EFFECT OF VARIOUS FORMULATIONS OF PESTE DES PETITS RUMINANTS VIRUS VACCINES ON THE DURATION OF IMMUNITY IN GOATS

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ABSTRACT: *Pesti des Petits Ruminants (PPR) is an acute highly contagious viral disease of small ruminants that cause heavy economic losses especially to the goat industry and is endemic in Pakistan. Mass scale vaccination and clamping strict bio-security measures are the only ways to control the disease. The present study was aimed to evaluate PPR virus (PPRV) vaccine with variable biological titer as well as role of adjuvant to induce protective immune response in beetal goats. Effect of boosting on the humoral immune response of the animals was also evaluated. PPRV vaccine with a biological titer of $10^{5.00}$ $TCID_{50}$ per dose provoked maximum antibody titer followed by the ones with a titer of $10^{4.00}$ or $10^{3.00}TCID_{50}$ which provoked nearly equivalent protective immune response while the animals inoculated with a vaccine having $10^{2.00}$ $TCID_{50}$ virus concentrations developed minimum antibody titer. The oil adjuvant PPRV vaccines elicited significantly higher immune response while gel based vaccines induced relatively less antibody titer but however minimum antibody titers were detectable in response to freeze dried vaccines. Although protective antibody level (≥ 10 neutralizing antibody units) was detectable in the animals vaccinated with either oil based, gel based or freeze dried vaccine containing biological titer of $10^{4.00}$ $TCID_{50}$ but however the extent and duration of immunity was found to be most superior in response to oil based vaccines. There was a significant difference in the antibody response of animals who received a booster dose of the vaccine in comparison to the ones who received single dose.*

INTRODUCTION

Livestock plays very important role in the Pakistan's economy as it adds about 11.5 % to the GDP of the country and more than 55.1 % of the Agriculture through value addition. Nearly 30-50 million people living in villages of Pakistan rear livestock like sheep and goat as additional occupation and 5-6 sheep/goats are owned by a single family. Pakistan has a total of 28.4 million sheep and 63.1 million goat population [1].Peste des petits ruminants (PPR) is an infectious and contagious viral disease of sheep and goats characterized by high fever, erosive lesions in the mouth, catarrhal inflammation in the ocular and nasal mucosa, conjunctivitis, pneumonia and gastroenteritis[2]. In the infected herds, about 100 % morbidity and 90 % mortality has been recorded [3] which might reaches up to 100 % in severe epidemics [4].Keeping in view of fatal nature and economic importance of the disease Office International des Epizooties (OIE) has categorized PPR as notifiable list-A disease [5]. Although there exists only one serotype of PPR virus (PPRV) but virulence varies among the strains isolated from various regions around the globe[6]. Occasionally PPR has been reported to be severe in goats than sheep but sometimes both species were proved to be equally susceptible [7, 8].

PPR is endemic in various parts of Africa and Asia including subcontinent. The disease was first time recognized in Pakistan in 1991 following an epidemic in the Punjab provinces but the laboratory based confirmation and initial characterization of the virus was performed in 1994[9]. Since then the disease is endemic in Pakistan [10]. A number of times the disease has been confirmed from the clinical specimen obtained from the effected herds placed at various geographical regions of the country. In some of the outbreaks the disease has been confirmed through the isolation or detection of the virus in the clinical specimen. But however, most of the reports are based upon detection of antibodies in the serum samples collected from the suspected herds.

The disease can be effectively controlled by large scale vaccination of the susceptible herds. Initially a killed virus vaccine was employed for the control of disease [11] which was replaced by an attenuated PPRV vaccine [12]. In Pakistan, homologous PPRV vaccines are being produced using Nigeria 75/I strain of the virus [13]. It is a routine practice to administer single PPRV vaccine shot per year to the animals in the country. Limited information is available about the efficacy of such vaccines in the indigenous breeds of goats. Most of the published reports are based upon testing the immune response in the inoculated animals for 3-4 months duration. The present study focused to test the extent and duration of immunity in the goats vaccinated with either single or booster dose of live attenuated, gel based and oil adjuvant PPRV vaccines.

