# CHAPTER I

# INTRODUCTION

## 1.1 Rabies Virus History

Rabies is regarded as a serious health hazard and as enzootic disease in many parts of the world.

Rabies has a very long history, rabies is Latin word come from an old Sanskrit word "Rabhas" means "to do violence," the Greeks called rabies Lyssa or Lytta which meant madness. The disease in man was described as hydrophobia in which the sick person is tormented at the same time with thirst and fear of water.

Democritus is thought to have made the first recorded description of canine rabies some 500 years. B.C. Aristotles, in 4$^{th}$ century B.C., described that dogs suffer from the madness. This causes them to become very irritable and all animals they bite become diseased. Others who mention rabies in ancient times were of the belief that not only caused by rabid dogs but also could spread by others mammalian The saliva contained the poisonous agent. In 1840 Professor Rey stated that they were able to transmit the disease through several animals by the inoculation of saliva. His idea was experimented by Zinke, by taking saliva from a mad dog and painted the saliva into incision on the foreleg of 1 year old dog, the dog had caused symptom in the day of seventh, by the tenth day the dog had overt rabies. Krugelstein reported on every phase of rabies in 1826, neither Krugelstein nor Zinke had any idea of living agent.

Fleming who believed and supported theory of the spontaneous generation, was a strong propenent by citing some bacterial infections in animals of spontaneous appearance of disease. He accepted the inoculation of rabies by a bite as the cause of disease but the origin of the virus took him fall back on spontaneous generation.

In 1879 Galtier reported his experiment on transmit rabies to rabbits and from rabbit to rabbit. The symptoms were paralysis and convulsions with average incubation period was 18 days, but he did not state his method. Another experiment by Maurice Raynaud, he took saliva from rabid patient and injected it subcutaneously into a rabbit's ear. The rabbit was paralyzed and died on the day of fourth.

In 1881 Pasteur reported his success experiment in producing rabies by injection CNS material and spinal fluid directly into the brain, the incubation peroid was shorted. Animals which recovered rabies symptoms was immune to later rabies inoculation, and he was unable to cultivate any bacteria from rabid brain, In 1885 he reported attenuated phenomenon of virus by passaged rabies virus from dog to monkey, and then from monkey to monkey, the virulence of virus fell off at each passage. The virus did not resume the virulence of street virus in dog or rabbit. Even intracerebrally inoculation might not produce disease. But if virus was passed back to the dog via into the bloodstream, it was much more virulent than street virus. When he passed street virus through successive rabbits intracerebrally inoculation, the incubation period gradually became shorter until it reached a fixed time of 7 days.

In 1903, Negri, who discovered the bodies which beared his name, he observed these bodies presented in the dogs brain at the horn of ammon. They were also found in spinal cord, and stained with eosin methylene

blue. In 1909, he described these bodies were parasites. In 1907 Bartarelli found that virus reached the saliva gland via peripheral nerves, Roux and Nocard found that rabies virus appeared in the saliva 2-3 days before the least of clinical symptom, and as long saliva virulent as 6 days before the animal showed any recognizable clinical signs of rabies (50.)

## 1.2 Morphology and Physiochemical Properties

The morphology of rabies virus has been studied in cell culture and tissues from infected animals. Rabies virus has been classified on a morphology basis as a Rhabdo virus, sero type I (42).

By electron microscopy, the virions appear as rod like particles usually with one round and one flat end. Like bullet-shape with a fairly constant diameter of 75-80 nm. And a length of about 180 nm. The virion is composed of a helical nucleocapsid surrounded by a membrane bearing surface spikes that have a knob-like structure at the distal end. The nucleocapsid is a single right-handed helix (1,15,42).

