



Detection of Haemagglutination inhibition antibody to Pandemic and Classical Swine Influenza Virus in Commercial Piggery in Lagos Nigeria

Meseko, C. A.¹; Odurinde, O.² and Calogero, T.³

¹Regional Laboratory for Animal Influenza and Transboundary Animal Diseases, Virology Department, National Veterinary Research Institute, Vom Nigeria; ²Virology Department, College of Medicine, University of Ibadan, Ibadan; ³FAO Reference Center for Animal influenza and Newcastle Disease, OIE and National Reference Laboratory for Avian Influenza and Newcastle Disease, Istituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy. *Corresponding author: Email: comeseko@yahoo.com; Tel No:+2348039183988.

INTRODUCTION

Nigeria with about 10 million pigs is the major pig producing country in Africa, accounting for over 30% of commercial and rural free range pig husbandry (FAOSTAT, 2011). Irrespective of the large population of pigs in Nigeria, studies on swine influenza is limited and the situation is the same for the rest of Africa (Girard *et al.*, 2010; Meseko *et al.*, 2013). Earlier publication by Olaleye *et al* (1989) described seroprevalence of swine influenza in south west Nigeria. Similarly Aiki-Raji (2004) also reported antibody detection in pigs among few records in literature that were specific on swine influenza. It was not until 2006 following widespread outbreak of avian influenza that attention was focussed on the importance of animal influenza in Nigeria (Fusaro *et al.* 2009). This could be partly due to the underestimation of the possible economic and public health implications of the virus compared to other prevailing swine diseases (El Hicheri *et al.*, 1998).

The emergence of swine origin influenza pandemic in 2009 (A (H1N1)pdm09)

stimulated global attention on the importance of pigs in influenza ecology and public health and it was shortly after Influenza A(H1N1)pdm09 outbreaks in human that infections were also reported in pigs in several countries (Smith *et al.*, 2009; Girard *et al.*, 2010). Because of the limited serological and virological data describing the status of swine influenza generally in Nigeria and following molecular detection and isolation of A(H1N1)pdm09 virus in the study area (Meseko *et al.*, 2014), we investigated the sero-prevalence of swine influenza in a major intensive piggery in Lagos state, Nigeria. This is in order to better understand the current burden and extent of exposure of both pandemic and classical swine influenza in the study area.

MATERIALS AND METHODS

High-density intensive piggery production in Lagos State Nigeria with about 800,000 pigs owned by many livestock cooperative groups in urban area were selected for this

investigation. Sera were conveniently sampled randomly based on availability and cooperation from butchers and staggered every month for 6 months (March to August 2012) at three slaughter slabs located in the piggery farm estate.

A total of 302 sera were obtained and all sera were first screened using ID Screen[®] (ID-Vet, Montpellier, France) competitive ELISA for influenza A [using nucleoprotein (NP) as antigen] according to manufacturer's instructions. The ELISA positive sera were subsequently tested after receptor-destroying enzyme (RDE) treatment and heat-inactivation, using the haemagglutination inhibition (HI) test. All assays were carried out at the WHO Collaborating Centre for Diseases at the Human-Animal Interface, Istituto Zooprofilattico Sperimentale delle Venezia (IZSVE) Padova Italy.

The HI tests were performed according to standard procedures of the World Animal Health Organization (2014), using 1% chicken red blood cells as indicator. Four viruses representative of different influenza strains commonly circulating in pig populations were used as HA antigens: A/swine/Italy/711/2006 (H1N1), A/swine/Italy/4159/2006 (H1N2), A/swine/Italy/716/2006 (H3N2) and pandemic A(H1N1)pdm09 virus A/Verona-Italy/2810/09 (H1N1pdm). They were propagated and titrated on Madin Darby Canine Kidney (MDCK) cells and viral titers were calculated by the Reed and Muench method (Reed and Muench, 1938). Haemagglutination Inhibition titers of 1:10 to 1:20 were considered indeterminate, while values of 1:40 or greater were considered as positive. If a sample scored positive for more than one antigen, due to sera cross-reaction, it was assigned to the subtype showing HI titer at least twofold higher than the other viral types. IBM SPSS Statistics for windows version 20.0 was used to compute data with the chi-square method

in invariant analyses. P value of ≤ 0.05 (95% C.I.) was considered significant.

RESULTS AND DISCUSSION

Out of a total of 302 analysed sera, 89 were positive for influenza A (29.4%). Haemagglutination subtyping showed that 88 were pandemic H1, one sample was weakly positive for classical H3, while 23 were both classical and pandemic H1. The result showed seroprevalence of 29% for pandemic H1 while the seroprevalence of classical swine influenza subtype H1 and H3 were 7.6% and 0.3% respectively. Twenty three sera cross reacted to both pandemic and classical swine influenza subtype H1. The HI titers obtained with each antigen used are summarized in Table 1.

