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## **Global Perspective of Rabies and Rabies Related Viruses: A Comprehensive Review**

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### **ABSTRACT**

Rabies, a fatal neurological disease of warm blooded animals, is not only a national but also a global problem. It is caused by a RNA virus under the genus *Lyssavirus* and family Rhabdoviridae. The disease is of paramount importance because of its global distribution, wide host range including a number of wild animals and extremely high case fatality rate. In spite of development of anti-rabies vaccine by Pasteur in 1885, the disease is still endemic in about 100 countries in the world where 2.5 thousand million people live. The dog is the main perpetuator of rabies in developing country including India, it is to be emphasized to bring all the dogs under immunization umbrella or to control the unauthorized stray dogs. Over the time, there is lot of development in the field of immunology, vaccinology and diagnostic arena, the disease is still endemic particularly in developing countries. Few countries viz., USA, Canada, France have employed the recombinant vaccinia virus based bait and succeeded in controlling the wildlife rabies to a great extent. However, cooperation and collaboration of people from the different field should work in a coordinated manner to control the rabies in animals particularly the stray dogs, main source of infection to animals and human beings.

**Key words:** Rabies, rabies related viruses, *Lyssavirus*, zoonosis, neurological disease

### **INTRODUCTION**

Rabies is an acute fatal encephalomyelitis caused by a virus under the genus *Lyssavirus* and family Rhabdoviridae. It is the most important viral zoonosis recognized today because of its global distribution, incidence, veterinary and human health costs and extremely high case fatality rate. The etiological agent of rabies encephalitis was believed to be unique until 1956, when first rabies related viruses were isolated in Africa and Europe. The name rabies comes from the Latin word Ravidus for frenzy. The disease in human beings is called hydrophobia because the patient exhibits fear of water, being incapable of drinking in spite of having intolerable thirst. Rabies in animals is not called hydrophobia because they do not have this peculiar feature.

In India, about 0.7 million people undergo anti-rabies treatment annually with an estimated death toll of 0.025 to 0.030 million per year. The disease is commonly transmitted by the bite of rabid animals usually carnivorous animals. In human beings 90% cases occur due to the bite of rabid dogs and 10% are due to the bite of other animals, aerosol transmission and corneal

transplantation (Nagarajan *et al.*, 2006). Rabies infections also prevail in wild animals like wolf, fox, mongoose, jackal, hyenas etc. Dogs are presumed to be the main transmitter in India due to high density of dog population. It is estimated that the dog population is around 25 million in India and 3/4th of all human rabies cases occur in villages and the incidence is about 5 times more in males when compared with females (Sudarshan, 2004). The picture of rabies has been complicated further with the evolvement of a number of rabies related viruses. Most of the rabies related viruses have the capability to produce rabies like symptoms in human beings. However, there is no vaccine based on the homologous rabies related viruses available in the market. So, it is the question in the mind of every individual whether the presently available anti-rabies vaccines are capable enough to provide protection against the newly discovered rabies related viruses or not (John, 1997; Hanlon *et al.*, 2005).

**Etiology:** The etiological agent of rabies encephalitis was believed to be unique until 1956, when the first rabies related viruses were isolated in Africa and Europe. To account for this increasing diversity the cross reactivity of internal antigens (the ribonucleoprotein complex) was used to identify the *Lyssavirus* genus within the Rhabdoviridae family. All the rabies related viruses are bullet shaped like rabies virus and have been placed under the genus *Lyssavirus* and family Rhabdoviridae but different genotype or serotype or phylogroup. Virus neutralizing antibodies (VNABs) which recognize the membrane glycoprotein (G) or MABs subdivided the genus into six serotypes (Badrane *et al.*, 2001). Comparison of the viral nucleoprotein gene (N) sequences delineated 7 genotypes namely the classical rabies virus itself (RABV, genotype 1, serotype 1), Lagos bat virus (LBV, genotype 2, serotype 2), Mokola virus (MOKV, genotype 3, serotype 3) and Duvenhage virus (DUVV, genotype 4, serotype 4) (Amengual *et al.*, 1997; Badrane *et al.*, 2001; Davis *et al.*, 2005). The European bat lyssaviruses (EBLV), subdivided into two biotypes (EBLV1, genotype 5 and EBLV2, genotype 6) and the Australian bat lyssavirus (ABLV, genotype 7), isolated in Australia, are also members of the *Lyssavirus* genus, but are not yet classified into serotypes (Ming *et al.*, 2007). Viruses of serotypes 2-4, EBLV and ABLV are known as rabies-related viruses. The genetic diversity of representative members of the *Lyssavirus* genus (rabies and rabies related viruses) using the sequence of the gene encoding transmembrane glycoprotein revealed two major phylogroups (Black *et al.*, 2000). Phylogroup I comprises the worldwide genotype 1 (RABV), the genotypes 5 (EBLV1) and 6 (EBLV2), the African genotype 4 (DUVV) and the genotype 7 (ABLV) (Guyatt *et al.*, 2003). Phylogroup II comprises the divergent African genotypes 2 (LBV) and 3 (MOKV). Both EBLV1 and EBLV2 have been further subdivided into EBLV-1a, EBLV-1b and EBLV-2a, EBLV-2b, respectively (Bourhy *et al.*, 1993; Davis *et al.*, 2005). Based on molecular epidemiology of rabies virus in different species, only classical rabies virus is present in India but not other rabies related viruses (Nagarajan *et al.*, 2006). All the rabies related viruses except the genotype 2 have the property to produce disease and implicated in human/animal death. The details of the rabies and rabies related virus have been given in the Table 1.