Rabies virus contains 5 major structural protein components detectable by electrophoretic fractionation (1,12,24,42). The first component with the molecular weight (m.w.) of 80,000 amu. is a glycoprotein-G that constitutes the protein moiety of the spikes protruding from the viral envelope. The second has mw. of 62,000 amu. is polypeptide-N corresponds to the protein of the viral nucleocapsid, this protein is identical with N-protein moiety isolated from infected cells. The third is protein-$M_1$ (mw. 40,000 amu.) associated with the nucleocapsid and the largest polypeptide-L having a mw. of 190,000 amu. The fifth has mw. 25,000 amu., the protein-$M_2$

The five distinct polypeptides, four of which have been shown recently to be distinct antigens,

1.2.1 The glycoprotein-G and membrane protein-$M_2$ antigen are located on outer surface.

1.2.2 The nucleoprotein-N and the protein-$M_1$ are internal antigens closely associated with the virus-RNA which, together with the largest protein-L, form the so-called replicative complex of the virus.

But glycoprotein-G was shown to be the only antigen capable of inducing the formation of virus-neutralizing antibodies, and of protecting animals against subsequent challenge with rabies virus (1,16,17,42).

A virion - associated transcriptase synthesising m-RNA at the early stage of infection. Enzyme activity correlated with the content of L-protein and was highest when virus was grown at 33°C (3,19,25,42).

Rabies virus has a single-stranded RNA, with mw. of $4.6 \times 10^6$ amu. And a sedimentation coefficient of 45 S (1,42).

The gross chemical composition (from flury HFP) has been estimated to be approximately 76 % protein, 1 % RNA, 22 % lipid, and 3 % carbohydrates. Of the total protein about 1/3 is L-protein, almost half with glycoprotein, and the rest with the two remaining membrane protein.

Rabies virus is rapidly inactivated by lipid solvent and 0.1 % trypsin. The virus is relatively stable at pH 5-10 at 40°C and about 35 seconds at 60°C, but inactivated rapidly at pH 3 or 11. Rabies virus is inactivated when suspended in 0.1 % bovine serum albumin at near neutral pH with a half-life of about 4 hr. at 40°C, and about 35 seconds at 60°C, but it is stable for several days at 0-4°C and for several years when frozen at 70°C or freeze-dried and held at 0-4°C (42).

The cycle of virus replication may be as short as 6 hours (22, 42).

1.3 Pathogenesis

The susceptibility of the animal to rabies infection not only by quantity of virus introduced into the animal but also by the site of the bite, the age of animal, and by the properties of virus strain (22).

In human,causes of infection frequently by bitting of rabid animals, some cases reported infection by aerosol has resulted from natural exposure (freetail bat virus) as well as from laboratory exposure oral infection of some rodents, insectivores, foxes and skunks has been demonstrated in the laboratory (10,42).

The virus from rabid animal usually introduced into a bitten wound. Occasionally the virus gain entrance through abrasion of skin or mucous membrane. The location of rabies virions can be detected within axons of animals, then invades along nerve fiber and spreads to spinals cord and to brain by passing centripetally. The rate of virus travelling along nerve fibre is roughly 3 mm. per hour (13). Propagation of the virus seem to occur passively, through axoplasm of peripheral nervous system to CNS (22,52,53,55). It is generally accepted that local virus replication occurs in mesenchyma tissue at the site of inoculation of the virus (22). The entry of rabies virus into the nervous system through sensory endings in muscle and spindles is well established (19,48), and the accumulation of rabies virus at nicotinic acetylcholine receptors of motor nerve end-plate, in the initial stages of nervous system penetration (31, 55). But the uptake mechanism of rabies virus by nervous system from the accumulated site is still unknown, the available evidence suggests that there are high density or affinity of surface virus receptors, and

Table I : Animal susceptibility to rabies infection (42)

| Susceptibility | | | |
|---|---|---|---|
| Extremely high | High | Moderate | Low |
| Foxes | Syrian hamsters | Man* | opossum |
| Coyotes | Skunks | Dogs | |
| Jackals and wolves | Raccoons | Sheep,goats | |
| Kangaroo rats | Domestic cats | and horses | |
| Cotton rats | Bats | Non-human primates | |
| Common field voles | Bobcats | Cattle | |
| Weasels | Mongooses | Field hamsters | |
| | Rabbit | Ferrets | |
| | | Squirrels | |

* Epidemiological evidence only based on the intramuscularly inoculated dose required to infect at least 50 % of animals..

