The Journal of Animal & Plant Sciences, 24(3): 2014, Page: 749-754
ISSN: 1018-7081

IN PROCESS QUALITY CONTROL FACTORS EFFECTING POTENCY OF PESTE DES PETITS RUMINANTS VIRUS VACCINES IN GOATS

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ABSTRACT

Pesti des Petits Ruminants (PPR) is an acute contagious viral disease of small ruminants and is causing heavy economic losses to goat industry. It is controlled by mass vaccination and bio-security measures. In present study, *in process quality control factors* such as variable biological titer, different adjuvants and shelf life of the PPR vaccines affecting antibody response of the goats were evaluated. The freeze dried 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 antibody response while the goats inoculated with a vaccine having $10^{2.00}$ $TCID_{50}$ virus concentrations developed minimum antibody titer. The oil based PPR virus vaccines elicited significantly higher antibody response ($p<0.05$) while gel based vaccines induced relatively less antibody titer but freeze dried PPR vaccine induced minimum antibody titers. Each of the adjuvant vaccines (oil or gel) and non- adjuvant vaccine containing biological titer of $10^{4.00}$ $TCID_{50}$ induced more than 50 Percent Inhibition (PI) values (protective antibody titer). The freeze dried vaccine stored at 4 °C did not show any significant drop in the biological activity of the virus even after 12 months of the storage. Moreover, both the freeze dried and adjuvant vaccines when stored for 12 months at 4 °C did not show any effect on their antibody response in the vaccinated goats. It is concluded that oil adjuvant vaccine with $10^{4.00}$ $TCID_{50}$ units of biological titer of virus induced the highest antibody titer in goats that persisted for more than 10 months post priming.

Keywords: PPR vaccine, goats, adjuvant, antibody response.

INTRODUCTION

Peste des petits ruminants (PPR) is an infectious viral disease of small ruminants. It is characterized by high fever, erosive lesions in the oral cavity, and catarrhal inflammation in the ocular and nasal mucosa, conjunctivitis, pneumonia and gastroenteritis (Munir *et al.,* 2013). There is only one serotype of the PPR virus but virulence varies among strains of the different regions. The disease is thought to be more severe in goats than sheep but both species are equally susceptible (Taylor *et al.,* 2002; Wang *et al.,* 2009). Nucleotide sequence of the virus and genetic makeup of the host species are important factors for severity of the disease (Couacy-Hymann *et al.,* 2007). The disease is endemic in parts of Asia and Africa, and has been prevailing in Pakistan since 1991. The causative agent of the ailment in several districts of the Punjab province has been confirmed through RT-PCR (Amjad *et al.,* 1996; Ayaz *et al.,* 1997; Ahmad *et al.,* 2005; Khan *et al.,* 2009). Chemically inactivated PPR virus infected lymph nodes and spleen homogenates induce protective immunity against the challenge infection (Braide, 1981). Due to antigenic cross reactivity between PPR virus and rinderpest virus, tissue culture based attenuated rinderpest vaccine was used to immunize the animals against PPR. Later on PPR virus (Nigeria 75/1 strain) was attenuated after 63 passages on the Vero cells (Diallo *et al.,* 1989). This vaccine induces solid immunity in vaccinated goats against PPR strains of all lineages and proved safe in protecting animals against the field challenge infection (Couacy-Hymann *et al.,* 1995).

The disease is causing heavy economic losses to the small ruminant livestock industry and induces sever consternation to the poor farmers in Pakistan. Mass vaccination is the only option to control the disease in the endemic areas. Present study is designed to improve its potency through adjusting optimum level of the virus immunogen and suitable adjuvant. Shelf life of the vaccine was also evaluated.