Rabies virus is distributed worldwide among terrestrial mammals and bats, presents the most comprehensive collection of isolates and has been extensively studied due to its health and economic significance. Rabies related viruses have so far been isolated in limited geographical regions. Lagosbat, Mokola and Duvenhage viruses have been isolated in subequatorial and Southern African countries mostly from frugivorous megachiropterans (*Eidolon* and *Epomophorus* sp.), micromammals and insectivorous microchiropterans (*Miniopterus* and *Nycteris* sp.), respectively (Foggin, 1983; Knobel *et al.*, 2005). The LBV was first isolated in Nigeria from the frugivorous bat

Table 1: Rabies and rabies related viruses under genus *Lyssavirus* and family Rhabdoviridae

| Genotype | Serotype/<br>phylogroup | Virus name                                      | Distribution   | Source species  | Human deaths                              | Other known<br>susceptible<br>mammalian host |
|----------|-------------------------|---|--|---|---|--|
| 1        | 1/I                     | Rabies virus                                    | Worldwide except Australia, Britain, Iceland, Ireland, New Zealand, Scandinavia                          | Dogs, foxes, raccoons, bats in the Americas and others  | 70,000 per year                           | Wide range of mammals                        |
| 2        | 2/II                    | Lagos bat virus                                 | Central African Republic, Nigeria, Senegal, South Africa, Senegal South Africa, Zimbabwe Ethiopia        | Fruit bats: <i>Eidolon helvum</i> , <i>Micropterum pusillus</i> , <i>Epomophorus wahlbergi</i><br>Insectivorous bats: <i>Nycteris gambiensis</i> cats dogs        | Not detected in human being               | Dogs, cats                                   |
| 3        | 3/II                    | Mokola virus                                    | Cameroon, Nigeria, Ethiopia, S.Africa, Zimbabwe<br>Central African Republic                              | Shrews, Domestic cats<br>Dogs, Rodents  | 2 (Nigeria, 1969, 1971)                   | Shrews, rodents, dogs and cats               |
| 4        | 4/I                     | Duvenhage virus                                 | S. Africa<br>Zimbabwe  | Insect. Bats: <i>Miniopterus schreibersii</i> , <i>Nycteris thebaica</i>  | 1(S Africa, 1971)                         | None detected                                |
| 5        | 5/I                     | European bat <i>Lyssavirus</i> EBL 1a<br>EBL 1b | Deumark, France, Germany, Hungary, Netherlands, Poland, Russian Federation<br>France, Netherlands, Spain | Insect. Bats : <i>Eptesicus serotinus</i>   | 2 (Russia, 1985; Sheep and Ukraine, 1977) | stone marten                                 |
| 6        | 6/I                     | European bat <i>Lyssavirus</i> EBL 2a<br>EBL 2b | Germany, Netherlands, Ukraine, UK Switzerland  | Insect. Bats: <i>Myotis dasycneme</i> and <i>M. daubertonii</i>   | 2 (Finland, 1985; Scotland, 2002)         | None detected                                |
| 7        | 1/I                     | Australian bat Lyssa virus<br>ABLV              | Australia<br>Philippines   | Insect. Bats ( <i>Saccolaimus flaviventris</i> ) Fruit bats : <i>Pteropus alecto</i><br><i>P. poliocephalus</i><br><i>P. scapulatus</i> , <i>P.conspicillatus</i> | 2 (Australia, 1997, 1998)                 | None detected                                |

(*Eidolon helvum*) in 1956. The virus was also isolated from the bat *Micropteropus pussilus* in Central Africa Republic, from the bat *Epomophorus wahlbergi* in South Africa, cat in South Africa and Zimbabwe and from a dog in Ethiopia. MOKV was first isolated from shrews in Nigeria in 1968. Thereafter, MOKV was detected in shrews from Nigeria and Cameroon, humans in Nigeria, domestic cats in Zimbabwe, Ethiopia and South Africa, a domestic dog in Zimbabwe and a rodent (*Lophuromys sikapusi*) from the Central Africa Republic. DUVV was isolated from a human, who died after a bat bite in 1970 in South Africa. It has also been identified in the insectivorous bat (*Miniopterus* sp.) in South Africa and another bat sp. (*Nycteris thebaica*) in Zimbabwe (Foggin, 1988; Katie *et al.*, 2007).

Since, the discovery in 1968, EBLV-1 has been isolated from a number of European countries from Russia to Spain, mainly in coastal regions. EBLVs in bats have been reported from Germany, Yugoslavia, Ukraine, Turkey, Greece, Poland, Netherlands, Denmark, France, Spain, Switzerland, Czechoslovakia, Slovak Republic, Hungary and UK (Amengual *et al.*, 1997; Botvinkin *et al.*, 2003; Davis *et al.*, 2005). They preferentially infect insectivorous micro-chiropterans of *Eptesicus* and *Myotis* sp., respectively. One case of EBLV1 infection in human occurred following a bat bite in Russia in 1985. EBLV2 was isolated in Finland from a biologist in 1986 that died of rabies and later

isolated from bats (*Myotis* sp.). Phylogenetic analysis has demonstrated that EBLV1 and EBLV2 form homogenous clusters and each may be subdivided further into two lineages a and b. EBLV1 (types 1a and 1b, GT 5) and EBLV2 (types 2a and 2b, GT 6) are genetically and antigenically related to RABV. Both EBLVs are however, significantly different from each other. EBLV-1 appears to be the more prevalent virus infecting 95% of all EBLV positive bats (Bourhy *et al.*, 1999; Black *et al.*, 2000; Fooks *et al.*, 2003).

