A new approach to develop a vaccine against capripox infection in sheep and goats using a new strain of sheep pox virus in Iran

Sadri, R.*; Fallahi, R.

Razi Vaccine and Serum Research Institute, Department of Animal viral Disease Research and Diagnosis , Shahied Beheshti Road / Hessarak, Karaj, Iran.

Key Words:
Single vaccine; potency; immunogenicity; control; cell culture.

Correspondence
Sadri, R.,
Razi Vaccine and Serum Research Institute, Department of Animal viral Disease Research and Diagnosis , shahied Beheshti Road / Hessarak
P.O. Box: 31975-148, Karaj, Iran.
Tel: +98(261)4570038
Fax: +98(261)4552194
Email: R.sadri@rvsri.ir

Received: 24 October 2009,
Accepted: 30 May 2010

Abstract

This report describes the preparation of a safe and immunogenic single vaccine against capripox disease in sheep and goats using two different types of cells (primary lamb kidney and vero, a bovine turbinate cell line). This used the 0240 pox strain isolated from sheep during an outbreak. The prepared vaccine was administered subcutaneously and induced complete protection against an experimental sheep and goat pox challenge virus. For the final evaluation, the efficacy of the prepared vaccine was used in a field trial under the supervision of veterinary clinicians. This vaccine was proved to be more efficacious than the routine vaccines that are currently produced.

Introduction

Pox disease is an acute to chronic disease of sheep and goats, which is highly contagious and characterized by generalized pox lesions. These diseases are not serologically distinguishable due to a high degree of antigenic homology between them (Davies, 1976) Various strains of sheep and goat pox virus (SGPV) cause disease only in sheep, others only in goats, and some in both sheep and goat (Davies *et al.*, 1982). Goat pox virus is highly host-specific, infecting only goats, but host specificity varies from isolate to isolate. It is possible that the host preference shown by different strains is due to their adaptation to the presence of either goats or sheep alone in a restricted geographical area. Isolates of Capripoxvirus are not host-specific; sheep, goats and cattle who have recovered from infection with Capripoxvirus isolates from a heterologous host are immune to any challenge with a virulent homologous virus (Kitching *et al*., 1989).

Sharma and Dhanda (1971) considered the possibility of a single vaccine if a strain of capripox could be identified that was equally pathogenic for sheep and goats; nowadays, pox virus disease is effectively controlled by routine vaccination using different strains of pox virus, and in recent years, intensive methods of management have played a principal role in making viral pox disease a relatively uncommon disease. Nevertheless, foci of infection have persisted in most of the northwestern and southeastern provinces of Iran. Therefore, mass vaccination of ruminants against capripox disease is still carried out continuously.

SGPV have been propagated successfully in various cell culture systems. Recently, a novel sheep isolate was collected in an outbreak and attenuated by serial passages in a cell system. The present study describes this new strain during the development of a single vaccine (Kitching, 1983).

Materials and Methods

Viral strain

The viral strain used in this survey was a novel strain of pox virus isolated from sheep during an outbreak. This strain was used to develop a single vaccine at the Razi Institute, producing a titer of $10^{-6.7}$ TCID50/ml.

Cell culture preparation

Two type cells both primary lamb kidney sub-culture (P1LK), the vero and bovine turbinate cell line (BTC), were prepared in Roux bottles as described by Baxendale in 1971 using Derby minimum essential media (DMEM) and Hanks media that contained 5-10% fetal calf serum (FCS) supplemented with triptose phosphate broth (TPB). The amount of FCS was reduced to 2-3% when a confluent monolayer formed for capripox growth, and excluded completely when cells were used for the growth of the vaccine virus.

Inoculation of the new strain of pox virus

After 24-48 hours of incubating cells at 37ºC, cells formed a confluent monolayer (Figure 1). The new strain of virus was inoculated into these cells and adsorbed at 37ºC for one hour. Cells were then maintained in an incubator for 7-10 days; cells usually degenerated during this period. At first, the cytopathogenic effects (CPE) appeared as small islands; they then gradually attached to each other and degenerated (Figure 2). The culture was then frozen and thawed between two and three times to release the virus completely. The suspension was centrifuged (1,800 rpm) for 20 min. The supernatant was inoculated in the next cell culture. On the final passage after 90-100% CPE was formed, the cultures were frozen at -70ºC and then aliquotted into 1.2 ml with 2% lactalbumin hydrolysate and sucrose as a preservative for the vaccine. Vaccine samples were then lyophilized and stored at -20ºC.