MATERIALS AND METHODS

Source of Vero cell line and PPR virus: Vero cells used for the propagation of virus were procured from the culture bank of the Department of Microbiology. PPR virus (Nigeria 75/1 strain) as a freeze dried vaccine was obtained from Veterinary Research Institute Lahore Cantt, Lahore. The cells were grown in T-175 cell culture flasks and fed with growth medium: Dulbecco`s modified Eagle Medium (DMEM) containing 10 % fetal calf serum (FCS) (Freshney, 2011). For the virus propagation, 50 ml of the freshly sub-cultured cells adjusted to final count of 4×10^5 cells per ml were poured in the flask. The vaccine

virus was reconstituted in the medium and used to infect the cells at the multiplicity of infection (MOI) value of 0.001 $TCID_{50}$ per cell. The virus infected flasks were incubated at 37 °C and 5 % CO_2, examined under inverted microscope after every 24 hours to check cell monolayer and detect any cytopathogenic effect (CPE). Harvesting of the virus was initially performed when CPE were 40-50 % evident in the cell monolayer. The cell culture supernatant containing virus was collected in a sterile bottle and stored at -80° C. The remaining cells were once again fed with 50 ml of the maintenance medium (DMEM supplemented with 2 % FCS) and incubated at 37 °C and 5 % CO_2 for another 48 hours till 80 % CPE were detected. The flasks were subjected to 2 freeze–thaw cycles for the cell lysis and recovered high virus infectivity titer. All of the harvested virus suspension was pooled as a single batch and centrifuged at 5000 Xg for 15 minutes at 4 °C. The clear supernatant was collected and virus infectivity was calculated as mean tissue culture infective $dose_{50}$ ($TCID_{50}$) (Villegas, 1998). The harvested virus was stored at -80 °C till use in subsequent experiments.

Vaccine preparation: Live PPR virus (PPRV) vaccines were prepared by diluting the virus suspension in sterile PBS to achieve $10^{5.00}$, $10^{4.00}$, $10^{3.00}$, and $10^{2.00}$ $TCID_{50}$/ml of the virus concentration. To prepare gel based vaccine sterile aluminium hydroxide gel was added at a final concentration of 10 % in the PPR virus suspension with a titer of 10^4 $TCID_{50}$ per ml. Oil based vaccine was prepared using montanide oil ISA-70 in a ratio of 60:40 (60 % adjuvant and 40 % antigen) as per manufacturer's instructions. The dose of the virus immunogen 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. Thiomersal sodium was added in the adjuvant containing vaccine in the final concentration of 0.01%. All of the vaccines were tested for their sterility by culturing on tryptose soya agar plates.

Experimental design: Live PPRV vaccines containing immunogen levels of $10^{5.00}$, $10^{4.00}$, $10^{3.00}$, and $10^{2.00}$ $TCID_{50}$/ml were inoculated to the group of five beetle goats. Similarly, each of the wet (fresh cell culture harvest), freeze dried, gel based and oil adjuvant PPRV vaccines were inoculated to a group of five goats. All of the animals were bled at the beginning of the trial and afterwards at one month interval for a total of four months. The serum samples were separated and stored at -20°C till further processing. Antibody titers were measured through competitive ELISA (cELISA) using commercial diagnostic kit (IDVET Innovative diagnostics®, Montpellier, France) based upon PPR virus nucleoproteins coated in the plates (Libeau *et al.,* 1995)

In order to determine their shelf life, all of the three PPRV vaccines (live freeze dried, gel based and oil adjuvant) were stored at 4 °C for a period of one year. Each of the vaccine was inoculated into a group of five goats at 0, 4, 8, and 12 months post storage. The serum samples were collected from the goats at 0, 14, 28, and 42 days post vaccination and processed for the antibody detection through cELISA.

Statistical analysis: The results were subjected to statistical analysis by one way ANOVA using SPSS software for windows, Rel.13.00 (IBM corporation, Chicago, USA).

RESULTS AND DISCUSSION

PPR is a viral disease of goat and sheep. It is controlled through mass vaccination program. Antibody response of goats to PPRV vaccines was measured through cELISA. The test was selected as it is the only recommended immunological method by OIE to test sero-conversion of the immunized animals against PPRV vaccines. Historically, AGID and other serological techniques were used to test the antibodies against PPR in the past but however, cELISA and serum neutralization test (SNT) are preferred methods as having advantage to differentiate between antibodies against PPR and rinderpest (another member of the genus *Morbillivirus* and the antibodies against which usually interfere with the anti PPRV antibodies) (Libeau *et al.,* 1995; OIE 2008). Although sensitivity and specificity of SNT is relatively higher than the cELISA but the later one is easy to perform under limited resources without the availability of cell culture facilities.

