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Participatory Epidemiology and Sero-Surveillance for Avian Influenza in Local Chicken at Live bird Market and House Holds in Enugu State, Nigeria

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Abstract

Enugu state has previously reported outbreak of avian influenza in three local governments (Igoetiti, Nkanu west, and Udi) in 2016.Trace back investigation on affected farms showed a direct link of confirmed outbreaks to the presence of apparently healthy asymptomatic local chicken roaming free as resident local chicken within intensive farms, however there was paucity of data to support this hypothesis. Two hundred (200) serum samples were obtained from apparently healthy local chicken at the live bird market (Ibagwa Nkwo Nsukka, N 06°91462', E 008.39664') and selected household within Enugu. Serology for Avian influenza specific antibodies was conducted using Agar Gel immune-diffusion test, Semi structured questionnaires (120) copies were developed using Participatory epidemiology modules (PEM), to access the level of awareness of respondents about avian influenza (AI), management practices that may be associated with suspected cases of avian influenza and accessibility to veterinary care within the study area. The level of awareness for avian influenza was low, 31(48.4) %,there was no association between gender, educational status of respondent and level of awareness of avian influenza within the study area (P=0.348, χ^2=1.035 df =2), (P=0.329, χ^2=2.225; df=2) respectively. Veterinary care are available, 53(82.8%)however,21(39.6%) reported that paucity of fund was a militating factor in accessing it services. Risk factors to avian influenza zoonoses includes consumption of dead poultry "*Morta"*,2 2 (34.4%),open disposal of carcasses,23(35.9%) and mixing of local chickens with known reservoirs of avian influenza, Ducks,76(17.2%), Pigeons, 119(26.9%) were very common. Serological findings shows that all 200 samples (serum) were negative for avian influenza antibodies, premised on this finding we concluded that local chickens may not serve as reservoirs of avian influenza in the study area within the time frame.

Keywords: Highly Pathogenic Avian influenza, live bird market, sero-surveilance, Nigeria

Introduction

Highly pathogenic avian influenza,(H5N1) was first reported in Nigeria in 2006,it persisted till 2008, resulting in one fetal human case in Lagos .it affected 25 states across 97 local government areas in Nigeria, About 1,525 suspected cases of HPAI was reported during this period and 300 of these cases confirmed as HPAI (FMA&RD,2013). Nigeria was declared free of avian influenza in 2013 by office of international epizootics (FMA&RD, 2013). However, a year after this declaration,(Coker et al, 2014), reported circulating level of low pathogenic avian influenza in duck at "SHASHA" live bird market in Ibadan southwest Nigeria. Enugu state have reported five suspected cases of HPAI, H5N1 since the 2015 resurgence, three of these cases were confirmed as highly pathogenic avian influenza. Trace back investigation in affected poultry farms showed a direct link of outbreaks to presence of apparently healthy asymptomatic resident local chickens within the intensive poultry farms. As at the first quarter of 2017, sporadic outbreak of avian influenza is still being reported in certain states of the federation with the emergence of H5N8 in Kano, and Ogun State south west Nigeria (OIE, 2016, OIE, 2017). At present, 26 states are currently affected with over 700 hundred million naira paid as compensation to affected farmers and another 1 billion naira as outstanding claims (FDVS&PCS, 2017).

This has brought to the fore the need for a comprehensive review of the current control policies of the Federal government of Nigeria toward attaining food safety.

Materials and Methods

Enugu state lies between latitude.6°30N and longitude .7°30E. It's bordered by Abia and Imo States to the south, Ebonyi State to the East, Benue state to the North East, Kogi state to the northwest and Anambra state to the west. It has an approximate land mass of 7.161km2, (2,765 sq mL), located on the tropical savannah zone of Nigeria, with a population of 3,267,837 people, (NPC, 2006). Enugu state has 17 local government areas (LGAs) divided into 6 agricultural zones, (Agwu, Agbani, Enugu, Nsukka, Enugu Ezike and Udi agricultural zones), Crop and livestock farming is the major occupation of the people(Chah *et al.,*2014).