1.4 Rabies Occurrence and Epidemiology

Now rabies is still a health problem as an enzootic disease, particularly in developing countries, rabies is widespread in most of the country of Central and South America, Africa and Asia. This disease has not occured or has been rarely eliminated in U.S.A., Canada, Europe, Oceania and many islands in the oceans and Mediterranean Sea. There are only few reports on successful rabies control, because the appearance of changing ecological conditions and in particular the influence of settlement areas, and the increasing density or proximity of dog populations which is closely correlation to the density of human population and occurrence rabies in most developing countries. As a consequence dog population able to sustain rabies are no longer restricted to major urban areas but seem to built up rapidly in the rural environment. This depends not only on the increase human population, but also on factors such as the increasing use of the dog for protection and hunting, another reasons is about the shortage of modern vaccines for man and animals. In developing countries dog rabies is spreading into new areas and frequency of cases is increasing in infected area. This make World Health Organization to programme not only to eliminated reservoir (dogs) but been mainly directed towards improved post-exposure treatment in man as well (2,4,12,15,42).

Thailand is also considered to be highly endemic area for rabies, the incidence of the last 3 years reported human death of rabies about 212 cases in 1981, 204 cases in 1982, and 201 cases of 50 weeks cumulated in 1983. The most common carrier of rabies in Thailand is dog (18,40), from brain specimens which received for rabies diagnosis were reported 9,952 cases, positive 6,209 cases in 1981, in 1982 about 10,833 cases positive 6,684, and in 1983 up to October 9,999 cases positive 5,939, and

more than 90 % of specimens were dogs (18).

Some investigators reported rabies carrier in Thailand is especially stray dogs, by examined the brain specimen of killed stray dogs, about 3 % of them found rabies antigen (40,51).

1.5 Rabies Prevention and Therapy

In the ancient times, Celsus, physician who lived in the first century, treated the patients those bitten by rabid dogs by burning, cupping and also sucking the wounds, when disease appeared, the patient was thrown into a pond to allow the patient to sink times after times, for thus both the thirst and dread of water was removed. Fleming used cock's brain or cock's comb applied to the wound, the flesh of a mad dog was salted and taken with food as a remedy.

Any methods for therapy and prevention would not satisfied, since the day of Pasteur, about 1885, when he discovered fixed virus in rabbit after passages, the rabid rabbit cord was broth and preserved in a flask of dry air, then carried out by injection 50 dogs ,all dogs were immune, or refractory to rabies, then he tried in a boy of 9 years, Josep Meister with 13 successive inoculation increasing virulence, beginning with one dried long enough to be avirulent. The boy never developed rabies. These practice of immunization is still continued and each year between 500,000 and 1,500,000 throughout the world receive a course of treatment.

1880 Fermi, reported to point out various defects in Pasteur's vaccine. He introduced new vaccine which treated with carbolic acid which more advantage than Pasteur's one, this vaccine attenuated type can be preserved and sent anywhere

1919 Sir David semple introduced a dead carbolic vaccine.

1940 Dawson and Klingler found that the growing of rabies virus in the developing chick embryo after many passages the virulence greatly reduced. Later H. Roprowski and H.R.Cox (1948) adapted these methods for production of chick embryo origin vaccine.

1960, the first tissue culture vaccine were produced in primary of Hamster Kidney cells by Fenje, and 1965 Cabasso et al., described the production of Flury low egg passages (LEP) chick embryo fibroblast, and later another tissue culture vaccine was developed such as HDCV in 1964 (12,50).

