PRODUCTION OF TISSUE CULTURE BASED PESTE DES PETITS RUMINANTS (PPR) VACCINE


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ABSTRACT

Peste des petits ruminants (PPR) is an acute disease of small ruminants causes low productivity as well as great economic losses in many countries including Pakistan. There has been a pressing need to contain this disease through a vaccine in Pakistan. The objective of this study was to prepare an efficacious vaccine against PPR disease particularly in goats in Pakistan. Tissue culture based live freeze dried PPR virus (PPR 75-1) vaccine was produced using Vero cell line at the Center for Advance Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta in 2007. The vaccine was checked for the validation, safety, and sterility using the standard procedures set by Office International des Epizooties (OIE) terrestrial manual for vaccine production. In total 3.1 million doses have been produced from 2007 to 2010. The PPR vaccine of 0.0923 million dose have been given free of cost to Livestock and Dairy Development Department, Government of Balochistan, Pakistan for immuno-prophylaxis against PPR disease in goats in various districts of Balochistan. Moreover approximately 3 million doses were also distributed commercially to different organizations nationwide. It is anticipated that the PPR vaccine prepared at CASVAB would be an effective tool to limit PPR disease in goats in Pakistan as well as by reducing economic losses due to the disease.

Key words: Peste des petits ruminants (PPR), tissue culture, vaccine, safety, sterility, potency

INTRODUCTION

Pakistan is gifted with a large population of small ruminant including 58.3 and 27.4 million heads of goats and sheep respectively (GOP, 2010). Peste des petits ruminants (PPR) is an acute and contagious (Dhar et al., 2002; Asim et al., 2008 and 2009) viral disease of small ruminants characterized by fever, ocular nasal discharges, stomatitis, diarrhoea and pneumonia with foul offensive breath (Lefeuvre and Diallo, 1990). PPR virus (PPRV) is a Morbillivirus which belongs to the Paramyxoviridae family (Barrett et al., 2005). The natural disease mainly affects goats and sheep, but is usually reported more severe in goats where it inflicts heavy losses. The morbidity can be up to 100% while mortality rate up to 100% can be seen in severe cases. However, this may not exceed 50% during milder outbreaks (OIE, 2006). Clinical picture of PPR includes pyrexia up to 41°C, anorexia, dry muzzle, serous ocular nasal discharge which become progressively mucopurulent, respiratory distress, ulceration of mucous membranes and gastroenteritis (Dhar et al., 2002 and Ozkul et al., 2002). The disease was reported in Pakistan in 1994 (Amjad et al., 1994) and outbreaks of PPR were further confirmed in the country in 2002. (Hussain et al., 2003). PPR vaccine is not recommended to vaccinate animals under four months in endemic area because they might have colostral PPR antibodies which could neutralize the vaccinal virus. The antibodies produced as the result of PPR vaccine persist for at least three years (OIE, 2006).

Little work has so far been reported on the immuno-prophylaxis against PPR in Pakistan (Asim et al., 2008; Asim et al., 2009), while mass scale vaccine production of PPR is probably not reported from this part of the world. The aim of the present study was to prepare an efficacious PPR vaccine using PPR 75-1 strain of PPR virus.

MATERIALS AND METHODS

Master seed of PPR vaccine (PPR 75-1 LK6 Vero 74 batch 1-960823) with 74 successive passages on Vero (African Green Monkey Kidney) cell culture of PPR virus isolated in Nigeria in 1975 (Taylor and Abegunde, 1979) was obtained from CIRAD-emvt (Campus International de Baillarguet, UPR15, TA30/G, Campus International de Baillarguet, 34398 Montpellier cedex 5, France). The master seed was stored at -20°C until further use.

Vaccine production: Vero cells, free from all bacterial, viral and fungal contaminants were used for PPR vaccine production. Minimum Essential Medium, supplemented with antibiotic (Penicillin 100 units/ml, Streptomycin 100 microgram/ml and Mycostatin 50 microgram/ml) and foetal born calf serum (10%) was used for the growth and maintenance of vero cells. Vero cells were grown in the tissue culture flasks and incubated at 37°C for 72 hours to get complete monolayer of the cells.
Inoculation of cells with PPR virus: Tissue culture flasks with complete monolayer of vero cells were used for inoculation with the PPR virus. Freeze dried virus seed was reconstituted in 10ml of cell culture medium without serum, medium was discarded from the flasks prior to inoculation with PPR virus (10^3 TCID_{50}/ml). After even distribution of virus onto the cells, the flasks were incubated at 37°C for 30 minutes in order to allow viral attachment. Fresh tissue culture growth medium enriched with 5% serum was then added to each of the flasks and incubated at 37°C. The vero cells infected with PPR virus were regularly examined to detect any cytopathogenic effect (CPE). Growth medium was also regularly examined and replaced by fresh growth medium as and when required. Cells were incubated until 70-80% CPE was observed. All the flasks with desired CPE were freeze at -70°C. Random samples from the stored flasks having CPE were checked for any contaminants and titrated for PPR virus by Reed and Muench (1938) method.

