

Live pig markets are hotspots for spread of African swine fever virus in Nigeria

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Abstract

Livestock trading through live animal markets are potential pathways for the introduction and spread of economically important pathogens like the African swine fever virus (ASFV) to new areas in several countries. Due to the high demand for live pigs in Nigeria both for restocking and slaughter, live pigs are sold at designated live pig markets (LPM) in the country. This involves movement of pigs over long distances. Despite, reports of ASF outbreaks following restocking of pigs bought from LPMs, there is paucity of information on the role of LPMs in the epidemiology of ASF. In this study, data and pig samples (whole blood, sera, tissue) were collected from 4 selected LPMs in Nigeria (Dawaki, Katsit, Numan & Pandam) between 2019 and 2020. Samples were analysed by polymerase chain reaction (PCR) and Enzyme-linked Immunosorbent Assay (ELISA). Four genes of ASFV positive samples were characterized to identify the circulating genotypes. Results revealed trade activities involving transportation of pigs from these selected markets to 42 major cities and towns in thirteen (13) States of Nigeria. PCR results revealed an overall ASF prevalence of 10.77% (66/613). ASFV was confirmed by PCR in all the selected LPMs with a prevalence rate of 3.13%-23.81%. The phylogeny revealed genotype I and serogroup 4 based on the *p72* protein that encodes the *B646L* gene and the *EP402R* gene encoding the CD2V. While sequence analysis of CVR of *B602L* gene revealed 8 tetrameric repeats variants, six of which have never been reported in Nigeria. Analysis of sera samples recorded a seroprevalence of 6.9% (16/217) within the study period. Findings from this study show that LPM are hotspots and channels for transmission and continuous spread of ASFV in Nigeria. Therefore, for ASF to be controlled in Nigeria, disease surveillance and regulation at LPMs are critical.

Introduction

Trading livestock through live animal markets is an important part of the economy of many developing countries (Fèvre et al., 2006; Leslie et al., 2015; Perry et al., 2005). Demand for live pigs is high in pig-producing areas of Nigeria, because these animals play a vital role in the culture and tradition of the people as they are used for celebrations and festivities (Fasina et al., 2010). Pig marketing in Nigeria consists largely of live animal sales which are acquired from multiple sources either directly from farmers at farm gates or fellow traders at live pig markets (LPMs) (Ajala & Adeshehinwa, 2008; Weka et al., 2021). Hence, LPMs are hubs for the congregation of animals from a wide range of locations some of which are reporting outbreak diseases. In addition, poor biosecurity practices, limited government regulation, and poor infrastructure enhance the ease of disease spread at these markets. Likewise, the trading and movement of sick pigs have been identified as a crucial pathway to the spread of transboundary diseases like African swine fever (ASF) in endemic countries such as Nigeria (Costard et al., 2013; Olugasa & Ijagbone, 2007). Pig husbandry system in Nigeria is differentiated into mostly free-roaming/extensive, semi-intensive and intensive pig production systems in pig producing areas in Northern Nigeria and intensive commercial/ communal pig estates in

