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Full Length Research Article

SEROPREVALENCE OF SWINE INFLUENZA A VIRUS CIRCULATING IN PIGS FROM SOUTHERN KADUNA, NIGERIA

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ABSTRACT
Influenza virus is shared between humans and swine since the 1918 Spanish flu pandemic. It is a virus of public health implication. Pigs play an important role in the ecology of Influenza as the mixing vessel for the emergence of a novel pandemic strains. Three factors supporting the mixing vessel hypothesis are: susceptibility of swine to avian and human viruses; reassortment of swine/avian/human viruses' occurring in pigs which can transmit reassortant influenza virus to humans through occupational exposures. This study provides information on the serotypes of some influenza virus in the pig market in Kaduna State. A total of 305 samples were collected from December, 2017 to early February and in June, 2018. Rectal temperature before slaughter was recorded and blood were collected from exsanguinated pigs at Katsit slaughter slab. Serum were separated and kept at -20°C until further analysis. The sera were tested for swine influenza by competitive ELISA. The result showed overall seroprevalence of 28.20% (n=86). Of this, male animals' represented 9.84 % and the female 18.36% of the total percentage. The breakdown results for each month was as follows: [December; 11 positives (3.61%), January; 22 positives (7.21%), February 20 positives (6.56%), June; 33 positives (10.82%)]. This study showed that Swine Influenza A virus is in circulation among pigs in Southern Kaduna with prevalence peaking at two seasons which coincides with the harmattan in January and the onset of rains in June. The high prevalence means possible transmission of the virus to humans who interface with Swine. There was no correlation between the influenza status and the sex of the swine under study. Also temperature distribution does not show difference between sexes, although there appears a slight difference in temperature distribution between statuses.

Keywords: Influenza, Seroprevalence, Swine, Kaduna State, Subtypes

INTRODUCTION

Influenza viruses (IVs) are enveloped, single stranded RNA viruses of the family Orthomyxoviridae, which comprises of influenza A, B and C viruses (Tong *et al.,* 2012). Influenza A virus is the causative agent of swine flu- a highly contagious respiratory disease (Sreta *et al.,* 2009). Subtypes of Influenza A virus (IAV) include H1N1, H1N2, H2N3, H3N1 and H3N2. Influenza B virus (IBV) has not been reported in swine, contrary to IAV, IBVs are restricted to human hosts (Zell *et al.,* 2007; Koutsakos *et al.,* 2016).The virus has been shared between humans and pigs since at least the 1918 Spanish flu pandemic (Garten *et al.,* 2009), and has since then been of public health importance because it undergoes antigenic shift and drift unlike B and C which are relatively stable (Anjorin *et al.,* 2012; Brooks *et al.,* 2007).

Pigs serve as an important host within which new serotypes can arise through genetic reassortment (Steel *et al.,* 2014). The swine-origin 2009 H1N1 pandemic virus, now called H1N1pdm09, contained genes from avian, swine, and human influenza A viruses(Bautista *et al.,*2010). The potential to generate novel IVs has resulted in swine being dubbed "mixing vessels" (Zell *et al.,* 2012). In Nigeria, Swine influenza occurs most often during the Harmattan period (November to January) and earlier (April to May), peaking in the raining season. However, numerous studies of respiratory diseases, including those caused by influenza viruses, have suggested a closer correlation with the dry Harmattan season (Adeola and Adeniji 2010).

The locations under study are specifically chosen for several reasons. First, the region is known for its high pig production in Nigeria (Ajala *et al.,* 2004), and second, the area plays host to the largest pig market in the country. The Katsit (Kafanchan) weekly pig market is the largest of its kind in Nigeria and the market plays hosts to the surrounding towns of Kwoi, Manchok, Kagoro, Zonkwa and Kachia in the southern part of Kaduna state. The Kafanchan pig market has remained an important pig market center since colonial days (Ajala *et al.,* 2008). This studies was targeted at swine influenza A antibodies circulating in pigs-the mixing vessels for pandemic influenza outbreak, it also provided data on swine influenza in the study area.

