

AMPLIFICATION OF VIRAL FUSION PROTEIN GENE BY PCR FOR THE DETECTION OF GOATPOX VIRUS FROM FIELD OUTBREAKS

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Goat pox is a severe pox disease of goats, characterized by pyrexia, generalized skin and internal pox lesions and lymphadenopathy (Kitching and Taylor, 1985). It causes severe economic losses, due to heavy mortality, birth defects in offsprings, abortions, mastitis and skin defects leading to carcass condemnation thus causing a major obstacle in the intensive rearing of goat and also greatly hampers international trade. Goat pox is distributed almost throughout the world. The disease has also been reported from many states of India (Joshi et al., 1999). Hence a rapid and specific diagnosis is of vital importance. In this study the usefulness of the polymerase chain reaction (PCR) assay was assessed to diagnose goat pox virus from field outbreak samples.

Scab materials received by this department during three suspected goat pox disease outbreaks among goat flocks reported during the last two years from Nagapattinam, Metcheri and Salem districts of Tamil Nadu were used for this study. Fifteen such crusted scab and skin samples collected from animals affected with goat pox, which had been stored at -20°C were taken and processed. One gram of tissue was ground with sterile sand and made into 10 percent suspension with phosphate buffered saline. The mixture was clarified at 1800 rpm for 10 minutes and then transferred to a micro centrifuge tube, which was further, subjected to DNA extraction. Known positive sheep pox virus (SPV) and Orf virus infected scab samples were taken as negative controls.

Two hundred microlitre of sample was used for the extraction of DNA using the DNeasy Tissue kit protocol given by the manufacturer (Qiagen, Cat.

No,69504, Germany). A 25 µl PCR reaction mix (12.5 µl of 2x PCR Master mix (Bangalore Genei PCR master mix, Cat. No 105908), 1µl (10 pico.mol) of each primer, 2 µl of DNA template sample and 8.5 µl of water) was prepared. Amplification was carried out as per the method of Ireland and Binopal (1998), which is as follows : an initial cycle of 94°C for 5 min, 50°C for 30s, 72°C for 1 min followed by 34 cycles of 94°C for 1min, 50°C for 30s, 72°C for 1 min with a final elongation step of 72°C for 5 min. The primers used in the PCR assay were specific to the viral fusion protein gene of the goat pox virus (Ireland and Binopal, loc.cit). These primers were synthesized and supplied by Sigma Aldrich Chemicals, India and the primer sequence were :Forward primer: 5' atg gac aga gct tta tca 3' ; Reverse primer: 5' tca tag tgt tgt act tcg 3' Eight µl of each amplified product were analyzed by agarose gel electrophoresis on a 2% agarose (Gibco, BRL) containing 1 µg / ml ethidium in TBE buffer and visualized on an UV transilluminator.

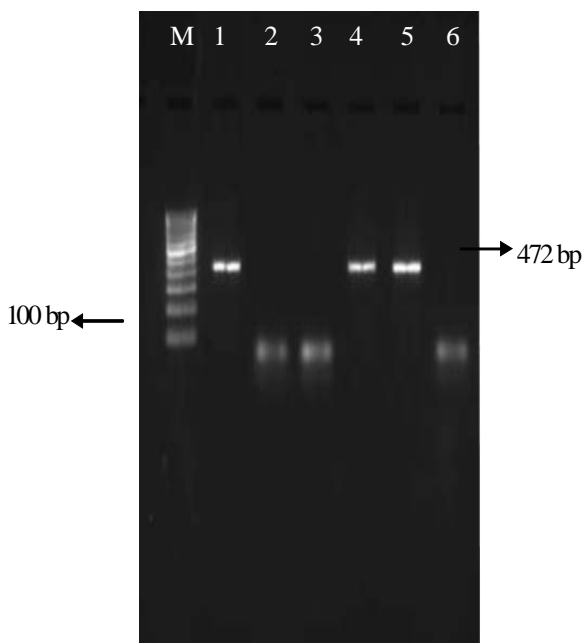
Out of 15 samples tested for GPV, 12 samples were positive by PCR showing the predicted PCR amplicon size of 472 bp (Fig.1). The three samples that did not show the predicted 472 bp bands were taken as negative for GPV infection. Under the PCR condition described in this report the negative control DNAs of sheep pox and Orf viruses that were used in this assay gave negative results. This showed that the primers targeting the viral fusion gene are specific for goat pox and does not cross-react with sheep pox and Orf viruses. However sequencing of these positive goat pox samples and a complete study on the molecular

epidemiology would throw light on the genetic relationship between GPV and SPV. The infections caused by GPV and SPV cannot be distinguished either serologically or on the basis of the clinical signs as the viruses are very closely related. It appears that the host preference shown by these viruses with respect to either sheep or goats, accompanied by the case history may be regarded as partially affirmative for either sheep pox or goat pox, but accurate identification requires laboratory studies (Rao and Bandyopadhyay, 2000). Ireland and Binopal, (1998) used this fusion protein gene along with restriction endonuclease *Dra* I to detect and characterize the goatpox isolates of India, Bangladesh and Iraq. The present study revealed that the amplification of viral fusion protein gene of goat pox virus by PCR assay could be used as a diagnostic tool for the differential diagnosis of goat pox outbreaks.

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Figure 1 : Agarose gel electrophoresis showing PCR amplicons obtained from Goatpox virus positive samples



- M - 100 bp maker
 Lane 1 - Known goat pox virus positive control
 Lane 2 & 3 - Known negative controls
 Lane 4 & 5 - PCR amplicons (472 bp) from goat pox suspected scabs
 Lane 6 - PCR amplification with negative samples