>5, 18</sup>. The numbers of carrier state animals or humans with subclinical infection are very negligible. To date, six persons have survived rabies virus infection<sup>33</sup>. Also, a few numbers of dogs which produced rabies in humans, and were shedding the virus, have been found to be normal even after years<sup>34</sup>. Similarly, a significant number of wildlife reservoirs have been found to be carrying subclinical infection<sup>16</sup>.



**Figure 6 Transsynaptic spread and retrograde axonal migration of rabies virus (from Dietzschold, 2005<sup>15</sup>).**

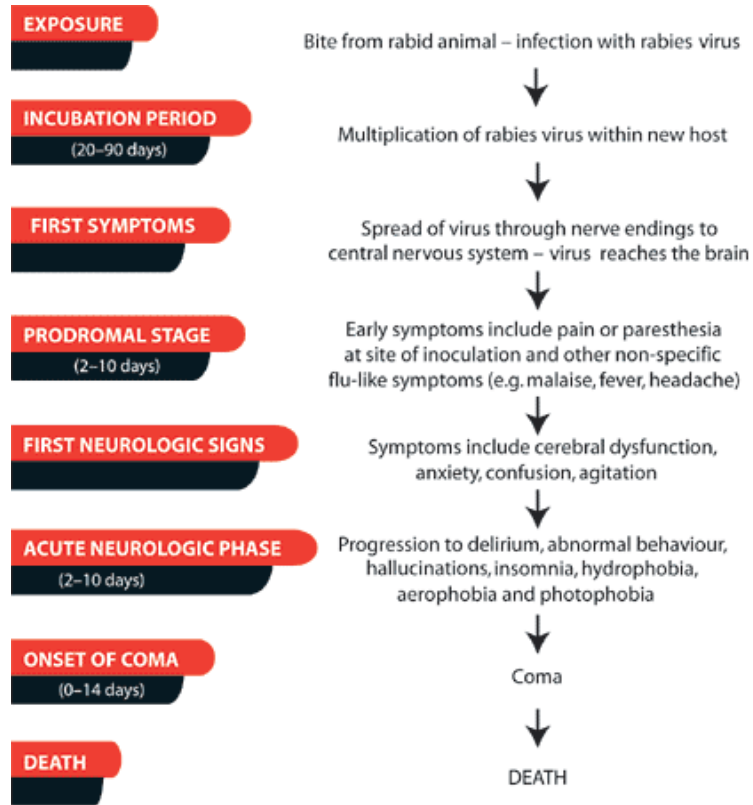
### 3.3 Clinical manifestations

The clinical symptoms vary with the species of infected animal. However, the symptoms like; low-grade fever, lack of appetite, paraesthesias, ataxia, anxiety, altered mentation, paralysis, coma, and death- are common for all species<sup>2</sup>.

#### 3.3.1 In Humans

The initial signs of rabies in humans are non-specific, but usually involve paraesthesias, generalized fatigue, and flu-like conditions<sup>33</sup>. Within days, the disease progresses to an acute neurologic phase with common symptoms being cerebral dysfunction, anxiety, confusion, agitation, progressing to delirium, abnormal behaviors, hallucinations and insomnia<sup>18, 26, 35</sup>. As the disease progresses further, two major clinical presentations- furious form (classic), and paralytic form (dumb) - are evident<sup>1</sup>. These symptoms last approximately 2-10 days and, once they develop, death is certain<sup>18</sup>. Furious form of rabies is seen in about 80% of the humans, and paralytic form in the remaining 20%<sup>18</sup>. Hydrophobia is the most common manifestation in furious form of rabies in humans, followed by aerophobia, photophobia, hyperactivity, abnormal behavior and convulsions. In paralytic form, as a result of paralysis of the laryngeal muscles, speech will be inhibited. Excitation is minimal and phobic spasm occurs in a small number of victims<sup>5</sup>.

The series of events in rabies leading to the clinical manifestations and death in humans is shown in figure 7.



**Figure 7 The infectious progression of rabies (from Anon, 2004<sup>18</sup>)**

### 3.3.2 In dogs

The average incubation period of rabies in canines is 3-8 weeks, but may range from 10 days to 6 months<sup>34</sup>. The clinical course may be divided into three phases - the prodromal, the excitative, and the paralytic phase. The latter two phases correspond with the ‘furious’ and ‘dumb’ form of rabies.

The prodromal phase, which lasts for 2-3 days, starts with fever and loss of appetite. This will be followed by change in temperament –becoming more affectionate or more irritable than usual<sup>34</sup>. The symptoms observed in excitative phases are similar to those seen in furious form of rabies in humans with one exception, i.e., hydrophobia is not commonly observed in dogs<sup>13</sup>. As the animal becomes excessively vicious and restless, it may wander around aimlessly, biting whatever comes in its way. Towards the end of this stage, incoordination of limbs occur due to convulsive seizures. The furious form of rabies is seen in about 25% of the cases<sup>13</sup>. Some dogs die during this furious/excitative form of rabies, and those that survive, enter into the

paralytic/dumb form. The manifestations of paralytic form of rabies are the results of paralysis and incoordination of major muscles. The characteristic signs include dropped jaw and drooling of saliva, and alteration of voice referred to as the 'rabies howl'. However, these clinical manifestations (in the paralytic form) are not commonly observed, making clinical diagnosis difficult<sup>34</sup>.

## **4 Laboratory diagnosis**

Rabies can't be diagnosed in animals or humans before the onset of clinical symptoms<sup>18, 36</sup>. But once the clinical signs become evident, diagnosis can be done on the basis of clinical symptoms coupled with history of exposure and laboratory tests<sup>2</sup>. Laboratory tests for rabies may be undertaken for three purposes: (1) diagnosis in humans; (2) diagnosis in animals; and (3) screening of an animal population for epidemiological studies. Antemortem diagnosis is done mostly in humans, and for epidemiological studies in animals, whereas postmortem diagnosis is useful all three purposes. Postmortem tests to identify rabies in animals are mainly useful for making decisions of postexposure immunization in the exposed humans; and for understanding the disease epidemiology which facilitates stopping of further human infections.

