ASSESSMENT OF POST-EXPOSURE ANTI-RABIES THERAPY IN GOATS

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DECLARATION

I hereby declare that the thesis entitled **"ASSESSMENT OF POST-EXPOSURE ANTI-RABIES THERAPY IN GOATS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"ASSESSMENT OF POST-EXPOSURE ANTI-RABIES THERAPY IN GOATS"** is a record of research work done independently by **Raji James,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

The goat is considered a convenient domestic species for commerce and research due to its biological and managemental advantages over other farm animals. The goat population in Kerala is estimated to be about two million whereas in India it is 114 million. It is well known that the goat plays a pivotal role in the rural economy of Kerala. Amongst the infectious diseases, with public health significance, rabies in goats is observed to be an alarming problem in Kerala.

Although rabies remains untreatable, the infection is preventable. The discovery of anti-rabies vaccine by Louis Pasteur in 1885 and the subsequent development of tissue culture vaccine and rabies immunoglobulin are effective in humans and animals for prophylactic as well as post-exposure anti-rabies therapy.

Post- exposure therapy of rabies is the combination of local treatment of wounds, passive immunoglobulin and vaccination (WHO, 1992). In selecting a vaccine for immunizing domestic animals, three factors must be considered: the vaccine's ability to protect animals against exposure, its cost and the duration of immunity, which it confers (Dierks, 1981). In the last 10-15 years, considerable progress has been made in improving the quality of vaccine. An outstanding achievement has been made in the production of high quality tissue culture vaccine for veterinary use. Tissue culture vaccines are the most popular amongst various types of rabies vaccine and studies have shown that inactivated tissue culture vaccines elicited good immunological response in all animals.

Sufficient information about the structure and controlled dissection of rabies virus resulted in the development of new anti-rabies vaccine. Now we are in the era of DNA vaccines for rabies virus. Studies have shown that DNA rabies vaccines comprising of plasmid DNA encoding rabies virus surface glycoprotein protect mice, dog and non-human primate against rabies virus infection. Since DNA vaccines can be produced at a low cost and are stable at room temperature, they are ideally suited for immunization in developing countries (Rangarajan *et al.*, 2000). This type of vaccine does not have the problems associated with live or

recombinant vaccines, such as safety, possible reversion to virulence, risk of contamination with adventitious agents and lack of stability and are superior to the inactivated vaccines (Woldehiwet, 2002).

There are no definite guidelines for post-exposure therapy of goats. However, previous studies had shown that post-exposure therapy with tissue culture rabies vaccination was effective in sheep (Soria and Blancou, 1995), cattle (Basheer *et al.*, 1997a), pig (Mitmoonpitak *et al.*, 2002) and in dog (Cho and Lawson, 1989).

Hence, the present study is conducted with the following objectives

- To assess the efficacy of two anti-rabies vaccines and two different schedules for post-exposure therapy in goats.

Review of Literature

2. REVIEW OF LITERATURE

2.1 HISTORY

The origin of rabies virus appears to be in Africa and this disease was first described in the Sumerian law code from the city of Eshunna in Mesopotamia dating from about 1885 B.C. (Beran, 1981).

Rabies was described in dogs by the philosopher Democritus (500 BC) and in human patients by Hippocrates during 400 BC (Smith, 1996).

Rabies recognized throughout the world as one of the earliest disease of mankind and the human implications of the disease were found in early Egyptian hieroglyphics and the writings of Asclepiadae, Democritus, Aristotle and others (Dreesen, 1997).

According to Fu (1997) it was an Italian savant, Girolamo Fracastoro, who really knew about rabies as we know the disease today and put the true nature of rabies on the record in his treaties ' the incurable wound', dated 1584.

2.2 ETIOLOGY

The virus of rabies is the prototype of the genus Lyssa virus in the family Rhabdoviridae (Greene and Dreesen, 1998).

2.3 VIRUS PROPERTIES

According to Kissling (1958) both fixed and street virus strains were propagated serially in hamster kidney tissue cultures, but no cytopathic changes were evident in these cultures.

Cell culture grown rabies virus displayed hemagglutination activity for goose erythrocyte in mixtures held at 0 to 4^0C at a pH of 6.2 to 6.4 (Halonen and Murphy, 1968).

The virion consists of helical ribo nucleoprotein capsid enclosed within a lipoprotein membrane or envelops which in turn appears to be covered with a fringe of short projections, probably a glycoprotein in nature (Aaslestad, 1975).

According to Murphy (1975) in thin section electron microscopy, rabies virus appears to be bullet shaped and the mean dimension of particles was 180nm length and a diameter of 75 nm including surface projections.

Five proteins have been identified following disruption of rabies virus with sodium dodecyl suphate. The ribonucleoprotein contains the genomic RNA associated with three internal proteins, the transcriptase, the nucleoprotein and a phosphoprotein, which controls both transcription and replication. The other structural proteins were the matrix protein, which was located on the inner side of the virus envelope and the glycoprotein, which forms the surface projection (WHO, 1992).

A biological characteristic of many of the Lyssa viruses was that on primary isolation from animals they displayed a wide range of incubation period, but following several passages, the incubation period of these street viruses became shorter and of fixed duration (King and Turner, 1993).

According to Greene and Dreesen (1998) as an enveloped virus, rabies was destroyed by various concentrations of formalin, phenol, halogen, mercurial, minerals acids and other disinfectants and extremely labile when exposed to UV light and heat.

According to Jallet *et al.* (1999) the basis of nucleotide sequence comparison and phylogenitic analysis, Lyssavirus genus had been divided in to six genotypes. GT1 includes the classical rabies virus and vaccine strains, whereas GT2 to GT6 correspond to rabies related viruses, including Lagos bat virus (GT2), Mokola virus (GT3), Duvenhage virus (GT4), European bat lyssa virus I (EBLI) (GT5), and EBL 2 (GT6). A new lyssa virus that may belong to new genotype (GT7) has recently been reported in Australia.

Classical rabies generally a fatal encephalitis of all mammals, caused by Lyssa virus genotype 1 has a genomic structure of single stranded, negative sense, nonsegmented RNA which codes for five separate proteins designated nucleoprotein (N), phosphoprotein (M_1 or NS), matrix protein (M2 or M), glycoprotein (G) and polymerase (L) (Davies and Lowings, 2000a).

Badrane *et al.* (2001) evaluated the genetic diversity of the lyssa virus genus using the gene encoding the transmembrane glycoprotein involved in virus host interaction, immunogenicity, and pathogenicity and classified the seven genus lyssa virus in two distinct phylogroup in which phylogroup I comprises genotype 1, genotype 5 (EBL1), genotype 6 (EBL2), genotype 4 (Duvenhage virus) and genotype 7 (Australian bat lyssa virus), while phylogroup II comprises the divergent African genotype 2 (Lagos bat virus) and genotype 3 (Mokola virus).

Rabies virus glycoprotein was the major contributor to pathogenicity of the virus, but was also the major antigen responsible for the induction of protective immunity (Faber *et al.*, 2002).

2.4 EPIDEMIOLOGY

Rabies in goats was usually sporadic, but appears to occur whenever rabid dog or wildlife may attack them (Beran, 1981).

Forman (1993) pointed out that stable cycle of rabies were present in Africa and Asia, where the dog was the main vector and most human death occur in India as spillover from the urban cycle.

Enzootic rabies existed throughout the world, with the exception of only a few regions or countries such as Antarctica, Australia, New Zealand and Hawaii (King and Turner, 1993).

In India, rabies is endemic except the Lakshadweep, Andaman and Nicobar, which were free of it (Saseendranath, 1996).

Many countries such as in India and China, the only important vectors of rabies were the dog (Fu, 1997).

Greene and Dreesen (1998) reported that throughout the world, in most of the Northern hemisphere, rabies was predominantly a disease of wildlife, whereas in Southern hemisphere, the dog was the primary species involved in the transmission of disease.

Davies and Lowings (2000a) reported that although most mammals were susceptible to rabies infection, classical rabies was particularly well adapted to small carnivores, which were the reservoir species throughout the world.

The major foci of rabies in the world today were the Indian Subcontinent, Southeast Asia and most of Africa (Plotkin, 2000).

Wilde *et al.* (2005) reported that wild life rabies played a minor role in south and southeast Asia, but existed in some species.

2.4.1 Susceptibility

Goats were considered to be of moderate susceptibility to rabies (Beran, 1981).

Cattle, rabbit, fox, skunk were highly susceptible to infection whereas goat was moderately susceptible (Choudhuri, 1995).

Greene and Dreesen (1998) stated that all warm-blooded animals were vulnerable to infection with rabies and susceptibility was affected by factors such as the viral variant, the quantity of virus inoculated and the site of bite.

2.4.2 Transmission

According to Charlton and Casey (1978) infection following ingestion of infective material would be unlikely to occur through intact buccal mucosa.

Goat to goat transmission probably not occurs since the rabid animals did not bite (Beran, 1981).

Hernandezaragoza and Ramirez (1984) isolated rabies virus from the salivary gland and brain of sheep.

Aerosol transmission of rabies has occurred, but only under specialized conditions in which the air contains a high concentration of suspended particles or droplet carrying virus particles (Clark, 1988).

Transmission of rabies was nearly always due to bite of an infected animal that has rabies virus in its saliva (Greene and Dreesen, 1998).

The usual route of infection was the transdermal inoculation of saliva from infected victims (Davies and Lowings, 2000a).

Vural *et al.* (2001) demonstrated rabies viral antigens in the nasal mucosa, trachea, lung, urinary bladder, oral and stomach mucosa of a goat experimentally infected with fixed rabies virus.

2.5 POST –EXPOSURE THERAPY

Cho and Lawson (1989) in an experiment proved that animals could be protected from rabies by proper post exposure treatment schedule. In his trial, exposed dog after treatment with human rabies immunoglobulin and vaccine protected all animals against rabies, whereas none of the animals receiving vaccine alone were protected.

The combination of local treatment of wounds, passive immunization with rabies immunoglobulin and vaccination was recommended for all severe exposures to rabies (WHO, 1992).

Lodmell *et al.* (2002) opined that the brisk induction of high level of neutralizing antibody correlated with protection in post-exposure therapy against severe rabies exposure. However, mere induction of high level of antibody or the absence of neutralizing antibody may not directly correlate with protection.

The efficacy of post-exposure treatment depended on the efficient, immediate, accurate delivery of the recommended treatment; the competency of the host immune response and the susceptibility of the infecting virus to the immunity induced by the vaccine (Warrell and Warrell, 2004).

2.5.1 Local Treatment of Wounds

According to Dean (1975) in rabies post-exposure therapy, immediate suturing of a wound was not generally advised, since it may contribute to the development of rabies.

Prompt local treatment of all bite wounds and scratches that may be contaminated with rabies virus was very important in post-exposure therapy. Recommended procedures were immediate thorough flushing and washing of the wound with soap and water, detergent or other substances of proven lethal effect on rabies virus (WHO, 1992).

Lack of proper wound care, was one of the possible reason for rabies vaccination failure (Lodmell *et al.*, 2002).

Hendekli (2005) stated that the local treatment of wounds was probably the most important step in post-exposure therapy and this step significantly reduced the amount of virus that enters the body and rabies could even be prevented by following this basic procedure. This should be done for a minimum period of 15-20 minutes.

Mc Kay and Wallis (2005) stated that immunoprophylaxis could not be ignored or avoided by local wound treatment, but the risk and needs were greatly reduced by appropriate wound care.

2.5.2 Passive Immunization

Cabasso (1975) reported that abundant evidence in animals and man showed that survival rate was increased when vaccine treatment was augmented by rabies antiserum.

Rabies immunoglobulin should be given for all category III exposures, irrespective of the interval between exposure and beginning of the treatment. As much as possible of the recommended dose (20 IU/ kg body weight of HRIG or 40 IU/kg body weight of ERIG) should be infiltrated around the wounds if

anatomically feasible. The remainder should be administered intramuscularly into gluteal region in single dose (WHO, 1992).

Jackson *et al.* (2003) pointed out that in rabies post-exposure prophylaxis; rabies immunoglobulin neutralized the virus before its invasion of the nervous system.

Immunoglobulin, administered at the onset of post-exposure treatment and injected into potential inoculation site, represent a safety net for the patient till vaccine induced endogenous antibodies were formed (Wilde *et al.*, 2005).

2.5.2.1 Monoclonal Antibodies

Schumacher *et al.* (1989) reported that immunization of mice and hamster with monoclonal antibody specific for rabies virus nucleocapsid protein and glycoprotein protected animals in post exposure situation.

Monoclonal antibodies were shown to protect Syrian hamsters against rabies when given intramuscularly 24 hours or more after intramuscular challenge with a field strain and can be recommended for post exposure treatment of humans and animals (WHO, 1992).

Prosniak *et al.* (2003) developed a cocktail of recombinant expressed human rabies virus neutralizing monoclonal antibodies and the post exposure prophylaxis efficacy obtained in mouse model comparable to that obtained with rabies immunoglobulin suggested that such a reagent should replace the immunoglobulin currently used in rabies post exposure prophylaxis, which include passive immunization and active immunization.

2.5.3 Post-exposure Vaccination

Louis Pasteur developed the first vaccine for post exposure treatment and the vaccination of nine-year-old Joseph Meister in 1885 initiated the modern era of prevention of rabies (Hoenig, 1986).

According to Lodmell and Ewalt (1987) antiglycoprotein antibody was markedly effective in inhibiting the cell to cell spread of rabies virus in fibroblast and epithelial cells (BHK 21and CER) in vitro, suggested that the inhibition of viral spread by antibody in vivo would more likely occur at an initial site of exposure and before nerves were infected.

Soria and Blancou (1995) conducted a study to analyze the efficacy of vaccinations as a method of post infection treatment in sheep experimentally infected with rabies virus. They found that of the seven sheep given vaccine alone, four were protected while all animals given immunoglobulin and vaccine were protected. They pointed out that no apparently healthy carrier animals were produced by any of the treatment.

An effective post-exposure schedule for domestic animals included immediate rabies vaccination, with a minimum of one booster vaccination, and 90 days strict isolation (Clark and Wilson, 1996).

Post-exposure vaccination of domestic animal found to be 99.8 per cent effective through their four-year study period in Texas and indicated that effective post exposure protocol for unvaccinated domestic animals exposed to rabies include immediate vaccination against rabies, a strict isolation period of 90 days and administration of booster vaccination during third and eighth week of the isolation period (Wilson and Clark, 2001).

Woldehiwet (2002) stated that because rabies had a long incubation period it was possible to prevent the development of clinical disease by vaccination after exposure, provided vaccination takes place, before the virus has gained access to the central nervous system.

Rabies immunoglobulin alone when administered at recommended dose, were almost undetectable in the blood, and therefore could not provide protection by themselves and these products had proved their efficiency when administered in the site of virus entry in association with rabies vaccine (Servat *et al.*, 2003).

2.6 VACCINES

2.6.1 Nervous Tissue Vaccine

Lavender (1970) prepared a purified suckling rat brain rabies vaccine free from encephalitogenic activity and this purified product passed the NIH potency test for rabies vaccine and produced high titre serum neutralizing antibody.

Inactivated nervous tissue vaccines produced from the brains of lamb or newborn mice had been shown to be effective in mass canine immunization program in North America (Lamb brain vaccine) as well as Latin America and the Caribbean (suckling mouse brain vaccine) (WHO, 1992).

Suckling mouse brain vaccines were known to contain low quantities of rabies glycoprotein inducing lower levels of neutralizing antibodies and induced immune responses directed mainly against rabies virus ribonucleoproteins with a high synthesis of anti-nucleocapsid non-neutralizing antibodies (Zanetti *et al.*, 1998a).

Passos *et al.* (2001) reported that suckling mouse brain vaccine was able to induce immune response in the capuchin monkeys, but protection was short lived. Eight capuchin monkeys were vaccinated against rabies with inactivated suckling mouse brain vaccine and blood samples were collected at 0, 30, 60, 90,150, 210, 240, 365 days and were tested by simplified fluorescence inhibition to titre neutralizing antibodies. All the monkeys developed neutralizing antibodies with titre >0.5 IU /ml after vaccination, but the immune response persisted only for 122.3$\pm$32.6 days.

According to Warrell (2003) nervous tissue vaccines were weak antigens and failure of treatment occur.

Fuenzalida- Palacios rabies vaccine, which used inactivated virus, prepared from suckling mouse brains, remained a good alternative for post exposure rabies prophylaxis; given its comparable efficacy and much lower cost. The risk of neuroparalytic adverse reactions associated with nervous tissue vaccines were rare

for the Fuenzalida-Palacios vaccine, which by using suckling mouse brain as substrate has decreased myelin content (Bonito *et al.*, 2004).