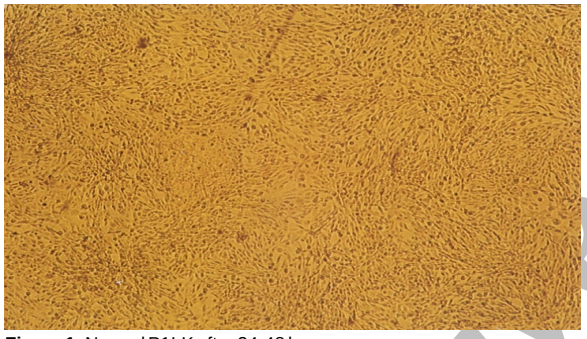


Figure 1: Normal P1LK after 24-48 hr.

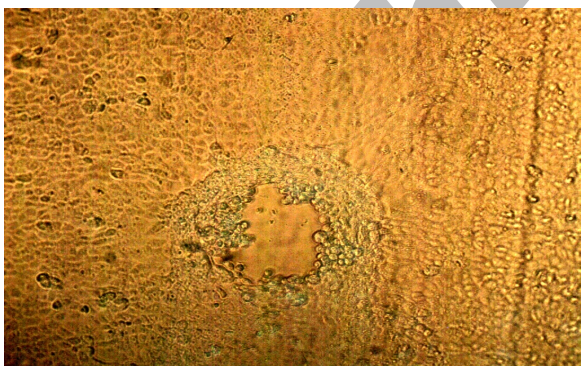


Figure 2: Cytopathic effects in the vero cell line 7-10 days.

Virus titration and minimum infecting dose

Titrations were carried out with ten-fold dilutions of experimentally-prepared vaccine using a range of dilutions from 10^{-1} to 10^{-7}ml in Hanks balanced salt solution without serum in both tubes and microtiter plates before and after freeze-drying with lactalbumin hydrolysate. Then, 0.1 ml of each dilution was inoculated into previously prepared sub-cultures. The cells were checked daily for the appearance of CPE and the end point. TCID50/ml values were calculated by two methods according to Reed and Muench and Karber (Reed and Munench, 1938).

Vaccine preparation

Two-three batches of vaccine were prepared from this new strain of capripox virus grown on primary lamb kidneys, vero and BTC. These batches were prepared in final passages individually. The vaccines were freeze–thawed using the conventional method. Titers of this experimental new vaccine were approximately $10^{-6.7}$ TCID50/ml.

Safety and potency tests

Vaccines from each final batch with a 10^{-4} dilution were prepared and given as a single vaccine dose. Three groups of sheep and goats (1-yr-old) from different Iranian races were selected and injected by a subcutaneous method; 1 ml was given to each sheep and goat as follows: Group 1: 10 sheep and 10 goats were given the single vaccine prepared in primary lamb kidney cell culture; Group 2: 10 sheep and 10 goats received the single vaccine prepared in the vero cell line; and Group 3: 20 susceptible sheep and goats were kept unvaccinated as controls in the same battery throughout the experiment. The inoculation site of all sheep and goats were examined daily for evidence of the effects of the vaccine, adverse or otherwise, for a period of 21 days. All animals, including controls, were challenged via 1 ml with a field strain of the virulent virus with a titer of 10^{-6} TCID50/ml by intradermal injection into the right flank of small ruminants.

Stability of single vaccine

Vials of the freeze-dried vaccine were stored at -20ºC for 18 months and for six months at 4ºC. Samples were then titrated at three month intervals with both the tube and microtiter plate methods. Titers of the single vaccine were stable for up to approximately $10^{-6.7}$ for a whole year. The results were suggested that this single vaccine will be able to produce solid immunity in sheep and goats.

Results

The new strain of sheep pox grew strongly in the P1LK, vero and BTC cell cultures and the CPE of the infected cell cultures showed that the initial rounding of the cells was followed by complete destruction of the cells. These cells detached from the Roux bottles after 7-10 days. The virus-induced CPE was observed earlier in primary lamb kidney cultures than the vero or bovine BTC, usually after a week or ten days post-inoculation (Plowright *et al*., 1958). The titer of the virus was increased gradually and reached a maximum of between $10^{-6.5}$ and $10^{-6.7}$ during the final passages. Titration was usually performed in both the tubes and microtiter plates, and calculations were performed with

both the Karber and Reed and the Muench methods. (Reed *et al*., 1938). The response curve of the single vaccine virus in both sheep and goats is shown in Figure 3. The results of the intradermal challenge of the vaccinated sheep and goats with the single vaccine showed that animals developed an inflammatory reaction or mild hypersensitivity at the inoculation site within five days after injection; this disappeared after a period of time. Control animals developed a 2-5 cm diameter papule at the site of the intradermal challenge by day 5 after infection. The superficial lymph nodes became enlarged and two groups of control animals became pyrexic and developed generalized papules with clinical signs of pox disease (Plowright *et al*., 1959), also the results of the SDS-PAGE test showed a similarity between proteins bonds of both single and conventional pox vaccine (Figure 4). The neutralization index of sera were collected on days 21, 168, 292 and 359 from vaccinated sheep and goats; these showed that the level of neutralizing Index (NI) against Capripoxvirus remained stable at 0.8 ± 0.5 unit in sheep and 1.0 ± 0.5 unit in goats.

