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Prevalence of antibodies to influenza viruses among handlers of live pigs at three locations in Ibadan, Nigeria

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Summary

The authors investigated the prevalence of haemagglutination inhibition (HI) antibodies to four strains of influenza viruses among handlers of live pigs in Ibadan, Nigeria. Venous blood specimens were collected from thirty pig handlers (out of a total of forty-eight) at three locations in Ibadan in April and May 2008. The overall prevalence of antibodies to influenza viruses was 100%, while those of influenza A and B viruses were 68.3% and 58.3%, respectively. The prevalence of influenza A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), B/Shanghai/361/2002-like and B/Malaysia/2506/2004-like was 46.7%, 90.0%, 76.7% and 40.0%, respectively. A total of 96.7% ($n = 30$) of pig handlers tested had polytypic influenza antibody reactions. This is the first report to document the prevalence of influenza antibodies among pig handlers in Nigeria and shows that humans who have regular and direct contact with live pigs in Ibadan are exposed to different strains of influenza viruses.

Keywords

Antibody, Ibadan, Influenza, Nigeria, Pig, Pig handler, Prevalence, Virus.

Prevalenza di anticorpi anti virus influenzali tra gli allevatori di suini nella regione dell'Ibadan, in Nigeria

Riassunto

Gli autori hanno valutato, con la tecnica dell'inibizione dell'emo-agglutinazione (HI), la prevalenza sierologia nei confronti di quattro ceppi di virus influenzali in allevatori di suini nella regione dell'Ibadan, in Nigeria. Quarantotto campioni di sangue sono stati prelevati, nel periodo aprile-maggio 2008, ad allevatori di trenta allevamenti. La prevalenza di anticorpi contro i virus influenzali è stata del 100%, quella relativa al virus dell'influenza A del 68,3% e al virus dell'influenza B del 58,3%. Le prevalenze relative all'influenza A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), B/Shanghai/361/2002-like e B/Malaysia/2506/2004 sono state rispettivamente del 46,7%, 90,0%, 76,7% e 40,0%. Il 96,7% (n = 30) di allevatori ha mostrato anticorpi nei confronti dei diversi sottotipi del virus dell'influenza. Questo è il primo rapporto che documenta la prevalenza di anticorpi anti influenza tra gli allevatori di maiali in Nigeria. Lo studio ha dimostrato che gli esseri umani soggetti a contatti regolari e diretti con i suini vivi nella regione dell'Ibadan sono esposti a diversi ceppi di virus influenzali.

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Parole chiave
Allevatore, Anticorpo, Ibadan, Influenza, Maiale, Nigeria, Prevalenza, Virus.

Introduction

Influenza viruses do not cause persistent or latent infections; they are maintained in human populations by direct person-to-person spread during acute infections (20). The most effective means of spread among humans are aerosols. The human infectious dose of influenza A virus infection is 0.6 to 3 $TCID_{50}$ when delivered by aerosol, but 127 to 320 $TCID_{50}$ when delivered intranasally (3). Globally, influenza virus activity is detectable throughout the year and viruses can be isolated in large cities at all times of the year. In the northern hemisphere, epidemics generally peak between January and April, but may occur as early as December or as late as May. In the southern hemisphere, outbreaks occur between May and September. Seasonality in tropical and subtropical climates is believed to coincide with the onset of the rainy season (20). In Nigeria, influenza occurs most often during the Harmattan period (November to January) and earlier (April to May), peaking in the raining season (7, 11).

The domestic pig is the animal species that can be naturally infected with both avian and human influenza viruses and allow productive viral replication (4, 13). This is because the epithelial cells in the trachea of the pig contain both NeuAc-2,3Gal and NeuAc-2,6Gal receptors (8, 21) required by avian and human influenza viruses, respectively (6, 10). Pigs also appear to have a relatively weak species-specific barrier against infection by avian and human influenza A viruses (16). Furthermore, there is evidence that some strains of influenza viruses persist in pigs many years after they have disappeared from the human population (18).

Therefore, while it is important to periodically investigate the prevalence of antibodies to influenza viruses circulating among humans, in the light of the role of the pig as an important 'mixing vessel' for influenza virus reassortment and generation of pandemic strains (15), it is probably of even greater significance to conduct periodic serological surveillance for influenza viruses among humans who have regular and direct contact with live pigs. This study focused on determining the prevalence of antibodies specific to four strains of influenza viruses among pig handlers in Ibadan, south-west Nigeria.