The available laboratory test for rabies can be grouped under the following five headings:

- 1) Immunological methods: E.g. Fluorescent antibody test (FAT), Enzyme linked immunosorbent assay (ELISA), Virus neutralization test (VNT), and Latex agglutination test (LAT),
- 2) Histopathology: E.g. Demonstration of Negri bodies, Immunohistochemistry
- 3) Molecular methods: E.g. Reverse transcriptase PCR
- 4) Virus isolation methods; followed by inoculation in cell lines or mouse brain,
- 5) Electron microscopy (EM).

The specimens for antemortem diagnosis in humans include serum, tear, cerebrospinal fluid, saliva, and tissue, such as skin rich in nerve supply<sup>2, 5</sup>. The most important specimen for postmortem examination of rabies in humans and animals is the brain tissue as the virus reaches its highest titer in the brain<sup>18</sup>. None of the above listed tests should be considered definitive when done alone<sup>18, 32</sup>.

#### 4.1 Direct fluorescent antibody test (DFA)

Direct fluorescent antibody test (DFA) involves the demonstration rabies virus antigen in the tissues examined<sup>18</sup>. Fluorescently-labelled anti-rabies antibody - e.g. with fluorescein isothiocyanate (FITC) - is incubated with rabies-suspect brain tissue, allowing the binding of antibody with the antigen. Unbound antibody will be washed away. In positive tests, areas where antigen was present can be visualized as fluorescent-apple-green areas using a fluorescence microscope<sup>2</sup> (figure 8). This test is performed on the brain tissue of the rabid animals postmortem. Routinely this test is performed on animals suspected of being rabid. Either fresh or frozen brain tissues may be used with similar sensitivity and specificity<sup>37</sup>. The thalamus, hippocampus, cerebellum, medulla oblongata, pons and medulla are the most reliable parts of the brain that are recommended to include in DFA tests<sup>10, 38</sup>. This method has been standard test over the last 40 years, and is still considered to be of the gold standard by the OIE, WHO, and the CDC<sup>1, 10, 14</sup>. The sensitivity of DFA test is 98–100%<sup>10</sup>. Several other tests are standardized by comparing against DFA test.

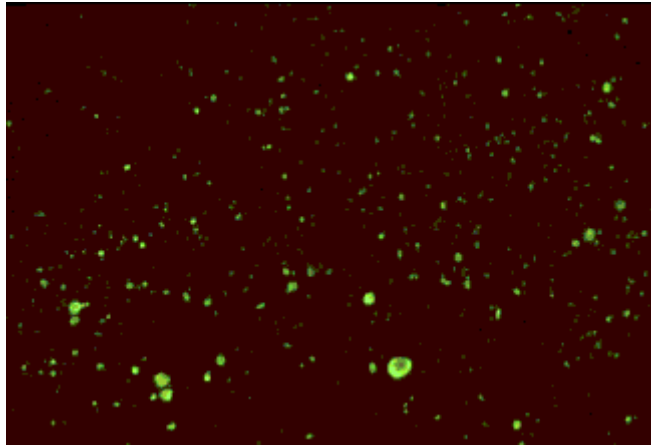


Figure 8 Diagram showing positive DFA test (from CDC, 2005<sup>14</sup>).

#### 4.2 Histological methods

Two routinely used histological methods are demonstration of Negri bodies, and Immunohistochemistry. The first test involves the demonstration of infected neurons by revealing the Negri bodies in the cytoplasm of neurons<sup>10</sup>. When brain tissue from rabies virus-infected animals are stained with a histologic stain - such as Seller's stain, hematoxylin or eosin - evidence of encephalomyelitis may be recognized<sup>14</sup>. This test was used, along with clinical signs, in the past to make confirmative diagnosis of rabies. However, since the sensitivity of this method is very low, about only 50%, it is no longer recommended for use<sup>10, 14</sup>.

Immunohistochemistry (IHC) involves the use of specific antibodies to detect rabies virus inclusion in formalin-fixed tissues, and is more sensitive than histologic staining methods like demonstration of Negri-bodies<sup>14</sup>. Typing of virus variants may also be done by IHC using specific monoclonal antibodies<sup>14</sup>.

### **4.3 Virus isolation (VI) methods**

In these methods, suspected samples- usually saliva or oral swabs- are inoculated onto suitable culture medium, and the virus obtained is further characterized to confirm the results of other tests. There are two commonly used VI techniques; Mouse inoculation test (MIT), and Cell culture test (CCT). Both of these methods are efficient enough for detecting small amount of virus<sup>1</sup>.

**MIT** involves intracranial inoculation of mice. Specific pathogen free (SPF) mice of the age 3-4 weeks old are generally recommended to use<sup>10</sup>. When a more rapid result is necessary, suckling mice (less than 3 days old) are preferred as they are more susceptible to rabies virus<sup>1</sup>. The observation period of about 28 days may be shortened by DFA test of brains of inoculated mice killed 3-4 days after inoculation<sup>1</sup>.

**CCT** involves inoculation of virus on to cell cultures (usually neuroblastoma cells). Commonly used cell lines are Murine neuroblastoma (NA C1300) cells as they are more susceptible to field isolates of rabies virus<sup>1</sup>. The cells are grown in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal calf serum (FCS), incubated at 36°C with 5% CO<sub>2</sub><sup>10</sup>. A monolayer culture of susceptible cells is inoculated with the suspected infective material. DFA test done after incubation period of 18 hours to 4 days will demonstrate the presence or absence of viral antigen. The sensitivity of CCT in neuroblastoma cells is higher than 98%, and some false negative results may be obtained with decomposed brains<sup>1</sup>. Wherever possible, virus isolation in cell culture should replace MIT as CCT is less expensive and gives more rapid results, and is also as sensitive as MIT<sup>10</sup>.