Southern Nigeria (Bourn et al., 1994; Ajala et al., 2006; Saka et al., 2010; Nwanta et al., 2011). In Nigeria, farmers restock their farms from LPMs and there have been reports of ASF outbreaks in pig farms following introduction of pigs from these markets. Also, due to high demand and need for better prices by farmers and traders, pigs reared in the northern parts of Nigeria are transported to southern parts of the country, the majority of such transactions is via LPMs. ASF is a lethal transboundary disease of pigs caused by the ASF virus (ASFV) which is the only member of the genus *Asfivirus* and family *Asfarviridae* (Dixon et al., 2020). The disease is endemic and restricted to countries in sub-Saharan Africa, but due to the highly transboundary nature of ASFV, the disease has spread to domestic and wild pigs in Europe, Asia, and Oceania (Penrith, 2020; Sauter-Louis et al., 2020). Genetic typing of the virus is based on characterization of the p72 capsid protein of *B646L*, the full length of the p54 of *E183L*, and further differentiation using the central variable region of *B602L* (Bastos et al., 2003; Gallardo et al., 2009). Based on the *p72 p* rotein capsid, 24 genotypes of ASFV have been identified, most of which are restricted to East and Southern Africa (Bastos et al., 2003; Quembo et al., 2018). Genotyping methods are important tools for tracing the origin of ASFVs and quickly differentiate or related virus strains during ASF outbreaks (Malogolovkin et al., 2015a). The serogrouping-identification method can be used to further discriminate ASFV strains. Based on this method, 8 ASFV serogroups have been identified based on the *EP402R* gene encoding the CD2V protein (Malogolovkin et al., 2015a). ASF was first introduced into Nigeria in 1997 via the trading of pigs by communities along the international border with the Benin Republic (Odemuyiwa et al., 2000). Thereafter, the disease spread to other pig-producing areas of the country through livestock trading routes (Fasina et al., 2010; Luka et al., 2016; Owolodun, et al., 2010a). ASFV genotype I was the only circulating genotype in Nigeria, until recently when genotype II was introduced into the country (Adedeji et al., 2021). Despite the importance of live pig markets (LPM) in the pig value chain in Nigeria, there are no extensive studies on the role of these markets in the epidemiology of ASF in Nigeria (Fasina et al., 2010; Luka et al., 2016; Owolodun, Bastos, et al., 2010). Though, previous studies have detected ASFV and swine influenza virus in a live pig market in Nigeria (Dennis et al., 2019; Luka et al., 2016; Meseko et al., 2014). However, these studies involved only one pig market with limited information on activities and pig movements in and out of the market. Studies in Nigeria have revealed that live bird markets play an important role in the ecology and epidemiology of Avian influenza in Nigeria (Coker et al., 2014; Fusaro et al., 2009; Sulaiman et al., 2021). Similarly, it has been reported that livestock farmers in Nigeria sell their animals through live animal markets to mitigate economic losses during disease outbreaks (Bolajoko et al., 2019). This further emphasizes the importance of live animal markets in the epidemiology of transboundary livestock diseases in Nigeria. This study assessed the role of LPMs as hotspots for transmission and continuous spread of ASFV in Nigeria

Materials and methods

Study area: Nigeria is located in West Africa, bordered by the Benin Republic to the west, the Niger Republic to the north, Cameroun to the east, and the Atlantic Ocean to the south. The country is divided into 36 States and the Federal Capital Territory (FCT) Abuja. The 36 States are further divided into Local government areas (LGA) with a total of 774 LGAs. Pigs are reared in 30 States of the country. Nigeria has two seasonal periods, the rainy season which is from April to October, and the dry season from November to March. The common breeds of pigs in Nigeria are the Iberian type Nigerian Indigenous Pig (NIP) which is usually black or pied, others are exotic breeds including, Large White, Landrace, Duroc, Hampshire, and their crossbreeds (Fadiga et al, 2013).

Selection and description of live pig markets: Organized LPMs are common in northern parts of Nigeria, while in the southern part of Nigeria, pigs are sold live at farm gates or as processed pork at stores. Due to limited information on the LPM operation in Nigeria, selection of LPM sites were done purposively based on information from pig farmers, easy access, literature search, logistics, visit to LPM, the network of distribution, security challenges, and the number of live pigs traded at markets visited. Hence, four (4) live pig markets were purposefully selected, all located in Northern Nigeria namely: Katsit pig market (also called Kafanchan pig market) in Zagon Kataf LGA of Kaduna State, Pandam pig market in Qua Pan LGA, and Dawaki pig market in Kanke LGA both located in Plateau State and Numan pig market in Numan LGA of Adamawa State (Fig 1). Katsit pig market is located on latitude N 9°. 34255 and Longitude E 8°.164936

at the outskirts of Kafanchan, Kaduna State. It is the largest LPM in Nigeria, Table 1), it is once a week market with trading activities taking place on Thursdays only. Located within the premises of the Katsit pig market is a slaughter slab where pigs bought within the market are slaughtered and sold to traders and the surrounding communities. The second market is Pandam pig market located on latitude N9⁰.52519 and longitude E8⁰.50510, it is also a once in week market with sales of pigs taking place on Mondays (Table 1). There is a slaughter point 500m to the market. The third market is Dawaki pig market, which is located on latitude N9.2625956 deg and longitude E 9.3401578 deg. Apart from pigs, other animals sold at the Dawaki pig market including sheep, goats, chickens, and dogs. The Dawaki pig market holds weekly on Thursdays. The slaughter point at Dawaki pig market is located directly behind the perimeter fence of the market with an access point for free movement of butchers and traders. The fourth market is the Numan Pig market located in Numan LGA of Adamawa State, the market holds on Tuesdays with GPS co-ordinates N 09⁰.27440' E12 .002043'

Table 1: Selected live pig markets located in Nigeria where this study was carried out.

