Transboundary and Emerging Diseases

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ORIGINAL ARTICLE

Surveillance for African Swine Fever in Nigeria, 2006–2009

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Summary

African swine fever (ASF) has had significant economic and social impact in Nigeria since 1997. However, there has been no effective national response to bring it under control. In this report, we confirm that ASF is still prevalent and widespread in Nigeria. Results from both serosurveillance and virological analyses indicated that ASF is present in most of the agro-ecological zones of the country. Nine per cent (9%) of serum samples and 48% of tissue samples were positive for ASF virus antibody and genome, respectively. Areas with high pig-related activities (marketing, consumption and farming) have higher prevalences compared with areas with less pig activities. Farm-gate buyers, marketing systems and transport of untested pigs within the country assist with the circulation of the virus. Only by putting in place a comprehensive routine surveillance and testing system, reorganizing the market and transportation systems for pigs, implementing on-farm bio-security protocols and considering the option of compensation will it be possible to achieve a significant reduction in ASF prevalence in Nigeria.

Introduction

Pigs play a major role in the socio-economic life of the people of Nigeria. They not only serve as a source of income especially for the rural population but also fulfil an important role in culture and food security. A substantial portion of the country's pig populations is resident in key pig producing, consuming and marketing areas of the country. The country's pig population has risen steadily from about 2 million to over 7 million from 1984 to 1997 (El-Hicheri, 1998). From 1997, widespread outbreaks of African swine fever (ASF) were experienced.

ASF is a haemorrhagic disease of domestic pigs caused by a DNA arbovirus of the genus Asfivirus and family Asfarviridae. The ASF virus (ASFV) has a double-stranded DNA genome, with a variable size of between 170 and 190 kb because of deletions and insertions occurring in the terminal regions of the genome and within a coding gene region of the central region of the genome, termed the central variable region (CVR) (Dixon et al., 2005; Owolodun et al., 2010). All ASF viruses belong to a single serotype. Differentiation is on the basis of genotypes (p72 genotyping and CVR sub-typing), and to date, only one p72 genotype and 6 CVR sub-genotypes have been identified in Nigeria (Bastos et al., 2003, 2004; Rowlands et al., 2008; Owolodun et al., 2010).

Three types of epidemiological cycles of maintenance for ASF virus have been described: a sylvatic cycle that occurs in southern and eastern Africa involving warthogs (*Phacochoerus aethiopicus*) and argasid ticks of the genus Ornithodoros with occasional spill-over to domestic pigs; a cycle in domestic pigs with the involvement of

Ornithodoros ticks inhabiting pig sties and a cycle in domestic pigs without the involvement of other hosts or vectors (Bastos et al., 2004). So far, only the cycle in domestic pigs, without known involvement of other hosts and vectors, has been implicated in the ASF endemicity in Nigeria. Although ASF was responsible for large numbers of fatalities amongst the intensively managed and free-range pig populations in Nigeria from September 1997, the cause of the disease was not confirmed until November 1997 (El-Hicheri, 1998). Prior to this, the other west African countries including Cote d'Ivoire, Togo, Benin and Gambia reported outbreaks of ASF and warning signals were sent to other west and central African countries (El-Hicheri, 1998). Notwithstanding the above, Nigeria remained ill prepared to respond to and control outbreaks of the disease. Consequently, ASF spread rapidly in Nigeria, causing high mortalities during September 1997 and October 1998 (El-Hicheri, 1998). An estimated 125 000 pigs from nine states were reported dead in this initial wave (El-Hicheri, 1998). Since the time of this initial epizootic, sporadic outbreaks have persisted in Nigeria with devastating impacts on both subsistence and commercial pig activities (El-Hicheri, 1998; Babalobi et al., 2007; Olugasa et al., 2007).

To date, despite the widespread losses caused by the epizootics of ASF in Nigeria, and their accompanying socio-economic effects, little progress has been made on the control and eradication strategy (Babalobi et al., 2007).

As there is no vaccine available for ASF, stringent bio-security and prompt diagnosis are the options for an efficient eradication programme for ASF in Nigeria (El-Hicheri, 1998). The PCR is an important diagnostic tool for ASF, particularly when animals are dying of acute disease and do not elicit a measurable immune response. In this report, we discuss results of ASF field, serological and virological surveillance in Nigeria, employing visual appraisal and laboratory-based techniques, respectively.