Materials and methods

Sampling method and specimen collection

Venous blood was obtained aseptically, using stratified random sampling, from the median cubital vein of thirty out of forty-eight pig handlers (30/48) at three different locations in Ibadan, south-west Nigeria in April and May 2008. The samples were tested at the Department of Virology, College of Medicine, University of Ibadan. These included 23 out of 35 males and 7 out of 13 females. The locations were as follows:

- Commercial Pig Farm Unit, University of Ibadan (6/11)
- University Research Farm, University of Ibadan (10/17)
- Pig Farmers' Centre in Monatan, Ibadan (14/20).

The history obtained from these pig handlers revealed that they had never received influenza vaccines. About 5 ml of blood was collected from each human into labelled sample bottles (without anticoagulants) and allowed to clot. These were centrifuged in the laboratory at 3 000 rpm_for 10 min. Sera were then collected using Pasteur pipettes and stored in labelled Eppendorf tubes at –20°C before analysis.

Virus strains and reference antisera

The virus strains used were as follows:

- influenza A/Brisbane/59/2007 (H1N1)
- A/Brisbane/10/2007 (H3N2)
- B/Shanghai/361/2002-like (B/Yamagata/16/88 lineage)
- B/Malaysia/2506/2004-like (B/Victoria/2/87 lineage).

These consisted of egg-grown viruses which had been concentrated, partially purified and inactivated by treatment with betapropiolactone (19). Sheep influenza A/Brisbane/59/2007 (H1N1) (homologous HI [haemagglutination inhibition] titre 1024/4HA), A/Brisbane/10/2007 (H3N2) (homologous HI titre 256/4HA), B/Shanghai/361/2002-like (B/Yamagata/16/88 lineage) (homologous HI titre 128/4HA) and B/Malaysia/2506/2004-like (B/Victoria/2/87 lineage) (homologous HI titre 128/4HA) CDC reference antisera were used as positive serum controls.

Influenza haemagglutination inhibition antibody detection

An HI assay was used for antibody detection. This was performed in accordance with the World Health Organization (WHO) *Manual on animal influenza diagnosis and surveillance* (18). Non-specific inhibitors of haemagglutination were removed from all test sera and positive serum controls by receptor destroying enzyme (RDE) treatment, to obtain a 1:10 dilution which had one volume serum, three volumes RDE and six volumes physiological saline. The RDE was obtained, along with the reference antisera and control antigens, from the WHO Collaborating Center for the Surveillance, Epidemiology and Control of Influenza, at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia.

The HI procedure was as follows: dilutions which contained 4 haemagglutination units (HAU) units/25 µl of reference antigens were obtained before each test and a serial-titration of the 4 HAU was performed to verify its accuracy. The 1:10 dilution of each test serum and control serum was then prepared through RDE treatment. Two rows of wells in a V-bottom microtitre plate were labelled for each test and control serum, and 25 µl of phosphate buffer saline (PBS) was added to wells 2 to 12 in each row. A total of 50 µl of each treated serum was then added to the first well labelled for it, from which 25 µl was serially diluted two-fold across the row and discarded after well 10 to give a dilution of 1:20 to 1:5 120. The last two columns were used as red blood cell (RBC) control wells; 25 µl of standardised reference antigens were then added to the appropriate wells. Plates were agitated manually and incubated at room temperature for 15 min, after which 25 µl of 0.5% of the chicken RBC suspension was added to all wells. Plates were agitated manually and incubated at room temperature (25°C) for 30 min.

Interpretation of results

Endpoints of serum dilutions which showed complete inhibition of haemagglutination of 4 HAU of the virus with a 0.5% solution of chicken RBCs were determined and the reciprocal of the endpoint of such serum specimens were recorded as the HI titre. Results obtained were analysed using the two-way analysis of variance (ANOVA) and Student's t-test, using GraphPad Prism (GraphPad Software Inc., San Diego). Values of $p<0.05$ were considered significant.

Results

The overall prevalence of HI antibodies to influenza viruses in pig handlers during this period was 100% ($n = 30$). There was no significant difference ($p>0.05$) in the values obtained among different age groups and locations or between gender and months of sampling. However, as shown in Figure 1, the prevalence of HI antibodies to the four influenza viruses tested varied significantly ($p<0.05$) within each age group, gender, location, and month of sampling. Antibodies to influenza A (H3) and influenza B/Shanghai/361/2002-like were more prevalent (90.0% and 76.7%, respectively) than those of influenza A (H1) and influenza B/Malaysia/2506/2004-like (46.7% and 40.0%, respectively). These results are shown in Tables I and II, respectively.