This result is consistent with earlier isolation of A (H1N1)pdm09 virus in the study area (Meseko *et al.*, 2014). Although some levels of cross reactivity were observed between classical and pandemic H1; nevertheless, the HI titre of pandemic H1 was significantly higher, $p < 0.05$ (at least 2 folds higher than classical H1). From these results, classical swine influenza virus activity in the study area was apparently low based on seroprevalence of 7.6% until A (H1N1)pdm09 was probably introduced to the farm complex during the WHO declared global outbreak of the 20th Century and that A (H1N1)pdm09 at 29% seroprevalence dominates other subtypes of influenza virus in this study.

Due to lack of records prior to 2009 of antibodies/virus detection of influenza A(H1N1)pdm09 in the study area and that A(H1N1)pdm09 circulation is a recent global phenomenon, It is our opinion that the virus may have been recently introduced to the tested farms possibly through transmission from human to pigs. This scenario is similar to report from Australia, where swine influenza was not described in pigs in that country before the A (H1N1)pdm09 was detected (Holyoake *et al.*, 2011). In the same vein classical swine

TABLE 1: Serological titres of influenza A viruses obtained with HI reaction in intensive piggery complex in Lagos state, Nigeria

Virus strain	HI Titre								No of Positive sample
	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	
H1N1pdm09	8	10	19	22	20	6	2	1	88
H1N1	6	10	4	3	0	0	0	0	23
H1N2	0	0	0	0	0	0	0	0	0
H3N2	1	0	0	0	0	0	0	0	1

influenza viruses was hardly described in the area under investigation, and there are limited evidence in literature. Though, previous studies performed before 2009 (Olaleye *et al.* 1989; Aiki-Raji *et al.* 2004) recorded a seroprevalence of 86 to 94% in pigs in neighboring Oyo State, but those were not antibodies due to A(H1N1)pdm09. Prior study in the West African sub-region (Ghana, Togo and the Republic of Benin) with close proximity to study area observed a very low sero-prevalence of swine influenza (Couacy-Hymann *et al.*, 2012), suggesting the absence of infection in this region. That finding was before 2009 showing that exposure to swine influenza in West Africa increased only after 2009. Hence our study in confirmed heighten influenza activity in pigs post 2009 pandemic. Our findings were also in agreement with a more recent investigation by Snoeck *et al.* (2015) where 27.4% of samples tested were found to have antibodies to A (H1N1)pdm09 in Ibadan Nigeria. Snoeck and co-workers also agreed with our observation that pigs investigated rarely had antibodies to swine and human H3N2 or human H1N1. Seroarchaeology of samples collected prior to 2009 pandemic may be able shed more light on whether pigs in Nigeria were previously exposed to A(H1N1)pdm09 strain or not.

In a published review on the status of swine influenza worldwide, Vincent *et al.* (2014)

posits that the sparse population density of pigs in Africa may contribute to the relatively low incidence of swine influenza in the region. However, Nigeria, with its high concentration of pig population compared to other countries in Africa, may likely have higher chances of swine influenza virus circulating thereby requiring greater monitoring for zoonotic and pandemic strain.

Continuous circulation of pandemic A (H1N1) pdm09 may also cause the virus to become endemic in a country with known avian and human influenza outbreaks (Monne *et al.*, 2015). The consequences of interspecies transmission and reassortment are neither good for profitable livestock husbandry or public health interests. Nigeria's reputation as the major pig producing country in Africa and as a hotspot for transboundary transmission of zoonotic disease requires close monitoring (FAOSTAT, 2011, Grace *et al.*, 2012).

In conclusion, this study showed evidence of both pandemic and classical swine influenza virus in pigs in Nigeria with its attendant economic and public health risks. Extensive molecular epidemiology of swine influenza is recommended in order to determine the genetic diversity and monitor the emergence of novel zoonotic or pandemic strain for rapid and effective control.

ACKNOWLEDGEMENTS

We thank Dr O. Allison and Mr A. Lanloye for their assistance in sample collection and Lagos State Ministry of Agriculture for permission to carry out this investigation in the piggery estate.