2.6.2 Tissue Culture Vaccine

The tissue culture fluid harvested from rabies virus infected BHK cell cultures and inactivated by BPL could be used for immunization against rabies and such virion free preparations showed a high complement fixing and good immunogenic activity (Wiktor *et al.*, 1969).

Chapman *et al.* (1973) stated that inactivated rabies vaccine for veterinary use could be prepared on a large scale by using LEP strain of virus grown in suspension cultures of BHK21 cells.

Abelseth (1975) reported the growth of a rabies virus in primary pig kidney tissue culture and subsequently reported on its use as a vaccine for domestic animals, with extensive studies which indicated that it was safe and effective.

Inactivated stable economic and easy to prepare rabies vaccine of high potency could be produced in BHK cell cultures (Larghi *et al.*, 1976).

Baer and Yager (1977) demonstrated that the vaccine prepared from BHK cells resulted in the production of serum interferon and neutralizing antibody and was highly effective in reducing mortality in a mouse for post-exposure rabies prophylaxis.

The inactivated tissue culture vaccines were good antigen and they were safer for all species; store well and may be conveniently blended with other veterinary vaccines such as foot and mouth disease vaccine for use in cattle (Crick, 1978).

According to Turner (1978) Ig G antibodies could significantly protect against rabies while early appearing Ig M antibodies were ineffective and cell culture vaccines were early inducers of IgG than nervous tissue vaccines.

Gaudry (1983) found out that sheep vaccinated with inactivated adjuvanted BHK 21 tissue culture vaccine resisted challenge administered 38-44 months after vaccination.

Wild ruminants responded well to rabies vaccination using cell culture vaccine and the responses proved better in animals, which were kept under optimum management conditions (Prosperi *et al.,* 1983).

Merry and Kolar (1984) demonstrated that inactivated tissue culture vaccine of murine cell culture, porcine and feline cell lines stimulated antibody responses in dogs to a level and duration surpassing those of a modified live vaccine.

Prosperi *et al.* (1984) validated the use of inactivated rabies vaccine in the prophylaxis of rabies in cattle. In his study, group of animals received attenuated tissue culture vaccine and inactivated tissue culture vaccine developed an almost identical antibody response. However, there were a higher number of seropositive animals in the group vaccinated with the killed vaccine.

Rabies vaccine produced in continuous cell lines originated from baby hamster kidney cells are potentially oncogenic, and applied only to immunization of animals (Perrin *et al.*, 1990).

Ramanna *et al.* (1991a) reported that a tissue culture inactivated rabies vaccine developed using tissue adapted CVS virus in BHK 21 cells was safe, potent and a satisfactory seroconversion observed in vaccinated dogs.

Post-exposure therapy with tissue culture rabies vaccine at the appropriate time interval augmented the immune response to resist infection and reduced the risk of transmission of rabies to human being (Ramanna *et al.*, 1991b).

A tissue culture rabies vaccine induced satisfactory immune response in cattle and mean antibody titres of log 10, 2.02 and 1.27 were observed at two and 17 months after vaccination respectively (Ramanna and Srinivasan, 1992).

Vaccines prepared in cell cultures should replace those derived from nervous tissue and baby hamster kidney cells were the most commonly used continuous cell line for the production of vaccine for animals (WHO, 1992).

Basheer *et al.* (1997a) proved that post-exposure vaccination against rabies in bovines with BHK21 cell culture derived inactivated vaccine "Raksharab" with schedule of vaccination on days 0, 3, 7, 14, 28 and 90 was superior to nervous tissue and Vero cell culture vaccine in eliciting immune response.

Basheer *et al.* (1997b) reported that post-exposure administration of BHK21 cell culture anti-rabies vaccine in cattle produced higher antibody response than the brain tissue culture vaccine.

Lontai (1997) reported that after two doses of chick embryo cell vaccine, there was higher seroconversion and much higher antibody level than that achieved after complete treatment with the nervous tissue vaccine.

Inactivated tissue culture rabies vaccine induced a satisfactory immune response in camel when tested for a period of 48 months (Kalanidhi *et al.*, 1998).

The virus neutralizing antibody obtained with cell culture rabies vaccine were always much higher than those obtained with suckling mouse brain vaccine. In their study, they showed that, although pre-exposure anti-rabies treatment with suckling mouse brain vaccine elicited high seroconversion rate, the response was short lived. The superior performance of rabies vaccine produced in cell culture emphasis the need to use them instead of those produced in nervous tissue (Zanetti *et al.*, 1998b).

Tissue culture rabies vaccine effectively immunized target species under field conditions and induced satisfactory antibody response during the three years period of study in dogs (Reddy and Srinivasan, 1999).

High titres of rabies neutralizing antibody and the persistence of anamnestic booster response supported the value of Vero cell rabies vaccine for pre-exposure prophylaxis (Sabehareon *et al.*, 1999).

According to Plotkin (2000) the advantages of cell culture vaccines were freedom from heterologous protein and a high level of immunogenicity that permits a rational dosing schedule.

Rodrigues *et al.* (2000) confirmed that inactivated tissue culture adjuvanted rabies vaccine could afford high virus neutralizing antibodies and seropositivity in bovines than attenuated rabies vaccine. They also pointed out that although they have not challenged the animals, neutralizing antibody levels was largely accepted as evidence of immunity.

An inactivated tissue culture rabies vaccine derived from Vero cell line was safe and immunogenic in humans (Sampath *et al.*, 2000).

2.6.3 Recombinant Vaccine

Wiktor *et al.* (1984) evaluated the immunogenicity of purified inactivated vaccinia virus recombinant containing rabies glycoprotein and found that this preparation induced high levels of neutralizing antibody and protected mice against intracerebral challenge with rabies virus.

Kieny *et al.* (1987) reported that vaccinia virus recombinant virus expressing rabies glycoprotein was a powerful immunogen, able to prime the animal for both the antibody and cytotoxic T cell responses against rabies.

Vaccinia recombinant virus expressing the rabies virus glycoprotein was potent enough to produce very high levels of rabies virus neutralizing antibodies in several wild life species and proved safe (Artois *et al.*, 1990).

An E I deleted, replication defective adenovirus recombinant of the human strain 5 expressing the rabies virus glycoprotein resulted in a long lasting protective immune response to rabies virus in neonatal mice, suggesting that this type of vaccine could be useful for immunization shortly after birth (Wang *et al.*, 1997).

2.6.4 DNA Vaccine

The production and application of isolated G protein as a vaccine, that the glycoprotein was the only structural protein of the virus that induced the formation of virus neutralizing antibodies and isolated G protein devoid of lipids and other protein have the same neutralizing capacity as the intact virus (Cox *et al.*, 1977).

Xiang *et al.* (1995b) in his study proved that a plasmid vector expressing full length rabies virus glycoprotein under the control of the simian virus 40 promoter has been shown to induce, upon intramuscular inoculation into mice a specific B and T cell mediated immune response and protection against challenge with a virulent strain of virus. He also pointed out that DNA vaccination was found to induce long lasting immunity to rabies virus without apparent negative side effects such as development of T cell tolerance or generation of anti-DNA antibodies.

DNA based immunization overcame the risk of attenuated vaccines reverting to virulence and elicited full protection against intracerebral challenges with various lyssa viruses and a range of antigen specific and nonspecific immune responses. A single intramuscular injection of plasmid was sufficient to induce continuous high level of virus neutralizing antibody (Bahloul *et al.*, 1998).

The immune response of mice, vaccinated as neonates in the presence of maternal immunity or up on passive immunization to rabies virus, with DNA vaccine was only marginally affected (Wang *et al.*, 1998).

Osorio *et al.* (1999) suggested that DNA immunization represented an alternative mode of administration for veterinary rabies vaccine. This study results were clearly encouraging with the identification of a novel and simple intramuscular DNA delivery technology that could induce strong immune responses, with a single inoculation of a relatively small amount of DNA, and would greatly facilitate commercial DNA vaccine development for both human and veterinary application.

DNA vaccines were associated with exceptionally prolonged humoral and cellular immunity, although the antibody response was slower to develop than that elicited after conventional vaccination (Davis and Lowings, 2000b).

Lodmell and Ewalt (2000) demonstrated that diploid cell vaccine booster elicited early and robust increase in antibody titres of mice that had received a primary vaccination with DNA rabies vaccine and stressed that combination of vaccines did result in long term duration of higher levels of neutralizing antibody.

Perrin *et al.* (2000) reported that a strong enough immune response obtained in dog after a single administration of DNA rabies vaccine may be due to the constant stimulation of immune system due to low persistent level of glycoprotein production by the injected plasmid.

DNA vaccination was especially attractive for disease such as rabies, which was a major problem in developing countries, not because an effective vaccine was not available, but because these vaccines could be produced more economically than cell culture derived vaccines and they did not require a cold chain and intramuscular immunization appeared to be effective in all mammalian species (Rangarajan *et al.*, 2000).

The intramuscular inoculation of rabies DNA vaccine induced significant levels of virus neutralizing antibody and anamnestic B cell response was seen in both mice and monkeys following the administration of booster dose. They reported that DNA vaccination was a viable and economical method of immunization for rabies prophylaxis programmes in countries such as India (Biswas *et al.*, 2001a).

Biswas *et al.* (2001b) stated that co-inoculation of DNA vaccine and a low dose of inactivated virus vaccine (CRV) could be developed as a novel cost effective vaccination strategy for combating rabies in particular, and infectious disease. Combined rabies vaccine induced higher anamnestic antibody response than DNA rabies vaccine in mice as well as cattle.

Lodmell and Ewalt (2001) showed that DNA vaccine might eventually represent a potential alternative to tissue culture based vaccines for post-exposure

protection of mice against rabies virus. They found that the intradermal injection of DNA into the ear pinnae on five consecutive days resulted in much stronger antibody response and neutralizing antibody was detected in seven days after vaccination than mice that had received human diploid cell vaccine. They also showed that a long rest period between vaccinations was not necessary for the elevation of antibody response.

The anti-rabies virus-neutralizing antibody elicited by plasmid DNA vaccination cross-neutralized a global spectrum of rabies virus variants (Rai and Yadav, 2001).

Lodmell *et al.* (2002) revealed that gene gun vaccination above axillary and inguinal lymph nodes or ear pinnae generated high levels of neutralizing antibody and concurrent booster after three days of primary vaccination accelerated detectable neutralizing antibody and increased the durability of response which supported the use of DNA vaccination for post exposure anti- rabies therapy.

A single inoculation of a plasmid encoding the rabies glycoprotein in to mice was shown to induce a rapid and a strong antibody response as five injections of cell culture derived vaccine and stressed the possibility that DNA based vaccination might be an effective post-exposure treatment for rabies (Bahloul *et al.*, 2003).

Fischer *et al.* (2003) proved that rabies DNA vaccination was feasible in horses and suggested that properly formulated DNA vaccines could generate immune response in large veterinary species at a level comparable to the responses achieved with conventional vaccine.

Lodmell *et al.* (2003) demonstrated that single dose rabies intradermal DNA vaccination into the ear pinnae of dog elicited elevated levels of anti-rabies neutralizing antibody that persisted for an extended interval and this method should be considered as a solution for control of canine rabies in developing countries.

The induction of virus neutralizing antibody following DNA rabies immunization was usually slow and DNA vaccines might not be suitable for use in a post-exposure vaccination regimen (Vanniasinkam and Ertl, 2004).

2.7 VACCINE REGIMEN

The post-exposure regimen in humans consisted of a simultaneous inoculation of vaccine and human rabies immunoglobulin on day zero followed by injections of vaccine on days 3, 7, 14, 28 and 90 (WHO, 1984).

The 2-1-1 schedules, one dose was given in the right arm, one dose in the left arm on day 0, and one dose applied in the deltoid muscle on day 7 and 21. The 2-1-1 schedules induced an early antibody response and may be particularly effective when post-exposure treatment did not include administration of rabies immunoglobulin in humans (WHO, 1992).

Vodopija *et al.* (1997) reported that 2-1-1 schedule had the advantage of early antibody induction, early peak of antibody titre and greater economy of rabies post-exposure treatment over earlier five post-exposure schedules.

Briggs *et al.* (2000) stated that WHO intradermal regimen using two-site method (2-2-2-0-1-1) for post-exposure therapy required only 15 per cent of the amount of vaccine needed to treat a patient by the "Essen" intramuscular regimen. This regimen was an attractive option for physicians in developing countries with limited resources.

The immunity produced by the tissue culture vaccine was associated with the quantity of antigen in the vaccine. So cattle received booster doses, demonstrated a considerable rise in rabies titers reaching much higher levels than found in animals vaccinated with a single dose (Oliveira *et al.*, 2000).

Mitmoonpitak *et al.* (2002) conducted a study to determine the efficacy of post-exposure rabies treatment in pigs using "Essen" schedule with inactivated tissue culture vaccine. All the animals developed detectable neutralizing antibody on day seven and levels over 0.5 IU/ml on day 14. This study suggested that post-

exposure therapy using a proven "Essen" schedule, applied to valuable farm animals could be safe and effective.

The intradermal vaccination of purified chicken embryo cell culture vaccine using the 2-2-2-0-1-1 regimen produced rabies antibody titer above 0.5 IU/ml by day 14 in humans and stated that this regimen an attractive option for resource starved countries (Mala *et al.*, 2005).

2.8 MEASUREMENT OF IMMUNE RESPONSE

Vaccination must induce an immune effector mechanism, which was able to limit the viral spread throughout the immunologically privileged site that was comparatively inaccessible to the immune system, and protection against challenge was clearly correlated with antibody titre (Xiang *et al.*, 1995a).

Glycoprotein (G) and nucleocapsid proteins (N) were dominant antigens in anti-rabies virus immune response and G protein induced virus-neutralizing antibodies and N Protein had dominant T helper epitopes (Gore, 2004).

2.8.1 Mouse Neutralization Test

Mouse neutralization test provided the most biologically relevant assessment of antibody activity (Kitala *et al.*, 1990).

The use of large number of mice with variable susceptibility to the virus and a long observation period were the disadvantages of mouse neutralization test (Pandit *et al.*, 1991).

The serum neutralization test in mice was the first neutralization test developed and its long and wide spread use has made it the standard by which other tests were evaluated (Smith, 1991).

Virus neutralization test on mice was time demanding, expensive and unpractical for routine use in virological laboratories (Ondrejkova *et al.*, 2002).

Cliquet *et al.* (2003) reported that for the evaluation of the immunogenicity of human and animal rabies vaccines, the recommended standard procedure was the mouse neutralization test.

2.8.2 Rapid Fluorescent Focus Inhibition Test

Tissue culture fluorescent antibody technique for the measurement of rabies neutralizing antibody was found to be reliable and comparable to the standard mouse serum neutralization test. This test was performed with BHK21 cells infected with the ERA vaccine virus strain in Lab tek tissue culture chamber slides (Debbie *et al.*, 1972).

Atanasiu *et al.* (1974) demonstrated that the neutralization test by the fluorescent antibody technique in cell culture was found to be more sensitive than inoculation test in mice for determination of rabies antibody titre in horse serum.

The antibody assay in the sera of immunized mice using rapid fluorescent focus inhibition test and antigenic value generated by NIH on the same vaccine showed correlation in results (Fitzgerald *et al.*, 1978).

Blancou *et al.* (1983) compared four different serological tests such as mouse neutralization test, RFFIT, plaque reduction test and immunoenzymatic test for the determination of antibody level against rabies virus in vaccinated dogs and results obtained with each of the last three methods were compared with those obtained with mouse inoculation test. Correlation coefficient was 0.810, 0.812 and 0.682 respectively and recommended that the three techniques could be used as an alternative to mouse neutralization test for routine titration.

For the assessment of immune response after rabies vaccination, RFFIT was a significantly better reproducible test system than mouse neutralization test and excellently correlated with the latter method (Kurz *et al.*, 1986).

Evaluation of vaccines based up on the antibody measurement could be divided into methods which titrate the level of virus neutralizing antibody elicited by vaccination and methods which titrate antibody irrespective of their capacity to

neutralize rabies virus. RFFIT, the method that determine the virus neutralizing antibodies were much preferred than methods such as hemagglutination, complement fixation test, radioimmunoassay or ELISA (Kieny *et al.*, 1987).

Pandit *et al.* (1991) demonstrated that a rapid fluorescent focus inhibition test (RFFIT) for rabies antibody estimation in murine neuroblastoma cell line, showed 94 per cent correlations with mouse neutralization test, while in 6 per cent of the sera tested, the RFFIT was found to be more sensitive than the mouse neutralization test.

