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Antibody response of dogs to ethiorab rabies vaccine

Abstract

Rabies is 100% fatal, but it is preventable. More than 95% of human rabies cases occur in improperly treated individuals. This is partly due to the fact that modern post-exposure rabies prophylaxis is expensive and therefore not readily available in many endemic regions. Nervous tissue vaccine has been in use for more than 100yrs. These vaccines have now been superseded in purity, potency, immunogenicity and safety. The efficacy and immunogenicity of inactivated tissue culture vaccine, produced in Ethiopia was evaluated. Twelve experimental dogs from local breed were duly conditioned during a quarantine period and assigned to two groups randomly. Animals in group I (cases) were vaccinated subcutaneously with 1 ml of our experimental vaccine. Dogs in group II served as non-vaccinated controls. The immune response of each dog was monitored for 120days. On the day 120 after final sampling, all dogs were challenged in the masseter muscle with a rabies street virus of canine origin. To evaluate the titer of the rabies virus neutralizing antibodies (VNA), sera were analyzed by Fluorescent Antibody Virus Neutralization (FAVN) Test. Geometric Mean Titers (GMT) to rabies virus was determined at days 7, 15, 21, 30, 60, 90 and 120. Geometric mean titers were equal to 1.59, 1.73, 2.19, 3.58, 3.17, 3.35 and 3.56IU/ml respectively. All dogs showed VNA titers higher than the 0.5IU/ml mandated WHO recommended threshold. 83.3% vaccinated dogs, survived the challenge virus. In contrast, all dogs in the control (non-vaccinated group), developed rabies and died. This study indicated cell culture-based anti-rabies vaccine manufactured in Ethiopia is efficacious and immunogenic.

Keywords: challenge, efficacy, ethiorab, GMT, immunogenicity

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Introduction

Rabies is a fatal disease. It is caused by RNA virus classified under the family *Rhabdoviridae* and genus *Lyssavirus*, which includes 14 genotypes.[1] Although canine rabies was successfully eliminated in the most developed countries, it is still widespread in over 80 developing countries.[2]

Vaccination of dogs against rabies effectively controls this zoonosis and prevents its transmission to humans.[3] RABV is highly immunogenic, and efficient inactivated virus vaccines for humans and live-attenuated vaccines for wildlife have enabled control of rabies in developed countries. Still, however, rabies encephalitis causes tens of thousands of human deaths each year in developing countries.[4,5]

Rabies in Ethiopia is believed to result in a significant loss of human lives annually; however, comprehensive surveillance data are lacking. Use of nerve tissue vaccine remains widespread in Ethiopia, contributing about 1000 DALYs per annum. Algeria still produces nerve tissue vaccines, but the situation in some other countries in North Africa and in the horn of Africa is unknown. In the last ten years, a minimum of 6,263 and a maximum of 36,162 doses of the human rabies vaccine were produced and distributed every year in Ethiopia.

Anti-rabies vaccines produced from original Pasteur virus strain and its derivative strains like Pasteur Virus, Challenge Virus Standard, Pitman-Moore, etc., and strains isolated more recently (Flury, Street-Alabama-Dufferin (SAD), Vnukovo and Kelev), protect against all strains of phylogroup 1. Classical anti-rabies vaccines may give inadequate cross-protection against other lyssaviruses, especially in phylogroup II; there is no evidence for immune response to protect from Mokola virus[6] and the West Caucasian Bat Virus.[7] Cross neutralization using conventional rabies virus vaccines has been demonstrated against two phylogroup 1 viruses: EBLV type-1 and EBLV type-2.[8]

Imported vaccines produced by cell culture techniques are very expensive and unaffordable for majority of Ethiopians. In developing world, the limitation of budgets to produce tissue culture vaccine and the commitment of government are obstacles to replace nervous tissue vaccine. Currently the Ethiopian government has heavily invested in upgrading the facilities required to produce cell culture based anti-rabies vaccine fulfilling the minimum WHO requirements.[9]

Rabies antibody response (greater than or equal to 0.5IU/ml serum) is quite enough to protect the development of the disease. The correlate of protection, as demonstrated by the effectiveness of post-vaccination challenge and numerous experimental studies, correlates with the presence of virus neutralizing antibody.[10] Although there have been a small number of cases of survivors following infection with rabies.[11] The vast majority of humans, who develop rabies, die as a consequence of infection. In Ethiopia most people believe in traditional healers. The individuals who are exposed to rabies virus often see traditional healers for the diagnosis and treatment of the disease. This practice is the barrier in getting on time post exposure prophylaxis.[12]

The anti-rabies vaccine used in this study was produced by multiplication of Pasteur Virus (PV) using Vero cell line. It was inactivated with formalin and formulated as liquid form.