Until 1996, Australia and the Antarctic were the only two continents recognized as being free of rabies and rabies related viruses. However, in June 1996, a lyssa virus infection was diagnosed in a frugivorous bat belonging to the Megachiroptera family (*Pteropus alecto*) in Australia. The presence of rabies related virus was then demonstrated in another species of insectivorous bat (*Taphozous flaviventris*). ABLV resembles the genotype 1 strain in terms of antigen and has been placed in genotype 7 in the *Lyssavirus* genus. The distribution of ABLV corresponds to the geographical distribution of frugivorous and insectivorous bats in mainland Australia. Since 1996, two cases of human infection due to ABLV have been reported in Australia. Neither of these persons had been vaccinated against rabies (Fraser *et al.*, 1996; Guyatt *et al.*, 2003).

**Prevalence and host range:** Rabies is a viral zoonosis and carnivores such as foxes and raccoons as well as many bat species are wildlife hosts of the rabies virus in nature. Globally, in terms of human disease, dogs represent the most important reservoir. Infection of humans usually follows bites by rabid animals and is almost invariably fatal once signs of disease occur. More than 2.5 thousand million people live in about 100 countries in the world where rabies is endemic. It is estimated that each year at least 55,000 people die from rabies and more than 10-12 million receive post exposure vaccination against this disease around the world. More than 99% of all human deaths from rabies occur in Africa, Asia and South America (Cliquet and Picard-Meyer, 2004; Tang *et al.*, 2005; Wilde *et al.*, 2005). Some islands such as Iceland, Japan and the UK and the European countries such as Belgium, Finland, France, Greece, Norway, Portugal, Spain, Sweden and Switzerland now considered as free of rabies. Until 1995, Australia was considered to be rabies free, but in 1996, rabies related *Lyssavirus* (genotype 7) was discovered in flying foxes, a bat species (Nandi and Maiti, 1994; Fraser *et al.*, 1996; Guyatt *et al.*, 2003).

India has approximately 25 million dogs, with an estimated dog: man ratio of 1:36. Most animal bites in India (91.5%) are by dogs, of which about 60% are strays and 40% pets. The incidence of animal bites is 17.4 per 1000 population. A person is bitten every 2 seconds and someone dies from rabies every 30 min. The annual number of person-days lost because of animal bites is 38 million and the cost of post bite treatment is about \$25 million (Sudarshan, 2004; Singh and Sandhu, 2007). Among human infections, rabies is believed to be the 10<sup>th</sup> most common cause of death. Once clinical symptoms have occurred the disease is almost invariably fatal. However, reporting is often incomplete and the estimated 5,000 deaths per year may be an underestimate. Asia accounts for more than 90% of all rabies fatalities. India alone reports 30,000 deaths per year i.e. an annual incidence of approximately 3 deaths per year i.e., 3 per 100, 000 populations. Annual incidences of 0.01 -0.2 deaths per 100, 000 are reported from Latin America and 0.01-13 deaths per 100,000 from Africa (Chaudhuri, 2005; Wilde *et al.*, 2005).

Although, all age groups are susceptible, rabies is most common in people aged under 15 years, with 30-50% of post exposure treatments given to children aged 5-14 years, the majority of being male. The susceptibility of rabies shows considerable variation among species. In southern Africa, parts of Caribbean, North America and Europe, the principal mammalian reservoirs are wild

carnivores (Foggin, 1988; Cliquet and Picard-Meyer, 2004). In Europe and the Arctic and sub-Arctic regions, the main wildlife vector is the fox. In North America, striped skunks, raccoons, coyotes and insectivorous bats also transmit rabies (Donald *et al.*, 1998). In Africa, animal reservoirs include the mongoose and jackal, in western Asia, the wolf and in Latin America the vampire bats. However, in Asia, parts of Latin America and large parts of Africa, dogs remain the principal host and transmitter of rabies to humans (Tang *et al.*, 2005; Schneider *et al.*, 2007).

In North America and Europe, rabies in domestic animals is well under control. However, in both Canada and the United States, the rapid spread of rabies in raccoons has become a major concern (Donald *et al.*, 1998). In Europe, 20 years of oral fox vaccination programmes have reduced the annual number of infected animals from over 20,000 to approximately 6,000 with countries in central and eastern Europe accounting for most cases now a days. EBLV 1 and EBLV 2 are restricted to insectivorous bats in Europe. LBV, MOKV and DUVV are restricted geographically to Africa. LBV is the only *Lyssavirus* that has never been reported to infect man. Human fatalities caused by MOKV, DUVV and ABLV are rare. All the seven genotypes, with one exception (MOKV) have been isolated from bats. The Netherland is the only country from which both EBLV1 and EBLV2 have been isolated (Davis *et al.*, 2005; Wilde *et al.*, 2005).

All mammals are susceptible to rabies infection though differences in susceptibility exist across the species. Cattle, cats and foxes are highly susceptible, whereas, skunks, opossums and fowl are relatively resistant. Human and dogs occupy an intermediate position. Pups are more susceptible than adult dogs. Experimental infection can be produced in any laboratory animal but mice are the animals of choice. They can be infected by any route. After intracerebral inoculation they develop encephalitis and die within 5-30 days (Nandi, 1995; Burton *et al.*, 2005).

**Transmission:** Species to species transmission of the rabies virus occurs readily. This virus is usually transmitted in the saliva, when an infected animal bites another. Less often, it is spread by any contact between infectious saliva or neurological tissues and mucous membranes or breaks in the skin. The rabies virus is not transmitted through intact skin. There are also rare reports of transmission by other routes. A few cases have been reported after corneal, liver or kidney transplantation. Aerosol transmission has been documented in special circumstances, such as in laboratories and bat caves with a high density of aerosolized virus particles (Nandi and Maiti, 1994). Rabies viruses have been transmitted by ingestion in laboratory animals and there is anecdotal evidence of transmission in milk in animals. Although there is some speculation that ingestion could play a role in wild animals, there are no records of human disease acquired by this route (Slater, 2001; Nandi *et al.*, 2006).

