

RISK FACTORS ASSOCIATED WITH AFRICAN SWINE FEVER VIRUS IN APPARENTLY HEALTHY DOMESTIC PIGS IN BENUE STATE, NIGERIA

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ABSTRACT

A 12 months cross sectional study (September 2022 to September 2023) was conducted to identify the predisposing factors associated with the spread of African swine fever virus (ASFV) from pigs in Benue state. A total of 250 samples were collected from pigs and examined for molecular detection of ASFV using Polymerase chain reaction (PCR). 20 (8.0%) of the pooled tissues were positive. Of all risk factors studied, Age ($p=0.02$), Nature of faeces ($p=0.00$), and source of sample ($p=0.00$), were statistically significant. Pigs managed extensively had 4.0% positive cases, semi- intensive (2.8%) and intensive (1.2%). 2.8% of the cross bred were positive, local breed (3.6%) and exotic breeds (1.6%). 2.8% had firm while 5.2% were loose. 5.2% were in the rainy season (May-October), while 2.8% in the dry season (November-April). 1.6% had ticks on them while 6.4% had no ticks on them. 8.0% were collected from abattoir/slaughter slabs, whereas 0.0% collected from piggeries. 7.2% had abattoirs in their communities while only 0.8% had no abattoirs in pig communities. 6.0% were fed with swill feeds, while 2.0% are fed on processed feed. 3.6% are males while 4.4% are females. 1.6% are of the age range between 0-7months, 2.8% (7-18months), while 3.6% are >18months. The presentation of sick/unthrifty pigs for slaughter, farmers' inadvertent role, an infected neighbourhood, a pig to pig contact, vectors (soft ticks) may contribute to the transmission of the virus. Whereas tick control, disinfection of tools, food and water control, and separation of sick pigs reduces the likelihood of infections.

Keywords: *Ascaridia galli*, economic loss, parasitic infections

INTRODUCTION

African swine fever (ASF) is a devastating viral disease currently threatening the pig industry worldwide (Ayoade & Adeyemi, 2003). It is a notifiable, lethal disease of domestic pigs (Rahimi *et al.*, 2010). Delta State, Nigeria recorded death rates spanning from 50 to 100% in various herds. (Otesile *et al.*, 2005).

Intensive efforts have been made in the use of genetic epidemiology to evaluate the African swine fever virus (ASFV) circulating in different parts of Africa in order to gain an significant understanding of the relation between and geographic spread of each circulating genotype (Bastos *et al.*,

2003; Bastos *et al.*, 2004; Lubisi *et al.*, 2005; Boshoff *et al.*, 2007; Lubisi *et al.*, 2007; Gallardo *et al.*, 2009; Owolodun *et al.*, 2010; Gallardo *et al.*, 2011). However, the causes/factors that support the continued circulation of ASF viruses in pig herds in various parts of Africa in general and in Nigerian pig populations in particular remain poorly understood or at best hypothetical. (Fasina *et al.*, 2012)

Serologically, ASF prevalence has been set at 7.5% and 12.8% for Plateau and Kaduna states respectively (Luther *et al.*, 2002); 49.7% for Plateau state (Owolodun *et al.*, 2005); 50% , 52.5%, 59.8%, 60.7% and 70% for Ondo, Oyo, Lagos, Ogun and Osun states respectively (Olugasa, 2007) and 55%

for Kebbi state (Bala *et al.*, 2009). Similarly, pooled tissue samples have indicated that ASF is prevalent in at least 14 states of Nigeria with an overall prevalence figure as high as 51% being suggested (Majiyagbe *et al.*, 2004; Owolodun *et al.*, 2007). Asambe *et al.* (2017) reported a seroprevalence of Africa Swine Fever virus (ASFv) of 2.8% as well as 48.2% by Adenaike *et al.* (2023) which has established the presence of the disease in pigs in the State.