Market	Village/LGA	State	Market days	Presence of pig slaughter slab within/by
Dawaki Pig market	Dawaki/Kanke	Plateau	Thursdays	Yes
Katsit Pig market	Katsit/Zangon/Kataf	Kaduna	Thursdays	Yes
Numan Pig Market	Numan/Numan	Adamawa	Tuesdays	No
Pandam Pig market	Pandam/Quan Pan	Plateau	Mondays	No

Data collection: Data was collected based on key informant interviews of government and pig traders' union officials at the market sites. Data collected were: average weekly population of pigs in the markets. Towns and villages from where pigs are brought into the markets and locations where the pig purchased are transported to. Government regulations in the markets and attached slaughter slabs and the fate of pigs unsold on at the market. Data on the average number of pigs slaughtered at the slabs were also collected.

Field observation: Due to poor records at the selected LPMs, observations of activities of the value chain actors (traders, farmers, transporters) and slaughter slab operators were carried out using a checklist. These include pre-entry inspections of pigs going in and out of the market site, mode of transportation of the pigs, holding facilities at the markets e.g pens, activities of the slaughter slab operators, and access of people and other animals to the market sites. Others include common breeds of pigs at the market, estimated age of pigs brought to the market, sanitation, and hygiene at the market sites. To carry out this aspect of the study, arrival at market sites was from 7 am to 8 am depending on location to maximize observation of activities, sample collections, and travel time back to the laboratory. At least 2 cycles of visits were planned per month for data and sample collection.

Figure 1 : Map of Northern part of Nigeria, showing the movement of pigs from towns and villages into the four selected live pigs markets

Figure 2 : Map of Nigeria showing movement and outflows of pigs from four selected pig markets to various destinations in the country

Sample size determination and collections: From April 2019- Feb 2020, October-November, 2020 samples were collected at the Dawaki, Katsit, Numan, and Pandam pig markets in Nigeria. With a population of 7million pigs, the minimum recommended sample size for such a population using a confidence level of 95% and a margin of error of 5% gives a calculated sample size of 384 (Dohoo *et al.*, 2009). The samples were collected using a convenient sampling method, based on the cooperation of the pig traders and slaughter slab operators. The samples collected were whole blood, tissue, and sera samples. 5mls of blood in heparinized and plain sample bottles were collected from pigs at LPMs and their slaughter slabs. Tissue samples (liver, spleen, and mesenteric nodes) were collected in labelled sample bottles from all pigs slaughtered at the slaughter slabs within the premises of the LPMs. Criteria for sample collection at the slaughter slab was pigs bought at LPMs, pigs from external sources were excluded. Both apparently healthy and sick pigs

were sampled. Sample collection was disrupted between March 2020 and September 2020 due to COVID-19 lockdown restrictions in Nigeria.

Detection of African swine fever antibodies : Sera samples collected were analyzed using commercially available ID Screen ASF Indirect kit (IDVet, Grabels, France) specific for the presence of antibodies against ASFV *p32*, *p62*, and *p72* proteins according to the manufacturer's instructions.

Detection of African swine fever virus. Total DNA was extracted from whole blood and homogenized tissue samples using the Qiagen Mini kit (Qiagen, Hilden Germany) following the manufacturer's protocol. A 257 bp region corresponding to the C-terminal region of the *B646* gene encoding the *p72* major capsid protein was amplified using the diagnostic primers PPA1 and PPA2 as previously reported to confirm the presence of the ASFV genome (Agüero et al., 2003).

