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Detection of African swine fever virus in archived Formalin fixed Paraffin embedded swine tissue sections in Uganda using Immunohistochemistry (IHC) and Polymerase chain reaction (PCR)

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Abstract

Background African swine fever (ASF), a devastating viral disease of pigs, is one of the main hindrances to pig farming in sub-Saharan Africa. Sporadic outbreaks of the disease occur annually in various regions of Uganda most of which remain undocumented. Confirmatory diagnosis of ASF is vital for instituting the disease prevention and control measures. Unfortunately, laboratory confirmatory diagnosis of ASF in Uganda is limited due to the high costs and expertise required. The aim of this study was to compare the level of agreement for positive detection of ASF virus by PCR and immunohistochemistry in archived formalin fixed, paraffin-embedded pig tissues from clinically suspected ASF cases in Uganda.

Results More than a third of the archived pig lymphoid tissues, (lymph nodes 33.3%, 95/285 and spleen; 42.8%, 120/280) had lesions suggestive of ASF. On average, 17% and 15% of the archived samples had ASF viral DNA and antigen respectively. The positivity rate for ASF in the tested tissue samples was higher in spleen (PCR, 17.5% and IHC, 16.1%) than in other tissues. More than half (51.1%, 48/94) of the lymph nodes and 41.9% (49/117) of spleen tissues that had lesions suggestive of ASF tested positive for ASF using conventional PCR. There was a strong agreement (*kappa* = 0.93913 at 95% CI; 0.88634–0.99193) between IHC and PCR in diagnosis of ASF in formalin fixed paraffin embedded pig tissue samples examined.

Conclusion ASF is still wide spread in Uganda and is one of the major threats to pig production in the country. PCR remain the most effective and reliable technique for diagnosing ASF in tissues even after a long period of archiving.

Keywords African swine fever, Pigs, Archived tissues, Immunohistochemistry, PCR, Diagnosis

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Background

Pig production contributes significantly to meet an ever-increasing global demand for meat. In Uganda, up to 17.8% of households rear pigs [1] and most of the pigs in rural areas are tethered in wet seasons and are free roaming during dry seasons. Commercial pig farming is on the rise in Uganda as pig farming has been considered as one of the viable enterprises for poverty reduction. As

such many governmental and non-governmental initiatives in Uganda have recently been promoting piggeries among low-income families. Unfortunately, pig production in Uganda is constraint by several factors including but not limited to; rampant diseases, unstable availability of feed resources, and limited access to timely extension services. Among the diseases of pigs, African swine Fever (ASF) is one of the main threats to the development of the pig industry in Uganda [2]. African swine fever is the most economically important disease of pigs due to its high morbidity and mortality as well as the restrictions often placed on trade of pig products when outbreak occurs. ASF is endemic in Uganda, and outbreaks have been reported throughout the year in different parts of the country. In most of these outbreaks, the epidemics take a natural course, often reaching climax and dying out, without serious interventions.

African swine fever, a devastating, trans-boundary viral hemorrhagic disease of pigs, presents in various forms as acute fatal disease or sub-acute and sometimes as a chronic disease with low mortalities [3, 4]. Previous report showed that pigs that survive ASF tend to develop some level of resistance against the same viral isolates, however they do not produce classical neutralizing antibody against the ASF virus [5]. In recent years, the continued occurrence of ASF virus of low virulence has resulted in an increased prevalence of sub-acute, chronic and or in-apparent infections. More so, in Uganda, ASF is endemic and outbreaks of the disease occurred sporadically in several districts of the country over the years [2, 6]. However, chronic and subclinical forms of ASF do occur and this is often confused with various diseases, hence the need for confirmatory diagnosis [3]. At present, there is no effective vaccines and therapy for ASF. Control and eradication of the disease depends on rapid and accurate laboratory diagnosis followed by institution of strict sanitary measures [7, 8]. These circumstances require reliable diagnostic tests for institution of an effective disease prevention measures [6].

One of the problems faced by farmers and local authorities in Uganda where disease outbreaks occur is the lack of confirmatory diagnostics. Although a number of diagnostic tests have been developed to detect ASF in infected pigs [3, 9, 10], there is still limited access to an effective rapid, relatively cheap and reliable diagnostic test for early detection of the disease in Uganda. More so, sample preservation still remains a challenge to Ugandan field veterinarians due to lack of consistent power supply and logistical challenges in many parts of the country. Fixation of pig tissue samples in buffered formalin seems to be an appropriate alternative for sample preservation and diagnosis of ASF. Because of the varied presentations of ASF in endemic areas, cases of African swine fever are frequently mistaken as other diseases. Hence veterinary clinicians and some lead farmers submit tissue samples to pathology laboratory for diagnosis of varied disease conditions. Under such circumstances, diagnosis of suspected ASF cases could be by histopathology and confirmed based on IHC, in situ hybridization (ISH) or PCR. Unfortunately, these techniques have their own drawbacks more especially following formalin fixation and this requires comparative studies which are very hard to come by [3, 11, 12]. In developing countries such as Uganda, veterinary confirmatory laboratory facilities are not readily available, hence veterinary clinicians and pathologist often rely on macroscopic and histopathology for tentative diagnosis of ASF. However, the level of diagnostic agreement between histopathology, IHC and PCR on different tissue samples from the different regions of Uganda have not been established.