Traditionally the dog and to a minor extent the cat, have been the main source of infection. However, native fauna, including foxes, skunks, wolves, coyotes, vampire, insect and fruit eating bats, raccoons, mongoose and squirrels provides the major source of infection in countries where domestic carnivores are well controlled (Nandi, 2008). In general, foxes are less dangerous than dogs. Foxes usually bite only one or two animals in a group while dogs will often bite a proportion of a herd or flock. Recent examples of epidemics include a fox rabies epidemic that moved slowly west in Europe and a raccoon rabies epidemic that moved north along the east coast of the US (Kihm *et al.*, 1992; Pastoret and Brochier, 1999; Katie *et al.*, 2007).

Bats are the important species in which subclinical carriers occur. Multiplication of the virus without invasion of the nervous system is known to occur in fatty tissues in bats and may be the basis of the reservoiring mechanism in this species. Violent behaviour is rare in rabid bats. Bats

represent a serious threat of spread of rabies because of their migratory habits. Most spread is within the species but also in humans and animals. Although rodent can be infected with the rabies virus they are not thought to play any role in the epidemiology of rabies either as multipliers or simply as physical carriers of the virus. The virus may be present in saliva of the infected animal for a period up to 5 days before symptoms are evident. The spread of the disease is often seasonal with the highest incidence in the late summer and autumn because of large scale movements of wild animals at mating time and in pursuit of food (Nandi and Maiti, 1994).

**Carrier state:** When the isolates of the carrier rabies viruses were inoculated into mice or dogs, they were as virulent as other street rabies virus strains. Experimentally infected dogs could become carrier of rabies virus after recovery from clinical infection and could transmit the virus for several months. In some carrier dogs which have been recovered from rabies, the virus is sequestered in the tonsil in spite of high levels of virus neutralizing antibody in serum and cerebrospinal fluid. Several species viz., dogs, monkey, raccoons, foxes and skunks are more likely to harbour the virus in the tonsil than the submaxillary salivary gland. However, tonsil isolate is always different from the virus isolated from the saliva of the same animal. This may be due to the fact that virus sequestered in the tonsil may be under different immune pressure than virus present in the brain. So, it is inferred that a wide array of tissues can harbour rabies virus during the course of infection. If organ and tissue specific variants of rabies virus exist with or without immune pressure then a dog infected with a low dose of virus and having a relatively long incubation period may be an ideal source for the selection or adaptation of new forms of the virus (Burton *et al.*, 2005; Nandi, 2008).

**Pathogenesis:** Most infections of animals and human beings with rabies occur following a bite by a rabid animal besides infections through aerosols and transplantation of organs. Oral and nasal infections are rare but mice and other animals can be infected by these routes experimentally. An epizootic that killed thousands of kudu antelopes in Namibia was thought to be spread through oral route. The usual route of rabies infection is transdermal inoculation of infected saliva (Nandi *et al.*, 2006).

After entering into the body, viruses remain latent before advancing rapidly to the spinal cord along the peripheral nerves. It has been shown that virus may replicate in muscle fibers before invading the nervous system which is necessary amplification stage to yield sufficient virus to invade peripheral nerves. The virus does not start to replicate in the surrounding muscle fibers immediately and there is a delay with the virus apparently in a latent form and not stimulating an effective immune response. The virus attaches to the nerve cells through acetylcholine receptors at the neuro-muscular junctions. These are the same receptors used by the some of the neurotoxins produced by some poisonous snakes. Once the virus enters the peripheral nerves it travels towards CNS via the motor and sensory axons. Within the CNS, the virus infects neurons and dendrites, with virus budding occurring from neuronal cell surfaces and synapses (Jackson, 2002). Virus dissemination occurs through retrograde axoplasmic flow, cell to cell transmission via synaptic junctions and free passage of virus within intercellular spaces. The virus appears to spread progressively by infecting areas adjacent to the entry site until most areas of the brain and spinal cord are affected. It has been estimated that the virus travels towards the CNS within motor and sensory axons at a rate of about 50-100 nm per day. The neurons with long axons may enhance the spread of virus to distant areas. Successive cycles of axoplasmic transmission and replication

in the perikarya and dendrites and transneuronal spread result in widespread dissemination. Although, several cell types of nervous system have been shown to harbor virus but infection of glial cells is uncommon. Eventually there is also a centrifugal spread of the virus in the axons of peripheral nerves and infection and possible replication in the salivary glands, the skin, heart and other tissues. It multiplies in the salivary glands and is shed in the saliva. The presence of the virus in the saliva and the irritability and aggression brought on by the encephalitis ensures the transmission and survival of the virus in nature (Pleasure and Fischbein, 2000).

The virus ultimately reaches virtually every tissue in the body though the centrifugal dissemination may be interrupted at any stage by death. The virus is almost invariably present in the cornea and the facial skin of patients because of their proximity to the brain. This provides a method for antemortem diagnosis of human rabies. The virus may also be shed in milk and urine. Viraemia is not clinically significant though it has been demonstrated under experimental conditions. The disease is characterized by little pathological changes, but apoptosis has been demonstrated in cell culture system and is experimentally infected mice. About 80% of patients develop an encephalitic or furious form of rabies and 20% have a paralytic or dumb form of the disease (Jackson, 2002; Nandi, 2008).

