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RABIES REVIEW

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INTRODUCTION

Rabies is an acute viral disease of the central nervous system (CNS) that affects all warm-blooded animals including mammals.[1] It is an acute infectious encephalomyelitis, caused by a number of *lyssaviruses* including: rabies virus and Australian bat *lyssavirus*.[2] Rabies occurs in more than 150 countries and territories. More than 55 000 people die of rabies every year mostly in Asia and Africa. 40% of people who are bitten by suspect rabid animals are children under 15 years of age. Dogs are the source of the vast majority of human rabies deaths. Wound cleansing and immunization within a few hours after contact with a suspect rabid animal can prevent the onset of rabies and death. Every year, more than 15 million people worldwide receive a post-exposure vaccination to prevent the disease– this is estimated to prevent hundreds of thousands of rabies deaths annually.[1] Several indirect estimates [3, 4] have suggested that modern. India has more rabid dog bites and human rabies deaths than any other country.

Historical aspect

Rabies is probably the oldest recorded of mankind. Rabies comes from the Latin word rabere. Rabere means to rage or rave. This Latin word rabere may have roots in a Sanskrit word rabhas. Rabhas means to do violence. The Greeks called rabies lyssa or lytta, which means frenzy or madness. They named human rabies hydrophobia, which means fear of water, a symptom shown by rabies victims. 400 BC Aristotle writes that *"dogs suffer from the madness. This causes them to become very irritable and all animals they bite*

become diseased." By now, the Greeks have two special rabies gods; one to prevent rabies, (*Arisaeus*, son of Apollo) and a one to heal rabies, (*Artemis*).

In 1679, Morgagni noted that the onset of clinical rabies was often preceded by paraesthesiae at the site of wound, and postulated a neural rather than hematogenous sprearl of the virus. Zinke in 1804 and Magendie in 1821 substantiated John I hinter's premise that the disease might be transmissible experimentally by inoculation of infectious saliva from animals and humans.[5, 6]

The nineteenth century witnessed a growing awareness of the neurotropic nature of tine virus, which culminated in the 1880s with Pasteur's epoch making experiments and post-exposure treatment, [7] Remarkably little was known of the disease agent until the early twentieth century, when Negri first visualized darkly staining inclusions in certain nerve cells in the brains of rabid animals, which he incorrectly considered to be a protozoan. [8] Remlingor's filtration experiments in 1903 established the agent to have ultramicroscopic properties. [6] In 1912, Babes published Traite de la Rage which still remains one of the most complete documents available for our understanding of the disease.[9] Matusmoto first observed rabies virus by electron microscopy and described its physical appearance in 1962. [10] Presence of viral antigen in Negri bodies was proven by several techniques including immunofluorescence, ferritin labeling and electron microscopy, and immunoperoxidase staining. The RNA nature of the rabies virus was confirmed in 1963.[11]

Although rabies virus has been successfully transmitted in experimental animals since the early 1800s.[12, 13] propagation of rabies virus in cell culture has been a relatively recent achievement. Rabies virus was first propagated in cell culture in 1936 and in chick embryo in 1938. [14] The propagation of rabies virus either in primary cells or in continuous cell lines was not accomplished until 1958 by Kissling.'[15] This accomplishment was relevant in that it lead to the development of tissue culture vaccines in the post- exposure prophylaxis of rabies.

Epidemiology

The epidemiology of human rabies is parallel to that of rabies infections of domestic animal populations worldwide and the degree of human contact with these animals. From the view point of public health, the dog or other canine species are the only important vectors for humans, being responsible for most infections in Asia, Africa and Latin America. Dogs are

the principal reservoirs of rabies in these countries arid dog bites are responsible for 95% of human rabies.[16] The predominance of cases attributable to dogs also reflects the high incidence of dog bites, estimated between 200 and 800 per 100,000 in many countries. [17] In developed countries like United States, Canada, and Western Europe where domestic rabies control programs are effective. Human exposure to rabies virus may result from bites of wild animals such as foxes, skunks, bats; bites of non-immunized domestic cats, aerosol inhalation (laboratory workers) and human to human transmission by corneal Transplant. [18, 19] The principal wild life vectors of rabies include the mongoose and jackal in Africa; the fox in Europe, Canada and the Arctic arid sub-Arctic regions; the wolf in Western Asia and the vampire bat in Latin America.[20]

Rabid bats have become the predominant rabies risk to humans in the United States because of a combination of virological, biological and ecological factors. In particular, the silver haired and eastern pipistrelles bats have been prominent recently because the virus recovered from them may infect human skin more easily than other rabies virus strains and because the small teeth of the bat may leave little evidence of a bite, Moreover, human exposures appeal to be more frequent, perhaps due to increasing encroachment of human habitation in formerly rural areas.[21, 22]

The major foci of rabies in the world today are the Indian subcontinent, South-east Asia, and most of Africa. Rates of human rabies are highest in Asia. More than 3 cases of rabies per 100,000 per year were reported in India arid Sri Lanka in the 1970s, [16, 23] although rates in most Asian countries are now probably between per 100,000 and 1 per million and those in Latin America are 1 per million or less. Data from India suggest that there are 30,000 cases each year and an estimate of the death rate associated with rabies is 35.5 deaths per one million people. The World Health Organization estimates that there are 50,000 cases of fatal rabies in entire world each year.[21] The annual mortality figures for rabies in various countries are given in (Table-1).[24] Before canine rabies was con Rolled in the United States in the 1940s and 1950s, there were about 50 (0.2 per 4 million cases of human rabies most from dog bites each year. As the number of cases in dogs decreased, the number of humans with rabies declined to an average of less than two per year in the 1960s and 1970s.[25]