### **4.4 Molecular methods**

Several reverse transcriptional-polymerase chain reaction (RT-PCR) based amplification methods have been developed in the recent years to detect the rabies virus RNA from a wide range of samples like saliva, CSF, tears, skin biopsy sample, urine, or brain tissue<sup>2, 39-41</sup>. In spite of being highly sensitive and specific, as these methods demand stringent quality control and

may produce false positive or false negative results, they are recommended to be used along with other conventional tests, for routine diagnostic purpose<sup>1, 2, 42</sup>. These molecular methods are mainly useful for epidemiological surveys as these tests can identify the geographical origin and reservoir host of a particular virus isolate, and also distinguish between field and vaccine strain<sup>1, 42-45</sup>. Molecular method like in situ hybridization (ISH) has been developed for the viral typing of formalin-fixed rabies virus-infected brain tissue<sup>46</sup>. Some other PCR-based techniques (TaqMan PCR) can even detect the concentration of infectious virus<sup>47</sup>. The usefulness of PCR-based methods, compared to DFA, in diagnosis of rabies and typing of the virus is limited by several factors in most situations. These are like the need for universal primers for all lyssaviruses, the need to sequence the PCR products, the cost of the equipments and expertise, and the need for stringent quality control<sup>2</sup>.

#### **4.5 Serological methods**

These tests involve the demonstration of antigens or antibodies in the body-fluids of the infected animals, or antigenic characterization of the isolated virus. Serological tests can't be used for routine diagnostic purposes as the seroconversion occurs lately, and moreover, the rabid animals rarely survive<sup>10</sup>. They are however useful for sero-prevalence surveys and to evaluate the immunization programs in animals<sup>10</sup>. Even in surveillance studies, the demonstration of antibodies merely reflects past exposure to the virus and is thus not a valid indicator of active infection. Monoclonal antibodies are commonly used for antigenic characterization of isolated viruses, which may reveal the geographical and temporal distribution of rabies virus variants<sup>45</sup>. Virus neutralization (VN) tests like the rapid fluorescent focus inhibition test (RFFIT) or the fluorescent antibody virus neutralization (FAVN) test can be used to measure the neutralizing antibodies in the serum or CSF of non-vaccinated patients<sup>1</sup>. It takes about 8 days for the virus-neutralizing antibodies to appear in serum (1).

Several enzyme-immunoassay (EIA) based methods have been developed over the past years, and are commercially available. ELISAs like rapid rabies enzyme immunodiagnosis test (RREID) allows quantitative detection of rabies antibodies in individual dogs and cats serum samples following vaccination<sup>10</sup>, and their results are highly correlated with DFA test, i.e. 95%-99%<sup>10</sup>. A latex-agglutination based test has been developed for detection of rabies virus antigen in dog saliva antemortem<sup>48</sup>. This test is said to be highly sensitive (95%) and specific (99%) compared to the result of DFA test.

## 5 Rabies in Nepal

Nepal is a small landlocked country of south Asia situated between India and China, at 80° 04'E to 88° 12'E longitude and 26° 22' N to 30° 27' N latitudes. It has an area of 14.7 million hectares and is rectangular, extending from east to west. The human population is about 23 millions. According to the FAO statistics of the year 2002, there are approximately 7.0 million cattle, 3.6 million buffaloes, 6.5 million goats, 0.9 million pigs, and 0.8 million sheep in Nepal. There are 347 registered veterinarians working currently in Nepal, most of them on governmental services. Three geographical variations are found in the country: the northern mountainous belt, the middle hills and the southern plains called the Terai belt. Politically, the country has been divided into five administrative regions; Far-western, Mid-western, Western, Central and the Eastern region. Among the four borders, three of them, namely, southern, western and eastern borders share that with India. Since there is no strict restriction of animal or human movement across the border between Indian and Nepal, and the ecological and socio-economic conditions are identical between these countries, it is believed that the epidemiological status of rabies is also similar. Similar situation of animal movement exists along the border with China, however only in limited territories.



**Figure 9** Map of Nepal showing sharing of border with India in East, West and South and with China in the north.

The current statistics of incidences animal bites and human rabies, rabies in animal population are scanty, unreliable and controversial due to poor surveillance and reporting system.

Recently an extensive study was conducted to assess the burden of rabies in India<sup>9</sup>. Believing  
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that the situation might be similar in the neighboring county, relevant information has been extrapolated from that study.

### 5.1.1.1 History

Rabies has been endemic in the Indian Subcontinent since time immemorial. The word “mad-dog” has been mentioned in the Mahabharata <sup>49</sup>—one of the oldest Sanskrit epic- which dates back to around 3300 BC. It is generally accepted that the word “rabies” is derived from the Sanskrit word "rabhas" which means "to do violence" <sup>50</sup>. No information is available regarding when rabies was first detected in Nepal. The earliest available record mentions that 625 people had received antirabies treatment in 1958; and in 1965, a leopard cub imported into Britain was found to be rabid <sup>17</sup>. Only after 1990s, official reporting has been started<sup>51</sup>. Since then, the country is witnessing a significant number of human and animal deaths annually, and in the later years, the overall trend has not changed much <sup>7</sup>.

### 5.1.1.2 Human rabies Incidence

More than 90% of approximately 150 human rabies deaths each year in Nepal are the result of bites by unvaccinated dogs <sup>7</sup>. Out of the remaining 10%, 4% exposures are through wild carnivores and 2% through other domestic animals. On an average, about 30,000-35,000 people receive postexposure immunization in Nepal annually <sup>7, 51, 52</sup>. According to some unofficial reports, every month, 350-400 cases of animal bites are reported at the Sukraraj Tropical and Infectious Disease Hospital at Kathmandu, and more than 250 of them being dog bites. The incidence of dog bites is estimated at 8 per 10000 people <sup>53</sup>. The number of animal-bite victims who are not given any postexposure prophylaxis is unknown, but is believed to be large.

Quoting the secretary of Health, it was stated in “The Rising Nepal” that around 15 people each year die of rabies in Kathmandu. There are frequent reports of public life being terrorized when a rabid dog or jackal bites a lot of people at once. Table 3 shows such recent incidents of animal bites published in the leading newspapers of Nepal. Table 2 shows the officially reported human rabies cases in Nepal. As mentioned earlier, these officially reported cases hardly depict the real burden which is believed to be 10-20 times higher than the reported figures.