**Molecular basis of virulence:** The rabies virus is bullet shaped and covered with spikes or peplomers. It has a single negative sense SS RNA (12 kb) which contains 5 genes each codes for one protein namely N, NS, M, G and L. Among all the proteins, G protein is important as it possess biological (cell surface receptors) and immunological (antibody binding) functions and defines the virus serotype. The N protein determines group specificity of Lyssaviruses. The rabies virus G protein is composed of an endodomain (ENDO) which interacts with internal protein, a transmembrane region (TM) involved in tropism and pathogenicity and an ectodomain (ECTO) protruding from the viral membrane. The ectodomain carries B and T cell antigenic sites and regions responsible for receptor recognition and membrane fusion. Sequence homology between external part of G protein and the receptor binding site of snake venom neurotoxins, it is reported that rabies virus binds to the nicotinic acetylcholine receptor. This hypothesis may apply to muscular cells. For neuronal and fibroblastic cells, it has shown that oligosaccharides and lipoprotein components such as the sialic acids of gangliosides may also be involved. The rabies virus receptor appears to be complex and may vary from one cell type to another (Bourhy *et al.*, 1993; Kissi *et al.*, 1995; Nadin-Davies *et al.*, 2001; Nandi, 2008).

The G protein is also involved in the pathogenesis of rabies and is believed to assume at least part of the neurotropism of the virus. In genotype 1 viruses, neurovirulence seems to be directly related to the maintenance of an arginine (for lysine) residue in position 333. Mutants with other amino acid in this position cannot infect certain types of neurons presumably because they are unable to recognize the receptor. However, several attenuated vaccinal strains such as HEP or Kelev are mutated in position 333. Mokola virus (genotype 3) which is highly neuropathogenic in mice and more severe encephalitis than rabies virus in dogs and monkeys, possesses an aspartate residue in position 333. So, the tissue specificity is probably very complex (Smith *et al.*, 1995; Bourhy *et al.*, 1999; Nadin-Davies *et al.*, 2001).

Serotyping and genotyping are the two methods widely used to differentiate between lyssaviruses. Serotyping showed a limitation when EBLVs were isolated and genotyping lacks biological significance. The ectodomain of the G protein of rabies virus considerably influence viral pathogenicity. It has been seen that an R 333 (or K333) in the glycoprotein is essential for the



virulence of ERA and CVS strains of rabies virus. Phylogroup I viruses possess R333 whereas, phylogroup II members have an R333D replacement. Viruses of phylogroup I and II were fully pathogenic when injected by I/C route to adult BALB/C and C3H mice. However, when  $10^5$  to  $10^7$  LD<sub>50</sub> (I/C) were injected by the I/M route, phylogroup I viruses were pathogenic but phylogroup II viruses were not. So, the natural absence of R333 has a negative effect on the pathogenicity of lyssaviruses. Two positively charged residues within the antigenic site III, K330 and R333 have been shown to considerably influence viral pathogenicity (Smith *et al.*, 1995; Bourhy *et al.*, 1999; Nadin-Davies *et al.*, 2001; Nandi, 2008). Selected antigenic mutants of rabies virus laboratory strains in which R333 has been replaced with another residue (except lysine) were totally apathogenic for adult mice (I/C and I/M routes). This was possibly due to a restriction in the type of infected neurons and in the transmission at interneurons. An antigenic double mutant (K330N and R333N) was unable to penetrate either motor or sensory neurons. Phylogroup II members were found in nature to carry similar replacements. In fact viruses from genotype 3 resemble a single (R333D) mutant and viruses from genotype 2 resemble a double mutant (K330L and R333D) (Kissi *et al.*, 1995; Bourhy *et al.*, 1999). In a genotype I ectodomain background, both types of mutants would be completely apathogenic for adult mice (I/C and I/M routes). Thus it appears that mutations in positions 330 and 333 have different pathological consequences. They are less deleterious in a phylogroup II (I/C pathogenic) than in a genotype I (both I/C and I/M apathogenic) ectodomain background. This modulation may be due to the local amino acid context. It has recently been proposed that regions 319 to 340 (including both the K330 and R333 residues) are involved in the recognition of high affinity neuron specific receptors (Smith *et al.*, 1995; Nadin-Davies *et al.*, 2001). It seems that the conservation of at least one positively charged residues is necessary and sufficient for receptor recognition and penetration into sensory and motor neurons. Although, the presence of an arginine (or lysine) at position 333 is crucial, genotype 2 and 3 viruses still remain pathogenic by the I/C route because they possess compensatory positively charged residues at position 331 or 334 not present in the glycoprotein of phylogroup I viruses. On the basis of amino acid homology of the ectodomain of G protein, it is noted that within the phylogroup it is > 74% and between the phylogroups it is <64%. Genotype 4, 5 and 6 are more homogenous (97-98.5% amino acid homology) and genotypes 2 and 1 are more heterogenous (Kissi *et al.*, 1995; Bourhy *et al.*, 1999; Nadin-Davies *et al.*, 2001).

The molecular analysis employing the monoclonal antibodies against G protein exhibited that there is minor differences among the classical rabies virus strains of field origin. However, these differences would not have any major impact on the performance of classical rabies virus vaccine strain in eliciting the immune response against the prevailing field virus strain as protective immunity is developed in the vaccinated animals and humans (Hanlon *et al.*, 2005). Recently, the epidemiological studies of rabies virus by sequencing the PCR amplified genes showed there is little genetic divergence (2%) between isolates. Contrastingly, the street isolates diverged from the current fixed vaccine strains by about 14%. Such divergence is considered unsurprising since most of the vaccine strain were isolated between 40 to 100 years ago (Nadin-Davies *et al.*, 2001). Although nervous tissue vaccines derived from animal brain particularly of ovine origin formed the mainstay in the pre and post exposure immunoprophylaxis against rabies in human beings and animals in developing countries like India, a variety of antirabies vaccines of cell culture (Vero, BHK21, CEF, WI-38, MRC-5 etc) origin besides the DNA vaccine, vaccinia virus vector based bait vaccine and gene deletion mutant vaccines have revolutionized the concept of vaccination against rabies (Hanlon *et al.*, 2005; Cleaveland *et al.*, 2006). The vaccine strain used in preparation

of anti-rabies vaccines is still capable of providing full protection in vaccinated animals and humans. However, time has come to reattenuate some of the modern field strains or to replace the existing vaccine strain with the current strain in order to achieve the fool-proof protection and to control the disease in an effective manner (Rupprecht and Gibbons, 2004; Wilde and Hemachudha, 2006).