Materials and Methods

Location

Nigeria lies within the latitude 4°–14°N and longitude 2°–15°E with a land area of 923 763 km^2 and a human population of about 150 million. It is located in west Africa and bounded on the west by Benin Republic, on the north by Niger and Chad, on the east by Cameroon and on the south by the Atlantic ocean (Fig. 1a). Seventeen states were selected to represent the different agro-ecological zones of the country (Fig. 1b). These agro-ecological zones were aligned within the six geo-political zones of the country for ease of reference (Table 1). Stratified sampling with random sampling within clusters of each stratum was used in farm site and slaughter slab/abattoir selections. There is currently no comprehensive database for pig farms in Nigeria, and the pig population figure of approximately 9 million is an estimation (Federal Ministry of Agriculture, Abuja, Nigeria, 2007). We marked all major pig producing, consuming and marketing areas by generating multiple random points using the geographic information systems software and visited such locations; requested the average number of pig herds/farms available from heads of local pig farmers associations, local agricultural officers and extension agents; listed out the numbers and sizes (in ranges, e.g. 1–20 (small), 21–50 (small-medium), 51–100 (medium), >100 (large)); conducted random selections of small, medium and large farms; and carried out random sampling within each selected farm. Not more than five samples were collected per farm. Market and abattoirs were included in the surveillance because they appear to be extensions of farms because no testing is performed for pigs before they are transported to the markets/abattoirs. Not more that five samples were collected from an abattoir/market at any point in time. As it was difficult to determine the actual numbers of pigs per location, the sampling was opportunistic at each location.

A key factor in the selection of sites was to include the main pig producing, pig marketing and pig consuming areas of the country, especially areas where there have been previous reports of ASF outbreaks. In certain regions/states of Nigeria, cultural and religious factors prevent close association with pigs and these were not included. Thirty-six major towns were visited apart from their surrounding villages and suburbs (Table 1).

Sample collection

A total of 1276 sera and 322 groups of tissue samples (894 individual tissues – livers, spleens, lungs, lymph nodes and kidneys) were collected between October 2006 and April 2009 (Table 1). The samples were collected from different breeds including crosses of Large White, Landrace, Hampshire and Duroc under free-ranged and intensively managed systems. The age of the pigs sampled ranged from 3 months to approximately 4 years. In addition, a few local pig breeds mainly from Kebbi and Wukari (≤30) were sampled. While the sera were collected mainly by venipuncture, with some coming directly from collection at the point of slaughter during decapitation, tissues were collected by humane killing (stunning and decapitation) of pigs, which enabled necropsy and tissue collections. All sera and tissues harvested in the field were transported on wet ice (+4°C) and stored at −20°C in the laboratory at the Viral Research Division of the National Veterinary Research Institute (NVRI), Nigeria, until used. All sera

Fig. 1. (a) Map of Africa. (b) Map of Nigeria showing the different geopolitical zones.

Table 1. ASFV serological and virological resultsof the major pig trading establishments in Nigeria located within 17 states and over 35 localities. Specimens were collected between October 2006 and April 2009. ELISA and IB were utilized for antibody detection, while PCR and VI were employed for antigen screening and viable virus isolation, respectively

			Antibody detection					DNA demonstration					Virus isolation				
				Positives		Negatives			Positives		Negatives			Positives		Negatives	
Region	Study area state and period of collection	Location	Total	*N°*	%	*N°*	%	Total	*N°*	%	*N°*	%	Total	*N°*	%	N°	%
South-west	LAGOS (September–October 2008)	OKEARO	10	5	50	5	50	30	29	97	1	3	29	25	86	4	14
		AGEGE	9	0	0	9	100										
		IKORODU	8	0	0	8	100										
		Subtotal	27	5	19	22	81	30	29	97	1	1	29	25	86	4	14
	OGUN (September–November 2008)	IFO	33	0	0	33	100	3	1	33	2	67	1	1	100	0	0
		OWODE EGBA	9	0	0	9	100										
		IJEBU ODE	8	0	0	8	100	5	3	60	2	40	3	1	33	2	67
		Subtotal	50	0	0	50	100	8	4	50	4	50	4	2	50	2	50
	OYO(September 2008–April 2009)	AKINSAWE	14	3	21	11	79										
		BODIJA	134	6	4	128	96	11	4	36	7	64	4	1	25	3	75
		Subtotal	148	9	6	139	94	11	4	36	7	64	4	1	25	3	75
Regional subtotal			225	14	6	211	94	49	37	76	12	24	41	30	73	11	27
North-east	TARABA(October–December 2008)	USSA	10	0	0	10	100										
		TAKUM	31	5	16	26	84										
		WUKARI	3	0	0	3	100										
		LAU	16	0	0	16	100										
		ZING	17	0	0	17	100										
		Subtotal	77	5	6	72	94										
	ADAMAWA (October–December, 2008)	YOLA	8	0	0	8	100	2	0	0	2	100					
		NUMAN	42	23	55	19	45	1	0	0	1	100					
		Subtotal	50	23	46	27	54	3	0	0	3	100					
	GOMBE (January–April 2009)	GOMBE	62	0	0	62	100										
		Subtotal	62	0	0	62	100										
Regional subtotal			189	28	15	161	85	3	0	0	3	100	0	0	0	0	0
South-south	CROSS RIVER (September–November 2008)	CALABAR	32	6	19	26	81	19	10	53	9	47	10	4	40	6	60
	AKWA IBOM (September–November 2008)	UYO	16	0	0	16	100										
	EDO (September–November 2008)	BENIN	24	0	0	24	100	32	0	0	32	100					
	DELTA (March–October 2006)	20 LOCATIONS	122	17	14	105	86	5	3	60	2	40	3	1	33	2	67
Regional subtotal			194	23	12	171	88	56	13	23	43	77	13	5	38	8	62