Virology

Rabies virus belongs to the order Mononegavirales, viruses with nonsegmented, negative-stranded RNA genomes. Within this group, viruses with a distinct "bullet" shape are

classified in the Rhabdoviridae family, which includes at least three genera of animal viruses, Lyssavirus, Ephemerovirus, and Vesiculovirus. The genus Lyssavirus includes rabies virus, Lagos bat, Mokola virus, Duvenhage virus, European bat virus 1 & 2 and Australian bat virus.[26] (Table 2)

Structure

Rabiies virions are bullet-shaped with 10-nm spike-like glycoprotein peplomers covering the surface. The ribonucleoprotein is composed of RNA encased in nucleoprotein-(), phosphorylated or phosphoprotein.(Figure 1).

Rhabdoviruses are approximately 180 nm long and 75 nm wide. The rabies genome encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L). All rhabdoviruses have two major structural components: a helical ribonucleoprotein core (RNP) and a surrounding envelope. In the RNP, genomic RNA is tightly encased by the nucleoprotein. Two other viral proteins, the phospoprotein and the large protein (L-protein or polymerase) are associated with the RNP. The glycoprotein forms approximately 400 trimeric spikes which are tightly arranged on the surface of the virus. The M protein is associated both with the envelope and the RNP and may be the central protein of rhabdovirus assembly. The basic structure and composition of rabies virus is depicted in the longitudinal diagram. (Figure 2).

Rabies is an RNA virus. The genome encodes 5 proteins designated as N, P, M, G, and L. The order and relative size of the genes in the genome are shown in the figure below. The arrangement of these proteins and the RNA genome determine the structure of the rabies virus.

Cycle of Infection and Replication

Rabies virus genome is single stranded, antisense, nonsegmented RNA of approximately 12kb. There is a leader-sequence (LDR) of approximately 50 nucleotides, followed by N,P,M,G and L genes.(Figure 3).

The fusion of rabies virus envelop to host cell membrane (adsorption) initiates the infection process. The interaction of the G protein and specific cell surface receptors may be involved. After adsorption, the virus penetrates the host cell and enters the cytoplasm by pinocytosis (via clathrin-coated pits). The virions aggregate in the large endosomes

(cytoplasmic vesicles). The viral membranes fuse to the endosomal membranes, causing the release of viral RNP into the cytoplasm (uncoating). Because lyssavirus have a linear single negative stranded ribonucleic acid (RNA) genome, messenger RNAs (mRNAs) must be transcribed to permit virus replication.

A viral-encoded polymerase (L gene) transcribes the genomic strand of rabies RNA into leader RNA and five capped and polyadenylated mRNAs, which are translated into proteins. Translation, which involves the synthesis of the N, P, M, G and L proteins, occurs on free ribosomes in the cytoplasm. Although G protein synthesis is initiated on free ribosomes, completion of synthesis and glycosylation (processing of the glycoprotein), occurs in the endoplamsic reticulum (ER) and Golgi apparatus. The intracellular ratio of leader RNA to N protein regulates the switch from transcription to replication. When this switch is activated, replication of the viral genome begins. The first step in viral replication is synthesis of full-length copies (postive strands) of the viral genome. When the switch to replication occurs, RNA transcription becomes "non-stop" and stop codons are ignored. The viral polymerase enters a single site on the 3’ end of the genome, and proceeds to synthesize full-length copies of the genome. These positive strands of rabies RNA serve as templates for synthesis of full-length negative strands of the viral genome.

During the assembly process, the N-P-L complex encapsulates negative-stranded genomic RNA to form the RNP core, and the M protein forms a capsule, or matrix, around the RNP. The RNP-M complex migrates to an area of the plasma membrane containing glycoprotein inserts, and the M-protein initiates coiling. The M-RNP complex binds with the glycoprotein, and the completed virus buds from the plasma membrane (Figure 4). Within the central nervous system (CNS), there is preferential viral budding from plasma membranes. Conversely, virus in the salivary glands buds primarily from the cell membrane into the acinar lumen. Viral budding into the salivary gland and virus-induced aggressive biting-behavior in the host animal maximize chances of viral infection of a new host. [27]

Pathogenesis

The pathogenesis of rabies must be considered according to the two routes of acquisition of infection, namely “bite” and “non-bite”. Most cases of human rabies occur following animal bites. The first event in rabies is the introduction of live virus through the epidermis or onto a mucous membrane. Early after implantation the virus is cell-free, since both washing of the wound and injection of antiserum reduce infection. The precise events during the