**Symptoms:** The length of incubation period will vary and depends on several factors, including the amount of the virus transmitted and the location on the body where exposure occurred. If the injury site is closer to the brain (face, neck etc.), the incubation period would be lesser compared to the injury in the legs. Not all the animals or humans exposed to the virus contract the disease. However, once symptoms become evident the disease usually is fatal. Symptoms in animals and humans can be similar but usually are highly variable and numerous. In humans initial symptoms typically appear within 30-60 days following exposure and can include mild fever, pain, itching at the site of the virus entrance into the body, restlessness, headache, fever, nausea, sore throat and anorexia. Increased production of saliva, muscle stiffness, sensitivity to light or sounds, irrational excitement or convulsions occurs as the infection progresses. Other symptoms are anxiety, convulsion, agitation, delirium and display of abnormal behavior. In animals, the symptoms of rabies include change in behavior, loss of appetite, fever, change in sound of a dog's bark, greater excitement, aggression and paralysis (especially lower jaw and excessive salivation) (Nandi *et al.*, 2006).

Although, 3 different phases of the disease often are described, they are not always observed. Each phase has a different set of visible symptoms. The first phase or prodromal phase occurs early and the virus replicates and begins to pass through the nervous system. Normal behavioural patterns are reversed in this phase. For example, if an animal is shy, it may become more aggressive whereas an aggressive animal may become more timid. In wild animals particularly those are active during day (diurnal) become active at night and nocturnal animals such as raccoons and bats are observed moving during day time (Pleasure and Fischbein, 2000).

In the second (furious) phase the animals becomes extremely irritable and aggressive often lunging at or biting anything that moves near it. Infected animals may produce excessive amounts of saliva. However, not all the animals display the furious stage or aggression at all. The final (dumb) stage is manifested by the onset of paralysis mostly in the lower jaw and extremities. Hence this stage is also called paralytic phase. Individuals eventually lose the ability to chew and swallow, walk normally, right themselves when they fall, maintain a standing position or in the case of bats maintain flight. The term hydrophobia or fear from water is due to the inability of the humans to swallow the water due to the paralysis of the pharyngeal muscle. Death usually follows the development of these dumb symptoms (Pleasure and Fischbein, 2000; Burton *et al.*, 2005).

**Immunity:** The kinetics of immune responses to rabies virus have been widely studied in experimentally infected mice, in vaccinated dogs and in humans received post exposure vaccination. But very little information on immune responses in naturally infected animals. It is not certain whether or not the saliva of the rabid animal plays an immunomodulating role allowing the virus to elude the host's immune response but salivary gland and brain homogenates have been shown to be immunosuppressive (Nandi and Chauhan, 2006). Experimental studies have shown that the immune response and possible immune suppression are largely influenced by strain, dose and route of inoculation. In mice that resist challenge, virus specific antibodies were first detected 4-6 days

after experimental infection reaching peak levels 2 weeks after infection. In animal that survive infection the antibody titre remain high whereas in mice that succumb to lethal experimental infection depletion of B and T cells in the spleen and thymus is noticed. The antigen specific suppression of CMI is mediated by pathogenic *Lyssaviruses* but not by the non-pathogenic rabies related viruses or inactivated pathogenic rabies viruses (Hanlon *et al.*, 2005).

Two types of tests are routinely used for the quantitative assay of humoral immune responses: the Virus Neutralization Test (VNT) and the Rapid Fluorescent Focus Inhibition Test (RFFIT) based on cell culture. High titres of VNT or RFFIT antibodies are indicative of a protective humoral immune response but animals with very low titre have been shown to resist challenge suggesting the cellular immune responses may play an important role in protection against challenge (Moore *et al.*, 2005). Experimental infection in nude mice (deficient in T cells) had shown that the cellular arm of immune system is very important. Immunization of man with anti-rabies vaccine has shown that cell mediated immune response as measured by lymphocyte proliferation assay also plays an important role. The cell mediated response can be detected for prolonged periods after vaccination. Cytotoxic T lymphocytes and T helper cell specific to epitopes in the viral glycoprotein or ribonucleoprotein of the rabies virus have been detected in peripheral blood of animals infected with the virus and in vaccinated humans. Mice that are naturally resistant to rabies virus are rendered susceptible by the depletion of CD4+ but CD8+ cell had no effect (Rupprecht and Gibbons, 2004; Wilde and Hemachudha, 2006).

**Vaccination:** Vaccination is the most effective and economical way to control a disease. Considerable attention about the main sources of infection and the main reservoirs of infection of rabies for man should be given. Although the main threat to humans and domestic animals is classical rabies virus, it is also important to consider the other members of the genus *Lyssavirus* that may infect animals. The conventional vaccines currently used for the vaccination of humans and domestic and free living animals are derived from fixed type virus of genotype 1 and serotype 1. Nervous tissue vaccines derived from animal brain particularly of ovine origin formed the mainstay in the pre and post exposure immuno- prophylaxis against rabies in human beings and animals in developing countries like India, Thailand etc., (Chulasugandha *et al.*, 2006; Kumar *et al.*, 2009). These vaccines provide good protection against classical rabies virus but may not confer good protection against serotype 4 and 6. Conventional rabies vaccines are not very efficient against infections caused by the bat viruses (EBL-1 and EBL-2) and Duvenhage virus isolates (Cleaveland *et al.*, 2006).

