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Short Communication

DETECTION OF AFRICAN SWINE FEVER ANTIBODIES BY IMMUNOBLOTTING ASSAY

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INTRODUCTION

The detection of African swine fever virus antibody by immunoblotting assay on samples of swine sera collected from the Livestock Investigation Department (LID), National Veterinary Research Institute's Pig farm is reported.

African swine fever (ASF) was first described in Kenya at the beginning of the nineteenth century (Montgomery, 1921). Since then the disease has been reported in a large number of African Countries South of the equator (Wilkinson, 1981; Plowright 1984). Although the disease was once reported in Nigeria in Ogun State in 1973, it resurfaced again in 1997 through the South Western Part of the country most probably by cross boarder contact with infected pig/pig products smuggled from neighboring Benin and Cameroon Republics (Anon, 1998; Empres, 1998; Majiyagbe, 1999). Within a short period, the disease became widely distributed through pig producing areas of Nigeria and has been causing alot of devastation to the National Pig herd. One of the earliest cases reported in Nigeria was an outbreak at the cooperative farm, Oko Oba, Agege Lagos, between mid 1997 and early 1998 where extensive mortality occurred (Odemuyiwa *et al*, 2000). At present there is no known cure for infected pigs nor vaccine available to protect surviving pigs. Control of the disease is based on accurate diagnosis and mass slaughter of infected and in contact pigs in order to prevent further disease spread (Wilkinson, 1989).

Previous reports on the serodiagnosis of ASF in Nigeria relied on the use of indirect enzyme linked immunosorbent assay (I ELISA) and hemadsorption (HAD) test (Majiyagbe, 1999; Luther 2001; Luther *et al.*, 2002). There are no published reports on the use of immunoblotting assay for ASF diagnosis in Nigeria. In this work, we report the application of immunoblotting assay for ASF virus antibody detection on swine sera collected from the LID of the National Veterinary Research Institute's pig farm, which previously tested positive to ASF virus antibody by I ELISA.

MATERIALS AND METHODS

Blood samples were collected from 29 pigs at the LID. Sera were collected and further clarified at 3000rpm for ten minutes and stored at 20°C until tested. Demographic information on each pig was also recorded. Also five, previously tested and well preserved ELISA negative sera from the same pigs were included in the assay.

Immunoblotting assay was performed as detailed in the Instituto Nacional de Investigaciones Agarias (INIA) bench protocols supplied with the immunoblotting kits from Valdeolmos, Madrid Spain. In brief, electrophoretically seprarated ASF viral protein resolved in 17 percent polyacrylamide gel (PAGE) were transferred under constant current intensity unto nitrocellulose filters (Hawkes, *et al.*, 1982). The filters were cut into strips then placed into plastic trough and blocked using 2 percent non fat dried milk in phosphate buffer saline (PBS) at pH 7.5 for 30 minutes in order to saturate the remaining free protein binding sites. Half a ml of blocking buffer (PBS/Milk) per strip was dispensed into the trough. After discarding the blocking solution, 0.5ml of test and ASF positive control serum were added at a dilution of 1:30 in PBS/2% milk at pH 7.2 and incubated at 37^{0}C for 45 minutes with continuous agitation. Penultimate to this was a washing step of at least four times using PBS/2% milk, pH 7.2. Stock solution of protein A Horse radish peroxidase conjugate at 1:500 was prepared and 0.5ml of this diluted conjugate was dispensed into each strip in the plastic trough and incubated for 45 minutes with continuous agitation. The strips were again washed four times in PBS/2% milk, pH 7.2. If specific ASF antibodies are present in the serum, the antigens in the strip will react with the serum antibodies resulting to the formation of an immuno complex, which will be developed following the addition of A peroxidase conjugate and 4-chloro-1-naphtol as substrate. The reaction was stopped using equal volume of tap water. Complex is visualized as bands on the nitrocelullose filters.

RESULTS AND DISCUSSION

African swine fever virus antibody was detected in 15 (51.7%) out of the 29 pigs sera tested. The ASF positive control serum and the test sera that had antibody to African swine fever produced 5 bands on each strip, corresponding to the antibodies against the proteins transferred to the filters i.e. infectious proteins (IP) 12, 23.5, 25.5, 30 and 34.

African swine fever virus antibodies were detected in pigs from all the pens. The prevalence was highest in pen 7 and lowest in pen 4 (Table I). Out of the five I ELISA negative sera included in the assay, 3 reacted with at least 2 viral proteins on the strips showing two bands on the filters. Photographs of the bands were not taken.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) has found wide application in most types of biochemical and immunological reactions to identify and characterize proteins by their relative molecular weights. It has also been possible to transfer electrophoretically separated proteins from SDS gels to filters through immunoblot assay (Towbin, *et al.,* 1979; Neal, 1981) without disrupting the antigenic properties of the proteins. In these assays, the antigen in question is transferred from a gel to a filter, which is then incubated with a reagent to block non specific binding sites. Immunoblotting techniques have been increasingly used to detect the presence of serum antibodies against specific antigens (Towbin and Gordon, 1984).

From the results obtained in this study, the ASF positive control serum and the test sera that had antibody to African swine fever virus formed immuno complexes with all the infectious proteins of African swine fever virus i.e. IP 12, IP 23.5, IP 25.5, IP 30 and IP 34. It was observed that IP 30 was the most reactive protein. Pastor *et al* (1989) also confirmed this observation in the detection of antibody to African swine fever by the immunoblotting assay where MS cells (Monkey Kidney Cell Line) infected at a multiplicity of infection of ten was used as soluble cytoplasmic ASFV antigen. Using this technique, for the five ELISA negative sera included in the assay, only three of the sera reacted and formed immuno complexes with 2 proteins: IP 12 and IP 30. These proteins are early proteins with low molecular weights and induce high antibody response (Escribano *et al.*, 1990). This finding suggest immunoblotting assay to be more sensitive in the detection of African swine fever virus infections at the early stages than ELISA. This finding corroborated previous works by Escribano *et al,* (1990) who also reported the

use of immunoblotting assay to confirm reactivity in ELISA. The fact that immuno complexes were formed with the 2 early proteins (IP 12 and IP 30) probably, indicate that the initial ELISA negative sera corresponded to sera obtained during the early stages of infection with the virus. It is clear from this finding that pigs in the early stages of infection probably lack sufficient levels of antibody against African swine fever virus induced proteins to be reactive in ELISA. This finding suggests the technique as being ideal for the objective confirmation of positive sera by ELISA.

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