There are 6 Abattiors in Enugu located at (Enugu ezike, 9th mile, Nenwe, Adani, Ogbette, and Nsukka), one Pig Market at Orie Oba. Live bird markets include, Artisan poultry market and Ugwuoba live bird market. There are two control posts at (Obollo Afor interstate control post, Ugwu Oba control post)

Sample size was determined using mean prevalence of 4.2%, (Ameji *et al.,* 2016), 13% (Coker *et al.,* 2014) and 2.9%, (Gugong *et al.,* 2012) as outlined by Thrusfield (1997).

$$N = \frac{Z^2 \times p \times q}{d^2}$$

Where q = 1-P
N = sample size
P = anticipated prevalence
d = desired precision
Z = appropriate value from the normal for the desired confidence (1.96)
α=0.05.

$$N = \frac{1.96 \times 1.96 \times 6.7\ \% \times (100-6.7)\ \%}{(5\%)^2}$$

N =96 local chicken: household poultry +LBM=96+96=192

A Multi stage sampling techniques was used in selection of live bird markets and cross-sectional survey was conducted at the live bird markets were local chickens were selected at random from their cages, similarly households within Enugu and Nsukka, were local chickens are reared were purposively selected, and local chicken picked at random. Laboratory test (Agar gel immunodiffusion test) was used in analysis of the serum samples obtained from selected local chicken; participatory epidemiological technique was used for collection of information from study group using semi structured questionnaires on avian influenza. Systematic random sampling technique of 1:3 was used to select ten cages containing local birds at the live bird markets; a random sample of 10 chickens was made from each cage to make a total of 100 birds. Households were local chicken were kept were selected purposively, chicken were subsequently picked at random.

Blood sample was collected after proper restraint the brachial vein was sterilized using 70% alcohol; 2-3ml of blood was collected using 5ml syringe with 21 gauge needles. Blood sample was left slanted for 2hrs in syringe to allow blood clot and separation of sera, subsequently sera was decanted into an Eppendorf tube, which was stored in a Coleman pack and transported to the avian influenza reference laboratory NVRI, Vom Jos, were it was analyzed. Inactivated avian influenza (H5) antigen and antiserum were strain A/duck/Potsdam/1402/86 obtained from Avian influenza reference Lab. Podova Italy, were used as the standard avian influenza antigen and antibodies respectively, Agar gel immune-diffusion test was conducted as described by OIE (2014).

Agar gel immune-diffusion test procedure was done using1% (w/v) Agarose and 8% (w/v) Nacl in 0.01M phosphate buffer solution PH 7.2 was dissolved in 100 mil distilled water the solution is stirred gently and incubated in a humidified chamber for 10 minute. Subsequently 15mil of the solution was dispensed into 9cm petri dishes and allow to cool at room temperature. Using a template cutter well of approximately, 5mm in diameter were cut into the gel on the petri dish as shown below.

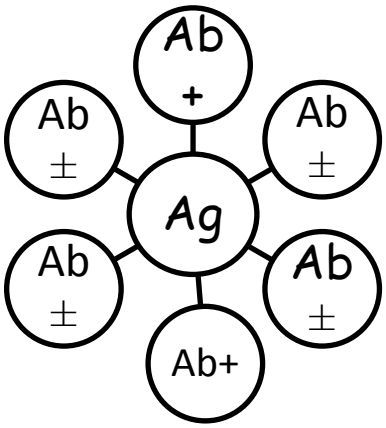




Fig II: (a)Ag **–**standardantigen **(b)** Ab± test sera **(c)** Ab+ standard positive antisera

The pattern of well is such about 50µ of each test serum samples is dispensed into the peripheral wells (Ab±) and the known avian influenza antigen is dispensed into the central well (Ag+), all corresponding to the meniscus. Sample was incubated for 24 hrs. and viewed in a dark background using oblique light