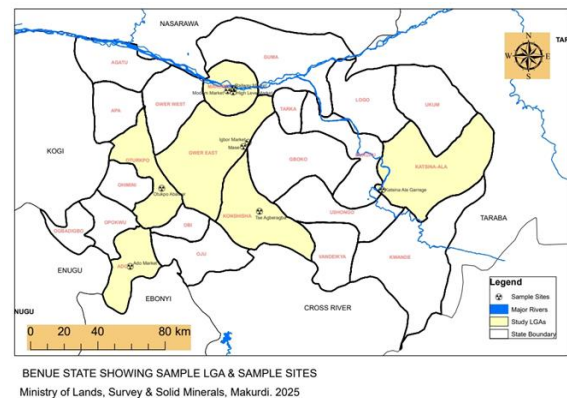
Risk factors and risk analysis studies for African Swine Fever (ASF) have been carried out by many authors with important outcomes (Fasina *et al.*, 2012). The abundance of ticks of the genus *Ornithodoros* combined with the availability of wild suids has been known to complicate the epidemiology of African swine fever in regions where they co-occur (Anderson *et al.*, 1998; Haresnape, Wilkinson & Mellor, 1988; Roger *et al.*, 2001). As previously emphasised, the vectors as well as the wild pigs have not been proven to have a role in the spread of the disease in West Africa, as of today (Fasina *et al.*, 2012). Other studies shows that herd size is a possible risk factor for pig diseases including the ASF, however, confirmation for this should be correlated with the true population at risk, husbandry-related factors, herd density, biological plausibility and herd distributions (Gardner *et al.*, 2002). In the case of West Africa, as is the case *et al.*, 2010; Nwanta *et al.*, 2011), hence, herd size may not truly reflect as a major risk factor for ASF in the sub-region. Although, husbandry method has also been associated with seropositivity in pig herds (Madec & Rose, 2003) and Mannelli *et al.* (1997) proved that free-range pigs are five times more likely to be seropositive to ASF virus antibodies compared with pigs kept in intensive facilities, this factor has yet to be investigated in West Africa. In addition, the payment of compensation was suggested to have a positive impact on prompt disease reporting and a negative influence on subsequent disease spread following infection in Sardinia (Mannelli *et al.*, 1998). However, such compensation schemes are largely lacking in West Africa and may thus serve as a risk factor for the spread of the disease. The legal and illegal sales of pig and pork products have been implicated in the transmission of ASF. Fresh pork, partially cooked and cured ham have been known to harbour the virus (Mebus *et al.*, 1993; Woolridge *et al.*, 2006). In Kenya, grazing management and proximity to the wildlife parks are identified risk factors. These factors shows the role of wild in the spread of ASF in East/ West Africa where wild pig populations are dwindling and presence of *Ornithodoros moubata* complex ticks have not yet established (Okoth *et al.*, 2009). Within the European Union, the free-range pigs are at higher risks of infection with ASF virus and this risk is associated with swill feeding (EFSA, 2010). In Denmark, inter-farm transportation and unrestricted movement associated with lack of barriers between the animal area and the loading bays have been identified as risk factors for contamination of herds (Boklund *et al.*, 2005). In the course of the outbreaks in West Africa, poor enforcement of movement restrictions, sale of pig and pig products and lack of a ban on live pig markets encouraged the continued redistribution and rediffusion of potentially infected pigs and meat arising and increased the potential of contaminating "clean" farms (El Hicheri *et al.*, 1998; LeFevre, 1998).

Similarly, the indiscriminate disposal of slaughtered pig viscera is a potential source of contamination to naïve herds and feral/free-range pigs can regularly contract infections from these infected viscera and disseminate the infection to other pigs. These are all activities that are likely to have played a role in the epidemiology of the disease in the sub-region. Finally, considering the volume of illegal meat transportation within and outside the sub-region, ASF will continue to be a constant threat to other parts of the world (Woolridge *et al.*, 2006; Chaber *et al.*, 2010)

MATERIALS AND METHODS

STUDY AREA

North Central state in Nigeria which derived its name from the Benue River as the second largest river in Nigeria after the River Niger. Its geographic coordinates are longitude 7° 47' and 10° 0' East. Latitude 6° 25' and 8° 8' North; and shares boundaries with five other states namely: Nasarawa State to the north for 231 km (partly across the Benue River), Tara2nd ed. VER Inc.; Taraba State to the east for 235 km, Cross-River State to the south for 96 km, Enugu State to the south-west for 84 km, and Kogi State to the west for 153 km. The state also shares a common boundary for 22 km with the Nord-Ouest Province, claimed by both Ambazonia and the Republic of Cameroon on the south-east and occupies a landmass of 34,059 square kilometres (Wikipedia, 2020).



