

African swine fever antibody detection and status in pigs in Apa, Ohimini and Okpokwu local government areas of Benue State, Nigeria

*¹Adenaike, E. A., ²Tekdek. L. B., ³Kazeem. H. M. and ⁴Simon A. Y.

¹Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State,

²Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State

³Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State

⁴Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Kaduna State

*Corresponding author: adenaike.lara@gmail.com, 08026580689



Abstract

Since African Swine Fever (ASF) is a notifiable, highly contagious, lethal haemorrhagic disease in domestic pigs. studies were undertaken to investigate the presence of antibody against African Swine Fever Virus (ASFV) among pig population in Apa, Ohimini and Okpokwu LGAs of Benue State, Nigeria . Eight, five, and seven serum were sampled from suspected pigs' populations in Apa, Ohimini and Okpokwu Local Governments Areas of Benue state respectively. One serum sample from Ohimini tested positive (ie 20%) but none of the samples from Apa and Okpokwu LGAs tested positive for the test (ie 0%). Since pigs with demonstrable antibody are normally considered as chronic carriers of the virus because it is doubtful if true recovery ever occurred. Control measures such as use of quarantine , test and culling of animals that tested positive for ASF antibody with commensurable level of compensation.

Keywords: ASF Antibody, Serology, Pigs, Benue state



Détection et statut des anticorps contre la peste porcine africaine chez les porcs dans les zones de gouvernement local d'Apa, Ohimini et Okpokwu, dans l'État de Benue, au Nigéria

Résumé

La peste porcine africaine (PPA) étant une maladie hémorragique à déclaration obligatoire, hautement contagieuse et mortelle chez les porcs domestiques. des études ont été entreprises pour étudier la présence d'anticorps contre le virus de la peste porcine africaine (VPPA) parmi la population porcine des LGA d'Apa, Ohimini et Okpokwu de l'État de Benue, au Nigéria. Huit, cinq et sept sérums ont été échantillonnés dans des populations de porcs suspectées respectivement dans les zones de gouvernement local d'Apa, d'Ohimini et d'Okpokwu, dans l'État de Benue. Un échantillon de sérum d'Ohimini s'est révélé positif (soit 20 %), mais aucun des échantillons des LGA d'Apa et d'Okpokwu n'a été testé positif (soit 0 %). Étant donné que les porcs présentant des anticorps démontrables sont normalement considérés comme des porteurs chroniques du virus, car il est douteux qu'une véritable guérison ne se soit jamais produite. Des mesures de contrôle telles que le recours à la quarantaine, les tests et l'abattage des animaux testés positifs aux anticorps anti-PPA avec un niveau de compensation proportionné.

Mots-clés : Anticorps PPA, Sérologie, Porcs, État de Benue

Introduction

African swine fever (ASF) is a notifiable, highly contagious, lethal hemorrhagic disease of

domestic pigs (Rahimi *et al.*, 2010). The disease is a devastating viral disease which is currently threatening the pig industry worldwide (Ayoade

and Adeyemi, 2003, It is a transboundary animal disease (TAD), resulting in serious economic losses, and a threat to the world food security and estimated losses of over USD 2.9 billion per annum as been ascribed to the disease in sub-Saharan Africa alone (Fadiga *et al.*, 2013). Mortality range of 50 to 100% in various herds, was recorded in Delta State, Nigeria (Otesile *et al.*, 2005). A total of 125,000 pigs died of ASF from September, 1997 to October, 1998 in Lagos, Ogun, Kaduna, Benue, Enugu, Akwa Ibom, Rivers, Plateau and Delta states in Nigeria (Hicheri, 1998). Besides the economic cost, the spread of the disease also has a devastating social effect, especially in smallholder farms in developing countries, pushing families into poverty by reducing their purchasing capacity, their sources of protein and even their capacity to pay health and education expenses (Cooper *et al.*, 2019). The disease is caused by African swine fever virus (ASFV) which is an enveloped double stranded deoxyribonucleic acid (DNA) virus belonging to genus *Asfivirus*. Maintenance and transmission of ASFV involve cycling of virus between soft ticks of the genus *Ornithodoros* and wild pigs such as warthogs, bush pigs, and giant forest boars (Murphy *et al.*, 1999). The virus can also be acquired through ingestion of contaminated feed (Rahimi *et al.*, 2010). Different strains of AFSV vary in their ability to cause disease, but there is one serotype of the virus detectable by blood antibody test (EU, 2010). The virus belongs to the member of the family *Asfavidae* and is a double stranded DNA virus that measures 200-220nm in diameter. The DNA of the virus is circular and has 170-190 kbp with the nucleoprotein core surrounded by an icosahedral sheet and possesses an outer envelope. The viral genome codes for about 34 structural protein and some nonstructural proteins (Ekwe and Wilkinson, 2000; Sarma, 2012). The virus survives in chilled carcasses or in frozen meat for several weeks (Merchant and Parker, 2005). In uncooked products, such as dried

