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SEROSURVEILLANCE FOR AVIAN INFLUENZA IN LOCAL CHICKENS IN HOUSEHOLDS AND LIVE BIRD MARKETS IN ENUGU STATE, NIGERIA

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ABSTRACT

Enugu State, Nigeria has previously reported an outbreak of avian influenza in three local governments namely Igoetiti, Nkanu west and Udi following the resurgence of avian influenza in 2015. Traceback investigation on affected farms showed a direct link of confirmed outbreaks to the presence of apparently healthy asymptomatic local chicken roaming freely as resident local chicken within intensive farms. However, there was a 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) and purposively selected households within Enugu state. Serology for Avian influenza-specific antibodies was conducted using the Agar Gel immunodiffusion test. Semi-structured questionnaires were used to access the level of awareness of respondents to 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, education of respondents and level of awareness about avian influenza within the study area, ($P = 0.348$, $\chi^2 = 1.035$; $df = 2$), ($P = 0.329$, $\chi^2 = 2.225$; $df = 2$). 53(82.8%) of the respondents reported to be aware of the availability of veterinary care; however, 21(39.6%) reported that limited fund was militating factor in accessing the services. Risk factors to avian influenza zoonosis included consumption of dead poultry "Morta" (22, 34.4%); open disposal of carcasses (23, 35.9%) and mixing of local chickens with known reservoirs of avian influenza where ducks (76, 17.2%) and pigeons (119, 26.9%) were very common. Serological findings showed that all 200 samples (serum) were negative for avian influenza antibodies, premised on this finding, it was concluded that local chickens may not serve as reservoirs of avian influenza in the study area within the time frame.

