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## Footprints of swine influenza H1N1 and H3N2 in pigs from southern Kaduna, Nigeria

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**Abstract**

Influenza A virus presents a significant public health burden worldwide, with the 1918 Spanish flu pandemic being the most dramatic example. Swine influenza viruses can be transmitted to humans through occupational exposures and in live pig markets. Novel variants can emerge in pigs because they can be infected by human, avian and swine strains. This study was carried out to determine the seroprevalence and serotypes of swine influenza in pigs from a major slaughter slab in southern Kaduna. Using competitive ELISA and haemagglutination-inhibition (HI) assays, 305 swine sera were analysed. The result showed an overall seroprevalence of 28.20% (n=86), with H3N2 7.87% (n=24) emerging as the most dominant subtype in circulation. Concurrent antibody detection of H1N1 in 5.26% (n=16) was also detected in boar 2.62% (n=8) and sows 2.62% (n=8). This study revealed swine Influenza H1N1 and H3N2 serotypes are in circulation in pigs in Kaduna State, and that reassortment in the instance of co-infection of swine host is possible.

**Keywords:** Co-seroprevalence, H1N1, H3N2, Serotypes, Swine Influenza, Kaduna



Drive Westbrook, Maine 04092 USA) and interpretation of the result was done according to the ELISA kit manual. All sera samples that tested positive to IAV and the positive reference antiserum for H1N1 and H3N2 were treated using Receptor Destroying Enzymes (RDE) before serotyping using an H1N1 and H3N2 reference antigen and antiserum using haemagglutination inhibition (HI) assay.

*Viral antigen and antiserum*

Swine Influenza antigens H1N1 and H3N2 with the corresponding antiserum were obtained from *Istituto Zooprofilattico Sperimentale delleVenezie* (IZSVe) Legnaro, Italy and were used in the present study.

*Influenza haemagglutination (HA) and haemagglutination inhibition assay*

HI test was carried out to detect Swine Influenza Virus (SIV) antibodies against H1N1 and H3N2subtypes. Haemagglutination (HA) and Haemagglutination inhibition assays were performed following OIE Terrestrial Manual (OIE, 2018).

*Haemagglutination inhibition (HI) assay*

The HI assay was also conducted as described by the OIE (2018). A 25 µL PBS was dispensed into each well of a plastic V-bottomed microtitre plate, and 25 µL of serum was later placed into the first well of each plate. Two-fold dilutions of 0.025 ml volumes of the sera were made across the plate. 4 HAU virus/antigen in 0.025 mL was added to each well, and the plate was left for 40 min at room temperature (20°C). Later, 0.025 mL of 1% (v/v) chicken red blood cells (RBCs) were added to each well and mixed gently, and the RBCs were allowed to settle to a distinct button for about 40 min at room temperature (20°C). The Haemagglutination Inhibition (HI) titre was read from the highest dilution of serum, causing complete

inhibition of 4HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs streamed at the same rate as the control wells (positive serum, virus/antigen and PBS controls) were considered to show inhibition. The validity of this result was assessed against a negative control. Serum titres greater than or equal to 1:4 (2log2) were considered positive. All data obtained were analysed using SPSS version 23.

**Results**

Out of 305 sera samples collected over four months, an overall prevalence of 28.20% (n=86) was recorded for Swine influenza A virus (Table 1). Monthly seroprevalence of 19.64 %, 29.73 %, 21.28 %, 40.74 % for December, January, February and June, respectively, were recorded in the study. The months of June and December had the highest and the least seroprevalence, respectively (Table 1). Serotyping of the ELISA positive SIV by HI test reveals H3N2 (7.87%; n=24) serotype is more prevalent than H1N1 (5.25%; n=16) (Table 2), with co-seroconversion of 2.62% (n=8) in boars and 2.62% (n=8) in sows for H1N1 recorded. While H3N2 subtype seroprevalence of 3.61% (n=11) in boars and 4.26% (n=13) for sows was recorded (Table.2). Out of the total prevalence of 28.2 % recorded, 5.25% representing 16 Swine samples tested positive for H1N1 and H3N2 with the same values of 8 (2.62%) observed for both males and females.

**Discussion**

The results of this study confirm the presence of Swine Influenza A virus, with a seroprevalence of 28.2% and an H1 and H3 co-seroconversion of 5.25% (n=16) in pigs from the live animal market in Kaduna State. However, some limitations should be noted. Firstly, due to time constraints and available resources, the convenience sampling technique

Table 1: Monthly Seroprevalence of swine influenza A antibodies in southern Kaduna

	Dec	Jan	Feb	Jun	Total
Number of pigs sampled	56	74	94	81	305
Number positive	11	22	20	33	86
Percentage (%)	19.64	29.73	21.28	40.74	28.20

Table 2: Distribution of swine influenza A serotypes based on sex and co-seroconversion

Gender	H1N1 Subtype (%)	H3N2 Subtype (%)	Co-seroconversion (%)
Male	8(2.62)	11(3.61)	8(2.62)
Female	8(2.62)	13(4.26)	8(2.62)
Total	16(5.25)	24(7.87)	16(5.25)

employed hinged on the assumption that as the largest pig market in the state, swine brought to the slabs were pooled from the communities of the surrounding Local Government Areas; as such, data collected may need to be replicated in future studies to minimise bias. Secondly, the sample size lacked enough power and so more study with a larger sample size might be needed to corroborate the findings of this study.

