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Prevalence of African swine fever virus in Warthogs in the Serengeti ecosystem, Tanzania

Bugwesa Z. Katale, Robert D. Fyumagwa, Maulid L. Mdaki, and Richard Hoare

Tanzania Wildlife Research Institute (TAWIRI) Veterinary Programme P.O BOX 707, Arusha, Tanzania

Abstract

African swine fever (ASF) is an endemic disease of swine in Tanzania, involving complex transmission cycles between domestic pigs, African wild suids and ticks. ASFV usually induces an asymptomatic infection in wild African suids. The two wild herbivores are ubiquitous due to extensive protected areas in the country of about 30% of the land cover. A study was conducted to evaluate the epidemiological situation and status of exposure of warthogs to ASFV in this natural park. Whole blood was collected from immobilized animals and organs from hunted animals in the Serengeti ecosystem. Serum was tested for ASF antibodies by indirect ELISA (OIE-ELISA) and later on confirmed using immunoblotting (OIE-IB). Tissue specimens including EDTA blood and organs, from ELISA antibody-positive samples were analyzed by polymerase chain reaction (PCR) for detection of ASFV genetic material. Results from indirect ELISA showed that 100% (34/34) of warthogs in the ecosystem were sero-positive against ASFV. Furthermore, analysis of organs from sero-positive samples by PCR showed that, 8.8% (3/34) of the warthog population showed a weak positive result. However no virus could be isolated in serum and tissues after three passages in cell cultures. Results obtained adds a confirmation for the ASFV presence in the Serengeti Ecosystem, and correlate to the previously described situation in eastern African countries where serological studies indicate a high prevalence of ASF in adult warthogs due to a previous exposure to ASFV, although they are typically nonviremic.

Keywords: African swine fever virus, Serengeti ecosystem, warthogs

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Introduction

African swine fever (ASF) is a highly contagious viral disease of domestic and wild suids of all breeds and ages. It is caused by a complex large enveloped DNA virus of 170 to 190 kbp in size, belonging to Asfarviridae family (Dixon et al., 2005). The disease has a significant socio-economic impact constraining pig keeping by livestock farmers in Sub-Saharan African countries, including Tanzania. In eastern African countries, the disease occurs through complex transmission cycles involving domestic pigs, soft ticks and wild African pigs, warthogs (*Phaecochoerus africanus*) (Lubisi, 2005). However, the role of the bush pig in the epidemiology of ASF if any has not been elucidated (Anderson et al., 1998; FAO, 2001; Jori & Bastos, 2009; Costard et al., 2009; Ravaomanana et al., 2011). Soft ticks (*Ornithodoros moubata*) also act as reservoirs and vectors for virus transmission (Jori & Bastos, 2009).

ASFV usually induces an asymptomatic infection in the wild African pigs (Colgrove et al., 1969; Mebus, 1988; Plowright et al., 1994; Zsaki et al., 1998). These suids and soft ticks serve as natural host and source of infection to domestic pigs (Jori & Bastos, 2009). The consequences of the disease are devastating. Acute forms result in lethal haemorraghic disease and high mortality rate, few days after infection. Mortality due to ASFV may reach up to 100% causing important economic losses to the pig smallholders in rural areas, representing a major challenge to the development of these countries.

Corresponding author: Bugwesa Z. Katale, Tanzania Wildlife Research Institute (TAWIRI) Veterinary Programme P.O BOX 707, Arusha, Tanzania

