RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
ISSN 2221-1896
www.roavs.com

Prevalence of African swine fever viral antigens in slaughter pigs at Nalukolongo abattoir, Kampala, Uganda

P. Sajjakambwe[1], J. Okwee-Acai[2] and L. Ojok[1]

[1]Department of Veterinary Pathology, [2]Department of Veterinary Surgery & Reproduction, School of Veterinary Medicine, Makerere University, P. O Box 7062, Kampala Uganda

Abstract

The underdevelopment of the African pig industry is widely attributed to African swine fever (ASF). Outbreaks of the disease occur in different parts of Uganda almost annually although cases are rarely confirmed. We conducted an abattoir based survey of ASF associated lymph node lesions to establish the status of the disease in apparently healthy pigs. Highly suspicious lesions were subjected to immunohistochemistry for viral antigen detection. Most lymph nodes with follicular necrosis, parenchymal haemorrhage and lymphoid depletion were positive to ASF antigens. Up to 22 (0.1%) of the 258 pigs from which samples were collected were positive to ASF viral antigens. We conclude that domestic pigs in Uganda can act as reservoirs of the disease i.e. sustenance of the disease in pig populations may not be entirely dependent on the sylvatic cycle.

Keywords: Uganda, African Swine Fever, Slaughter Pigs

Introduction

Agriculture is the main stay of Uganda's economy. The national Agricultural contribution to GDP is about 30% while 85% of total exports are agro-based (Niringiye, 2009). Livestock production is an important component of Uganda's agricultural sector contributing 17% of agricultural GDP (Niringiye, 2009). Pig production is a key component of the livestock industry; especially in the subsistence sub-sector of the economy (Nissen et al., 2011).

Globally, pig production plays a key role in food security especially, amongst the urban poor (Speedy, 2003). Pork is actually, one of the cheapest forms of animal protein available to the less privileged urban communities (Delgado, 2003). Development of the pig industry is therefore vital in improving the plight of the poor in urban and peri-urban settlements globally.

The biggest challenge to development of the African pig industry is posed by African swine fever (Wilkinson, 1984; Jori and Bastos, 2009). African swine fever (ASF) is a serious and notifiable viral disease of pigs, endemic in Africa (Mebus, 1988; Jori and Bastos, 2009). The swine fever virus is highly contagious, and spreads rapidly in pig populations by direct or indirect contact (Costard et al., 2009). This DNA virus persists for long in pig products and the environment. During outbreaks, morbidity and mortality may reach as high as 100% (Penrith and Nyakahuma, 2000; Babalobi et al., 2007).

Sporadic outbreaks of ASF are reported in Uganda almost annually but cases are usually not confirmed in reference laboratories (Vizcaíno, 2007). For instance, in 1998, an estimated 60,000 pigs were lost to a single outbreak of the disease in Eastern Uganda. This particular outbreak is feared to have spread to neighbouring Kenya (Rutebarika and Nantima, 2002). Since there is no vaccine available for control of ASF, strict zoo-sanitary measures are essential in prevention and containment of outbreaks (Penrith and Vosolo, 2009). Such measures must be supported by a well planned and executed national epidemiological surveillance programme (Penrith and Vosolo, 2009). In Uganda, however, standard diagnostic techniques are not in place hence, suspected ASF cases are diagnosed principally on the basis of clinical signs and post-mortem lesions. The few confirmed outbreaks are based on samples sent to reference laboratories in Europe (Vizcaíno, 2007).

Because of lack of diagnostic capacity within the country, almost no epidemiological surveillance is being done to monitor and control ASF in Uganda. The status of the disease in the Ugandan pig populations is therefore not accurately known. We hence, conducted an abattoir based survey of ASF associated lymph node lesions as a rapid screening technique. Lesions so

consistent with ASF were subjected to immunohisto-chemistry for confirmation of ASF viral antigen.

