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IMMUNE RESPONSE OF GOATS TO A VERO CELL ADAPTED LIVE ATTENUATED HOMOLOGOUS PPR VACCINE

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Peste des Petits Ruminants (PPR) is an acute, febrile and highly contagious viral disease of small ruminants. For controlling PPR, live attenuated vaccines and diagnostics are available to launch national PPR vaccination campaign. One such live attenuated Vero cell adapted PPR vaccine developed at Indian Veterinary Research Institute (IVRI), Mukteswar, is being used extensively in India. The objective of this study was to assess the duration of protective immune response in vaccinated goats and to recommend vaccine strategy for field use. This is the first report on long term immune response to PPR vaccine in goats.

Materials and Methods

Cells and viruses : Vero cells (ATCC CCL-81), live attenuated PPR vaccine (PPRV Sungri 1996 isolate) developed at IVRI, Mukteswar (Sreenivasa *et al.*, 2000) were used in this study.

Vaccination of goats : Four goats (two male and two female) of local hill breed of 10 month age, free from PPRV antibodies were vaccinated each with 1 ml of reconstituted (one field dose = 1000 $TCID_{50}$) PPR vaccine (P61) by s/c injection under cold chain as part of PPR vaccine testing at IVRI, Mukteswar. Two goats were kept as control. All the goats were observed during the post vaccination period and bled at 0d, 7d, 14d, 21d, 28d, 2m, 3m, 4m, 5m, 6m and then at quarterly intervals to collect serum samples till 6 and half years.

Preparation of virus for Serum Neutralisation test (SNT): Virus titer was quantified in Vero cells by estimating the 50% tissue culture infective doses (TCID50) in 96 well micro-titre plates as per the standard procedures (Burleson *et al.*, 1992). Briefly, the virus was 10 fold diluted in serum free Eagle's Minimum Essential Medium (EMEM) and 100µl of diluted virus was added to 100µl of cell suspension (10^6 cells/ml) per well and four replicates per virus dilution with cell control in micro-titre plates. The plates were incubated at 37^0C with 5% CO_2 and medium was changed on every 48 h with EMEM (2% FBS). The plates were checked for Cytopathic effect (CPE) daily from 3rd day onwards. The final reading was completed on day 8. The vaccine titres were calculated using Reed and Muench (1938) formula and stored in aliquots at -80^0C for use in SNT.

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Assessment of protective immune response in PPR vaccinated goats : The sera collected from vaccinated goats were heat inactivated and SNT was performed for assessing the neutralizing PPRV antibody titre as per the method described by Rossiter *et al.* (1985). The quantity of anti-PPRV antibody in the vaccinated goat serum samples was determined by either a monoclonal antibody (MAb) based PPR Competitive ELISA (C-ELISA) or polyclonal antibody based Indirect ELISA (I-ELISA) as per the procedure of Singh *et al.*, (2004) and Balamurugan *et al.*, (2007), respectively.

Results and Discussion

The PPR vaccine developed at IVRI Mukteswar as per the recommendations of OIE manual of standards for diagnostic tests and vaccines for PPR (OIE, 2000). The SN titre of $\geq$ 1:8 is considered the protective virus neutralizing titre which can withstand challenge of any field virus as per OIE (*loc. cit*).

All goats in this study were free from PPRV antibodies as their pre-vaccinate serum samples showed $\leq$ 1:2 titre in SNT and they also tested negative in PPR ELISA and I-ELISA. The immune response in terms of protective antibody titre was observed till six and half years in three goats (G41F, G52M and G50M) as one goat (G46F) died at 21 months due to a fall and lacerated injuries. The post-mortem report of the dead goat did not reveal any PPR lesions and none of the internal organs tested positive for PPR antigen in a monoclonal antibody based PPR Sandwich ELISA. Further, the goat (G46F) showed protective SN titre and was strongly positive for PPRV antibodies from 2nd week to 21 months. Two goats kept as control without vaccination for a period of one year showed SN titre of $\leq$ 1:2 indicating no protective levels of PPRV antibody in their sera.

Similarly, in PPR C-ELISA, the percentage inhibition (PI) in the first week was 26-38% (below the positive PI of 40%), in second week the PI has increased to 72-79% and from third week to six and half years the PI ranged from 81 to 100% suggesting strong positivity of the serum samples for PPR antibody as detected in C-ELISA. The correlation of C-ELISA titre was 1-3 $\log_2$ of SN titre (Singh *et al.*, 2004). The serum samples collected at quarterly intervals and tested in C-ELISA were in agreement with the SNT results in terms of PPRV antibody levels. In addition to C-ELISA, the serum samples were also tested using the I-ELISA and there was a steady increase in the antibody titre observed after 1st week in all the four goats, which crossed the Positive - Negative cut-off value 0.36 = (Mean (0.337) + 2xSD (0.013) in 2nd week post vaccination and maintained high antibody level above the cut off throughout the observation period in three goats.

The results from this study suggest that there is adequate protective antibody titre in all the goats tested. In India, the average life span of sheep and goats ranges from 8-10 and 10-15 years, respectively (Banerjee, 1992). The goats are normally reared by the farmers for not more than 3 to 4 years for meat purposes or 8 years for breeding. The goats and sheep are slaughtered for good quality meat when they are 1-2 years of age. Sheep are also kept for wool till 7-10 years, a longer period compared to goats. The PPR vaccine, that has provided immunity for six and half years would be sufficient to protect goats from PPR during their life span, indicating a single dose of PPR vaccine is sufficient without any booster dose. The cost of vaccine is

economical, thereby making the option of vaccination under Indian field conditions a technically viable and economically feasible preposition to the farmers to utilize this vaccine to protect their small ruminants.

Summary

The protective immune response of a Vero cell based live attenuated PPR vaccine was studied using SNT, C-ELISA and I-ELISA in goats (n=4) to ascertain its efficiency. All the three tests showed high levels of PPRV antibody in goats from 2nd week to six and half years after a single shot vaccination. The protective immune response was reflected by increase in the SN titre from the first week, which reached protective level (≥ 1:8 titre) in 2nd week and maintained upto six and half years in all vaccinated goats. It is clearly evident from this study that the PPR vaccine could possibly confer life time protection akin to tissue culture Rinderpest vaccine after a single shot immunization.

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