MATERIALS AND METHODS

Virus and Cell culture

PPRV strain Nigeria 75/1 used in the present study wasobtained from the Veterinary Research Institute Lahore Cantt, Lahore. Vero cells were used as a host system for the cultivation of PPRV. The cells were procured from the culture bank of the Department of Microbiology and propagated in vented T-75 cell culture flasks. The cells were fed with growth medium: Dulbecco`s modified Eagle Medium (DMEM) containing 10 % fetal calf serum (FCS)

and grown at 37 °C and 5 % CO_2. In order to propagate the virus, 20 ml of the freshly cultured cells adjusted to final count of 4×10^5 cells per ml were added in the flask and simultaneously infected with the virus at the multiplicity of infection (MOI) value of 0.001 $TCID_{50}$ per cell. Flasks were incubated at 37 °C in a CO_2 incubator adjusted to 5 % gas level and examined under inverted microscope every 24 hours to check cell monolayer and detect any cytopathic effects (CPE). Virus harvesting was performed at successive intervals initially when CPE were 40-50 % evident and finally following the destruction of complete cell monolayer. Harvested virus suspension was pooled as a single batch and centrifuged at 5000 *xg* for 15 minutes at 4 °C.Virus quantitation as mean tissue culture infective $dose_{50}$ ($TCID_{50}$) was calculated by the Spearman-Kärber method [14]. PPRV as a cell culture supernatant was stored in 15 ml aliquots at -80 °C till further uses.

Vaccine preparation

Live PPRV vaccines containing immunogen levels of $10^{5.00}$, $10^{4.00}$, $10^{3.00}$, and $10^{2.00}$ $TCID_{50}$/ml were prepared by diluting the freshly harvested virus in the sterile phosphate buffered saline to achieve the required virus concentration. To prepare gel based vaccine, sterile aluminium hydroxide gel was addedat a final concentration of 10 % in the virus suspension having a titer of 10^4 $TCID_{50}$ per ml. Oil based vaccine was prepared using montanide oil ISA-70 in a ratio of 70:30 (0.70ml adjuvant and 0.30ml virus per dose) as per manufacturer's instructions. The dose of antigen was adjusted to have a final virus concentration of 10^4 $TCID_{50}$ per ml of the vaccine. The oil and virus suspension was properly homogenized after adding thiomersal sodium in a final concentration of 0.01%. All of the vaccines were tested for their sterility by culturing on tryptone soya agar plates.

Effect of immunogen level and adjuvant on the duration of immunity

Live PPRV vaccines containing immunogen levels of $10^{5.00}$, $10^{4.00}$, $10^{3.00}$, and $10^{2.00}$ $TCID_{50}$/ml were each inoculated at dose rate of 1 ml per animal to a group of five beetle goats. The serum samples were collected from the goats at 3, 6, 9, and 12 months intervals post vaccination. Similarly, each of the wet,gel based and oil adjuvant PPRV vaccines containing virus concentration of $10^{4.00}TCID_{50}$/dose were inoculated to a group of five goats. All of the animals were bled at 3, 6, 9, and 12 months post vaccination and serum samples were separated. All of the serum samples were stored at -20 °C till further processing.

Effect of boosting on the antibody response of the goats

To test the possible effect of boosting on the antibody response of goats all of the three PPRV vaccines (wet, aluminium hydroxide gel based and oil adjuvant) were inoculated to a group of 5 animals. A booster dose of the vaccine was injected after 30 days of priming. Serum samples were collected from the animals at 3, 6, 9, and 12 months post priming and stored at -20 °C till further processing.

Antibody level in all of the serum samples against PPRV were detected through serum neutralization test (SNT). Frozen sera were heated at 56 °C for 30 minutes and filtered through 0.20 µm pore size syringe filters before processing. SNT was performed by constant virus diluted serum method according to the procedure as described before [15].The results were subjected to statistical analysis by one way ANOVA and t-test using SPSS software for windows, Rel.13.00 (IBM corporation, Chicago, USA).