sausage and ham the virus can persist for 3 to 6 months. The virus can be viable in garbage containing meat scraps that have not been heated to 65°C for 1 hr. The virus is sensitive to lipid solvents and can be inactivated rapidly by 2% NaOH (Sarma, 2012). The dried virus is not destroyed by exposure to 40°C in 15 days. It is preserved in 0.5% phenol in 50% per cent glycerin mixture at room temperature for 536 days. It is quite stable and since it remains viable for 11 days at room temperature, 15 weeks in carcasses, 5 months in processed hams, and 6 months in bone marrow. It has been suggested that multiple antigenic type of virus exist in Africa but only one in Europe (Merchant and Parker, 2005).

Benue State is known for swine production in Nigeria (RIMS, 1992). If swine production is affected by ASFV in the LGAs, it will affect animal protein consumption and also affect southern part of Nigeria. It is therefore of utmost importance to assess the ASF status of pigs in the area so that control and preventive measures can be instituted so that the pig population in the area will be safeguarded.

Materials and Methods

Study Area

The study area where samples were collected were Apa, Ohimini and Okpokwu Local Government Areas of Benue State of Nigeria. Blood samples were collected from pig population from both commercially managed and traditionally managed pigs in Apa, Ohimini and Okpokwu LGAs. in Benue State of Nigeria. Benue state lies in North central geopolitical zone of Nigeria between latitude Latitude 6°25'N and 8°8'N, and longitudes 7°47'E and 10°°E. It covers an area of 30,955km² (Agada and Nirupama, 2015) The state is bounded by 6 states (Nasarawa to the north, Taraba to the east, Kogi to the west, and Enugu, Ebonyi and Cross River to the south) Including an International border with Cameroon republic to the southeast (Agada and Nirupama, 2015). The capital of Benue State is Makurdi.

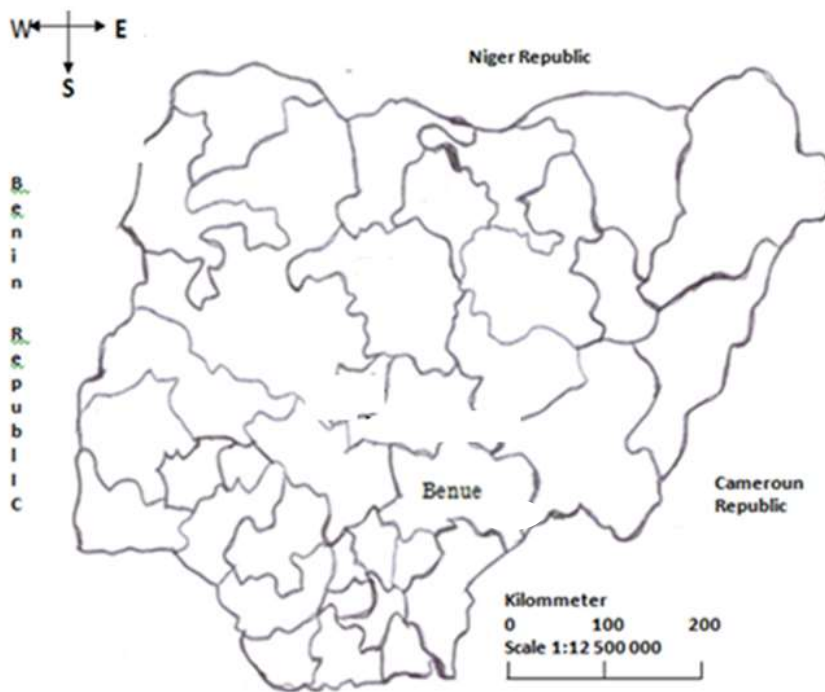


Figure 1 Map of Nigeria showing Benue State (Dada et al., ,2010) used for the study.

