PRODUCTION OF PPR VACCINE IN THE SUDAN

By

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INTRODUCTION

Outbreaks of peste des petits ruminants (PPR) have been reported in Sudan since 1971, (Babiker El Hag Ali, 1973 and Babiker El Hag Ali, and Taylor, 1984). To control the occurrence of these epizootics, vaccination against PPR was carried out using the rinderpest (RP) tissue culture vaccine (Babiker El Hag, 1973) based on the principal of antigenic relationship that exists between PRR and RP viruses. However, the use of rinderpest vaccine against PPR was avoided in Sudan when rinderpest seromonitoring studies were performed. This in turn necessitated the production of PPR virus vaccine in the Sudan for control of PPR disease and to support goats and sheep trade. The purpose of this paper is to record the techniques used for the production of experimental batches and subsequent large scale production of PPR vaccine.

MATERIALS AND METHODS

Cells: PPR vaccine is produced in vero cells which must be free from all bacterial, fungal and extraneous viral contamination. The cells (VERO-E6) used here were obtained from the Veterinary Vacc-ine and Sera Research Institute –Cairo-Egypt.

Culture Medium: Glasgow Minimum Essential Medium (GMEM) supplemented with antibiotics (100i.u/ml penicillin, 50µg/ml streptomycin, 10µg/ml Gentamycin) and mycostatin at a concentration of 50i.u/ml. The medium, enriched with 10% foetal calf serum was used as a complete medium for cell growth. Once the monolayer was confluent a maintenance medium that contained 2% calf serum was used.

PPR Vaccine Seed Virus; Reference PPR virus vaccine strain [PPR 75/1] which was originally isolated in Nigeria 1975 and attenuated in VERO cell culture (Diallo *et al.*, 1989) was used as it is recommended by OIE to control PPR disease in many African countries.

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Three vials of (PPR 75/13ILK6 VER76) freeze-dried PPR vaccine seed cultured in VERO cells were obtained from PANVAC (Debrizeit-Ethiopia).

Working Bank Production: The freeze dried contents of PPR seed bank vaccine vials were each reconstituted with two ml of sterile cell culture medium without serum, pooled and then thoroughly mixed with VERO cell suspension in complete culture medium to provide at least $10^{2.5}$ $TCID_{50}$ per ml. One tenth of the VERO cell suspension was kept to serve as un-inoculated cell culture control.

The inoculated cell culture was agitated and distributed in $75cm^2$ culture bottles and incubated stationary at 37°C. Culture medium was renewed 3-4 days later with maintenance medium containing 2% serum. The cultured cells were examined daily to detect cytopathic effect (CPE) and the virus was first harvested when there was 50-60% cytopathogenic effect (CPE), and the harvested virus was stored at –20°C. Successive harvests were made every two days until the CPE reached 80-90% when the final harvest was made. All successive harvests (generally two or three) were frozen and thawed three times and then pooled to form a single batch. The pooled harvest was centrifuged in a cold centrifuge (3000 rpm/30min.) to get rid of cell debris and the supernatant was kept at 4°C.

Freeze Drying: A freeze-drying cryoprotectant containing 5% lactalbumin hydrolysate and 10% sucrose was prepared using distilled deionized water by heating to 80°C in water bath. The protectant was then sterilized by filtration (Babiker El Hag Ali, 1971). The supernatant of the harvest containing culture fluid was mixed with an equal volume of chilled freeze drying protectant and distributed in freeze drying vials by automatic syringe. Freeze-drying was carried out for 26 hours in Edwards. Mini Fast mod D0.5 freeze dried machine the vials were sealed under final vacuum of 0.01 mmHg and stored in –20°C.

Production of Experimental Batch and Vaccine Batches: These were produced and tested as for the working seed bank above.

Virus Titration: Ten fold virus dilutions (0.5ml +4.5ml medium without serum) ranging from 10^{-3} to 10^{-5} were prepared under chilled condition. Each viral dilution was then delivered into five cell culture tubes at rate of 0.2 ml per tube. One ml of VERO cell culture

suspension in growth medium with 10% foetal calf serum was immediately added to each tube and the tubes were closed and incubated stationary at 37°C.

After 3-4 days the cell culture fluid was changed to maintenance medium. The maintenance medium was then changed every two days and tubes were microscopically examined for appearance of CPE at intervals of two days up to the 15th day post inoculation. Virus titers were calculated by Spearman-Kãrber method and expressed as $TCID_{50}$ per ml.

Identification of Virus and Detection of Contaminating Cytopathic Agents: Freeze-dried vials of PPR vaccine working bank were tested for vacuum, reconstituted in chilled phosphate buffered saline and diluted to contain approximately 10^{-4} $TCID_{50}$ per ml. Two-fold dilutions were prepared from each of foetal calf serum free of PPR antibodies and PPR rabbit hyperimmune serum in one ml quantities in disposable tubes with covers. An equal volume of diluted PPRvaccine (10^{-4} $TCID_{50}$)was added to each serum dilution and the mixtures were incubated in stirred water bath at 37°C for one hour. Each serum, virus mixture was then dispensed into five tissue culture tubes at the rate of 0.2ml. and each tube received one ml of cell culture suspension in GMEM medium containing 10% foetal calf serum and antibiotics. The tubes were securely closed, incubated stationary at 37°C and examined as for virus titration.