Freeze drying: The virus harvested from all the flasks was pooled together and mixed with freeze drying medium (1:1 ratio). Freeze drying medium (pH 7.2) contains lactalbumin hydrolysate (2.5%), sucrose (5%) and sodium glutamate (1%). The final vaccine product was homogenized, freeze dried and stored at -20°C.

Validation of PPR vaccine: In order to confirm the presence of PPR virus in the final vaccine product, anti-PPR serum was used to neutralize the virus in the cell culture (OIE 2006). The anti-PPR serum was obtained from CIRAD-emvt (Campus International de Baillarguet, UPR15, TA30/G, Campus International de Baillarguet, 34398 Montpellier cedex 5, France).

Safety and sterility test: Randomly selected (5%) samples were checked for the safety and sterility. Safety in mice and guinea pigs was checked by injecting 0.5 ml PPR vaccine intramuscularly into hind limb of two guinea-pigs, 0.5 ml into the peritoneal cavity of two guinea-pigs, and 0.1 ml into the peritoneal cavity of six mice. Two guinea-pigs and four mice were kept as uninoculated controls. These animals were observed for 3 weeks. (OIE, 2006). Potency test for the PPR vaccine in small ruminants was not carried out due to the unavailability of wild (field) strain of PPR virus.

RESULTS AND DISCUSSION

Vero cells grown in standard conditions yielded complete monolayer of cells in tissue culture flasks in 72 hours. Vero cells were spindle shaped, appeared as single cells as well as patches of cells which later developed into complete monolayer. The infected vero cells with PPRV were observed with 70-80% CPE in 5 days. CPE was characteristic with rounding and aggregation of vero cells on 4th day whereas generalization of CPE was observed on 5th day post infection. Formation of syncytia is reported by Lefevre and Diallo (1990), Mohan (2004) and John et al. (2006) which was not seen in the infected vero cells in the present study. The present finding is further supported by the research documented in OIE (2006) stating that it is sometimes difficult to see the syncytia in vero cells infected with PPR virus, if they exist, they are very small. Titer of pooled PPR virus harvested was 10^5.5 tissue culture infective dose 50 (TCID_{50}) /ml in the present study which is in close agreement with the TCID_{50} of 5.83/ml titer for Sungri strain of PPR virus as reported by Raveendra et al. (2009). Conversely TCID_{50} of 7.37/ml titer for Arasur strain of PPR virus was also documented by the same research group. During 3 weeks of quality control observation, none of the mice and guinea pigs showed any harmful effects and was found in good health. All the laboratory animals killed at the end of 3 weeks for post-mortem examination were observed with no significant post-mortem lesions. Similar observation for the quality control of PPR vaccine was also reported for mice and guinea pigs (Asim et al., 2009). Challenge and protection study for PPR vaccine have been reported in sheep (Dhar et al., 2002; Ozkul et al., 2002) but in the present study sheep were not used for checking the potency of the vaccine due to the unavailability of wild (field) strain of PPR virus. The results of Virus neutralization test for the present PPR vaccine with anti-PPR serum using vero cells have shown no CPE even after 5 days of incubation. In the present study overall 3.1 million doses of PPR vaccine have been produced from 2007 to 2010. The major part of these doses has been distributed to the small ruminant livestock holders as well as Government sheep and goat farms of Balochistan. The PPR vaccine of 0.0923 million dose have been given free of cost to Livestock and Dairy Development Department, Government of Balochistan, Pakistan for immunoprophylaxis against PPR disease in goats in various districts of Balochistan. Moreover approximately 3 million doses were also distributed commercially to non-government organizations nationwide. So far the response from the user of this vaccine in Pakistan has been promising, and the frequency of the natural clinical cases of PPR diseases in goats seems to be declined. Further studies are needed to compare the present vaccine with the vaccine prepared from the locally isolated and attenuated PPR virus from the natural cases of PPR in goats in Pakistan.

Acknowledgement: The authors are thankful to Strengthening of Livestock Services in Pakistan (SLSP), Islamabad for catering training and provision of vaccinal strain of PPR virus from CIRAD, France.
REFERENCES