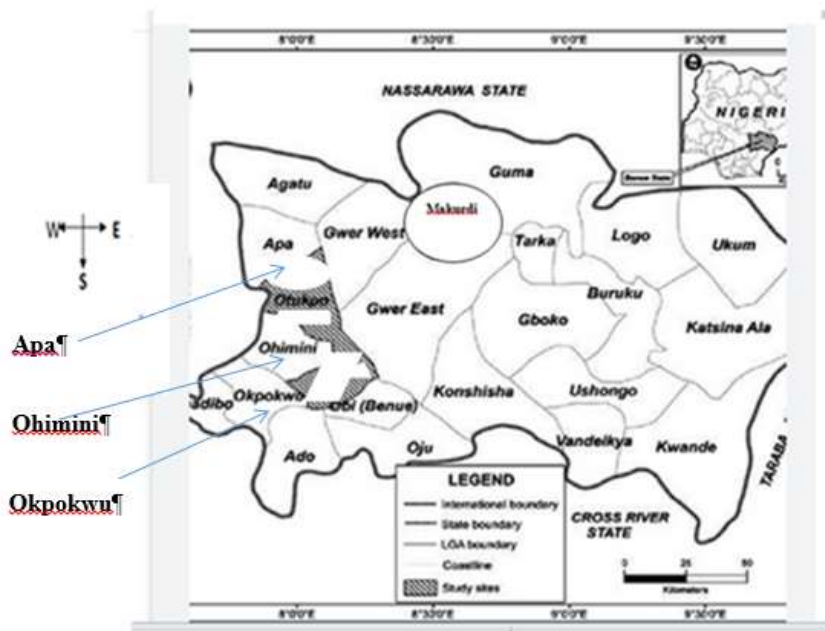


Figure 2 Map of Benue State showing Apa, Ohimini and Okpokwu Local Government Areas of Benue State of Nigeria (Dada et al., ,2010) used for the study.

Activity before the collection of samples for analysis

The following were taken from live animals or before animals: The breed or breed trait and sex of each animal were recorded; the weights of the animals were taken mostly with the use of weighing band. The body of each animal was carefully examined with magnifying glass or hand lens for any lesion on the skin and to see if there were any ectoparasites (eg Mites or ticks).

Sample collection

A total number of 20 blood samples (5 to 10 mls) from each animal was collected from Apa, Ohimini and Okpokwu LGAs of Benue state of Nigeria. The blood was collected mainly from live animals through the jugular vein in two different tubes for each animal (one tube containing anticoagulant and vacutainer tube without anticoagulant. All the samples collected were placed in a cooler and covered with ice pack. The samples were analyzed in the Departments of Veterinary Pathology, Veterinary Public Health and Preventive Medicine and the National Animal Production Research Institute Ahmadu Bello University, Zaria, Kaduna State.

Antibody detection test

The detection of antibody against ASFV antibody was carried out by indirect Enzyme-linked immunosorbent assays (ELISA) as described by manufacturer (Innovative Diagnostics IDvet, 310, rue Louis Pasteur-Grabels-FRANCE). It is an indirect ELISA for the detection of anti-African Swine fever antibodies in porcine serum and plasma samples or blood filter samples.

Description and principles of Indirect ELISA for the detection of anti-African swine fever

antibodies in porcine serum and plasma samples or blood filter samples

Even-numbered microwells were coated with p32, p62 and p72 ASFV recombinant proteins, odd-numbered wells are uncoated. Serum samples to be tested and controls were added to even and odd-numbered wells. Anti-ASFV antibodies with the aid of micropipette, if present it will form an antigen-antibody complex. After washing, an anti-multi-species horseradish peroxidase (HRP) conjugate is added to the wells. It fixes to the antibodies, forming an antigen-antibody-conjugate-HRP in the presence of antibodies, blue solutions which become yellow after addition of the stop solution. In the absence of antibodies, no coloration appears. The microplates were read at 450 nm. ELISA recorder and computer will help in recording, and result sheets for recording all the result shown by the ELISA recorder and computer.