Most surveillance studies on swine influenza focus on H1N1 and H3N2 due to the fact that these two subtypes are often implicated in the ecology of swine influenza more than any other subtypes, and studies have shown that antibodies to the virus were detectable all year round, with peaks observed in the dry Harmattan seasons, from November to January and in April and May in Nigeria (David-West & Cooke, 1974; Kabantiyok *et al.*, 2019), which coincides with the period in which this study was carried out (Table 1). In a two-year surveillance study, Meseko *et al.* (2014) reported a prevalence of 13.7% of swine influenza A in pigs from commercial piggery farms in Lagos state. When compared with the prevalence observed in this study, factors such as strict maintenance of biosecurity and good farm management could explain the reason for the sharp difference in the prevalence because the farms sampled are purely intensively managed. The prevalence (28.2%) observed in this study means there is a high possibility of transmission between pig herds and humans living in a close association which is seen by the high prevalence reported by Adeola *et al.* (2010), who reported a prevalence of 68.3% among pig handlers.

Swine are cited as a major concern in the spread of novel IAVs to humans due to the expression of more  $\alpha$  2, 6-sialic acid (SA) receptors, which is shared in both the respiratory epithelia of swine and humans. The presence of antibodies to swine IAV means that subjects might have at one point or the other had direct contact with pigs because the virus has limited sustained human to human transmission history (Garten *et al.*, 2009; Lewis *et al.*, 2016; Kabantiyok *et al.*, 2019).

Influenza A H1N1 and H3N2 viruses circulate widely among pigs and forms part of the dominant subtypes of Influenza virus infecting pigs (Kothalawala *et al.*, 2006; Vincent *et al.*, 2014). The findings of this study in Table 2 corroborate this as a high prevalence of 7.87% (n=24) for H3N2 and 5.25% (n=16) for H1N1 was observed. The H3N2 subtype is particularly known to circulate widely in pigs. It is antigenically related to the human type of influenza that is inclined

to the procuring of internal protein genes from an avian virus. The literature on swine influenza is replete with data on the circulation of H1N1 and H3N2 in swine and human populations (Adeola *et al.*, 2010; Odun-Ayo *et al.*, 2018). Most studies show a higher H1N1 (Jolaoluwa *et al.*, 2013) prevalence than for H3N2. The high prevalence of H3N2 in this study may reflect a shift in the dominant subtype among pigs in this region, although the antibody subtype in circulation is likely to be largely affected by the previous composition of vaccine (Choi *et al.*, 2002) and for reasons unknown, the epidemiological status of certain SIV serotypes might vary from region to region (López-Robles *et al.*, 2014). The former is ruled out as the government of Nigeria has always maintained a no influenza vaccine policy in livestock. More research into the epidemiological distribution of these subtypes in swine is required to better understand factors responsible for the variations of SIV subtypes in regions of unvaccinated swine populations.

This study recorded a 5.25% (n=16) co-seroconversion of H1N1 and H3N2s Table 2. Concurrent infection of different strains in swine provides an enabling environment for reassortment, which may culminate in the emergence of new subtypes and even the potential for a recent pandemic outbreak. H3N2 is widespread in US swine, and this strain which has genes from human lineages, swine lineages, and avian lineages, was generated through reassortment (Pantin-Jackwood *et al.*, 2014). Although this study limits itself to H1N1 and H3N2, there remain other subtypes that were not reported. Human interaction with pigs form dominant interface for the transfer of human type swine flu to pigs (Choi *et al.*, 2002) as more human origin influenza viruses are responsible for the development of novel strain than avian origin influenza virus (Van Poucke *et al.*, 2010) this could be attributed to the abundance of  $\alpha$ 2-6 SA linkages on the pigs' respiratory epithelia which is highly conserved for attachment to IAV of human origin. In a study on the prevalence of swine influenza virus subtypes on swine farms in the United States, Choi *et al.* (2002) reported a co-infection of 1.5% for H1N1 and H3N2 subtypes, which is lower than the 5.25% recorded in this study. The high value recorded in this study compared to the 1.5% recorded by Choi *et al.* (2002) could be accounted for because most swine used for this study are kept free-range where they interact with their environment and other pigs. Suriya *et al.* (2008), in a study to determine the risk factors associated with the influenza A virus in

pigs, recorded a very high co-seroconversion rate of 41.1%.

Normally, the reassortment of gene segments between different subtypes of the virus plays an important role in the emergence of a novel strain. Co-infection sets the stage for it, and this process is highly efficient within a coinfecting cell. Co-infection studies have shown that synchronous co-infection at moderate or high doses can give rise to ~60 to 70% of progeny shed from an animal host (Tao *et al.*, 2015), so the presence of co-infection even as low as 5.25% is important in predicting the virus ecology.

This study reveals a previous infection of swine influenza virus H1N1 and H3N2 subtypes in southern Kaduna. In addition, a concurrent seroconversion of 5.25% was also detected in boars and sows sampled. Therefore, we recommend more awareness to all pig stakeholders on the risk of the virus and surveillance to identify all the subtypes circulating in the area and the country at large to adopt suitable prevention and control measures.

#### Conflict of Interest

The authors declare that there is no conflict of interest.

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