Materials and Methods

Lymph node samples were purposively collected from a total of 258 pig carcases at Nalukolongo Pig abattoir in Kampala, Uganda. This abattoir receives slaughter pigs from different regions of Uganda. The samples were collected from January to March, 2009. Samples were collected from all carcases on each day of sampling. The inguinal, mesenteric and renal lymph nodes were particularly targeted since they are easily located without mutilating the carcass. Each lymph node sample was placed in a transparent polythene bag and labelled with a laboratory number and place of origin of the slaughter pig. The labelled samples were immediately put in a cold box for transportation to the Pathology Laboratory, School of Veterinary Medicine, Makerere University, Kampala for gross, histopathological and immunohistochemical analysis.

Grossly, the lymph nodes were examined by visual observation and palpation (Herenda, 1994). Any gross lesions such as gross enlargement, haemorrhage, congestion and consistence on palpation were noted. After gross examination, reasonable tissue samples were trimmed off each lymph node and fixed in 10% buffered formalin for histopathological analysis (Luna, 1968). Paraffin wax embedded tissue sections were stained using H&E and microscopically examined (Luna, 1968).

Samples with gross and histopathological changes consistent with ASF were processed for immunohistochemical analysis. Paraffin-embedded tissue sections were placed on a water bath at 42^{o}C for 2-5 minutes. They were then picked using adhesive coated microscope slides (086-PLUS, SDL, Illinois, USA). Antigen retrieval and immunohistochemical staining of tissue sections were based on the microwave oven heating technique described by Shi et al., (1991). Primary polyclonal ASF antibodies (ASF antiserum, Plum Island, USA) were used.

Statistical analysis

Data was entered and validated in Excel spreadsheets. It was then exported to SPSS for analysis. Descriptive statistics were generated using the frequency procedures of SPSS.

Results

Majority (68%) of slaughter pigs originated from the central Uganda districts of Kayunga, Masaka, Kiboga, Sembabule and Luwero. Only 32% of the pigs originated from the eastern districts of Jinja and Soroti (Table 1). There were no slaughter pigs originating from the northern and western regions of the country.

Haemorrhage (34.4%) and necrosis (29.4%) were the commonest gross lesions seen (Table 2). Some lymph nodes showed atrophy (12.4%), oedema (6.6%) and gross enlargement (2.4%). Hemorrhagic lymph nodes were distinctly deep red in colour (Figure 2).

Table 1: Origin of slaughter pigs at Nalukolongo abattoir

Region	District	Percentage by District (%)	Percentage by Region (%)
Central	Kayunga	19	68*
	Kiboga	16	
	Luwero	04	
	Masaka	18	
Eastern	Jinja	16	32
	Soroti	16	

*Most slaughter pigs at Nalukolongo abattoir originated from Central Uganda

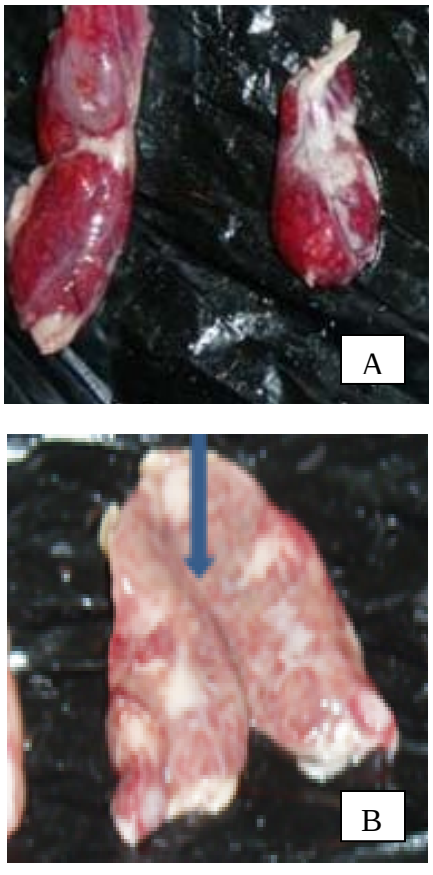

Figure 2: Photograph showing hemorrhagic lymph nodes

Note the characteristic reddening of the node (A) and diffused bleeding in the parenchyma (B)