The study was carried out in Benue state which is divided into three zones namely; Zone C (Otukpo, Ado), Zone B (Makurdi, Gwer-East) and Zone A (Katsina-Ala, Konshisha). The study sites (Local government areas) where chosen using simple random sampling technique. Availability of Live pig markets/abattoirs/ slaughter slabs, insecurity and pig population were also considered in our choice.

SAMPLE COLLECTION

Ethical approval was sought and granted by the animal care and use ethical committee of the college of Veterinary Medicine, JOSTUM (JOSTUM/CVM/ETHICS/2024/13) before the commencement of study.

A total of forty-five (45) sera, one hundred and seventy five (175) pooled tissue samples (livers, spleens, lungs, lymph nodes and intestines) and thirty (30) faecal samples totalling two hundred and fifty samples (250) were collected between September 2022 and September 2023 (Table I). The samples were collected from different breeds including crosses of

Large White, Landrace, Hampshire, Duroc and locals under extensive, semi intensive and intensively managed systems. The ages of the pigs sampled ranged from less than 3 months to approximately 9 months and above. 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 slaughter of pigs, which enabled necropsy and tissue collections while faecal samples were collected via the rectum using sterile polythene bags. All samples were transported to the Biotechnology Division of the National Veterinary Research Institute Vom, Plateau State, Biotechnology laboratory in cold boxes where they were stored at -4°C until processed for molecular detection of African swine fever virus (ASFV) using Polymerase chain reaction (PCR).

RESULTS

The details of all the 250 pigs used for this study and the results of PCR for African swine fever virus (ASFV) in Benue State are presented in (Tables I). 9 (3.6%) males and 11 (4.4%) females pigs were positive with 9 (3.6%) local pigs, 7 (2.8%) crossed and 4 (1.6%) exotic pigs. 4 (1.6%) pigs between the ages of 0-7months, 7 (2.8%) pigs between the ages of 7-18months and 9 (3.6%) pigs over 18 months were positive and showed statistical significance. 13 (5.2%) pigs with loose faeces and 7 (2.8%) pigs with firm faeces were positive and showed statistical significance. 4 (1.6%) pigs with ticks on them were positive while 16 (6.4%) that had no ticks were positive. 13 (5.2%) pigs were positive during the wet season while 7 (2.8%) pigs were positive in the dry season. 15 (6.0%) pigs fed on swilled feed were positive while 5 (2.0%) pigs fed on processed feed were positive. 20 (8.0%) of the pooled tissue samples collected from slaughter animals in abattoirs/slaughter slabs were positive while faecal and serum samples from piggeries were all negative and this showed statistical significance. Only 20 pigs representing 8.0% of the total pig samples in Benue State analysed were positive while the remaining 230 that represented 92.0% were negative.

DISCUSSION

During the investigation of 250 samples (tissues, faeces and sera) collected from pigs and examined for the presence of African swine fever virus (ASFV). Data shown from Tables Ia and Ib, that the ages of the animal (adult pigs were more susceptible). The high number of positive in older pigs (3.6 %) compared to younger pigs (1.6% and 2.8%) is in agreement to work done by Bisimwa *et al.* (2020) which states that most likely, the longer lifespan and increased risk of exposure to repeated African Swine Fever virus (ASFV) infections would lead to the persistence of antibodies and suggesting the circulation of a low virulence of African Swine Fever virus (ASFV) genotype strains. Also in agreement with Adenaike *et al.* (2023), with the fact that adult sows are traditionally not easily sold out or slaughtered by farmers which gives room for fast proliferation of the virus.