RESULTS AND DISCUSSION

Live PPRV vaccines having higher immunogen level (10^5 $TCID_{50}$) induced maximum antibody response (Figure-1) in comparison to the vaccines containing relatively lower immunogen level (10^4, 10^3 and 10^2TCID_{50}). Similar observations were recorded in past [16, 17] where immune response of the goats to freeze dried PPRV vaccineswas in linear relation with the virus concentration in the vaccines. Such immunogen dependent antibody response of the host has also been recorded in other animal species like buffalo and poultry in response to numerous vaccines [18, 19, 20].Present study describes that goats inoculated with PPRV vaccine having 10^3TCID_{50} or higher virus concentration developed reasonable immune response with a mean neutralizing antibody (MNA) of more than 4.00 (log_2) that persisted for a period of one year. A previous study described that antibody level of more than 16 neutralizing units against PPRV in the goats was supposed to be protective against clinical disease[21]. That observation in conjunction with the findings of present study indicates that live PPRV vaccines with a minimum virus concentration of10^3TCID_{50} can provide protective immunity for up to one year duration in the inoculated animals. Several other researchers also evaluated the efficacy of PPRV vaccines by testing the immune response in the inoculated goats. Most of these reports tested the immune response for shorter time duration of up to 6 months [22]which indicates that $10^{3.00}$ $TCID_{50}$ virus titer in the vaccine is quite enough to induce reasonable antibody level in the animals. Immune response of animals against PPRV vaccines was measured for several months through cELISA and showed that animals had detectable antibodies for 18 months duration [23, 24] but however, none of them detected neutralizing antibodies which is most appropriate indication of measuring protective immunity in the animals.

Animals inoculated with adjuvanted PPRV vaccines displayed relatively superior immune response than the ones inoculated with non-adjuvanted vaccines (Figure 2). Gel based PPRV vaccine showed significantly higher anti PPRV MNA titer than that of the wet vaccine over entire study period of one year ($p<0.5$). However, oil based PPRV vaccine proved superior to all of the other vaccines. Similarly, oil based FMD virus vaccine induced higher antibody titer in sheep as compared to the gel based or non-adjuvantFMD virus vaccines[19]. Moreover, oil based vaccinesinducehigh antibody titers and for longer durations than the vaccines containing other adjuvants or without adjuvants[25, 26, 27].

Priming of the goats with PPRV vaccine (wet, gel based and oil adjuvant) induced humoral immune response. Such humoral response was the result of cytokine activated

PPRVimmunogen specific plasma cells. The cytokines were released from the immunogen stimulated CD4+ Th cells [28]. PPRV primed goats when received the booster dose of the respective vaccine showed higher antibody response than the primed goats after 30 days post boosting (Figure 3). This

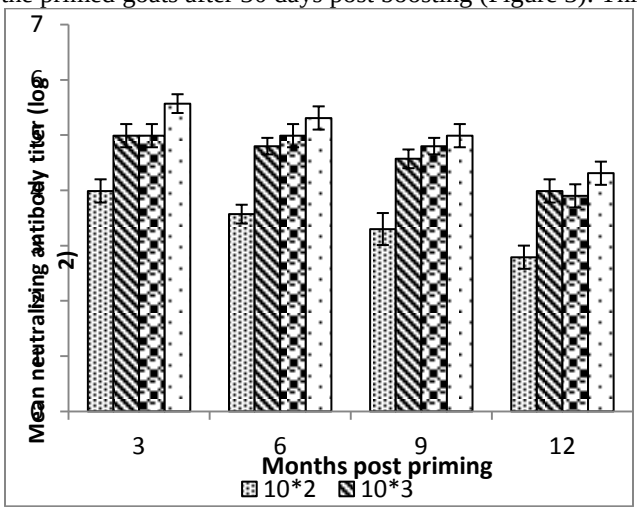

Figure 1: Effect of PPR virus immunogen in its freeze dried vaccine on duration of antibody (Anti-PPRV-SN Ab) titer in goats