Rapid fluorescent focus inhibition test was found to be slightly more sensitive than mouse neutralization test in detecting virus-neutralizing antibodies in post vaccinal sera (Smith, 1991).

Zavadova *et al.* (1996) found that for monitoring efficiency of anti-rabies vaccination, the quantity of virus neutralizing antibodies by virus neutralization test, RFFIT and virus hemagglutination showed comparable result.

Briggs *et al.* (1998) compared two serological methods such as RFFIT and FAVN for detecting the immune response after vaccination in dogs and cats and found that the two showed same sensitivity and specificity.

Ondrejkova *et al.* (2002) compared RFFIT, virus neutralization test on mice and fluorescent antibody virus neutralization test for the detection and quantification of rabies antibodies in canine sera and titres showed 87 per cent correspondence in virus neutralization test on mice and fluorescent antibody virus neutralization test whereas RFFIT and fluorescent antibody virus neutralization test showed 95 per cent correspondence.

Rapid fluorescent focus inhibition test was the gold standard for rabies immunology and a WHO requirement for immunogenicity testing of new vaccines (Wilde *et al.*, 2005).

2.8.3 Enzyme Linked Immunosorbant Assay

Nicholson and Prestage (1982) developed an enzyme linked immuno assay for detecting rabies antibodies from subjects immunized with human diploid cell vaccine. This assay was highly reproducible and close agreement with standard mouse neutralization test.

Heberling *et al.* (1987) developed a dot immunobinding assay that used inactivated antigen for the detection of rabies viral antibodies. The study showed a good correlation between the rapid fluorescent focus inhibition test and DIA results for determining the antibody status of vaccinated humans and dogs.

WHO (1987) recommended ELISA as a satisfactory test for testing the potency of rabies vaccines in licensing institute.

An indirect ELISA for the measurement of rabies specific antibodies in the sera of domestic and wildlife reservoirs found that this assay was rapid, economical reproducible and considered it to be a favorable alternative to the fluorescent focus inhibition test (Barton and Campbell, 1988).

ELISA based on glycoprotein antigen of rabies virus was found to be simpler and allowed more samples to be assayed in a shorter time than the neutralization assay in mice and the RFFIT in cell culture (Grassi *et al.*, 1989).

Kitala *et al.* (1990) developed an enzyme immunoassay (INH-EAI), which was comparable and more reproducible than the rapid fluorescent focus inhibition test for the determination of antibody response after vaccination.

Dot-ELISA could be used as an alternative to serum neutralization test to monitor antibody response after rabies vaccination to determine the efficacy of vaccination (Bhattacharya and Narayan, 1995).

Sugiyama *et al.* (1997) developed a competitive ELISA as an alternative to the virus neutralization test for rapid and simple detection of antibodies to rabies virus in dogs. The c- ELISA was a convenient and practical assay for detecting and

measuring antibodies to rabies virus at titres similar to those obtained by virus neutralization test.

Liquid phase blocking ELISA may be of value in rabies seroepidemiological studies and could be developed as a reference technique for the detection of rabies antibodies in vaccinated dogs (Cleaveland *et al.*, 1999).

According to Davis and Lowings (2000b), rabies antibody ELISA 'S was relatively inexpensive, quick and easy to perform and did not require the use of live virus.

Arai *et al.* (2002) investigated the immunogenicity of rabies vaccine using neutralization test and ELISA and found a clear correlation between neutralizing antibody titre and ELISA titre.

Cliquet *et al.* (2003) demonstrated that indirect ELISA was a suitable tool for evaluating the seroconversion rate in fox population following rabies vaccination and this technique was highly correlated with conventional seroneutralization test on cell cultures.

ELISA assay would be a valuable screening tool for the detection of rabies antibodies from vaccinated domestic animals and could be compared with the fluorescent antibody neutralization test (Cliquet *et al.*, 2004).

2.8.4 Other Tests

Grandien (1977) proved the usefulness of the mixed hemadsorption test for rabies antibody estimation after vaccination. This test measured only antibodies of the IgG class and it was sensitive and gave no false positive result. Compared with neutralization test, this test was technically more feasible and gave reliable result in two days.

Diaz and Myers (1981) observed that the sensitivity of counter immunoelectrophoresis for assessing the immune status of a patient after rabies post-exposure treatment was comparable to that of serum neutralization test and

permitted the quantitative determination of titers of rabies serum antibodies in a rapid manner.

The modified immunoadherence hemagglutination test (IAHA) was used for rapid detection and measurement of rabies antibody in human serum. On comparison with the RFFIT, the IAHA test was faster and could be used for rapid screening of vaccinated subjects (Budzko *et al.*, 1983).

Hemagglutination inhibition test was expected to be a simple and reliable method for measuring protective antibody against rabies (Mannen *et al.*, 1984).

Madhusudana *et al.* (2001) demonstrated that indirect immunofluorescence test was a rapid and cost effective technique for assaying rabies antibodies and showed significant correlation with mouse neutralization test.

Materials and Methods

3. MATERIALS AND METHODS

The study was carried out at the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary & Animal Sciences, Mannuthy between November 2003 and November 2004.

3.1 MATERIALS

3.1.1 Glassware and Reagents

All glasswares used were of either Borosil or Vensil brand and chemicals were of analytic or guaranteed reagent grade. All materials were processed by standard procedures and sterilized by either keeping in hot air oven at 160°C for 60 minute or autoclaving at 121°C for 15 minutes at 15 lbs pressure, depending on the materials sterilized.

3.1.2 Experimental Animals

Sixty goats reported with the history of rabid animal bite in and around Thrissur district were subjected to the study. They were grouped in to four groups

3.1.3 Vaccines

The following vaccines were employed (Fig. 1).

a. Inactivated Tissue Culture anti-rabies Vaccine (Raksharab®). Vaccine prepared with fixed rabies virus (CVS-11) grown on BHK-21 cell line inactivated by aziridine, adjuvanted with aluminium hydroxide, manufactured by M/s Indian Immunologicals Ltd, Hyderabad.

b. DNA Combined Tissue culture anti-rabies vaccine (Dinarab®). Manufactured by M/s Indian Immunnologicals, Hyderabad.

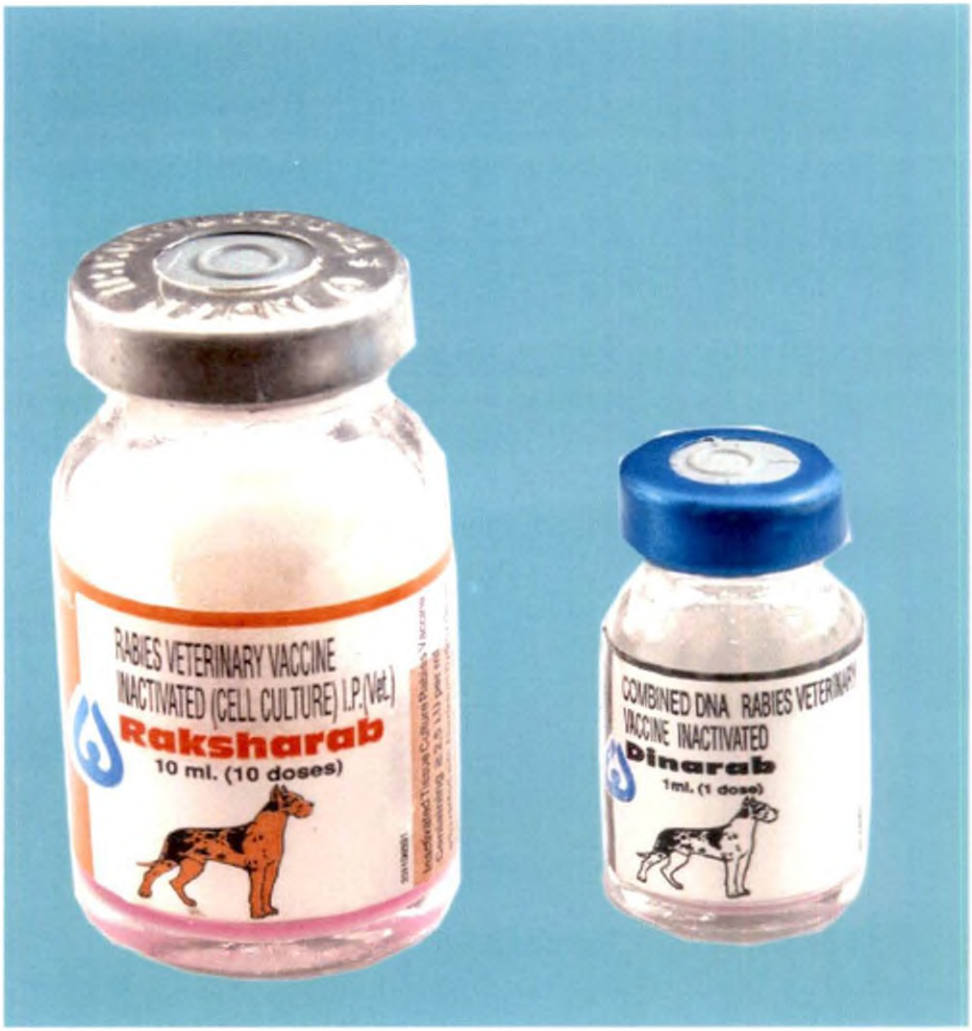

Fig. 1. Anti-rabies vaccines: Raksharab and Dinarab

3.1.4 Rapid Fluorescent Focus Inhibition Test (RFFIT)

3.1.4.1 Lab -Tek Chamber slides with cover slip

Lab-Tek chamber slide with cover, eight well slides, sterile (Nalge Nunc international).

3.1.4.2 Reagents

a. Diagnostic conjugate: Fluorescein isothiocyante (FIFC) conjugated anti-rabies virus antibodies, Bio-Rad, France.
b. Acetone: Acetone AXO 120-6 (500ml) GRACS.
c. Cell culture media

(i) Distilled Water: Distilled deionized water, sterile. Category No: 25-055CV (500ml).

(ii) Fetal bovine serum: Fetal bovine serum (500ml), 40nm filtered. FBS should be heat inactivated just before use.

(iii) Minimum essential medium: Contains Earle's salts but no L-glutamin or sodium bicarbonate.

Components	**Concentration (mg/l)**
Inorganic salt	2000.00
Calcium Chloride	4000.00
Potassium Chloride	976.70
Sodium Chloride	68000.00
Sodium Phosphate H_2O	1400.00
Other Components	
D-Glucose	10000.00
Phenol red	100.00

Amino acids	
L-Arginine – HCl	1260.00
L-cystine – 2 Na.	286.00
L-Histidine	420.00
L-isoleucine	520.00
L-leucine	520.00
L-Lysine	720.00
L-Methionine	150.00
L-Phenylalanine	320.00
L-Threonine	480.00
L-Tryptophan	100.00
L-Tyrosine	520.00
L-Valine	460.00
Vitamines	
D-Ca pantothenate	10.00
Choline chloride	10.00
Folic acid	10.00
i-inositol	20.00

(iv) MEM vitamin solution liquid: Prepared in 0.85 per cent Nacl; P^H 7.0 to 7.4

Components	**Concentration (mg/l)**
NaCl	8500.00
D-Ca Pantothenate	100.00
Cholin Chloride	100.00
Folic acid	100.00
i-inositol	200.00
Nicotinamide	100.00
Pyridoxal Hcl	100.00
Riboflavin	10.00
Thiamin Hcl	100.00

(v) Antibiotics – Antimycotic (100x) Invitrogen Life technologies, Gibco contains 10,000 units of penicillin, 10,000 μg of streptomycin and 25μg of amphotericin B as fungizone®. Antimycotic in 0.85 per cent Saline.

(vi) Sodium bicarbonate: Sodium bicarbonate solution, 100ml liquid 7.5 per cent (w/v). Invitrogen life technologies, Gibco.

d. Trypsin: Trypsin – EDTA (0.05 per cent) Trypsin, 0.53mM EDTA 4 Na (10x) Invitrogen life technologies, Gibco.

e. Phosphate buffered saline: Two PBS formulas are used for RFFIT.

(i) For rinsing cell monolayers, Ca^{2+} and Mg^{2+} free PBS, P^H 7.4.

Sodium chloride	8.0g
Potassium chloride	0.2g
Sodium phosphate dibasic anhydrous	1.15g
Potassium phosphate monobasic anhydrous	0.12g
Water reagent – Grade type I, QS	1000.0ml
Adjusted to P^H 7.4 with hydrochloric acid	

(ii) For immunofluorescene 0.01m, P^H 7.4-7.6

Sodium chloride	8.5g
Potassium phosphate monobasic	0.23g
Potassium phosphate dibasic	1.46g
Water, Reagent grade Type I, QS	1000.0ml
Adjusted to P^H 7.5 with hydrochloric acid.	

f. Dimethyl sulfoxide (DMSO) for Cryopreservation of cell line.

3.1.4.3 Cell culture flask

Flask, 75cm^2 cell culture flask, treated, non-pyrogenic, polystyrene and sterile.

3.1.4.4 Equipments

a. Fluorescent microscope: Zeiss, Axioskop with 200x.

b. Co_2 incubator, Co_2 water-jacketed incubator. Forma scientific, Inc.

3.1.4.5 Standards and references

(a) Mouse neuroblastoma cells: The cell culture line of mouse neuroblastoma (MNA) cells used at the Centre for Disease Control (CDC) was originally obtained from the Wistar institute, Rabies section, Philadelphia, USA.

(b) Rabies challenge virus: Rabies challenge virus standard (CVS-11) strain used at the CDC obtained from the Laboratory of standards and testing.

(c) Reference serum standard: US standard Rabies immune Globulin obtained from the laboratory of standards and testing.

The standards and references kept at M/s Indian Immunologicals, Laboratory Hyderabad, were used for the present study.

3.2 METHODS

3.2.1 History of Cases

Sixty goats after exposure to a rabid animal were included in the present study. Exposures included being bitten by a rabid animal.

3.2.2 Treatment Regimen

Wound cleaning with soap and water and antisepsis, followed by vaccination was carried out as outlined in WHO recommendations for human post exposure treatment (WHO, 1992). The exposed goats were randomly grouped in to four groups.

3.2.2.1 Immunization

Post exposure immunization was carried out in all the four-study group of goats in two different schedules with two vaccines.

3.2.2.1.1 Schedule of Vaccination

The schedule I and schedule II were derived based on preliminary studies conducted in the Department of Veterinary Epidemiology and Preventive Medicine.

Schedule I : Essen schedule of post exposure regimen

1st dose	:	On 0 day (day of first injection)
2nd dose	:	3rd day
3rd dose	:	7th day
4th dose	:	14th day
5th dose	:	28th day
Dose	:	1 ml
Route & vaccination	:	Intramuscular injection

Schedule II:

1st dose	:	On 0 day
2nd dose	:	1st day
3rd dose	:	2nd day
4th dose	:	3rd day
5th dose	:	4th day
Dose	:	1 ml
Route & vaccination	:	Intramuscular injection

Group I (17 Animals) : Inactivated Tissue Culture Anti-Rabies Vaccine; Schedule I

Group II (14 Animals) : DNA Combined Tissue Culture Anti-Rabies Vaccine; Schedule I

Group III (15 Animals): Inactivated Tissue Culture Anti - Rabies Vaccine; Schedule II

Group IV (14 Animals): DNA Combined Tissue Culture Anti-Rabies Vaccine; Schedule II

3.2.3 Collection of Serum Samples

The whole blood samples were collected from the experimental animals without anticoagulants for separation of sera starting from day zero and subsequently on 7, 14, 28, 60 and 90th day. The serum samples were inactivated at 56°C for 30 minutes in water bath and kept at –20°C until tested.

3.2.4 Rapid Fluorescent Focus Inhibition Test (RFFIT)

Rabies virus neutralizing antibody levels were measured by the rapid fluorescent focus inhibition test. The procedure was carried out as per Smith *et al.* (1996).

3.2.4.1 *Dilution of the test sera in Minimum Essential Medium – 10*

Serum end titrations were routinely tested at eight serial five-fold dilutions in MEM-10 using an eight well Tissue-Tek slide (Fig.2). To the first well, 0.075ml of MEM-10 was added using a microtitre pipette and 0.1ml of MEM-10 to the seven other wells of the slide. To the first well, 0.05ml of test serum was added (1: 5 dilution) and mixed several times and 0.025ml transferred to the second well and continued to transfer 0.025ml to each consecutive well up to the final dilution, (1:390625) discarding 0.025ml at the end.