Titres of HI antibodies to influenza A (H3) (mean = 124.1) and B/Shanghai/361/2002-like (mean = 237.4) were also significantly higher ($p<0.05$) than those of influenza A (H1) (mean = 23.6) and B/Malaysia/2506/2004-like (mean = 38.3). These results are given in Figure 2. Figure 3 shows that 29 (96.7%) of pig handlers in this study had polytypic reactions, as 14 (46.7%), 13 (43.3%) and 2 (6.7%) had

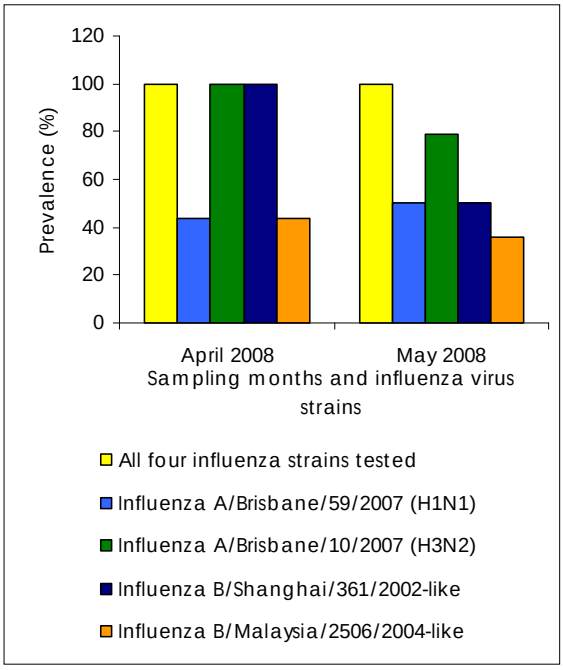

Figure1
Prevalence of influenza haemagglutination inhibition antibody to different strains tested in the study
Observed differences are significant $p<0.05$

detectable antibodies to two, three and all four influenza viruses, respectively.

Discussion

Influenza occurs most often in Nigeria during the Harmattan period (November to January) and earlier (April/May), peaking during the raining season (7, 11). Results of antibody detection obtained in this study which was conducted during one of the 'influenza seasons' (April/May), indicate that the pig handlers had been exposed to all the four influenza viruses tested. The overall prevalence of HI antibodies to influenza viruses in humans tested in this study was 100%, while those of influenza A and B viruses were 68.3% and 58.3%, respectively. The present study is the first report of influenza antibody prevalence among pig handlers in Nigeria. Results obtained from previous studies that reported on the prevalence of influenza antibodies among humans in Nigeria, had similar high values to those obtained in this study. Olaleye *et al.* (11) reported 90% prevalence for influenza A

Table I
Prevalence of haemagglutination inhibition antibody to influenza viruses at different locations in Nigeria

Species	Location	Number positive(%)*				
		Overall	A (H1N1)	A (H3N2)	B/Shanghai	B/Malaysia
Human	1	6 (100.0)	2 (33.3)	5 (83.3)	6 (100.0)	3 (50.0)
	2	10 (100.0)	4 (40.0)	10 (100.0)	9 (90.0)	4 (40.0)
	3	14 (100.0)	8 (57.1)	12 (85.7)	8 (57.1)	5 (35.7)
Total		30 (100.0)	14 (46.7)	27 (90.0)	23 (76.7)	12 (40.0)

* differences observed across each row are significant ($p<0.05$)

1 Commercial Pig Farm Unit, University of Ibadan
2 University Research Farm, University of Ibadan
3 Pig Farmers' Centre, Monatan, Ibadan

Table II
Gender distribution of humans who had detectable haemagglutination inhibition antibody titres to four influenza strains

Criteria	Number positive (%)*				
	Overall	A(H1N1)	A(H3N2)	B/Shanghai	B/Malaysia
Gender					
Male	23 (100.0)	9 (39.1)	20 (87.0)	17 (73.9)	10 (43.5)
Female	7 (100.0)	5 (71.4)	7 (100.0)	6 (85.7)	2 (28.6)
Total	30 (100.0)	14 (46.7)	27 (90.0)	23 (76.7)	12 (40.0)

* differences observed across each row are significant ($p<0.05$)