MATERIALS AND METHOD

Study Design

Sampling Techniques/Sample Size

The pigs used for this study were exsanguinated pigs sourced from LGA's in Southern Kaduna and brought to the Katsit Pig market slaughter slab. The difficulty in bleeding pigs due to deep veins informed the choice of sampling from slaughter slabs; as such the pigs were selected by convenience sampling. An average of 35 pigs was sampled for each visit from December 2017 to February and in June 2018.

Sample Collection

Body temperature (rectal temperature before slaughter), nasopharyngeal (NP) swabs and 5 mls of blood were collected from exsanguinated pigs in plain sample bottles from December 2017 to February and in June 2018 at the Katsit slaughter slab. Serum was separated and transported on ice to the Regional Laboratory for Avian Influenza and Transboundary Animal Diseases for further analysis.

Study Population and Sample size was calculated by using Slovin formula:

$N = Z^2 \times P \times q/L^2$
Prevalence of Awosanya *et al.* (2013) was used to determine sample size.

ELISA Assay for Detection of Antibody in Animal Serum
Preparation of Wash Solution
The wash concentration was brought to 26°C and then mixed to ensure dissolution of any precipitated salts. The Wash Concentrate was diluted in 1/10 distilled/deionized water before used (e.g., 30mL of Wash Concentration (10X) plus 270mL of water per plate to be assayed).

Preparation of Samples
Test samples were diluted (1/10) in Dilute Buffer prior to being assayed (e.g. by diluting 15µL of sample with 13µL of Dilution Buffer). The controls were not diluted. (IDEXX Laboratories, Inc. One Drive Westbrook, Maine 04092 USA).Test procedure was carried out according to the user manual and interpretation of result was done according to the ELISA kit manual. Sera positive to Influenza A virus were used for serotyping. The viruses used were obtained from Instituto Zooprofilattico delle Venezie in Italy. These consist of egg-grown viruses which had been concentrated, partially purified and inactivated by treatment with betapropiolactone (OIE, 2015).

RESULTS

ELISA Result
ELISA result from a total of 305 sample collected in the four month period is presented in **Table 1**. Here a prevalence of 28.20% represented 86 samples positive for Swine influenza virus. A breakdown of monthly collection reveals that a monthly prevalence of 3.61%, 7.21%, 6.56% and 10.56% for the months of December, January, February and June respectively. The month of June carrying the highest data on seroprevalence.
In **Fig1** is a pie chart showing in brief the prevalence of data for each of the months samples were collected.

Table 1: Distribution of Swine Influenza Virus Antibody Based on Collection Months

	Dec	Jan	Feb	Jun	Total
Number Sampled	56	74	94	81	305
Number Positives (%)	11 (3.61)	22 (7.21)	20 (6.56)	33 (10.82)	86 (28.20)

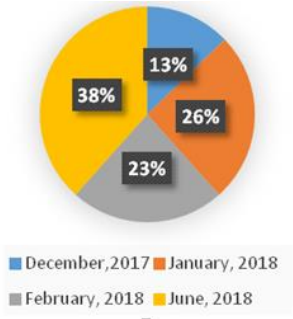

Fig.1 Pie Chat Representation of Serum Positive Samples for December 2017, January, February and June 2018

Status Distribution by Sex
Seroprevalence distribution by sex is represented on **Table. 2** Here out of 106 males captured in the investigation 30 males were positive which represented 9.84%. From a total of 199 females, 56 were positive for Swine influenza representing 18.36% prevalence. Kruskal Wallis test for significance shows distribution of swine influenza antibody is the same across sex.

Table 2 Distribution of Swine Influenza A antibody by Sex of Pigs Sampled

Sex	Number Sampled	Number Positive (%)
Male	106	30 (9.84)
Female	199	56 (18.36)
Total	305	86 (28.20)

(P value= 0.586, using a one way two tailed ANOVA and test of significance at of 0.05)

Swine Temperature against Status
The box plot diagram below shows mean temperature values and ranges for both serum positive and serum negative status of the samples.