REFERENCES

- AIKI-RAJI, C., OYEDELE, I., AYOADE, G., FAGBOHUN, O. and ODERINU, T. (2004). Detection Of Haemagglutination–Inhibition Antibodies Against Human H 1 n 1 Strains Of Influenza A Viruses In Swine In Ibadan, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 5, 278-279.
- COUACY-HYMAN, E., KOUAKOU, V. A., APLOGAN, G. L., AWOUME, F., KOUAKOU, C. K., KAKPO, L., SHARP, B. R., MCCLENAGHAN, L., MCKENZIE, P. and WEBSTER, R. G. (2012). Surveillance for influenza viruses in poultry and swine, west Africa, 2006–2008. *Emerging infectious diseases*, 18, 1446.
- EL HICHERI, K., GOMEZ-TEJEDOR, C., PENRITH, M., DAVIES, G., DOUATI, A., EDOUKOU, G. and WOJCIECHOWSKI, K. (1998). The 1996 epizootic of African swine fever in the Ivory Coast. *Revue scientifique et technique (International Office of Epizootics)*, 17, 660-673.
- FAOSTAT 2011. FAO statistical databases. Food and Agriculture Organisation of the United Nations. Rome, Italy. Available at : <http://faostat.fao.org> (accesses 9th December, 2015)
- FUSARO, A JOANNIS, T., MONNE, I., SALVIATO, A., YAKUBU, B., MESEKO, C., OLADOKUN, T., FASSINA, S., CAPUA, I., AND CATTOLI, G. (2009). Introduction into Nigeria of a Distinct Genotype of Avian Influenza Virus (H5N1) *Emerging Infectious Diseases* 15(3): 445-447.
- GIRARD, M. P., TAM, J. S., ASSOSSOU, O. M. and KIENY, M. P. (2010). The 2009 A (H1N1) influenza virus pandemic: A review. *Vaccine*, 28, 4895-4902.
- GRACE, D., MUTUA, F., OCHUNGO, P., KRUSKA, R., JONES, K., BRIERLEY, L., LAPAR, L., SAID, M., HERRERO, M. and PHUC, P.M., THAO, N.B., AKUKU, I. and OGUTU, F. (2012). Mapping of poverty and likely zoonoses hotspots. Zoonoses, ILRI ; Projct 4. Report to the UK Department for International Development. Nairobi, Kenya.
- HOLYOAKE, P., KIRKLAND, P., DAVIS, R., ARZEY, K., WATSON, J., LUNT, R., WANG, J., WONG, F., MOLONEY, B. and DUNN, S. (2011). The first identified case of pandemic H1N1 influenza in pigs in Australia. *Australian Veterinary Journal*, 89, 427-431.
- MESEKO, C., OLALEYE, D., CAPUA, I., AND CATTOLI, G. (2013). Swine influenza in Sub Saharan Africa, current knowledge and emerging insights. *Zoonosis and Public Health*. 61(4): 229-237.
- MESEKO, C., ODAIBO, G. and OLALEYE, D. (2014). Detection and isolation of 2009 pandemic influenza A/H1N1 virus in commercial piggery, Lagos Nigeria. *Veterinary Microbiology*, 168, 197-201.
- MONNE, I., MESEKO, C., JOANNIS, T., SHITTU, I., AHMED, M., TASSONI, L., FUSARO, A. and CATTOLI, G. 2015. Highly Pathogenic Avian Influenza A(H5N1) Virus in Poultry, Nigeria, (2015). *Emerging Infectious Diseases*, 21, 1275-1277.

- OLALEYE, O., OMILABU, S., BABA, S. and FAGBAMI, A. (1989). Haemagglutination-inhibiting (HI) antibodies against strains of influenza A virus in horse and pig sera in Nigeria. *Journal of hygiene, epidemiology, microbiology, and immunology*, 34, 365-370.
- REED, L. J. and MUENCH, H. (1938). A simple method of estimating fifty per cent endpoints. *American journal of epidemiology*, 27, 493-497.
- SMITH, G. J., VIJAYKRISHNA, D., BAHL, J., LYCETT, S. J., WOROBEY, M., PYBUS, O. G., MA, S. K., CHEUNG, C. L., RAGHWANI, J. and BHATT, S. (2009). Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature*, 459, 1122-1125.
- SNOECK, C. J., ABIOLA, O. J., SAUSY, A., OKWEN, M. P., OLUBAYO, A. G., OWOADE, A. A. and MULLER, C. P. (2015). Serological evidence of pandemic (H1N1) 2009 virus in pigs, West and Central Africa. *Veterinary Microbiology*, 176, 165-171.
- VINCENT, A., AWADA, L., BROWN, I., CHEN, H., CLAES, F., DAUPHIN, G., DONIS, R., CULHANE, M., HAMILTON, K. and LEWIS, N. (2014). Review of influenza A virus in swine worldwide: a call for increased surveillance and research. *Zoonoses and public health*, 61, 4-17.
- WORLD ORGANISATION FOR ANIMAL HEALTH (2014): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 7th ed. OIE Chapter 2.8.8. Swine Influenza.