Wash solution preparation

Wash concentrate (20X) was brought to room temperature and mixed thoroughly to ensure that the wash concentrate (20X) is completely solubilized. The Solution 1X was prepared by diluting the wash concentrate (20X) to 1/20 in distilled/deionised water.

Procedure for testing of serum samples

All reagents were allowed to come to room temperature ($21^{\circ}\text{C} \pm 5^{\circ}\text{C}$) before use. All reagents were homogenized by inversion or vortex. Each sample was deposited twice (adjacently in even and odd-numbered wells).

Arrangement of serum samples

	No Ag 1	Ag 2	No Ag3	Ag 4	No Ag5	Ag 6	No Ag 7	Ag 8	No Ag9	Ag 10	No Ag 11	Ag 12
A	NC	NC	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	NC	NC	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	PC	PC	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	PC	PC	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

Figure 3. Showing plate map. Samples above are deposited in duplicate in adjacent Even and odd- numbered wells. No Ag=Uncoated well Ag=Coated well, NC=Negative control, PC = Positive control, S= sample

The following were added as instructed by the manufacturer of the kits.

100 ul of Dilution Buffer 14 to each well. 10ul of the negative control to wells A1, B1 and A2, and B2,

10 u lof the positive control to wells C1, D1 and C2, D2. 10ul of each sample to be tested to the remaining wells. Each sample was deposited twice (adjacently in even and odd-numbered wells)

It was later incubated for 45 min at 21° C (± 5°C). The wells were emptied and washed 3 times with approximately 300 ul of washed solution. Drying of the wells was avoided during washing. Conjugate 1X was prepared by diluting the concentrated conjugate 10X to 1/10 in dilution buffer 100 ul of the conjugate 1X was added to

each well It was incubated for 3 min at 21° C (± 5°C).

The wells were emptied and washed 3 times with approximately 300 ul of washed solution. Drying of the wells was avoided during washing.

100 ul of the substrate solution was added to each well. It was incubated for 15 min ± 2min at 21° C (± 5°C) in the dark.

100 ul of the Stop Solution was added to each well in order to stop the reaction.

The O.D was read and recorded at 450 nm.

Validation

O.D result was calculated as follows

O.D net = O. D even well-O.D odd well

Any OD value that is negative, the sample was given O.D net as value of Zero

Interpretation

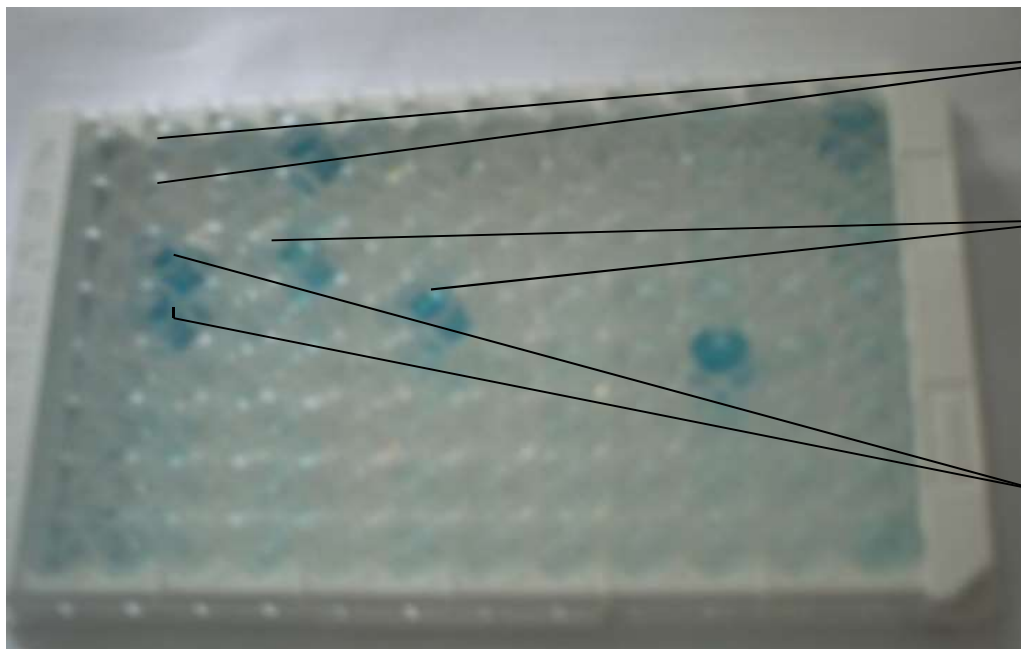
S/P percentage (S/p%) was calculated

$$S/P\% = \frac{\text{Net OD sample}}{\text{Net OD PC}} \times 100$$

Samples with a S/p%

With less than or equal to 60% are considered negative.

