SPECIAL ARTICLE

FACTORS INFLUENCING THE PERFORMANCE OF ANTI RABIES VACCINES IN DOGS

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Abstract

India is considered to be endemic for rabies with dogs playing a major role as vectors in it's transmission to human beings and other animals with special reference to the livestock. Thus, to control rabies in dogs, vaccination is the most practical approach. Cell culture based inactivated rabies vaccines are being used to vaccinate the dogs and the anti-rabies vaccinal efficacy is determined by Rapid fluorescent focus inhibition (RFFIT)/ Fluorescent antibody virus neutralization (FAVN) test. A titer of 0.5 IU/ ml of the serum or greater is considered to be sufficient for conferring protection whereas, titre below 0.5 IU/ml is considered as insufficient, leaving the dog less likely to be protected from rabies. A plethora of factors have been implicated with vaccination failure *viz*., quality of vaccine, age of animal, duration after vaccination, breed and gender of dogs, health status, nutrition and management, endo parasitism, neutering status of dogs, genetic constitution, status of booster vaccination, storage of vaccines and stress. For a successful rabies vaccination all these factors need to be considered along with frequent monitoring for presence of vaccinal antibodies.

Key words: Factors affecting rabies vaccination, Vaccination, Immune response, Rabies

Introduction

Rabies is a well-known zoonoses that continues to pose global public health challenges. It is an acute viral encephalitis in humans and other mammals caused by Lyssa virus of the family *Rhabdoviridae*, and is nearly always fatal[1]. The annual number of human deaths caused by rabies is estimated to be 55,000 worldwide with about 32000 in Asia[2]. Prevalence of rabies is particularly high in India[3] and stray dogs play an important role as reservoir and transmitters of disease to humans and domestic animals[4].Despite the availability of various brands of vaccines in India, the disease prevails. There is a need for a much more coordinated vaccination programmes and it's implementation in a larger scale covering wider area.

After they have been vaccinated against rabies, dogs are protected primarily through the generation of rabies virus-specific neutralizing antibodies[5].Establishment of pre-exposure immunity will protect individual animals from contracting rabies and thus preventing further spread to humans or other domestic animals. However, such vaccinal immunity is influenced by various factors. In this review, the factors that could affect the post-vaccinal immune response in dogs are discussed.

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Saga of development of anti rabies vaccines

Vaccination is the only method to prevent rabies and Louis Pasteur pioneered this approach in 1885 using desiccated spinal cords derived from rabies-infected rabbits. Subsequent vaccines were also derived from neural tissues from a variety of animal sources and were effective and affordable throughout the world. Later, the Semple's anti rabies vaccine was developed by David Semple in India in 1911[6]. World Health Organization (WHO) has recommended discontinuation of this vaccine from 1993, because of high content of myelin basic proteins[7]. Alternatives to this approach included inactivation of chick embryo propagated rabies virus (RABV)[8] or inactivation of suckling mouse brain propagated RABV that has a lower level of myelin compared to the adult brain[9].

A new paradigm for rabies vaccines in general followed the development of cell culture for virus propagation. The first tissue culture vaccine was derived from virus grown in primary hamster kidney cells[10,11], followed by growth of fixed RABV in a human diploid cell(HDC) line[12]. An alternative to HDCV was the use of purified chick embryo cells. These vaccines are now used successfully worldwide. Vos and others[13] conducted safety studies on SAD B19, an attenuated oral rabies vaccine and concluded that it can be used for oral vaccination campaigns. Preparation of rabies vaccine using Vero cells was standardized for the purpose of large scale production and thus comparatively an economical rabies vaccine.

Despite the undoubted success of current commercial vaccines against rabies, there have been numerous attempts to develop alternatives, based on the genetic manipulation. The key target for antibodies is virus glycoprotein, which is a surface-exposed protein. A number of antigenic sites to which neutralizing monoclonal antibodies bind have been identified on this protein[14,15].Recently, new live attenuated Rabies virus vaccine candidates have been designed through reverse genetics approach by employing gene mutations [16], duplication or triplication of the G gene of rabies virus[17,18]. Some such attempts have been reported to be promising as these candidates have been found to be non pathogenic and immunogenic in animal models. However, one of the limitations of such DNA vaccines is the need of multiple immunizations using high doses of the DNA to achieve sufficient immunity. In addition to such multiple administration of vaccines resulting in immunotolerance, need of adjuvants to improve the efficacy may be a safety concern[19].Although various recombinant rabies vaccines have been developed using several viral vector systems [20,21,22,23], their efficacy, safety and utility for both pre and post exposure vaccinations have to be well established.