incubation period of highly variable length are unknown but the virus is thought to be present at or near the site of the bite during most of the period. [28] There is infection of muscle fibers, which may be an obligatory pathogenetic step. Initial viral replication appears to occur within striated muscle cells at the site of the inoculation. The nicotinic acetylcholine receptor is a putative binding site for viral glycoproteins.[29, 30] The virus travels towards the CNS (centripetal spread) within motor and sensory axons by retrograde fast axonal transport at a rate of about 50-100 mm per day.[31-34] severing the nerve tract prevents transmission of virus from peripheral sites of inoculation to the spinal ganglia.[35] The virus attaches to neural axons through lipoprotein receptors. Recent preliminary evidence indicates that the rabies virus phosphoprotein interacts with dynein LC8, which is important in both actin and microtubule based transport in neurons. [36-38] Other receptors, such as the neural cell adhesion molecule (NCAM) and p75 neurotrophic receptor may also be involved. [39-41] Neurons are infected in the spinal cord and subsequently there is spread from neuron to neuron within axons in the CNS by fast axonal transport along neuroanatomical connections. Many neuronal cell types are infected in a wide spread distribution in the CNS, while glial infection is usually relatively uncommon. Vermin has been documented in experimental conditions, but is thought not to play a role in naturally acquired disease. Once the virus reaches the CNS, it replicates almost exclusively within the gray matter. The early localization of the virus in the limbic system, with cortical sparing, correlates clinically with behavioral and emotional changes seen in an alert and cognitively intact patient, and may facilitate transmission by biting in rabies vectors. There is spread of rabies virus from the CNS (centrifugal spread) along neuronal pathways, particularly it involves the parasympathetic nervous system, which arc responsible for infection of the salivary glands, skin, heart, adrenal medulla, kidneys, lungs, liver and skeletal muscles, Passage of the virus into salivary glands and viral replication in mucinogenic acinar cells facilitates further transmission via infected saliva.[42, 43]

The likelihood that a bite by a rabid animal will result in rabies is now believed to be related to a number of factors such as: 1) Infecting strain of the virus, 2) Host's genetic background, 3) Concentration of nicotinic acetylcholine receptors in skeletal muscle,4) Size of the inoculums, 5) Degree of innervations of the site of the bite and 6) Distance between the site of the bite and the central nervous system.[43-47]

The risk is 'better with an unprovoked attack than with a provoked attack. Bites incurred in the course of feeding or handling any animals should he regarded as provoked. Though care takers of patients with rabies who comes in a contact with infectious materials (saliva, central nervous system specimens, tears or possibly, respiratory track secretions) are theoretically at risk for infection, transmission under such circumstances has not been reported. There is an accidental report of man-to-man transmission.[48]

The pathogenesis of rabies virus infection resulting "non-bite" acquisition requires viral replication at site of initial inoculation. Specifically for those who acquire infection from an aerosol, replication of the virus probably occurs in the olfactory epithelium with direct transmission to the brain by the olfactory tracts. [49] With local acquisition of infection there is direct retrograde addition of virus from the eye to the brain.[19]

Immunology

The role of cell-mediated immunity in the action of rabies virus propagation is not clear. Current data indicate that cell-mediated immune system probably plays a minor role in pathogenesis. [50-52] However, there is evidence from animals of rabies and other neurotrophic infections that is established in central nervous system, antibody can clear virus and prevent death.[53] Administration of immunoglobulin products or the generation of humeral antibody responses by vaccine administration, circulating neutralizing antibodies certainly decreases mortality.[54,55]

During the incubation period, rabies virus is evidently segregated from the immune system, since no antibody responses are seen. After the development of neurological symptoms antibodies appear in the serum and later in the CSF. Antibodies in the CSF reflect active local production in the CNS, as well as passive transfer, and are absent after vaccination.

Immunopathologic studies in mice show that neutralizing antibodies are essential component of the protective response. However, "knock out" mice which are unable to mount T cell mediated immune response, do less well than control mice, despite the presence of antibodies, and it is evident that cellular immunity plays at least a minor role in protection.[21]

Pathology

The pathology is usually resembles to that of other viral diseases of the CNS hyperemia, varying degrees of chromatolysis and nuclear pyknosis of nerve cells along with neuronophagia; infiltration by lymphocytes and plasma cells of the Virchow-Robin space; microglial infiltration; and parenchymal areas of nerve cell destruction.[56] The inflammatory changes that accompany rabies encephalitis are non-specific; perivascular cutting with mononuclear cells is nearly always found and microglial nodules (Babes nodules) are present in almost half the cases.[57] Encephalitis most commonly involves the medulla, pons and spinal cord, with each site affected in slightly more than one third of the cases, Pathological changes predominantly affect the gray substance rather than the white, and this has been considered to be the single most important point in distinguishing rabies from an allergic encephalomyelitis secondary to rabies vaccine. [58]

The most characteristic pathologic finding of rabies in the CNS is the formation of cytoplasmic inclusions called Negri bodies within the neurons. Negri bodies are well-defined, often oval or elongated, eosinophilic cytoplasmic inclusions in neurons. They may be present anywhere in the cytoplasm or in its dendrite, and there may be more than one in a single cell.[59] Negri thought them to be a stage in the life cycle of the virus; however, immunofluorescent staining has resulted in detection of the viral antigen within the Nogri body.[60] The number of Negri bodies increases with the duration of the disease. Negri bodies are not demonstrated in at least 20% of cases of rabies, and their absence from the brain material does not rule out the diagnosis. Negri bodies are common in cerebellar Purkinje cells, hippocampal pyramidal cells and neocortical neurons. Smaller, less well defined inclusion bodies, previously termed “lyssa bodies” are often more numerous than Negri bodies and also represent its accumulation of the virus. ‘These findings are therefore as pathogenomics Negri bodies.[61]

Degenerative neuronal changes are not usually prominent, which suggests that neuronal dysfunction of unknown cause is likely important in the pathogenesis of rabies, Rabies virus infection has been associated with apoptosis both in cell culture and in neurons in rodent models under specific experimental conditions.[62, 63] In some experimental situations apoptosis of virus infected neurons may be an altruistic response and serve to limit viral spread in the host, while in other situations apoptosis of virus infected neurons may be detrimental to the host and lead to increased neuronal injury.[64] However, the relevance of

observations in experimental models of apoptosis to natural rabies, in which features of neural apoptosis are not prominent, is not clear.