**Table 2 Incidence of human rabies in Nepal, 1991-2003 (data source Gongal and Rai, 2001<sup>7</sup>, <sup>51</sup>; RabNet, 2003<sup>51</sup>).**

Year	Reported cases (total deaths)	Source of exposure		Postexposure treatment received cases
		Dog	Cat	
				--

1991	23	--	--	--
1992	30	--	--	--
1993	51	--	--	--
1993	49	48	--	22,258
1994	49	--	--	--
1995	22	--	--	--
1995	34	34	--	17,929
1996	29	--	--	15,832
1997	--	--	--	20,020
1998	155	150	4	35,000
1999	163	159	1	35,000
2000	--	--	--	24,998
2001	--	--	--	17,231
2002	--	--	--	--
2003	44	--	--	14, 642

**Table 3 Recent incidents of animal bites and rabies cases covered in leading news papers**

Date	Region	Exposure	Details	Source (Newspapers)
March 13, 2001	Mid-western	Dog bite	A 30 yr old man died, 55 others bitten	The Kathmandu Post www.kantipuronline.com
June 22 2001	Eastern	Jackal bite	Jackal bit 45 people and 10 animals	The Kathmandu Post
October 21 2002	Central	Dog bite	3 people- father, son and mother-died from a same family, more than 25 people died since last July	The Rising Nepal www.gorkhaparta.org.np
June 20 2003	Western	Meat	10 buffaloes died, people consumed the meat, later 125 of them were given PET, no death in humans	Jha et al., 2003 <sup>54</sup>
January 07 2004	Eastern	Milk	About 60 people drank unpasteurized milk from a rabid cow, all were given PET <sup>1</sup>	Nepanews.com www.nepalnews.com
March 30 2004	Eastern	Probably dog	Seven cattle died, 55 rabid-suspected dogs killed	The Kathmandu Post www.kantipuronline.com
June 21 2004	Eastern	Dog bite	60 people were bitten, receive PET	Nepali Times www.nepalnews.com
February 23 2005	Central	Dog bite	50 people were bitten, 30 livestock dead	The Rising Nepal www.gorkhaparta.org.np
	Central	Monkey	A lot of people bitten by monkey (number not known), 20 received PEP	www.environmentnepal.com
August 17 2005	Eastern	Dog bite	3 people, 21 cattle, and a large number of goats and dogs died, 65 people were bitten	The Rising Nepal www.gorkhapatra.org.np

### 5.1.1.3 Risk groups

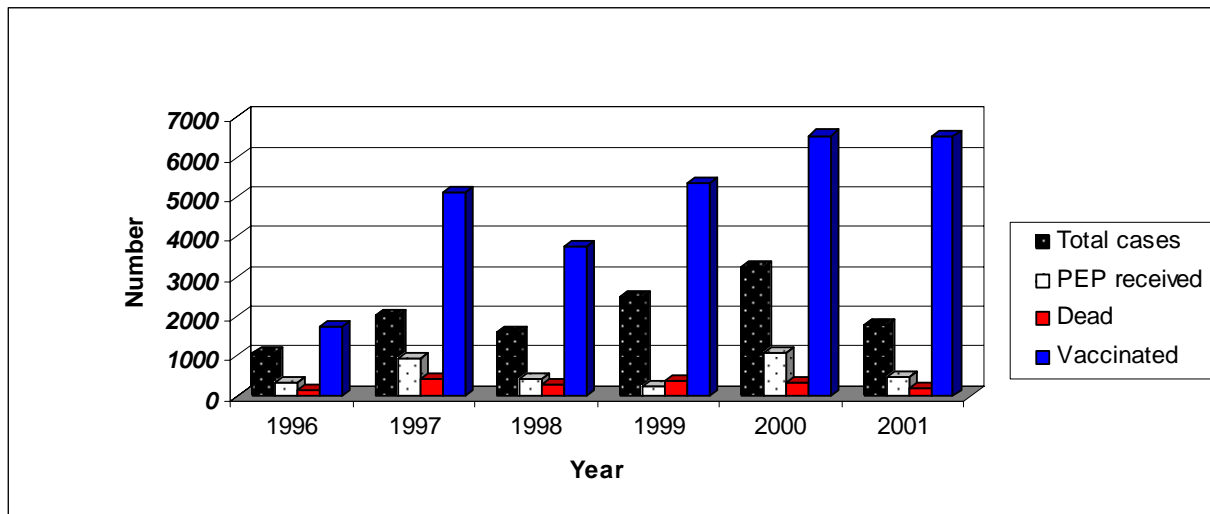
Regarding risks of dog bites in Nepal, children are the most vulnerable age groups (age <15), and males are more prone than females (4:1)<sup>35, 51</sup>. Low income people are most frequently bitten by dogs. Common biting sites are the lower limbs (56.2%), upper limb (20.9%) and hands (17%)<sup>9</sup>.

<sup>1</sup> Post-exposure treatment

A high proportion of victims seek treatments from traditional healers. Tourists visiting Nepal are also considered as potential risk group for exposure to rabies<sup>55</sup>.

#### 5.1.1.4 Animal reservoirs and virus ecology

Dogs and cats are the main reservoirs of rabies virus in Nepal, and other animals like cattle, buffalo, horses, sheep and goats are also found to be affected<sup>17, 51, 54</sup>. Dog bites are still the most common form of animal bites and canine rabies is the major epidemiological pattern of the disease in Nepal<sup>56</sup>. As in most parts of the world, the virus is maintained as two different epidemiological entities: urban rabies, and sylvatic rabies<sup>45</sup>. Both of these cycles are overlapping in Nepal<sup>51</sup>. In the whole Indian Subcontinent, it has been well-established fact that more than 90% of the human rabies cases are due to bite of rabid dog<sup>1, 2, 9</sup>. In Nepal too, dog is the principal reservoir and transmitter of the urban cycle, whereas no particular animal species has been identified as maintaining and as transmitter of the sylvatic cycle. It is not known whether there are separate sylvatic rabies cycles in Nepal. Figure 10 shows the cases of animal rabies and animal vaccinations in Nepal.



