

Short Communication

POLYMERASE CHAIN REACTION (PCR) DETECTION OF THE GENOME OF AFRICAN SWINE FEVER VIRUS (ASFV) FROM NATURAL INFECTION IN A NIGERIAN BABY WARTHOG (*Phacochoereus aethiopicus*)

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INTRODUCTION

The National Veterinary Research Institute, Vom procured baby warthog (*Phacochoereus aethiopicus*) caught by hunters in the forest of Adamawa state of Nigeria to be screened for the presence of genome to African swine fever virus (ASFV). The Spleen, lymph nodes and liver tissues obtained from the warthog were accessed for the presence of African swine fever virus genomic DNA by the polymerase chain reaction (PCR). DNA extracted from these tissues were amplified for a region of ASFV DNA genome by the PCR using specific primers of 20bp (base pair) from a highly conserved region of the ASFV DNA genome. The amplified genome was visualized following gel electrophoresis of PCR products using 1.5% agarose gel in 1x tris-acetate buffer (TAE buffer). A single, discrete and specific band of expected size (278bp) when measured against 200bp (base pair) DNA molecular marker (Roche) was observed from all the three tissues tested. The detection of these bands confirms virus presence. This finding therefore, provides current evidence of the presence of ASFV in Nigerian warthogs. African swine fever virus (ASFV), the causative agent of African swine fever (ASF) is a unique, complex, icosahedral DNA arbovirus belonging to the genus asfivirus in the family asfaviridae (Dixon *et al.*, 2000). The virus multiplies in the cytoplasm of the infected cells. In nature, maintenance and transmission of this virus involves the cycling of virus between argasid soft ticks (*Ornithodoros moubata* complex) and wild pig population (warthogs and bush pigs) in sub-Saharan Africa. This association is unlikely ever to be eliminated, hence making it potentially very difficult to eradicate the disease in Africa. And it appears that this continent will be the main reservoir of ASFV for the foreseeable future (Majiyagbe *et al.*, 2004). The ASFV genome comprised of a linear double stranded DNA molecule which are covalently closed at both ends by a 37 nucleotide-long hair pin loop composed almost entirely of partially paired adenine (A) and thymine (T) residues (Dixon *et al.*, 2000). Depending upon the virus isolate, the size of the DNA molecule oscillates between 170 and 190-kilo base pair (Kbp) (Murphy *et al.*, 1995). ASF, a highly significant disease of domestic swine occurs in several disease forms ranging from per acute to sub-clinical or inapparent forms (Mebus and Dardiri, 1980 and Mebus, 1988). The disease entered Nigeria around 1997 through the southwestern region of the country most probably through cross border contacts with infected pigs/or pig products smuggled through the Nigerian border from neighboring Benin Republic who had earlier reported the outbreak of the disease (Anon, 1998a). Within a short period the disease became widely distributed through pig producing areas of Nigeria (Majiyagbe, 1999 and Luther, 2001) and has been causing a lot of devastation to the national pig herd. The present situations of ASF in Nigeria revealed that virtually all the pig producing centres are affected. Of the 36 states of the Federation, the disease has been confirmed through laboratory test in 18, covering the south-west, south-east and central states of Nigeria (Majiyagbe *et al.*, 2004). Fast and accurate detection of ASF outbreaks is required to limit the spread of the disease because the disease resembles several other haemorrhagic diseases of pigs both clinically and pathologically. The application by PCR for the detection of regions of ASFV genomic DNA presents a sensitive and specific method of identifying the virus. The technique consists essentially of the enzymatic synthesis of millions of copies of a target DNA sequence (Saiki *et al.*, 1985). Using a thermostable DNA polymerase (Saiki *et al.*, 1988), and a succession of cycles that includes denaturation of the template DNA, hybridization of specific DNA primers to the template

and extension of the primers, it becomes possible to enzymatically generate several copies of the target region. Using this highly sensitive technique, it became possible to detect the presence of ASFV genomic DNA in a Nigerian warthog. This communication herein reported is the first documented report of the detection of ASFV from a Nigerian warthog reported hitherto only in eastern and southern-African countries (Scott, 1965 and Plowright *et al.*, 1969).