Objective

The main objective of this study is to evaluate the efficacy and immunogenicity of the rabies vaccine produced in the Ethiopian Public Health Institute using Vero cells with the Pasteur rabbit fixed rabies (PV).

Materials and methods

Dogs

Twelve dogs of local breed, 4-5months old, were used in this study. Before vaccinated with our experimental vaccine all dogs were de-parasitized, de-wormed, and vaccinated against the major canine infectious diseases (DHPPiL, CANVAC).

Vaccine

The experimental vaccine is produced by multiplication of PV rabies strain on Vero cell line grown on roller bottle. Purification of the virus is done by filtration through 0.2µm filter and it is inactivated by formalin (1:4000) ratio. The vaccine contains 1IU/ml in a single dose.

Challenge virus preparation

The rabies virus was obtained locally from rabid dog in Addis Ababa, Lafto sub-city. The isolated virus was continuously passaged on the mice brain until the titer of the virus reached the recommended challenge virus titer; i.e. 10-6.

Experimental design

The experimental design for this experiment is Random Clinical Trial (RCT). Dogs were randomly divided into two groups of 6 dogs each. Dogs assigned to the two groups, were bled at day zero (pre-vaccination). Dogs assigned to group I were vaccinated via the subcutaneous route with 1 IU/ml of Ethiorab vaccine. Dogs in group II served as non-vaccinated controls. Dogs in Group I were bled at days 7, 15, 21, 30, 60, 90 and 120 post-vaccination to determine rabies neutralizing antibody titers. Dogs were inoculated with challenge virus intramuscularly in mandibular area from both sides on the 120th day and observed for 45days.

Serum sample collection

About four milliliters of blood from dogs were drawn on days 0 (pre-vaccination sample), 7, 15, 21, 30, 60, 90 and 120 by a veterinarian using plain vacutainer tubes. Vacutainer tubes were labeled immediately prior to blood sample collection. The collected blood samples were left at room temperature for 30minutes to two hours for clotting and then centrifuged at 1500rpm for 10minutes to separate the sera. The serum samples were coded, and kept frozen at minus 20°C throughout the study period.

Challenge of dogs

Dogs were inoculated with rabies virus clinical isolate intramuscularly in mandibular area from both sides on day 120. All animals, vaccinates and controls, were challenged at the same time, i.e. a control, followed by a vaccinated group. A separate needle and a separate syringe were used for each dog. Challenged dogs, retained in isolation, were observed for 45days before euthanasia, and FAT of their brains.

Statistical analysis

Microsoft Excel Version 2007 was used to determine the GMT values on day 0, 7, 15, 21, 30, 60, 90 and 120. Median lethal dose and the effective dose of challenge virus were calculated by Spearman-Kärber formula.[13]

Ethical clearance

The study was performed after obtaining Ethical Clearance from Ethiopian Public Health Institute, Scientific and Ethical Review Committee.

Results

Antibody responses

On day zero (pre-vaccination), none of the 12 dogs used in this experiment were sero-positive (VNA titer: <0.5IU/ml), indicating that they had never been in contact with rabies virus. All experimental dogs assigned to the respective group responded to Ethiorab rabies vaccine and developed rabies Virus Neutralization Antibody. 1.59IU/ml GMT of VNA was recorded on the seventh day after test vaccine administration. Fifteen days after the vaccination of the vaccine the rabies antibodies response showed an increase of mean titer of VNA, the mean titer was 1.73IU/ml. At day 21 post-vaccination, all vaccinated animals except one dog showed titer increase (Table 1).

Table 1 Geometric Mean Titer antibody response to rabies vaccine

Days Post-Vaccination	7	15	21	30	60	90	120
Geometric Mean titer (IU/ml)	1.59	1.73	2.19	3.58	3.17	3.35	3.56

At day30, all dogs showed a tremendous increase virus neutralization antibody response to experimental rabies vaccine with a GMT of 3.58IU/ml and this result is the highest antibody titer response to the test vaccine. Even though antibody response started to decline at day 60, all experimental dogs showed an increment on day 90th.

