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PRODUCTION OF HOMOLOGOUS LIVE ATTENUATED CELL CULTURE VACCINE FOR THE CONTROL OF *PESTE DES PETITS RUMINANTS* IN SMALL RUMINANTS

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ABSTRACT

Antibody response of a live-attenuated *Peste des Petits Ruminants* (PPR) cell culture vaccine was studied at Veterinary Research Institute, Lahore, Pakistan. For this purpose, one group of five sheep and 5 goats each was vaccinated subcutaneously with 1 ml reconstituted PPR vaccine and second group of five sheep and 5 goats was inoculated with 1 ml saline solution. Blood samples were collected before and after vaccination, sera were obtained and analyzed for antibodies against PPR by competitive ELISA (cELISA). Findings suggested that antibody titres at day zero, 21 and 45 were 24.762 ± 2.69, 65.467 ± 2.29 and 83.012 ± 2.11 in sheep and 18.723 ± 2.27, 59.162 ± 1.53 and 72.176 ± 2.93 in goats, respectively. No untoward reactions were observed following vaccination. All vaccinated animals developed high titre of antibodies (PI>50).

Keywords: *Peste des Petits Ruminants*, cell culture, vaccine, live attenuated.

INTRODUCTION

Peste des Petits Ruminants (PPR) is a disease of major economic importance and imposes a significant constraint upon sheep and goats production owing to its high mortality rate. The disease is characterized by fever, necrotic-stomatitis, gastroenteritis and pneumonia (Khan *et al*., 2007). Infection rates in enzootic areas are generally high (above 50%) and can be upto 90% during an outbreak (Radostits *et al.*, 2007). Case fatality rates are also higher in goats (55-85%) than in sheep (less than 10%) (Abu-Elzein *et al.*, 1990). The disease appeared to localize mainly in west and central Africa (Scott, 1981), parts of Middle East and the Indian sub-continent (Taylor, 1984). The existence of PPR has been recognized in Pakistan since 1991, when it gave rise to an epidemic in Punjab province (Athar *et al.*, 1995). A recent study (Khan *et al*., 2007) revealed that the sero-prevalence of PPR virus in small ruminants in Punjab province of Pakistan was 43.33%.

After the ban on the use of rinderpest vaccine under global rinderpest eradication programme (GREP), there was an urgent need for a safe and efficacious vaccine to combat the menace of PPR disease in Pakistan. The objective of this study was to produce and evaluate a live attenuated cell culture vaccine for providing protection against PPR disease to small ruminants which are the species most susceptible to PPR virus.

MATERIALS AND METHODS

Cell line and virus

African green monkey kidney (Vero) cells were maintained in minimal essential medium supplemented with 10% foetal calf serum. PPR virus Nigeria 75/I (PPR 75-1 LK 6 Vero 75) was procured from Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD), France.

Preparation and lyophilization of PPR vaccine

PPR vaccine was produced in Vero cells by culture method in roller bottles. For this purpose, Vero cells were propagated into roller culture bottles at a concentration of $5x10^4$–10^5 cells/sq-cm. The fresh trypsined cells were infected in suspension in roller bottles with 10^{-3} $TCID_{50}$ multiplicity of infection (MOI) and incubated at 37°C in the roller apparatus.

Growth medium in the roller bottle was replaced with maintenance medium containing 2% foetal calf serum. The bottles were examined regularly to detect cytopathic effect (OIE, 2004).

For lyophilization, equal volume of vaccine and stabilizer (Weybridge medium) were mixed. One milliliter of the mixture was dispensed in sterilized 5ml capacity glass vial and freeze dried. The final product was subjected to the tests, as described earlier (OIE, 2004).

Virus titration

Three freeze-dried PPR vaccine vials were reconstituted separately and micro titration assay was carried out to check the median tissue culture infectivity dose ($TCID_{50}$) on 96 well microtitre plate (Mark *et al.*, 1994). Presence of PPR virus in the product was confirmed by the method described earlier (OIE, 2004).

Quality control tests

Pooled vaccine sample from randomly selected vials was inoculated into nutrient both, thioglycolate media and soybean casein digest medium. Safety test (OIE, 2004) was done in rodents in order to detect any

non specific toxicity associated with PPR vaccine. For this purpose, one vial of PPR cell culture vaccine (100 doses) was reconstituted in 100 ml saline solution. Two guinea pigs (weighing 300g each) were injected 0.5 ml of vaccine intramuscularly into the hind limb, while another two guinea pigs were given the same dose into peritoneal cavity. Similarly, six un-weaned mice (weighing 20g each) were injected 0.1 ml vaccine into the peritoneal cavity. Two guinea-pigs and four mice were kept as uninoculated controls. The animals were monitored for any kind of illness for 3 weeks.