Table 2: Prevalence of gross lesions in slaughter pigs at Nalukolongo abattoir

Gross Lesion	Prevalence (%)
Haemorrhage	34.4*
Necrosis	29.4*
Atrophy	12.4
Oedema	6.6
Gross enlargement	2.4

*Haemorrhage and necrosis were the most prevalent gross lesions

On histopathology, parenchymal hemorrhage (50.8%), follicular necrosis (48.8%), follicular hyperplasia (48.1%), sub-capsular hemorrhage (37.6%) and lymphoid proliferation (33.3%), were common. Eosinophilic infiltration (18.6%) and lymphoid depletion (8.5%) were also seen. Immunohisto-chemistry showed that most lymph nodes with follicular necrosis (86.4%), parenchymal haemorrhage (81.8%) and lymphoid depletion (63.6%), were positive to ASF antigens (Table 3). The photomicrographs (Figure 2) show a typical parenchymal haemorrhage and positive immunohistochemical response.

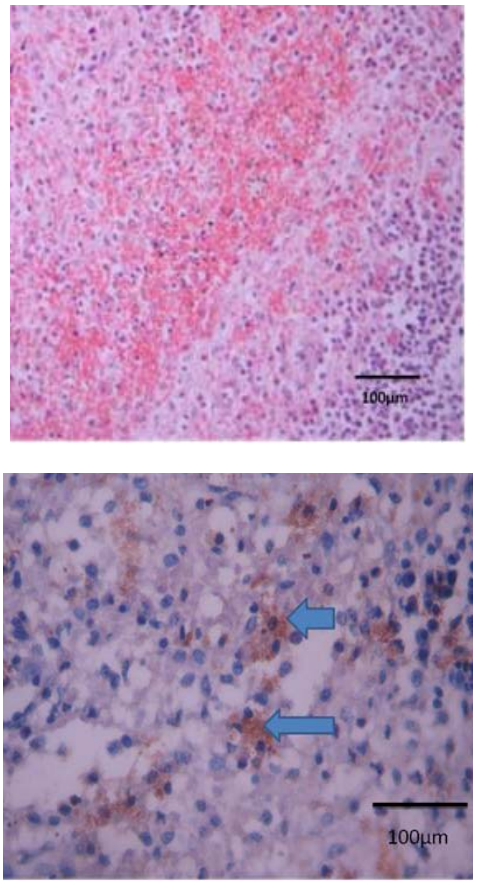

Fig. 3: Photomicrograph showing haemorrhage and a positive immunohistochemical response.

Note the diffused bleeding (left photo) and the antigen-antibody reaction sites (arrows).

Table 3: Prevalence of histopathological lesions

Histopathological Lesion	Frequency (%)	No. positive to ASF antigens (%)
Follicular necrosis	48.8	86.4*
Parenchymal hemorrhage	50.8	81.8*
Lymphoid depletion	8.5	63.6*
Sub-capsular hemorrhage	37.6	36.4
Follicular hyperplasia	48.1	09.1
Lymphoid proliferation	33.3	04.5
Eosinophilic infiltration	18.6	00.0

*Most lymph nodes with follicular necrosis, parenchymal haemorrhage and lymphoid depletion were positive to ASF antigens

Up to 22 (0.09%) of slaughter pigs at Nalukolongo Abattoir tested positive to ASF antigen (Figure 5). The regional prevalence were: central (0.081%) and eastern (0.094%) as shown in Table 4.