The freeze dried PPRV vaccine containing 10^5 units of $TCID_{50}$ induced maximum antibody titer in goats (Figure 1). The titer was significantly higher than that induced by the vaccine containing either 10^4or 10^3 units of the infectivity titer ($p<0.5$). The vaccine containing 10^2 units of $TCID_{50}$ induced minimum anti- PPRV ELISA antibody titer. It is clear from the results that antibody response of the goat is directly proportional to the amount of immunogen or infectivity titer in the vaccine. The goats show immunogen level dependent immune response to PPRV vaccine (Matrencher *et al.,* 1991 and Rashwan *et al.,* 2012). Other animal species such as buffalo and poultry also show immunogen dependent antibody response (Noreen, 2012; Khan *et al.,* 2011). Antibody response of buffalo calves to foot and mouth disease (FMD) virus is directly proportional to amount of the immunogen in montanide based trivalent FMD virus vaccine (Tariq, 2007; Akram, 2012; Altaf *et al.,* 2012). Minimum $10^{2.50}$ $TCID_{50}$ units biological titer of PPRV is a standard amount of the immunogen per dose of the freeze dried vaccine which induces protective immunity in the vaccinated animals (Diallo *et al.,* 1989; Khan *et al.,* 2009; Rashwan *et al.,* 2012).

Adjuvant such as montanide oil, aluminium hydroxide gel and others are commonly used in veterinary vaccines (Aucouturier *et al.*, 2001). Antibody response of goats to montanide ISA-70 PPRV vaccine was significantly higher than that of the gel based, freeze dried, and wet PPRV vaccines ($p>0.5$). Similarly antibody response of the goats to gel based vaccine was significantly higher than that of freeze dried vaccine (Figure 2). Montanide oil based FMD virus ("Asia 1" and "O") vaccine induced higher antibody response in sheep as compared to the gel based or non-adjuvanted FMD virus vaccine (Altaf *et al.*, 2012). Oil based adjuvant such as montanide ISA-70 increases immunogenic potential by inducing inflammation at the injection site, recruitment of antigen presenting cells, protect the immunogen from tissue degradation and increasing retention time of the immunogen (Dupuis *et al.*, 2006; Roestenberg *et al.*, 2008). In contrast to this, aluminium hydroxide gel retained antigen for short period of time. The antibody response to gel based vaccines starts rapidly and decline thereafter earlier. Moreover, gel directly activates monocytes to produce cytokines that activate T cells. Cytokines of the activated Th cells increase the expression of MHC class II molecules on antigen presenting cells (APCs). The increase in the expression of antigen-presenting and costimulatory molecules leads to enhanced accessory functions of monocytes. These properties of the gel may be reponsible for higher antibody response in the vaccinated animals as compared to that of live virus vaccines (Ulanova *et al.*, 2001).