However, the concept has been changed and people are gradually switching over to the most potent efficacious and safe vaccines of cell culture origin to control rabies due to the weak immunogenicity and neuroparalytic complications of nervous tissue vaccines. In humans, cell culture based rabies vaccine comprises HDCS (Human diploid cell strain), MRC-5, WI-38, chicken embryo fibroblast cells and Vero cells are the most commonly used. BHK-21 is the most commonly used continuous cell lines for the production of vaccines for animals (Hanlon *et al.*, 2005; Wilde and Hemachudha, 2006).

A live canarypox virus that expresses the rabies virus glycoprotein has been licensed in the USA as a parenteral monovalent vaccine for cats and as combination rabies vaccines for cats with feline panleukopenia virus, feline parvovirus, feline calicivirus vaccines included in the product (Kieny *et al.*, 1990). A recombinant adenovirus vectored vaccine expressing rabies virus glycoprotein was shown to be capable of inducing antibody response in greyhound dogs immunized

S/C or I/M. This vaccine holds promise as a rabies virus vaccine for dogs (Hu *et al.*, 2006). A recombinant vaccinia virus vector in which the G gene of the Evelyn-Rokitniki-Abelseth (ERA) strain of rabies virus was inserted into the thymidine kinase region of the vaccinia virus genome has been developed for immunization against rabies. The vaccinia-rabies glycoprotein (V-RG) recombinant virus induced a rapid virus-neutralizing antibody (VNA) response in mice, both by subcutaneous inoculation and by oral administration and the animals were protected against a lethal rabies virus challenge (Kieny *et al.*, 1990; Blanton *et al.*, 2007). The vaccine was used in USA and France to immunize raccoons, coyotes, wild dogs, fox, jackals etc. with high success and by 1995 the vaccine was conditionally licensed in the US for use in Oral Rabies Vaccination (ORV) programs (Blanton *et al.*, 2007).

Mass vaccination of dogs remains the main strategy for controlling urban rabies in endemic areas. In order to avoid maternally derived immunity, vaccines are better given when the animal is young but not less than 3 months of age in dogs (Coleman and Dye, 1996). Primary vaccination can be a single injection (live attenuated vaccines) or two inoculations one month apart. After that vaccines are given annually, biannually or triennially to boost their immunity depending on the efficacy of the vaccine (Bourhy *et al.*, 1993).

**Efficacy of anti-rabies vaccines against rabies related viruses:** Commercial vaccines for rabies (human and veterinary) are based on and protect against RABV. For RABV, DUVV, EBLV and ABLV, conserved antigenic sites on the surface glycoproteins allow cross neutralization and cross-protective immunity to be elicited by rabies vaccination. No person administered with pre-exposure immunization or timely post exposure treatment has died of rabies regardless of the source and genotype of the virus. This suggests that the RABV vaccines induce antibodies that should be capable of cross neutralizing and cross protecting against at least some *Lyssavirus* genotypes. The level of protection as determined in mice that survived after 28 days following a heterologous virus challenge using a non-genotype 1 *Lyssavirus* appears to depend on the virus strain used in the vaccine e.g., Pasteur Virus (PV) or Pitman-Moore (PM) strain and the genotype of the challenge virus. Generally rabies vaccines based on the PM and LEP strain induced weaker protection against EBLV 1 than the PV strain and few data are available for EBLV 2. Mice immunized intraperitoneally with the inactivated adjuvanted veterinary vaccines (Rabisin and Rabiffa) were protected against EBLV 1 but only partial protection against EBLV-2 (Rupprecht and Gibbons, 2004; Hanlon *et al.*, 2005; Wilde and Hemachudha, 2006). The PM virus vaccine induced neutralizing antibodies against EBLV-1 in 73% patients only and Challenge Virus Standard (CVS) in 100% patients. Little or no cross-protection against infection with MOKV or LBV is elicited by rabies vaccination and most anti-rabies virus antisera do not neutralize these *Lyssaviruses* (Fekadu *et al.*, 1988). Four new rabies-related viruses (Aravan, Khujand, Irkut and West Caucasian bat viruses) have been isolated recently from Eurasian bats and are described as new putative *Lyssavirus* species. There is a reduced protection with pre-exposure vaccination and with conventional rabies post-exposure prophylaxis against all four new bat variants of rabies virus (Hanlon *et al.*, 2005; Cleaveland *et al.*, 2006; Wilde and Hemachudha, 2006).

**Diagnosis:** The diagnosis of rabies is challenging because of the long incubation period (20-60 days on average) and the lack of specificity of early prodromal symptoms and neurologic symptoms, including paresthesias, pruritis and pain at the site of viral entry. The infection eventually evolves into a viral encephalitis (furious rabies), with classic symptoms of hydrophobia, aerophobia, hyper-excitability and autonomic dysfunction (Nandi and Chauhan, 2006).

The laboratory diagnosis of the disease is based on the detection of typical intracytoplasmic inclusion bodies, Negri bodies in the neurons. It has been subsequently replaced by the detection of specific antigens by the direct or indirect immunofluorescent antibody (IFA) technique. Bilateral smears of samples from the hippocampus (Ammon's horn) brain stem and the cerebellum exhibit the Negri bodies in Sella's staining or antigens in IFA technique or IPT or ELISA (Bourhy *et al.*, 1989; Nandi *et al.*, 1990). At a magnification of 3400 to 31,000, rabies antigen appears as dust-like particles, 1  $\mu$ m in diameter and/or large, round to oval masses and strings 2 to 10  $\mu$ m in diameter. These intracytoplasmic inclusions appear smooth with very bright margins and a somewhat less intensely stained central area (Sureau *et al.*, 1991; Nandi *et al.*, 2006).

