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CONFIRMATORY DIAGNOSIS OF AFRICAN SWINE FEVER IN SOUTHERN NIGERIA BY IMMUNOBLOTTING ASSAY

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Abstract

When a swine epizootic occurred in groups of pigs in south western Nigeria in 2001, a need for detailed investigation and confirmatory diagnosis arose at the University of Ibadan. A class of epizootiology students at the university was involved in the investigation. Comprehensive information about the nature and causation of the disease was provided. Four diagnostic techniques were used for confirmatory diagnosis, including Indirect Enzyme Linked Immunosorbent Assay (I-ELISA), immunoblotting, polymerase chain reaction (PCR), and virus isolation. Only immunoblotting assay was used at the University of Ibadan, while samples were sent to the National Veterinary Research Institute (NVRI) in Vom, Nigeria for I-ELISA and to two reference laboratories for African swine fever in South Africa and Madrid, Spain for PCR and virus isolation respectively. The results obtained from these laboratories using the other three diagnostic techniques were compared with that of the immunoblotting assay. Immunoblotting assay confirmed all samples that tested positive on PCR and virus isolation. It was also relatively easier to use, highly sensitive and specific for confirmatory diagnosis. It was however costlier in application for large sample size testing compared to I-ELISA.

Key words: African swine fever; confirmatory diagnosis; immunoblotting assay

Introduction

Definition of African swine fever immunoblotting assay

African swine fever immunoblotting assay is a serological diagnostic procedure that detects antibodies against African swine fever virus that are present in test serum samples. The toolkit is made up of nitrocellulose filters containing pre-labeled African swine fever (ASF) proteins, standard positive and negative sera samples, and the indicator system. The nitrocellulose filters were produced from the complete and quantitative transfer of African swine fever (ASF) proteins from sodium dodecyl sulphate (SDS)-gels, while preserving the antigenic properties of the proteins under denatured conditions (Pastor et al, 1989).

Elaboration on African swine fever confirmatory diagnosis

Commencing from June 2001, outbreaks of a disease suspected to be ASF were reported at the University of Ibadan (Ibadan) Teaching and Research Farm and other parts of

Ibadan city (Latitude $7^{0}23^{1}$N and Longitude 3^{0} 56^{1}E). The disease was characterized by anorexia lasting one to three days, shivering, labored breathing, swollen eyelids, staggering gait, abortion of life fetuses and blood stained frothy discharges from the mouth, eyes, ears, anus and vulva, and sudden death. Post mortem tests would not provide confirmatory proofs of a specific disease. In this case, laboratory diagnosis needed to be directed towards isolation of the virus and if possible carry out the detection of genome DNA by the polymerase chain reaction (PCR) especially in case of Nigeria that already suspected its presence. (OIE, 1999). The National Veterinary Research Institute in Nigeria had facilities for I-ELISA and PCR, thus tissue samples from affected pigs were sent to the laboratory for these procedures. Immunoblotting technique was used at Ibadan. Tissue samples including mesenteric lymph node, gastro hepatic lymph node, renal lymph node, spleen, heart, liver, lung and kidney were collected and sent to Onderstepoort Veterinary Institute (OVI), South Africa (Permit No13/1/130/4-017) and to Instituto Nacional de Investigacion y Technologia Agraria y Alimentaria (INIA) in Madrid, Spain (Permit CISA-INIA of 19 October 2001). Samples for PCR and virus isolation were from University of Ibadan Teaching and Research farm and Alabata farm. The results were compared to assess the reliability of the immunoblotting assay.