Factors Affecting Vaccinal Performance

After vaccination against rabies,to assess whether a dog has adequate immunological protection,the standard method of testing is estimation of the presence of virus neutralizing antibodies (VNA) by the Fluorescent Antibody Virus Neutralization test (FAVN) / Rapid Fluorescent Focus Inhibition Test (RFFIT) at officially recognized laboratories[24]. A serum titre of 0.5 IU/ml and above of rabies virus-specific antibodies is considered adequate protection against rabies. A titre below this level is considered as vaccination failure, leaving the dog less likely to be protected from the rabies virus[25].Such vaccination failures could be attributed to the following factors;

1.Age: Age is one of the important factors that influence immunological responses to vaccines, especially in the extreme ages of life. In general, young puppies and very old dogs do not show a sound immune response to vaccination unlike adult dogs. As per a study by Mansfield *et al.,* dogs less than six months and cats more than 14 years of age had lower antibody titres[26].The pre-vaccination rabies titre was higher in old dogs than in young dogs [27]. This could be attributed to more number of boosters / annual vaccinations in old dogs than the young ones. Where as young puppies are at greater risk of not attaining protective antibody titers after their first anti rabies vaccination probably due to the neutralization of vaccine virus by the maternally derived antibodies. However, this risk can be minimized by administering a second vaccination and blood sampling to assess the neutralizing antibody titre [28].

2.Duration after vaccination: Window period is the time between the vaccination and the appearance of detectable antibodies to the virus. In case of rabies, the window period of serum sample collection, in general has been recommended to be 20-50 days with an average of 28 days after vaccination. After one year of vaccination with booster, only 54.7% of the dogs maintained protective antibody levels against rabies [29]. Non-adjuvant recombinant rabies vaccines have been reported to induce excellent antibody responses in previously vaccinated dogs 14 days after administration [30].

3.Breed: In a study by Kennedy *et al.,* breeds of dogs that are small in size elicited higher antibody levels than larger breeds. Another observation was that the magnitude of response immediately following vaccination and duration of immunity varied between breeds of dog [31.].This is because genetic variations across breeds are large, whereas within breed, variation is much more limited. However Jakel *et. al.,*[28] could not find any differences in antibody response between breeds.

4.Management: Aghomo *et al.,* reported that puppies from non-vaccinated female dogs responded well to vaccine after 4th week of age, showing progressive increase in virus neutralizing antibodies as measured by RFFIT. But puppies from vaccinated female dogs responded only at 10th week of age although maternal antibody levels had decreased by 6th week of age [32]. The importance of the interval between vaccination and antibody testing was demonstrated by Cliquet *et. al.*,[24] and Mansfield *et. al*.,[26], showing that the risk of test failure significantly increased when dogs were tested beyond six weeks after vaccination. Primo-vaccinated pets had significantly lower rabies antibodies than dogs vaccinated twice or more, and a rapid decrease of rabies antibodies was seen in primo vaccinated dogs[24]. The choice of the vaccine and the timing of blood tests are critical factors in achieving successful serological test results after rabies vaccination[33].Regular vaccination, exercise, companionship, neutering and annual booster of vaccination favoured long duration of immunity in the dog [34].

5.Gender: In a study by Rife et al., castrated mice responded to antigenic stimulation than uncastrated mice and had twice the number of T lymphocytes [35]. It is suggested that testosterone may affect the immune system through enhancement of suppressive activity in testosterone injected animals. Due to late thymus involution, immune system in females works longer and effectively against parasitic and infectious diseases [36]. Mansfield *et al*., reported that neutered animals responded better by maintaining a protective antibody titer to anti rabies vaccination than unneutered animals [26].In contrast to this, Kennedy et.al.[31]demonstrated that gender of the dog does not have any effect on immune response to anti rabies vaccination.

