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Research Article

Comparison of DNA-Extraction Methods on Detection of *Capripoxvirus* by *Vap gene*-PCR

Ahmed Zein Elabdeen Mahmoud[1]*, Abdelmalik Ibrahim Khalafalla[2] and Muaz Magzob Abdellatif[3]

[1*]Hail Veterinary Laboratory, Ministry of Agriculture. Saudi Arabia
[2]Abu Dhabi Food Control Authority (ADFCA)
[3]Northern Border University, Saudi Arabia

[*]**Corresponding author:** ahmedzein78@yahoo.com

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ABSTRACT:

Polymerase chain reaction (PCR) have been developed to amplify DNA of viral pathogens as conventional methods may be tedious, laborious, time consuming and/or less sensitive. For comparative investigation, DNA suitability for amplification of CaPV from scab suspension (n=20), cell culture harvest, phenol chloroform and commercial PUREGENE®kit was evaluated for the detection of CaPV by *vap gene*-PCR. PUREGENE®DNA kit demonstrated satisfactory extraction of DNA from all scabs compared to that of the Phenol Chloroform Isoamyl alcohol extraction (75%) and cell culture suspension (50%), scab homogenates used directly as template failed to yield amplifiable DNA. Thus, we recommend the use of PUREGENE®DNA Kit for the extraction of the virus DNA for successful PCR.

Keywords: DNA extraction - Capripoxvirus - vap gene-PCR

INTRODUCTION

Capripoxvirus (CaPV) is the genus of the family Poxviridae[1], it causes a serious economic impact on small ruminant production systems, causing losses in productivity, mortality, damaging skins and hides, as well as inflicting international trade restrictions[2,34]. CaPVs infections are transboundary that affect animal trade and have to be notified to the OIE[3,4,5]. It is distributed in Northern and Central Africa, the Middle East and most of the Asian continent[4,5]. Outbreaks was regularly reported in different parts of the Sudan[6,7,8,9,10,11].

Routine diagnosis is limited because clinical findings can easily be confused with other diseases, virus isolation is time consuming, laborious requiring serial passages, electron microscopy is often not available and serology is hindered by low level of antibody response and shortcomings of the available methods of antigen detection. PCR has become the method of choice for detection and differentiation of GaPVs[12,13,14,15,16]. High quality of DNA represents a challenging step for PCR, during the last decades various protocols have been described

including the phenol chloroform-based approaches[17].

A rapid detection of CaPV would be invaluable for controlling outbreaks as well as for disease surveillance. The aim of this work is to define the adequate, relatively easy, reliable and cheap method to extract high quality and quantity of DNA template for subsequent vap gene-PCR for detection of CaPV.

MATERIALS AND METHODS

Collection of samples and processing

Skin scabs (n=20) were collected from clinically affected sheep and goats at Khartoum State. Scabs were collected aseptically in 50% glycerol for virus isolation and without a preservative for PCR. The homogenates (20%) were centrifuged at 1500rpm for 15min, supernatant was collected into sterile bottles and treated with antibiotics (1000 IU penicillin - 250mg of streptomycin) and 5.000 IU of mycostatin; the fluids were left for 90min at 4°C and then stored at –20°C until used.

Virus isolation

Suspensions were inoculated (0.2ml) into semi confluent primary lamb testis cell culture (LT) prepared as described by Plowright and Ferris[18], incubated for 1 hour at 37˚C. Flasks were refed with Glasgow Modified Eagles Medium (GMEM), monitored for 7 days for appearance of cytopathic effect (CPE), monolayer was then splitted using trypsin-versin solution and the cells were reseeded again. The procedure was repeated for six passages, cultures were then harvested by freezing and thawing three times, centrifuged at 1000rpm for 5min, preserved at –20˚C till used.

Virus neutralization

The alpha neutralization procedure (VNT) was performed as described by Beared[19].