Table 1. (*Continued*)

			Antibody detection					DNA demonstration					Virus isolation				
				Positives		Negatives			Positives		Negatives			Positives		Negatives	
Region	Study area state and period of collection	Location	Total	*N°*	%	*N°*	%	Total	*N°*	%	*N°*	%	Total	*N°*	%	N°	%
North-central	PLATEAU	JOS 2006–07	54	4	7	50	93	77	36	47	41	53	36	10	28	26	72
	(2006–2007,	JOS 2008	394	36	9	358	91										
	March–December	Pankshin	2	1	50	1	50										
	2008)	Subtotal	450	41	9	409	91	77	36	47	41	53	36	10	28	26	72
	BENUE	MAKURDI	50	1	2	49	98	70	31	44	39	56	31	7	23	24	77
	(September–	GBOKO	25	0	0	25	100	25	20	80	5	20	20	13	65	7	35
	December 2008)	Subtotal	75	1	1	74	99	95	51	54	44	46	51	20	39	31	61
Regional subtotal			525	41	8	482	92	172	87	51	85	49	87	30	34	57	66
North-west	KADUNA	KADUNA	20	1	5	19	95	19	16	84	3	16	16	14	88	2	13
	(December	CHUKUN	12	0	0	12	100										
	2008–January	CHIDA	7	0	0	7	100										
	2009)	KAFANCHAN	13	1	8	12	92										
		Subtotal	52	2	4	50	96	19	16	84	3	16	16	14	88	2	13
	KEBBI	ZURU	22	0	0	22	100										
	(December 2008)																
Regional subtotal			74	2	3	72	97	19	16	84	3	16	16	14	88	2	13
South-east	ABIA (October–	UMUAHIA	5	0	0	5	100	11	0	0	11	100					
	November 2008)	UMUDIKE	12	0	0	12	100										
		ABA	7	3	43	4	57										
		Subtotal	24	3	13	21	88	11	0	0	11	100					
	IMO (October–	MBAISE	11	0	0	11	100	5	0	0	5	100					
	November 2008)	OWERRI	10	0	0	10	100										
		Subtotal	21	0	0	21	100	5	0	0	5	100					
	ENUGU (October–	ENUGU	4	0	0	4	100	7	0	0	7	100					
	December, 2008)	EMENNE	20	0	0	20	100										
		Subtotal	24	0	0	24	100										
Regional subtotal			69	3	4	66	96	23	0	0	23	100					
National total (2006–2009)			1276	111	9	1163	91	322	153	48	169	52	153	79	50	76	50

ASFV, African swine fever virus; IB, immunoblotting.

and tissues were initially tested in Nigeria by indirect ELISA and PCR, and later sent to the EU Community ASF Reference Laboratory [Centro de Investigación en Sanidad Animal – Instituto Nacional de Investigación y tecnologia Agraria y Alimentaria, (CISA-INIA)] for confirmatory ASF serological and virological diagnoses.

