The Bangladesh Veterinarian (2016) 33(1): 23 – 27

Evaluation of antibody response to Goat Pox cell culture vaccine in goats in India

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Abstract

Goat Pox is an important contagious viral disease that causes serious economic loss in small ruminants. The disease causes high morbidity, mortality and trade restriction. Several outbreaks of the disease are reported in Africa and Europe. In India also, the disease has high economic significance. Present study was undertaken to estimate specific antibody response to Live Goat Pox Vaccine in goats. Thirty-five healthy goats were vaccinated with single dose and 10 goats were kept as control. Blood was collected at defined intervals and sera were separated. Virus neutralization test was performed to estimate neutralization index as marker of antibody levels in serum. The study revealed that vaccine could induce immunity against the disease within seven days of vaccination, and reached a peak at 21 days. The titre remained at protective level throughout the one-year study. Further study can be done to estimate the total duration of immunity through extended study, and the response to challenge with virus. (*Bangl. vet*. 2016. Vol. 33, No. 1, 23 – 27)

Introduction

Goat Pox Disease (GPD) is one of the most significant viral disease infecting small ruminants. The causative agent is Goat Pox Virus, a dsDNA virus in the genus Capripoxvirus, subfamily *Chordopoxviridae* of *Poxviridae* family (Van Regenmortel *et al*., 2000). GPD is highly contagious, affecting goats and sheep and causing huge economic losses (Kamran *et al*., 2015).

The virus enters via the respiratory tract, and is transmitted through close contact (Kitching, 2004; Radostits *et al*., 2006). Incubation period is 4-14 days. Within 24 hours of appearance of papules on the body, animals develop rhinitis, conjunctivitis and enlargement of superficial lymph nodes. High mucosal discharge causes difficult and noisy breathing. In acute cases, animals die of respiratory complication and anorexia. Skin lesions due to formation of thrombi persist for up to six weeks, leaving small scars. The acuteness and severity of capripoxvirus infection may vary depending upon the host and strain of virus.

The disease has been reported in North and Central Africa, Middle Eastern countries, Asia and former Soviet Union (Kamran *et al*., 2015). Recent outbreaks have been reported in Mongolia, Kazakhstan, Azerbaijan, Bulgaria, Greece and Turkey (Beard *et al*., 2010; Kamran *et al*., 2015). Several outbreaks of GPD are reported in India,

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including Karnataka state (Bhanuprakash *et al.,* 2005). Control of GPD is crucial for alleviation of poverty in endemic regions.

Available vaccines for GPD induce immunity after a single injection up to at least the economic life of animals, around three years (Martrenchar *et al.,* 1999; Sreenivasa *et al.,* 2000; Pandey, 2004). Satisfactory serological studies have been performed by many researchers, but the duration of the studies was limited. In the present study, specific antibody response to GPV was studied through an extended period of one year.

Materials and Methods

Grouping and rearing of goats

Forty-five healthy Jamunapuri goats of one year of age were divided into two groups:

Group 1: Thirty-five goatswere vaccinated with Live Goat Pox Vaccine (Uttarkashi strain) from Hester Biosciences Limited, India.

Group 2: Ten goats were unvaccinated controls.

Vaccinated goats were kept at Merda-Adraj village, Gujarat, India. Unvaccinated goats were kept at Jetpura village, Gujarat, India. All animals received a free supply of feed and water and were observed daily.

Vaccination, blood collection and testing

Animals from group 1 were vaccinated subcutaneously in cool atmosphere with one dose of Live Goat Pox Vaccine (Hester Biosciences Limited, Gujarat, India). Blood samples were collected from 10 animals/group from both vaccinated and unvaccinated groups at 0, 7, 14, 21, 28, 35, 42, 49 and 56 days; followed by 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months. At the time of blood collection, no signs of illness were observed. Serum was separated and stored at -20°C.

All samples were subjected to Virus Neutralization Test at Hester Biosciences Limited, Anand laboratory (OIE, 2008). Test sera and controls were diluted five times in Eagles/HEPES (N-2-hydroxyethylpiperazine, N-2-ethanesulphonic acid) and inactivated at 56°C for 30 minutes. Inactivated 50 µL serum was added to columns 1 and 2 in A to H rows of 96-well flat-bottomed tissue culture grade microtitre plates. Second serum was added in columns 3 and 4. In columns 5 and 6, third serum was added. Positive and negative controls were added in columns 7 and 8, 9 and 10, respectively. Eagle's/HEPES (50 µL) was added in columns 11 and 12, and whole row of H. Goat Pox virus reference strain was diluted in Eagle's/HEPES in a series of log dilutions of 5, 4, 3. 5, 3, 2.5, 2 and 1.5 $TCID_{50}$/mL. Log 1.5 dilution virus (50 µL) was added in row G and other dilutions were added each row upwards, till log 5 dilution in row A. The plate was covered and incubated at 37°C. Cell suspension was prepared in 10^5 cell/mL concentration in Eagle's medium with 2% fetal calf serum. After incubation of plate, 100 µL cell suspensions were added to each well, except H11 and

H12. Again the plate was covered and incubated at 37°C for 9 days. Cytopathic effect was observed and recorded for calculation of neutralization index.

