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Serosurvey of antibody to highly pathogenic avian influenza (H5N1) in pigs, north central Nigeria

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Abstract
Avian influenza is a disease of economic and public health importance that has been described in most domestic animals and humans. Highly pathogenic avian influenza H5N1 epidemic in Nigeria was observed in agro-ecological zones where pigs and chickens are raised in shared environment with chances of interspecies transmission. We investigate the likelihood of transmission of the disease to pigs in North Central Nigeria where there were several cases of highly pathogenic avian influenza H5N1 in both commercial and free range poultry. Two hundred and twenty swine sera collected in Kaduna and Plateau states were screened for influenza A/H5N1 antibody by haemagglutination inhibition test according to standard protocol. All the sera tested were negative for influenza A/H5 antibody (HA titre < 2^2). Our inability to detect appreciable antibody level to avian influenza A/H5N1 therefore may be due to lack of infection because of low susceptibility of pigs to Influenza A/H5N1. We recommend wider serological and virological studies to identify other circulating influenza virus in pigs in different agro-ecological zones to provide useful epidemiological data on evolving influenza virus.

Keywords: antibody, avian influenza, H5N1, Nigeria, pigs.

Introduction

The Highly Pathogenic Avian influenza (HPAI) H5N1 epizootic that started in Hong Kong in 1997, and subsequently spread across many countries including Nigeria was detected in various avian and mammalian species (Enserink & Kaiser, 2004; Keawcharoen *et al*., 2004; Choi *et al*., 2005; Yuen *et al*., 1998) with potential for transmission of different strains of the virus from these species to humans (Robinson *et al*., 2007). The influenza A/H5N1 virus being highly pathogenic to poultry had also acquired increased capacity for interspecies transmission (Kuiken *et al*., 2004). Apart from pigs being susceptible, they also serve as mixing vessel because their respiratory epithelial cells contain the sialic acid receptors for both human and avian influenza viruses, which allows for co-infection and subsequent recombination events (Capua & Alexander, 2002; Olsen, 2002). The chances of interspecies transmission is further enhanced by mixing of different species of animals as commonly practiced in semi intensive and rural farming in Nigeria where pigs, chickens, humans and other species inter-mingle. Several cases of HPAI H5N1 outbreak in Nigeria occurred in mixed farms and in rural communities (where different species of poultry and pigs freely scavenge with chances of shared infections including HPAI). Many pig farming operations in Kaduna and Plateau states (North Central Nigeria) are located in HPAI H5N1 outbreak zone which may result in pigs acquiring the subtype or other avian influenza from poultry thereby serving as 'mixing vessel'. This study was designed to screen swine sera for highly pathogenic avian influenza virus infection through antibody detection by haemagglutination inhibition (HI) test.

Materials and methods

Blood samples were taken from free range and farmed pigs at weekly intervals in Jos abattoir, Plateau State. Samples were also collected from

an interstate live pig market in Kafanchan, Kaduna State where pigs bred in different locations in North Central Nigeria are traded and presented for slaughter at slabs within the mark*et* Sera were collected over four months from April to August, 2008. The sera were pre-treated according to standard procedures with modification (WHO, 2011) and as described by Pirtle *et al* (1976) by heating at 56 ^{0}C in a water bath for 30 minutes to inactivate the sera. Thereafter, the serum was diluted 1:4 with 40% kaolin (in place of receptor destroying enzymes, RDE) and held at 37^{o}C for 12 to 18 hours in water bath to remove non-specific inhibitors of haemagglutination. This was followed by adsorption of serum in 1:1 dilution of sample (sera) and 10% chicken RBCs incubated at 25 degree Celsius for 30 minutes to complete the removal of non-specific haemagglutinin as described by Pensaert *et al*., 1981.

Chicken red blood cells (cRBC) used throughout the test was derived from blood collected from specific antibody negative Yarkon white cocks pooled in an equal volume of Alsever's solution (anticoagulant). Red blood cells were washed three times in phosphate buffered saline (PBS) pH 7.4 before being used as 10% and 1% (packed cell v/v) suspension, as appropriate. The HI test was performed as previously (OIE, 2008). Using a V-bottomed microtitre plate, each swine serum was tested against 4 haemaglutinating units (HAU) of H5N1 antigens. Positive and negative control antigens and antisera were run with each test. All reference antigen and antisera were sourced from FAO/OIE reference laboratory for avian influenza and Newcastle disease, [Instituto Zooprofillatico Sperimentale Venezie (IZSVe) Padua, Italy]. HI positive titres were defined by reciprocal of the highest dilution of serum that completely inhibited haemagglutination of 4 HAU of the virus with 1% solution of chicken red blood cells. Sera with antibody to A/H5N1 less than four reciprocal of dilutions were recorded as negative (WHO, 2011).

Results

Two hundred and twenty sera were collected over the period. The HI results showed that all the swine sera tested were negative for A/H5N1 antibody as the titres were < 2^{2}.

Discussion

None of the 220 samples collected from pigs in the study area were positive for detectable level of antibody to avian influenza subtype H5N1. Our inability to detect antibody to avian influenza may be due to lack of infection or low susceptibility of pigs to H5N1. This was corroborated by serological investigation in Cambodia (Rith *et al*., 2012) where no evidence of H5N1 antibody was found in 150 randomly tested samples. In a similar report (Shieh *et al*., 2008), 9883 serum samples collected from 1974 pig farms all tested negative for H5 and H7; but in the same study, 1234 sera that were positive for influenza A by agar gel immunodiffusion (AGID) showed 1.9% and 1.0% HI positive rates for H1 and H3 subtypes respectively. This is an indication that other subtypes of influenza A viruses circulate in pigs. Therefore, the findings in Egypt by El-Sayed *et al*. (2010) where 1.6% and 4.6% of 240 tested sera were positive, using non local and locally produced antigens respectively, is equally instructive. Furthermore previous studies have shown that pigs may produce antibody in response to infection by avian influenza (Bronitki *et al*., 1965) and that avian influenza has been detected in pigs by virus isolation and molecular methods in the past (Pensaert *et al*., 1981; Choi *et al*., 2005).

Experimental studies, has shown that domestic pigs have very low susceptibility to HPAI H5N1, where pigs inoculated with influenza A/H5N1 were asymptomatic or had mild illness in contrast to mouse and ferret mammal models where the virus was highly pathogenic and replicated systemically (Lipatov *et al*., 2008). The work did not indicate if the animals seroconverted or not. Shittu *et al*. (2010) also showed an insensitivity of pig red blood cells to H5N1, which may be an indication of a poor host's receptor adaptation. In our opinion, the development of capacity for production of antigen/antiserum with locally isolated viruses may improve the sensitivity of diagnostic tests in the country as was the case in Egypt. This observation also has implications for the degree of protection conferred by locally produced (versus imported) vaccines in disease control (El Sayed *et al*., 2010). Investigation for other subtypes of influenza A virus apart from H5N1 that may be circulating in domestic pigs is highly recommended. Furthermore, elaborate serological and virological studies to identify circulating swine influenza virus in pigs in different agro-ecological zones in the country would be useful. This information is vital as pigs are known to have the highest epidemiological role in the evolution of new influenza viruses (Ma *et al*., 2008).

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