ASF antibody detection

Indirect enzyme-linked immunosorbent assay OIE –ELISA

The details of the test are fully described in the World Organisation for Animal Health (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees) (2008). Briefly, ELISA microtitre plates (Nunc Polysorp® Immunoplates Nunc, Roskilde, Denmark) were incubated at 4°C overnight with 100 μl/well of ASFV soluble cytoplasmic antigen at a previously determined optimal concentration, in coating buffer (0.1 M carbonate buffer, pH 9,6). The coated plates were washed with phosphate buffered saline (PBS) (pH 7.5) containing 0.05% (v/v) Tween 20 (PBS-T) and used immediately or stored at −20°C until use. Porcine sera were added to the plates at a 1 : 30 dilution in PBS-T and incubated for 1 h at 37°C. Reference sera were included on each plate. Horseradish peroxidase (HRP)-labelled protein A (HRP-protein A, PIERCE) was diluted 1 : 5000 in PBS-T and added to the plates and incubated for 1 h at 37°C. After washing the plates, 0.2 ml of 3-dimethylaminobenzoic acid+3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (DMBA-MBTH) substrate (Sigma) were added per well. The reactions were stopped by the addition of 50 μl of 3N H_2SO_4, and the optical density (OD) was measured at 620 nm using a spectrophotometer Multiskan EX® (Thermos Scientific, Vantaa, Finland) after incubation for 10 min at room temperature.

The cut-off was determined using the following formula: [Optical density-negative serum × 1] + [Optical density-positive serum × 0.2].

Immunobloting

Sera yielding positive and doubtful results on ELISA were retested using the OIE (2008) recommended confirmatory test, the immunoblotting (IB) assay. Briefly, ASF vp30 blotted nitrocellulose strips were incubated by complete immersion in separate partitions in a plastic trough in 2% (w/v) blocking buffer (non-fat dried milk in PBS; pH 7.2), for 30 min with continuous agitation (Gallardo and Arias, 2009). The blocking buffer was discarded, and 1/40 dilutions of each test and control sera in fresh buffer was added per strip respectively. This mixture was incubated for 45 min at 37°C, with continuous agitation. The content was discarded, and the single strips and wells were thoroughly washed four times in blocking buffer. A 1 : 1000 dilution of HRP-protein A conjugate was added (0.5 ml), and the content was incubated for 45 min at 37°C, with continuous agitation. The washing steps were repeated, and 0.5 ml of the substrate (4-chloronaphtol/methanol) solution was added to each well of the trough. The reaction was stopped after 15 min by running cold water over the strips. Sera were regarded as positive only if the strips they were incubated with displayed bands of identical size and position as those that reacted with the positive control sera.

ASF virological detection

Nucleic acid extraction and genomic DNA amplification

DNA was extracted directly from a 10% saline buffer suspension of each of the 322 pooled tissues using a nucleic acid extraction kit (Nucleospin/ Machery-Nagel–Cultek) following the manufacturer's procedures. A PCR assay using the ASF diagnostic primers PPA1 [5′-AGTTATG GGAAACCCGACCC-3′], PPA2 [5′- CCCTGAATCGGAG CATCCT-3′] that generate an amplicon of 257 bp within the p72 gene (Agüero et al., 2003) was used to confirm the presence of ASFV DNA. For the amplification of DNA, 23 μl of the previously prepared PCR mix (Roche) was added to 2 μl of the DNA template in a 0.2-ml reaction tube. A similar preparation was made for the positive (Spain '70) and negative controls (nuclease-free distilled water) templates. A DNA marker was added to one lane on each side of the gel. The reaction mixture was treated as follows: (i) incubated for 10 min at 95°C; (ii) subjected to 40 cycles of PCR, with 1 cycle consisting of 15 s at 95°C, 30 s at 62°C and 30 s at 72°C; and (iii) incubated for 7 min at 72°C. The PCR products were analysed by electrophoresis through 2% agarose gel and visualized under UV light.

The results were only taken as valid if all the negative controls showed no bands while the positive controls displayed bands at the 257 bp region of the DNA marker.

Virus isolation

Antibody-negative/naïve pigs were used for the preparation of primary leucocyte cultures (PLC) as previously described (Malmquist and Hay, 1960). These PLC were used for the isolation of viruses from ASF antigen–positive samples. Briefly, cells were seeded into 96-well tissue culture grade microtitre plates (200 μl; 300 000 cells per well) in homologous swine serum and incubated in a humidified atmosphere containing 5% CO_2 at 37°C. Three-day cultures were infected by quadriplicates of a 10% suspension of PCR-positive ground tissues supplemented with 5 μg/ml gentamycin sulphate (BioWhittaker) and incubated for 24 h at 37°C. After inoculation, a 20-μl

preparation of 1% homologous red blood cells (RBC) in buffered saline was added to each well. The plates were examined for haemadsorption and cytopathic effects over a 6-day period. The negative samples were blind passaged three times.