Existing reports on IHC tests done on ASF cases in Uganda only focused on demonstration of the presence and distribution of viral antigens in pig tissues on limited number of cases during field and abattoir studies [13, 14]. Similarly, molecular techniques such as PCR have been used independently for confirmation of the disease outbreaks in Uganda. The aim of this study was to compare the level of agreement between IHC and PCR for positive detection of ASFV in archived formalin fixed paraffin embedded pig tissue samples from clinically suspected cases of ASF.

Materials and methods

The study area

This study was conducted in Uganda, involving the four regions of the country, namely; Central, Eastern, Northern and Western regions. All districts that submitted pig tissue samples for disease diagnosis at central veterinary diagnostic laboratory (CDL) and veterinary pathology teaching laboratory of Makerere University from 2013 to 2018 were considered in the study. Samples used in the current study were from the following districts: Kampala, Wakiso, Mukono, Mpigi and Masaka districts, Tororo, Soroti, Jinja, Lira Gulu, Amuru, Moyo Adjumani, Masindi, Kagadi, Kabarole, Kabale and Kamwenge.

Study design/study population and data analysis

This was a cross-sectional laboratory based ASF diagnostic study that established cases of ASF among pigs in districts that submitted tissue samples to CDL for disease diagnosis during the study period (2013–2018). The archived pig tissue samples were processed for PCR, histopathology and immunohistochemistry (IHC) with the aim of detecting presence of ASF. The data generated were sorted, cleaned and exported into Stata version 18 for analysis. The test results were compared using

Pearson Chi- squire tests and level of agreement between tests used determined using Cohen kappa test.

Sampling criteria

Well preserved pig tissue samples from lymph nodes, spleen, lungs and liver were used in the study. For consistency, samples from other organs and tissues other than spleen, lymph nodes, liver and lungs were left out in the current study due to their limited numbers. Pig tissue samples collected and submitted to CDL for purposes of swine diseases diagnosis only within the study period (2013–2018) were considered.

Swine tissue sample preparation and histopathology

Pig tissue samples were fixed in 10% buffered formalin in the ratio of 1:10 (v/v) for at least 48 h at room temperature (25 °C). The formalin fixed tissue samples (lymph nodes, spleen, lungs and liver) were then trimmed, placed in cassettes and processed in semi-automated tissue processor following established histotechnology protocol [15]. In brief, the tissues in cassettes were dehydrated in three changes of graded isopropyl alcohol (75%, 80%, 90% and absolute). Followed by clearance of the alcohol from the tissues by two changes of xylene. Extra internal strength to the tissues were provided by infiltration of paraffin wax and later the samples were embedded in paraffin wax and cut into blocks. Tissue samples were then sectioned to a thickness of 5 µm. The sections were floated on warm tap water at 45 °C and fished out onto clean microscope slides and adhered firmly to the slides by heating in an oven at 56 °C overnight. The sections were then stained using Hematoxylin and Eosin (H&E) stain and examined under light microscope. Histopathologic lesions observed were, recorded, described and scored as indicated in Table 1.

Immunohistochemistry on formalin fixed paraffin embedded pig tissue sections

Immunohistochemistry technique was carried out to detect ASF virus antigen in formalin fixed paraffin embedded swine tissues based on an established protocol [15]. Briefly, formalin fixed paraffin embedded tissue samples were sectioned to 4 µm thickness and adhered onto clean labeled siliconized glass slides using poly –L- lysine. Sample adhesion to the slide was further enhanced by heating in an oven at 54–56 °C, overnight. Paraffin embedded sections were de-waxed and rehydrated through a series of alcohol dilutions. Sections were then permeabilized in 0.05% (w/v) trypsin at 37 °C for 15 min and then washed thrice. Primary polyclonal antibody (a kind donation from Dr. Carmina Gallado, EURL Madrid, Spain) was added to the sections and incubated at 37 °C for 30 min at room temperature and non-specific binding sites were blocked with bovine serum (1% (v:v) in PBS). Endogenous peroxidase was quenched by incubating the sections for 30 min at 37 °C in Immunu-Pure Peroxidase Suppressor (Pierce, UK). Following repeated washing, secondary antibody (Goat anti-rabbit biotinylated antibody from Thermo Fisher Scientific, USA, Catalog # 31,820) diluted 1% v/v in 2% normal swine serum in PBS was applied to the sections for 30 min. The sections were then washed and the ABC biotin: streptavidin: HRP mix (Vector ABC ELITE kit) was applied to the sections for 30 min followed by 3,3-diaminobenzidine tetrahydrochloride (DAB) substrate. After colour development, the sections were counterstained with hematoxylin, dehydrated mounted and left to dry at room temperature. The slides were then examined under light microscope and microphotographs captured.