Virus challenge

All vaccinated and control dogs were challenged with 1 ml (100 LD50) of the locally isolated rabies virus. The first control dog started to show rabies symptoms on day 15th after challenging and died on the same day. Two control dogs were died after showing rabies symptom on day 18th after challenging. Again one dog from the control group died on 19th day. The other dog from the control group died on 30thday. One dog from the control group didn't show any type of rabies symptom. The brain of this dog was removed on day 45 and FAT was done. All control group except that survived the challenge virus showed one or more of the typical rabies symptom (hypersensitive to touch, paralysis of the throat, jaw muscles and legs, hide in dark places, disorientation, in coordination and staggering, weakness, seizures and sudden death). The brains of the dead dogs (control group) were removed and FAT was done to confirm that their death was due to infection by rabies virus. Accordingly, all dead dogs were confirmed rabies virus positive (FAT positive). One of vaccinated dog was dead of rabies after challenged with challenge virus within 45 days follow up period.

This data is in line to those reported by[14,15] in which all dogs challenged with rabies street virus of canine origin, survived in the group of vaccinated dogs and 80% of control dogs succumbed to the virus challenge. The finding of this study demonstrates the immunogenicity and protectivity of candidate vaccine. All dogs received rabies vaccine showed high antibody titer after challenge. The results confirm that rabies vaccine can protect against local rabies in dogs. The survival of the vaccinated dogs after challenged with rabies virus clinical isolate showed as the immunogenicity and efficacy of the vaccine. Figure 1 shows rabies neutralizing antibody after challenged with 100LD50 rabies virus clinical isolate.

The viral challenge boosted the antibody response in five dogs, vaccinated dogs further increasing the antibody response of rabies vaccine to protect dogs if they were exposed to a natural challenge.

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These results is in agreement with those reported by[15,16] who showed that virus challenge of dogs boosted the immunity of the inoculated dog after vaccine regardless of the absolute titer at the time of exposure.

Discussion

Curing rabies is a challenging task as no effective treatment is yet available. Therefore, vaccination has irreplaceable role. The development of adequate level of antibody (greater than or equal to 0.5IU/ml) is necessary for protection against the disease. Tissue culture anti-rabies vaccines are extremely potent, immunogenic and safe compared to nerve tissue vaccines.

In this study, all dogs vaccinated with PV tissue culture vaccine develop virus neutralizing antibody. Five dogs out of six i.e. 83.3% vaccinated with the above vaccine survived the challenge virus. One of the six dogs which died following challenge was sero-positive. The failure of immunity to vaccinated dogs may be due to a heavy challenge overwhelming host defense. As reported by Sikes[17] elsewhere, groups of dogs with high percentage of sero-conversion will have the highest probability of surviving challenge. In this study, as many others, presence of virus neutralizing antibodies to rabies at a time of challenge did not indicate protection for all of the animals. Similarly, absence of neutralizing antibodies in serum at the time of challenge did not mean the animals were unprotected. However, there was strong statistical ($P<0.1$) that animals with neutralizing antibodies at the time of challenge were better protected than those with no detectable neutralizing antibodies. Studies done by Aubert[18] also support correlation between neutralizing antibodies and protection against lethal challenge.

All vaccinated dogs survived when they were challenged with a street rabies virus of canine origin. In contrast, 83.3% of the non-vaccinated dogs succumbed to the challenge virus of canine origin. This result demonstrates the ability of experimental vaccine to effectively protect dogs against a challenge virus of a wild origin, as it would happen in field conditions.[19]

This experiment showed that the test vaccine used in this experiment is able induces the virus neutralizing antibody titer higher than 0.5IU/ml in all dogs used in the experiment. In addition, despite the decline of VNA on day 60 post vaccination, still it's by far greater than the WHO minimum acceptability.

Moreover, the survival of vaccinated dogs after inoculation with challenge virus of canine origin indicates that the vaccine is efficacious and efficiently acceptable for vaccinations against rabies in dogs. From this result, it can be concluded that this vaccine can be used for mass vaccination of animals after licensing.

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Conflicts of interest

Author declares there are no conflicts of interest.

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