Table 4: Prevalence of ASF antigens in slaughter pigs at Nalukolongo abattoir

Region	Prevalence (%)
Central region (n=173)	0.081
Eastern region (n= 85)	0.094
Overall prevalence (n=258)	0.090

Discussion

Most (68%) of the pigs examined at Nalukolongo abattoir originated from central Uganda. Traditionally, Central Uganda is known to have the highest pig populations than any region of Uganda (UBOS, 2008). Actually, almost half of the 3.2 million pigs in Uganda are kept by farmers in the three central Uganda districts of Kampala, Mukono and Masaka (UBOS, 2008). The current study was conducted at Nalukolngo abattoir which itself is located in the central region. This is far off from other regions, especially northern and south-western Uganda and may explain these regions were not represented in the studied samples. A more extensive survey is therefore necessary to assess the national prevalence of ASF.

This study showed that most lymph nodes with follicular necrosis, parenchymal haemorrhage and lymphoid depletion were positive to ASF antigens. Similar histopathology studies of ASF infected pig tissue have revealed that the disease is characterized by haemorrhage, intravascular coagulation and lymphopenia, due to extensive apoptosis in lymph nodes (Wardley, 1982; Vallee et al., 2001). Our findings further support and extend earlier observations that classically, the principal pathogenic features of

ASF in domestic pigs are haemorrhage and destruction of lymphoid tissues (Oura et al., 1988). This study also strengthens the philosophy that though not pathogonomic, the lymphoid lesions combined with other changes such as petechiation of the kidneys and marked congestion/enlargement of the spleen, may be employed in the preliminary investigation of ASF outbreaks in endemic areas (Penrith and Nyakahuma; Lubisi et al., 2009). Virus isolation or antigen detection is however essential in confirmation of a diagnosis and epidemiological survey.

The prevalence of ASF in apparently healthy slaughter pigs was 0.09%. In a field/farm survey in Rakai, Central Uganda, Bjornheden (2010), reported a much higher prevalence of 2.1% in apparently healthy pigs. The current study relied on antigen detection using immunohistochemistry while the Rakai survey involved antibody detection using ELISA. The sensitivity and specificity of the two tests in diagnosis of ASF could be different. The most probable reason for the low prevalence observed in the current study is that, lymph nodes with ASF-like lesions were the only ones subjected to immunohistochemical analysis for antigen detection. Some of the samples without ASF-like lesions could have tested positive to the viral antigens had they been analysed.

Since there was no report of a very recent outbreak of ASF in central and eastern Uganda, detection of the viral antigens in apparently healthy pigs demonstrated that domestic pigs can act as reservoirs of the disease. A similar conclusion was arrived at by Bjornheden (2010). Our results also provide additional evidence that ASF is endemic to Uganda with epidemic outbreaks being sustained by domestic, intermediate and sylvatic cycles (Lubisi et al., 2005; Bjornheden, 2010).

We conclude that ASF virus in Uganda is probably maintained by sub-clinically infected pigs that serve as reservoirs of the disease. In other words, sustenance of the disease in pig populations may not be entirely dependent on the sylvatic cycle of transmission.

Acknowledgments

We appreciate Assistant Professor Robert Barigye, North Dakota State University, Prof JC Gomez Villamandos, The University of Cordoba Spain, and Dr. Rahana Dwark, Ondesteport Veterinary Institute for all their generous support to this study.

References

Babalobi, O.O., Olugasa, B.O., Oluweyelo, D.O., Ijagbone, I.F., Ayoade, G.O. and Agbede, S.A. 2007. Analysis and evaluation of mortality losses of the 2001 African swine fever outbreak, Ibadan, Nigeria. *Tropical Animal Health and production.* 39 (7): 533-542

Bjornheden, L. 2001. A study of domestic pigs, wild suids and ticks as reservoirs of ASF virus in Uganda. MSc. Dissertation, Uppsala, Sweden.

Costard, S., Wieland, B., Glanville, W. and Jori, F. 2009. African swine fever: How can global spread be prevented? *Philosophical Transactions of Royal Society of Biological Sciences,* 364:2683-2696

Delgado, C.L. 2003. Rising consumption of meat and milk in developing countries has created a new food revolution. *The Journal of Nutrition*, 133: 39075-39105.