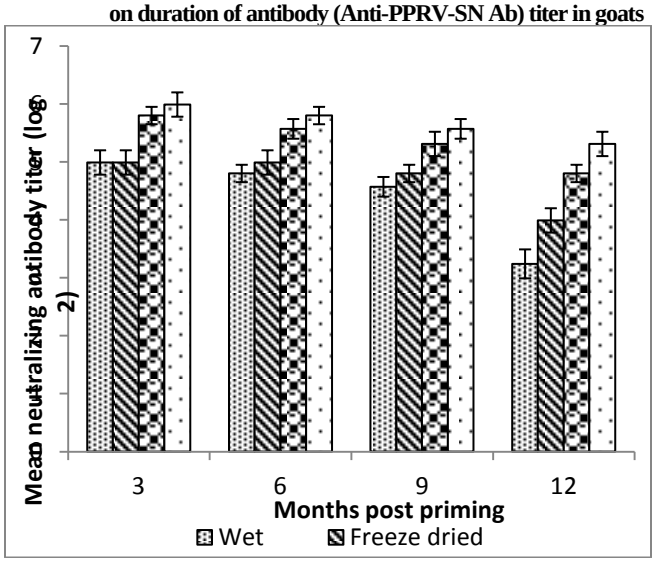

Figure 2:Effect of adjuvant in PPR virus vaccine on duration of anti-PPR serum neutralizing antibody response of goats

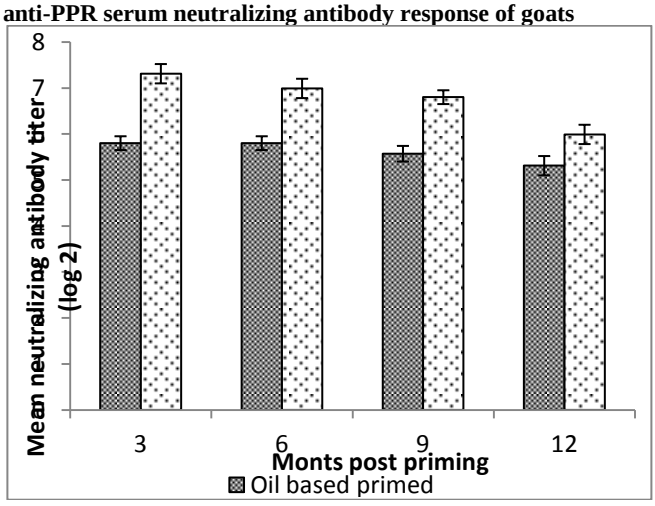

Figure 3: Effect of boosting with oil based PPR virus vaccine on duration of antibody response of the goats primed with the same vaccine

booster antibody response persisted significantly higher for 12 months ($p>0.05$). PPRV vaccines in the primed goats produce memory immunocytes of B cell or T helper cell lineage that may persist over long period of time and are responsible for an enhanced immune response following the administration of second dose of the vaccine [21, 29]. A number of other studies also favors the observation that booster dose of various kinds of vaccinescan induce stronger and long lasting immune response in the inoculated animals. It is evident from MNA response of the goats that a single dose of adjuvantPPRV vaccine is enough to induce reasonable antibody level for up to one year duration while a booster dose of the vaccine is needed for non-adjuvantPPRV vaccine.

CONCLUSIONS

In order to develop a protective immune response, goats must be inoculated with PPRV vaccine having 10^3 $TCID_{50}$ or higher virus concentration. Adjuvant containing vaccines induce better immune response in the goats than the ones without adjuvant. A single dose of adjuvant containing vaccine can induce protective antibody level for up to one year duration while a booster dose of the non-adjuvant vaccine is needed for such response.

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