Paralytic rabies is characterized by perivascular inflammatory infiltrates and neuronal destruction that is primarily restricted to the lower brain stem and spinal cord. There may be lymphocytic, leptomeningeal inflammation and, within the cord, neuronal degeneration. The brains stem and cerebrum may show neuronophagia and presence of microgliâl nodules. [65] The histopathology of peripheral nerve discloses presence of segmental demyelination, remyelination and Wallerian degeneration. Segmental demyelination and remnyelination predominate over Wallerian degeneration and can be present alone. It is suggested that peripheral nerve involvement in paralytic form may be due to cross reaction of some protein component of virus with nerve myelin causing demyelinalion of peripheral nerve. [66]

Clinical Features

Incubation period

The infected individual remains asymptomatic during this period. The average duration of incubation is 20-90 days. Usually it varies from few days to months and also depends upon types of exposure. The rabies virus is segregated from the immune system during this period, and no antibody response is observed.

Prodromal period

The virus enters the CNS. The duration of this period is 2-10 days. Nonspecific symptoms develop like malaise, anorexia, headaches, fever, pharyngitis, nausea, vomiting, diarrhea, anxiety, depression. Few signs such as paresthesia, pain, or intense itching at the inoculation site is pathognomonic for rabies and occurs in 50% of cases during this phase; this may be the individual's only presenting sign.

Acute neurologic period

This period is associated with objective signs of developing CNS disease. The duration is 2-7 days. Symptoms include muscle fasciculation, priapism, and focal or generalized convulsions. Patients may die immediately or may progress to paralysis, which may be present only in the bitten limb at first but usually becomes diffuse.

The form of rabies known as furious rabies may develop during this period. Patients develop agitation, hyperactivity, restlessness, thrashing, biting, confusion, or hallucinations. After

several hours to days, this becomes episodic and interspersed with calm, cooperative, lucid periods. Furious episodes last less than 5 minutes. Episodes may be triggered by visual, auditory, or tactile stimuli or may be spontaneous. Seizures may occur. This phase may end in cardiorespiratory arrest or may progress to paralysis.

Another form of rabies, paralytic rabies, is also known as dumb rabies or apathetic rabies, because the patient is relatively quiet compared with a person with the furious form. Twenty percent of patients do not develop the furious form. Paralysis occurs from the outset, and fever and headache are prominent.

Coma

This begins within 10 days of onset, and the duration varies. Without intensive supportive care, respiratory depression, arrest, and death occur shortly after coma.

Physical Examination

Neurologic period

With furious rabies, patients present with episodic delirium, psychosis, restlessness, thrashing, muscular fasciculation, seizures, and aphasia.

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

Autonomic instability is observed with furious rabies, with various symptoms like, fever, tachycardia, hypertension, hyperventilation, anisocoria, fixed pupillary dilation (“blown pupil”), optic neuritis,facial palsy, mydriasis, lacrimation, excessive salivation, perspiration, postural hypotension,.

In patients with paralytic rabies, fever and nuchal rigidity may occur. Paralysis is symmetrical and may be either generalized or ascending and may be mistaken for Guillain-Barré syndrome. The sensory system is usually spared. Calm clarity gradually progresses to delirium, stupor, and then coma.

Coma and Death

Respiratory failure occurs within 1 week of neurologic symptoms. Hypoventilation and metabolic acidosis predominate. Acute respiratory distress syndrome is common. Bradycardia and cardiac arrest occur resulting into death. [66]

DIFFERENTIAL DIAGNOSES

Differential diagnosis of rabies includes: encephalitis caused by arboviruses such as Japanese, Eastern equine and West Nile viruses, enteroviruses, Nepah virus, and Herpes virus; acute hepatic porphyria with neuropsychiatric disturbances and signs of autonomic dysfunction; substance abuse like alcohol withdrawal or delirium tremens; acute serotonin syndrome due to administration of serotonin uptake inhibitors; tetanus; Guillain-Barré syndrome; transverse myelitis; neuroparalytic accidents, when the patient is from a country where nerve-type vaccines are used; psychiatric disturbances; cerebrovascular accidents; epilepsy; poisoning by atropine-like compounds; poliomyelitis; and intracranial mass.[67]

Laboratory diagnosis

Although there are a few reports of imaging studies, CT and MR imaging of the brain have generally been reported not to show specific abnormalities in rabies.[68-72] similarly electroencephalography usually shows only non-specific abnormalities and is not Diagnostically helpful.[73] CSF analysis may show a mononuclear pleocytosis, especially after a few days of the neurologic illness. Anderson et al.[74] found a CSF pleocytosis in 59% of patients in the first week of illness and in 87% after the first week. Infectious rabies virus has only rarely been cultured from CSF.

The diagnosis of rabies is made by isolation of the virus from tissue or secretions, detection of viral antigen in tissue samples (corneal smears, brain or rarely CSF), serologic evidence of acute infection or molecular techniques such as PCR or genetic probes [75-78] Since not all specimens are positive in every patient, particularly early illness of the disease, a skin biopsy specimen, saliva, cerebrospinal fluid, and corneal impression should be examined for virus. The optimal site of skin biopsy is time densely innervated nape of the neck, where a 5 mm punch biopsy above the hairline provides ample cutaneous nerves around hair follicles. A corneal impression is made by vigorously pressing a microscopic slide against the eyeball. The sensitivity of skin biopsy for diagnosis of rabies is in excess of 50% and may be as high as 90%. Specificity is nearly 100 percent.[79] Neck biopsy has replaced corneal

smears as the diagnostic method of choice, since fluorescent staining of conical cells is associated with a higher percentage of negative results.