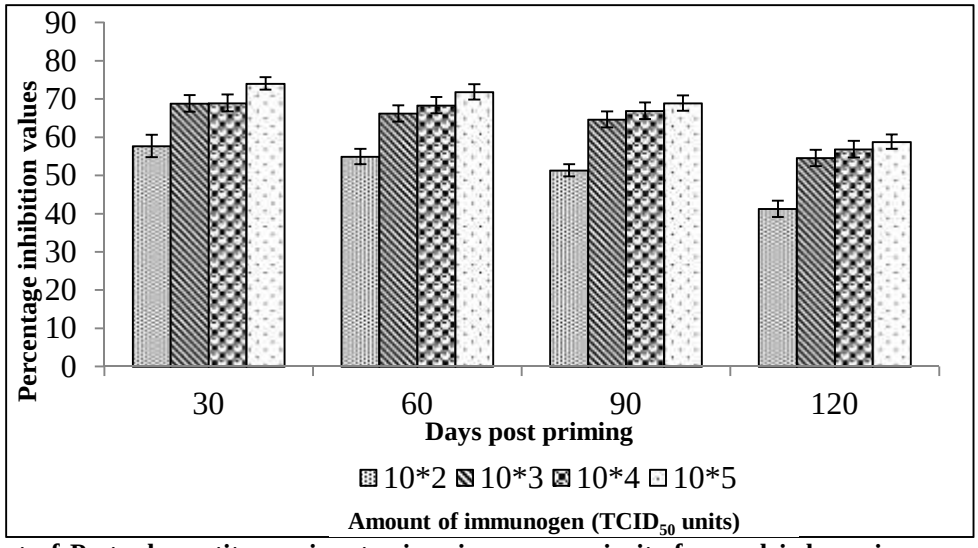

Figure 1. Effect of Peste des petits ruminants virus immunogen in its freeze dried vaccine on antibody (Anti-PPRV-cELISA Ab) response of goats.

cELISA: competitive enzyme linked immunosorbent assay, PI: Percentage inhibition, * Tissue culture infective dose 50,

Infectivity titer of the freeze dried PPRV vaccine remained unaffected following storage at low temperature (4°C) for 12 months. Some other reports also show that immunogen of PPRV vaccine remains stable for one year as indicated by consistent biological titer of the virus recovered from the vaccines after storage at low temperature (Rashwan *et al.*, 2000; OIE, 2012). Antigenicity of freshly prepared freeze dried, gel or oil based PPRV vaccine was not significantly different from the vaccines stored at low temperature for 12 months (Figure 3) which corroborates with the findings of Muhammad *et al.* (2011) and Akram (2012) who showed that aluminium gel, oil, and lanolin based FMD vaccine were antigenically stable for six months when stored at 4±2 °C. Immunogen of vaccines may be protein, lipo-polysaccharide or polysaccharide. These macromolecules degrade persistently. The rate of decay is enhanced by high temperature, sun light or exo-enzymes of microbial contaminants. Growth of the microbial containment in the vaccine during production process is controlled by adding antimicrobial agents such as thiomersal sodium before mixing with adjuvant (Birner and Garnet, 1964; Stone, 1985; Dorea, 2011). Low temperature for vaccine storage has minimal degrading effect on the immunogen. This is why the vaccines are stored or moved at low temperature from point of production to door step of end users (Turner *et al.*, 2011; D'Onise *et al.*, 2012; Shrivastava *et al.*, 2012).

It is concluded that each of the adjuvant vaccines (oil or gel) and non- adjuvant vaccine containing biological titer of $10^{4.00}$ $TCID_{50}$ induced more than 50% percent inhibition (PI) that is considered as protective antibody titer. The freeze dried vaccine kept at 4 °C did not show any significant drop in the biological activity of the virus even after 12 months of storage. Moreover both the freeze dried and adjuvant vaccines when stored for 12 months at 4 °C did not show any effect on their antibody

response in the vaccinated goats. Oil adjuvant vaccine with $10^{4.00}$ $TCID_{50}$ units of biological titer of virus induced the highest antibody titer in goats that persisted for more than 10 months post priming.

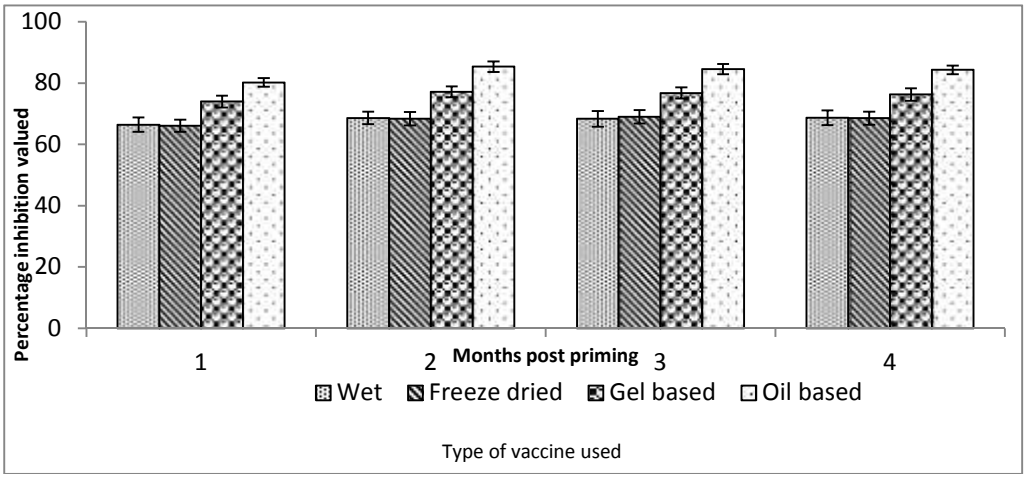

Figure 2. Effect of adjuvant on anti-PPR virus cELISA antibody response of goats

cELISA: competitive enzyme linked immunosorbent assay, PI: Percentage inhibition, * Tissue culture infective dose 50,