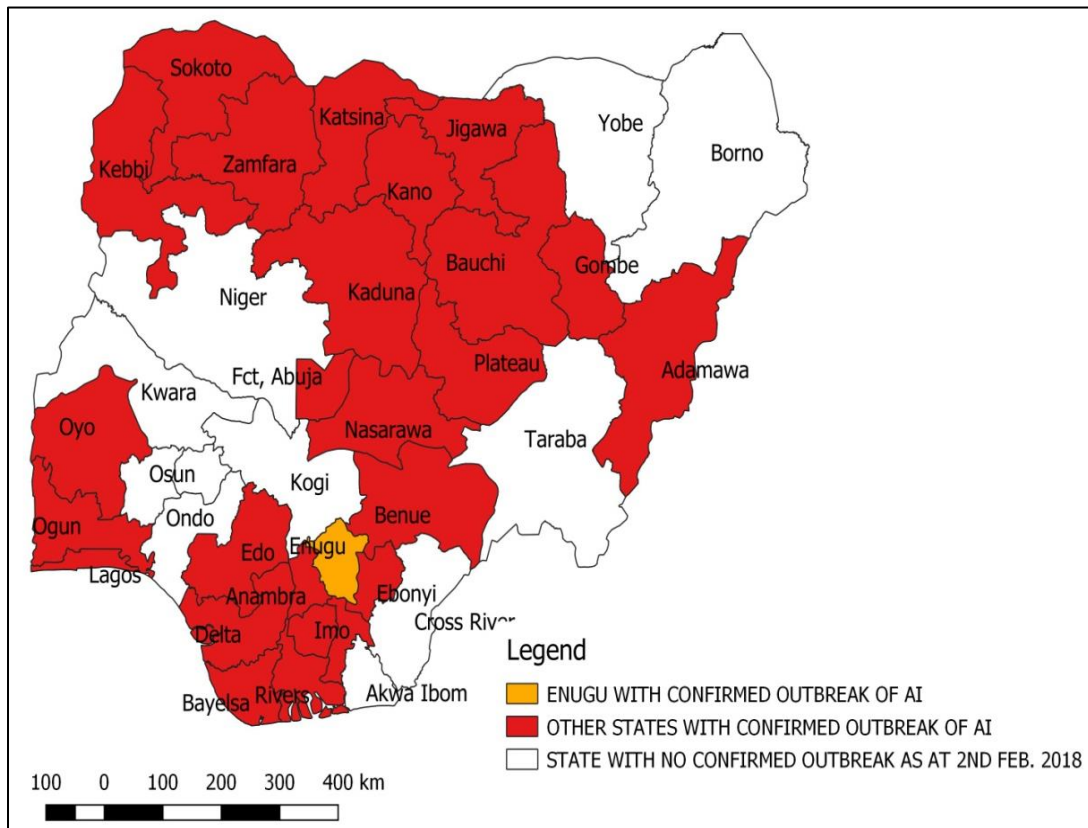
INTRODUCTION

Highly pathogenic avian influenza (H5N1) was first reported in Nigeria in 2006 where it persisted until 2008, resulting in one AI case in a woman in Lagos, (Monne *et al.*, 2007), 25 across 97 local government areas were affected (Fusaro *et al.*, 2009). About 1,525 suspected cases of HPAI were reported during this period; however, Nigeria was declared free of avian influenza in 2013 (Nyager, 2013). A year after this declaration, Coker *et al.* (2014) reported a circulating level of low pathogenic avian influenza in duck at the *Shasha* live bird market in Ibadan southwest Nigeria. In 2015, a resurgence of highly pathogenic avian influenza was reported following increased mortality at a live bird market in Lagos and backyard poultry farms in Kano state (Monne *et al.*, 2015). The possible emergence of a novel influenza strain with no prior human or animal immunity has remained a talking point within the scientific circle. The isolation of influenza A subtype H5N8, in Kano State (OIE, 2016), and Oyo State (OIE, 2017) has brought to the fore the urgent need for a

comprehensive review of the current control policies of the Federal Ministry of Agriculture towards stamping out of avian influenza in Nigeria and ensuring food safety.

Enugu state-reported five suspected cases of HPAI and H5N1 between 2015-2018, three of these cases were confirmed to be highly pathogenic avian influenza. As in the first quarter of 2018, a sporadic outbreak of avian influenza was still being reported in certain states of the federation with the detection of H5N8 in Kano state in northwest Nigeria (OIE, 2016). As of the first quarter of 2018, 26 states had reported an outbreak of HPAI with over ₦700 million paid as compensation to affected farmers and another ₦1.6 billion as outstanding claims, (FDLPCS, 2017). This research work aimed to identify remote and immediate factors responsible for the outbreak of avian influenza in Nigeria and provide data for policy development towards findings a long-lasting solution to the outbreaks and spread of Avian influenza within Nigeria thus ensuring and enhancing public health and food safety.