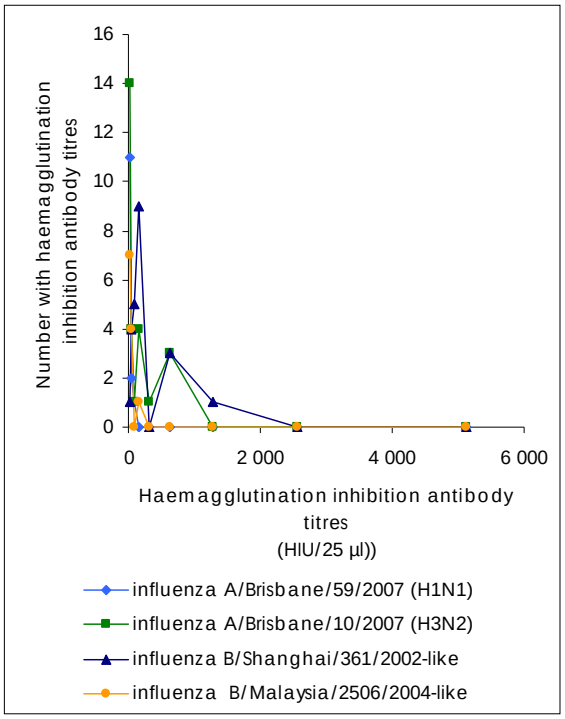

Figure 2
Titres of haemagglutination inhibition antibody to influenza viruses in humans

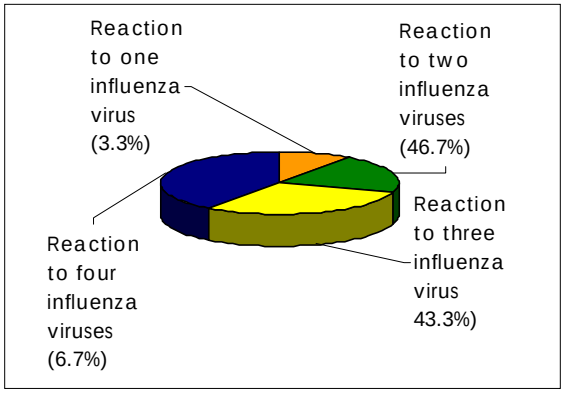

Figure 3
Frequency of pig handlers who had serum haemagglutination inhibition reactions to one or more influenza virus strains

viruses, while Olaleye *et al.* (12) obtained a prevalence rate of 71.6% from humans of different age groups in Nigeria. In our study, as the history of these humans revealed the absence of prior influenza vaccination, this high overall prevalence (100%) indicates a very high level of either previous or current influenza virus infection. However, unlike the generally high prevalence observed, our results also indicated that a majority of the pig handlers had low HI antibody titres (≤20). This could indicate waning influenza antibody levels, after an exposure that occurred a long time ago.

The prevalence of antibodies to influenza viruses in humans has been found to vary with age (12, 13, 14). However, results from this study revealed that the prevalence of antibodies to influenza A and B viruses did not vary significantly ($p>0.05$) with the age of the pig handlers.

Moreover, differences in prevalence of antibodies to influenza viruses due to gender, location and month of sampling were not significant ($p>0.05$). However, the prevalence of antibodies to the different strains tested in this study varied significantly ($p<0.05$) within each age group, gender, location and month of sampling, with prevalence of HI antibodies to influenza A(H3) and B/Shanghai/361/2002-like remaining the highest.

This study showed that different strains of influenza viruses co-circulate in people who have regular and direct contact with live pigs in Ibadan. Previous serological surveillance studies revealed that prevailing human influenza H1N1 and H3N2 strains were readily transmitted to pigs (1, 5). Human influenza-like A (H3N2) viruses have also been isolated from pigs (2, 17, 21), although they are not usually maintained in pigs independently of the human population (9). Therefore, the likelihood of human-to-swine transmission of influenza viruses in Ibadan, a city which has witnessed increasing commercial swine production coupled with poor biosecurity and environmental hygiene, is very high. Furthermore, the possibility of co-infection of these pigs with avian influenza viruses is very significant in the study area, especially when considered along with the observation that two of the pig farms used for this study were within 5-10 km radius of commercial poultry farms, and 5 pig handlers (16.6%) had regular contact with commercial poultry farms. These co-circulating strains of human and swine, and possibly avian, influenza viruses may therefore recombine in these pigs to form novel, reassortant viruses which could be

highly virulent when established in human, swine, or avian populations.

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