**Figure 10 Incidence of animal rabies and animal preexposure vaccination in Nepal, 1996-2001 (data source RabNet, 2003<sup>7</sup> and personal communications).**

Other domestic animals identified as reservoirs of rabies virus in India (by DFA test)<sup>9</sup> are cat, cattle, sheep, goat, horse, pig, monkey, and camel. Donkeys are also suspected to play a role of reservoir. Wild animals reservoirs identified in India are mongoose, jackal, fox, deer, bear, wolf, and lion. Tigers, leopards and cheetah are also suspected to act as reservoirs. In Nepal, besides

dogs, cats, mongooses, and jackals are also found to act as the source of human exposures<sup>51</sup>. In a few instances, cattle, buffalo and monkey have also been implicated as a source of exposure<sup>54</sup>. The role of wild animals in maintaining rabies virus in Asia has not been clearly established<sup>57</sup>. The importance of wild animals in the maintenance and spread of the infection has not been seriously studied.

#### **5.1.1.5 Dog population and ecology**

The dog population in Nepal, as in most of the Asian countries<sup>57</sup>, comprises of three groups; owned dogs (dogs with owners), community dogs and stray dogs. A large number of stray dogs occur in Sub Indian continent, mainly in the big cities, besides community owned dogs. The estimated dog population in Indian is 25 millions<sup>57</sup>. Majority (62.9%) of the biting dogs in India are stray dogs. In one survey done in Kathmandu valley- the capital city of Nepal-, human to dog population ratio was found to be 1:4.7 and the stray dog density was 2,930 stray dogs/km<sup>2</sup><sup>58</sup>. It has been mentioned by Gongal and Rai<sup>51</sup> that 70-80% of the total population of dogs in Nepal are stray, and 90% of these stray dogs are found in the city areas. The reason may be due to abundance of garbage on which they survive.

Corresponding with the ongoing Maoist insurgence and displacement of people in Nepal, the number of abandoned dogs is on the rise<sup>52</sup>. This is increasing the risk of human exposure to rabid dogs, as with increased population there, will be a more regular supply of susceptible hosts, which enables the host to carry viruses with shorter time of infection and higher virulence (cited in Hyun et al.<sup>11</sup>). Similar fear has been observed in Sri Lanka aftermath of Tsunami as the number of stray dogs has increased dramatically<sup>5</sup>. There are some other examples mentioning human displacement contributes to increase in the number of stray animals<sup>32</sup>.

#### **5.1.1.6 Spatiotemporal distribution**

Although rabies has been reported from the entire territory of Nepal, the distribution is not uniform. The densely populated urban areas in the Terai belt and Midhills are frequently reporting rabies<sup>56</sup>, where stray dog are mainly responsible for disease transmission. On the other hand, sylvatic rabies is more common in the regions (Parsa, Chitwan, Nawalparasi, Bardiya, Kanchanpur) having national parks<sup>51</sup>. Table 3 lists the recent incidents of human exposure to rabies in different part of Nepal.

Rabies was found to be endemic throughout the year in most parts of India<sup>59</sup>. Similar situation is believed to exist in Nepal. However, Gongal and Rai mention that the incidences of dogs bites

are higher in Nepal during autumn season corresponding with their breeding season<sup>51</sup>. The higher number of dog bites during this time period may also be due to increased contact of people with dog for adorning them (in “dog festival”).

### **5.1.1.7 Molecular epidemiology**

To date, none of the viruses isolated from Nepal, neither from humans nor from animals, has been typed or genome is sequenced. There is little information on the genomic sequence of virus prevalent in Nepal. In one instance, rabies virus was isolated from a US citizen who was bitten by a dog while visiting Nepal<sup>60, 61</sup>. After returning to the US, the person fell sick and the disease was diagnosed to be rabies. Phylogenetic analysis of the isolated virus revealed that it was closely related to the virus type prevailing in India<sup>62</sup>. Gongal and Rai<sup>51</sup> however have mentioned that the strain circulating in Nepal resembles to arctic fox strain. As compared to the African countries, the rabies viruses prevalent in the Asian counties are less diverse<sup>62</sup>, which is believed to be due to difference in the pattern of livestock movement/farming system in these continents. These clues suggest that restrictions to domestic animal movement can be exploited in rabies-control efforts in Asia.

### **5.1.1.8 Diagnostics available**

Commonly used laboratory techniques for rabies diagnosis in humans in Nepal are the DFA, MIT, and histopathological staining techniques. The latter two techniques are also used for animal rabies diagnosis. DFA test for rabies diagnosis was introduced at the central level at the end of 1996. Due to the lack of trained manpower and facilities, most animal rabies cases at the regional level are diagnosed only clinically<sup>51</sup>.

## **6 Prevention and Control**

Rabies is an incurable disease after the onset of clinical illness, **yet is preventable**. As almost >90% of human rabies is acquired via dog bite, control of infection in this species of animal is of special importance. Prevention of rabies in humans may be achieved by prevention of rabies in reservoir hosts, proper administration of pre- and postexposure immunizations, and by education of the general public about the disease.

### **6.1 Animal rabies control**

Successful rabies controls have been achieved in a number of countries of the Western Europe, Japan, North America, and many areas in South America<sup>1</sup>. The WHO has recommended that

canine rabies control programs should incorporate the three basic elements, with priorities varying according to the local conditions. These elements are: (1) epidemiological surveillance; (2) mass immunization; and (3) control of dog population. The preliminary results of dog control programs (in the countries like Argentina, South Korea, India and Sri Lanka) which involves combination of parenteral vaccination, oral vaccination, and sterilization of owned dogs- are encouraging<sup>57 63 11 45</sup>.