Rabies virus infection can be diagnosed serologically based on the presence of serum neutralizing antibodies against rabies virus in previously unvaccinated patients, but these antibodies are not usually present until the second week of illness and they may not be present even by the time of death. If the patient has not been immunized against rabies, a fourfold rise in titer of neutralizing antibody to rabies virus in serial serum samples is diagnostic. If the patient has been vaccinated, a clue to the diagnosis may be obtained from the absolute titers of serum neutralizing antibody and the presence of neutralizing antibody in CSF Postexposure rabies prophylaxis rarely produces CSF neutralizing antibody to rabies virus. If present after prophylaxis, it is usually found at a low titer (<1:64), whereas CSF titers in human rabies may vary from 1:200 to 1:1,60,000. Active disease produces high titers (>1:5000), which is helpful for diagnosing acute rabies from post vaccination encephalomyelitis associated with vaccines derived from animal neural tissue.[45, 74]

The best diagnostic method for laboratory confirmation for rabies is detection of rabies virus RNA in saliva by reverse transcription polymerase chain reaction (RT-PCR), which has high sensitivity and specificity. RT-PCR amplification is a relatively new advancement in antemortem diagnosis of rabies.[78, 80] Using this technique, rabies virus RNA may be detected in saliva, skin biopsies, CSF' and brain tissue. Rabies virus RNA was detected in saliva in ten out of ten patients, the United States who were assessed with this technique.[22] The nucleic acid sequence based amplification (NASBA) technique is relatively easy to use and the whole process from extraction through amplification to detection takes about 4 hours. However, not every sample of saliva or CSF was positive; hence every pith must have both saliva and CSF tested, and preferably more than one saliva sample.[81]

Samples of brain tissue obtained either on postmortem examination or at brain biopsy should be subjected to: (1) mouse Inoculation studies for virus isolation, (2) fluorescent antibody (FA) staining for viral antigen, and (3) histologic and/or electron microscopic examination for Negri bodies or RTPCR for detection of rabies virus RNA. While mouse inoculation studies for virus isolation and direct FA staining, for viral antigen are quite reliable and sensitive, "autosterilisation" may occur and these tests may be negative If the patients' life has been prolonged and high levels of neutralizing antibody are present in serum and CSF.[82, 83] It Is Important to remember that the absence of Negri bodies does not

exclude rabies. The use of fluorescence histochemistry can demonstrate rabies viral antigen. Keep high index of suspicion in such cases."

Treatment

There is no specific treatment for rabies.[84] Therapy of rabies has been unsuccessful except. In four reported cases in which rabies vaccination was completed prior to the onset of disease. [85-89] In all other reported cases, both antiviral therapy with cytosine arabinoside or ribavirin and Immunotherapy's with antiracism virus hyper immune serum or Interferon-alpha given either by intrathecal or intramuscular routes have been unsuccessful. Therapy is supportive and adequate sedation and analgesic is very important for palliation. Intensive supportive medical care can clearly prolong the lives of patients with rabies, but the cost and inevitably fatal outcome have led clinicians to question its use.

Prevention

Rabies is the only disease where we can start prophylaxis alter the patient has been exposed. The reason for this is that it has a long incubation period hence we get enough time to take protective measures. The opportunities available for the destruction of the virus.[90] Extensive experience in various areas of the world indicates that, where appropriately administered the combination of local wound treatment, passive Immunization and vaccination is uniformly effective. All 3 elements are essential, since rabies has occurred when one of the elements was omitted.[91]

Local wound therapy

The wound should be scrubbed with soap and then flushed with water. Both mechanical and chemical cleansing is important. Quaternary ammonium compounds such as 1 to 4% benzalkonium chloride or 1% cetrimonium bromide are useful because they inactivate the rabies virus. However, 0.1% benzalkonium solutions are less effective than 20% soap solutions. The choice of the cleaning solution is less important than the copious of the fluid administered.[92]

Pre-exposure prophylaxis

The people who are considered as high risk group need pre-exposure prophylaxis. These groups includes; aveterinarian, animal handlers and laboratory workers; bthe people whose activities bring them in contact with rabies virus or rabid animals; c-international travelers

likely to come in contact of the animals in the rabies threaten areas. All these groups should be treated with rabies vaccines to avoid the chances of sudden infection (Table 3).

Post-exposure prophylaxis

If a person is bitten by an animal, the wound and scratches should be washed thoroughly with soap and water to decrease the chances of infection. Post-exposure prophylaxis involved one dose of rabies immune globulin and five doses of rabies vaccine within the 28 days period. Rabies immune globulin contains antibodies from blood donors who were given rabies vaccine. The rabies vaccine works by stimulating a person's immune system to produce antibodies that neutralize the virus (Table 4).[93]

Passive immunization

Human rabies immune globulin (HRIG) is preferred because equine antirables serum (ARS) may cause serum sickness. The recommended dose of HRIG is 20 lU/kg while that of ARS is 40 lU/kg. Fifty per cent of the total dose should be infiltrated locally In the area of the wound while the rest should be administered intramuscularly in the gluteal area. Because serum may participate to suppress active production of the antibody, larger than recommended doses should not be given. [94, 95] The WHO is currently considering an amendment which suggests that firstly as much of the HRIG dose as possible should he infiltrated around the wounds; secondly that the HRIG should be diluted if there is insufficient volume to infiltrate all the bite sites and thirdly, that the remainder is administered into the muscle of the upper thigh rather than the gluteal muscle.

