

PRODUCTION OF GOAT POX VIRUS VACCINE FROM A LIVE ATTENUATED GOAT POX VIRUS STRAIN

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ABSTRACT

Kid kidney cells form less than 2 months of age were grown, 80% confluent monolayers were obtained after 10-12 days. Live attenuated goat pox virus vaccine (Gorgan 55, Iranian) was used to infect the confluent monolayers. Eighty percent cytopathic effect (CPE) was obtained on day 5 of virus inoculation. TCID₅₀ calculated for the pool virus was 10^{5.5}/ml. Vaccine was found free from any bacterial and mycoplasma contamination. All mice and guinea pigs were found healthy after 21 days post vaccinal administration. Vaccinated goats challenged after 14 days with 1 ml of virulent goat pox virus (TCID₅₀ 10^{4.5}/ml) did not show any unwanted reaction while controls challenged with 1 ml of virulent goat pox virus (TCID₅₀ 10^{3.5}/ml) showed pox lesions and rise in body temperature. The main objective of this study was to produce and study the efficacy of this vaccine.

Key words: attenuated, goat pox virus, confluent, monolayer, virulent, challenge.

INTRODUCTION

Goat pox is a viral disease of goats caused by a capripox virus and characterized by fever, generalized papules or nodules, vesicles (rarely), internal lesions particularly in the lungs, and death. According to Davies and Mbugwa (1985) the affected goats show rhinitis, and temperature rises up to 42°C. Mahmood *et al.* (1989) have reported that the disease can cause significant economic losses especially in young animals in the form of morbidity, reduced productivity, and colossal loss to the hide and skin industry. In Pakistanistan goat pox disease has been recorded in the province of Punjab and Khaiber Pakistanthoonkhwa since 1975. Cases of goat pox are reported from different areas of Balochistan as well (Mahmood *et al.*, 1989).

Capripoxvirus grow in tissue culture of bovine, ovine or caprine origin, although primary or secondary cultures of lamb testis or lamb kidney cells are considered to be the best (Carn, 1995 and Davies and Mbugwa 1985). About 90% confluent cells are infected with the virus and allowed at for absorption 37°C and further incubated at the same temperature for virus growth in the presence of suitable growth medium. Cytopathic effect (CPE) is observed in next 24-72 hours, the CPE consists of retraction of the cell membrane from surrounding cells and eventually rounding of the cells with margination of nuclear chromatin (Davies and Mbugwa, 1985).

Capripoxvirus infections can be controlled through vaccination (Carn, 1993). All strains of capripoxvirus share a major neutralization site, cross

protection and antigenic relationship between sheep and goat pox viruses (Kitching and Taylor 1985). Cross reaction between sheep and goat pox viruses using immunofluorescence and virus neutralization tests do exist (Soad *et al.*, 1996). Attenuated goat pox vaccine has been used against sheep pox intradermally and is reported to be protective (Wang and Jiang, 1988). Inactivated vaccines used against capripox, sheep pox and contagious ecthyma virus provide short term immunity (Yadav *et al.*, 1987). Attenuated Capri pox virus produced by passages on fetal muscle cells (Davies and Mbugwa 1985) and used as a vaccine, has been found protective against challenge.

The main objective of this study was to produce vaccine from a live attenuated goat pox virus (Gorgan 55, Iranian strain) using cell culture technique and to use it for controlling the disease caused by indigenous virulent goat pox virus in Balochistan.

MATERIALS AND METHODS

Activation of virus inoculums: Kid kidney cortex cells adapted goat pox virus strain (Gorgan 55) was obtained from Razi Institute Tehran, Iran. To activate and prepare virus for harvesting, kid kidney cells and testis were grown in tissue culture flasks at 37°C in the growth medium containing 10% foetal calf serum, 1% fungizone (250ug/ml), 0.1 g/ liter streptomycin, 100000 I.U/ liter penicillin. Cell monolayers were infected with the virus and passaged blindly three times and CPE of about 70-80% was obtained. This virus was collected and saved at -20°C.

Preparation of primary kidney cell culture and virus propagation:

Cells were grown using the technique described for chicken embryo liver cells in tissue culture flasks by Villegas (1989). Briefly, kidneys were aseptically obtained from a kid less than 2 months of age. After decapsulation of the kidney, cortical tissues were removed under aseptic conditions and collected in a beaker containing PBS. The cortical tissues were chopped and washed 3-4 times with PBS. Trypsinization of the cortical tissues was performed with 0.25% of trypsin solution for 20 minutes in a trypsinizing flask and repeated 4 times to obtain maximum cells. The trypsinized cells were centrifuged at 1500 rpm for 20 minutes and pellet was collected and suspended in growth medium containing 10% foetal calf serum, 0.1 g/ liter streptomycin, 100000 IU / liter penicillin, and 1% fungizone (250 ug/ml). Cells were then grown in tissue culture flasks at 37° C. After 5 days of incubation, media from the tissue culture flasks containing confluent monolayers was discarded and the flasks were washed with PBS. The cells were then infected with the Gorgan 55, Iranian goat pox virus and incubated at 37°C for 30 minutes for virus adsorption. Tissue culture flasks were refed with growth medium after 30 minutes and reincubated at 37°C till a good cytopathic effect (CPE) of the virus was obtained. Some flasks were kept as control those were uninfected with the virus. Cells were examined daily under the inverted microscope for the virus growth and compared with controlled flasks. Cells from the flasks showing good CPEs were collected with a rubber policeman, pooled and stored at -20°C.