### 6.1.1 Immunization campaign

Effective animal vaccines that offer a significant duration of immunity have been developed and mass parenteral immunization programmes remain the basis of canine rabies control<sup>1</sup>. Main concern of the developing countries, where most of the canine rabies occurs, is presence of large number of stray dogs which are not easily accessible for vaccination, resulting in low vaccination coverage. Low vaccination coverage is also due to insufficient community participation as well as lack of resources<sup>63, 64</sup>. However, it has been revealed that, in these countries, at least 60-75% of the dogs can be accessed for vaccination which will be sufficient to control canine rabies<sup>1, 65, 66</sup>. The WHO has recommended that, for mass parenteral vaccination campaigns, only inactivated and adjuvanted rabies vaccine should be used<sup>1</sup>. The vaccination campaigns can be made more effective by coupling of parenteral vaccination in the accessible reservoir hosts along with oral vaccination in the inaccessible animals like the stray dogs and the wild carnivores. Oral immunization is done by administration of bait-laden vaccines to the reservoir hosts. To date, seven European have eradicated rabies from wild animals by oral vaccination programs<sup>1</sup>. The available parenteral and oral vaccine types for use in animals are listed in the following table.

**Table 4 Vaccines for animal use**<sup>1, 67-69</sup>

Vaccine	Description
<b>Injectable inactivated animal vaccines</b>	
Nervous tissue vaccine	Produced from the brains of lambs or suckling mice, produce some adverse reactions, being replaced with cell-culture vaccines
Modified live-virus vaccines	Not recommended for parenteral immunization, rabies infection may occur as a result of the vaccine strain
Cell-culture vaccines	Most preferred vaccine for dog immunization
Combined vaccines	Combined with other disease vaccines like canine distemper virus, canine adenovirus type 1, <i>Leptospira</i> and canine parvovirus.
<b>Oral animal vaccines</b>	
Modified live-virus vaccine	Several types have been developed for oral immunization of wildlife, e.g., SAD, SAG strain rabies virus vaccine

Live recombinant vaccines	Developed by inserting glycoprotein gene of rabies virus (VRG) into vaccinia virus, stable in high temperatures (56°C), more than 75 million doses has been used for fox rabies control in Europe
DNA vaccines	Not available commercially. Trials being done with promising results, for parenteral or oral route, found to be stable, inexpensive to produce, easy to construct and induce a full spectrum of long-lasting humoral and cellular immune responses

The immunization method of canine rabies control suffers from a few serious drawbacks. The periodic vaccination campaigns are not sustainable because of rapid population turnover of stray dogs. Also, a single vaccine injection may not result in long lasting virus neutralizing antibodies<sup>64</sup>. As the very young dogs cannot be vaccinated against rabies but are in more close contact with humans, especially children, they continue to remain risk of exposure. Also the costs associated with periodic vaccinations cannot be borne by the least developed countries that are suffering from rabies the most. The drawbacks of oral-vaccinations are its degradation in hot climates<sup>64</sup>, and safety issues to non-targeted species, including humans. R

### 6.1.2 Dog population management

According to the WHO, dog population for rabies control can be managed by combination of three strategies. These are: (1) control of reproduction; (2) control of habitat; and (3) restriction of movement. Surgical sterilization is believed to work best when supplemented with immunization campaigns. The main rationale of coupling of surgical sterilization with immunization is that there will be reduction of population turn-over, and also individuals susceptible to rabies. Also sterilization alters the behaviour of male dogs limiting their mobility and aggressiveness which would otherwise facilitate the spread of rabies<sup>1</sup>. Although not yet fully evaluated, the preliminary results of artificial birth control strategies are found to be encouraging in canine rabies control<sup>1</sup>.

Stray dog population may also be reduced by limiting their food availability. As these dogs feed mostly on garbage wastes of urban areas, proper management of garbage disposal system making it inaccessible to dogs would greatly help to reduce the population of stray dogs<sup>65</sup>.

One controversial strategy is mass slaughter for reducing the population of dogs. Although some countries like Singapore eradicated dog rabies in the past (1892) by mass slaughter<sup>13</sup>, this method is no longer acceptable for ethical, ecological and economical reasons<sup>70</sup>. Elimination of dogs alone does not have a significant impact on dog population densities or the spread of rabies. The population turnover of dogs may be so high that the dog population is easily compensated by

increased survival rates, immigration from the periphery, and increased fecundity of adult dogs<sup>1, 65</sup>. This method – mass killing alone- is still in practice in most of the rabies endemic countries where strychnine hydrochloride is used most often for killing. This strategy, if used for humane killing of a small proportion of unvaccinated dogs may prove useful, when coupled with mass vaccination<sup>1</sup>.

### 6.1.3 Control in wild animals

Although, number of humans acquiring rabies directly from wild animals is very low, these animals may serve an important role of maintaining the sylvatic cycle of rabies, and spreading the infection to urban cycle. Surveillance for identification of important wild animal reservoirs and their immunization by bait (oral) vaccines is the recommended strategy which has been successful in a number of European countries<sup>1</sup>

### 6.1.4 Surveillance

Rabies surveillance plays an important role in the planning, implementation and evaluation of rabies control programmes. Because of the lack of effective surveillance programs, the public health impact of rabies is highly underestimated in the developing world<sup>1</sup>. Even in the countries like India where highest annual deaths occurs due to rabies, active-surveillance system for rabies hardly exists, and rabies is not a notifiable disease<sup>9</sup>. This underreporting resulting in underestimation is a major factor in the low level of political commitment to rabies control.

According to the WHO, the surveillance programs for assessing the impact of rabies should include: estimation of incidence in humans and animals, temporal and spatial distribution of cases by species, proportion of cases diagnosed and quality of diagnosis, number of human PETs and their costs, and rabies-control costs in dogs<sup>71</sup>. Active surveillance systems are necessary for estimation of incidence in humans and animals. The passive-surveillance data are highly underestimating the true incidence and burden of rabies. In one study, it was found that the incidence of canine rabies estimated from passive-surveillance was approximately 72 times less than the estimate from active-surveillance<sup>72</sup>.

Population estimation is crucial for designing control programs and for estimation of the costs involved. Dog population may be estimated by standard methods like the “Capture-Mark-Reobservation” technique<sup>73</sup>. Serological surveillances like the MIT, RFFIT, and FVAN can be done periodically to monitor the antirabies antibody titer for the evaluation of vaccination programs<sup>11, 74</sup>. Antigenic and genetic typing of virus isolates should be done to determine

epidemiological patterns and the source of infection in cases occurring before, during and after vaccination campaigns <sup>1</sup>.