Results and Discussion

The level of awareness of highly pathogenic avian influenza (HPAI) was low,31(48.4%), 33(51.6%) has no knowledge of the disease. However only 16(51.6%) of those knowledgeable about AI can identify at least three clinical signs associated with suspected cases of HPAI, i.e. (purple discoloration of the wattle, and comb, bleeding ovaries, unusually high mortality), while the remaining 15(48.4)% of the respondent could identify at least two of the clinical signs, (Swollen head, pin point hemorrhages on the shank). Furthermore, 11(17.2%) of the respondents prefer veterinary intervention when their flock shows symptoms of diseases, 42(65.6) % of the respondent reported selling their sick birds, while 11(17.2%) prefer to slaughter and consume them. There is no association between gender and level of awareness of avian influenza (P=0.348, X^2=1.035 df =2). Male respondents were more informed about avian influenza, 12(52.17%), females 11(47.83%) respectively. There was no significant association between the educational status of respondent and their level of awareness about avian influenza, (P=0.329,χ^2=2.225; df=2) although level of awareness is higher in respondents with secondary education 14(60.87) %, tertiary education, 8(34.78) % and primary education (4.34) %. There is no association between occupation of the respondents and their level of awareness about avian influenza (P=0.954) (χ^2=0.33;df=3), although farmers were more informed, 12(53.33%), civil servants 7(23.33%), students 5(10%) and 6(13.34%) was not applicable to any chore.

Over the last decades, collection of existing veterinary knowledge through surveys has become an integral means of identification and prioritization of animal health problem within the community *(*McCorkle and Mathias-Mundy,1992). Most of the respondents interviewed practices extensive poultry farming 58(90.6) %, and 6(9.4%) practice intensive poultry farming. About 41(64.06%) of the respondent supplement their feed with waste from the live bird market, 19(29.69%) of the respondent feed their poultry from waste from commercial farms, and 4(6.30%) purchase commercial feed for their poultry stock. Considering the sources of poultry, 47(73.4%) reported they obtain their flock from free range poultry, 17(26.6%) purchases their stock from commercial poultry market. On disposal of poultry carcass and waste 23(35.9%) of the respondent dump dead carcass in public bin, 19(29.7%) bury dead carcass, while 22(34.4%) reports that they consume dead chicken.

The possible emergence of a novel influenza strain with no prior human or animal immunity has remained a talking point within the scientific circle. Consequently, isolation of influenza A subtype H5N8, in Kano state, *(*OIE,*2016),* and Oyo State (OIE 2017), has brought to the fore the need for a comprehensive review of the current control policies of the Federal government of Nigeria toward stamping out avian influenza in the Country. Considering management practices with high risk of avian influenza zoonoses or disease outbreak within the study area, 23(35.94%) stated that they dispose of their sick or dead birds in refuse dump, 22(34.38%) consume sick or dead chicken, "Morta" and 19(29.69%) bury carcasses in deep pit. The rationale behind this behavior is chiefly due to economic reasons and the attendant lack of information on the public health risk.

The source, quality and quantity of poultry feeds are integral to good animal husbandry; it improves resistance to infection (Mavale, 2001). 41(64.1%) of the respondent supplement their feeds with food waste from the live bird market, 19(29.7%) with waste from commercial farms while 4(6.3%) give commercial feed. Feed waste obtained from the live bird market which may provide suitable milieu for viral or infectious agent. There was no antibodies to influenza A viruses in serum of apparently healthy local chicken at the live bird markets and household in Enugu state Nigeria, which was in contrast to findings by Ameji *et al.* (2016) who reported presence of antibodies to influenza A Viruses in backyard and rural poultry chicken in Kogi State, El-Yuguda and Baba (2002) who reported the presence of antibodies to influenza A in village chicken in Borno State. Thus non detection of viral antibody in the serum of local chickens within the study area and time frame laid credence to the fact that local chickens may not be carriers/reservoirs of avian influenza viruses during the period.