Figure 1: Cases of Avian Influenza Between 2015- 2nd February 2018



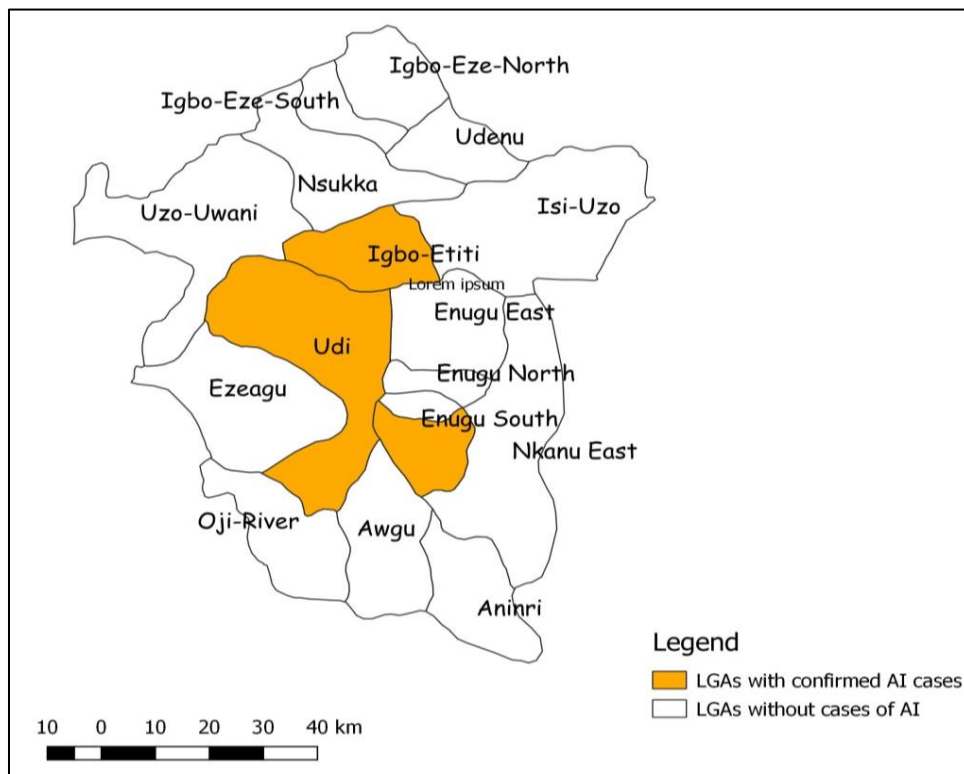
MATERIALS AND METHODS

Study Area

Enugu State was created in 1991 from the old Anambra State; it is bordered by Abia and Imo State 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 lies between latitude 6° 30N and longitude 7° 30E with an approximate land mass of 7.161 Km² (2,765 sq. mile). It is located in the tropical savannah zone of Nigeria. It has a population of 3,267,837 people

(NPC, 2006). The total number of poultry in Nigeria is estimated to be 145 million birds (NASS, 2015). Enugu state has 17 local government areas (LGAs) subdivided into 6 agricultural zones namely Agwu, Agbani, Enugu, Nsukka, Enugu Ezike, and Udi. Crop and livestock farming is the major occupation of the people (Chah et al., 2014). There are 6 slaughterhouses in Enugu at Enugu ezike, 9th mile, Nenwe, Adani, Ogbette, and Nsukka; one pig market at Orié Oba; artisan poultry market and Ugwu Oba live bird market. There are two control posts at Obollo Afor interstate control post and Ugwu Oba control post.

Figure 2: Outbreak of Avian Influenza Between (2015-2018) by LGAs in Enugu State



Agar gel Immunodiffusion Test and Procedure

The 100 ml Agar used was prepared where 8g of NaCl and 1g Agarose was weighed the salt mixture is then poured into a sterile bottle (500 ml) and later 100 ml of PBS (pH adjusted to 7.2) was added. The heat was supplied to the mixture until it completely dissolves. Dispense 15 ml of the melted Agar into 100×15 mm sterile Petri dishes placed on level surface open until the Agar is set. The plate was incubated and stored at +4 °C until use

Surveillance Team

The team members include: (a) the Researcher as the facilitator (b) AI Desk Officer for LGA in Enugu (d) one additional animal health worker in the locality. These participants were retrained on PE and assigned roles.

Study Design

A cross-sectional survey design was adopted for the study interviews were conducted using a semi-

structured questionnaire was used to obtain information from local poultry keepers. Parameters accessed by a questionnaire includes poultry management system, level of awareness of respondents to avian influenza, clinical signs associated with suspected cases of AI, method of disposal of carcasses, source of feed and availability of veterinary care amongst others. Similarly, a serological test to determine the prevalence of avian influenza in local chicken was conducted using the Agar gel immunodiffusion test.

The study population included local chickens in households, local chicken at the live bird markets and surveillance point agents within the study area. The sample size was determined using a mean prevalence of 4.2%, (Ameji *et al.*, 2016), 13% (Coker *et al.*, 2014) and 2.9%, (Gugong *et al.*, 2012); by (Thrusfield, 1997). The following formula was used: $n = \frac{z^2pq}{d^2}$ where $q = 1-p$, $n =$ sample size $p =$ anticipated prevalence (6.7) $d =$ desired precision (0.05) and $z =$ appropriate value from the normal for the desired confidence (1.96). A sample size of 96 local chicken was determined since samples are to be collected at two main units LBM and household, it follows that $96+96 = 192$

Sampling Technique

A systematic random sampling technique of 1:3 was used to select ten cages containing local

chicken at the live bird markets where a random sample of 10 chickens was made from each cage to make a total of 100 birds. Purposive sampling technique was used to select households keeping local chickens and samples were collected from chicken selected at random.