DNA extraction

Phenol-chloroform

Phenol-chloroform extraction was done according to the method described by Tripathy and Reed[20]. Briefly, 200µl of cell lysis solution (10mM Tris-Hcl, pH7.4, 100mM NaCl, 10mM EDTA, 0.5% SDS and 2% β mercaptoethanol) was added to 100mg scab, the mixture was then homogenized, 15µl of protinase K (20mg/ml) was added and incubated at 55˚C for 4 hours. 1.5µl of RNase (4mg/ml) was added, incubated at 37˚C for 15min. DNA was extracted from the digest in the presence of equal volumes of phenol-chloroform-isoamyalchohol (50:2:48). The aqueous phase was removed to another tube containing phenol-chloroform, repeated for three times. The aqueous phase was then transferred to another tube containing 3M Na-acetate in a volume equivalent to 1:10 and an equal volume of isopropanol alcohol was mixed and stored at –20˚C overnight. DNA was pelleted by 13000rpm for 45min at 4˚C, washed with 300µl 70% alcohol. 50µl of DNA hydration was added, incubated at 65˚C for 1 hour and stored at 4°C till used.

PUREGENE®kit

PUREGENE®DNA extraction kit (Gentra System, Minneapolis, USA) was used according to the manufacturer instructions. Briefly, 500µl cell lysis solution was added to 100mg scab, after it was homogenized, 2µl of proteinase K (20mg/ml) was added and the mixture was incubated at 55˚C for 60min, followed by addition of 1.5µl RNase (4mg/ml). the procedure was then completed as described by the manufacturer.

DNA amplification

The sequences of the primers and PCR were used according to Ireland and Binepa[12]. Scab homogenates (20µl) and culture suspension (20µl) were added to the PCR mix minus the polymerase, heated at 100ºC for 10min before amplification, purified DNA (5µl) extracted by Phenol-chloroform and PUREGENE® kit (5µl) were used as templates. Virus strain (0240) kindly provided by the Central Veterinary Research Laboratories (CVRL). Khartoum and Double distilled water (DDW) were used as controls.

Amplicons were separated in 1.5% agarose gel (SIGMA) containing Ethidium bromide (1µl/40ml agarose) (PROMEGA, Madison, USA, 10mg/ml).

RESULTS

Virus isolation

Ten isolates (50%) were obtained onto LT, identified as capripox virus by VNT. CPE appeared at passage four 7 days post inoculation (PI), it reached 90% at passage six 7 days PI.

PCR

Amplification succeed from culture suspensions (50%), phenol-chloroform (75%), PUREGENE® kit (100%) and control positive that correspond exactly to the expected size of the vap-gene (192bp). No band was detected when scab homogenates and DDW were used as templates (Data not shown).

DISCUSSION

Control and eradication of CaPV infections depend on rapid, sensitive and economic diagnostic tool for mass screening of affected animals. Antigen detection assays is less sensitive less reproducible, interpretation of results is more subjective than molecular techniques. PCR is considered one of the best alternatives[12,21,22]. The study was designed to compare the effect of different DNA Extraction on the detection of CaPV by vap gene-based PCR. Virus isolation on LT succeeds at 4th passage 7 days post inoculation from scabs (50%). Mangana-Vougiouka[21] declared that CaPVs isolation is difficult; it grows slowly or requires additional passages, even if cultured in most sensitive LT. PCR was performed using scab suspension and culture harvest directly, purified DNA extracted by phenol-chloroform and PUREGENE®kit as templates. No product was detected when scab homogenates were used as template, amplicons were visualized from culture suspensions (50%), phenol-chloroform (75%) and PUREGENE®kit (100%). The results obtained correspond with developed PCR[13,14,15,23,24,25.] However, our findings are contrary to that published by Ireland and Binepal[12] who succeed in amplification from biopsy and cell culture, which may be attributed to the use of Gene Releaser or to the variation in viral titer in clinical samples used. According to the present study we recommend the use of PUREGENE®kit for successful vap gene based-PCR for detection of CaPV.

CONCLUSION

DNA extraction is essential for successful *Vap gene*-PCR for detection of *Capripoxvirus*. According to the present study PUREGENE®kit is preferred for nucleic acid extraction.

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