Prevention of Rabies in Men

A. Pre-exposure Persons who run a high risk of repeated exposure, such as laboratory staff working with rabies virus, veterinarians, animal handlers and wildlife officers, should be protected by immunization consist of 3 injections of a potent rabies vaccine (at least 2.5 IU.) on the days 0,7 and 28, or, 0,28 and 56 (42). The protective rabies antibodies titre may reach 0.5 IU/ml.(59). Further booster injection should be administered at annually (42).

B. Post-exposure Initiation of treatment in severely exposed persons should be immediate and never await the results of laboratory diagnosis in any event, local treatment is usually completed and serum and/or vaccine therapy is administrated. The report from a reliable laboratory indicating absence of rabies usually justifies cessation of treatment.

Vaccine application

Vaccination application schedule recommended in a given situation depends on the type and potency of vaccine, by including booster doses which serve to overcome interference from serum or globulin and to prolong duration of serum (42,54,57).

A. Nervous-tissue vaccine (NTV)

Brain tissue vaccine : Semple vaccine, by vaccination 14-21 doses plus booster doses 30 and 90 days after first dose of vaccine.

Suckling mouse brain vaccine : with 14 daily doses plus booster doses 10 and 20 days after the primary series. Reduced schedules are used in several Latin America and France, consisting generally of 7 daily doses and booster on the day 10, 20 and 90, requirement potency of reduced schedules ; should be 1.3 IU ; furthermore, the complete schedule should be used when application with serum.

The disadvantage of nervous tissue vaccine is known of the incidence of neuroparalytic following a course of vaccination is about 1 : 160, with fatal termination in 1 : 11,000 (3,42), because of adult brain contains an encephalitogen associated with myelin, this factor could be noted that very young animals considered to be free from paralytic factor (21), but it had reported cases of neurological disease from suckling mouse brain vaccination (12,23,42).

B. Vaccines prepared from avian embryo

The successful adaptation of rabies virus to chicken embryo, two of best known types, the Flury low egg passages (LEP) and Flury high egg passage (HEP). Both vaccine are live attenuated, a course of vaccination is 21 doses plus 3 booster doses 10,20 and 90 days after completion

of the vaccine schedule (42).

Another similar vaccine, a fixed virus strain is grown in duck embryo (DEV) (9). The DEV is regarded as safer and antibody was produced more rapidly than NTV. On the otherhands, potency level of DEV is consistency lower than those of NTV (12,42).

DEV also gives disappointingly low antibody levels, and the incidence of neurological complications is 1 : 25,000. Only one death in about 172,000 vaccinees (12). DEV is used for pre-and post-exposure in U.S.A. (12,42).

C. Tissue culture vaccine (concentrated, minimum potency 2.5 IU). Six doses on day 0,3,7,14,30 and 90. The 90 day dose is not used in some countries (42).

From the first experiment tissue culture vaccine were produced in primary cultures of hamster kidney cells (12,50), after that Flury LEP vaccines were produced in chick embryo fibroblasts (12,50), Fenje's virus (ERA) strain was passaged in chick embryo and then adapted to pig kidney cell. These vaccines were used for animal immunization.

Tissue culture vaccine suggested for human use include hamster kidney cell, syrian hamster kidney cells and sheep embryo kidney, chicken embryo fibroblast, rhesus monkey kidney cells, and the human diploid cells strain WI-38 (4,12,42,50).

All tissue culture types give high level of antibody and have been free of serious adverse effect, although occasional vaccinees may be caused some illness.

Antirabies Serum

A. Heterologous origin (equine, rabbit, etc), were globulin fractions of purifying and concentrating serum. Serum should be administered intramuscularly in a single dose of 40 IU/kg. body weight followed by a complete course of vaccine.

B. Human origin globulin. The dose recommended is a single administration of 20 IU/kg. body weight followed a complete course of vaccine.

Serum sickness occures in approximately 15-45 % of person given heterologous serum, it is less frequent in person below 15 years of age. It was found that passively administered immune serum tends to inhibit the active immunity stimulated by vaccination (42,53,57).