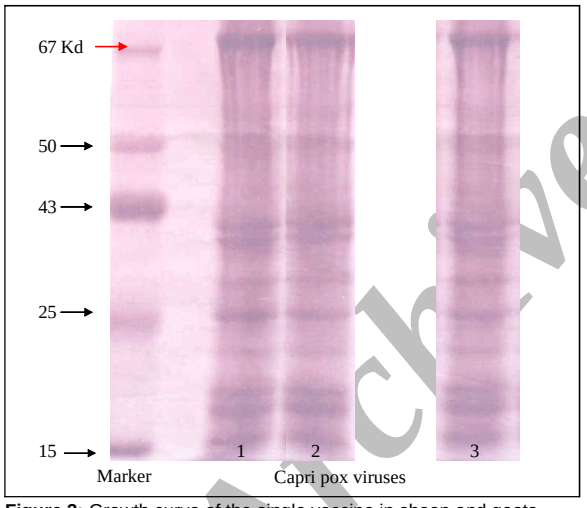
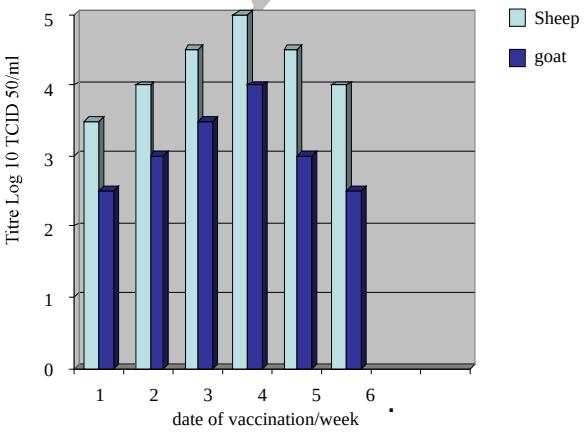


Figure 3: Growth curve of the single vaccine in sheep and goats

Figure 4: SDS-PAGE between new and conventional pox vaccine.



Discussion

The new strain virus was propagated in vero, BTC and P1Lk cells. The vaccine virus was passaged blindly for several cycles in order to adapt the virus to the cell culture and CPE were evident after five days and completed within ten days (Ramyar., 1987) The single vaccine subsequently developed had significant differences in titers from the conventional vaccines with titers of $10^{-5.9}$, meaning that the TCID50/ml was 0.6-0.8 log higher than the conventional vaccine that has been prepared in the Razi Institute. It was found that the titer was 10^{-3}, and the TCID50/ml was the minimum titer that could protect sheep and goats against a virulent field virus (OIE, 2008). The vaccine was tested for safety and potency by the intradermal method and vaccinated animals were observed for three weeks. The developed vaccine protected both sheep and goats against virulent strains of Capripoxvirus for at least 12 months and produced no adverse reactions in the susceptible Iranian breeds of sheep and goats (Kitching *et al*., 1987). However, sheep and goats responded to vaccination by developing precipitating antibodies and resisting viral challenges (Caren, 1995). The type of challenge used in this experiment was intended to be severe and it is unlikely that a vaccinated animal would encounter such a challenge in a field situation.

There have been numerous unsuccessful attempts to replace live capripox vaccine with dead preparations. However, the shortage of information with regards to the immunity to pox virus infections makes a rational assessment of whether effective dead pox vaccine could become a reality impossible. Epidemiological surveys have shown that single strains of capripox virus cause epidemic disease in both sheep and goats in mixed flocks, which are usually seen in countries, such as Iran, India and Turkey. Even if clinical disease is only seen in goats, it is possible that subclinical disease caused by the same strain is also seen in sheep (Murray *et al*., 1973); therefore, the application of a single vaccine for the protection of the whole flock and the prevention of the presence of sub-clinical pox virus within the flock, has many advantages. Accordingly, this method may produce a vaccine that can control the re-occurrence of this economically important disease and eradicate Capripoxvirus from areas or even whole countries through an annual vaccine program.

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