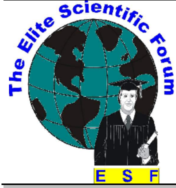

Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH NOTE

Production of Tissue Culture Based Peste Des Petits Ruminants (PPR) Vaccine at CASVAB, Quetta, Pakistan

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ARTICLE INFO
Received: Nov 24, 2011
Accepted: Feb 18, 2012
Online: April 12, 2012

Keywords
Peste des petits ruminants
Potency
Safety & Sterility
Tissue culture
Vaccine

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ABSTRACT

Peste des petits ruminant (PPR) is an acute disease of small ruminants which causes low productivity as well as great economic losses in many countries including Pakistan. There has been pressing need to control this disease through vaccination in Pakistan. The aim of this discussion is to convey the production of an efficacious vaccine against PPR disease 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 validation, safety, sterility and efficacy 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 doses 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 as well as to reduce economic losses due to this disease in Pakistan.

INTRODUCTION

Pakistan is gifted with a large population of small ruminant including 58.3 and 27.4 million heads of goats and sheep respectively (Anonymous, 2010). PPR is an acute and contagious (Dhar et al., 2002; OIE, 2008) viral disease of small ruminants characterized by fever, oculonasal discharges, stomatitis, diarrhea and pneumonia with foul offensive breath (Lefevre and Diallo, 1990). Peste des petits ruminant 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, 2008). Clinical picture of PPR includes pyrexia up to 41°C, anorexia, dry muzzle, serous oculonasal discharge which become progressively mucopurulent, respiratory distress, ulceration of mucous membranes and gastroenteritis (Dhar et al., 2002; Ozkul et al., 2002). PPR disease is reported to be detrimental to small ruminant welfare and causes substantial economic losses, thereby affecting the livelihood of poor farmers and pastoralists (Megersa et al., 2011). Similarly PPR is accounted as a most important disease affecting small ruminant's productivity in Sudan during a cross-sectional survey (Abdalla et al., 2012). The existence of PPR has been recognized in Pakistan since 1991 (Ather et al., 1995), while further outbreaks of PPR were also documented (Pervez et al*.,* 1993; Hussain et al*.,* 1998; Tahir et al., 1998; Hussain et al., 2003; Zahur et al., 2008). The Immunocapture ELISA (ICE) was reported as a rapid, sensitive and specific test for the antigen detection of PPRV as well as is able to differentiate between rinder pest virus (RPV) and PPRV (Abubakar et al., 2008). The competitive ELISA (cELISA) is suggested as a most suitable choice because of its high diagnostic sensitivity (99.8%), specificity (90.5%) and

reliability (Anderson and McKay, 1994; Choi et al., 2005; Khan et al., 2007; Khan et al., 2008; Abubakar et al., 2009). A sero-survey of PPR through cELISA in the small ruminant of Pakistan was carried out. The researchers reported that this virus has become an endemic infection of sheep and goats and is widely distributed all across the country (Zahur et al., 2011). Besides the serological assays the use of reverse transcriptase polymerase chain reaction to analyze suspected field samples collected from sheep and goats in Nigeria was found very helpful in the rapid diagnosis of PPR (Ularamu et al., 2012). Further the use of homologous and recombinant vaccines has been suggested to control the PPR in Pakistan as other PPR containing measures are not economically viable (Abubakar et al., 2011a).

The optimal age for PPR vaccination in lambs and kids was suggested from 75 to 90 days after birth (Sanne et al., 2006). The antibodies produced as the result of PPR vaccine persist for at least three years (OIE, 2008). Another study on the PPR vaccine efficacy in field conditions in sheep and goats using cELISA was reported by Khan et al., (2009). Mean antibodies titers of 37, 65 and 91 while 43, 78 and 86 at 10, 30 and 45 days post-vaccination were observed in sheep and goats respectively. Recently it has been reported that all the experimental goats vaccinated with PPR vaccine were found positive for antibodies one month post vaccination and even after one and half year post-vaccination (Abubakar et al., 2011b).

Little work has so far been reported on the immuno-prophylaxis against PPR in Pakistan (Asim et al, 2008, 2009; Khan et al., 2009; Abubakar et al., 2011c), 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 demonstrate the preparation of 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 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 PPRV. Freeze dried virus seed was reconstituted in 10ml of cell culture medium without serum, medium was discarded from the flasks prior to inoculation with PPRV (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 PPRV 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 freezed at -70°C. Random samples from the stored flasks having CPE were checked for any contaminants and titrated for PPRV 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 PPRV 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 un-inoculated controls. These animals were observed for 3 weeks (OIE, 2008). Potency test for the PPR vaccine in small ruminants was not carried out due to the unavailability of wild (field) strain of PPRV.

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 roundening 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 (2006), 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 (2008) stating that it is sometimes difficult to see the syncytia in vero cells infected with PPRV, if they exist, they are very small. Titer of pooled PPRV 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 PPRV 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 PPRV. 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 farm of Balochistan. Furthermore, the PPR vaccine has also been sold to the Government, non-government organization of other provinces. So far the response from the use 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 PPRV 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 PPRV from CIRAD, France.

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