Efficacy test in small ruminants

Ten adult Beetal goats and 10 adult Lohi sheep found negative for PPR virus antibodies by competitive enzyme linked inmunosorbent assay (cELISA) were selected. Five goats and five sheep were each inoculated subcutaneously with 1 ml of reconstituted live attenuated PPR cell culture vaccine. The remaining five goats and five sheep were inoculated with 1ml of saline solution as placebo. Blood samples were collected by jugular vain puncture from each animal before vaccination and at day 21 and 45 post vaccination. Sera were obtained and subjected to cELISA for calculation of optic density (OD) (Libeau *et al*., 1995). Percentage inhibition (PI) values in cELISA were generated using ELISA data interchange (EDI) software developed by IAEA (Jeggo and Anderson, 1992):

$$PI = 100 - \left(\frac{\text{OD of test sample}}{\text{OD of monoclonal control}} \times 100\right)$$

RESULTS AND DISCUSSION

Cells and virus used for the vaccine production fulfilled all the OIE and FAO standards. Working seed, freeze dried vaccine and all other ingredients used during the vaccine production were tested for sterility. Nutrient broth, thioglycolate medium and soybean casein digest medium inoculated with the vaccine were negative for any aerobic, anaerobic and fungal growth.

Harvesting of virus

Cells were found to be healthy till day 3 post inoculation. On fourth day, initiation of cytopathic effects was observed in the form of rounding of the cells which progressed gradually to aggregation of cells leading to formation of syncytia. Infected cells were first harvested when CPE was 60% on day six post inoculation, while second harvest was taken when more than 80% CPE was present.

Medium tissue culture infective dose ($TCID_{50}$) of the vaccine

The titre of the PPR freeze dried vaccine in Vero cells was $10^{5.2}$ $TCID_{50}$/ml, which fulfilled the requirement of OIE and FAO standards. The vacuum in the freeze dried vaccine vials was ranging from 98 to 99%.

PPRV identity test

Regarding to PPR virus identity test, CPE was present in the wells containing cells infected with the mixture of virus and cell culture medium, whereas cells in wells with the mixture of viral suspension and PPR antiserum remained compact and devoid of any evidence of CPE. This was according to OIE terrestrial manual (OIE, 2004).

Safety test in laboratory animals

Guinea pigs and mice inoculated with live attenuated PPR vaccine showed no adverse local or systemic reactions and remained healthy during the period of observation.

Safety and efficacy test in small ruminants

Animals remained healthy during the period of observation and no untoward effects were observed. Regarding the efficacy in vaccinated animals, antibody response to PPR vaccine increased from day 21 to 45. The antibody titres at day zero, 21 and 45 were 24.762 ± 2.69, 65.467 ± 2.29 and 83.012 ± 2.11 in vaccinated sheep and 18.723 ± 2.27, 59.162 ± 1.53 and 72.176 ± 2.93 in vaccinated goats, respectively (Fig. 1). These findings are in accordance to OIE terrestrial manual (OIE, 2004). In non-vaccinated animals, percentage inhibition (PI) value of sera remained lower than 50 (Fig. 1).

In conclusion, live attenuated PPR cell culture vaccine produced in this institute can be safely used to immunize small ruminants against PPR disease and thus huge economical losses can be minimized.

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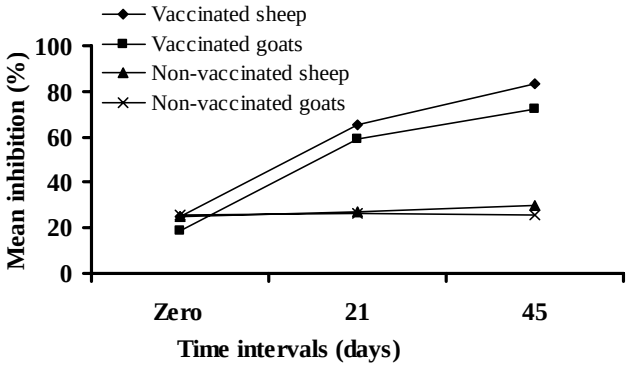

Fig. 1: Antibody titre in vaccinated and non vaccinated sheep and goats at different time intervals.

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