ASFV genotyping, serogrouping and CVR sequence analysis

Representative ASFV positive samples from the 4 selected LPMs were selected for molecular characterization targeting four regions of the virus. The regions were the C-terminal end of the *B646L* gene encoding the *p72* protein, the full length of *E183L* gene encoding *p54* protein, central variable region (CVR) within the *B602L* and CD2v protein encoding the *EP402R* gene (Bastos et al., 2003; Gallardo et al., 2009; Malogolovkin et al., 2015b). ASFV sequences were generated commercially by MacroGen Inc. (Netherlands Europe) and LGC Genomic (Germany). The raw sequence data were assembled using Staden Package (<http://staden.sourceforge.net/>) and multiple alignments were performed using the CLUSTALX algorithm implemented in MEGA X (Kumar et al., 2018). Sequences for four gene segments of ASFV were compared to available sequences in the GenBank using the Basic Local Alignment Search Tool (<https://blast.ncbi.nlm.nih.gov/Blast>) with default settings. MEGA X was used for phylogenetic analysis to a Neighbor-joining tree inferred using the Maximum Composite Likelihood method for *p72* protein with data resampled 1,000 times in the bootstrap method. For the *p54* protein encoded in *E183L* gene, a Minimum Evolution method tree was inferred using the Maximum Composite Likelihood method and data resampled 1,000 times in the bootstrap method (Kumar et al., 2018). The CD2v protein sequences were inferred using Neighbor-joining method and data resampled 1,000 times in the bootstrap method. Sequences of ASFV genotypes and serogroups were retrieved from the Genbank for the construction of the phylogenetic trees for both *p72*, *p54* and CD2v protein genes. The tandem repeat sequences (TRS) of CVR (*B602L* gene) from this study were translated to protein sequences using ExPASy (<https://web.expasy.org/translate/>) with default software settings. The translated amino acid tetramers were matched with previously reported codes (Achenbach et al., 2017; Nix et al., 2006). ASFV TRS from this study were compared with 21 TRS of CVR previously reported in Nigeria and other West African countries. Sequences were deposited into GenBank with the following accession numbers: OL692379- OL692391 (*p72* gene), OL692397-OL692406 (*p54*), OL692410-OL692419 (*B602L* gene) and OL780773- OL780778 (*CD2v* gene).

Data analysis and mapping of pig movement

Results of data and analyzed samples collected were tabulated, maps of pig movements were generated to show the inflows and outflows of pigs at the 4 selected LPMs in Nigeria.

Ethics approval

This study was approved by the National Veterinary Research Institute Animal Ethics Committee Vom, Nigeria (AEC/03/26/16).

Results

Data on weekly population and destinations of pigs at the four selected live pig markets

A total of 34 visits were carried out to the four selected LPMs for data and sample collection within 13 months period. The common breeds at the four selected live pig markets visited were the crossbreed of Nigeria Indigenous Pig (NIP), Large White, Landrace and Duroc with an age range from 6 months- 5 years. Weekly population of pigs at the selected LPMs was as follows: Katsit (3,000-6,000 pigs) (Fig1A, Table 2),

Pandam (1,500-3000 pigs) (Table 2, Fig 3B), Dawaki (400-500 pigs), and Numan pig markets (1,000- 1,500). The pig population on a market day depends on the season of the year, there are more pigs during the dry season compared to the rainy season. Overall, it is estimated that 500,000 -700,000 pigs transit through these markets yearly. Data collected based on the interview of the pig market officials shows that the pigs are also brought to the market and transported to several locations (Fig 1-2). Pigs bought from Katsit pig market are supplied to 18 towns or cities in 13 States of Nigeria, (Table 2, Fig 2). Pigs are brought into Katsit pig market from several towns or villages in Kaduna (14), Niger (4), Nasarawa (3), and Plateau (2) States (Table 2, Fig 1). While pigs at the Pandam market are sourced from villages or towns in Plateau State (20) and Nasarawa State (4). Interestingly, pigs from Pandam pig market are also transported to the Katsit pig market. Furthermore, pigs sold at Dawaki pig market are mostly sourced from villages and towns in Bauchi (2) and Plateau (8) States, and transported to 5 States in Nigeria (Table 2). Live pigs traded at the Numan pig market are sourced from towns and villages in Adamawa (9), Gombe (7), and Taraba (2) States as well as the Republic of Cameroon. From the Numan pig market, the pigs are transported to 5 States of Nigeria (Table 2, Fig 2). Based on the data collected at the slaughter slabs within or closed to the LPMS, averagely, 15-40 pigs are slaughtered at Katsit, while 4-10 pigs are slaughtered at Dawaki, Numan, and Pandam Markets. The number of pigs slaughtered is dependent on the season of the year and disease outbreak. Postmortem meat inspection is usually carried out by a veterinary assistant with emphasis on zoonotic diseases like tuberculosis and porcine cysticercosis which are partially or wholly condemned for destruction.