The higher molecular detection rate observed in female than male pigs were not significant and contrasted the findings of Abwage *et al.* (2015) who reported higher prevalence in male

pigs in Taraba state. The higher African Swine Fever virus (ASFV) antibody detection rate in female pigs could be explained by the fact that more female pigs are kept for breeding purposes and stay for longer period in the herds, while the male pigs are fattened and sold off, except for a few that are kept as breeding boars and for other reasons (Lammers *et al.*, 2007).

Table Ia; Distribution of African Swine Fever Virus (ASFV) in pigs in Benue State, Nigeria (September 2022 to September, 2023).

VARIABLE	NO SAMPLED (%)	ASF STATUS POSITIVE PCR (%)	P-Value	OR(95% CI)
SEX				
Male	90(36%)	9(3.6%)		
Female	160(64%)	11(4.4%)	0.47	1.51 (0.60-3.78)
BREED				
Local	77(30.8%)	9(3.6%)	Ref	
Cross	97(38.8%)	7(2.8%)	0.43	1.7 (0.60-4.8)
Exotic	76(30.4%)	4(1.6%)	0.25	2.38 (0.70-8.10)
AGE				
0-6 months	106(42.4%)	4(1.6%)	Ref	
6-18 months	44(17.6%)	7(2.8%)	*0.02	4.8 (1.34-17.44)
>18 months	100(40.0%)	9(3.6%)	0.25	2.27 (0.68-7.62)
NATURE OF FAECES				
Loose	63(25.2)	13(5.2%)		
Firm	187(74.8%)	7(2.8%)	*0.00	6.69 (2.53-17.65)
PRESENCE OF SOFT TICKS ON THE PIGS				
Present	30(12.0%)	4(1.6%)		
Absent	220(88.0%)	16(6.4%)	0.28	1.96 (0.61-6.32)
HUSBANDRY OF PIGS				
Extensive	110(44.0%)	10(4.0%)	Ref	
Semi-intensive	111(44.4%)	7(2.8%)	0.46	1.47 (0.54-4.06)
Intensive	29(11.6%)	3(1.2%)	0.73	1.15 (0.30-4.50)
SOURCE OF SAMPLE				
Abattoir/Slaughter slabs	175(70%)	20(8.0%)		
Piggery	75(30%)	0(0.0%)	*0.00	19.9 (1.19-333.8)

Table 1b Distribution of African Swine Fever Virus in pigs in Benue State, Nigeria (September 2022 to September, 2023)

VARIABLE	NO SAMPLED (%)	ASF STATUS POSITIV E PCR (%)	P- Value	OR(95%CI)
PRESENCE OF ABBATTOIR IN PIG COMMUNITIES				
YES	227(90.8%)	18(7.2%)		
NO	23(9.2%)	2(0.8%)	0.89	1.11 (0.24 – 5.1)
SEASON OF SAMPLING				
Wet (May-Oct.)	130(52.0%)	13(5.2%)		
Dry (Nov.-April)	120(48.0%)	7(2.8%)	0.25	1.79 (0.69-4.66)
GEOGRAPHICAL LOCATION				
Otuokpo	41(16.4%)	1(0.4%)	Ref	
Ado	42(16.8%)	1(0.4%)	1.00	1.03(0.06-16.97)
Makurdi	42(16.8%)	7(2.8%)	0.05	8.0 (0.94-68.29)
Gwer-East	42(16.8%)	5(2.0%)	0.20	5.4 (0.60-48.47)
Konshisha	41(16.4%)	4(1.6%)	0.36	4.32 (0.46-40.50)
Katsina-Ala	42(16.8%)	2(0.8%)	1.00	2.0 (0.17-22.96)
TYPE OF FEED				
Swill	150 (60.0%)	15(6.0%)		
Processed	100 (40.0%)	5(2.0%)	0.23	2.11 (0.74-6.010)

The local breed (3.6%) had a higher prevalence than the crossed (2.8%) and exotic (1.6%). This observation agrees with earlier reports by Fasina *et al.* (2012) where he observed that the infection rate was found to be higher in indigenous breeds compared to cross bred animals. Because most times local breeds are managed extensively and the move around and are most like to be in contact with infected animals or materials most especially for pigs around pig abattoirs/slaughter slabs where there is indiscriminate disposal of slaughtered pig viscera which serve as a potential source of contamination to naïve herds and feral/free-range pigs that can regularly contract infections from these infected viscera and disseminate the infection to other pigs.