Table 1 Lesion score sheet

Score	Indication
1	No observable lesions in tissue sample
2	Mild to moderate morphological changes seen in tissues
3	Severe localized morphological changes seen in tissues
4	Extensive severe morphological changes seen in tissues

Genomic DNA extraction and PCR Amplification of ASF viral DNA in formalin fixed paraffin embedded (FFPE) tissues

DNA from formalin fixed paraffin embedded (FFPE) pig tissue blocks were extracted from each organ namely; spleen, lymph node, liver and lungs. Four sections of 5 µm thickness from each sampled tissue were pooled for DNA extraction. The pre-extraction process involved, addition of 1 ml of xylene octane to sections of FFPE tissue and vortexed for 20 s. Then 100 µl of methanol was added and vortexed for 20 s. The resultant suspension was centrifuged at 18.405 × g for two minutes and supernatant discarded. The pellet was incubated over night at 55 °C while shaking. The tissues were digested by addition of 20 µl of proteinase K (20 mg/ml) and vortexed for half a minute. The DNA was then extracted using a commercial DNA extraction kit for formalin fixed-paraffin embedded tissue (QIAMP DSP DNA FFPE tissue kit, Qiagen GmbH, Hilden, Germany) following the manufacturer's established protocol.

ASF viral DNA amplification and detection by PCR

A 257 bp region of ASF viral genome that is consistent to the central portion of the viral protein 72 (p72) was amplified following established protocol [9, 16]. The amplification of the genomic DNA was done in a total reaction volume of 25 µl, consisting of 12.5 µl 2X Green

GoTaq® G2 reaction buffer (Promega, USA), 1 µl each of forward and reverse primers, 1.5 µl of MgCl, 2 µl of DNA template and 7 µl of PCR water. DNA from ASF viral isolate, UG 12, Kampala, genotype XVIII [16] was used as positive control. The PCR consisted of an initial denaturation at 95 °C for 6 min followed by 30 cycles of final denaturation at 94 °C for a minute. Annealing occurred at 55 °C for a minute and extension at 72 °C for 10 min. The PCR products were then run in 1.5% agarose gel against 100 bp DNA ladder (BioLab, Singapore) at 125 V in Tris–Acetate-EDTA (TAE) buffer containing 0.5 µg/µl Ethidium bromide for 35 min. The stained gels were visualized in gel documentation system and images captured.

Results

Origin of the archived pig tissue samples used in this study

Pig tissue samples received from veterinary clinicians and some farmers were fixed in 10% buffered formalin and processed into paraffin blocks and archived in Veterinary pathology diagnostic laboratory, Makerere University, Uganda. The archived tissues examined in this study originated from all the four regions of Uganda. The central region contributed the highest percentage 36.2% (105/290) of the tissue samples, followed by the Northern, Eastern, and Western regions. Among the 19 districts from which samples were obtained and archived, Wakiso district in central Uganda submitted the highest proportion of the samples 12.1% (35/290) while Kabarole district contributed the least percentage 1.38% (4/290). Swine tissue samples commonly submitted for disease diagnosis at the diagnostic laboratory between 2013 and 2018 included, spleen, lymph nodes, liver and lungs. The sample reception records are in Table 2.

Severity scores of histopathologic lesions suggestive of ASF in archived pig tissues

In this study, microscopic lesions suggestive of ASF were commonly observed in lymphoid tissues (spleen and lymph nodes), followed by those in lung sections while liver lesions were not consistent hence could not be used for presumptive diagnosis of ASF. More than a third of the archived pig lymphoid tissues, (lymph nodes 33.3%, 95/285 and spleen; 42.8%, 120/280) had lesions suggestive of ASF. The lesion severity in lymphoid tissues ranged from severe (score ≥ 3) hemorrhagic lesion with lymphoid follicular degeneration

Table 2 Origin of pigs included in the study by region and districts of Uganda

S/No	Sample origin		number of pigs studied	Percentage (%) of the total number of samples studied
	Region	District		
1	Central Region	Kampala	23	7.93
		Wakiso	35	12.07
		Mukono	22	7.59
		Mpigi	18	6.2
		Masaka	7	2.41
	Sub total		**105**	**36.2**
2	Eastern Region	Kumi	15	5.17
		Tororo	19	6.55
		Soroti	18	6.2
		Jinja	10	3.45
	Sub total		**62**	**21.4**
3	Northern Region	Lira	13	4.48
		Gulu	12	4.14
		Amuru	20	6.9
		Moyo	11	3.79
		Adjumani	9	3.1
	Sub total		**65**	**22.4**
4	Western Region	Masindi	14	4.83
		Kagadi	7	2.41
		Kabarole	4	1.38
		Kabale	15	5.17
		Kamwenge	18	6.2
	Sub total		**58**	**20**
	Total number of samples		**290**	**100%**