KEY WORDS: PCR, ASF, ASFV, Oligonucleotide primers, Warthog

MATERIALS AND METHODS

The warthog was purchased from professionally licensed hunters resident in Adamawa state of Nigeria which has a total land area of 42,159 square kilometers and located between latitude 11° and 8° North of the Equator and Longitude 11° 42' West and 13° 34' East (Anon, 1992b). The vegetation zone is the sub Sudan zone, which is marked by short grasses, interspersed by short trees commonly found in the northern part and the northern Guinea Savannah zone (Anon, 1992b). Each piece of the warthog's tissues (spleen, lymph nodes and liver) was ground separately in a sterile mortar. A 1:10 dilution of each macerated tissue sample was made in high-performance liquid chromatography grade water (HPLC H₂O) and centrifuged at 2000 rpm for five minutes. Thereafter, an aliquot of 100µl of the supernatant for each tissue was taken and transferred separately into sterile 1.5ml Eppendorf tube and stored at -20°C until used.

DNA extraction was done using guanidinium thiocyanate method as modified by Bloom *et al* (1990). Reagents and buffers used for both the DNA extraction and PCR were of molecular grade and sourced from Onderstepoort Veterinary Institute for Exotic Diseases, Republic of South Africa. Preparation of PCR master mix with final reaction volume of 25µl for each extracted DNA was done using special PCR tubes as follows: 2.0µl of extracted DNA template, 17.5µl of HPLC H₂O, 1.0µl of both up and down stream oligonucleotide primers, PAS₁, and PAS₂ respectively (with the following sequences PAS₁, 5'-ATG GAT ACC GAG GGA ATA GC-3'; PAS₂, 5'-CTT ACC GAT GAA AAT GAT AC-3'), 2.5µl of 10 x buffer (50mM KCl, 10mM Tris HCl (pH 8.3) and 1.5mM MgCl₂), 0.5µl of enzyme taq polymerase (S.A Taq-MD (PTY) Ltd. South Africa) and 0.5µl 100mM deoxyribonucleosides triphosphates (dNTPs). Each tube was overlaid with 60.0µl of mineral oil

so as to prevent evaporation of the reaction mixture during PCR. Each PCR master mix of each tube was placed in an automated PCR thermal cycler (Techne Cyclogene, FPHC3CD) for amplification for 30 cycles as follows: Denaturation at 94°C for 15 seconds, annealing at 62°C for 15 seconds and extension at 72°C for 15 seconds. Gel electrophoresis of 3.0µl of each amplified PCR products was carried out using 1.5% agarose gel in 1X TAE buffer containing ethidium bromide at a final concentration of 0.5 µg/ml, and 2.0µl of 0.25% orange G in aqueous solution of 30% glycerol was used as loading buffer. Electrophoresis was carried out for 20 minutes at a constant Voltage of 120 millivolts. Finally, the gel was visualized under ultraviolet (UV) light and photographed using a Polaroid MP4 land Camera (D11105B).

RESULTS AND DISCUSSION

Single discrete and specific bands of expected size (278bp) when measured against a 200bp (base pair) DNA molecular marker (Roche) were observed from all the three tissues tested as represented in figure 1. Band of this size specificity was also seen in the known positive control tissue of Lane 6. However, the negative control tissue in Lane 5, and the water control of Lane 4, as expected, produced no band. This result was reproducible as the PCR was repeated at least three times and bands of this size specificity were observed in all the three assays.

The association of African swine fever virus with wild squids in Africa is an established age-long relationship of host-parasite association whereby prolonged infection occurs without any manifestation of the disease. ASFV has been recovered from wild pigs mainly

warthogs in countries of the south and east African Sub-regions (Scott, 1965 and Plowright *et al.*, 1969) and less frequently from red river hog (*Potamochoerus porcus*) and giant forest hog (*Hylochoerus meinertzhageni*) (Heuschele and Coggins, 1965 and Detray, 1967). Cox (1963) reported the isolation of ASFV from hippopotamus, porcupine and hyena.