Table 2: Live pigs markets in Nigeria and the locations where pigs are sourced and destinations where they are transported to.

Live Pig Market	Weekly pig population	States and towns where Pigs are sourced	State and towns where they are transported to after being purchased	Average number of pigs slaughtered at Slab per Market day
Katsit pig Market, Kaduna State	Dry Season: 4,000-6,000 Rainy Season: 3,000-3,500	Plateau (Jos, Pandam) Nasarawa (Keffi, Makera, Garaku Assakio) Kaduna (Kachia, Kaduna town, Birin Gwari, Fadan Karshi, Gwantu, Kudumi, Kagoro, Sabon Gida, Gidan Waya, Kufana, Kwoi, Kachia, Zagon Kataf, Manchok, Zaria), Niger (Kuchi, Zazagga, Guni, Sarkin Pawa)	Enugu (Enugu, Obolla-afor) Benue (Makurdi, Gboko, Otukpo, Eke) Edo (Benin, Auchi), Enugu (Obolla-afor, Enugu) Kaduna (Kaduna) Cross-river (Calabar) Akwa Ibom (Uyo) Rivers (Port Harcourt) Nasarawa (Assakio) Delta (Asaba, Warri) Lagos (Badagry) Anambra (Onitsha) Abuja Bayelsa (Yenagoa)	15-40 pigs

Live Pig Market	Weekly pig population	States and towns where Pigs are sourced	State and towns where they are transported to after being purchased	Average number of pigs slaughtered at Slab per Market day
Pandam Pig Market Plateau State	Dry Season: 2,500-3000 Rainy Season: 1,500-2,000	Plateau (Mangu, Langtang, Mabudi, Bokkos, Shendam, Panshin, Tunkus, Zomo, Amper, Gazum, Pajat, Kabwir, Shishiri, Pilgani, Kerang, Ampang, Kwanpe, Tashan Sule, Shishiri, Pil-Gani, Pajat) Nasaraw State (Assakio)	Plateau (Jos) Nassarawa (Lafiya, Awe, Assakio) Kaduna (Kaduna, Kafanchan) Benue (Makurdi, Oturkpo, Vandeikya Cross-River (Calabar, Ikom, Ogoja, Obudu) Enugu (Enugu, Obollaofar) Taraba (Wukari) Kogi (Lokoja) River (Port Harcourt, Bonny) Delta (Warri) Bayelsa (Yenagoa)	4-10 pigs
Dawaki Pig Market Plateau State	400-500	Plateau (Mangu, Panshin, Amper, Lantang, Dasham, Dawaki, Kwal, Kabwir) Bauchi (Tafawa Balewa, Bogoro)	Cross River (Ikom, Ogoja, Obudu, Obubura) Plateau (Jos, Pandam, Langtang, Shendam) , Nasarawa (Assakio) Benue (Makurdi) Enugu (Enugu)	4-10 pigs
Numan Pig Market Adamawa State	1,000-1,500	Adamawa (Numan, Michika, Bazza, Watu, Banganje, Dong, Guyuk, Kwaddai, Ngbalang) Gombe (Kalungo, tal, Todi, Banganje, Ture, Pobauli, Kalaring) Taraba State (Zing, Mayo-lope) Cameroon	Cross River (Ikom, Ogoja, Biase, Calabar) Benue State (Zaki Biam, Vandeikya) Delta State (Warri) Ebonyi State (Abakaliki) Adamawa State (Yola)	

Table 3: Monthly distribution of samples collected at the four selected live pig markets in Nigeria and the results of samples analyzed by the polymerase chain reaction

Month & Year	Tissue samples	Blood samples	Total samples	Samples positive by PCR
April 2019	5	9	14	5
May, 2019	17	55	72	14

Month & Year	Tissue samples	Blood samples	Total samples	Samples positive by PCR
June 2019	6	0	6	1
July 2019	18	11	29	4
August 2019	67	54	121	5
September 2019	15	9	24	4
October 2019	47	36	83	3
November 2019	16	12	28	7
December 2019	20	14	34	0
January 2020	51	32	83	15
February 2020	27	19	46	2
March 2020- Sept 2020	0	0	0	0
October 2020	39	26	65	10
November 2020	27	10	37	0
Total	355	258	613	66