Statistical analysis

Analyses were performed using StatGraphics v2.0 (Statpoint technologies Inc., Warrenton, Virginia, USA), and data were entered on the nominal scale. Pearson chi square (χ^2) tests were used to determine the associations between the results of the laboratory tests and the regions/locations where the samples originated. Antibody detections and DNA demonstrations were initially cross-tabulated separately with the regions/locations of samples. Virus isolation results were excluded from similar statistics because of wide disparities in data available from the different regions. The percentage distributions of the test results relative to the regions and locations were generated, and the contingency coefficient was then used to measure the strength of the association, if any. The results were exported into Microsoft Excel® (Microsoft Corporation, Redmond, Washington, USA) worksheet and rounded off to the nearest whole values.

Results

Serology

One thousand two hundred and seventy-six serum samples were classified as positive or negative by indirect ELISA followed by confirmatory immunoblotting. From the sera analysed, 111 (9%) tested ASF antibody positive and 1163 (91%) yielded negative results. Specifically, 189 sera tested positive for ASF antibody by indirect ELISA but only 111 were confirmed by immunoblotting assay (Fig. 2a). The remaining 78 ambiguous/doubtful sera, which were spread across geographical regions, were negative by immunoblotting assay. The regional prevalence of ASF antibodies varied as follows: south-west (6%), north-east (15%), south-south (12%), north-central (8%), north-west (3%) and south-east (4%) (Fig. 3). At $P < 0.05$, the χ^2 value was 18.232 and contingency coefficient was 0.2.

DNA demonstration and virus isolation

PCR was performed on 322 organ pools from domestic pigs. Following ASFV DNA amplification from the pooled samples, a single major amplicon of approximately 260 bp was generated from 153 (48%) of the samples (Fig. 2b). The specificity of the amplicons obtained was confirmed by *BsmaI* restriction analyses previously

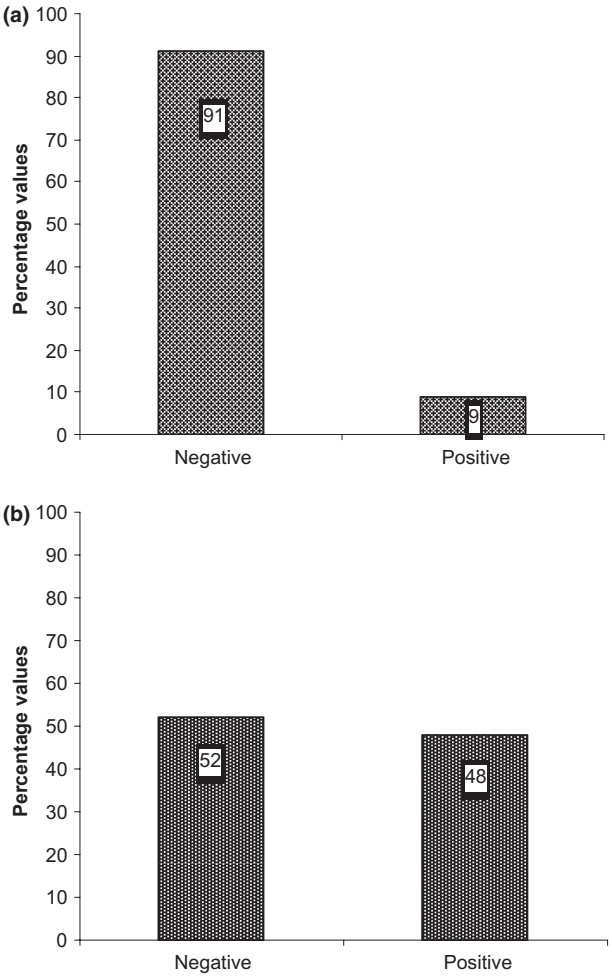

Fig. 2. Comparative African swine fever antibody (a) and African swine fever virus genome detection (b) in samples collected in Nigeria states.

described by Agüero et al. (2003). The regional prevalence of ASFV genome was as follows: south-west (76%), north-east (0%), south-south (23%), north-central (51%), north-west (84%) and south-east (0%) (Fig. 3) ($P < 0.05$; χ^2 value = 63.844; contingency coefficient = 0.2.