Greater than or equal to 60% are considered positive



Negative

Antigen-

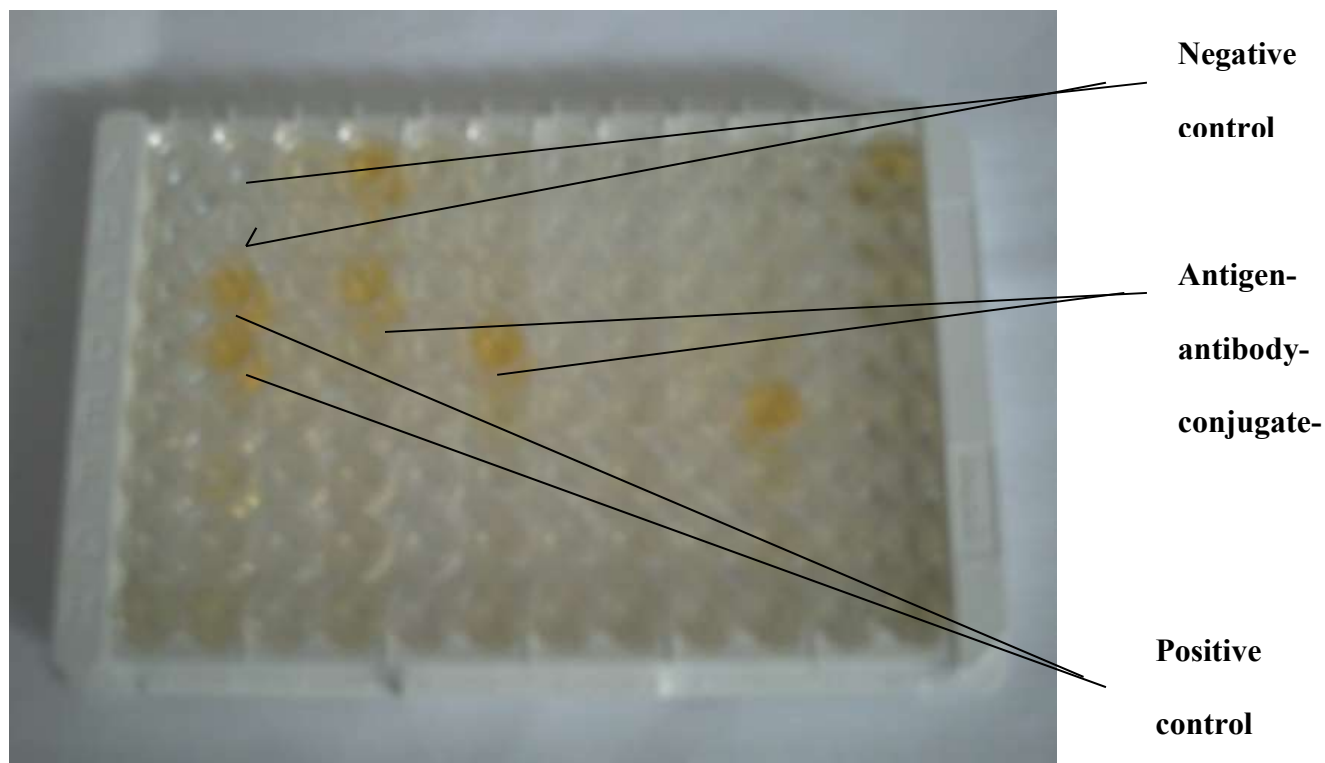
antibody-

conjugate-

Positive

control

Plate1 Picture showing African swine fever antigen-antibody-conjugate-HRP Complex which is shown (above) by blue coloration.



PlateII Picture showing presence of African swine fever antibodies in the serum, the blue solution which become yellow after addition of the stop solution.