Data analysis

Neutralization index of ≥ 1.5 was considered as positive. All data were entered into Microsoft Office Excel Worksheet (2013, Microsoft Corporation) and represented as Mean ± SEM. The data were analyzed by single factor analysis of variance method and $p<0.05$ was considered as significant difference between the groups.

Results and Discussion

The initial neutralization index was 0.1 ± 0.04 (Group 1) and 0.06 ± 0.04 (Group 2). Neutralization index increased gradually in vaccinated goats. At 21 days, it was highest (2.22 ± 0.07), which was significantly different from 0 days. After 21 days, the index decreased gradually, remaining in the range of 1.82 ± 0.05 to 2.11 ± 0.05 throughout the year. In unvaccinated goats of group 2, neutralization index was 0.02 ± 0.01 to 0.09 ± 0.06 throughout the study (Fig. 1).

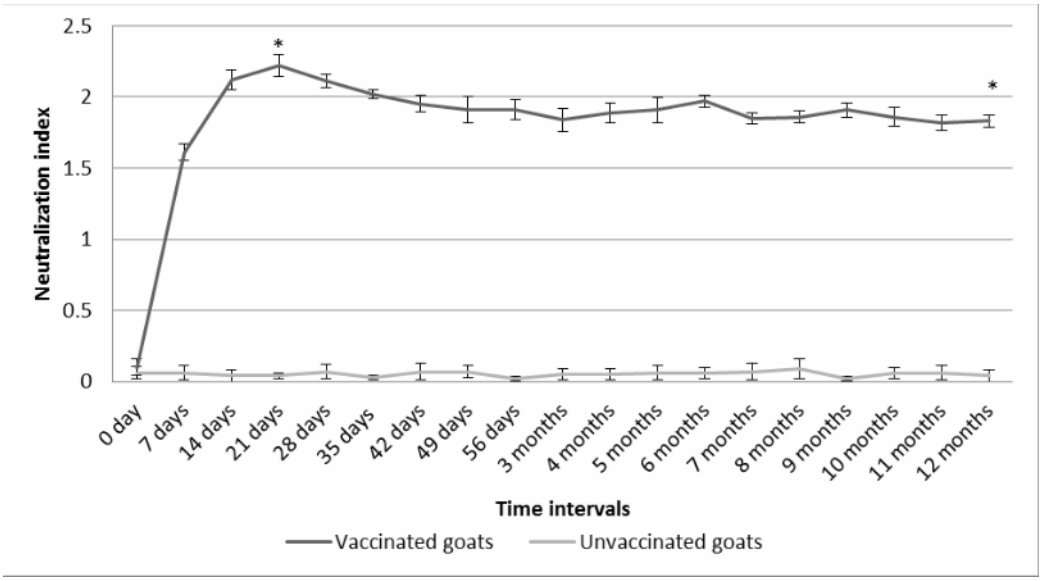

Fig. 1. Neutralization index (Vaccinated and unvaccinated goats)

The data are expressed as mean ± SEM; *P<0.05, significantly different as compared to 0 day

Virus neutralization test was selected to perform serological test as per OIE terrestrial manual, 2008. It is a reliable and accurate method for the measurement of specific antibodies against Goat Pox virus (Sadri *et al.,* 2002; Gelagay *et al.,* 2012). The antibody levels were considered directly proportional to the neutralization index (Zeidan *et al.,* 2016).

After vaccination, within 7 days only, index value rose rapidly to 1.61 ± 0.06 in vaccinated goats, which indicates that vaccine could stimulate the production of specific antibodies to protective level within seven days. Further, the index value rose to maximum after 21 days, i.e., 2.22 ± 0.07. Similar observation was reported by Fakri *et al.* (2015), where neutralization index reached maximum 21 days after vaccination.

Zeidan *et al.* (2016) performed similar study with optical density as a unit of measurement of specific antibodies: they reported maximum immune response at 10 days. Other researchers performed similar study using virus neutralization test, but the study periods were not more than 28 days (Gelagay *et al.,* 2012). After 21 days of vaccination, the titre remained protective up to one year. Further investigations can be done to understand the protective behaviour of the vaccine extended than one year.

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