All samples that tested positive on PCR underwent virus isolation in pig leucocyte cultures because the OIE regards the test as a gold standard in ASF diagnosis. From the 153 PCR-positive samples, only 77 (50%) yielded positive virus isolation results. Only haemadsorbing ASFV was isolated.

Combined serological and virological ASF diagnostic results are summarized in Table 1 and indicate the prevalence of ASF in target Nigerian states. Certain patterns were observed in the prevalence of ASF using the serological and virological analyses. Oke-Aro in Lagos State, a facility that has a holding capacity of up to 300 000 pigs

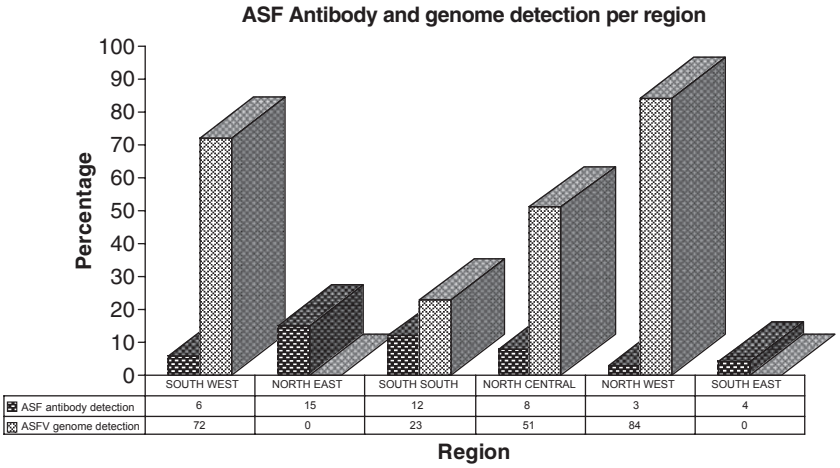

Fig. 3. Regional comparison of African swine fever virus antibody and genome prevalence between October 2006 and April 2009, involving 17 states and over 35 locations in Nigeria.

and many pig abattoirs, presented with high seroprevalence (50%) (ELISA and immunoblotting) and virus prevalence, as shown by both PCR and virus isolation (86%) results. In Numan in Adamawa State, a major pig trading centre, the ASF surveillance also showed high seroprevalence of ASF (55%), although insufficient tissues were collected to perform a comprehensive analysis including PCR and VI. In contrast, in Gboko in Benue state and Kaduna city (Kaduna State), a higher prevalence of ASF virus was observed (65% and 88%, respectively) compared with positive serological results (0% and 5%) However, in the course of the surveillance, it was impossible to obtain significant numbers of sera from Kafanchan International pig market, another important pig marketing area, and therefore results from Kafanchan (Kaduna State) may be biased when compared to the overall results.

Similarly, the following locations had evidence of significant virus activity based on PCR results: Ijebu-Ode, Ogun State 3/5 (60%); Delta, Delta State 3/5 (60%); Calabar, Cross River 10/19 (53%); Jos, Plateau State 36/77 (47%); Makurdi, Benue State 31/70 (44%)and Bodija, Oyo State 4/11 (36%) (Fig. 1). Figure 3 shows comparative serological and PCR regional results indicating the higher virus prevalence in north and south-western regions in contrast with zero percentage (0%) prevalences recorded from the south and north-eastern parts of the country.

Discussion

The results show that ASF is still a problem in the Nigerian pig industry. We are aware that our surveillance system is subject to certain limitations. The surveillance was carried out in selected locations, and some regions may have been under-represented based on the estimated pig population as verbally communicated by stakeholders, and the targeting of only high pig concentration areas for sampling, because the distribution of pigs varies due to religious and cultural differences in human population. Within the target areas, the sampling was startified and randomized as described earlier. Similarly, sample size was not as good in some areas as others because we depended largely on the cooperation of ordinary farmers, farmer groups, assistance from the State, Local Government Authorities and other relevant bodies to carry out surveillance in the field. Some farmers expressed dissatisfaction with the government and did not allow sampling in their farms because no compensation was paid for pigs that had died as a result of ASF.

Although no stamping out was carried out, farmers were of the opinion that outbreaks should have been controlled by culling and conpensation paid as was the case in the control of HPAI H5N1. The surveillance results reported here represent the most comprehensive study on the ASF situation in the west African country to date despite reports from other investigators (Babalobi et al., 2007; Luther et al., 2007a,b; Olugasa, 2007; Olugasa et al., 2007 and Owolodun et al., 2007).