and necrosis (lymph node, 28.4%, and spleen, 25.7%) to mild or moderate lesions. Pulmonary congestion and oedema were common findings among samples from pigs that died of ASF, nonetheless the severity of the lung lesions varied from moderate (19.3%) to severe/extensive haemorrhagic pneumonia and lung edema (24.5%). Haemorrhagic pneumonia with areas of lung tissue consolidation was also frequently observed among pigs that succumbed to ASF. Hepatic haemorrhages, congestion and periportal necrosis were also observed in several samples that tested positive for ASF. In addition to the lesions due to vascular disturbances attributed to haemorrhagic diseases such as ASF, other lesions that could not be attributed to ASF were recorded in this study. The other lesions included; cysts in lymphoid and hepatic tissues, abnormal pigmentation, haemosiderosis and necrosis with mineralization in lungs and lymphoid tissues including multifocal hepatic necrosis, fibrosis, lipidosis and abscess. The detailed lesion description and the corresponding scores were as in Table 3.

Confirmatory diagnosis of ASF using PCR from archived pig tissue samples from the four regions of Uganda

Confirmatory molecular diagnostic test (PCR) revealed that 17% (50/290) of the archived pig tissue samples from all the regions of Uganda had ASF viral DNA. Central region had the highest ASF positivity rate (20%, 21/105) while the Western region had the least sample submission and ASF positivity rate (13.8%, 8/58). Comparison of ASF positivity rate using chi-squire test (at 95% CI) between regions of Uganda showed that there was no statistically significant difference ($X^2 = 1.306$, df = 3, $p = 0.728$) between the regions. More so, there wasn't any statistically significant difference in ASF PCR positivity rate between the districts ($X^2 = 7.902$, df = 18, $p = 0.980$). The findings were as presented in Table 4, while representative gel for PCR product and immunohistochemistry micrographs was as shown in Fig. 1 and 2 respectively.

Comparative detection of ASF in pig tissues using Histopathology, Immunohistochemistry (IHC) and PCR

Histopathology, IHC and conventional PCR were conducted on archived FFPE pig tissue samples from spleen,

Table 3 Lesion severity score in pig tissues archived at Pathology laboratory, Makerere University

Tissues examined	Histopathology Lesion description	Lesion severity Score	Number of tissue samples with such score	% of tissues with such Score
Lymph node	No observable lesion in lymph node tissues	1	33	11.6
	Moderate congestion and Hemorrhages	2	14	4.9
	Severe congestion, edema, hemorrhages and lymphoid follicles necrosis	3	81	28.4
	Other lesions (cysts, melanosis, pigment of brown atrophy, haemosiderosis, mineralization)	2	157	55.1
	Sub total		**285**	
Spleen	No observable lesion in spleen tissues	1	16	5.7
	Mild to moderate congestion and lymphocytes depletion in the follicles	2	48	17.1
	Severe congestion and extensive lymphoid follicular necrosis	4	72	25.7
	Other lesions (lymphoid hyperplasia, cysts, fibrosis, pigment of brown atrophy)	2	144	51.4
	Sub total		**280**	
Lungs	No observable lesion in lung tissues	1	44	15.2
	Moderate pulmonary congestion and edema	2	56	19.3
	Severe pulmonary congestion, edema and areas of lung tissue consolidation	3	71	24.5
	Extensive pulmonary congestion, edema and lung tissue consolidation	4	12	4.1
	Other lesions (melanosis, granulomas, fibrinous pneumonia, purulent pneumonia)	2	107	36.9
	Sub total		**290**	
Liver	No observable lesion in liver tissue	1	22	8.5
	Moderate Hepatic congestion and hemorrhages	2	43	16.5
	Severe hepatic congestion, hemorrhages and periportal lymphocytic hepatitis	3	66	25.4
	Other lesions (pigmentation, fibrosis, multifocal necrosis, lipidosis, abscess)	2	129	49.6
	Sub total		**260**	

Table 4 PCR detection of ASFV genome in archived pig tissue samples from Uganda

Region of Uganda	Number of samples examined	Number positive for ASF with PCR	Positivity rate (%)
Central	105	21	20.0
Eastern	62	9	14.5
Northern	65	12	18.5
Western	58	8	13.8
Total	290	50	17

lymph nodes, liver and lungs. On average, 17% (50/290) of the pig tissue samples examined had ASF viral DNA while approximately 15% (45/290) of the samples tested positive for ASF using IHC. The positivity rate for ASF was higher on spleen samples by both PCR and IHC (PCR =17.5%, and IHC =16.1%) than in other tissues (shown in Table 5). More than half (51.5%, 48/95) of the lymph node tissues and 41.9% (49/117) of spleen that had lesions suggestive of ASF based on the histopathology, tested positive for ASF using conventional PCR. However, the positivity rate was relatively low among lung and liver tissues compared to lymphoid tissue from same carcasses. Ten pig tissue samples (10/151, 6.6%) that apparently did not have histopathology lesions suggestive of ASF, tested positive for ASF using PCR. However, the positivity rate among pig samples that did not have observable histopathologic lesions but tested positive for ASF was not statistically significant ($p = 0.187$). The details are as shown in Table 5.