## 6.2 Human rabies prevention

In spite of being incurable after onset of clinical symptoms, postexposure immunizations initiated at an early stage is generally 100% effective in preventing death in humans <sup>18</sup>. Rabies is a vaccine preventable disease and immunization can be done before or after the exposure. Immunization may be achieved by using two types of biologicals: rabies vaccines and rabies immunoglobulins (RIGs) (Table 5).

**Table 5 Rabies vaccines and immunoglobulins currently available for human use** <sup>1, 2, 18, 75</sup>

Name	Description
<b>Rabies Vaccines</b>	
Nerve-tissue vaccines (NTVs)	Inactivated vaccine prepared from goat or sheep brain (Semple), or from mouse brain (Fuenzalida), have adverse effects (in an estimated 0.3 to 0.8 per 1000 cases), not suitable for pre-exposure immunizations, being phase out by 2006, in use in most of the rabies endemic Asian countries
Human diploid cell vaccines (HDCV)	First-generation modern cell-culture rabies vaccine, virus grown on human diploid cells, good immunogenicity and tolerability, for both purposes: pre- and post-exposure immunization
Purified Vero cell rabies vaccines (PVRV)	Second-generation modern cell-culture rabies vaccine, virus grown on monkey kidney cells, good immunogenicity and tolerability, unknown risk of oncogenesis
Purified chick embryo cell-culture vaccine (PCECV)	Second-generation modern cell-culture rabies vaccine, prepared in primary chick embryo cells, good immunogenicity and tolerability, high purity and potency, approved by the US FDA
<b>Rabies Immunoglobulins (RIGs)</b>	
Human rabies immunoglobulins (HRIGs)	Use for passive immunization, prepared from pooled serum from rabies virus-immune human donors and horses, should be used concomitantly with rabies vaccines for PEP following exposure, are expensive and may be in short supply or non-existent in most developing countries, some individuals may be allergic
Equine rabies immunoglobulins (ERIGs)	

### 6.2.1 Preexposure immunization

Preexposure immunization has been recommended for people who are at increased risk of exposure with rabies virus and animal bites, like the veterinarians, zookeepers, laboratory staffs working with rabies, vaccine producers, wildlife workers and travellers to rabies endemic countries (1,2,14). Cell culture or embryonated egg vaccine has been recommended for

preexposure immunization (1). The nerve-tissue vaccine (NTV), which is still in use in some rabies endemic countries (76), should not be used because of its high content of myelin and risks of producing severe side effects like neuroparalysis (1 in 5,500 to 1 in 11,000) (1). NTVs may also transmit Creutzfeldt-Jakob Disease (CJD) if made from infected sheep brain (77). The preexposure vaccination schedule consists of administration of three doses of vaccine on days 0, 7, and 21 or 28 (1,2). Persons received preexposure immunization should have a serum sample tested for antibody every 6 months and receive booster vaccine, when necessary. Preexposure vaccination however does not eliminate the need for postexposure prophylaxis after rabies exposure (14). However, the persons may need only few doses of post exposure vaccines.

### 6.2.2 Post-exposure management and prophylaxis

The strategy for post-exposure management of human patients consists of: (1) cleaning of wounds; (2) observation of the animal; and (3) immunization using vaccines and immunoglobulins. The treatment regimen depends on the degree of exposure (table 6).

**Table 6 Guide for postexposure treatment in humans**<sup>78</sup>

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

The WHO and CDC has recommended the following treatment regimen<sup>5, 14</sup>: Treatment should start with thorough cleaning of wound with virucidal antiseptics. This is should be followed by administration of RIG and cell-culture or purified embryonated egg rabies vaccines. This decision about immunization should be made based on extent of exposure (Table 6). The same guideline should be followed for exposures to immunized dogs as vaccination failures may sometimes occur. Immunization schedule comprises of administration of one dose of RIG and five doses of rabies vaccine over a period of 28 days. The first dose of vaccine should be given

along with RIG as soon as possible. The remaining doses of rabies vaccine should be given on days 3, 7, 14, and 28 after the first vaccination.

In some least developed countries, where brain-tissue vaccines are still in use, the treatment regimen constitutes administration of rabies vaccine subcutaneously in seven daily doses with additional doses on days 10, 20, and 90 <sup>2</sup>.

### **6.3 Education campaigns**

The two target groups of rabies education campaign are the general public and the healthcare professionals.

#### **6.3.1 Education of general public**

General public should be educated about fatal outcome of the disease. Dissemination of knowledge about responsible pet ownership, proper method of keeping dogs, and timely vaccinations- is especially important in developing countries. The public may also be educated about proper garbage disposal system making them aware that improper disposal of garbage containing food left-over will indirectly help to increase the population of stray dogs. Also, people should be discouraged the feeding of stay dogs. They should be educated about emergency procedures to be followed after being bitten by a suspected rabid animal, and ways of avoiding animal bites. Since children are more vulnerable, the adoption of health education program as a part of the formal primary school curriculum may be done <sup>58</sup>. Besides, to attain high vaccination coverage in vaccination campaigns, well-designed educational programs that increase public participation are important <sup>1</sup>.

#### **6.3.2 Education of healthcare professionals**

Healthcare professionals should be given regular training on epidemiology and management of animal bites, emphasizing on definition of exposure and urgency of treatment-need for each type of exposures. They should be competent enough to decide whether PEP in a particular situation is necessary to execute. Also training on the proper administration of vaccines is important in developing countries as some deaths occurred recently because of inappropriate treatment or advice of “treatment not needed” <sup>35</sup>. As medical personnel are lacking knowledge on the history of exposure, most of them prescribe PEP in any type of exposures because of the fatal nature of disease. This is adding burden of rabies in developing countries.