3.2.4.2 Preparation of control slide

Control slides were prepared by using reference serum control, a virus back titration and a cell control. Added 0.075ml of MEM-10 to the first well of the reference serum dilution on the left of the slide and 0.1ml of MEM-10 to the remaining wells of the reference serum dilution wells (1:25 to 1:625) and to the 3 wells of the virus back titration. The cell control well received 0.2ml of MEM-10. To the 1: 5 dilutions well on the bottom left of the slide, 0.05ml of reference serum containing 2 IU/ml was added. Mixed well and transferred 0.025ml of the 1:5 dilution of reference serum to the 1:25 dilution well and continued through to the 1:625 dilution well, discarding 0.025ml at the end.

3.2.4.3 *Preparation of challenge virus and back titration*

The amount of virus used in the test was 50 FFD_{50}/0.1ml. Two serial 10 fold dilutions of CVS – 11 from the 50 FFD_{50}/ 0.1 as 5 FFD_{50} and 0.5 FFD_{50}/0.1ml was made by using MEM-10 as a diluent and added in sequential chambers. The virus preparation containing 50 FFD_{50}/0.1ml added to all chambers of the test sera and reference serum dilution and kept all slides for 30 minutes at 37°C in a CO_2 incubator with 0.5 per cent CO_2.

3.2.4.4 Preparation of the mouse neuroblastoma cells

Suspension of MNA cells in 10ml of MEM -10 (Fig. 3) was transferred to a 25ml conical centrifuge tube and counted the cell using a hemocytometer. Added 0.2ml of 6×10^5 cells/ml to each chamber of the slides, starting with the cell control slide. The slides were kept at 37°C in a 0.5% CO_2 incubator for 20 hours.

3.2.4.5 *Fixation of slides*

After 20 h., removed the slides from the incubator and poured off the medium into viricidal solution. The slides were rinsed once in PBS and then fixed for 10 minutes at room temperature in cold acetone (-20°C). After fixation, remove the slides from acetone and all dried at room temperature for 10 minutes.

3.2.4.6 *Staining of slides*

Rabies conjugate was added to each chamber sufficient to cover the entire monolayer (approximately hundred microlitter per well) (Fig. 4). The slides were incubated in a humidity chamber at 37°C for 30 minutes. Following 30-minute incubation, decanted the conjugate from the slides and rinsed in PBS (4588) for 10 minutes, and observed the slides under fluorescent microscope.

3.2.5 Interpretation

Each of the eight well tissue tek slide chamber had 25 to 50 distinct microscopic fields when observed at 160-200 times magnification. Observed 20 microscopic fields in each chamber and counted the number of fields, which

which contain fluorescing cells. Absence of neutralization was indicated by the presence of numerous foci of virus specific fluorescence (Fig. 6 & 7). The results of the RFFIT can be expressed as a serum titre or in international units (IU) of antibody using the control slide and test serum values.

3.2.6 Statistical analysis

Statistical analysis of the results obtained was done by Kruskal Wallace method as per Snedecor and Cochran (1985).

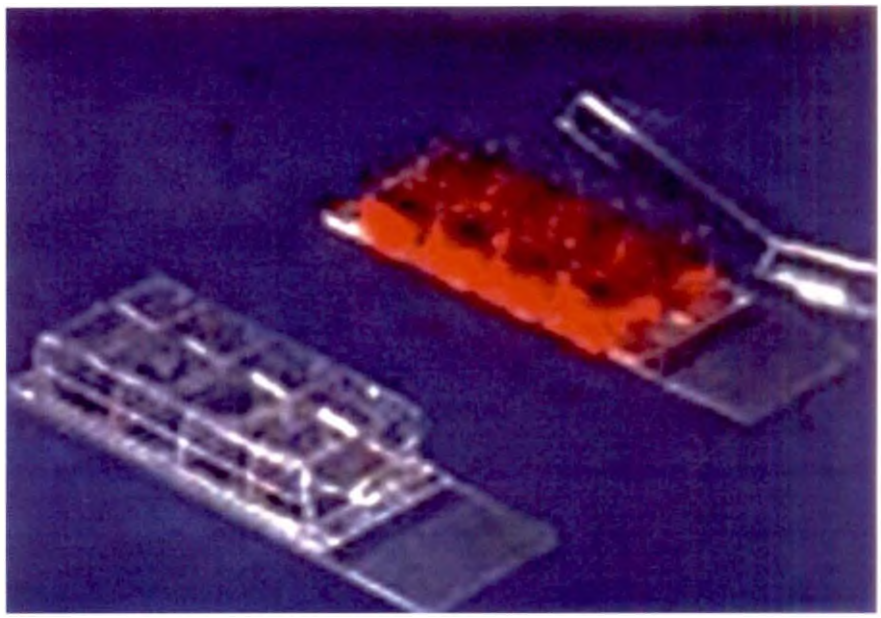

Fig. 2. Serum Samples diluted in MEM-10 in the consecutive wells of a eight – well Lab- Tek Chamber Slide

Fig. 3. Mouse neuroblastoma cells suspended in MEM –10 in a trypsinization flask

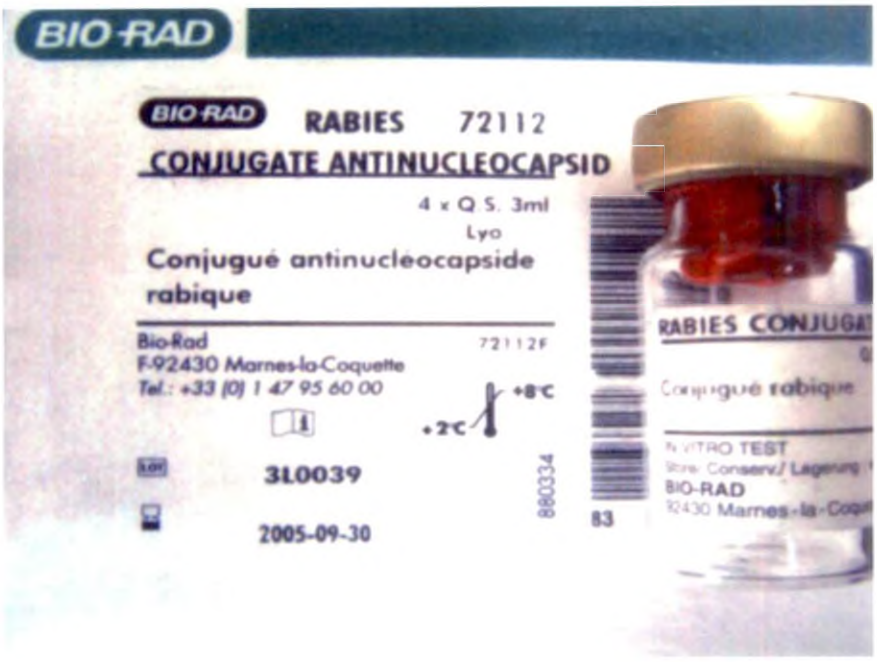

Fig. 4. Fluorescein Isothiocyanate conjugated anti-rabies serum

Fig. 5. Fluorescent microscope attached to the computer

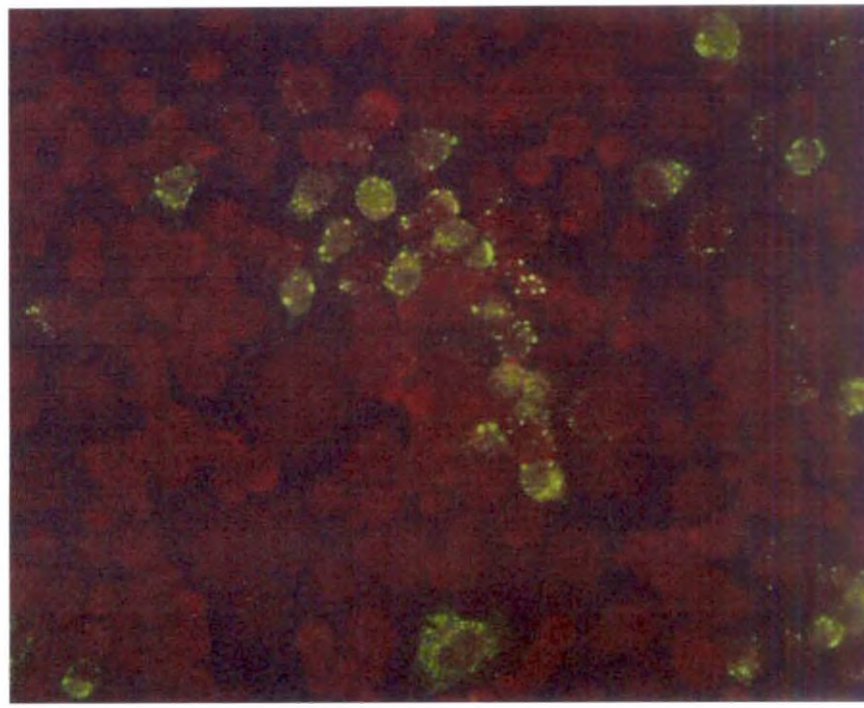

Fig. 6. Fluorescent foci observed under a fluorescent microscope

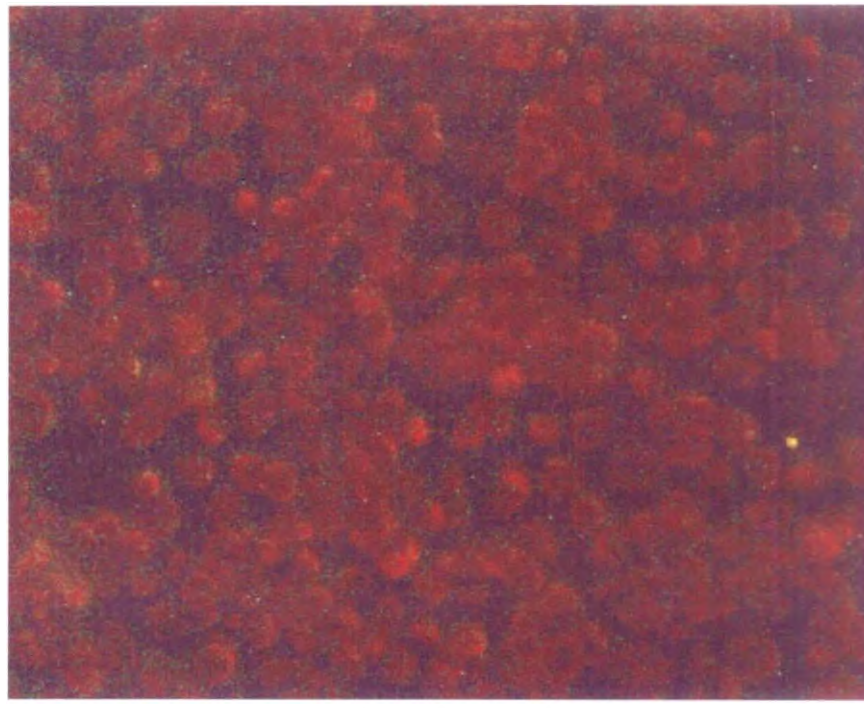

Fig. 7. No fluorescent foci observed under a fluorescent microscope

4. RESULTS

All the serum samples collected from sixty goats randomly divided in to four groups *viz.* group I, group II, group III and group IV were subjected to Rapid fluorescent focus inhibition test (RFFIT) for estimation of rabies virus neutralizing antibody titres and antibody titres are presented in the table (1 to 4). The results were expressed in international unit (IU/ml).

Comparisons of results within a group were done by students Paired t-test. Kruskal Wallace test was used for the pair wise comparison between treatment groups at each sampling day.

4.1 IMMUNE RESPONSE FOLLOWING POST-EXPOSURE VACCINATION IN THE FOUR GROUPS

4.1.1 Group I

The rabies virus neutralizing antibody titer of group I animals are shown in the table (1). The bitten animals were seronegative on the first day of treatment. On seventh day geometric mean antibody titre was 0.28 IU/ml and significant increase in the titre on day 14 (2.49 IU/ml) ($P<0.05$). The highest titre of 2.56 IU/ml was observed on day 28-post exposure. The titre was gradually decreased to 1.63 IU/ml on 60^{th} day and significant ($P<0.05$) decrease in the titre on 90^{th} day (Table 5). On 90^{th} day, geometric mean antibody titre was 0.56 IU/ml (Fig. 8).

4.1.2 Group II

The rabies virus neutralizing antibody response of group II animals are presented in table (2). On day seven, geometric mean titre was 1.19 IU/ml and the titre gradually increased on subsequent days of observation. On day 14, there was significant increase in the titre (6.37 IU/ml). The maximum rabies virus neutralizing antibody titre of 6.44 IU/ml had recorded on 28^{th} day following vaccination and significant ($P<0.05$) reduction of geometric mean rabies antibody titre was observed from 28^{th} day to 90^{th} day (Table 5 and Fig. 9).

4.1.3 Group III

The antibody titres of group III animals are shown in the table (3). The geometric mean rabies virus neutralizing antibody titre on seventh day after vaccination was 1.19 IU/ml and there was significant increase in the titre on 14th day ($P<0.05$). The maximum rabies virus neutralizing antibody titre was observed on 14th day (6.68 IU/ml). A fall in the geometric mean rabies virus neutralizing antibody titre from 6.68 IU/ml to 4.73 IU/ml during 14th day to 28th day of study was observed. A significant fall ($P<0.05$) in geometric mean rabies virus neutralizing antibody titre from 4.73 IU/ml to 1.03 IU/ml on 28th day to 60th day and from 1.03 IU/ml to 0.63 IU/ml ($P<0.05$) on 60th day to 90th day of study was observed, though the titre was above protective level (Table 5 and Fig. 10).

4.1.4 Group IV

The rabies virus neutralizing antibody titres of the animals are presented in table (4). The animals were seronegative before vaccination and reached 1.49 IU/ml on seventh day of study. After five consecutive vaccinations, the maximum geometric mean rabies virus neutralizing antibody titre of 3.61 IU/ml was observed at 14th day of observation. A significant fall ($P<0.05$) in the geometric mean rabies virus neutralizing antibody titre from 2.04 IU/ml to 0.66 IU/ml on 28th day to 60th day of study was observed (Table 5). The antibody titre remained above protective level even on 90th day (Fig. 11).

Table1. Rabies virus neutralizing antibody titre (IU/ml) of group I animals

Animal no.	Day of bleeding					
	zero	7	14	28	60	90
GT1- I	0.01	0.19	2.29	2.81	1.62	0.11
GT2- I	0.01	0.19	2.81	2.57	2.29	0.56
GT3- I	0.01	0.51	1.25	13.80	2.57	0.61
GT4- I	0.01	0.61	2.81	69.18	2.81	0.79
GT5- I	0.01	0.10	0.51	0.51	0.56	0.15
GT6- I	0.01	0.56	10.00	13.80	13.8	2.81
GT7- I	0.01	0.01	1.99	3.38	2.81	0.51
GT8- I	0.01	0.01	0.56	1.25	2.81	1.62
GT9- I	0.01	0.06	0.61	2.57	2.81	1.25
GT11-I	0.01	0.05	0.39	2.57	2.29	0.56
GT12-I	0.01	0.95	3.01	2.57	2.29	0.56
GT13-I	0.01	0.79	11.48	12.88	0.56	0.56
GT14-I	0.01	0.67	1.25	1.25	0.61	1.99
GT16-I	0.01	2.18	4.78	0.11	2.18	0.39
GT17-I	0.01	5.24	10.00	3.98	1.62	0.51
GT18-I	0.01	0.32	13.80	0.11	0.25	0.11
GT19-I	0.01	1.25	13.80	3.01	0.56	0.45
GMT*	0.01	0.28	2.49	2.56	1.63	0.56