The tissue samples were largely collected from the abattoirs, submitted to the central laboratory from farms or through direct purchase of live pigs from farms followed by humane slaughtering. The fact that the tissue samples yielded more positive results than sera could be attributed to farmers' practices: during an active outbreak, farmers often will not report to authorities but will rapidly sell off pigs before they die of ASF; furthermore, unthrifty and sick animals are culled first for slaughter.

This may also be because of the fact that animals were culled early in sickness or during the per-acute phase before a measurable humoral immune response can be generated. As PCR test detects only a fragment of genome sequence of the virus, the PCR may be positive, even when no infectious virus is detected by virus isolation, suggesting the possibility that some of the pigs may no longer be infectious.

Fasina et al., (2009) had previously reported higher seroprevalences around the abattoir/market areas when compared with farms. As pigs introduced to the markets/abattoirs rarely stay for more that 3 weeks before slaughter, it may be possible that antibodies are developed while still on farms, suggesting infection by mild strains of ASFV that permit the pigs to live longer and develop detectable antibodies. Most of the tissue samples used in this study came from the submitted samples, slaughter slabs, abattoir and meat shops, although a deliberate effort was made to buy whole animals from farms and slaughter these for tissue sample collection. The tissue samples from Delta State were collected during an active ASF outbreak, which lasted for about 7 months (March–October 2006), and this may be responsible for the higher antigen prevalence compared to other states within the region. The overall results indicate that a mild form of ASF virus may also be circulating in the field, resulting in a high number of persistently infected pigs. This may be particularly true in regions where both serological and virological results indicated high values. A similar report was recently published by Owolodun et al. (2010). In addition, it is highly likely that the differences noticed serologic and virologic results is linked to pigs that have better innate resistance to the pathogenic effects of the virus and recover completely. Penrith et al. (2004) had earlier described such resistance in an endemic pig population in Mozambique.

Lower prevalences were recorded from the south-eastern and south-southern states probably because these regions are not involved in trading pigs with other regions of the country, as reported by the eastern Nigerian farmers interviewed. A similar situation was obtained from the Zuru area of Kebbi State (north-west) and extremes of Taraba State (north-east), although Calabar, Cross River State (south-south) presented with a higher prevalence. It was, however, discovered that most of the pigs slaughtered in Calabar originate from Numan, Adamawa State (north-east) and parts of Nasarawa State (north-central).

From these analyses, it was suggested that the sale and circulation of infected animals is an important factor in keeping ASF virus in circulation. Other contributing factors are visits of farm-gate buyers who move from farm to farm, lack of compensation for compulsory slaughter in outbreak situations, lack of awareness by farmers and lack of/poor implementation of bio-security (El-Hicheri, 1998).

It is important to put in place a comprehensive routine surveillance and testing system, reorganize the market and transportation systems for pigs and consider the option of compensation for compulsory slaughter in outbreak situations to achieve a reduction in new ASF infections in Nigeria.

Finally, this surveillance has provided valuable baseline data on the probable role of trade movements in the epidemiology of ASF in Nigeria. It will, however, be important to consider the roles of husbandry systems (free-ranging/scavenging versus confined), swill feeding, possible role of wild suids and other factors that may contribute to the epidemiology of ASF in Nigeria.

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References

Agüero, M., J. Frenandez, L. Romero, C. Sanchez-Mascaraque, M. Arias, and J.M. Sanchez-Viscaino, 2003: Highly sensitive PCR assay for routine diagnosis of African swine fever virus in clinical samples. *J. Clin. Microbiol.* 41, 4431–4434.

Babalobi, O.O., B.O. Olugasa, D.O. Oluwayelu, I.F. Ijagbone, G.O. Ayoade, and S.A. Agbede, 2007: Analysis and evaluation of mortality losses of the 2001 African swine fever outbreak, Ibadan, Nigeria. *Trop. Anim. Health Prod.* 39, 533–542.

Bastos, A.D.S., M.-L. Penrith, C. Crucie're, J. Edrich, G. Hutchings, F. Roger, E. Couacy-Hymann, and G.R. Thomson, 2003: Genotyping field strains of African swine fever virus by partial *p72* gene characterization. *Arch. Virol.* 148, 693–706.