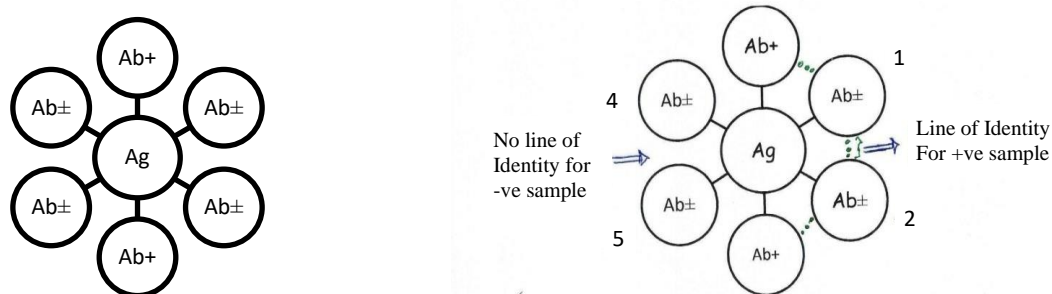
Blood Sample Collection

The brachial vein was sterilized using 70% alcohol; 2-3 ml of blood was collected using a 5 ml syringe with a 21-gauge needle. A blood sample was dispensed in a sterile test tube, slanted for 2 hours 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 laboratory.

Procedure for Agar gel immunodiffusion test

1% (w/v) Agarose and 8 % (w/v) NaCl in 0.01 M phosphate buffer solution PH 7.2 was dissolved in 100 ml distilled water, the solution was stirred gently and incubated in a humidified chamber for 10 minutes. Subsequently, 15 ml of the solution was dispensed into 9 cm diameter Petri dishes and allow cooling at room temperature. Using a template cutter, wells of approximately 5 mm in diameter were cut into the gel on the petri dish as shown below (OIE, 2014).

Figure 3: Representation of experimental landmark of Agar gel immunodiffusion medium showing positive and negative test after the result



Key: Ag –standard antigen; Ab± test sera; Ab+ standard positive antisera; °°° line of identity

The pattern of well is such that about 50 µm of each test serum samples were dispensed into the peripheral wells (Ab±) and the known avian influenza antigen is dispensed into the central well (Ag+), all corresponding to the meniscus. The sample was incubated for 24 hours and viewed in a dark background using oblique light. Regions of precipitation between the specific known avian influenza antigen and antibodies (Serum samples) is an indication of the positive test result and vice versa. Inactivated avian influenza (H5) antigen and antiserum were (strain A/duck/Potsdam/1402/86) obtained from the Avian influenza reference Lab. Padova Italy and used as the standard avian influenza antigen and antibodies respectively, Agar gel immunodiffusion test was conducted as described by (OIE, 2014).

RESULTS

Level of Awareness, Knowledge of Clinical Signs and Management Practice

The level of awareness of highly pathogenic avian influenza (HPAI) was low (31, 48.4%), 33(51.6%) had no knowledge of avian influenza. 6(51.6%) of those who were knowledgeable about AI identified at least three clinical/PM signs that could be associated with suspected cases of HPAI. These were purple discoloration of the wattle, comb, bleeding ovaries seen at slaughter unusually high mortality. 15(48.4%) of the knowledgeable respondents could identify at least two of the clinical signs that are associated with avian influenza namely swollen head and pin point haemorrhages on the shanks.

On availability of veterinary care, 53 (82.8%) of the respondents posited that veterinary care was available within the study area, where out of these, 32 (60.4%) reported that veterinary care was affordable while 21 (39.6%) claimed they were beyond their reach. Considering sex demography of the respondents (Table 1), female respondents accounted for 36 (62.5%) and male respondents, 28 (37.5%). There is no association between gender and level of awareness of avian influenza (P = 0.348; X² = 1.035 df=2). However, male respondents were more informed about avian influenza 12 (52.17%) than females 11 (47.83%) respondents.

Table 1: Distributions showing genders of respondent and awareness about AI

Gender	Awareness about AI	Not aware of AI	Total
Male	12	16	28
Female	11	25	36
Total	23	41	64

P= 0.348; χ²= 1.035 df = 2

Table 2 showed that there was no significant association (P=0.329) between the educational status of respondent and their level of awareness of avian influenza, although level of awareness is higher in respondents with secondary education 14 (60.87%), tertiary education, 8(34.78%) and primary education (4.34%) respectively.

Table 2: Distribution of educational levels and awareness about AI

Educational Status	Aware About AI	Not Aware About AI	Total
Secondary	14.	21	35
Primary	8	6	14
Tertiary	9	6	15
Total	31	33	64

P= 0.329 (χ²= 2.225; df= 2)

Furthermore, Table showed that farmers 12(53.33%) were more informed than civil servants 7(23.33%) and students 5(10%), however, there is no association (P=0.954) between the occupation of the respondents and their level of awareness.