The sylvatic cycle between warthogs and argasid ticks has been reported from Tanzania (Plowright et al., 1969) where ASF virus has been detected in warthogs (Plowright, 1977; Thomson 1985; Plowright et al., 1994). Warthogs (*Phacochoerus africanus*) and *ornithodoros* soft ticks vector constitute the ancient sylvatic virus cycle (Penrith et al., 2004a; Jori & Bastos, 2009). In Tanzania, ASFV transmission depends on maintenance of ASFV within and between the sylvatic (in wild suids) and domestic pig cycles. The first outbreak of ASF was reported in 1914, followed by second outbreak in 1962 (www.fao.org). From onward, outbreaks of ASF have emerged or re-emerged on a regular basis with major outbreaks in different regions. Major outbreaks with economic losses have occurred in Mbeya (2001), Arusha and Arumeru districts (2003), Dar es Salaam (2001), Kigoma (2004). Molecular epidemiological data indicated that outbreaks in Dar es Salaam, Mbeya, Kigoma and Arusha were caused by different clusters of ASF viruses and that ASF is not widespread in Tanzania (Wambura et al., 2006). Furthermore, phylogenetic analysis and sequence data of the isolates from the outbreak in Mbeya and Dar es Salaam in 2001 indicated 99% homology to virus isolates from Malawi in 1978, Mozambique in 1998 and Zambezi in 2001 with no evidence on the source of the ASFV that caused outbreak in Arusha region, Tanzania (Wambura et al., 2006). The presence of isolated epidemiological cycles in East Africa and recovery of multiple genotypes verify the epidemiological complexity of ASF in this region (Lubisi, 2006). Epidemiologically, as in other eastern African countries, wild suids and soft argasid ticks is though to be of significant importance in the transmission of ASFV to domestic pigs in Tanzania. In regions where *Ornithodoros* soft ticks are present, the detection of ASFV in these reservoirs contributes to a better understanding of the epidemiology of the disease and establishment of effective control and eradication programmes (Basto et al., 2006). Some studies have been carried out to date to improve knowledge about ASFV persistence in ticks and wild suids population in Africa (Montgomery, 1921; De Tray, 1963; Plowright et al., 1969; Plowright, 1977; Thomson, 1985; Plowright et al., 1994; Ravaomanana et al., 2011). Apparently, the role of the bush pig in the epidemiology of ASF if any has not been elucidated (Anderson et al., 1998; FAO, 2001; Jori & Bastos, 2009; Costard et al., 2009; Ravaomanana et al., 2011). Literature on wild pigs and AFS is however dispersed and scattered, and information sources amalgamating the epidemiological role of different species are presently lacking (Jori & Bastos, 2009). The persistence infection/exposure status of warthogs to ASFV in protected areas of Tanzania is unknown. In this paper, an epidemiological investigation has been performed to evaluate the ASFV presence and exposure status of warthogs to ASFV in the Serengeti ecosystem, Tanzania.

Serengeti National Park (SNP) and Ngorongoro Conservation Area (NCA) are part of the Serengeti ecosystem which occupy an area of 25,000 km² and is defined by annual movement of herds of ungulates interacting with high population of livestock. The ecosystem lies between 1° and 2° S; 34° and 36° E and has a diverse habitats ranging from vast open grassland plains to acacia straw savanna and rock outcrops. SNP (14,763 km²) is a protected area. Human settlement is not allowed except for park and lodges staff and tourists. The park is situated on the north-western edge of NCA stretching almost to the shores of Lake Victoria and extends up north to the border with Kenya and is contiguous to Maasai Mara National Reserve (Fig. 1). Two third of the park (northern and western) is bush or woodland and is the centre of the Serengeti ecosystem and roughly defined by the annual wildebeest migration. The southern part is dominated by short grass plains and the northern and western part is bush or woodland. The Ngorongoro Crater, the world's largest intact, inactive caldera occupies approximately 300 km^2 (4%) of the total NCA and is a multiple land use area where livestock, humans (mainly Maasai pastoralists) and wildlife legally interact. The rim is 2200m above sea level and the crater floor (250 km^2) is about 600m below, composed predominantly of grassland with small patches of swamp and A*cacia* riverine forest. Although a geographically distinct unit, it is part of the greater Serengeti ecosystem.