Herenda, D. 1994. Manual on meat inspection for developing countries. FAO Corporate Documents Repository, Rome.

Jori, F, and Bastos, D.S. 2009. Role of wild suids in the epidemiology of African swine fever. *Ecohealth,* 6(2): 296-310

Lubisi, B,A,, Bastos, A.D., Dwarka, R.M. and Vosloo, W. 2005. Molecular epidemiology of African swine fever in East Africa. *Archives of Virology*, 150(12):2439-2452

Lubisi, B.A., Dwarka, R.M., Meenowa, D. and Jaumally, R. 2009. An investigation into the first outbreak of african swine fever in the republic of mauritius. *Transboundary and Emerging Diseases*, 56: 178–188.

Luna, L.G. 1968. Manual of Histologic Staining: Methods of the Armed Forces Institute of Pathology (3rd ed.). McGraw-Hill, New York.

Mebus, C.A. 1988. African swine fever. Advances in Virus Research. 35: 257-269

Niringiye, A. 2009. Patterns of agricultural growth and overall growth of Ugandan Economy. *African Journal of Agricultural Research*, 4(12): 1364-1373

Nissen, S.,Poulsen, I.H., Nejsum, P., Olsen, A., Roepstorff, A., Rubaire-Akiiki, C. and Thromsborg, S.M. 2011. Prevalence of gastrointestinal nematodes in growing pigs in Kabala District in Uganda. *Tropical Animal Health and Production*, 43(3): 567-572.

Oura, C.A., Powel, P.P., Anderson, E. and parkhouse, R.M. 1998. The pathogenesis of ASF in the resistant bush pig. *Journal of General Virology*, 79: 1439-1443

Penrith, M.L. and Nyakahuma, D. 2000. Recognising African swine fever: A field manual. FAO corporate documents repository, Animal production and health Division, The FAO, Rome.

Penrith, M.L. and Voslo, W. 2009. Review of African swine fever: Transmission, Spread and Control. *Journal of the South African Veterinary Association*, 80 (2): 58-62.

Rutebarika, C. and Nantima. 2002. Uganda and neighbouring countries under serious threat of African swine fever. Emergency Prevention System for Transboundary Animal and Pest and Diseases. http://www.fao.org/AG/AGAInfo/ programmes/en /empres/earlywarning/ew13.htm

Shi, S.R., Key, M.E. and Kalra, K.L. 1991. Antigen retrieval in formalin fixed, paraffin-embedded tissues: An enhancement method for immunohisto chemical staining based on microwave oven heating of tissue sections. *Journal of Histochemistry & Cytochemistry*, 49 (8): 931-937.

Speedy, A.W. 2003. Global production and consumption of animal source foods. *The Journal of Nutrition*, 133: 40485-40535

UBOS .2008. National Livestock census, Uganda Bureau of Statistics (UBOS), Kampala Uganda. www.ubos.org

Vallee, I., Tait, S.W. and Powel, P.P. 2001. African swine fever virus infection of porcine aortic endothelial cells leads to inhibition of inflammatory response, activation of thrombotic state and apoptosis. *Journal of Virology*, 75 (21): 10372-10382

Vizcaino, J.M. 2007. African Swine Fever. Annual report of the OIE reference laboratories and collaborating centres; Valdeolmos, 28130-Madrid, SPAIN. http://oie.jouvesi.fr/fileadmin/Home/fr/Our_scientific_expertise/ reflabreports/2007reports/04

Wardley, R.C. 1981. Effect of African swine fever on lymphocyte mitogenesis. *Immunology*, 46:215-220

Wilkinson, P.J. 1984. The persistence of African swine fever in Africa and Mediterranean. *Preventive Veterinary Medicine*, 2 (1-4): 71-82.