* Geometric mean titre

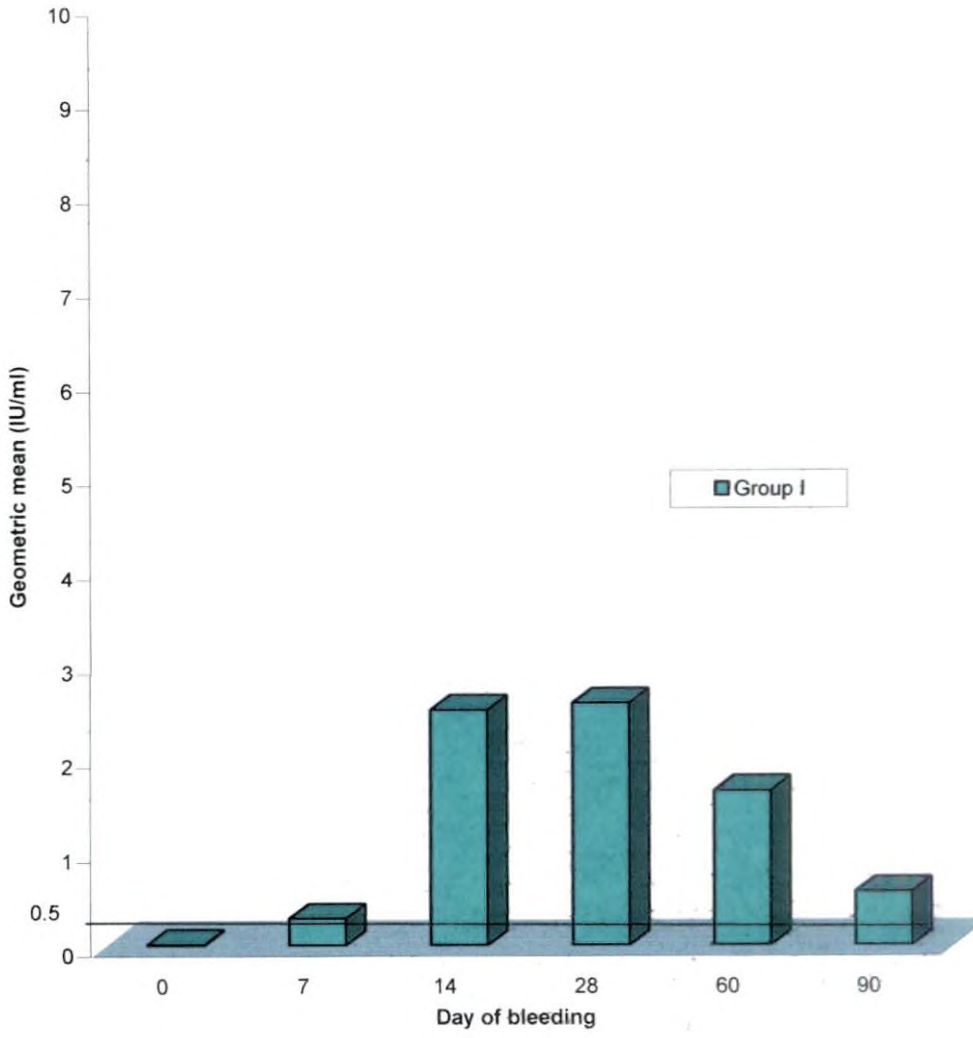

Fig.8. Geometric mean rabies virus neutralizing antibody titre (IU/ml) of group I (Black line indicate level of protection)

Table 2. Rabies virus neutralizing antibody titre (IU/ml) of group II animals

Animal no	Day of bleeding					
	zero	7	14	28	60	90
GDI- I	0.01	0.32	0.79	2.81	1.99	0.45
GD2-- I	0.01	2.81	38.90	3.01	2.81	0.95
GD3 - I	0.01	3.71	36.30	12.88	0.95	2.81
GD4- I	0.01	0.67	6.30	2.57	1.99	0.51
GD5- I	0.01	0.95	10.00	13.80	2.57	0.67
GD6- I	0.01	4.67	12.88	2.81	2.57	2.81
GD7- I	0.01	2.34	3.38	2.81	2.51	2.81
GD8- I	0.01	0.51	2.81	2.81	0.61	0.56
GD9- I	0.01	1.62	12.88	13.8	2.81	0.56
GD10-I	0.01	1.25	13.8	30.9	6.30	2.81
GD11-I	0.01	0.15	3.01	13.8	2.81	0.56
GD12-I	0.01	1.86	4.78	12.88	2.29	0.56
GD13-I	0.01	0.61	2.81	13.80	2.81	0.56
GD14-I	0.01	2.57	2.81	2.29	2.29	0.56
GMT*	0.01	1.19	6.37	6.44	2.24	0.92

* Geometric mean titre

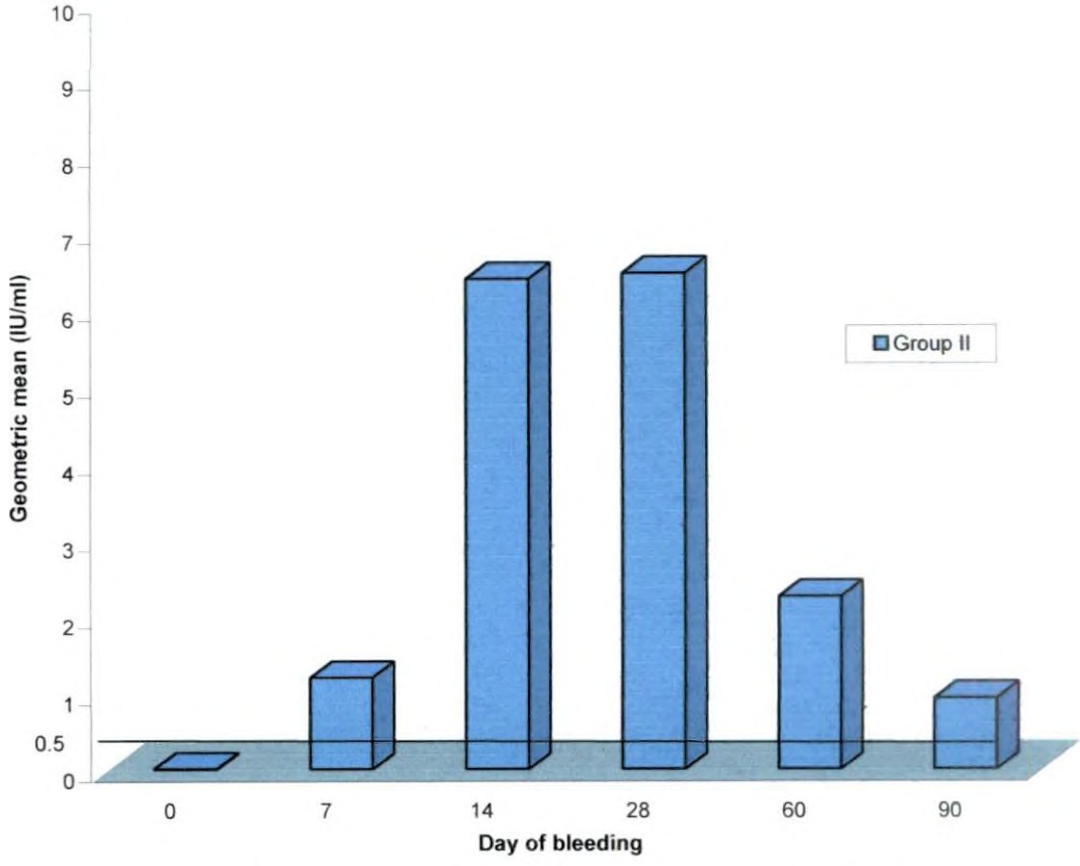

Fig.9. Geometric mean rabies virus neutralizing antibody titre (IU/ml) of group II (Black line indicate level of protection)

Table 3. Rabies virus neutralizing antibody titre (IU/ml) of group III animals

Animal no.	Day of bleeding					
	zero	7	14	28	60	90
GT1- II	0.01	10.42	10.00	12.88	2.81	2.29
GT2- II	0.01	1.99	11.48	13.80	2.81	2.29
GT4- II	0.01	2.57	13.80	10.00	0.61	0.56
GT5- II	0.01	0.12	3.71	11.48	2.81	1.62
GT6- II	0.01	0.39	2.57	0.56	0.10	0.11
GT7- II	0.01	3.16	30.90	2.81	2.57	2.29
GT8- II	0.01	1.25	3.09	0.56	0.51	0.11
GT9- II	0.01	1.99	3.09	13.8	2.81	0.79
GT10-II	0.01	0.14	0.95	2.29	0.56	0.56
GT11-II	0.01	0.39	23.98	2.57	0.56	0.56
GT13-II	0.01	1.62	12.88	2.81	0.45	0.11
GT14-II	0.01	0.95	12.88	2.81	0.56	0.39
GT15-II	0.01	3.54	13.80	12.88	2.29	2.81
GT16-II	0.01	1.25	30.90	13.8	2.81	0.56
GT17-II	0.01	1.44	10.00	6.30	0.56	0.95
GMT	0.01	1.19	6.68	4.73	1.03	0.63

* Geometric mean titre

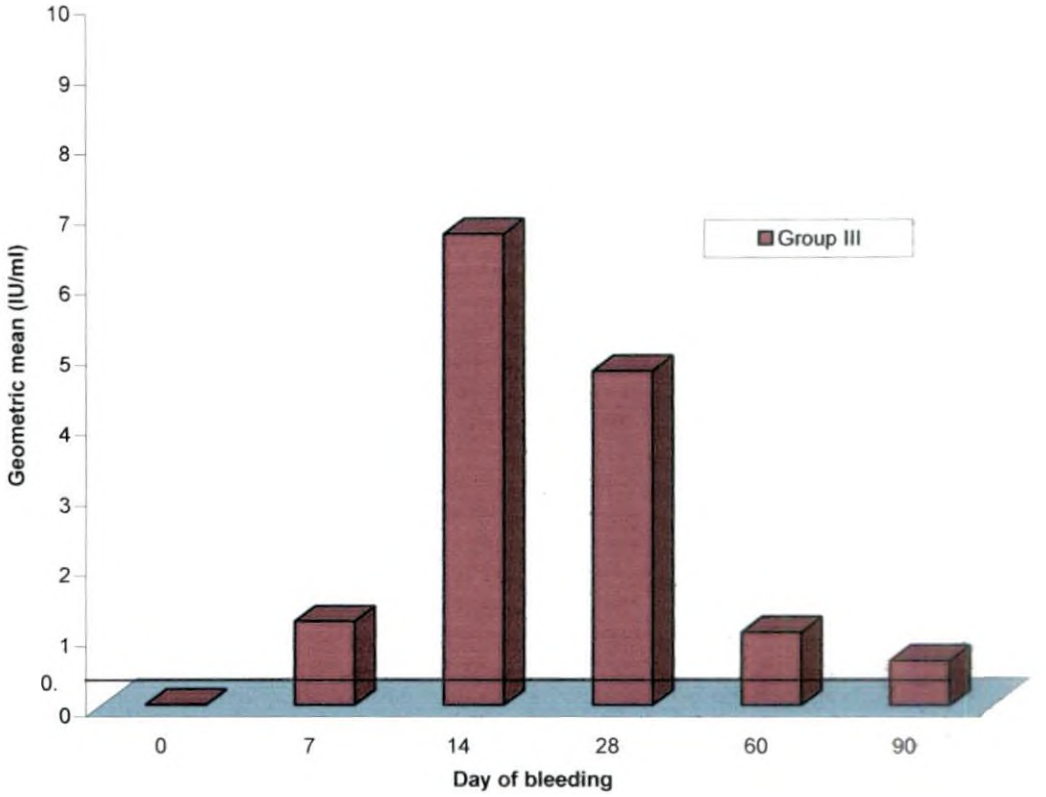

Fig.10. Geometric mean rabies virus neutralizing antibody titre (IU/ml) of group III (Black line indicate level of protection)

Table 4. Rabies virus neutralizing antibody titre (IU/ml) of group IV animals

Animal no	Day of bleeding					
	zero	7	14	28	60	90
GD1- II	0.01	1.25	8.12	1.25	0.56	0.56
GD2- II	0.01	1.25	2.57	2.81	0.56	0.45
GD3- II	0.01	2.29	0.79	2.81	2.57	0.56
GD4- II	0.01	4.16	2.81	2.57	0.51	0.56
GD5- II	0.01	2.51	3.98	2.57	0.39	0.56
GD6- II	0.01	2.29	3.98	2.57	0.39	0.56
GD7- II	0.01	0.56	2.51	0.61	0.56	0.45
GD8- II	0.01	0.51	8.12	1.25	0.45	0.56
GD9- II	0.01	2.29	12.88	12.88	2.81	2.81
GD10-II	0.01	10.00	2.29	3.98	2.81	1.62
GD11-II	0.01	0.95	1.25	0.56	0.15	0.13
GD12-II	0.01	5.24	8.12	10.00	2.81	1.62
GD13-II	0.01	1.99	2.29	2.57	0.56	0.45
GD14-II	0.01	0.12	6.30	0.61	0.45	0.13
GMT*	0.01	1.49	3.61	2.04	0.66	0.56

* Geometric mean titre

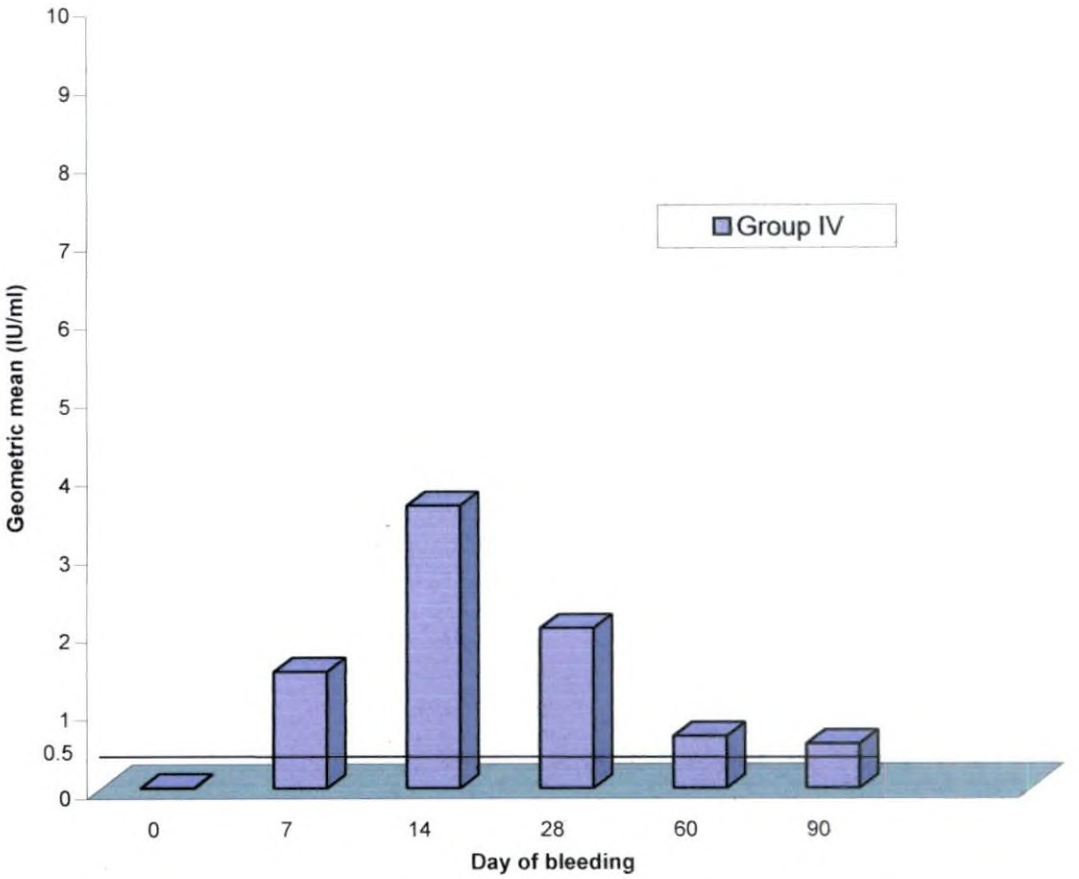

Fig.11. Geometric mean rabies virus neutralizing antibody titre (IU/ml) of group IV (Black line indicate level of protection)

Table5. Table of t-values between days in different treatment groups

Group	Days				
	0&7	7&14	14&28	28&60	60&90
I	-	3.62*	0.77	1.41	2.72*
II	-	2.98*	0.39	3.47*	3.51*
III	-	4.04*	1.80	4.90*	2.22*
IV	-	1.68	1.75	2.79*	2.01

* Significant at 5 per cent level

4.2 COMPARISON OF IMMUNE RESPONSE BETWEEN GROUPS

The comparisons of rabies virus neutralizing antibody titers of animals of all the four groups are presented in Table (6) and Figure (12).

4.2.1 Group I Vs Group II

Group I treated with inactivated tissue culture anti- rabies vaccine and group II treated with DNA combined tissue culture anti-rabies vaccine showed significant difference ($P<0.05$) in inducing the rabies virus neutralizing antibody titres in the initial days of observation (Table 6). Both groups received the vaccine as per the human "Essen" schedule. On first day of therapy, both groups had no antibodies. On day seven, neutralizing antibody titres of 0.28 IU/ml were present in-group I. In contrast, antibody titre was 1.19 IU/ml in-group II animals.

Both groups showed a consistent increase in the neutralizing antibody titer on day 14. These groups showed a statistically significant difference in the antibody response ($p<0.05$). Group I animals on day 14, had a titer of 2.49 IU/ml whereas group II had a titer of 6.37 IU/ml.