Materials and Method

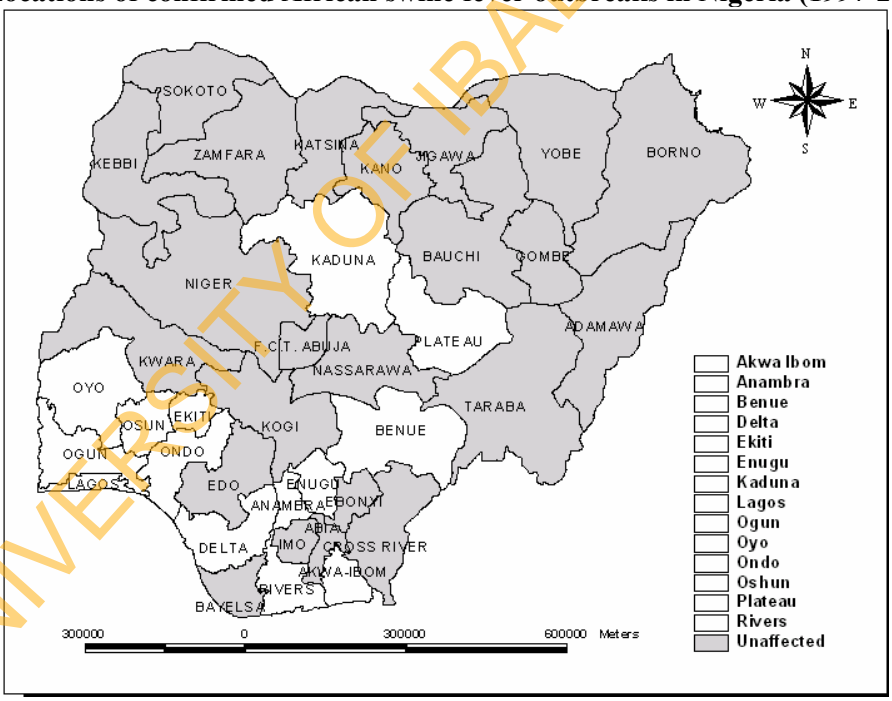
Fifty nitrocellulose strips for immunoblotting already labeled with African swine fever virus (ASFV) proteins, and standard positive and negative reference sera samples were obtained from Instituto Nacional de Investigacion y Technologia Agraria y Alimentaria (INIA) in Madrid, Spain. (Pastor et al (1989) was used in carrying out the test. Phosphate buffered saline (PBS) solution was prepared with PBS tablets (sigma), dissolved in 200 ml of distilled water. This provided a buffer solution pH 7.2. Two grams of non-fat dry milk was added to 100 ml of PBS pH 7.2. This PBS-milk 2% buffer was used to dilute sera samples and for blocking on immunoblotting antigen strips. A chromogen, 4-chloro-1-naphtol (Sigma-Aldrich) was used to detect presence of ASFV immunoglobulins in the reaction. 12 mg of 4-chloro-1-naphtol was dissolved in 4 ml of Methanol. The substrate of 4-chloro-1-naphtol – Methanol solution, with 8µ H_20_2 was used as chromogen. This was prepared just before use. Each test serum sample at 1:50 dilution in PBS–milk 2% solution was incubated at 37^{o}C for 45 minutes on an immunoblotting strip. The strip was washed 4 times with 0.5ml of PBS-milk 2% solution for 5 minutes at the last wash. Protein A-peroxidase conjugate was then added (0.5 ml at a 1:1000 dilution in PBS-milk 2% solution) and incubated at 37^{o}C, in

continuous agitation. The strip was washed 4 times. The substrate H_20_2 with 4-chloro-1-naphtol (0.5ml) was added to the strip. The reaction was stopped after 10 minutes with running water.

Results

A total of sixty seven sera samples were prepared from ten farms at the start of the outbreaks. From the preliminary results from OVI, five samples tested positive to ASF by PCR, two samples tested positive by ASF virus isolation at INIA, Madrid Spain, 32 out of 40 samples tested positive by I-ELISA at NVRI and 24 out of thirty five sera samples tested positive for ASF on immunoblotting assay at Ibadan. The pattern of ASF proteins identified by ASF virus that reacted with antisera were as shown in Figure 3.2 indicating ASF specific proteins IP23.5, IP25, IP25.5, IP30, IP31, IP34 and IP35 in positive cases.