Animal restraint and samples collection

A total number of 34 warthogs were randomly selected in target areas in the Serengeti ecosystem in 2004. Warthogs were restrained chemically by using etorphine hydrochloride (M99®) at 2-4 mg in combination with azaperone at 50-80 mg depending on body weight. Immobilized animals were reversed using natrexone hydrochloride at 5-10 mg (Kock et al., 2004). Blood samples were collected from immobilized animals in 10 ml plain tubes for serum, 5 ml EDTA tubes and FTA cards. Moreover, 14 tissue samples that include spleen, liver, kidney, and lymph nodes were collected opportunistically from naturally dead or road killed animals. Tissues, serum and whole blood samples were frozen at -196ºC in liquid nitrogen prior to submission for laboratory analyses to CISA-INIA, Valdeolmos, Madrid, Spain (FAO and European reference Laboratory for ASF).

Sample analysis

ASF antibody detection on 34 serum samples was performed using OIE prescribed antibody detection techniques as described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chapter

2.8.1. OIE, six edition, 2008). Briefly first screening was done using OIE- indirect ELISA using semi purified virus (E70) produced in MS cells as coated antigen and protein-A labelled to HRPO as indicator. Positive and doubtful results were confirmed by OIE Immunoblotting (OIE-IB). In addition, INGENASA ELISA commercial kit Ingezim PPA Compac (11.PPA k3) used was based on the use of the ASFV protein p73 as antigen.

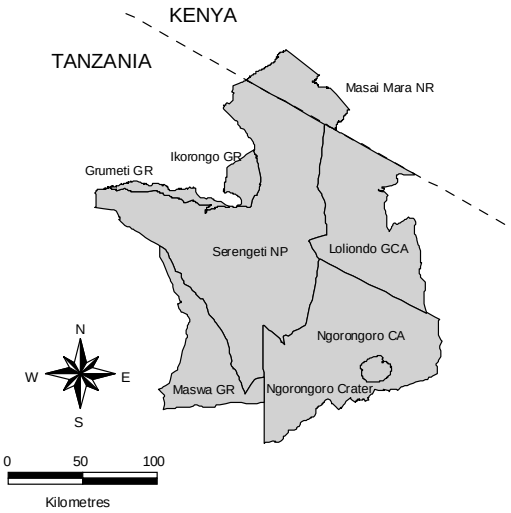

Fig 1: Map of Serengeti ecosystem showing the Serengeti National Park, Ngorongoro Conservation Area, Ikorongo-Grumeti Game Reserves, Maswa Game Reserve, Loliondo Game Controlled Area.

ASF virus detection was performed by p72-based diagnosis polymerase chain reaction (PCR) described by Aguero et al 2003. DNA was extracted directly from serum, whole blood, and 10% suspensions of each tissue using a nucleic acid extraction kit following manufacturer's instructions (High Pure template extraction kit ROCHE). A polymerase chain reaction (PCR) assay using the ASF diagnostic primers PPA1/PPA2 which generates an amplicon of 257 bp within the p72 protein was used to confirm the presence of ASFV DNA (Aguero et al. 2003). The PCR products were analyzed by electrophoresis through 2% agarose gels and the specificity of the amplicons obtained was confirmed using the *BsmAI* restriction endonuclease.

Porcine alveolar macrophages cultures were used for the isolation of PCR positive samples as previously described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Chapter 2.8.1. OIE, six edition, 2008). Briefly, cells were seeded into 96-well tissue culture grade microtitre plates (100µl; 200,000 cells per well) in Dulbecco's modified Eagle's medium (DMEM) containing 20% foetal bovine serum and were infected at a multiplicity of infection (moi) 1:10 with PCR positive serum or 10% suspensions of homogenenised PCR positive tissues supplemented with 5 µg/ml gentamicine sulphate (BioWhittaker). After inoculation, a preparation of 1% homologous red blood cells in buffered saline was added to each well and the plates were incubated for 7 days in a humidified atmosphere containing 5% CO2 at 37°C. The plates were examined for haemadsorption over a 6 day period.