Patten of trade at live pig markets: Apart from Numan and Katsit pig markets, there are no holding pens in all the 3 other LPMs. Pigs are kept under trees on the bare ground which is not cemented and they tethered to pegs very close to each other (Fig 3A-B). Although at Katsit LPM there is a private withhold facility, within the market, there are no pens. The traders transport their pigs in trucks, vans, and motorcycles to the LPMs. The pigs are collected from different communities or villages close to the markets. Pigs are bought based on physical appearance and size without weighting. There were no pre-entry antemortem inspections before pigs were allowed into the market' sites. Although, inside the LPMs antemortem inspection is usually carried by traders to rule out porcine cysticercosis by examination of the tongue of pigs before any transaction. All the four pig markets in this study were fenced, but the perimeter fencing of Dawaki and Katsit were partial, free-roaming pigs or dogs were observed at the two markets. Another observation is the washing of transport vehicles within the premises of the markets. Further observation is the slaughtering of moribund pigs in the premises of the market sites (Fig 3D). Other observations include free movement of slaughter slab operators between the slaughter slabs and the market area where live pigs are kept. Pigs are slaughtered on every market day and sales of pork were observed at all live pig market sites. Pigs showing clinical signs suggestive of ASF were observed at three LPMs (Dawaki, Katsit, Pandam) cyanotic skin and ASFV genome was detected by PCR in samples collected from some of these pigs (Fig 3C).

Laboratory results

ELISA results

A total of 217 sera samples were analyzed using ID Screen ASF Indirect (IDVet, Grabels, France), with a seroprevalence of 6.9% (16/217), which were as follows: Dawaki 5.6% (1/18), Katsit 4.2% (7/167), Numan 27.3% (6/22) and Pandam 10% (1/10).

Polymerase Chain Reaction results

A total of 613 samples were collected comprising 355 tissue and 258 whole blood samples with an overall prevalence of 10.77% (66/613). Of which, ASFV was detected in 13.52 % (48/355) and 6.98 % (18/258) of tissue and whole blood samples respectively by PCR (Table 2). Furthermore, the results showed Dawaki live pig market had the highest PCR positive samples of 23.81% (15/63), followed by Padam 18.18% (16/88), Katsit market had 8.13% (35/430), and 3.13% (1/32) in Numan pig markets respectively. ASFV was detected by PCR in at least one sample collected during 28 of 34 visits (Table 3).

Phylogenetic analysis of *B646L*, *E183L* and *EP402R* genes of African swine fever virus detected and sequence in samples collected

Accession number	Location	Year	Genotype	Sequence	Reference
Nig- NUM424	Live pig market Numan Adamawa State	2019	NA	ABNAAAACBN33AAAAACAACBNAAAAACBNNAFA	This study
Nig- KAF199	Live pig market Katsit Kafanchan Kaduna State	2019	NA	ABNAAAACBN30AAACBNAAAACBNNAFA	This study
Nig- KAF307	Live pig market Katsit Kafanchan Kaduna State	2020	NA	ABNAAAACBN32AAACBNAAAFA	This study
Nig- KAF194	Live pig market Katsit Kafanchan Kaduna State	2019	NA	ABNAAAACBN30AAACBNNAFA	This study
Nig- KAF163	Live pig market Katsit Kafanchan Kaduna State	2019	NA	ABNABNAAAA38ACBNNAFA	This study
Nig- KAF253	Live pig market Katsit Kafanchan Kaduna State	2020	NA	ABNAAAACBN33FA	This study
Nig- PDM288T	Live pig market Pandam, Plateau State	2020	NA	ABNAAAACBN33FA	This study
Nig- KAF41	Live pig market Katsit Kafanchan Kaduna State	2019	NA	BNDBNDBNAA0	This study