Table 3: Distribution showing occupation of respondents and awareness of AI

Occupation	Awareness of AI	Not aware of AI	Total
Farmer	12	15	27
Civil	7	8	15
Servant			
Student	5	6	11
Not Indicated	6	5	11

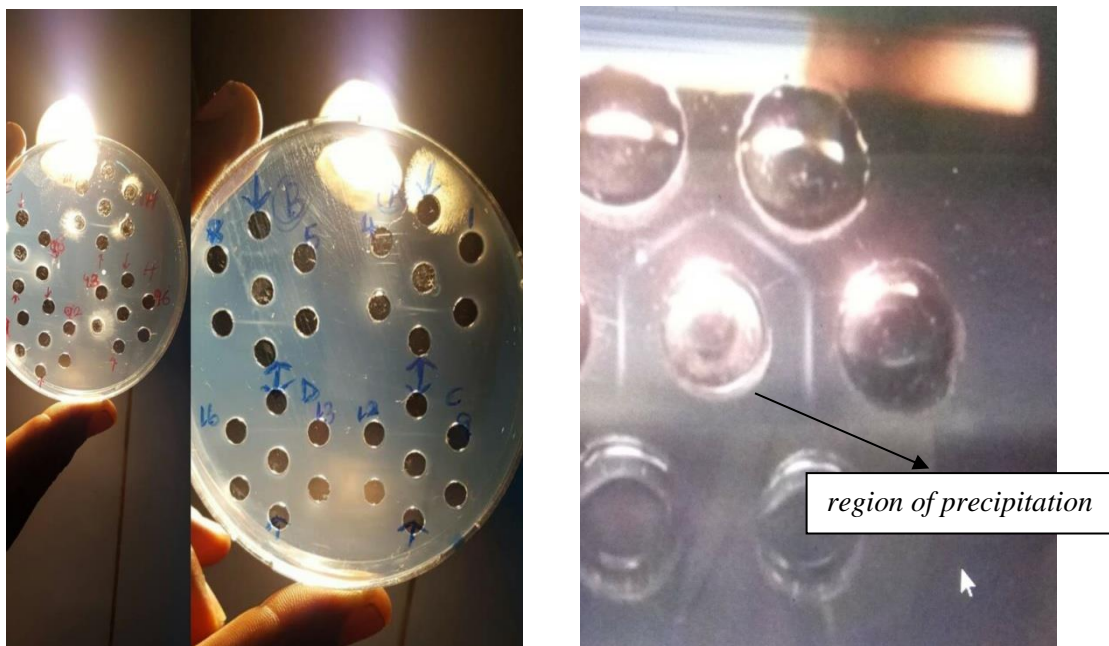
(P= 0.954) (χ²=0.33; df= 3

Result of Serology for Avian Influenza Antibodies

antibodies in local chicken using Agar gel immunodiffusion techniques, *Figure 4*.

There was no precipitation band formed in all 200 serum samples screened for avian influenza

Figure 4: Agar gel immunodiffusion test result with no region of precipitation (Negative text)



Agar gel immunodiffusion test.

Standard Control

Poultry Production with Associated Risk at the Live-bird Markets and Households

In Table 4, 41(64.06%) of the respondents supplemented their feeds with feed waste from the live bird market, 19(29.69%) of the respondent feed their poultry from waste from commercial farms

while 4(6.26%) purchased commercial feed for their poultry stock. The findings of the study further showed that Newcastle disease (5.3 ± 0.96), fowl pox (2.3 ± 0.96), Coccidiosis (4.8 ± 0.96), and flea infestation (1.8 ± 0.96), were the common diseases affecting poultry birds during the study period (see *Table 4*).

Table 4: Poultry diseases reported at Ibagwa Nkwo LBM between March-April, 2017

Local bird sellers	Fowl Pox (Kitipa)	Newcastle (Agbagu olu)	Coccidiosis (Qria nsi mmiri)	Flea infestation (Akpiri)
I	2	6	4	1
II	1	4	5	2
III	3	6	5	3
IV	3	5	6	1
Mean Score \pm SD	2.3 ± 0.96	5.3 ± 0.96	4.8 ± 0.96	1.8 ± 0.96

Table 5 described the level of mixing/rearing of poultry stock with other animals at Ibagwa Nkwo live bird market where a total of 443 poultry kept comprising 248(55.6%) local chicken, 119 (26.9%) pigeons, and 76(21.7%) ducks.