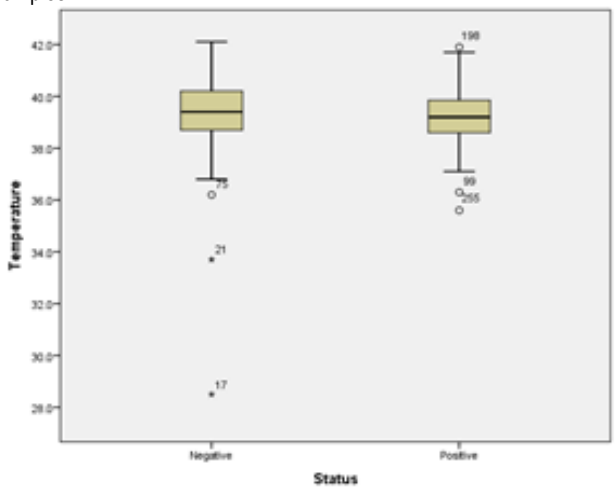

Figure 2: Box plot showing temperature distribution for temperature versus status of samples

DISCUSSION
This study reveals a seroprevalence of 28.2% in pigs from the live market. Similar seroprevalence studies on live pig market carried out by Adeola *et al,* 2010 reported a prevalence of 68.3% among pig handlers, showing that the presence of this virus means a high possibility of transmission in herds and humans living in close association with pigs. Although the time frame varies for both studies, literatures on swine influenza activity shows that antibody to the virus is detectable all year round (Normile, 2006) with peaks observed in the dry Harmattan seasons November to January and in April and May (David-West *el al.,* 1974).

The breakdown results for each month [December; 11 positives (3.61%), January; 22 positives (7.21%), February 20 positives (6.56%), June; 33 positives (10.82%) did not reveal any pattern save that it agrees with seasonality for the virus. Influenza virus in Nigeria normally peaks in the dry Harmattan season, however

result obtained in June produced the highest prevalence in this study. This is inconsistent with data on influenza seasonality for sub-Saharan countries particularly those near the equator of which Nigeria is one (Hirve *et al.,* 2016). Although data for June reflects the highest in the four month period, its plausibility is not in question as there is evidence of two seasonal peaks (November to January and April to May) for Swine Influenza in Nigeria as reported by (Odun-Ayo *et al.,* 2018 and David-West *et.,* 1974).

Seroprevalence based on sex distribution showed a higher prevalence in female 56(18.36%) when compared to the male with 30(9.84%). This result showed that female pigs are twice likely to have been exposed to the virus than male pigs because of an odd ratio (OR) of 1.86(approximately 2). In another study done in Bangladesh by Uddin *et al,* (2018), a differing result of a higher prevalence in males 84(14.28%) than in females 74 (9.46%) was reported. Statistical analysis from this study showed that there is no significant association between influenza infection and sex of the pig (P value= 0.586, using a one way two tailed ANOVA and test of significance at of 0.05). This is in agreement with a two year survey study carried out by Gonzalez-Reiche *et al,* (2017) that revealed an opposing data on distribution of IAV based on sex which produced 23(44.2%) and 29 (55.80%) for females and males respectively, hence the distribution varies between sexes. The differences in prevalence could mean the virus distribution lacks sex preference and could fall on either side of the divide.

The distribution of temperature is significantly different between sexes Kruskal Wallis test for significance showed that distribution of temperature is the same (P = 0.133) across categories of status (positive and negative status). Mean temperature for both positive and negative influenza status showed a slight difference but still falls within the normal pig temperature which ranges from 38-39°C; This is not surprising as elevated temperature in Swine is associated with pigs having a current Swine Influenza infection, and the live virus does not circulate persistently in adult pigs like it does in piglets and the pigs that made the slaughter slab were all adult pigs (Choi *et al.,* 2004). Sample negative status tend to have a more spread out temperature value when compared to positive status with sample negative having a dominant temperature value of 39°C while sample positive status showed a dominant temperature value of 38°C as can be seen on the box plot.

Conclusion

Swine influenza virus is in circulation among pigs in Southern Kaduna with a prevalence of 28.20%. Although not established in this particular work, there is likelihood of this subtype circulating in human population who are in close contact with pigs. There was no significant association between detection of swine influenza antibody and variables such as body temperature and sex of pigs in this study.

Recommendation

Surveillance on swine influenza should not stop at producing data but should reach out to farmers- especially those from the rural communities where majority of these pigs were pooled out, on the effect of the virus on the pigs and humans first and then its negative impact of the economic value of the pigs.

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