Animal Safety and Potency Tests: The purpose of these test was to check the absence of any virulent contaminating agent pathogenic for sheep and goats and the efficacy capacity of the vaccine batch. Ten of the freeze dried vaccine vials were diluted in buffered saline to provide 10^5 $TCID_{50}$/ml (100 doses / 1 ml.

The vaccine bank and the experimental batch were each used for vaccination of the sheep and goats which were more than one year of age. Two goat and two sheep were subcutaneously vaccinated with 10^5 $TCID_{50}$ per animal, another two sheep and two goats were vaccinated subcutaneously with 0.1 dose/animal, the remaining two goats and two sheep were kept as contact controls. The animals were observed daily for clinical signs and morning rectal [illegible]atures measurements were recorded for three weeks.

After three weeks post inoculation blood was collected from all animals for sera and all animals were subcutaneously inoculated with an estimated goat infectious dose (10^{-3} GID_{50} per animals) of a previously titrated locally isolated pathogenic PPR virus strain (Sennar, 1973 isolated by B.H. Ali). The animals were observed for clinical signs and morning rectal temperatures were recorded for two weeks.

Biological Test: The same undiluted vaccine fluid which was prepared for inoculation of goats and sheep was simultaneously inoculated into two adult guinea pigs, one with 0.1ml interperitoneally and the other with the same dose intramuscularly. In addition six white mice were each inoculated with 0.1ml of undiluted vaccine interperitoneally. Both guinea pigs and mice were observed daily for any abnormality in conjunction with inoculated goats and sheep.

Biological Sterility Test: Thioghycolate broth was prepared in screw cap Bijou bottles. Six bottles were each inoculated with 0.1ml of undiluted reconstitut-ed vaccine used in the previous tests. Three of the these bottles were left at room temperature and the other 3 bottles at 37°C incubator for seven days and observed for presence of bacteria or fungi.

RESULTS

A total of 220 vials were produced in the first working seed bank, then followed by another batch containing 1050 vials. The mean titre of these batches were $10^{4.85}$ and $10^{5.5}$ $TCID_{50}$ per ml respectively.

No cytopathic agent or contamination was detected in uninoculated control cultures for period of two weeks. All sheep and goats inoculated with the produced PPR vaccine did not show clinical signs or thermal reaction for the period of three weeks and after challenging with virulent PPR virus. The uninoculated control sheep and goats reacted thermally to the challenge. After 21 days the inoculated sheep and goats sera were found to contain PPR antibodies (ELISA) while the sera from uninoculated controls did not contain any detectable antibodies for PPR.

DISCUSSION

The prohibition of the use of RPV tissue culture vaccine as a basic requirement for the OIE pathway in order to declare the Sudan free of RP necessitated that a PPR virus vaccine to be produced for use in sheep and goats. The present paper records the successful local production of this vaccine in the Sudan. An increase in the incidence of field cases and high prevalence of PPR antibodies in sheep and goats have been recently reported from all over the country (Intisar, 2002). It is hoped that the local availability of the PPR vaccine will enable concerned authorities to carry out vaccination programs to curb the disease incidence in Sudan and at the same time avoid interference with serosurveillance efforts needed to declare the country free of rinderpest.

Over the last two years, more than 2 million doses of the PPR vaccine have been produced and used under field condition in various areas of the Sudan and in animals for export. Testing sera collected from some of the vaccinated animals for PPR antibodies using the PPR competitive ELISA (Pirbright Laboratory-England) indicated over 90% of an antibody conversion rate.

However, the demand for the vaccine is increasing, and close monitoring of it's performance under various conditions of use in the field is urgently required.

The Criterria for the Fitness of the Produced PPR Vaccine are as Follows:

a) To have a mean titre not less than 10^{-4} $TCID_{50}$.

b) Absence of cytopathic effect (CPE) in uninoculated control cell cultures during the first two weeks after seeding.

c) Complete neutralization of vaccine virus by specific PPR rabbit hyperimmune serum and absence of CPE which could otherwise be caused by contaminating cytopathic virus.

d) Demonstration of PPR neutralizing antibodies in post inoculation sera of vaccinated sheep and goats and absence of clinical signs and thermal reaction before and after challenge with virulent virus except in uninoculated controls.

e) Survival of mice and guinea pigs with absence of disease signs for a period of three weeks.

f) Negative results of sterility tests for bacteria fungi and mycoplasma.

g) The end vacuum pressure of the final product should be around 0.01 mm Hg before release to stores. Freeze dried vaccine vials are stored at –20°C or less than that.

These criteria are in accordance with international requirements of PPR vaccines (Anderson *et al*., 2000, Babiker El Hag Ali 1971).

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