## 7 Rabies prevention in Nepal

### 7.1 Control attempts

Rabies has been categorized as an important zoonotic disease prevailing in Nepal<sup>51</sup>. Rabies control activities in Nepal, initiated by the government, include production and distribution of antirabies vaccine and killing of dogs by poisoning. Antirabies vaccine production and animal rabies control activities are assigned to be administered by 'Rabies Control and Public Health Protection Section (RCPHPS)' under the Ministry of Agriculture. Rabies control programs at the national level are assigned to be coordinated by 'The Epidemiology and Disease Control Division' under the Ministry of Health. As yet, however, no rabies control program exists at national level.

Dog rabies control is being undertaken in some big cities by a number of non-governmental organizations (NGOs). These organizations are involved in education campaigns and in the immunization and management of dog population. Their work is however, limited to small areas, and far from producing any significant impact in reducing rabies. The works of these NGOs is providing very low vaccination coverage in dog population and has been largely ineffective in management of stray animals. Also their control programs are not appropriately designed as there is no information on dog population or ecology. Absolute lack of information about wildlife reservoirs is also hindering the designing effective control programs.

Public health authorities in Nepal still believe that rabies control can only be achieved by mass killing of the dogs<sup>56, 79</sup>. So, most municipalities carry out poisoning of dogs once a year or only when a rise in the dog population occurs. Strychnine sulphate is distributed periodically for the elimination of stray dogs endemic districts<sup>56</sup>.

Most of dog control programs have been implemented in Kathmandu- the capital city of Nepal- which is home for a large number of stray dogs. The exact number is known, but it is estimated to be at least 100,000 stray and community dogs occurring in this city alone. Every year 8,000 to 12,000 stray dogs are killed in Kathmandu valley, at the expense of Rs 200, 000 to 300, 000 (1 US\$=70 Rs) for poisoning only. Vaccination coverage in this dog population is very low. In 1999, in a mass vaccination campaign about 15000 dogs were vaccinated accounting for 10% coverage in Kathmandu city. Recently, Gongal<sup>52</sup> reported about 25,000 dogs receive preexposure vaccination annually.

Although there is no legislation for compulsory dog immunization, owned dogs and other livestock are being immunized commonly by Semple vaccines. These brain-tissue vaccines are produced inside the country and contain 20% and 5% carbolyzed sheep brain, and are used, respectively, for preexposure and postexposure immunizations<sup>59</sup>. In the 1990s, the amount of 5% and 20% Semple vaccine produced inside the country was 300 000 ml and 50 000 ml, respectively<sup>80</sup>, and later on in 1999 it was 38,399 doses<sup>81</sup>. Dogs are the most commonly vaccinated species, in which the first vaccination is given at the age of three months. The immunity produced by this Semple vaccine lasts for six months to one year. Modern tissue-culture vaccines are also available for use in dogs, mainly through ‘kennel clubs’.

Rabies outbreaks in animals are reported only in a few districts. In most parts of the country, many outbreaks go unnoticed. Hardly any surveillance system exists.

## **7.2 Treatment practices in humans**

Preexposure vaccinations are received only a small number of people; mainly veterinarians and zookeepers. A significant number of human rabies deaths in Nepal are due to inaccessibility of the postexposure treatments. Although modern vaccines (HDCV, PCEC, PDEV and PVRV) and RIGs are available in the country, Semple-type sheep-brain vaccines are most widely used<sup>51, 82</sup>. This is because these modern vaccines are very expensive and beyond the reach of poor income people who are most common dog-bite victims. In addition, the government provides these neural-tissue vaccines free of cost. Although the WHO is discouraging the use of brain tissue vaccines since it has side effects like local allergic reaction, neuritis, myelitis, post vaccination shock and encephalitis, in both humans and animals<sup>57, 59</sup>, Asian countries including Nepal still manufacture cheap vaccines produced from infected brains<sup>57</sup>. So, in spite of having several adverse reactions and being painful, neural vaccine is still in use. In those persons who received PEP using brain-tissue vaccines, incidences of adverse reactions and deaths due to vaccine failures are occasionally reported in Nepal<sup>83-86</sup>.

Shortages of vaccine are also frequently reported from different parts on Nepal. Although, 5% sheep-brain vaccine inactivated by Beta proprolactone (BPL) is being produced for human postexposure treatment<sup>80</sup>, the amount produced inside the country is not sufficient<sup>84</sup>. In 1999, the amount of sheep brain-tissue vaccine produced in Nepal was 94,175 doses<sup>81</sup>. By 2006, the government is planning to phase-out the production brain-tissue vaccine and introduce the production tissue-culture vaccines.

## **8 Discussion and conclusions**

Rabies is an important but neglected disease in Nepal. No national rabies control program exists since the disease is not considered a serious public health burden by the concerned authorities. This is mainly because of the lack of morbidity and motility data for both humans and animal rabies. So the first step in rabies control would be drawing the attention of national policy makers by presenting them with the real impact of the disease. This is possible only by effective surveillance programs and making rabies a notifiable disease. Effective surveillances demand well established laboratory facilities and expertise, which is lacking in the country. So, the first priority should be given for building of infrastructures and advancement of knowledge about epidemiology of the rabies virus and transmission patterns prevailing. Also the economic feasibility and sustainability of the prospective control program should be evaluated by some pilot surveys.

Studies done in neighboring countries and limited information available from Nepal itself suggest that canine rabies control should be the mainstay of rabies control program for Nepal. For rabies elimination in owned dogs, legislation of compulsory vaccination should be implemented. For the stray dogs, combinations of the strategies like parenteral vaccination, and sterilization of the accessible dogs, oral vaccination or slaughter of the inaccessible population seems to work best. Regarding sylvatic cycle, identification of the reservoir species and their immunizations by oral vaccines may be done.

The goal of rabies control by designing an effective program can only be achieved by multisectorial cooperation between public health and veterinary sectors. Also coordination at international level is necessary. While the open borders are shared with India and China, sustainable control is unlikely to be achieved in Nepal without coordination of control programs with these countries, particularly with India. As the magnitude of rabies burden seems negligible compared to other public health problems, it is highly unlikely in the near future that government of Nepal will initiate a control program. However, if funding is available and the international organizations like the World Health Organization (WHO) and the World Organization for Animal Health (OIE) are willing collaborate with the public health authorities in Nepal, this country may be made rabies-free.

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