Bastos, A.D.S., M.-L. Penrith, F. Macome, F. Pinto, and G.R. Thomson, 2004: Co-circulation of two genetically distinct viruses in an outbreak of African swine fever in

Mozambique: no evidence for individual co-infection. *Vet. Microbiol.* 103, 169–182.

Dixon, L.K., J.M. Escribano, C. Martins, D.L. Rock, M.L. Salas, and P.J. Wilkinson, 2005: Asfarviridae. In: Fauquet, C.M., M.A. Mayo, J. Maniloff, U. Desselberger, and L.A. Ball (eds), *Virus Taxonomy, VIIIth Report of the International Committee on Taxonomy of Viruses*, pp. 135–143.Elsevier Academic Press, London.

El-Hicheri, K.., 1998: Emergency assistance on control and eradication of an outbreak of African swine fever in Western Nigeria. Report of the FAO Consultancy Mission to Nigeria. TCP/NIR/7822(E). FAO, Rome, December, 1998.

Fasina, F.O., D. ShamakiA.A. Makinde, and L.H. Lombin; ASF PITT, 2009: Field surveillance and laboratory diagnoses of African swine fever in Nigeria. International Symposium on Sustainable Improvement of Animal Production and Health. Vienna Austria, IAEA-CN-174-151. Pp300-302. Also available at: http://www-naweb.iaea.org/nafa/aph/BookOfExtendedSynopses.pdf.

Gallardo, C., and M. Arias, 2009: *ASF Diagnosis Protocols*. CISA-INIA, Madrid, Spain.

Luther, N.J., K.A. Majiyagbe, C.I. Nwosuh, and D. Shamaki, 2007a: The epizootiology, prevalence and economic aspects of African swine fever in parts of Middle Belt central states of Nigeria. *Trop. Vet.* 25, 96–100.

Luther, N.J., D. Shamaki, K.A. Majiyagbe, and C.I. Nwosuh, 2007b: The prevalence of African swine fever in parts of the middle belt central states of Nigeria. *Bull. Anim. Prod. Health Afr.* 55, 79–81.

Malmquist, W., and D. Hay, 1960: Haemadsorption and cythopathic effect produced by African swine fever virus in bone marrow and buffy coat cultures. *Am. J. Vet. Res.* 21, 104–108.

Olugasa, B.O., 2007: Serological evidence of African swine fever virus infection in commercial pig herds in Southwest Nigeria. *Afr. J. Livest. Ext.* 5, 61–66.

Olugasa, B.O., I.F. Ijagbone, P. Calistri, A. Conte, J.E. Freier, and M.P. Ward, 2007: Pattern of spread of African swine fever in south-western Nigeria, 1997–2005. *Vet. Ital.* 43, 621–628.

Owolodun, O.A., B. Yakubu, J.F. Antiabong, O.K. Adefalujo, M.E. Ogedengbe, E.T. Obisakin, and D. Shamaki, 2007: Molecular assessment of African swine fever in North-Central Nigeria. *Bull. Anim. Prod. Health Afr.* 55, 96–103.

Owolodun, O.A., A.D.S. Bastos, J.F. Antiabong, M.E. Ogedengbe, P.S. Ekong, and B. Yakubu,. 2010: Molecular characterisation of African swine fever viruses from Nigeria (2003–2006) recovers multiple virus variants and reaffirms CVR epidemiological utility. *Virus Genes* DOI 10.1007/s11262-009-0444-0. Published online 6th January, 2010.

Penrith, M.L., G.R. Thomson, A.D. Bastos, O.C. Phiri, B.A. Lubisi, E.C. Du Plessis, F. Macome, F. Pinto, B. Botha, and J. Esterhuysen, 2004: An investigation into natural resistance to African swine fever in domestic pigs from an endemic area of South Africa. *Revue. sci. Tech. Off. Int. Epizoot.* 23, 665–677.

Rowlands, R.J., V. Michaud, L. Heath, G. Hutchings, C. Oura, W. Vosloo, R. Dwarka, T. Onashvili, E. Albina, and L.K. Dixon, 2008: African swine fever virus isolate, Georgia, 2007. *Emerg. Infect. Dis.* 14, 1870–1874.

World Organisation for Animal Health (OIE), 2008: African swine fever. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees)*. Chapter 2.8.1; Web (Sixth) edition. Available at http://www.oie.int/eng/normes/mmanual/2008/pdf/2.08.01_ASF.pdf. Accessed on 20th January, 2009.