Recently, Luther *et al* (2007) reported the incidental detection of ASFV genomic DNA from natural infection in a Nigerian red river hog (*Potamochoerus porcus*) at the Jos wildlife conservancy. Before this finding, previous available information on the study of ASFV carried out on Nigerian warthogs and bush pigs was an attempt to isolate ASFV and detect antibodies to ASFV from these species at the Yankari National Park (Taylor *et al.*, 1977); no virus nor antibodies to ASFV was found. Since then, changes may have occurred in the

epidemiology of the disease in Nigeria. From the findings observed in the Nigerian bush pigs, the interest of the authors was further stimulated to investigate the presence of ASFV in the warthogs. In this preliminary study, we were able to detect for the first time the genomic DNA of ASFV in Nigerian warthog using PCR. This finding as reported here is therefore very significant even though it involved only one warthog. It is the authors considered opinion therefore, that a similar survey of Nigerian warthogs be undertaken in an extensive manner to cover a larger area of the country in order to determine their involvement in the epidemiology of ASF in Nigeria. This research area will certainly open up a rich field for investigation as large numbers of warthogs and bush pigs abound in Nigeria whose disease status and degree of parasitism by soft ticks (*Ornithodoros moubata*) is unknown. Further studies are in progress in an attempt to isolate the virus.

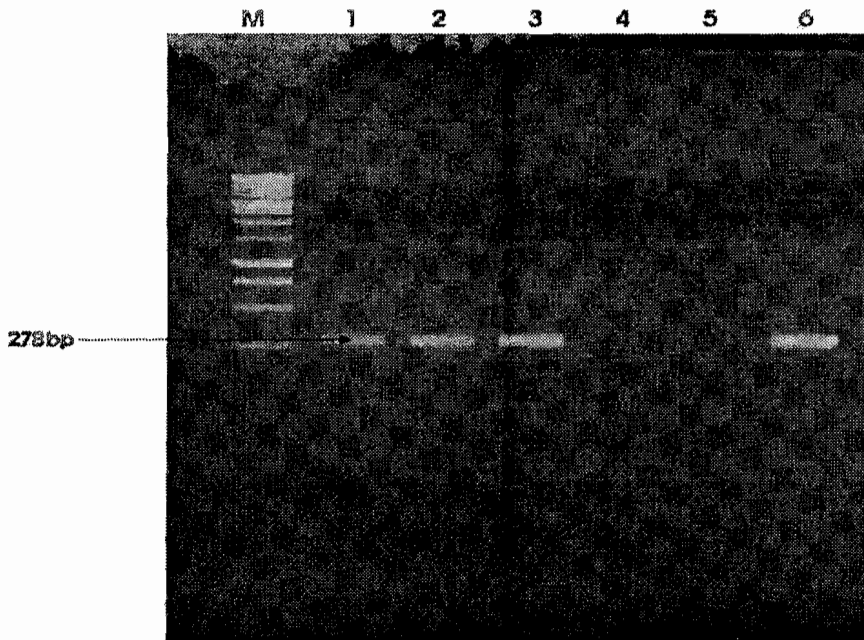


Fig 9: PCR fingerprints of ASF Virus

Lane M: 1kb Ladder, Lane 1-3 Tissue samples
Lane 4: Water control Lane 5 negative control
Lane 6: Positive control

Fig 1: PCR Analysis of DNA Extracted from Tissue Samples of Baby Warthog Showing a Single, Specific Band for all the 3 Tissues Tested (Lanes 1-3) when Measured Against a 200bp Molecular Weight Marker (Lane M)
Lane 1 Spleen, Lane 2 Prescapular Lymph node, Lane 3 Liver
Lane 4 Water Control, Lane 5 Negative Control, Lane 6 Known Positive Control (Dominican Republic, DR-1 Gene-bank accession number L27498)

Since it was only the tissues that were processed for the PCR, no serological test was carried out to detect antibodies for the virus. However, in southern and eastern Africa, extensive serological work carried out (Thomson *et al.*, 1983; Thomson, 1985 and Plowright *et al.*, 1994) showed that seroconversion rates in warthogs varied from one area to another, and that high rate of seropositivity were not always associated with the presence of *Ornithodoros moubata* complex ticks.

Therefore, it would not be safe to assume that on the basis of failure to find virus in the tissues of Nigerian warthogs or bush pigs by Taylor *et al* (1977) that Nigerian warthogs or bush pigs are less important in the maintaining of ASF virus. We suggest that it is necessary to examine more of these wild suids from different areas of Nigeria before they can be ruled out as a possible reservoir of the virus. This is particularly so as there is a need to establish the role of this wild animal species in the epidemiology of ASF in Nigeria. Such studies are extremely important as they may reveal the presence of more ASF-endemic foci in Nigeria. The existence of such new foci would then obviously modify the approach of outbreak investigation, prevention and control.

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