For further confirmation and characterization, virus is isolated in cell cultures from brain tissue and salivary glands on post mortem samples and saliva and the CSF for the *Intra vitam* diagnosis in human being and ante-mortem diagnosis in animals. When samples are obtained from a live animal a negative result is not conclusive. The virus may be absent from biopsies, saliva or CSF of some patients with clinical rabies and saliva has been shown to be positive only in a small number of animals whose brain are positive. Although, BHK21 and other cell lines are routinely used to cultivate the fixed rabies virus they are not ideal for the isolation of street rabies virus. The murine neuroblastoma (NAC1300) cell lines are reported to be the most susceptible for isolation and propagation of rabies virus (Rudd and Trimarchi, 1987; Bourhy *et al.*, 1989; Nandi *et al.*, 2006).

Two Serological tests were used to diagnose rabies: RFFIT and the indirect immunofluorescence assay. The RFFIT measures neutralizing antibody. An antibody titer of 1:5 or more, as defined by the reciprocal of the serum or cerebrospinal fluid dilution that reduces the challenge virus by 50%, was considered positive. An indirect immunofluorescence assay, using patient serum or cerebrospinal fluid diluted 1:4 or more, detects serum reactive with rabies antigen in infected cell cultures. The presence of antibody in serum was considered diagnostic if no vaccine or antirabies serum was given to the patient. Antibody in the cerebrospinal fluid, regardless of the rabies immunization history, was considered indicative of rabies virus infection (Nandi and Maiti, 1994; Moore *et al.*, 2005).

The rabies-specific nucleic acid sequences in brain tissue, in the saliva and the CSF of infected individuals can be amplified by RT-PCR. The RT-PCR is likely to reduce the time to establish diagnosis in few hours. Besides Northern blot can also be used to provide diagnosis using the radio-labeled or biotin labeled probes (Moore *et al.*, 2005; Rojas *et al.*, 2006). Hemi-nested reverse-transcriptase polymerase chain reaction (hnRT-PCR) can be used as a tool for rabies virus detection in stored and decomposed samples. The results of test show that the hnRT-PCR was more sensitive than RT-PCR. The hnRT-PCR has demonstrated efficacy in rabies virus detection in stored and decomposed materials, suggesting its application for retrospective epidemiological studies of rabies virus (Heaton *et al.*, 1997; Picard *et al.*, 2004).

**Control:** Each year more than 55,000 people are reported to die from rabies and the WHO estimates that 10-12 million receive post-exposure treatment. More than 90% of all human cases of rabies are believed to be associated with dog and rest with other domestic animals. The control of rabies largely depends on the prevention of infection of dogs and cats by vaccination in endemic areas and the control of their movement, including measures of quarantine and vaccination in rabies free countries. Eradication programmes also require the adoption of appropriate strategies for eliminating or reducing infection in wildlife reservoirs of the virus (Chaudhuri, 2005). In many parts of the world, where the main source of human infection is dogs, mass vaccination and

reduction of the number of stray dogs remain the most effective means of control. In Europe, control of rabies largely aims to eliminate infection in foxes by vaccination with recombinant vaccinia virus based bait vaccine. In South America mass vaccination of cattle, together with measures to reduce the number of vampire bats is often required to reduce the incidence of vampire bat rabies (Haupt, 1999; Schneider *et al.*, 2007).

Continuing molecular epidemiological and surveillance studies are necessary to trace spill over transmission from reservoir species to non-reservoir animals and humans and also to monitor the emergence of specific rabies strains into new species and geographical area, which to a large extent is often prompted by human activities viz., movement of wildlife and importation of animals. A further more ambitious goal is to co-ordinate all efforts from all sectors to increase rabies surveillance programmes in Africa and Asia and thus ultimately to decrease the incidence of rabies in the continents (Haupt, 1999; Slater, 2001).

## CONCLUSION

Rabies virus is continually evolving throughout the world. Significant advances have been made by the multi-disciplinary sectors of immunology, vaccinology, molecular virology and epidemiology thus allowing a far greater understanding of rabies virus circulation. Rabies is a disease for which all the necessary remedies exist unlike the situation with many other diseases. It is possible to prevent, control and treat rabies with the safe and effective cell culture derived anti-rabies vaccines or anti-rabies globulins. In spite of this, WHO records more than 55,000 human deaths from rabies each year mostly due to infection by GT 1 viruses from dogs. The number of cases in humans and animals is still believed to be underestimated. As epidemiological surveillance is absent, irregular or insufficient in several countries of Asia and Africa, the levels of underreporting are difficult to estimate.

Ideally there should be a national reference laboratory for rabies diagnosis with its branches in every state of the country. The destruction of all stray and feral dogs, which is usually very unpopular with the general public, does not in the long term constitute a realistic method of disease control. Pet dogs usually receive rabies vaccinations when they are 3 months old, but very often they become infected before they reach to this age. The stray and unauthorized dogs which are often not brought under the immunization campaign harbour the rabies virus. They act as carriers and transmit the rabies virus to other susceptible animals and humans. It has been reported that vaccination before the age of 3 months is effective, even in an animal with a maternal antibodies. Puppies should be vaccinated along with adult dogs during any mass parenteral vaccination campaign. This would help to broaden vaccine coverage and reduce the incidence of rabies in children.

In India, the rabies in animals and humans is a real problem and lot of efforts have been attempted to control the disease. A number of cases are still reported with a certain percentage of casualties. The isolation and sero-surveillance studies of the rabies related virus have not been carried out. Although, it can only be speculated that there may be prevalence of the rabies related virus infection, however the anti-rabies vaccine having the right strain should be properly and judiciously used in case of bat bite to prevent and control the rabies related virus infection if there is any.

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