On 28^{th} day post exposure, Group II animals had higher geometric mean neutralizing antibody response (6.44 IU/ml) compared to group I (2.56 IU/ml) ($p<0.05$).

Group I and group II animals had comparable antibody titer on day 60 (1.63 IU/ml and 2.24 IU/ml) and were not statistically significant ($p>0.05$). On day 90, there was no significant difference ($P>0.05$) in the antibody response between group I and group II animals (0.56 IU/ml and 0.92 IU/ml).

4.2.2 Group I Vs Group III

There was a significant difference (P<0.05) in the rabies virus neutralizing antibody response between these groups on seventh day of observation. Before vaccination, both groups were seronegative.

The schedule II induced higher rabies virus neutralization antibody titre than the schedule I by day seven. The Group III animals had a titre of 1.19 IU/ml whereas group I had a titre of 0.28 IU/ml. On day 14, group III animals had a significantly higher antibody response than group I animals (P<0.05). Group III animals had a titre of 6.68 IU/ml and group I animals had a titre of 2.49 IU/ml.

On day 28, Group III animals showed higher geometric mean rabies virus neutralizing antibody titre (4.73 IU/ml) compared to group I animals whose antibody titre was 2.56 IU/ml. (P<0.05). There was significant statistical difference (p<0.05) between these groups on 60th day of observation. The rabies virus neutralizing antibody titres between these groups were not statistically significant on day 90 (P>0.05). The group I and group III had a titre of 0.56 IU/ml and 0.63 IU/ml respectively. None of the animal developed rabies during the observation period of 180th day.

4.2.3 Group I Vs Group IV

The group I animals treated with inactivated tissue culture anti-rabies vaccine in "Essen" schedule and group IV animals treated with DNA combined tissue culture anti-rabies vaccine in five consecutive days differed significantly (P<0.05) in the antibody response in the initial days. Group IV animals showed a higher antibody response (1.49 IU/ml) than group I animals whose antibody level was 0.28 IU/ml on seventh day.

On day 14, rabies virus neutralizing antibody responses between these groups were not statistically significant (P> 0.05). On day 14, group I animals had a titer of 2.49 IU/ml, and group IV animals had higher neutralizing antibody response (3.61 IU/ml).

Group I animals showed gradual increase in the geometric mean neutralizing antibody titer on day 28 (2.56 IU/ml) whereas in-group IV animals, titer gradually reduced to 2.04 IU/ml. Though there was no statistically significant difference, group I showed better response. On day 90, both the group I and II had same geometric mean rabies virus neutralizing antibody titre (0.56 IU/ml).

4.2.4 Group II Vs Group III

On the first day of treatment, both groups had no antibody. On day seven, both groups showed rise in the antibody above protective level (1.19 IU/ml). However, there was no significant difference ($P>0.05$) in the rabies neutralizing antibody titer. Both groups had a comparable antibody response (6.37 IU/ml and 6.68 IU/ml) on day 14.

On day 28, rabies virus neutralizing antibody titer of group II animals and group III animals were not statistically significant ($P>0.05$). Reduction in the neutralizing activity in all animals in-group II (2.24 IU/ml and group III (1.03 IU/ml) were recorded. The geometric mean rabies neutralizing antibody titer of group II and group III animals were not statistically significant ($P>0.05$) on day 90 and all animals in-group II are better seroconverted and had higher geometric mean rabies neutralizing antibody titer (0.92 IU/ml) compared to group III animals (0.63 IU/ml), though it was above protective level.

4.2.5 Group II Vs Group IV

On day seven, antibody response was comparable in both groups irrespective of the schedule of vaccination followed. There was no significant difference in the rabies virus neutralizing antibody titre ($P>0.05$). On day seven, group II and group IV had shown rabies virus neutralizing antibody titre of 1.19 IU/ml and 1.49 IU/ml respectively.

Both groups showed an increase in the antibody response, on day 14. There was significant difference in the neutralizing antibody titer between these groups ($P<0.05$). Group II showed a higher geometric mean of rabies virus neutralizing antibody titre (6.37 IU/ml) compared to group IV (3.61 IU/ml).

Group II animals showed a consistent increase in the antibody response on day 28 whereas group IV animals had a reduced antibody response. Both groups showed a gradual decrease in the antibody response with time. The geometric mean of rabies virus neutralizing antibody titer of group II was 0.92 IU/ml whereas that of group IV was 0.56 IU/ml and there was no significant difference in the antibody response between these groups on day 90 ($P>0.05$). Though statistically not significant, the magnitude of response was higher for group II. The geometric mean rabies virus neutralizing antibody titre remained above the minimum acceptable level of antibody titre (0.5 IU/ml) in both group II and group IV till 90^{th} day of treatment.

4.2.6 Group III Vs Group IV

The geometric mean titer of group III and group IV animals were not statistically significant ($P>0.05$) on day seven, though the titres were above protective level. Both the group III and group IV had a titer of 1.19 IU/ml and 1.49 IU/ml respectively.

On day 14, geometric mean of rabies virus neutralizing antibody titer showed significant difference between group III and group IV animals ($p<0.05$).Both groups produced an increased antibody response and group III had a titer of 6.68 IU/ml and group IV had 3.61 IU/ml .There was significant ($P<0.05$) difference in the antibody titre on day 28 in group III and group IV(4.73 IU/ml and 2.04 IU/ml) The geometric mean of rabies virus neutralizing antibody titer gradually decreased in both groups with time. On day 90-post exposure, Group III and group IV showed a titer of 0.63 IU/ml and 0.56 IU/ml respectively and the differences were not statistically significant.

4.3 COMPARISON OF IMMUNE RESPONSE IN SCHEDULE I AND SCHEDULE II

Schedule I and Schedule II used in this study were effective in inducing a protective immune response and maintaining the mean rabies virus neutralizing antibody titre above protective level of titre (>0.5 IU/ml).

Group I animals with schedule I showed protective antibody titre (>0.5 IU/ml) from 14th day to 90th day of study period. Group I with schedule I produced a geometric mean rabies virus neutralizing antibody titre of 0.28 IU/ml on day seven whereas group III with schedule II produced antibody titre of 1.19 IU/ml. Though both groups were treated with same vaccine, group III produced protective antibody titre response on day seven itself.

Group II with schedule I induced protective antibody titre (0.5 IU/ml) from seventh day to 90th day. Group IV with schedule II had shown protective rabies virus neutralizing antibody titer from seventh day onwards. Group III and group IV animals with schedule II induced an earlier higher neutralizing antibody titre, but declined very rapidly by day 90.

4.4 OBSERVATION OF TREATED GROUPS

All the vaccinated animals were observed for the period of six months from the day of first vaccination for the development of any signs suggestive of rabies. Six months after exposure, all the study goats were alive and no animal had succumbed to rabies. The vaccines were well tolerated by the goats and there were no serious adverse effects. All the animals were found to be healthy during the monitoring period of six months in all four groups.

Table 6. Geometric mean rabies virus neutralizing antibody titres of different treatment groups

Day of bleeding	Group I		Group II		Group III		Group IV	
	No. of goats	Geometric mean (IU/ml)	No. of goat	Geometric mean (IU/ml)	No. of goat	Geometric mean (IU/ml)	No. of goats	Geometric mean (IU/ml)
0	17	0.01[a]	14	0.01[a]	15	0.01[a]	14	0.01[a]
7	17	0.28[a]	14	1.19[b]	15	1.19[b]	14	1.49[b]
14	17	2.49[a]	14	6.37[b]	15	6.68[b]	14	3.61[a]
28	17	2.56[a]	14	6.44[b]	15	4.73[b]	14	2.04[a]
60	17	1.63[a]	14	2.24[a]	15	1.03[b]	14	0.66[b]
90	17	0.56[a]	14	0.92[a]	15	0.63[a]	14	0.56[a]

a, b = Between treatment groups ,means with different superscripts are significantly different ($p<0.05$).

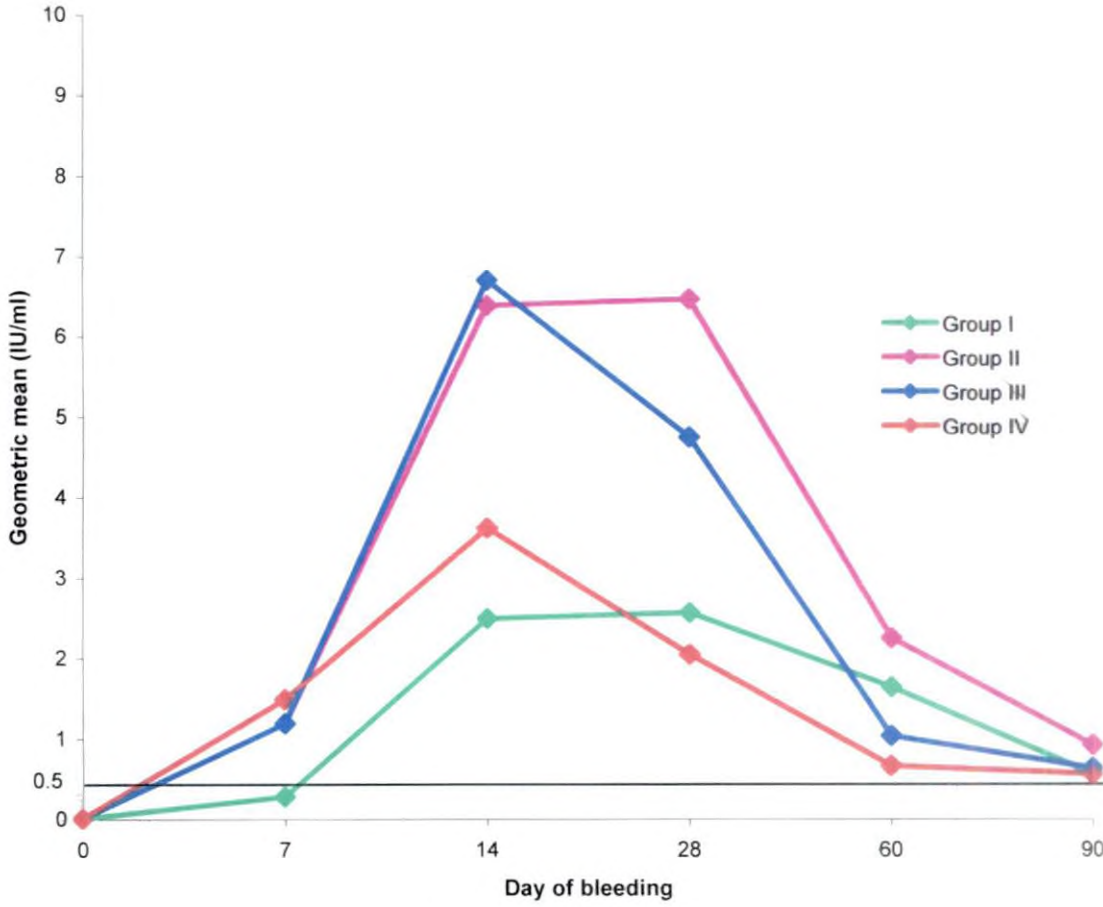

Fig.12. Comparison of geometric mean rabies virus neutralizing antibody titres (IU/ml) in four different groups (Black line indicate level of protection)

Discussion

5. DISCUSSION

Rabies is almost always fatal but preventable disease. Post-exposure therapy is the combination of local treatment of wound, passive immunization with rabies immunoglobulin and vaccination which is recommended in all severe exposures to rabies virus (WHO, 1992). An effective rabies post-exposure therapy must trigger a response as quickly as possible, to successfully compete with rabies virus as it progresses towards central nervous system. The sooner and stronger the immune response induced by the rabies vaccine, better the prognosis. The efficacy of post-exposure therapy depend on the efficient immediate delivery of the recommended treatment, the competency of host immune response and the susceptibility of the infecting virus to the immunity induced by the vaccine (Warrell and Warrell, 2004).

Even though domestic animals should be considered as cul-de-sacs, such dead end infections are always of public health relevance. So in unvaccinated animals exposed to a rabid animal, euthanasia is recommended. But the monetary and emotional value of the domestic animal cannot be disregarded. Additionally the World Health Organization has approved options that reduce post-exposure treatment in humans and has advocated the need to develop an effective and inexpensive post-exposure protocol for animals (WHO, 1992).

The major cause of failure of rabies eradication program is the unaffordability of vaccine rather than unavailability. DNA vaccines are especially attractive for diseases such as rabies because these vaccines can be produced more economically than cell culture vaccines and do not require a cold chain and intramuscular immunization appeared to be effective in all mammalian species (Rangarajan *et al.*, 2000).

The present study was undertaken to assess the efficacy of post-exposure vaccination in goats using two different vaccines *viz.* inactivated tissue culture anti-rabies vaccine and DNA combined tissue culture anti-rabies vaccine using "Essen" schedule and a new schedule of five continuous injections based on preliminary

studies conducted in the Department of Veterinary Epidemiology and Preventive Medicine.

5.1 IMMUNE RESPONSE FOLLOWING POST-EXPOSURE VACCINATION IN FOUR DIFFERENT GROUPS

5.1.1 Group I

This group consisted of 17 goats which were treated with inactivated tissue culture anti-rabies rabies vaccine in "Essen" schedule of anti-rabies post-exposure therapy (Table 1 and Fig 8). WHO (1992) set an arbitrary figure for the minimum titre of protective antibodies after vaccination against rabies at 0.5 IU/ml.

On day zero, none of the animals were seropositive indicating that they had never been vaccinated previously.

On day seven, geometric mean rabies virus neutralizing antibody titre was 0.28 IU/ml, which was below the protective titre recommended by WHO. This was in agreement with the findings of Basheer *et al.* (1997a) who demonstrated that cattle vaccinated with tissue culture vaccine in "Essen" schedule showed titre below 0.5 IU/ml on day ten. All the animals in the treatment group did not seroconvert satisfactorily on seventh day and the animals which showed titre above 0.5 IU/ml was 53 per cent. Some animals responded well while some had poor response on seventh day (0.01 IU/ml to 5.24 IU/ml) with geometric mean rabies virus neutralizing antibody titre of 0.28 IU/ml. Delgado and Carmenes (1997) suggested that factors such as genetics, nutrition or parasitic infections might contribute to poor immune response that was detected after rabies vaccination in dogs.

Oliveira *et al.* (2000) reported that a single dose of the tested vaccine does not induce detectable levels of antibodies in the majority of the cattle after first vaccination, whereas all animals developed antibody titres two weeks following booster. For guarantee of adequate immunity, booster doses of rabies vaccines were recommended by Benisek *et al.* (2000).

On day 14, it was found that these levels were further raised above protective titre (2.49 IU/ml) and assumed that booster doses have induced further rise in the antibody titres and titres reached maximum response on day 28. This was in agreement with the findings of Basheer *et al.* (1997a) who reported titres 2.33 IU/ml on day 20 post-exposure in cattle in "Essen" schedule. Post-exposure rabies vaccination using chicken embryo cell culture rabies vaccine induced protective levels of antibody on day 14-post vaccination and enhanced by day 30 in "Essen" schedule in humans (Natarajan *et al.*, 1992). In another study Selvakumar and John (1989) had shown that chicken embryo cell culture rabies vaccine in "Essen" schedule induced protective levels on day 14 post-exposure. Increase of antibody titres on day 7, 14, and 28 indicated that animals responded well to the booster injections in this study. Ramanna and Srinivasan (1992) reported that tissue culture rabies vaccine, inactivated with BEI and adjuvanted with aluminium hydroxide gel induced satisfactory immune response on single administration and exhibited anamnestic response on revaccination.

The antibody levels rose further with additional doses of the vaccine and reached peak titre on day 28 (2.56 IU/ml). This was in agreement with the findings that cattle vaccinated with Vero cell rabies vaccine in "Essen" schedule elicited an antibody titre of 2.83 IU/ml on day 30 (Basheer *et al.*, 1997a). According to Quiambao *et al.* (2000) purified Vero cell rabies vaccine produced peak geometric mean titre on day 28 in "Essen" schedule.

On day 60-post-exposure, the titre decreased but the animals maintained protective levels of antibody titre up to 90^{th} day of observation (0.56 IU/ml). Reddy and Srinivasan (1999) reported that tissue culture rabies vaccine effectively immunized the target species under field conditions and induced satisfactory antibody response during the three year period of study in dogs.