Table 5: Stocking of different poultry species and swine at Ibagwa Nkwo LBM, Nsukka

Categories	Local Chicken	Pigeon	Duck	Swine	Coordinates
A	20	8	12	6	N06°.91462,E008.39664.
B	31	14	6	-	N06°.96432,E008.49364
C	34	16	14	-	N06°.86231,E008.59564
D	15	6	6	13	N06°.56531,E008.56561
E	26	-	-	4	N06°.55531,E008.66511
F	21	-	9	9	N06°.45571,E008.67513
G	14	15	10	12	N06°.85571,E008.77513
H	34	9	-	-	N06°.95571,E008.77815
I	32	21	-	-	N06°.75971,E008.47835
J	21	15	19	8	N06°.65974,E008.43885
Total	248	119	76	52	

Table 6: Distribution of methods of management of sick poultry/poultry carcasses at the LBM and households

Management	Number and % of respondent
Veterinary intervention	11 (17.2)
Sell off sick birds	42 (65.6)
Slaughter and consume sick or dead poultry	11 (17.2)

DISCUSSION

There was general apathy amongst the poultry stakeholders to participate in the study sighting previous visits by a representative of the government with no justifiable outcome. However, the application of persuasive technique and confidence building encouraged some poultry farmers to participate in the study.

Respondents demonstrated a good degree of understanding of clinical signs associated with common local diseases, though the level of awareness of avian influenza was low 31(48.4%), in contrast to 87% reported by Durosilorun (2009). Veterinary care was available, 53 (82.8%), of these population, 21 (39.6%) reported that the high cost of veterinary care was a militating factor in accessing veterinary services. This may have contributed to the rising practices of ethno

veterinary practice within the study area which is in tandem with findings by Anzaku, (2013). This was in sharp contrast to (Ekue *et al.*, 2002) who concluded that the increasing practice of ethno veterinary practice was in direct response to inadequate extension services and low cost of ethno veterinary materials.

Considering management practices with high risk of avian influenza outbreak within the study area, 11 (17.2%) of the respondent slaughter and consume sick or dead chicken “(Morta)” 42(65.6%) sell off their moribund poultry to small scale eateries and homes while 11(17.2%) engage the services of veterinary practitioners in management of their flocks. The rationale behind this behaviour is chiefly due to economic reasons and the attendant lack of information on the public health implication of consumption of sick or dead poultry, as such individuals who consume such

poultry are continuously at risk of avian influenza zoonosis.

Since 2003, the World Health Organization has reported 387 confirmed human cases of HPAIV infection caused by the H5N1 virus. 245 (63%) persons involved in these confirmed cases died as a result of these diseases (WHO, 2008). Furthermore, developing economies may be at the receiving end of this pandemic (Mckibbin and Sidorenko, 2006), more so the poultry value chain in Nigeria remain poorly regulated, consumption of dead poultry, mixing of different poultry flock may enhance avian influenza viruses to be potentially better adapted to human (WHO, 2005). Considering the gender of the respondents, 28 (37.5%) and 36 (62.5%) were male and female, respectively. Male in the eastern part of Nigeria is mostly engaged in import of poultry equipment while female engage in small scale poultry ventures for economic reasons due to the ease of handling poultry as compared to other livestock. Pagani *et al.* (2008), in a similar study, *it was* reported that in the southwest of Nigeria the stakeholders of the poultry value chain were either male or female which is a sharp contrast to practices in Northern Nigeria, where males play a dominant role in the poultry subsector largely due to religious inclination. The family '*Anatidae*' (Duck, Water birds) has for long been known to play a major role in the transmission of avian influenza viruses between migratory birds and domestic chicken (Coker *et al.*, 2014). *Consequently*, mixing of these species of poultry with local poultry chicken at a different level of the poultry value chains may provide the needed milieu for adaptation, the emergence of reassortant strains and the outbreak of avian influenza.