Table I : Showing the information about of the animals from Apa LGA and the S/P% as calculated from ELISA result

S/N	G/N	Sex	Weight in Kg	Breed Trait	S/P%	ASF antibodies
1	1	F	25	DK	11.572	-
2	2	F	15	Lw/cr	12.815	-
3	3	M	15	Lw/cr	12.143	-
4	4	F	35	Lw/cr	15.724	-
5	5	M	25	Lw/cr	9.030	-
6	6	F	30	Lw/cr	10.898	-
7	7	M	30	Lw/cr	20.550	-
8	8	F	43	Lw/cr	10.431	-

Table II : Showing the information about of the animals from Ohimini Local Government Area of Benue State and the S/P% as calculated from ELISA result

S/N	G/N	Sex	Weight in Kg	Breed Trait	S/P%	ASF antibodies
9	1	M	50	Lw/cr	13.65	-
10	2	M	39	Lw/cr	21.69	-
11	3	F	39	Lw/cr	12.35	-
12	4	F	26	Lw/cr	36.12	-
13	5	M	32	Lw/cr	117.75	+

Table I|I : Showing the information about of the animals from from Okpokwu Local Government Area of Benue State and the S/P% as calculated from ELISA result

S/N	G/N	Sex	Weight in	Breed Trait	S/P%	ASF antibodies
14	1	F	45	Lw/cr	10.79	-
15	2	F	55	Lw/cr	15.88	-
16	3	M	60	Lw/cr	10.22	-
17	4	M	45	Lw/cr	07.37	-
18	5	F	38	Lw/cr	12.71	-
19	6	F	40	Lw/cr	10.22	-
20	7	M	44	Lw/cr	15.21	-

Lw/cr: Large white cross

Cros: Cross: Between local breeds and other undefined breeds.

S/N means serial number G/N means group number

The microplate was read at 450nm. The ELISA recorder and computer helped in recording, and result sheets for recording all the result shown by the ELISA recorder and computer.

Tables I and III record for indirect antibody ELISA shows that there all the samples collected from Apa and Okpokwu respectively were negative for the test. Table II record shows that 1(20%) of the samples collected from Ohimini LGA were positive, and 4(80%) were negative for the test.

DISCUSSION

African swine fever (ASF) is a notifiable, highly contagious, lethal hemorrhagic disease in domestic pigs. Ayoade and Adeyemi, (2003) described African Swine Fever (ASF) as a devastating viral disease currently threatening the

pig industry worldwide. This is so because outbreak of African Swine Fever (ASF) is characterized by a mortality ranging from 50 to 100% (Otesile *et al* 2005). In Benue State, for example the veterinary services reported 27,000 pig-owner families (Hicheri, 1998). This disease can be a very frustrating disease to most pig farmers and this can lead to decrease in animal protein production and consumption. This study that was undertaken to assess the level of African swine fever virus antibody status in some pigs in Ohimini local government in Benue state, Nigeria. Alternatively, if we are to consider Radostits *et al.*, 2007, that pigs with demonstrable antibody should be considered as chronic carriers of the virus as it is doubtful that true recovery ever occurs. This means that at least

20% of the pigs in Ohimini LGA are carriers of African swine fever virus. This has serious implication because according to Rahimi *et al.*, (2010).

Conclusion

This study has shown that African Swine Fever (ASFV) antibodies are present in pig population in Ohimini LGA of Benue state, Nigeria and this has confirmed the carrier status of the pigs in the local government areas.

Recommendations

We therefore recommend as follows;

- Biosecurity measures such as control of personnel from other pig farms, visitors, butchers and provision of foot deep. Farm equipment such as shovels and wears must not be lent out to other farms. Cooking all garbage or food left overs to be giving to pigs. The use of blood meal made from blood of pigs for other pig feed should be discouraged.
- Soft ticks of the genus *Ornithodoros* must be controlled. This will help in controlling the ticks that transmit the virus. Domestic pigs should not be allowed to have contact with the wild pigs (warthogs, bush pigs, and giant forest boars) since this will help in breaking the cycling of virus between soft ticks of the genus *Ornithodoros* and wild pigs.
- Prohibition of movement of pigs from one part of the country to another that is free of the disease unless the animals are confirmed by veterinarians to be free from the virus.
- Pig pens should be disinfected with strong solution of caustic soda 4 months before animals are reintroduced.
- Quarantine, compulsory slaughter of infected and in-contact animals and other animals at risk with adequate compensation to the owners.

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