Nig_-KAF164	Live pig market Katsit Kafanchan Kaduna State	2020	NA	BNDBNDBNAA0			This study
Katsit fetus 2	Live pig market Katsit Kafanchan Kaduna State	2005	NA	ABNAAAACBNAA	ABNAAAACBNAA	KTQ427185	(Owolodun, et al., 2010)
Nigeria_-ABD13KPM2_-A	Live pig market Katsit Kafanchan Kaduna State	2012	NA	ABNAAAAACBNAA	ABNABTDBNAFA		(Luka et al., 2016)
Nig11_-KAF_14	Live pig market Katsit Kafanchan Kaduna State	2014	NA	ABNABNAAAAA	ABNACBNA	KT961377	(Luka et al., 2016)
Nig13_-KAF_14	Live pig market Katsit Kafanchan Kaduna State	2014	NA	ABNABNAAAAA	ABNACBNAFA	KT961366	(Luka et al., 2016)
Nig12_-KAF_14	Live pig market Katsit Kafanchan Kaduna State	2014	NA	ABNAAAACBNAA	ABNAAAACBNAA	KT961378	(Luka et al., 2016)
PL03	K-Vom, Plateau State Nigeria	2016	Inflow	ABNABNAAAAA	ABNACBNA		Adedeji <i>et al</i> 2021
Nigeria_-CDP11SP	Delta/Ughelli	2007	Outflow	ABNABNAAAAA	ABNACBNAFA	KT150931	
Nig29_-LGTT5_-15	Plateau/Langtang	2015	Inflow	ABNAAAACAA	ABNACBNAFA	KT961375	
Nig6_-JS10_13	Plateau/Jos	2013	Inflow	ABNAAAACBNAA	ABNAAAACBNAA	KT961364	
NIG_-BNGBT2	Benue State Nigeria	2009	Outflow	ABNAAAAACBNAA	ABNABTDBNAFA	KT150924	Luka <i>et al</i> , 2015

NIG_- ABDPOSASF5	Jos Plateau State Nigeria	2011	Inflow	ABNAAAAACBNAABTDBNAFAH150923	Luka <i>et al.</i> , 2015
Makurdi pig	Benue, Nigeria	2005	Outflow	ABNAAAAACBNAAAAAACBNAFA427186	(Owolodun, et al., 2010)
Udi pig 1	Enugu State Nigeria	2003	Outflow	ABNAAAAACBNAAAAAACBNAACQ2B1AFA	(Owolodun, Bastos, et al., 2010)
OJ pig 1	Edo	2006	Outflow	ABNAAAAACBNAAAAAACBNAACQ4718BNAFA	(Owolodun, Bastos, et al., 2010)
Nig 01 CAM/89	Lagos Cameroon	2006 1989	Outflow Inflow	AAACBNAAAAACBNAAAAAACBNAAAAAB ABNAAAAACBNAAAAAACBNAFAF513045	
IC- SP/2014/3	Ivory Coast	2014	NA	ABNAAAAACBNAABTDBNAFAFMH836365	(Luka et al., 2016)
Ben09/AGL1	Benin	2009	NA	ABNAAAAACBNAAAAAACBNAFA	(Alkhamis et al., 2018)
Tog09/P1	Togo	2009	NA	ABNAAAAACBNAAAAAACBNAFA	(Alkhamis et al., 2018)

Discussion

This is the first comprehensive study on the possible role of LPMs in spread of ASFV in Nigeria. Field reports revealed that ASF outbreaks occur throughout the year with many unanswered questions on how the ASFV persists and continue to spread in the pig populations in the country. Findings from this study suggest that LPMs in Nigeria may play a key role in the unabated ASF outbreaks in the country. During the duration of this study which was conducted over a period of 13 months involving 34 visits. ASFV was detected by PCR in samples collected during at least one visit in the 4 selected LPMs. In addition, at one sample collected during 28 out of 34 visits was positive and ASFV was also detected in 12 out 13 months that samples were collected within the study period (Table 3). Although, visits to the markets were disrupted by COVID-19 lockdown restrictions in Nigeria between March 2020 and September 2020, however, samples were collected for the first 11 calendar months. The total number of samples collected was 613, with ASFV detected by PCR in 10.72% (66) of samples collected. In addition, ASFV was detected in 3.13% -23.81% of pig samples collected in all selected live pigs markets. Finding from this study indicates that ASF-infected pigs are sold at all the selected pig markets in Nigeria. This is worrisome because pigs from these markets are supplied to several cities and towns in Nigeria (Fig 2) both for slaughtering and restocking. Previously, ASFV has been detected in Katsit pig market, however, this is the first report of detection of ASFV in Dawaki, Numan, and Pandam LPMs (Luka et al., 2016; Owolodun et al., 2010; Owolodun, Yakubu, et al., 2010b). Phylogenetic analysis of characterized sequences *B646L* and *E183L* genes selected positive samples collected from the 4 LPMs revealed ASFV genotype I and Ia respectively (Fig 4 & 5). While, analysis of the sequence CD2v gene revealed Nigerian ASFV samples clustered in serogroup 4 (Fig 6). However, analysis of CVR sequences showed 8 TRS variants were recovered from the markets within the study period (Table 4). Six variants (Tet-10, Tet-13, Tet-18, Tet-26, Tet-33, Tet-41) are being reported for the first time in this study. These findings suggest that live pig markets are the most likely locations to recover the current circulating ASFV TRS variants in Nigeria. In addition, adequate attention and regulation of activities at live pig markets are important in the control of the disease in the country. Previously, 5 TRS variants (Tet-15, Tet-17a, Tet-20a, 20b, Tet-48) were recovered at Katsit market, the same variants were detected in outbreak cases in other parts of the country. Particularly, Tet-20b which was widely circulating in Nigeria between 2009 and