Free-range poultry is at a higher risk of coming in contact and roosting with wild birds in the absence of any form of housing, undue exposure to weather may aggravate disease outbreak in reservoir poultry. The demography of occupation of the respondents shows that 63.6 (53 %) were full-time farmers, 27.6 (23%) were civil servants, and 12 (10%) were students at different levels of the institution. Poultry farming provides a source of income to supplement the earnings of students and civil servants who are stakeholders, it may also serve as an indicator that the current drive of the Federal Government of Nigeria, to make the

agricultural venture profitable may be yielding dividend.

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 respondents supplement their feeds with food waste from the live bird market, 19 (29.7%) with waste from commercial farms and 4 (6.3%) give commercial feed. It's instructive to note that feed waste obtained from the live bird markets or commercial poultry farms may harbour infectious agents and provides a suitable medium for the growth of viral agents. Similarly, faecal contaminated feeds from reservoir poultry may lead to a disease outbreak in the naïve poultry population.

The poor economic status of poultry local chicken sellers, the paucity of information relating to the public health implication of such practices is the bane of effective disease control in the study area. The government can play a leading role in subsidizing the price of feed for peasant farmers, and revitalization of the moribund extension services may go a long way in ensuring food safety. The implications and improper management of poultry carcasses and sick flocks can never be overemphasized, open disposal of dead or sick poultry carcasses makes it possible for wild birds, free-range poultry, and scavenging mammals to feed on them and such birds may become sentinels for spread of new infection and by extension households with scavenging pets are continuously at risk of avian influenza zoonoses. There were no antibodies to influenza A viruses detected in sera of apparently healthy local chicken at the live bird markets and households in Enugu State Nigeria. This was in contrast with findings by Ameji *et al.*, (2016), who reported presence of antibodies to influenza A Viruses, (4.2%) in backyard and rural poultry chicken in Kogi State Nigeria, El-Yuguda and Baba (2002), reported the presence of antibodies to influenza A viruses in village chicken in Borno state, and (Gugong *et al.*, 2012) demonstrated the presence of influenza A subtype (H5) (2.9%) in local chicken in Kaduna state .

Local poultry are rarely vaccinated (Dipeolu *et al.*, 1998), the presence of antibodies to influenza A viruses in serum of bird may be due to natural

infection (Biswas *et al*, 2009; Wakawa *et al.*, 2009), exposure of naïve birds to reservoirs / carriers of avian influenza viruses, and prophylactic use of AI vaccines. It is instructive to note that following the resurgence of avian influenza in 2015, the federal government stepped up biosecurity measures across the country especially in states where outbreak has already been reported, the resultant (culling of infected poultry, carrier birds, decontamination of farms and recently compensation of affected farmers) these have played a major role in curbing spread of infection in poultry. This underscores the importance of continuous active disease surveillance, and biosecurity measures towards prevention and control of avian influenza outbreak (De Benedictis *et al.*, 2007), at every level of the poultry value chain. Survey of Ten (10) local chicken sellers who also keep other sentinel species at the live bird market shows 248 (50.1%) were local chickens (119, 24.4%), pigeon (76, 15.4%), and pigs (52, 10.5%). Rearing of pigeon with local poultry is a risk factor in the epidemiology of avian influenza (Coker *et al.*, 2014). Similarly, Pig has been reported as a potential sentinel for the adaptation of avian influenza viruses which may cause zoonotic disease's (Monne *et al.*, 2007), consequently rearing this species of animals together or in close proximity with local chicken should be discouraged

CONCLUSIONS AND RECOMMENDATIONS

This study has shown that local chickens may not have served as reservoirs of avian influenza in the study area within the time frame (April-May 2016). Consequently, consumption, rearing of local chickens may not pose any public health risk to the populace within the study period. Similarly, intensive farms where these birds 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 the human host. Mixing of different species of birds is very common within the live bird markets and this practice may provide the needed milieu for the emergence of new strains of influenza viruses. There should be sustained active

surveillance for avian influenza within Enugu State to compliment laboratory analysis and early detection and confirmation of suspected cases of avian influenza. Furthermore, 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 vigour on educating farmers on new approaches in tackling endemic and emerging diseases. Bearing in mind the level of mixing of different species of livestock at the live bird markets continuous active serosurveillance should be conducted with a view to determining possible new sentinels of infection. Rearing or mixing of different poultry birds and potential sentinel animal with local chicken should be discouraged toward stamping out of avian influenza from Nigeria and ensuring food safety.

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