The level of agreement between IHC and PCR, between PCR and histopathology and IHC and histopathology were determined using Cohen kappa analysis for reliability test. There was a strong agreement (*kappa* = 0.93913, standard error =0.026935 at 95% CI; 0.88634–0.99193) between IHC and PCR tests for diagnosis of ASF on the pig tissues examined. However, there was week agreement between histopathology and IHC (*kappa* = 0.30095, standard error =0.042924 at 95% CI; 0.21682–0.38508) and between histopathology and PCR (*kappa* = 0.29475,

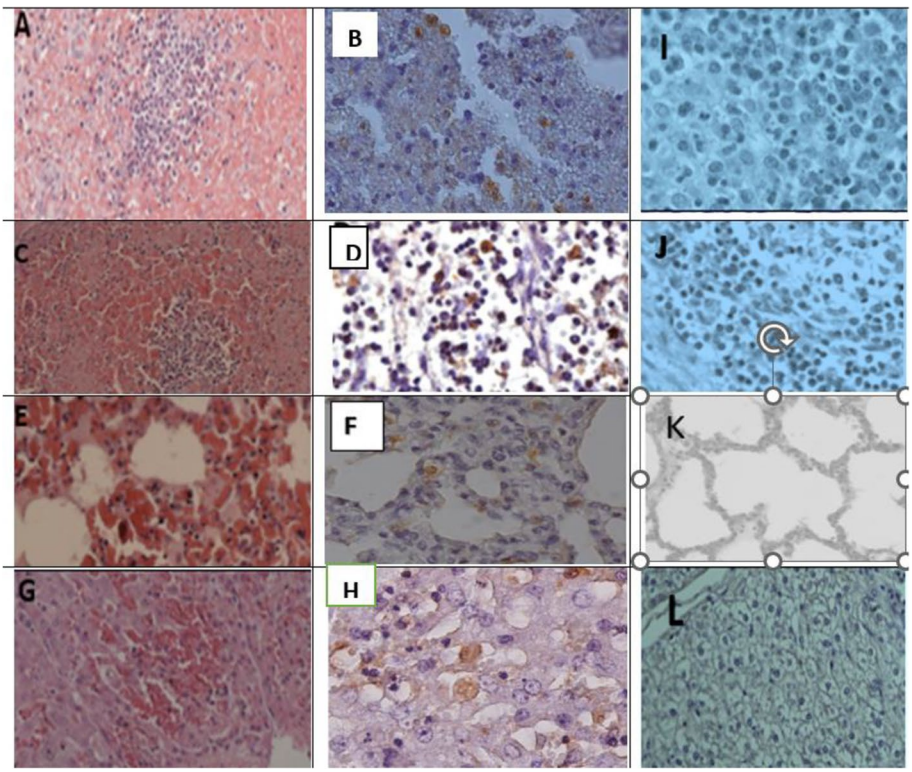

Fig. 1 Histopathology and positive Immunohistochemistry test results for ASF. Section (**A**) is Hematoxylin and Eosin stain of pig spleen, showing severe congestion & wide spread hemorrhages overshadowing the white pulp & lymphocytes depletion. **B**, is an IHC test image of the spleen section positive for ASF. **C**, is a lymph node section with severe perifollicular haemorrhages & the section tested positive for ASF using IHC as shown in slide **D**. **E**, is Haematoxylin and Eosin -stained section of lung tissue with extensive haemorrhages, congestion and oedema while **F** is IHC of the lung tissue positive for ASF. **G**, is Hematoxylin and Eosin stain of liver section with wide spread haemorrhages & (**H**) is the corresponding IHC positive results for ASF on the liver section. Sections I, J, K and L are negative controls for IHC test for ASF on Spleen, lymph node, lung and liver tissue sections respectively.