Active immunization

Active immunization against rabies should be started simultaneously with administration of antiserum. There are two types of vaccine available for active immunization: the nerve tissue vaccine and the tissue culture vaccines. Several vaccines are available:

> Semple vaccine and suckling mouse brain vaccine (SMBV), either phenolised 10% brain suspension or beta-propiolactone inactivated 5% brain suspension.

> Human diploid cell vaccine (IIDCV)

Purified chick embryo cell vaccine (PCECV)

> Purified Vero cell vaccine (PVCV)

> Purified duck embryo vaccine (PDEV)

Cell culture-derived vaccines are clearly the first choice. They are effective and well tolerated. The WHO recommends "the use of vaccines prepared in cell culture should replace those derived from brain tissue as soon as possible".

In India, about one million people each year are injected with rabies vaccine; two-thirds of these receive the Sample vaccine, and about one-third cell culture vaccines. The PCECV, Rabipur, is manufactured in India under license from Germany. About 11/2 million doses of this vaccine arc used and about 1/2 million closes of PVCV. The ethical dilemma is how to choose between these vaccines. The first one is cheap, but unsafe, The other 3 vaccines are extremely safe, but they are expensive The post-exposure prophylaxis with HDCV is out of reach except for the well-to-do; the cost of a course of either PCLCV or PVCV is equivalent to about one ninth's earnings for the average Indian citizen.[96]

Complications of vaccination

The incidence of neuroparalytic complications in the use of nerve tissue vaccines varies between 1 in 600 to 1 in 2500. [96, 97] The complications are divided into major (encephalitis, myelitis, polyradiculitis, meningitis) and minor (fever, myalgia, and skin reactions). Postvaccinial encephalomyelitis usually develops 8 and 15 days after the host inoculation; most patients develop complications during or soon alter the course of vaccination. There is no relation between severity of disease and number of injections received. The incidence of neuroparalytic accidents from Semple vaccine (pliemiol-inactivated rabies virus grown in sheep brain) is more frequent (1:400) as compared to the incidence of similar complications from suckling moose brain rabies vaccine (1:8000). This is because of the presence of lesser amount of animal in the later, thus reducing the chances of allergic encephalomyelitis. This adverse reaction may cause death in a small proportion of affected persons.

Although the HDCV remains the gold standard for assessing the performance of cell culture vaccines, hypersensitivity reactions are quit uncommon for the IIDCV licensed in the USA. About 6 of patients suffer a systemic allergic reaction following a booster dose of HDCV after primary immunization with the I 10CM Most of the reactions are probably due to the β-propiolactone used to inactive the virus as it may under the bovine albumin antigenic Serum reactions are not seen with the PCECM Rabies since the purification process evidently removes most human serum albumin in the cell growth medium urn before virus inactivation with in β-propiolactone.

Vaccination schedules

There are several dose recommends that have been shown to be effective studies and clinical reports suggest that all are effective if administered properly and timely after the exposure of rabies virus.

The most widely used WHO Eastern scheme calls for a single 1.0 ml dose Administered in the upper deltoid on days 0, 3, 7, 14 and 30 with in an optimal further dose on day 90. [98] Doses should be given intramuscularly in the deltoid or, in children, the anterolateral thigh region, but not in the gluteal area where lower antibody responses have been found. The vaccine should be given at the same site as the antiserum, Post-vaccination serologic testing may he used in unusual iris lances such as immunosuppressed patients. After the fifth dose, antibodies are always present, usually at a titre of >10 IU.

The 2-1-1 regimen (Zagreb scheme)[99] is an abbreviated multi-site schedule in which two 1.0 ml doses i.m. are administered in the upper deltoid (one in the left arm, one in the right arm) on day 0, with a single dose on days 7 and 21. This schedule induces an early antibody response and may be particularly effective if RIG cannot be given. It is in routine use in some countries.

The two-site intradermal regimen [100] is used primarily in Thailand and involves giving two 0.1ml doses of vaccine intradermal at two sites on days 0, 3 and 7 and one 0.1 ml dose on days 30 and 90. Although this regimen is effective when given correctly, the WHO cautions that only staff that has been trained in this technique should administer intradermal injections. Furthermore, as the lyophilized vaccine, once reconstituted, must be stored at 4-8 ^{0}C and used within a few hours, this scheme does not offer any cost savings unless more than one patient needs vaccination. Upon exposure of an immunized person to rabies virus, a single 1.0 ml dose of the vaccine is given i.m. in the upper deltoid on days 0 and 3, although this is currently under review. No human RIG is required.

Vaccination failures

Failure of post-exposure rabies prophylaxis may be due to several reasons; non-potent vaccine, faulty transport and storage, incomplete dissolution of the vaccine in the diluents, under-dosing, delay in treatment, wrong classification, inappropriate local wound management, underlying conditions like cirrhosis or alcoholism, concomitant administration of immunosuppressive drugs, intra gluteal administration. [101,102]

Raising Rabies Awareness

September 28 is World Rabies Day, a global health observance that seeks to raise awareness about rabies and enhance prevention and control efforts. Co-sponsored by CDC and the Alliance for Rabies Control (ARC) since 2007, World Rabies Day has been celebrated in countries throughout the world, including the U.S.