Titration of the virus: Virus titration was performed using Spearman-Kärber's method as described by Villegas and Purchase (1989). Cells were grown in 96-well tissue culture plates. About 0.1 ml of the virus from ten-fold dilutions ranging from 10⁻¹ to 10⁻⁸ was used to infect the cells in each well of the 96-well tissue culture plate. Tissue culture plates were incubated at 37°C and TCID₅₀ was calculated 5 days post- infection. Control (uninfected) wells were also kept for each dilution. Single vaccinal dose was determined to be 10^{2.7} TCID₅₀. For diluting the virus, PBS with 6% skimmed milk was used. Hundred doses of the vaccine in each vial were added before lyophilization. Lyophilization was performed for 14 hours.

Vaccine testing: A vial of lyophilized vaccine containing 100 doses from freezer was thawed and vortexed to disrupt the cells and release virus. Sterility test was performed as described by Mahmood *et al.* (1989) with slight modification to see for any bacterial, fungal, and mycoplasma contamination. Ten ml of PBS was added in a bottle of vaccine and 1 ml of the vaccinal content was used to inoculate pleuropneumonia like organism (PPLO) broth, reinforced clostridial medium (RCM), and blood agar plates. All the plates were incubated at 37°C for 10

days both in anaerobic and aerobic environment. Control plates (without viral inoculation) were also kept for each plate in the same manner. Guinea pigs and mice were also injected with the vaccine to see for any pathogenic contaminants. Four mice were given 1 vaccinal dose intradermally, 2 guinea pigs were given 5 vaccinal dose intraperitoneally and 2 guinea pigs were given 5 vaccinal dose intramuscularly. These animals were under observation for 21 days to look for any reaction. The safety and potency of the vaccine was also checked according to the method described by Mahmood *et al.* (1989) with some modification. Four groups of young goats (A, B, C and D) were made. Group A (3 goats) was given 100 vaccinal dose subcutaneously, group B (3 goats) 1 vaccinal dose subcutaneously and group C (2 goats) was given 1/10 vaccinal dose subcutaneously. Group D (4 goats) was kept as a control group (unvaccinated). All animals were kept under observation for 14 days to see for any reaction, pyrexia and anorexia.

After 14 days all vaccinated goats were challenged intradermally with 1 ml of virulent goat pox virus (TCID₅₀ 10^{4.5}/ ml). The control group was challenged intradermally with 1 ml of virulent goat pox virus with a TCID₅₀ of 10^{3.5}/ml. The animals including control group were kept under observation for 21 days for any reaction. Temperature and feeding were also recorded for these 21 days.

RESULTS AND DISCUSSION

After 3 passages of virus (goat pox virus vaccinal strain) on kidney and testes cells about 80% CPE was achieved. Complete monolayer of kidney cells in the tissue culture flasks was obtained after 10-12 days (Fig. 2). In some tissue culture flasks refeeding of the cells with the growth medium was done after 4 days to get rid of the unattached cells. Complete monolayers were infected with the goat pox virus (vaccinal strain). A good CPE that is strand formation, rounding, damage and clumping of the cells due to the virus was obtained 3-4 days post-infection (Fig. 1). An 80% CPE was obtained on 5th day post-infection. Infected cells were pooled and stored at -20°C. The TCID₅₀ calculated for the virus was 10^{5.5}/ml. All mice and guinea pigs received this vaccine were alive and did not show any reaction in 21 days of observation.

After 10 days of incubation of PPLO broth, RCM and blood agar plates inoculated with the vaccine at 37°C no aerobic or anaerobic bacterial growth was observed. No unwanted reaction due to the vaccine was observed during the 14 days in the vaccinated animals. The temperature and feeding were normal too. All vaccinated animals were found protective after being challenged with the virulent field strain of goat pox virus. Anti-viral antibodies subside the infection in hosts but according to Carn (1993) it is the cell-mediated immune

response that eliminates the infection. The feeding and temperature were normal and no reaction or lesions were observed in any vaccinated animal in 21 days post challenge. Live-attenuated vaccine with good protective efficacy is also reported by Davies and Mbugwa (1985). Both live and inactivated vaccines can be used against this disease but live vaccine provides long-lasting immunity and protection (OIE, 2009 and Babiuk *et al.*, 2008). Controls (unvaccinated animals) did show a rise in body temperature between 105 - 107 F° from day 5 to day 9 after challenge with virulent goat pox virus. The temperature became normal in controls from the 10th day onward. Local skin reaction (macules, papules) started in controls after 10th day of challenge. The skin reaction was oedematous which became necrosed in 5 to 6 days and later a hard scar was formed. Fever followed by macules and papules are characteristic of this disease (Mahmood *et al.*, 1989).

The study indicates that Gorgan 55, Iranian cell culture adapted goat pox virus, provides adequate protection against local virulent goat pox virus and that both the strains share major immunogenic determinants. All the strains of goat pox virus share major neutralizing site (OIE, 2009) hence vaccine produced against one strain provides protection against all strains. Vaccination is the easiest way to control the disease and improve food security. If the disease is not controlled in sheep and goats can lead to considerable socio-economic losses because the virus causes reduced milk yields, decreased weight gain, abortion and increased susceptibility to pneumonia (Babiuk *et al.*, 2008 and Yeruham *et al.*, 2007).



Fig 1: Infected kidney cells with goat pox virus showing CPE



Fig 2: Uninfected kidney cells

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