None of the animals in the treated group developed clinical signs for the observation period of six months; indicates that this "Essen" schedule is effective in controlling rabies among goats bitten by suspected rabid animals. Basheer *et al.* (1997b) in their study indicated that post-exposure therapy using cell culture rabies

vaccine with "Essen" schedule would be effective in preventing disease developing in infected animals. Mitmoonpitak *et al.* (2002) treated pigs exposed to rabid dog with tissue culture rabies vaccine in proven "Essen" schedule and proposed that this schedule applied to farm animals can be safe and effective. Cho and Lawson (1989) demonstrated the efficacy of human diploid cell rabies vaccine for post-exposure treatment in dogs in "Essen" schedule. Ramanna *et al.* (1991b) reported the protective role of tissue culture vaccine in experimental rabies infected sheep.

Under this group, the animals attained protective level on day 14 and reached maximum titre of 2.56 IU/ml on day 28-post-exposure and the protective level was maintained until 90th day of observation. None of the animals developed any sign of rabies during the period of 180 days observation.

5.1.2 Group II

This group of animals was vaccinated with novel DNA combined tissue culture anti- rabies vaccine (Table 2 and Fig 9). The vaccination was similar to the post-exposure vaccination protocol ("Essen" schedule) recommended by WHO for humans.

After one booster dose geometric mean titer of neutralizing antibody on day seven was 1.19 IU/ml and 86 per cent of animals had shown titre above 0.5 IU/ml. This was in agreement with the findings of Bahloul *et al.* (2003) who reported that DNA based vaccination was shown to induce a rapid and strong antibody response. In their study, mice received a single dose of DNA vaccine and had detectable levels of neutralizing antibodies (higher than 1 IU/ml) on day seven.

All the animals had an increase in the antibody response ranging from 0.79-38.9 IU/ml; geometric mean titer 6.37 IU/ml on day 14 post-exposure after three injections. This is in accordance with the findings of Biswas *et al.* (2001b) who reported that cattle immunized with DNA combined tissue culture vaccine induces a geometric mean rabies virus neutralizing antibody titre of 5.4 IU/ml on day 21 in two dose immunization regimen.

The anamnestic antibody response was higher for animals on day 28 after three booster doses. The titres on day 14 and 28 were almost comparable to each other (6.37 IU/ml and 6.44 IU/ml). Lodmell and Ewalt (2000) reported that combination of different rabies vaccines are important not only for the rapid elevation of anamnestic neutralizing antibody titre, but also for the long-term duration of higher levels of neutralizing antibody. Lodmell *et al.* (2002) demonstrated that DNA booster vaccination in "Essen" schedule in monkeys resulted in higher neutralizing antibody level on day 14 post-exposure and increased the durability of response. Vodopija *et al.* (1997) reported that booster doses would guarantee an efficient anamnestic antibody response and success of post-exposure vaccination.

On day 60 and 90, the titres were reduced to 2.24 IU/ml and 0.92 IU/ml respectively, but the levels were maintained above 0.5 IU/ml. Biswas *et al.* (2001a) demonstrated that monkeys vaccinated with DNA vaccine had maintained virus-neutralizing titre above 0.5 IU/ml up to five month in two dose regimens.

In the present study, geometric mean rabies virus neutralizing antibody titre was raised above protective level (1.19 IU/ml) on seventh day and maintained until the end of the study period (0.92 IU/ml). Perrin *et al.* (2000) reported that a single intramuscular injection of DNA vaccine in dog elicited significant neutralizing antibodies (>0.5 IU/ml) on day 28 and maintained until the boost on day 175 and observed that booster inoculations increased the rabies virus neutralizing antibody titre. Osorio *et al.* (1999) demonstrated significant levels of neutralizing antibody titre up to nine months after single intramuscular injection of DNA vaccine.

None of the animal developed rabies signs till 180 days of the observation period.

5.1.3 Group III

This group of animals was treated with inactivated tissue culture anti-rabies vaccine on five consecutive days (Table 3 and Fig 10). According to WHO (1984),

vaccination schedule recommended in a given situation depends on the type of vaccine and potency of vaccine used. The schedule should include booster doses in order to prolong the duration of antibodies. These experiments were performed to determine whether multiple booster vaccinations without rest period would accelerate the onset of the neutralizing antibody response.

After four booster doses, antibody titre on day seven was 1.19 IU/ml, which was above the protective level (0.5 IU/ml) recommended by WHO. The accelerated onset of the antibody response might have been due to the multiple booster vaccination in five consecutive days. This observation agrees with the opinion of Tizárd (2000) who described that repeated injection of antigen produced immune response with shorter lag period and for longer duration than single inoculation. Though the animals had geometric mean titre higher than the level considered adequately by WHO ($\geq$ 0.5 IU/ml), some animals in this group did not show this minimum acceptable level. The animals showed titre above protective level was 73 per cent. This may be explained by the fact that immune response being a biological process and is never equal in all members of a vaccinated population.

All animals had a rise in antibody level on day 14-post-exposure (0.95 IU/ml to 30.90 IU/ml; geometric mean, 6.68 IU/ml). The animals produced maximum neutralizing antibody titre on 14th day of post vaccination (6.68 IU/ml). This was in agreement with the finding that a long rest period between vaccinations is not required for the elevation of antibody response. It is suggested that the rest period is especially important for low expression vectors (Lodmell and Ewalt, 2001).

This group of animals showed significantly higher response on day 7, 14, and 28 indicated that repeated injection in five consecutive days had significant effect on early induction of higher rabies virus neutralizing antibody titre which is very essential in post- exposure therapy. Vodopija *et al.* (1999) stated that primary importance of the early induction of rabies neutralizing antibodies is to offset the risk of infection due to a possible short incubation period of disease.

The antibody titre was found to be decreased on subsequent sampling days ie, on day 60 and 90. Simani *et al.* (2004) opined that level of antibody titre decreases as the time between the last dose and the sampling increased. According to Oliveira *et al.* (2000), decline in titres may have occurred due to decrease in the number of memory cells. However, all animals maintained the protective titre (0.63 IU/ml) until 90th day of sampling. Sampath *et al.* (2000) demonstrated that purified Vero cell rabies vaccine showed geometric mean titre of more than 0.5 IU/ml on day 14, 30, 90 and 365 in five dose regimens. All animals were alive and active until 180th day of this study.

In this group of animals, antibody titre was raised to 1.19 IU/ml on seventh day, reached peak of 6.68 IU/ml on day 14 and maintained above protective level (0.63 IU/ml) up to 90th day. None of the animals developed rabies during the period of observation of 180th day. This schedule has the advantage of continuous vaccination. Often when following "Essen" schedule even in humans missing some injections in between leads to the breakdown of immunity resulting in rabies.

5.1.4 Group IV

This group of animals vaccinated with DNA combined tissue culture anti-rabies vaccine on days 0-1-2-3-4 schedule had protective levels of antibody titre throughout the study period (Table 4 and Fig 11). On day seven, they had a geometric mean rabies virus neutralizing antibody titre of 1.49 IU/ml and reached peak titre on day 14-post-exposure. On seventh day, 86 per cent of the animals seroconverted after receiving all five injections. This was in agreement with the findings of Lodmell and Ewalt (2001) who demonstrated that mice received five consecutive DNA vaccinations at 24-hour interval developed detectable levels of neutralizing antibody on day five post-exposure than those received three or only primary vaccination.

The animals showed maximum antibody response on day 14 (3.61 IU/ml) supported by the findings of Tizard (2000) who described that repeated injection of antigen produced immune response with shorter lag period. This stresses the point

that long rest period between vaccinations is not necessary for the rapid elevation of antibody response. On subsequent sampling day, ie, on day 28 and 60, geometric mean rabies virus neutralizing antibody titre observed was 2.04 IU/ml and 0.66 IU/ml respectively. Lodmell *et al.* (2002) opined that multiple DNA vaccination increased the durability of neutralizing antibody response. On day 90 post-exposure, antibody titre of >0.5 IU/ml were present in-group IV animals that had received multiple DNA combined tissue culture anti-rabies vaccine (0.56 IU/ml). Osorio *et al.* (1999) suggested that DNA rabies vaccine could induce durable and protective immune response against rabies infection in companion animals.

DNA combined tissue culture anti-rabies vaccine after continuous five days of injection, produced rabies virus neutralizing antibody titre of 1.49 IU/ml on seventh day, which rose to 3.61 IU/ml on day 14, then maintained to 0.56 IU/ml up to day 90 post exposure. The early rise of antibody titre is highly significant in the protection in post-exposure therapy.

5.2 COMPARISON OF IMMUNE RESPONSE BETWEEN DIFFERENT GROUPS

5.2.1 Group I vs. Group II

Group I treated with inactivated tissue culture anti-rabies vaccine and group II treated with DNA combined tissue culture anti-rabies vaccine showed significant difference in the rabies virus neutralizing antibodies in the initial days of observation. Both groups received the vaccine as per the human "Essen" schedule. On day seven, in-group I, 53 per cent of the animals had titer well above the minimum acceptable level (0.5 IU /ml) whereas group II it was 86 per cent. On day seven, group II had higher virus neutralizing antibody (1.19 IU/ml) compared to group I, where it had geometric mean rabies virus neutralizing antibody titre of 0.28 IU/ml which was lower than the protective titre (0.5 IU/ml). This observation endorses the view that co-inoculation of two vaccines might reduce the delay in the induction of antibody responses associated with prime boost immunization regimen (Lodmell and Ewalt, 2000; Biswas *et al.*, 2001b).

There was significant increase in the anamnestic antibody response in-group II. On day 14, titre was 6.37 IU/ml, and the rapidity with which the antibody production was triggered accounts for the subsequent protective effect. Both groups reached the peak titre on day 28 after three injections and the response was significantly higher ($P<0.05$) for group II. The titre obtained on day 28 in-group II was 6.44 IU/ml; whereas the peak antibody titre group I was 2.56 IU/ml. This was in agreement with the findings of Biswas *et al.* (2001b) who found that the potency of combined rabies vaccine is two fold higher than that of undiluted tissue culture rabies vaccine in two-dose immunization regimen. In their study, it was demonstrated that the undiluted veterinary tissue culture rabies vaccine was shown to induce rabies virus neutralizing antibody titre of 2.1 IU/ml on day 21-post immunization in cattle whereas the mean rabies virus neutralizing antibody titre in cattle immunized with veterinary combined rabies vaccine was 5.4 IU/ml. They also reported that five dose vaccination regimen induced higher anamnestic antibody response. Further Bahloul *et al.* (2003) reported that, the DNA vaccine was associated with slightly higher protection rate compared to cell culture-derived vaccine. Fischer *et al.* (2003) reported that properly formulated DNA vaccine could generate immune response in large species. The geometric mean rabies virus neutralizing antibody titre of animals vaccinated with DNA combined tissue culture rabies vaccine showed higher response on day 14, 28 post-exposure. This stressed the findings of Lodmell and Ewalt (2000) who opined that the combination of vaccines did result in long-term duration of higher levels of neutralizing antibody.

On day 60 and 90, magnitude of response was higher for group II than group I, though they are not statistically significant.

The enhanced efficiency of DNA combined tissue culture anti-rabies vaccine was measured based on their ability to induce higher anamnestic rabies virus neutralizing antibody response and better seroconversion efficiency immediately after vaccination. Since the most important factor in rabies post-exposure therapy is the rapidity to reach protective levels of antibodies that ensure efficient virus neutralization, these results stresses the possibility that this DNA combined tissue culture anti-rabies vaccine might be an effective post-exposure treatment of rabies.

5.2.2 Group I vs. group III

There was significant difference in the neutralizing antibody titre between animals in these groups with different schedule of vaccination ($P<0.05$). Antibody titres in the group III (1.19 IU/ml) who had received complete vaccination with four booster doses were significantly higher than that of group I (0.28 IU/ml) which received only one booster vaccination on day seven. The immunity produced by the inactivated tissue culture rabies vaccine was associated with the quantity of antigen in the vaccine so that the additional doses have significant effect on inducing adequate levels of rabies antibodies during the test period (Oliveira *et al.*, 2000). So group III animals demonstrated a considerable rise in rabies titres reaching much higher levels on initial days of observation than group I. Haviv *et al.* (1999) reported that increasing the first dose of vaccine might result in earlier and higher neutralizing antibody titres. In group I, 53 per cent of the animals showed titre above 0.5 IU/ml and that of group III was 74 per cent on seventh day.

There was significant difference in the geometric mean rabies virus neutralizing antibody titre on day 14, group III had a titre of 6.68 IU/ml (received five injections) and group I had 2.49 IU/ml. (received three injections). Warrell (2003) reported that larger initial dose results in greater immunogenicity of the vaccine. Takehara (1986) in his study demonstrated that the higher virus concentration of the inoculum, the more rapidly the antibodies was produced. Weanling mice injected with chicken embryo passaged rabies virus developed neutralizing antibodies much earlier and its titre became higher than those, which received lower amount of virus. As far as post-exposure therapy is concerned, protection critically depends on the rapid onset of the antibody responses rather than the final level reached by these responses.

Group III animals showed significantly higher antibody response on day 28 post exposure, but the antibody response was gradually reduced in both groups on day 60. On day 90, both groups had comparable geometric mean titer (0.56 IU/ml and 0.63 IU/ml) irrespective of the schedule of vaccination. All animals in both groups showed titres well above the protective levels of rabies virus neutralizing

antibody response (0.5 IU/ml) from day 14 onwards after three injections. This was in agreement with the findings of Quiambao *et al.* (2000) who reported that by day 14, all subjects that received Vero cell rabies vaccine had seroconverted, and antibody levels maintained above protective level.

Inactivated tissue culture anti-rabies vaccine in proven "Essen" schedule induced neutralizing antibody titre well above the protective level from 14th day onward to 90th day of study period and protected all animals from clinical rabies. This observation agrees with the findings of Basheer *et al.* (1997b). Repeated administration of vaccine on five consecutive days resulted in earlier and higher antibody titre and agrees with the findings of Lodmell and Ewalt (2001). They also reported that long rest period between vaccinations was not necessary for the elevation of antibody response and important only in case of low expression vectors.

Although the difference in the magnitude of responses were not statistically significant in the measurement of long term responses between these groups, evidence of enhanced efficiency of 0-1-2-3-4 schedule for inactivated tissue culture anti-rabies vaccine was obtained from the measurement of their ability to induce rapid onset of higher antibody response and seroconversion efficiency.

None of the animals in this group developed rabies for an observation period of 180 days.

5.2.3 Group I vs. Group IV

The group I animals treated with inactivated tissue culture anti-rabies vaccine in "Essen" schedule and group IV animals treated with DNA combined tissue culture anti-rabies vaccine on five consecutive days differed significantly ($P<0.05$) in the antibody response in the initial days. On day seven, group IV showed higher response (1.49 IU/ml) than group I (0.28 IU/ml).

Group IV showed peak antibody titre on day 14 (3.61 IU/ml) whereas group I on 14th day, the titre was 2.49 IU/ml after three injections. However, on day 28, the titre declined in group IV animals, whereas in group I, the titre rose to 2.56 IU/ml after three booster doses. Though the DNA combined tissue culture anti-rabies vaccine produced superior response on day seven, the level was not found to be maintained on subsequent observations.

On 60th day, inactivated tissue culture anti-rabies vaccine produced higher geometric mean rabies virus neutralizing antibody titre (1.63 IU/ml) than the DNA combined tissue culture anti-rabies vaccine (0.66 IU/ml). This was not in agreement with the earlier findings in which group that received combined rabies vaccine showed superior performance than group that received tissue culture rabies vaccine. This may be due to the different approach in the schedule of vaccination followed for DNA combined tissue culture anti-rabies vaccine. Both groups responded well to the booster inoculation. However, it is the ability to respond to booster immunization, not the magnitude of antibody titer following primary immunization, which can provide a rapid increase in the level of rabies virus neutralizing antibodies, providing protection from clinical rabies (Sabehareon *et al.*, 1999). After three months, both groups showed decrease in the antibody titre, though it was above the protective level. None of the animal developed rabies for an observation period of 180 days.

5.2.4 Group II and Group III

Group II and group III was treated with DNA combined tissue culture anti-rabies vaccine and inactivated tissue culture anti-rabies vaccine respectively. The schedule of vaccination also differed and group II followed the 0-3-7-14-28 whereas group III in 0-1-2-3-4 regimen. Group II animals that received two injection of DNA combined tissue culture anti-rabies vaccine and group III animals that received five injections of inactivated tissue culture anti-rabies vaccine have same geometric mean rabies virus neutralizing antibody response on day seven (1.19 IU/ml). This was in agreement with the findings of Biswas *et al.* (2001b)

who reported that booster inoculation did not have significant effect on early kinetics rabies virus neutralizing antibody induction.

