PCR based confirmation of sheeppox vaccine virus

Amitha R. Gomes*, Raveendra Hegde, S.M.Byregowda, Suryanarayana T, Ananda H, Yeshwant S. L., P. Giridhar and C. Renukaprasad.

Institute of Animal Health and Veterinary Biologicals,
Hebbal, Bangalore-24
* Corresponding author

Introduction

Sheeppox, a contagious viral disease of sheep, is the most severe of pox infections of animals. The disease is of economic importance, as it causes heavy mortality in lambs, abortion and mastitis in ewes and skin defects (Singh et al., 1979). Vaccination is an effective means of controlling losses from sheeppox. Modified live virus vaccine has been used extensively for protection against sheeppox. The strain was maintained in sheep lamb testicular cells. The virus was confirmed by serological techniques. But these techniques are less sensitive and are slow. In the present study an attempt was made to confirm the vaccine strain, Roumanian Fenar strain by PCR method which is considered to be rapid, accurate and more sensitive.

Material and methods

Sheeppox vaccine virus, Roumanian Fenar strain maintained in secondary lamb testicular cells were used in the study. DNA from the virus was extracted using Bacterial genomic DNA spin-50 kit procured from Chromus Biotech Pvt. Ltd., Bangalore with some modifications. Concentration of the DNA was determined by using Nanodrop.DNA amplification was performed using 2 µl of DNA (60 ng) in a 25 µl reaction mixture containing 12.5 µl of hotstart PCR DNA mix (Bangalore Genei) and 100 pmol of each forward and reverse primer. The thermal profile consisted of initial denaturation at 950C for 1 min, followed by 30 cycles of denaturation at 950C for 1 min, annealing at 470C for 1 min, and extension at 720C for 1 min. Final extension was carried out at 720C for 10 min. the primers used were group specific primers (Sigma) fro Capri pox virus genus that amplify 390 bp fragment of UTR +p32 gene. The sequence of the forward primer was CTAAAATTAGAGAGCTATACTTCTT and the reverse primer was CGATTTCCATAAACTAA AGTC. Post PCR analysis was carried out by gel electrophoresis using ten µl of the amplified DNA in 0.8 % agarose stained with ethidium bromide was loaded with amplified DNA using bromophenol dye as a tracking dye along with 100 bp DNA ladder. The gel was run for 90 min at 60 volts and visualized under ultraviolet transilluminator for the presence of specific bands. Then the image was captured under gel doc system. Appropriate controls were incorporated into the system.

Results and discussion

In the present study the viral DNA was extracted from the SPV-RF infected cell culture fluid using bacterial genomic DNA extraction kit. It showed that the kit can efficiently be used for extraction of DNA from viral samples also. The DNA concentration was estimated in Nanodrop. The concentration of the DNA was found to be 32 ng/µl. In the study DNA was used at a concentration of 60 ng per reaction. DNA fragment of expected size (390 bp) was observed on gel electrophoresis confirming the presence of capri pox virus. Similar observations with clinical samples from sheeppox have been done by Sunitha et al., 2008 and Sharma et al 2008. The results from the present study indicate that the PCR assay can be adopted as a rapid test for detection of sheeppox vaccine virus when compared to other serological tests which are comparatively slower and less sensitive.

References

1. Singh I. P., R. Pandey. And R. N. Srivastava. (1979) Sheeppox: a review. *Veterinary bulletin*. 49, 145-154.
2. Sunith. G., A. V. Krishna Mohan and G. Hanumantha Reddy. (2008) Capripox virus detection from the field samples by PCR assay. *Indian Veterinary Journal*. 85, 811-812.
3. Sharma, S., M. hosamani, R. K. Singh, H. Kumar, S. verma, D. Pan., Meenakshi and Mahajan. (2008) PCR based confirmation of outbreaks of sheeppox. Indian Veterinary Journal. 85, 1163-1165.

* * * * * * * *