World Rabies Day is an excellent time to take steps that can help prevent and control rabies, such as vaccinating pets including dogs and cats and providing education on how to avoid the animals that typically transmit rabies: raccoons, bats, skunks, and foxes.[103]

Future directions of rabies research

Today, in the 21st century, more than 100 years after the development of the first rabies vaccine, our challenges are still the development of cheap and safe vaccines for humans and the implementation of vaccination programs for domestic as well as wild life animals. Oral vaccination of wild life with attenuated as well as recombinant vaccines have successfully reduced or eliminated wild life rabies in foxes in Europe.[104] Edible plants expressing rabies virus antigens may provide the cheap source for mass vaccination against rabies in both humans and animals. Development of antisense oligodeoxynucleotides (ODNs) may inhibit rabies virus replication.[105] ODNs complementary to rabies virus genomic RNA have a strong ability to inhibit rabies virus infection in cell culture, although it is still too early to say whether this can eventually translate into therapy.

Death from rabies in India

This study is the first to provide an estimate of deaths from symptomatically identifiable furious rabies based on a representative sample of Indian deaths and to report the geographic, age and gender distributions of these deaths. While the MDS was not designed specifically to identify rabies deaths, its large size, and representative sampling make it suitable for identifying deaths due to relatively rare conditions and subsequently generating reliable estimation of population based rates. Our figure of 12,700 (99% CI 10,000 to 15,500) human deaths from rabies in 2005 is within the uncertainty ranges of a recent indirect estimate by Sudarshan and colleagues of 17,137 (95% CI 14,109–20,165) prior to the addition of 20% to account for paralytic/atypical forms of the disease. While the Sudarshan study also used verbal autopsies, it relied on case finding in communities located near large medical centers followed by interviews of people in the communities in which the cases originated and thus cannot be considered a truly nationally representative sample.

Similarly, the derivation of 19,713 (95% CI 4,192–39,733) human deaths using a dog-bite probability model is based on several assumptions, most notably that the epidemiology of canine rabies in India, where very few dogs are tested for rabies, is similar to that in Africa. To our knowledge, there have been no nationally representative studies of canine rabies in India. Despite these methodological challenges, the three studies together suggest a range of rabies deaths between 13,000–20,000 deaths. Although we did not report any rabies deaths in a small number of states (which represent less than 7% of India's population and total deaths), routine government hospital data and medically certified causes of death from urban areas from 1998 to 2004, would add only about an additional 100 to 500 rabies deaths from these states. Thus, the inclusion or exclusion of these states does not alter our national estimate of 12,700 deaths and lies well within the 99% confidence range of our estimates (10,000–15,500). While the MDS was not designed to examine rabies treatment, we were nonetheless able to extract useful information from the narratives. The completely treated cases probably received the Simple-type rabies vaccine that was still widely used in India during the study period (2001–03). The partially treated cases might have received Semple or cell culture rabies vaccine, tetanus toxoid, antibiotics, another drug, or a traditional remedy. Since treatment information was contained only in the narrative, we are not able to comment on the timing or specific contents of the injections received by the deceased. [106]

Table-1: Annual mortality figures for rabies

Country	Deaths/million population
USA	0.02
S. Africa	0.10
Vietnam	4.70
Thailand	4.50
Indonesia	11 .00
Bangladesh	18.00
India	35.5

Table 2: Virus Serologic Type

Rabies*	1	Mammals (dogs, cats, cattle, Bats, wild carnivores, humans)
Lagos bat	2	Bats
Mokola*	3	Humans, dogs, cats, shrews
Duvenhage*	4	Bats, humans
Kofonkan	5	Culicoides mosquitoes
Obodhiong	6	Mansonia mosquitoes
Rochamboau	7	Mosquitoes in French Guyana

(* Associated with human disease)

Table 3: Pre-exposure rabies vaccination

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

	known source -bite or non-bite exposure	in rabies epizootic areas	necessary
* Pre-exposure booster immunization consists of one dose of rabies culture cell vaccine (in Brazil, Vero cell rabies vaccine) injected into the deltoid area by intramuscular (0.5 ml) or intradermal (0.1 ml) route.			
Sources: Costa *et al.*, 1999 (20) and Brasil, Ministério da Saúde, 2004			

Table 4: Post-exposure rabies treatment

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

	observation period, stop treatment. If the animal disappears, dies or becomes rabid, continue treatment to complete 5 doses of rabies vaccine and use heterologous anti-rabies serum.	observation period, stop treatment. If the animal disappears, dies or becomes rabid, continue treatment to complete 5 doses of rabies vaccine and use heterologous anti-rabies serum.	
There are some specific differences between the Brazilian National Rabies Recommendations (13, 20) and those for São Paulo State, they are: * Start treatment with 2 doses of the vaccine, one on each of days 0 and 3. **Start treatment with 2 doses of the vaccine, one on each of days 0 and 3. † Start treatment with heterologous anti-rabies serum and vaccine. If the animal is healthy on the 10th day after the accident, treatment may be suspended.			
Source: Costa *et al*, 1999 (20).			