In-group III animals the peak antibody titre was reached on day 14 post-exposure (6.68 IU/ml) and it declined thereafter. Group II, with three injections produced a geometric mean rabies virus neutralizing antibody titre of 6.37 IU/ml on day 14, which is comparable to that of group III. This was in agreement with the findings of Bahloul *et al.* (2003) who found out that the immunogenicity and efficacy of a single injection of DNA rabies vaccine was as effective as five injection of cell culture derived vaccine. In their study, similar high levels of rabies antibodies were elicited on day 13 after single injection of DNA vaccine and three injections of cell culture derived vaccine in mice. DNA vaccine was able to elicit an antibody response as quickly as cell culture derived vaccine. Group II showed consistent increase in the titre on 28th day (6.44 IU/ml) whereas group III had a titre of 4.73 IU/ml. DNA vaccine was associated with slightly higher protection rate compared to cell culture vaccine, although the difference was not statistically significant. The duration of higher antibody response was longer for group II animals that received the vaccine in proven "Essen" schedule. Both groups showed reduction in the geometric mean rabies virus neutralizing antibody titre with time.

Although the ability to induce rapid onset of rabies virus neutralizing antibodies were comparable in both groups, evidence of enhanced efficiency of group II was obtained based on the measurement of their longer duration of higher antibody response.

5.2.5 Group II vs. Group IV

Both groups treated with DNA combined tissue culture anti-rabies vaccine with a different approach to the schedule of vaccination. On day seven, goats received five injections in Group IV and Group II goats received two injections of DNA combined tissue culture anti-rabies vaccine.They did not show significant difference in the geometric mean rabies virus neutralizing antibody titre (1.19 IU/ml and 1.49 IU/ml).This again demonstrated that booster injection did not have

significant effect on early kinetics of rabies virus neutralizing antibody response. However, the magnitude of response was higher for group IV animals. This was in agreement with the findings of Lodmell and Ewalt (2001) who reported that mice that had received five consecutive DNA vaccinations at 24-hour interval produced higher response on day 10 post-exposure, when compared to titres of mice that were vaccinated only once.

Analysis of antibody response on day 14, 28 and 60 post immunization indicated that group II induced a higher anamnestic antibody response than group IV. However, both groups responded well to the booster injections. Biswas *et al.* (2001a) in his study indicated that a rapid rise in rabies virus neutralizing antibody titre following the administration of the booster dose was seen in mice immunized with rabies DNA vaccine indicating the presence of vaccination induced memory cells. Group IV produced peak antibody titre on day 14 with geometric mean rabies virus neutralizing antibody titre of 3.61 IU/ml, but the titre was lower than that produced by group II on day 14 (6.37 IU/ml). Both groups treated with the same vaccine, but the difference in the geometric mean rabies virus neutralizing antibody titre may be due to the different schedule followed. This was in agreement with the findings of Biswas *et al.* (2001b) who found that a novel combination of rabies vaccine containing a low dose of cell culture derived inactivated rabies virus and DNA induced a higher level of rabies virus neutralizing antibody titre in cattle after a five dose vaccination regimen in "Essen" schedule. Further, though group IV animals had shown a lower titer compared to group II, both groups maintained a level higher than the minimum acceptable antibody titre on all sampling days. Geometric mean rabies virus neutralizing antibody titre of both groups reached protective levels on day seven itself supporting the findings of Lodmell and Ewalt (2001) that DNA vaccine if administered via different methods are very successful in elevating the antibody titer.

5.2.6 Group III and Group IV

Group III goats treated with inactivated tissue culture anti-rabies vaccine and group IV with DNA combined tissue culture anti-rabies vaccine, produced rabies virus neutralizing antibody titer higher than that require for minimum level

of protection (1.19 IU/ml and 1.49 IU/ml respectively) on day seven. Though geometric mean rabies virus neutralizing antibody titres were not significantly different, group IV animals showed higher magnitude of response. This agrees with the findings of Lodmell and Ewalt (2001) who demonstrated low titres of neutralizing antibody were detected at five days post-exposure in mice that received human diploid cell vaccination in a schedule (five consecutive days) identical to that of the DNA vaccinated mice.

Both groups had a consistent increase in the geometric mean rabies virus neutralizing antibody titre and group III showed a significantly higher response on day 14 and 28 (6.68 IU/ml and 4.73 IU/ml) compared to group IV (3.61 IU/ml and 2.04 IU/ml). Biswas *et al.* (2001b) stated that DNA combined tissue culture anti-rabies vaccine showed superior response than tissue culture vaccine alone. Here tissue culture vaccine showed superior response than the combined rabies vaccine and not in agreement with the previous observation. This may be due to the different schedule of vaccination followed for combined rabies vaccine. Simani *et al.* (2004) proved that, the failure in adhering to the common WHO protocols on rabies vaccination would be the main reason for undesired levels of antibody in high-risk individuals due to various intrinsic host factors.

5.3 COMPARISON OF IMMUNE RESPONSES IN SCHEDULE I AND SCHEDULE II

Schedule I and schedule II used in this study were effective in inducing protective immune response and maintained geometric mean neutralizing antibody titre above protective level (0.5 IU/ml). Group I animals treated with tissue culture anti-rabies vaccine in schedule I ("Essen" schedule) protected all animals supported by the findings of Ramanna *et al.* (1991b) that post-exposure therapy with tissue culture rabies vaccine at the appropriate time will augment the immune response to resist the infection. In their study, "Essen" schedule was found effective to protect animals exposed to rabid animal. According to Wilson and Clark (2001) post-exposure therapy in goats using rabies vaccine in "Essen" schedule was effective. Mitmoonpitak *et al.* (2002) proposed "Essen" schedule to farm animals exposed to

rabid dog. Cho and Lawson (1989) demonstrated the efficacy of rabies vaccine by simulating post-exposure treatment in dogs according to the schedule ("Essen" schedule) recommended by the WHO for human use.

Though group I and group II animals in schedule I elicited protective antibody level during the period of study, only group II treated with DNA combined tissue culture anti-rabies vaccine showed protective levels on seven day itself. This proved the earlier seroconversion efficiency of DNA combined tissue culture anti-rabies vaccine under schedule II. Group III treated with tissue culture anti-rabies vaccine and group IV treated with DNA combined tissue culture anti-rabies vaccine in schedule II elicited an earlier higher protection level supported the findings of Tizard (2000). This is also supported by the findings of Lodmell *et al.* (2002) that a lengthy rest period between vaccinations was not necessary to accelerate and augment the neutralizing antibody response.

Lodmell and Ewalt (2001) showed that long rest period between vaccinations was not necessary for the elevation of the antibody response. In their study, mice treated with DNA vaccine and human diploid cell vaccine on five consecutive days elicited neutralizing antibody on day seven post-exposure. Haviv *et al.* (1999) reported that increasing the first dose of rabies vaccine might result in earlier and higher neutralizing antibody titre. In his trial, in a severly-exposed patient, an additional dose of human diploid cell vaccine was administered about 20 hour after the first dose. Baer and Yager (1977) reported that numerous daily injections of vaccine are required to elicit an adequate antibody response.

5.4 OBSERVATION OF TREATED ANIMALS

All the vaccinated goats in the four groups were observed for a period of six months from the day of first vaccination (zero day) and found to be healthy without development of any signs suggestive of rabies. The result obtained in the present study correlates with the statement of Benisek *et al.* (2000) who opined that in cattle the rate of immunity onset after vaccination and preservation of sufficient levels of specific antibodies for a minimum period of six month is important. According to Selvakumar and John (1989), the ultimate proof of the efficacy of the

vaccine lies in its ability to prevent clinical disease, assessed by post treatment survival of the persons exposed to rabies virus.

According to Quiambao *et al.* (2000) in post-exposure treated patients, a survival assessment of six month is sufficient to establish the efficacy of rabies vaccine. This agrees with the observed result in all the vaccinated group which remained protective up to the six month study period and the vaccine used and schedule are protective for the post-exposure treatment in goats.

Basheer *et al.* (1997a) observed the post-exposure vaccinated animals for the period of five months to evaluate the efficacy of different vaccines and different post-exposure schedule. This correlates with the present result obtained from the observation of study animals for the period of six month.

While evaluating the efficacy and cost effectiveness of vaccine realistically, resultant complication also should be considered. Adverse effects were not observed in any of the goats that were vaccinated. No serious untoward local or systemic reactions were noticed. Ramanna *et al.* (1991a) reported that tissue culture vaccine did not produce untoward reactions in the vaccinated dogs and more safe, as they do not have significant nonspecific proteins. The vaccine was equally efficacious in pregnant animals and was safe with no untoward effects either on the dam or on the fetus. Varner *et al.* (1982) opined that pregnancy is not considered a contraindication to post-exposure prophylaxis.

CONCLUSION

Sixty goats immunized with inactivated tissue culture anti-rabies vaccine and DNA combined tissue culture anti-rabies vaccine in two different schedules were followed up for 90 days for the immune response and persistence of anti-rabies antibodies in the sera. No vaccine failure was observed among the treated goats, as all the goats had protective titres of $\geq$ 0.5 IU/ml until 90^{th} day of post-exposure therapy in all the four groups. Analysis of the result indicated that both inactivated tissue culture anti-rabies vaccine and DNA combined tissue culture anti-rabies vaccine were effective in inducing the protective titre up to day 90^{th} day of

observation. It was also observed that though both vaccines when administered as per schedule I ("Essen" schedule) produced better response throughout the study period, both groups under schedule II also induced better response from day seven to day 14 post-exposure and declined from 28th day onwards, though remaining well above protective titre till 90th day of observation.

It is concluded that both vaccines and schedule were protective. It was observed that both vaccines under schedule II produced good protection on seventh day, which is very significant as far as post-exposure therapy is concerned. As it is conducted continuously for five days, there is less chance of missing and making the vaccination schedule more user friendly. Out of the two vaccines used, DNA combined tissue culture anti-rabies vaccine induced higher geometric mean rabies virus neutralizing antibody titre from seventh day onwards in both schedule, though there was a drastic fall in titre on subsequent days, though the titre was maintained above the protection level. Hence DNA combined tissue culture anti-rabies vaccine in schedule I and schedule II or inactivated tissue culture anti-rabies vaccine in schedule II were observed as the best for post-exposure anti-rabies therapy for goats.

Summary

6. SUMMARY

Sixty goats exposed to rabid animal from Thrissur district were used in this study. These goats were randomly divided into four groups and two different anti-rabies vaccines were tried in two different schedules. Group I goats were treated with inactivated tissue culture anti-rabies vaccine and group II goats were treated with DNA combined tissue culture anti- rabies vaccine as per the WHO recommendations for the human post-exposure therapy using "Essen" schedule ie, on days '0', '3', '7', '14', and '28'. Group III animals were treated with inactivated tissue culture anti-rabies vaccine and group IV animals with DNA combined tissue culture anti-rabies vaccine in five consecutive days ie, on days '0', '1', '2', '3', and '4' - schedule derived from the preliminary studies in the department of Veterinary Epidemiology and Preventive medicine. The blood from all the animals were collected on 0, 7^{th}, 14^{th}, 28^{th}, 60^{th}, and 90^{th} of vaccination and rabies neutralizing antibody was titrated using rapid fluorescent focus inhibition test (RFFIT).

All animals in the treatment groups had no rabies virus neutralizing antibodies before treatment. Group I animals treated with inactivated tissue culture anti-rabies vaccine in schedule I reached peak titre on 28^{th} day of vaccination and protective titre in this group was observed on 14^{th} day (2.56 IU/ml). The titre gradually decreased on subsequent days of observaton. However, the animals maintained the protective level up to 90^{th} day of observation (0.56 IU/ml).

Group II animals treated with DNA combined tissue culture anti-rabies vaccine in schedule I produced protective titre (1.19 IU/ml) on day seven and induced higher anamnestic antibody response on day 14 (6.37 IU/ml). This group reached peak titer on 28^{th} day vaccination and maintained the protective titre during the entire study period.

Group III animals treated with inactivated tissue culture anti-rabies vaccine in schedule II produced maximum response on 14^{th} day of post vaccination and

maintained above protective level (0.63 IU/ml) up to 90th day of study. The protective titre was observed on day seventh (1.19 IU/ml).

Group IV animals treated with DNA combined tissue culture anti-rabies vaccine in schedule II reached protective titre on day seven (1.49 IU/ml) and produced peak titre on 14th day post vaccination (3.61 IU/ml). These animals maintained the protective titre up to 90th day of sampling.

Group II animals treated with DNA combined tissue culture anti-rabies vaccine showed higher anamnestic antibody response (6.44 IU/ml) and longer duration of higher antibody response than group I animals. There was significant difference in the geometric mean rabies virus neutralizing antibody titre between group I and group III animals during the first month of study period. Group III animals responded well to the booster injections and had reached higher levels on 14th day of post vaccination (6.68 IU/ml) whereas group I showed peak titre only on 28th day of vaccination (2.56 IU/ml). Group IV animals showed higher response (1.49 IU/ml) on day seven than group I animals, but the level was not found to be maintained on subsequent days of observation. The geometric mean rabies virus neutralizing antibody titre of group II and group III were comparable in all sampling days. However, the duration of higher antibody response was longer for group II animals. Group II and group IV showed significant difference in the antibody response, though both groups were treated with the same vaccine. Both had different schedule of vaccination. Group II with proven "Essen" schedule showed higher response (6.37 IU/ml) than group IV with 0-1-2-3-4 schedule (3.61IU/ml) on 14th day. Group III and group IV animals did not show significant difference in the early induction of rabies virus neutralizing antibody titer. However, on subsequent sampling days, i.e., 14th and 28th day, group III animals responded well.

Inactivated tissue culture anti-rabies vaccine in five daily injections responded well than the proven "Essen" schedule. The result makes it evident that five daily doses of tissue culture rabies vaccine can offer adequate protection against rabies, eliciting a response higher than 0.5 IU/ml. As it is conducted continuously for five days, there is less chance of missing and subsequent immunity

breakdown. The proven capability to induce early and high titred rabies neutralizing antibody response with a peak on day 14, the persistence of antibody, and the anamnestic booster response supported the value of DNA combined tissue culture anti- rabies vaccine in schedule I and schedule II for rabies post-exposure prophylaxis in goat.

No adverse reactions noticed in any of the vaccinated group. Decrease of the antibody titre more than 3 month after vaccination indicates that immunity is not maintained long enough. Pregnancy is not a contraindication to rabies post-exposure therapy in animals. All sixty goats with rabies exposure were alive 180 days after treatment, resulted in higher clinical vaccine efficacy.

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ASSESSMENT OF POST-EXPOSURE ANTI-RABIES THERAPY IN GOATS

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ABSTRACT

The effectiveness of post-exposure therapy against rabies in goats, with two different vaccines viz., inactivated tissue culture anti-rabies vaccine (Raksharab®) and DNA combined tissue culture anti-rabies vaccine (Dinarab®), was studied with different schedule of vaccination, in Thrissur, Kerala. The rabies virus neutralizing antibody titres were assessed by using rapid fluorescent focus inhibition test (RFFIT). Sixty unvaccinated goats exposed to rabid animal bite were selected for study. Group I animals were given inactivated tissue culture anti-rabies vaccine and group II animals were given DNA combined tissue culture anti-rabies vaccine in schedule I ("Essen"). Group III and group IV were treated with inactivated tissue culture anti-rabies vaccine and DNA combined tissue culture anti-rabies vaccine in schedule II whose five injections were given on 0, 1, 2, 3 and 4^{th} day. By day 14, all animals in-group I had achieved rabies antibody titre above the protective level recommended by WHO ($\geq$0.5 IU/ml) and reached peak titre on day 28. Group II animals that received DNA combined tissue culture anti-rabies vaccine elicited protective titre on day seven and reached peak titre on day 28. All the goats in group I and group II were maintaining the protective titre till 90^{th} day and none had developed rabies for a period of 180 days. Though both vaccines in schedule I ("Essen") produced geometric mean rabies virus neutralizing antibody titre above 0.5 IU/ml up to 90^{th} day of study period, DNA combined tissue culture anti-rabies vaccine induced earlier and higher virus neutralizing antibody titre. Both vaccines under schedule II produced good protection on seventh day. Inactivated tissue culture anti-rabies vaccine under schedule II elicited earlier and higher neutralizing antibody response than schedule I. Out of two vaccines used, DNA combined tissue culture anti-rabies vaccine induced higher geometric mean rabies virus neutralizing antibody titre in a rapid manner in both schedule. Animals were monitored monthly for six month after exposure and all treated groups were alive and no vaccine related serious adverse events occurred. It is concluded that DNA combined tissue culture anti-rabies vaccine in schedule I and schedule II or inactivated tissue culture anti-rabies vaccine in schedule II were observed as the best for recommending to the field for post-exposure anti-rabies therapy in goats.