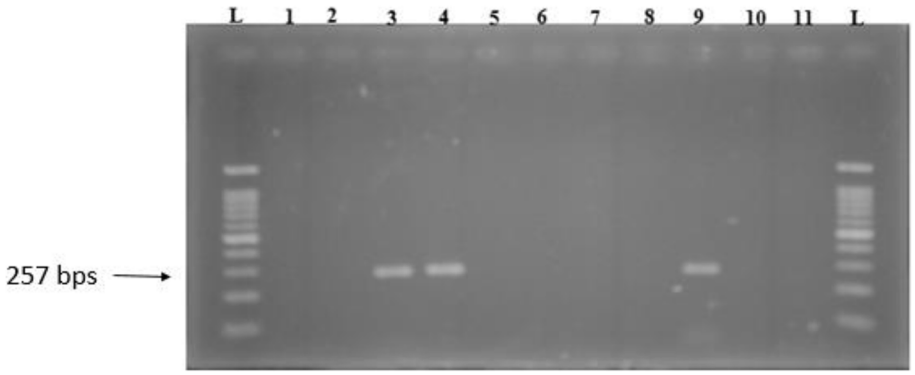

Fig. 2 Molecular (PCR) confirmation of ASF in formalin fixed paraffin embedded pig tissues. PCR result showing 257 bp fragment of ASFV p72 gene. Lane L had 100 bp molecular ladder, 1 & 2 Negative control, 3 had positive control, wells 4 & 9 had archived pig samples tested positive for ASF viral DNA while 5, 6, 7, 8, 10 & 11 were samples that tested negative for ASF

Table 5 ASF comparative detection and diagnostic positivity rates in selected pig tissue samples

Tissue sample	No. of archived & tested samples	Histopathology Lesions suggestive of ASF		IHC Test Positive for ASF		PCR Test Positive for ASF	
		#	%	#	%	#	%
Spleen	280	117	41.8	45	16.1	49	17.5
Lymph node	285	94	33.0	44	15.4	48	16.8
Liver	260	72	27.7	39	15.0	44	16.9
Lungs	290	139	47.9	45	15.5	50	17.2

standard error = 0.044623 at 95% CI; 0.20729–0.38221). The histopathology and IHC micrographs and representative PCR gel image for ASF positive and negative cases are in Figs. 1 and 2 respectively.

Discussion

African swine fever (ASF) is one of the serious threats to the pig industry in Uganda and sporadic outbreaks of the disease were reported in all regions of the country in recent years [6]). However, confirmatory diagnosis of the disease still remains a challenge in Uganda due to the limited number of veterinary diagnostic facilities.

This study used archived pig tissue samples from Veterinary pathology teaching laboratory and central diagnostic laboratory (CDL) to comparatively diagnose ASF using PCR and IHC. The samples for this study originated from all four geographic regions of Uganda namely; Northern, Eastern, Western and Central regions [6]. The commonly received and archived pig tissue samples used in the current study included, lymph nodes, spleen, liver and lungs. However other vital organs such as pharyngeal tonsils and kidneys were unfortunately often not included among samples submitted to diagnostic labs. Histopathologic examination of pig tissue samples in this study revealed that 33.3% of the lymph nodes and 42.8% of the spleen samples submitted had haemorrhages and necrosis which are quite common in pigs with ASF [17, 18]. The wide spread haemorrhages observed in the various tissues of pigs that died of ASF in this study suggests that the majority of the archived samples were probably from pigs that died of an acute or subacute ASF.

In the current study, lymphoid follicular degeneration and necrosis, coupled with wide spread haemorrhages were common findings in the spleen and lymph nodes of pig carcasses that had ASFV antigens. Previous studies also revealed that ASF was characterized by extensive lymphadenopathy and wide spread necrosis and apoptosis of lymphocytes and macrophages in lymphoid tissues of affected pigs [17, 19, 20]. In addition to what is reported in this study, Perez and colleagues reported hyperplasia of mononuclear phagocytic cells and increased number of immature lymphoid cells in lymph nodes and spleen of pigs experimentally infected with ASF virus. Therefore, severe lymphoid follicular necrosis and wide spread haemorrhages are key lesions that could be used for presumptive diagnosis of acute ASF.

In this study, ASF was comparatively diagnosed using conventional PCR and IHC on formalin fixed paraffin embedded pig tissues, namely spleen, lymph nodes, liver and lungs. The study reported a strong agreement

(*kappa* = 0.93913, at 95% CI; 0.88634–0.99193) between IHC and PCR tests in diagnosis of ASF on archived pig tissue samples examined. However, there was weak agreement between histopathology and IHC (*kappa* = 0.30095, at 95% CI; 0.21682–0.38508), and between histopathology and PCR (*kappa* = 0.29475, at 95% CI; 0.20729–0.38221). Similar to what is reported in the current study, Izzati and colleagues in 2020 reported a strong positive correlation between IHC and PCR results in diagnosis of Africa swine fever in Vietnam [19]. The advantage of immunohistochemistry over other tests is its ability to demonstrate viral antigen distributions in the infected tissues which is vital in understanding the pathogenesis of the disease [3]. Nevertheless, PCR remains the best diagnostic assay for ASF.

The current study also revealed that, 6.6% of the pig tissue samples that tested positive for ASF did not have observable histopathological lesions suggestive of the disease. However, this proportion was not statistically significant ($p = 0.187$) in comparisons to those samples with microscopic lesions. Similar to what is reported in the current study, Perez and others reported that some pigs that died of ASF lacked gross and or histopathologic lesions [21]. Histopathology is known to have low specificity and sensitivity hence should be used in combination with other confirmatory tests such as PCR and IHC [19, 20].