Conclusion and Recommendations

This study has shown that Local chickens may not serve as reservoirs of AI in the study area within the time frame. Consequently, consumption, rearing of local chicken may not pose any public health risk to the populace similarly; intensive farms were this chicken roam free as resident local chicken may not be at risk of infection. The level of awareness of avian influenza within the study area is low (48.4%).Consumption of dead chicken known as "Morta" in local parlance is very popular amongst the respondents, a risk factor in the epidemiology, and adaptation of avian influenza to human host. Mixing of different species of birds is very common within the live birds markets as such may provide the needed milieu for emergence of a new strains of influenza viruses.

There should be a sustained active surveillance for avian influenza within Enugu state, with compliment of laboratory analysis to aid early detection and confirmation of suspected cases of avian influenza. Similarly, the Enugu State government should subsidize the cost of veterinary services with a view to making it accessible to small scale farmers.Reactivation of the comatose extension department at the state and federal offices with a renewed vigor on educating farmer's on new approaches in tackling endemic and emerging diseases.Bearing the level of mixing of different species of livestock at the live bird markets continuous active sero-surveillance should be conducted with a view to determining possible new sentinels of infection.

References

Ameji, O.N., Sa`idu, L. and Abdu, P.A. (2016). Sero-prevalence of avian influenza in poultry in Kogi State, Nigeria. *Current Research in Poultry Science,* 6: 1-6.

Chah, J., Irohibe, I. and Itodo, C. (2014). Constraints to Indigenous Chicken Production in Enugu State, 2014 2nd International Conference on Sustainable Environment and Agricultural PCBEE vol. 76 IACSIT Press, Singapore DOI: 10.7763/IPCBEE. 2014. V76. 3.

Coker, T., Meseko, C., Odaibo, G., and Olaleye, D. (2014). Circulation of the low pathogenic avian influenza subtype H5N2 virus in ducks at a live bird market in Ibadan, Nigeria. *Infectious Diseases of Poverty*, *3*(1): 38.

El – Yuguda, A.D. and Baba, S.S. (2002). Prevalence of selected viral infection invarious age groups of village chickens in Borno state, Nigeria. *Nigerian Journalof Animal Production*, 29(2): 245 – 250.

FDV&PCS (2017). Federal department of veterinary and pest control services, Avian influenza daily notification (August 29th, 2017).

FMA&RD (2013). Self-declaration from Nigeria on its disease-free status from notifi able avian influenza Self-declaration. Resolution N'21 submitted to the OIE on 25 January 2013 by Joseph Nyager, Delegate Director, Livestock, Federal Ministry of Agriculture Abuja,Nigeria and adopted at the 8th OIE General session in, (May), 2013 pg. 56–57,

Gugong, V.T.,Ajogi, I., Junaidu, K.,Okolocha, E.C.,Ngbede, E.O.,Hambolu, S.E., Maurice, N.A. (2012). Avian influenza (H5 subtype) antibodies in village chickens in four local government areas of Kaduna state, Nigeria. *Vet. World,*5(12):713-717. doi;10.5455/vetword.2012.713-717

Mavel, A.P. (2001). Country report: Mozambique. In: Alders, R.G. and Spradbrow, P.B. (Ed.).*SADC planning workshop on Newcastle disease control in village chicken.Proceedings of an International Workshop.* 6-9 March 2000,Maputo,Mozambique.*ACIAR Proceeding,* 103:20-25.

McCorkle N. and Mathias-Mundy, E. (1992). *Journal of the International African Institute,*62(1): 59-93.

Mckibbon, W. and Sidorenko, A. (2006). Global macroeconomic consequences of pandemic influenza .Lowry institute for international policy, Sydney, Australia at: www.lowyinstitute.org/Publication.asp?pid=345

NPC (2006).National population Censors,http://www.population.gov.ng/index.php/censuses

Oie (2016).Update on avian influenza in animals (types H5 and H7).http://www.oie.int/animal-health-in-the-world/update-on-avian-influenza/2016/

Oie (2017). Oie situation report for avian influenza, http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/OIE_AI_situation_report/OIE_SituationReport_AI_10_7August2017.pdf

(Oie) W.O. for A.H. (2014). Avian Influenza. *OIE Terrestrial Manual*, http://doi.org/10.1159/000151603