6. Genetic factors: One strong genetic factor known to influence immune response to vaccination is the Major Histocompatibility Complex (MHC). It has been previously identified that dog leukocyte antigen (DLA) polymorphism is related to both autoimmune and infectious disease susceptibility [31].

7.Nutritional status: Nutritional status of a dog constitutes an important factor in the outcome of vaccination as malnourished animals may be immune suppressed. Cell-mediated and non specific immunity are more sensitive to nutritional deficiency than humoral immunity. Van loveren *et. al.,*[37] indicated that nutritional status as well as individual nutrients in food can affect vaccination titers.

8.Stress: Stress is known to suppress the normal immune response probably because of increased steroid production and is reported in many studies [37]. In general, stress in case of vaccinated animals has been reported to be responsible for certain extent of vaccination failure.

9.Parasitism: The infestation with ecto and endoparasites has been attributed as a factor responsible for vaccination failure. In puppies suffering from immune suppression due to heavy parasitism and not treated with anthelmintic, significantly lower specific antibody levels after anti rabies vaccination was demonstrated (Mojzisova *et. al*.[38]).

10.Multiple vaccinations: Boosting the immune system at a regular interval could result in better immune response. A study by Cliquet *et al*., revealed that Primo-vaccinated pets had significantly lower rabies antibodies than dogs vaccinated twice or more, and a rapid decrease of rabies antibodies was seen in primo vaccinated dogs [24].In a study by Hirayama *et. al*., where rabies vaccine was administered without the booster dose, the titers declined in

120 days, therefore 40% of the animals did not have protective titers [39]. Whereas, with booster, the drop of serum antibody titers (<0.5 IU/mL) in dogs occurred after 180 days [29]. Administration of the first vaccine followed by a booster vaccine after 3-4 weeks as a part of the primary immunization schedule for rabies could result in a better vaccination response. This schedule is practiced by most of the practitioners. Similar observations have been made by Hirayama *et al.,* 1990 [39].

11.Storage Condition of Vaccine: Generally, proper storage of anti rabies vaccine in the cold chain is recommended. This factor appears to be most influencing on the post anti rabies vaccinal antibody levels. A study in dogs receiving properly stored and improperly stored vaccine resulted in slight differences in antibody response [40]. Interestingly in yet another study carried out by Washington-led research team determined rabies vaccines stored at warmer temperatures still were found protective against the rabies.[41] This study provided the preliminary robust data that the antibody response of dogs vaccinated with Nobivac® Rabies vaccine stored for several months at high temperatures (up to 30 °C) was not inferior to that of dogs vaccinated with vaccine stored under recommended cold-chain conditions (2–8 °C). A controlled and randomized non-inferiority study was carried out comparing the four-week post vaccination serological responses of Tanzanian village dogs inoculated with vaccine which had been stored at elevated temperatures for different periods of time with those of dogs vaccinated with the same product stored according to label recommendations. Specifically, the neutralizing antibody response following the use of vaccine which had been stored for up to six months at 25 °C or for three months at 30 °C was not inferior to that following the use of cold-chain stored vaccine. These findings provide reassurance that the vaccine was likely to remain efficacious even if exposed to elevated temperatures for limited periods of time and, under these circumstances, it can safely be used and not necessarily destroyed or discarded. The availability of thermo tolerant vaccines has been an important factor in the success of several disease control and elimination programs and could greatly increase the capacity of rabies vaccination campaigns to access hard to reach communities in Africa and Asia [41].

Conclusion

Rabies is fatal but can be prevented by vaccination which induces immunity against the disease. Pre-exposure and post exposure vaccinations are mainly employed in control of rabies where a protective antibody response is generated. Several factors influence the immune response after vaccination. So, for a successful vaccination in dogs against rabies, factors such as type of vaccine, booster vaccinations, the breed, age at vaccination, and number of days after vaccination when the antibody titers are tested, nutritional and health status should be considered. A successful rabies vaccination needs booster at regular interval and frequent monitoring for neutralizing anti-rabies vaccinal antibody titre to ascertain protective levels. Further field based studies are necessary for checking the thermo tolerance of inactivated anti rabies vaccines.

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