This study further revealed that, approximately 17% and 15% of the archived pig tissue samples had ASF DNA and antigens respectively. Therefore, ASF was the likely cause of death of the pigs. All pig tissue samples that tested positive for ASF with IHC also were positive for the disease with convention PCR. However, PCR test was slightly more sensitive in detecting ASF in formalin fixed paraffin embedded pig tissue samples than IHC and histopathology. The high positivity rate among archived pig tissue samples shows that ASF was wide spread in Uganda and has remained one of the major hindrances to pig production in the country. Pig tissue samples that tested positive for ASF with PCR but negative with IHC in this study could have been obtained from chronic cases of the disease, leading to masking of viral antigens by anti-ASFV antibodies as explained by Oura and colleagues [3]. Although in the current study antigen retrieval was done using enzymatic digestion, it was likely that some of the targeted epitopes remained obscure, hence some false negative tests result with IHC reported in the study. Unfortunately, in the current study, the course of the disease was not established as the study used archived tissue samples from natural infections.

African swine fever viral antigens in the current study were detected in the spleen, lymph nodes, lungs and liver of the infected pig tissue samples. In addition to mononuclear phagocytic cells (MPCs), ASF virus is known also to replicate in many tissues and cell types such as endothelial cells [20], megakaryocytes and platelets [22] and hepatocytes [3, 23]. The majority of the infected cells in the current study had intracytoplasmic ASF viral antigens that stained brown with immunohistochemistry technique using 3, 3'-Diaminobenzidine (DAB) substrate. This observation was supported by the finding of Oura and colleagues which reported that ASF viral replication site is in the cytoplasm and tend to be more concentrated in perinuclear region of the infected cells [3].

Conclusion

African swine fever is widespread and endemic in Uganda and the disease is one of the major threats to pig production in the country. Timely and accurate diagnosis of ASF for institution of appropriate control measures to date is the reliable approach towards control of the disease.

PCR remains the most effective and reliable technique for diagnosing ASF in tissues even after a long period of archiving. The test outcomes of immunohistochemistry on formalin fixed paraffin embedded pig tissues strongly agreed with that of PCR in diagnosis of ASF, hence in the absence of PCR, IHC could be an effective technique for diagnosing ASF. The positive reactivity of IHC and the intensity of antigen staining was better on the lymphoid tissues (spleen & lymph nodes) than on other tissue samples.

Study limitations

This was comparative diagnostic study which did not sequence positive amplicons due to lack of funds, hence the genotype (s) of ASF virus detected in the study period was not established.

Recommendations

- Histopathologic examination of swine tissue samples should be accompanied with confirmatory diagnostic tests for ASF such as PCR, IHC, or immunofluorescence test.
- Formalin fixation of pig tissues is an appropriate method of tissue sample preservation for diagnosis of ASF especially in situations where fresh sample submission to diagnostic laboratories is unlikely.
- Lymphoid tissues should always be part of the variety tissue samples collected for ASF diagnosis.
- Continued community education and sensitization on measures to prevent or control outbreak of ASF is vital as the disease is still wide spread in Uganda.

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Authors' contributions
Mathias Afayoa (MA), Lonzy Ojok (LO) and Julius Boniface Okuni (JBO) conceived the study jointly. MA did laboratory work of tissue processing for histopathology, immunohistochemistry and PCR. The three authors; MA, LO, JBO read the slides and analysed the data. MA drafted the manuscript while LO and JBO edited the manuscript. All authors read and approved the manuscript for publication.

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Data availability
The data generated and/or analysed during this study are all included in this article. However raw data is available from the corresponding author on request.

Declarations

Ethical approval and consent to participate
This was a continuation of previous study and full ethical clearance for the study was obtained from Uganda National Council of Science and Technology (UNCST) under reference number VAB/REC/11/110. The study used archived pig tissue samples submitted to veterinary diagnostic laboratory in a period of five years, hence there was neither direct participation of farmers nor use of live animals.

Consent for publication
Not applicable for this study.

Competing interest
The authors declare no competing interests.