Results

As expected, infected warthogs showed no clinical signs of the diseases or post mortem lesions indicative of ASF (Thomson et al., 1980; Jori & Bastos, 2009), since the fact that they do not show any pathogenic effects that has been well documented. Among the 34 serum samples collected in target area in Serengeti National Park, all warthogs serum samples (34/34) showed positive antibodies response to ASFV using OIE prescribed ASF antibody detection techniques ELISA plus IB. Same result was obtained by the analyses of the samples using the blocking INGENASA commercial kit.

For ASFV detection, PCR (Aguero *et* al., 2003) was performed on 34 serum samples, 12 blood samples and also 14 individual tissues (4 mesenteric lympnodes, 5 spleen, and 5 kidney samples) from sampled animals. Following, PCR amplification, a single major amplicon of approximately 260 bp was generated from 3 of the 34 (8.8%) sera and 4 of the 14 tissue samples (4 mesenteric lympnodes, 5 spleen, and 5 kidney samples) examined. No positive result was obtained from the analyses of blood samples.

Attempts to isolate ASFV were performed by inoculation of cells derived from 2 PCR-positive tissues (2 kidneys) and 3 PCR-positive sera into porcine peripheral blood macrophages (PBM) cells. No virus could be isolated after three passages from PCR positive warthog serum and 2 kidney PCR positive samples.

Discussion

African swine fever virus (ASFV) does not cause clinical disease in wild suids, however, surveillance in warthogs/bushpigs in protected areas is important for understanding the epidemiology of the disease for prevention and control of the disease in domestic pigs. It is well known that warthogs are natural reservoirs for ASFV (Jori & Bastos, 2009), the considerable viral replication and viremia occurs in young animals (Thomson et al., 1980). In Tanzania, the role of warthogs as source of infection to domestic pigs around protected areas is still unknown. Lack of epidemiological role of different wild suids for transmission of ASF poses a challenge in disease

control. Findings from this study showed that all warthogs had positive antibodies against ASFV. Interestingly, only three ELISA sero-positive samples out of 34 were PCR weak positive. The amplification of ASFV nucleic acid from 14 tissue samples using PCR revealed two tissue samples from kidney were positive. However, no virus could be isolated after three passages in cell cultures. Previous study by Reis et al. (2007) revealed that positive detection of antibodies signifies previous exposure and not necessarily a current infection.

The Indirect ELISA is a sensitive diagnostic test, but it does not differentiate between past and current infection. In this study, all serum samples that tested positive for ELISA were also positive for OIE-immunoblotting. Results from this study are similar to the findings from Pastor et al. (1992) who reported that, all the ELISA positive sera were also positive by rapid dot immunobinding assay and Immunoblotting. Therefore, ELISA is still a promising diagnostic test for ASF. However, it is very important to subject animals that show a serological response against ASFV to PCR to detect ASF virus nucleic acid to see whether ASF virus could be isolated.

The findings from this study correlated with previous well defined situation in eastern African countries where serological studies indicate that in many warthog populations most of adult warthogs are sero-positive (Heuschele & Coggins, 1969; Jori & Bastos, 2009) and persistently infected although are seldom viremic. In this study, the ASF antibody prevalence in warthogs was 100%. A previous study by Reis et al. (2007) reported that, the immune response of domestic pigs to ASFV is complex and it involves both cellular and serological components. However, complete protection against ASFV requires a cellular component of the immune response (Reis et al. 2007).

High exposure status among warthogs to ASFV suggests that the infection rate is very high in warthog populations in Serengeti ecosystem. These findings add information to previously conducted study in the Serengeti ecosystem by Plowright *et al*., (1969) that ASF virus has been detected in wild pigs, warthogs and soft ticks since the 1960s. The results also suggest that domestic pigs adjacent to protected areas in northern Tanzania are at a very high risk of contracting the disease. It is speculated that, domestic pigs might be infected through remains of hunted warthogs including the skin being fed to domestic pigs (Thomas et al. 1980) as they may be some lymphal ticks on the skin.

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