2015 (Luka et al., 2016; Owolodun, et al., 2010). In addition to TRS variants recovered in this study, 21 variants have now been identified in Nigeria, 13 variants of which have been recovered from LPs in the country. The live animal market trade system plays a crucial role in the introduction of pathogens into new areas particularly long-distance disease spread (Martin et al., 2011; Zhou et al., 2015). Likewise, previous studies have described the important role of the live bird market in the epidemiology of avian influenza in Nigeria, but no report on the role of LPs in the epidemiology of ASF in the country (Coker et al., 2014; Sulaiman et al., 2021). This study has demonstrated that ASFV is circulating in LPs in Nigeria. These markets are possibly hotspots for the long-distance spread of the virus via the movement of ASF-infected pigs to several locations in the country from LPs. Though most of the pigs traded in these markets are for slaughter, however, farmers also buy pigs at these markets for restocking on their farms. In addition, unsold pigs are returned to owners' farms after the market day because of the unavailability of withholding facilities at these markets. Such practice further complicates the situations of ASF in Nigeria. It is important to note all the markets where this study was carried out, there is limited government regulation of market operators' activities. The apparent absence of antemortem inspection at market gates, lack of holding pens, close contact of pigs from different locations, and selling of pork within market premises enhances the ease of ASFV spread between pigs at market premises. Other poor biosecurity practices observed were free access to market sites by free-roaming pigs and dogs and unrestricted access of slaughter slab operators. Field reports in Nigeria and previous studies in Zambia have reported outbreaks following the introduction of pigs bought at LPs (Siamupa et al., 2018; Simulundu et al., 2018). This study also demonstrated the widespread free movement of pigs from surrounding communities to the four live pig markets and the movement of pigs from markets to various destinations in Nigeria (Fig 1 and Fig 2). In total, pigs are sourced from over 70 villages/towns located in 7 States of Nigeria and villages in Cameroon for trading at the market sites (Table 2, Fig 2), while pigs traded at the markets are supplied to 42 towns and cities in 13 States of Nigeria (Table 2, Fig 2). It is interesting to note that all these pig movements are done without movement permits or health certificates issued by the appropriate government agencies. In other countries like Zambia, Indonesia, and China, movement permits or health certificates are required before the movement of pigs between farms, live pig markets, districts, and regions (Gao et al., 2021; Leslie et al., 2016; Siamupa et al., 2018). Movement permits and certificates are important tools to track pig movements and reduce the risk of disease spread, but in Nigeria these movement permits/health certificates were not in use in all the live pig markets selected for this study. Results from this study estimate that nearly 500,000-700,000 pigs transit through these markets yearly, without appropriate restrictions or regulation to mitigate the spread of important transboundary diseases such as ASF. The pigs are transported to live pig markets using motorcycles, cars, buses, trucks, and on foot. The vehicles visit multiple farms, villages or towns, and livestock markets to collect and deliver the pigs to the markets without proper sanitary measures to prevent disease transmission. Also after delivery, the vehicles were washed at market premises leading to possible environmental contamination with the virus.

Conclusion

The study shows the possible role of LPs in spread of ASF. Of the samples collected, ASF was detected in 10.77% (66/613), while seroprevalence was 5.9%. Phylogenetic analysis of characterized samples collected from markets revealed ASFV genotype I. While sequence analysis of CVR within *B602L* recovered 8 TRS variants, six of which are new. For effective ASF control measures in Nigeria, disease surveillance and strict regulation at live pig markets must be put in place.

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