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References

1. UBOS, "Annual Agricultural Survey 2018 Statistical Release," 2018.
2. D. Kalenzi Atuhaire et al., "Epidemiological Overview of African Swine Fever in Uganda (2001–2012)," J. Vet. Med., vol. 2013, pp. 1–9, 2013, https://doi.org/10.1155/2013/949638.
3. C. A. L. Oura, P. P. Powell, and R. M. E. Parkhouse, "Detection of African swine fever virus in infected pig tissues by immunocytochemistry and in situ hybridisation," J. Virol. Methods, vol. 72, no. 2, 1998, https://doi.org/10.1016/S0166-0934(98)00029-9.
4. D. Beltran-Alcrudo, M. Arias, C. Gallardo, S. Kramer, and M. Penrith, African swine fever: detection and diagnosis – A manual for veterinarians, vol. 19. 2017.
5. Sánchez-Cordón PJ, Montoya M, Reis AL, Dixon LK. African swine fever: A re-emerging viral disease threatening the global pig industry. Vet J. 2018;233(2010):41–8. https://doi.org/10.1016/j.tvjl.2017.12.025.
6. F. Norbert Mwiine, J. Nkamwesiga, C. Ndekezi, and S. Ochwo, "Molecular characterization of african swine fever viruses from outbreaks in peri-urban Kampala, Uganda," Adv. Virol., vol. 2019, 2019, https://doi.org/10.1155/2019/1463245.
7. A. Catarina Urbano and F. Ferreira, "African swine fever control and prevention: an update on vaccine development," 2021, https://doi.org/10.1080/22221751.2022.2108342.
8. "OIE MANUAL forTerestrial Animals," in Manual, Paris, France: European Union Laboratory for African Swine Fever 2020.
9. Agüero M, Fernández J, Romero L, Mascaraque CS, Arias M, Sánchez-Vizcaíno JM. Highly sensitive PCR assay for routine diagnosis of African swine fever virus in clinical samples. J Clin Microbiol. 2003;41(9):4431–4. https://doi.org/10.1128/JCM.41.9.4431-4434.2003.
10. Hutchings GH, Ferris NP. Indirect sandwich ELISA for antigen detection of African swine fever virus: Comparison of polyclonal and monoclonal antibodies. J Virol Methods. 2006;131(2):213–7. https://doi.org/10.1016/j.jviromet.2005.08.009.
11. Afayoa M, et al. Comparison of immunoperoxidase monolayer assay, polymerase chain reaction and haemadsorption tests in the detection of African swine fever virus in cell cultures using Ugandan isolates. J Gen Mol Virol. 2014;6(4):36–45. https://doi.org/10.5897/JGMV2014.0062.
12. J. Pérez, J. M. de Las Mulas, J. C. Gómez-Villamandos, M. A. Sierra, F. Rodríguez, and A. Fernández, "Detection of African swine fever virus protein VP73 in tissues of experimentally and naturally infected pigs," J. Vet. Diagnostic Investig., vol. 6, no. 3, 1994, https://doi.org/10.1177/104063879400600314.
13. Sajjakambwe P, Okwee-Acai J, Ojok L. Prevalence of African swine fever viral antigens in slaughter pigs at Nalokolongo abattoir, kampala -Uganda. Research opinion in Animal and Veterinary Sciences. 2011;1(5):313–7.
14. J Ganowiak 2012. Patho-anatomic studies on Africa swine in Uganda. http://epsilon.slu.se. ISSN 1652 - 8697
15. Prophet Edna B, "Catalog Record: Laboratory methods in histotechnology | HathiTrust Digital Library," Washington DC, American Registry of Pathology, 1992. https://catalog.hathitrust.org/Record/002647795 (accessed Nov. 30, 2021).
16. K. A. David et al., "Comparative detection of African swine fever virus by loop-mediated isothermal amplification assay and polymerase chain reaction in domestic pigs in Uganda," African J. Microbiol. Res., vol. 8, no. 23, 2014, https://doi.org/10.5897/ajmr2014.6848.
17. F. J. Salguero, "Comparative Pathology and Pathogenesis of African Swine Fever Infection in Swine," Frontiers in Veterinary Science, vol. 7. 2020, https://doi.org/10.3389/fvets.2020.00282.
18. K. Belák et al., "Comparative studies on the pathogenicity and tissue distribution of three virulence variants of classical swine fever virus, two field isolates and one vaccine strain, with special regard to immunohistochemical investigations," Acta Vet. Scand., vol. 50, no. 1, 2008, https://doi.org/10.1186/1751-0147-50-34.
19. Izzati UZ, et al. Pathological investigation and viral antigen distribution of emerging African swine fever in Vietnam. Transbound Emerg Dis. 2021;68(4):2039–50. https://doi.org/10.1111/tbed.13851.
20. Sánchez-Cordón PJ, et al. Evaluation of lesions and viral antigen distribution in domestic pigs inoculated intranasally with african swine fever virus ken05/tk1 (Genotype x). Pathogens. 2021;10(6):1–23. https://doi.org/10.3390/pathogens10060768.
21. J. Pérez, F. Rodríguez, A. Fernández, J. Martín De Las Mulas, J. Carlos Gómez-Villamandos, and M. A. Sierra, "Detection of African swine fever virus protein VP73 in tissues of experimentally and naturally infected pigs," 1994.
22. Gómez-Villamandos JC, et al. A pathological study of the perisinusoidal unit of the liver in acute African swine fever. Res Vet Sci. 1995;59(2):146–51. https://doi.org/10.1016/0034-5288(95)90049-7.
23. Gomez-Villamandos JC, et al. Experimental African swine fever: Apoptosis of lymphocytes and virus replication in other cells. J Gen Virol. 1995;76(9):2399–405. https://doi.org/10.1099/0022-1317-76-9-2399/CITE/REFWORKS.

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