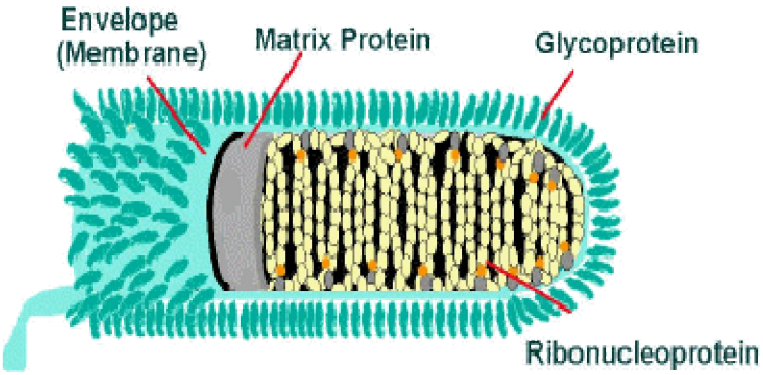

Figure 1. Structure of rabies virion

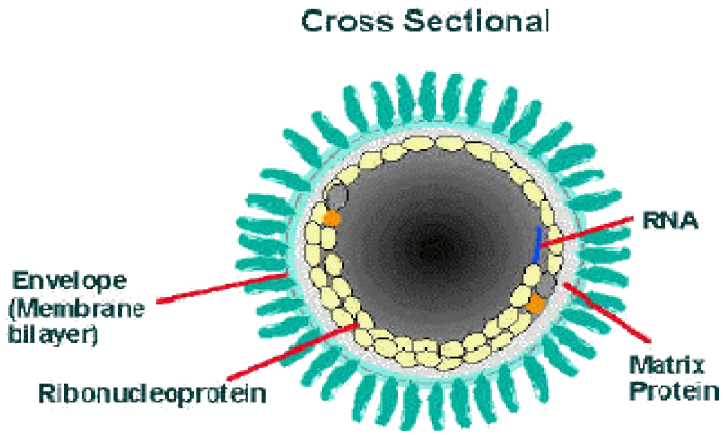

Figure 2. The cross-sectional diagram of rabies virus

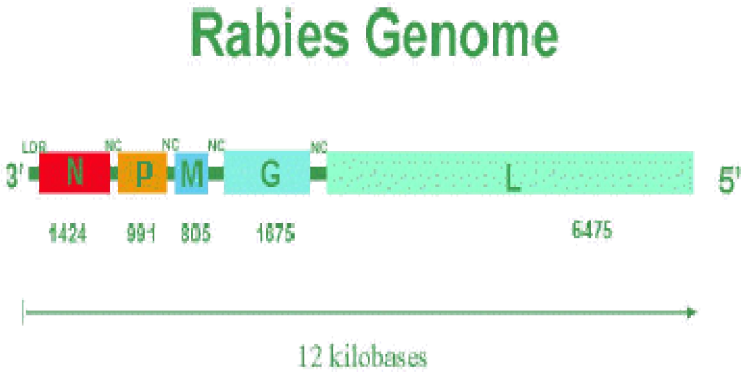

Figure 3. Rabies Genome

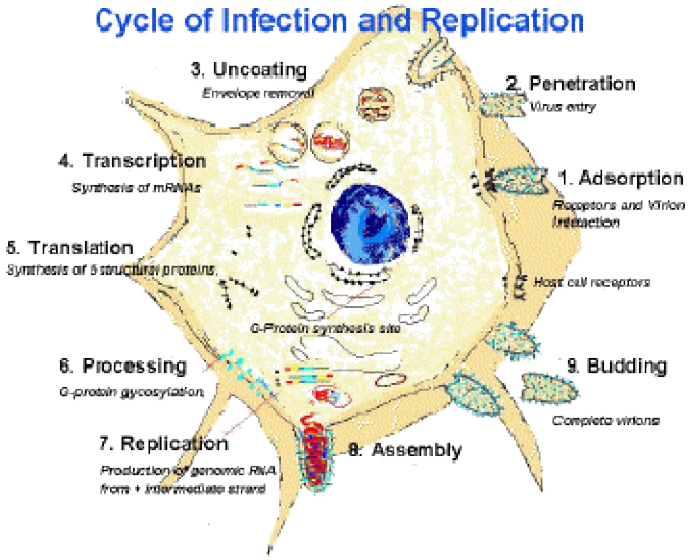

Figure 4. Cycle of Infection and Replication

CONCLUSION

Rabies is an ancient disease that remains a modern problem in much of the developing world. A growing body of knowledge about the rabies virus has not resulted in effective treatment, so prevention remains the key. Vaccination and control of rabies in domestic animals has reduced human rabies to almost negligible levels in developed countries. Further reduction in human mortality due to rabies seems unlikely in developed countries, although it may be possible to prevent some cases acquired abroad through proper education and the vaccination of travelers.

Extensive health education is required for the general public paramedical and medical staff in tropical countries, if any accident has to be made on this problem. The control of stray dogs would be an ideal deterrent. Unfortunately, the explosion of dog population makes

such a step highly unfeasible, but not impossible. The deplorable absence of a comprehensive national canine rabies control program in most tropical developing countries especially India, leaves vaccination of the exposed Individuals the only alternative. Ignoring this practice, as may happen because of social-cultural beliefs, may prove fatal and even minor dilations front the recommended regimens in countries where the risk of rabies is very high have led to a handful of cases of rabies despite immunization. Thus rabies remains a disease that is widely feared; yet readily prevented.

We estimate that there were 12,700 deaths due to symptomatically identifiable furious rabies in India in 2005. It is very important to note that this figure underestimates the total number of deaths due to rabies since paralytic and atypical cases would not have been detected by verbal autopsy. This study is the first to estimate rabies mortality based upon a nationally representative sample of deaths rather than modeling or from extrapolation from selected focal surveillance. Thus we provide previously unavailable regional and demographic information about human rabies deaths that can help to focus both human and canine rabies control programmes in the country and act as a baseline that can be used as comparison for future estimates of rabies mortality. Elimination of the canine reservoir of rabies is not likely in India at anytime in the near future. However, the concentrated geographic distribution of rabies in India suggests that a significant reduction in the number of human deaths or potentially even elimination of rabies deaths is possible and this study serves as a baseline against which future gains may be measured.

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