The prevalence, as observed in this study is higher in the dry season (5.2%) than the wet season (2.8%). This is in agreement with reports by Bisimwa *et al.* (2020) as well as Awosanya *et al.* (2015) that shows a higher prevalence in the dry season. Reason been that free- range system is common in certain areas where farmers lack financial resources capability to construct housing or purchase feeds especially during dry season when there is no crop farming activity. This situation results in pigs scavenging for food exposing them to the risk of disease transmission by increasing the likelihood of pig into contact with an infected animal, ingestion of contaminated viscera/swills (Dohoo *et al.*, 2009;

Nantima *et al.*, 2015). Also in agreement to work done by El Hicheri (1998) which shows that African Swine Fever (ASF) spread rapidly in Nigeria between September 1997 and October 1998. The result obtained observed that more farmers keep their pigs in an enclosure especially during the rains and open them to roam when the rains are over and crops have been harvested.

Husbandry system (pigs kept in the free-ranging system (4.0%) exhibited a higher prevalence of infection than those in intensive (1.2%) and semi-intensive (2.8%). This is in agreement to work-done by Mannelli *et al.* (1997) as well as Costard *et al.* (2009) who have similarly reported that free-range pigs and local pig movement were associated with the spread of African Swine Fever (ASF). Also Madec & Rose. (2003) proved that free-range pigs are five times more likely to be positive to African swine fever virus (ASFv) antibodies compared with pigs kept in intensive facilities. Our results were similar to those of Ironkwe & Amefule (2008) in Rivers state, south-south Nigeria who reported a very few (10%) respondent who practice intensive method. Management systems are closely related to feeding and source of feeds. However, pigs under intensive system perform better than extensive system (Rekwot *et al.*, 2003).

Pigs with soft ticks on their body had a lower prevalence of 1.6% than those without with a prevalence of 6.4%. This is in agreement to work done by Fasina *et al.* (2012) who reported that Domestic pig-to-pig contact remains the only proved means of transmission of African Swine Fever (ASF) in Nigeria and West Africa, no tick vector has been associated with African Swine Fever (ASF) in Nigeria till date.

Pigs fed with swill feeds had higher prevalence (6.0) than those on processed feed (2.0%). This is in agreement to a study by (Horst *et al.*, 1997; El-Hicheri, 1998) which shows that Food and water control significantly reduced the risk of ASF Since the introduction of food and swill are an important means of transmission of pig diseases.

Pig samples gotten from abattoir/slaughter slabs have a higher prevalence of 8.0% compared to those from the piggery (0.0%). This is in agreement with work done by Fasina *et al.* (2012) which suggest the fact that abattoir/slaughter samples yielded more positive 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 due to the fact that animals were culled early in sickness.

Samples of pigs that are gotten from communities where there are abattoirs have a higher prevalence of 7.2% than those without abattoirs where pigs roam. This in in agreement with work-done by Fasina *et al.* (2012) shows that pigs around pig abattoirs/slaughter slabs where there is indiscriminate disposal of slaughtered pig viscera which serve as a potential source of contamination to naïve herds and feral/free-range pigs that can regularly contract infections from these infected viscera and disseminate the infection to other pigs.

CONCLUSION

Our findings constitute the evidence demonstrating the presence of African swine fever virus (ASFV) from other

local governments in the Benue state besides Ado local government in apparently healthy domestic slaughtered in the abattoir/slaughter slabs. Our data will ultimately be useful in contributing to an improved ASF control strategy in the State. Molecular characterization will be required to know the genotypes of the ASFV.

RECOMMENDATIONS

We recommend that control measures such as use of biosecurity, test and slaughter of animals that are positive for African swine fever virus (ASFv) with adequate compensation as a policy should be adapted by the Government as a way to completely eradicate the disease in the State and the country at large.

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DECLARATIONS

Ethical approval was sought and granted by the animal care and use ethical committee of the college of Veterinary Medicine, JOSTUM (JOSTUM/CVM/ETHICS/2024/13) before the commencement of study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest among them.

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