Figure 3.1: Locations of confirmed African swine fever outbreaks in Nigeria (1997-2004)

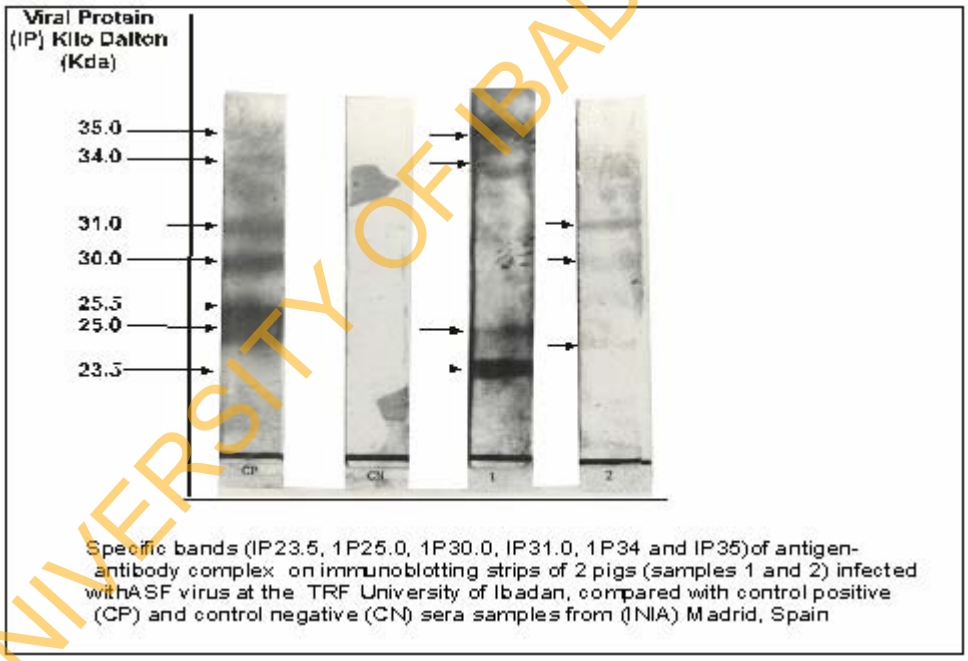


Map designed by B.O. Olugasa, based on Majiyagbe *et al,* 2004

Table 3.1: Farms from which blood samples were collected for immunoblotting assay

S/No	Name of Farm	Location of Farm	Local Government	Number of pigs bled	Number Tested	Number Positive
1.	Teaching and Research Farm	University of Ibadan	Ibadan North	15	10	7
2.	Murewa Farms	Ejioku Village	Lagelu	4	2	2
3.	Timade Farms	Ajibode Village	Akinyele	2	2	2
4.	Government Farm	Iwo Road, Ibadan	Lagelu	4	2	2
5.	Obiwale Farm	Ologuneru Village	Ido	4	2	1
6.	Alabata Farm	Alabata Village	Iseyin	10	5	3
7.	Oshigbesan Farms	Barika, Ibadan	Ibadan North	4	2	1
8.	Shaba Farm	Abadina, University Ibadan	Ibadan North	1	1	1
9.	Anon OBJ Farms	Ota, Ogun State	Ado-Odo/Ota	8	4	2
10.	Lords Farm	Moore Plantation, Ibadan	Ibadan south west	15	5	3
Total				**67**	**35**	**24**

Figure 3.2: Result of immunoblotting test carried out on serum samples from clinically ill pigs



Specific bands (IP23.5, 1P25.0, 1P30.0, IP31.0, 1P34 and IP35)of antigen-antibody complex on immunoblotting strips of 2 pigs (samples 1 and 2) infected withASF virus at the TRF University of Ibadan, compared with control positive (CP) and control negative (CN) sera samples from (INIA) Madrid, Spain

Discussion

Test results of immunoblotting assay, I-ELISA, PCR, and virus isolation ascertained cause of swine epizootic in southern Nigeria was due to ASF. ASF virus isolate was on samples from the University of Ibadan farm associated with 7 positive by immunoblotting assay. PCR positive result from Alabat farm associated with three positive cases out of five by immunoblotting assay. Use of the I-ELISA with its 96 WHO wells was more economic compared to individual nitrocellulose strips. Thus responsible for the larger coverage by

Majiyagbe *et al* using the I-ELISA compared with the small test coverage size at the University of Ibadan by immunoblotting assay.

Conclusion

Immunoblotting assay was sensitive and specific for ASF confirmatory diagnosis, but its use in large size screening was not economically advantageous.

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