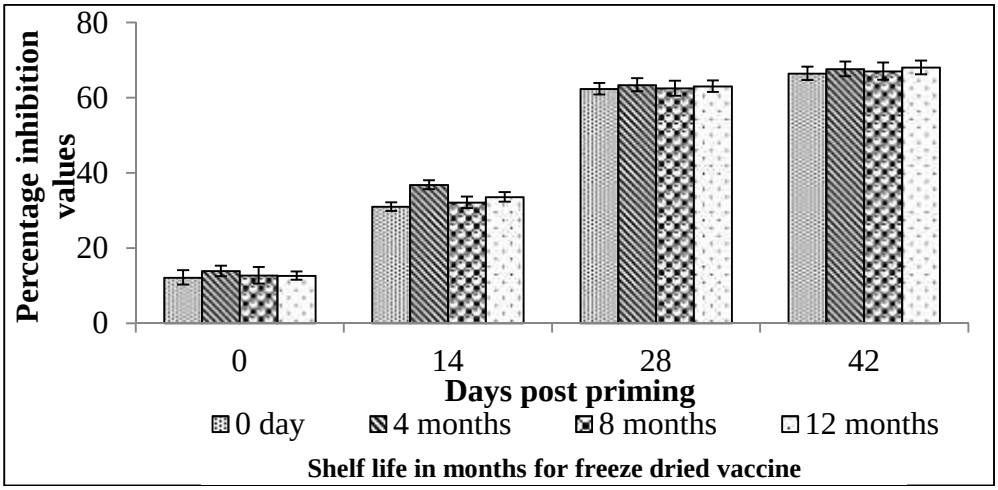

Figure 3: Effect of shelf life of freeze dried vaccine on anti-PPR virus cELISA antibody response of goats.

cELISA: competitive enzyme linked immunosorbent assay, PI: Percentage inhibition, * Tissue culture infective dose 50,

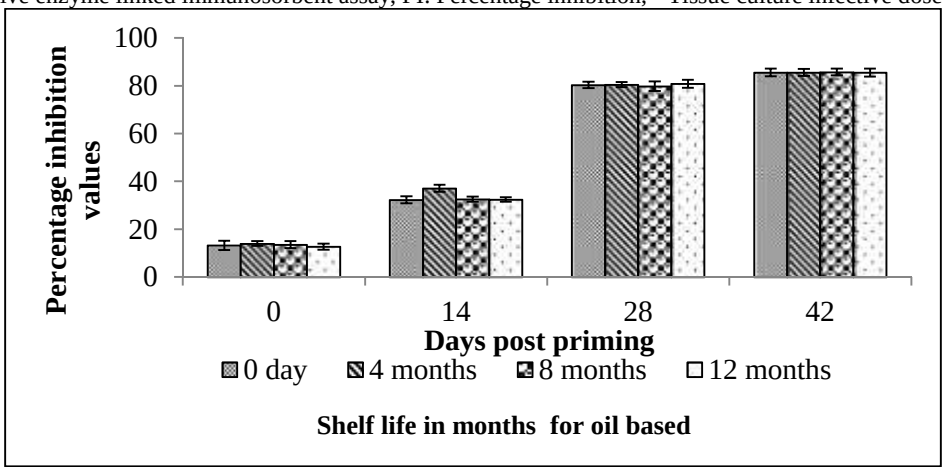

Figure 5. Effect of shelf life of oil based vaccine on anti-PPR virus neutralizing antibody response of goats.

cELISA: competitive enzyme linked immunosorbent assay, PI: Percentage inhibition, * Tissue culture infective dose 50,

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